Check for updates

Arteriosclerosis, Thrombosis, and Vascular Biology

REVIEW

Potential Mechanisms Underlying Bleeding During Infection With Hemorrhagic Fever Viruses

Megan V. Perkins, Nigel Mackman

ABSTRACT: Viral hemorrhagic fever (VHF) describes different diseases caused by several viruses from 6 virus families: *Filoviridae*, *Nairoviridae*, *Phenuiviridae*, *Hepadnaviridae*, *Arenaviridae*, and *Flaviviridae*. VHF was once considered a geographically localized problem, but due to expanding vector ranges and increased human contact with animal reservoirs and hosts, the number of VHF cases is increasing. As the name indicates, VHF is associated with bleeding. Both direct effects from viral infection of host cells and indirect effects caused by the host response to the virus contribute to dysregulation of the hemostatic system. Many studies have measured different parameters and various biomarkers in samples from infected humans and nonhuman primate models. For example, Ebola virus infection in a nonhuman primate model leads to increased TF (tissue factor) expression in peripheral blood mononuclear cells and extracellular vesicles. In dengue virus infection, thrombocytopenia and platelet dysfunction occur. There are likely both common and distinct mechanisms underlying bleeding in different VHFs, as sites of bleeding differ between the viruses. Herein, we discuss the potential mechanisms leading to bleeding during VHF, which include a consumptive coagulopathy, decreased coagulation factor production, thrombocytopenia and platelet dysfunction, and endothelial cell activation and damage, resulting in increased vascular permeability. While a significant body of work exists examining different aspects of the various viral infections that may lead to bleeding, there are still many open questions and areas for investigation. Therefore, more studies are needed to better understand the mechanisms underlying bleeding in VHF caused by different viruses.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: coagulation ■ platelets ■ tissue factor ■ vascular permeability ■ viral hemorrhagic fever

Downloaded from http://ahajournals.org by on January 27, 2026

Viral hemorrhagic fever (VHF) is a catch-all term for the disease induced by viruses designated as hemorrhagic fever viruses (HFVs). VHF generally begins with nonspecific, febrile symptoms that may progress to more severe disease, including hemorrhagic symptoms, with a variable clinical course. While all HFVs can induce hemorrhagic symptoms, including coagulopathy, thrombocytopenia, and bleeding, they do so to differing frequencies, severity, and possibly by different mechanisms. HFVs are from 6 virus families: *Filoviridae*, *Nairoviridae*, *Phenuiviridae*, *Hepadnaviridae*, *Arenaviridae*, and *Flaviviridae* (Figure 1). This review will discuss Ebola virus (EBOV), Marburg virus (MARV), Lassa fever virus (LASV), Rift Valley fever virus (RVFV), Crimean-Congo HFV (CCHFV), Hantaan virus (HTNV), Sin Nombre virus (SNV), yellow fever virus (YFV), and dengue virus (DENV). All of these viruses have small (<20 kilobases) RNA genomes. A brief overview of the virological information for each virus is presented in Table 1. The case presentation information is presented in Table 2. Importantly, the case presentation information for many of the viruses (eg, number of infections, number of cases presenting with severe and hemorrhagic manifestations, and case fatality rate [CFR]) is derived primarily from data on individuals presenting to a clinical setting or from epidemiological estimates. Notably, many individuals infected with some of these viruses experience subclinical disease or do not present to a clinic. Thus, these numbers should be considered as estimates based on available data. The geographic distribution of the different HFVs is shown in Figure 2.

Filoviridae: EBOV and MARV

EBOV belongs to the genus *Ebolavirus* that has 5 other species. Not all members of the *Ebolavirus* genus cause disease in humans. For those that do, the disease is

Correspondence to: Nigel Mackman, PhD, Department of Medicine, University of North Carolina at Chapel Hill, 116 Manning Dr 8004B Mary Ellen Jones Bldg, Chapel Hill, NC 27599. Email nmackman@med.unc.edu

For Sources of Funding and Disclosures, see page 371.

© 2025 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

CCHFV	Crimean-Congo hemorrhagic fever virus
CFR	case fatality rate
DF	dengue fever
EBOV	Ebola virus
EC	endothelial cell
EV	extracellular vesicle
EVD	Ebola virus disease
F	factor
F	factor
GP	glycoprotein
HCPS	Hantavirus cardiopulmonary syndrome
HFRS	hemorrhagic fever with renal syndrome
HFV	hemorrhagic fever virus
HTNV	Hantaan virus
ICAM-1	intercellular adhesion molecule-1
LASV	Lassa fever virus
MARV	Marburg virus
NHP	nonhuman primate
PAI	plasminogen activator inhibitor
RVFV	Rift Valley fever virus
s	soluble
SNV	Sin Nombre virus
TAM	TYRO3-AXL-MER
TF	tissue factor
tPA	tissue-type plasminogen activator
uPA	urokinase-type plasminogen activator
VCAM-1	vascular cell adhesion molecule-1
VE-cadherin	vascular endothelial cadherin
VEGF	vascular endothelial growth factor
VHF	viral hemorrhagic fever
vWF	von Willebrand Factor
YFV	yellow fever virus
ZO-1	zonula occluden-1

collectively called EBOV disease (EVD) and has a pooled CFR of ≈60% (Table 2).[24] Most individuals with EVD are symptomatic and experience severe disease (Table 2).[32] EVD can be separated into 3 phases: early, peak, and resolution. The early phase begins with febrile symptoms (eg, arthralgia, headache, fatigue, and rash) that progress to severe gastrointestinal symptoms (eg, nausea, vomiting, and diarrhea). During the early phase, hemorrhagic symptoms (eg, ecchymoses, bloody vomit or stool, bloody gums, conjunctival injection, nosebleeds, and bruising) may begin (Table 2). Hemorrhagic symptoms may occur in up to 50% of cases, but the incidence is highly variable (Table 2).[37] During the peak phase, about 4 to 12 days after disease

Highlights

- Viral hemorrhagic fever is a term collective referring to several different diseases caused by various viruses.
- Both direct effects from infection and the host immune response contribute to hemostatic dysregulation.
- We discuss herein the potential mechanisms underlying bleeding in viral hemorrhagic fever, including increased TF (tissue factor) expression, thrombocytopenia, platelet dysfunction, decreased coagulation factor synthesis, and endothelial cell activation and damage.

onset, viral load begins to peak. With an increase in viral load, symptoms, such as gastrointestinal and hemorrhagic symptoms, continue and may worsen. In addition, multiorgan dysfunction may be observed, including renal failure, respiratory failure, neurological symptoms (eg, meningoencephalitis and stroke), and cardiac problems (eg, myocarditis and pericarditis).[37] Individuals surviving EVD enter the resolution or convalescent phase and are more susceptible to secondary infections leading to sepsis. A significant proportion of surviving individuals also report long-term sequelae, including arthralgias, myalgias, visual and auditory changes, extreme fatigue, rashes, and gastrointestinal issues. The cause of such symptoms is unknown, but these secondary complications are debilitating for affected individuals.[44]

MARV is 1 of 2 species of the genus *Marburgvirus*. MARV causes MARV disease, which is separated into early, peak, and resolution phases and has a similar clinical course to EVD in terms of symptoms and overall timing.[3] There is no evidence of asymptomatic MARV disease currently. Similar to EBOV, MARV disease has a high proportion of cases with hemorrhagic manifestations (34%–83%) and an average CFR of 50% (Table 2).[25,28,38]

Arenaviridae: LASV

LASV belongs to the genus *Mammarenavirus*. There are 41 members of this genus, and only 8 of the members cause disease in humans, including LASV.[4] LASV is transmitted to humans primarily through contact with secretions from infected rodents (Table 1; Figure 2).[17] The primary rodent reservoir is the multimammate rat, which is found in Western Africa (Table 1).[17] The majority (≈80%) of individuals infected with LASV are asymptomatic, and only ≈20% of infected individuals progress to severe disease (Table 2).[17] Lassa fever progresses through 3 stages. The first is the viral prodrome, characterized by nonspecific febrile symptoms lasting 4 to 7 days. The peak phase occurs next and is characterized by a sore throat, chest pain, conjunctival injection, vomiting, diarrhea, and abdominal pain. These symptoms may then progress further to hypotension, shock, neurological symptoms (eg,

Downloaded from http://ahajournals.org by on January 27, 2026

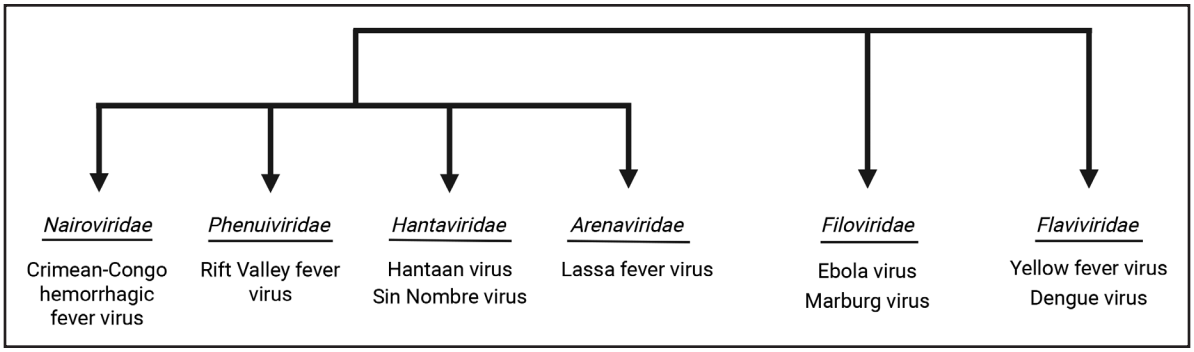

Figure 1. Overview of the phylogenetic relationship between viruses causing viral hemorrhagic fever.
An abbreviated phylogenetic relationship of the different viruses causing viral hemorrhagic fever is shown by virus family with each individual species shown below. The figure was made with BioRender.com.

altered consciousness, seizures, tremors, and hearing loss), head and neck edema, and hemorrhage from the mouth, nose, rectum, or vagina (Table 2). Overt bleeding can occur in up to 40% of clinical cases and has a strong association with mortality (Table 2).[17] The final phase is the convalescent phase. Survivors may suffer from long-term complications, such as hearing loss, seizures, cognitive impairment, and ophthalmic complications.[45]

Phenuiviridae: RVFV

RVFV belongs to the genus *Phlebovirus*, which has 67 species, but only 11 of those species, including RVFV, cause disease in humans.[46] RVFV geographic distribution follows that of its primary reservoir and vector, *Aedes* mosquitoes, and other mosquito and arthropod species that serve as secondary vectors (Table 1; Figure 2).[19] While mosquitoes can infect humans after taking a blood meal, most people become infected through contact with tissues and fluids from infected domesticated livestock.[19] RVFV induces severe and lethal disease in domesticated ruminates, causing large economic impacts in affected communities. Most humans infected with RVFV experience mild or subclinical symptoms.[19,21] Due to the mild nature of many RVFV infections in humans and differences between outbreaks, the exact incidence of RVFV is unclear. However, estimates suggest that half a million people were infected with RVFV between 1997 and

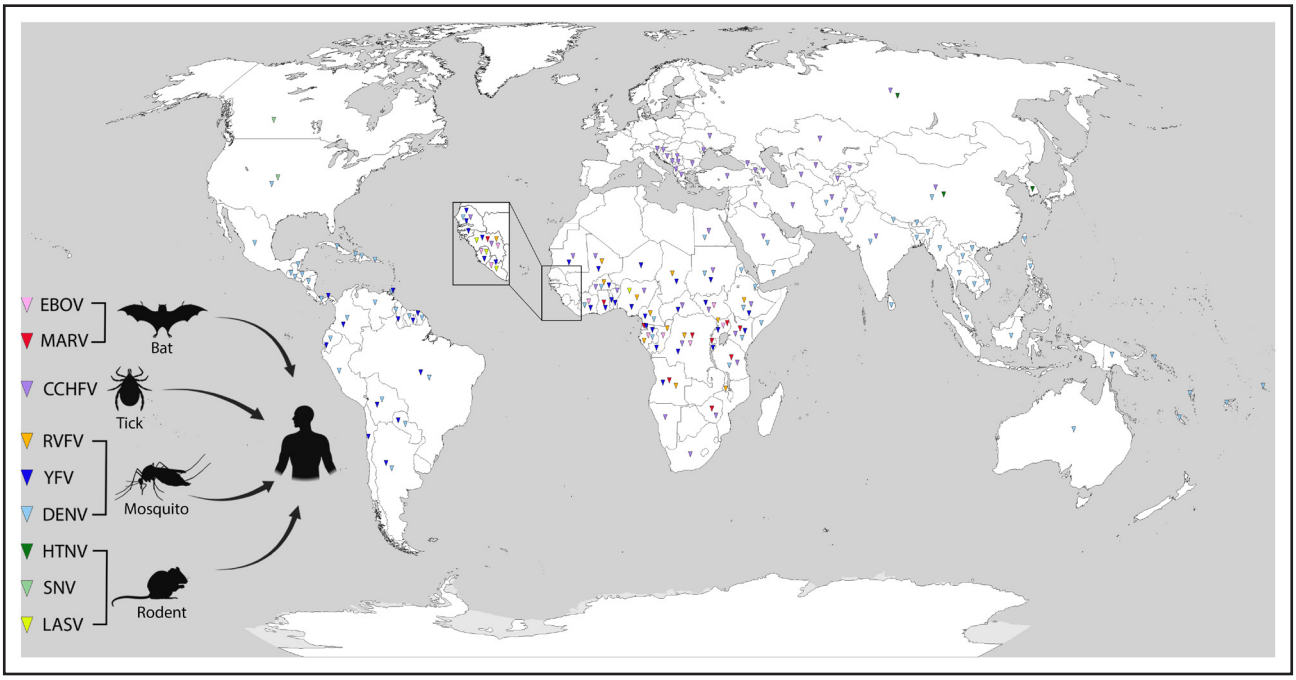

Figure 2. Geographic distribution of the different hemorrhagic fever viruses.
Inverted triangles color coded to represent each individual virus are shown on the map where the virus is known to circulate: Ebola virus (EBOV; pink), Marburg virus (MARV; red), Crimean-Congo hemorrhagic fever virus (CCHFV; purple), Rift Valley fever virus (RVFV; orange), yellow fever virus (YFV; dark blue), dengue virus (DENV; light blue), Hantaan virus (HTNV; dark green), Sin Nombre virus (SNV; light green), and Lassa fever virus (LASV; yellow green). The vectors and reservoirs for each virus are also shown, as the distribution of the viruses follows that of their vector and reservoir.

Downloaded from http://ahajournals.org by on January 27, 2026

Table 1. Information on the Viruses That Cause Viral Hemorrhagic Fever

	EBOV	MARV	LASV	RVFV	CCHFV	HTNV	SNV	YFV	DENV
Virus family, genus	*Filoviridae, Ebolavirus*	*Filoviridae, Marburgvirus*	*Arenaviridae, Mammarenavirus*	*Phenuiviridae, Phlebovirus*	*Nairoviridae, Orthonairovirus*	*Hantaviridae, Orthohantavirus*	*Hantaviridae, Orthohantavirus*	*Flaviviridae, Orthoflavivirus*	*Flaviviridae,* and *OOrthoflavivirus*
Primary cellular targets of the virus	Myeloid[1]	Myeloid[2,3]	Myeloid[4]	Hepatocytes Neurons Myeloid[5]	Hepatocytes Endothelial[6]	Endothelial Myeloid[7]	Endothelial Myeloid[7,8]	Hepatocytes Myeloid Endothelial[9,10]	Myeloid[11]
Organ(s) primarily affected	Liver, spleen, secondary lymphoid organs, and others later in infection[1]	Liver, spleen, secondary lymphoid organs, and others later in infection[3]	Liver and spleen[12,13]	Liver[14]	Liver[6]	Kidney[7]	Lung[15]	Liver[10]	Liver and spleen[16]
Geographic distribution	Sub-Saharan Africa[1]	Sub-Saharan Africa[3]	Western Africa[17]	Africa, Saudi Arabia, and Yemen[18,19]	Africa, Middle East, Southeast Asia, and Southern/Easter Europe[6]	Asia[7,20]	North America[7,20]	Sub-Saharan Africa and South America[10]	Sub-Saharan Africa, Central and South America, and Southeast Asia[16]
Mechanism of transmission	Contact with infected bodily fluids/tissues[1]	Contact with infected bodily fluids/tissues[3]	Contact with infected rodent/rodent secretions excretions (eg, urine and feces) Contact with infected bodily fluids[17]	Mosquito bite and contact with infected bodily fluids/tissues[19,21]	Tick bite Contact with infected bodily fluids[6]	Contact with infected rodent/rodent secretions excretions (eg, urine and feces)[7,20]	Contact with infected rodent/rodent secretions excretions (eg, urine and feces)[7,20]	Mosquito bite[10]	Mosquito bite[16]
Vector	N/A	N/A	N/A	*Culex* and *Aedes* spp.[19,21]	*Hyalomma* spp.[6]	*Apodemus agrarius*[7,20]	*Peromyscus maniculatus*[7,20]	*Aedes* spp. Haemagogus spp. Sabethes spp.[10]	*Aedes* spp.[16,22]
Reservoir host	Unknown: bats suspected[1]	*Rousettus aegyptiacus*[23]	Multimammate rat[17]	*Aedes* spp., rodents, wild ruminants, and bats[19]	*Hyalomma tick*[6]	*Apodemus agrarius*[7,20]	*Peromyscus maniculatus*[7,20]	Nonhuman primates[10]	Humans[22]

CCHFV indicates Crimean-Congo hemorrhagic fever virus; DENV, dengue virus; EBOV, Ebola virus; HTNV, Hantaan virus; LASV, Lassa fever virus; MARV, Marburg virus; RVFV, Rift Valley fever virus; SNV, Sin Nombre virus; and YFV, yellow fever virus.

2010.[18] Less than 1% of infected individuals develop hemorrhagic manifestations (Table 2).[19,21] Initial symptoms begin in a manner similar to other VHFs, with nonspecific febrile symptoms for 3 to 4 days. Hemorrhagic disease may begin about 1 week after symptom onset with the development of a macular rash, extended ecchymoses, gum or gastrointestinal bleeding, and bloody stools or vomit.[21] Hemorrhagic symptoms frequently portend a fatal outcome. While the overall CFR for RVFV is low (<1%), it can be up to 50% in cases with hemorrhagic symptoms (Table 2). Other severe disease manifestations for RVFV infection include ocular (up to 10% of cases) and encephalitic (<1% of cases) symptoms.[19,21]

Nairoviridae: CCHFV

The *Hyalomma* tick is the primary reservoir and vector of CCHFV (Table 1; Figure 2). Humans, livestock, and wild animals can become infected with CCHFV through the bite of an infected tick. Humans may also become infected upon handling parts or fluids of infected animals. Most infections (up to 88%) with CCHFV are thought to be asymptomatic.[33] Of the ≈12% of infections that are severe enough to cause individuals to seek medical help, hemorrhagic symptoms (eg, nosebleeds, ecchymoses, and blood vomit) can occur in up to 50% of individuals (<6% of all infections; Table 2).[33] As with other VHFs, initial symptoms of CCHFV infection are nonspecific and febrile.[6] A week or less after symptom onset, the hemorrhagic phase begins. Hemorrhagic disease is characterized by mucous membrane and skin petechiae and extended ecchymoses, nosebleeds, coughing up blood (hemoptysis), bleeding from injection sites, and bloody stools, vomit, or urine (Table 2). These symptoms usually persist for 2 to 3 days. Severe cases may develop disseminated intravascular coagulation, hypovolemic shock,

Downloaded from http://ahajournals.org by on January 27, 2026

or multiorgan failure, including that of the liver and lungs. Survivors can suffer from a variety of complications, including hypotension, arrhythmias, difficulty breathing, and issues with their vision, hearing, or memory. Long-term complications remain undefined.[6]

Hantaviridae: HTNV and SNV

Orthohantaviruses are divided into Old World (eg, HTNV) and New World (eg, SNV) based on their geographic distributions. Old World viruses are primarily found throughout Europe and Asia, while New World viruses are found primarily in the Americas (Table 1; Figure 2).[20] Of the 60 different viruses in the genus *Orthohantavirus*, 28 viruses, including HTNV and SNV, are clinically important.[7,20]

HTNV and other Old World *Orthohantaviruses* cause hemorrhagic fever with renal syndrome (HFRS). Moderate (50%) and severe disease (20%) account for up to 70% of clinical HTNV cases and are differentiated by the severity of symptoms, such as bleeding (Table 2).[34] The overall CFR for HTNV infection is about 1%.[7,20] HFRS can be divided into 5 phases.[7,34] The first is a nonspecific febrile phase lasting a week or less, which progresses into a hypotensive phase. Hemorrhagic manifestations occur in <30% of cases and may begin near the end of the febrile phase.[34,39] Hemorrhagic symptoms include conjunctival hemorrhages and petechiae in the mouth (Table 2).[7] The hypotensive phase is variable in duration (hours to a couple of days) and is characterized by hypotension that may lead to hypovolemic shock. Hemorrhagic signs may increase during this phase, including bruising, petechiae on the skin and mucosal surfaces, reddening of the conjunctiva, nose bleeds, intracranial hemorrhage, and bloody vomit, urine, or stools (Table 2).[7] Following this phase is the oliguric phase. This phase lasts less than a week and is characterized by temporarily decreased kidney function, which leads to low urine output (oliguria) or other abnormal characteristics (eg, no urine output, increased protein, or blood in the urine). Up to a third of individuals with severe HFRS die during the hypotensive phase, and about 50% die during the oliguric phase.[7] The next phase is the polyuric phase, where the body begins to return to normal, including a recovery of kidney function and urinary output. This phase can last up to several weeks. The final phase is convalescence, which can last up to half a year. Chronic renal failure and hypotension are rare complications of HFRS.[7]

SNV and other New World *Orthohantaviruses* cause Hantavirus cardiopulmonary syndrome (HCPS). Like HFRS and other VHFs, HCPS begins with a nonspecific febrile phase that is usually short (<1 week).[7] The cardiopulmonary phase onsets suddenly in >50% of cases with the rapid development of a cough, shortness of breath, noncardiac pulmonary edema, pleural effusions, hypotension, and tachycardia. Cardiogenic shock, lactic acidosis, respiratory failure, and decreased plasma volume may also occur in severe cases.[7] HCPS has a high CFR relative to HFRS (35% versus 1%; Table 2). Individuals surviving the cardiopulmonary phase enter the polyuric phase, where pulmonary edema resolves and the body begins to return to normal. Convalescence is the final stage. While recovery is slow, survivors do not typically have any long-term complications that are directly attributed to viral infection rather than intense hospitalization. Notably, while SNV is considered an HFV, severe bleeding is not a hallmark symptom of the pathology (Table 2).[7,20]

Flaviviridae: YFV and Dengue Virus

YFV is an *Orthoflavivirus*. There are 53 total *Orthoflaviviruses*, with over half causing disease in humans. Yellow fever begins with nonspecific febrile symptoms. Approximately 20% to 60% of individuals enter a remission phase for up to 2 days before progressing to the toxic phase, which is marked by yellowing of the skin or eyes (leading to the name yellow fever) caused by liver and renal dysfunction or failure, thrombocytopenia, and hemorrhagic symptoms (Table 2).[9,10] Hemorrhagic manifestations occur in ≈12% of cases and include petechiae, bruising, bloody gums, and bloody vomit. Before death, individuals may also experience encephalitic manifestations, such as confusion, seizures, and coma. For individuals surviving yellow fever, long-term complications are rarely reported.[9,10]

DENV is also an *Orthoflavivirus*. The disease caused by DENV has historically been broadly classified as an acute febrile illness (dengue fever [DF]), a severe illness with fever, hemorrhagic symptoms, thrombocytopenia, and evidence of plasma leakage, such as increased/progressively increasing hematocrit or decreased albumin in the plasma or blood (dengue hemorrhagic fever), or dengue hemorrhagic fever with additional circulatory failure (dengue shock syndrome). In 2008, the World Health Organization reclassified the DENV presentations as DF, DF with warning signs, and severe DF. The primary difference between DF with warning signs and severe DF is the severity of the symptoms, where individuals with severe DF have severe plasma leakage, which can lead to dengue shock syndrome, severe bleeding, and severe spleen and liver enlargement.[47] All types of DF can be broadly divided into the febrile, toxic, and recovery phases. Signs of severe disease occur during the toxic phase, including hemorrhagic symptoms. Hemorrhagic manifestations occur in 0.5% to 5% of clinically presenting DENV cases and include petechiae, bruising, and bloody vomit and stool (Table 2).[11,16] There are 4 distinct DENV serotypes, which can be thought of as distinct viruses. Importantly, most severe DENV infections occur upon reinfection with a different DENV serotype. This is due

Downloaded from http://ahajournals.org by on January 27, 2026

Table 2. Case Presentation of Viral Hemorrhagic Fever Caused by Different Viruses

	EBOV	MARV	LASV	RVFV	CCHFV	HTNV	SNV	YFV	DENV
Global incidence, cases/y	Variable, usually small <300*[3,24]	Variable, usually small <300[3,25]	Estimated 100 000–300 000[17]	Variable and outbreak-dependent[18]	Estimated 10 000–15 000†[26]	Estimated >10 000[20,27]	30[20]	200 000[10]	>100 000 000†[11]
Case fatality rate, %	60[24]	50[28]	1[29]	<1[30]	5–40[31]	≈1[7,20]	35[20]	>20[10]	<1[11]
Cases having severe manifestations, %	60–85[32]		20[17,29]	<5[30]	12[33]	≈20[34]	>50[35]	12[36]	0.5–5[11]
Cases having hemorrhagic manifestations, %	<50 (variable)[37]	34–83 (variable)[38]	40[17,29]	<1[30]	3.6–6[33]	<30 (variable)[34,39]	N/A	12[36]	0.5–5[11]
Case fatality rate for cases with hemorrhagic/severe manifestations, %			15–70[17,29]	<50[30]		5–15[7]	>50[35]	30–60[10,36,40]	Variable‡
Outward signs of hemorrhagic disease	Maculopapular rash Petechiae Bleeding/bruising Bloody vomit/stool Bloody/red conjunctiva[3,37]	See EBOV	Bleeding from the mouth, nose, rectum, and vagina[17]	Macular rash Bruising Blood gums, stools, or vomit[21]	Petechiae Bruising Nosebleed Coughing up blood Bloody stools, vomit, or urine[33]	Petechiae Bruising Bloody conjunctiva Bloody stools, vomit, or urine[7,20]	No outward signs of bleeding[7,20]	Jaundice Liver and renal dysfunction Petechiae Bruising Bloody gums or vomit[9,10]	Petechiae Bruising Bloody vomit/stool[16]
Approved vaccine available	Yes[41]	No[25]	No[42]	Only for livestock[14,19]	No[6]	No[20]	No[20]	Yes[9,10,36]	No§

CCHFV indicates Crimean-Congo hemorrhagic fever virus; DENV, dengue virus; EBOV, Ebola virus; HTNV, Hantaan virus; LASV, Lassa fever virus; MARV, Marburg virus; RVFV, Rift Valley fever virus; SNV, Sin Nombre virus; and YFV, yellow fever virus.

*The 2013–2016 outbreak was the largest on record (>28 000 cases).

†Many cases are asymptomatic, making true incidence difficult to estimate.

‡Mortality for severe dengue can be up to 20%, but, with proper supportive care, the mortality can be as low ≈1%.[11]

§There are 2 approved vaccines and 1 vaccine in phase 3 trials; however, the safety and efficacy of such vaccines are hotly debated.[16,43]

to antibody-dependent enhancement, in which antibodies from the first infection with 1 DENV serotype bind and enhance infection of a different DENV serotype by facilitating entry into target cells expressing Fcγ receptors. This aspect of DENV infection has also complicated vaccine development (Table 1).[43]

THE HEMOSTATIC SYSTEM

Hemostasis is defined as the process that stops bleeding following blood vessel injury. The hemostatic system has several different components, including platelets, the coagulation system, the fibrinolytic system, the endothelium, and the blood vessel wall. Under normal conditions, these components are in balance, leading to the formation of a hemostatic plug comprised of platelets and cross-linked fibrin that stops bleeding. However, during disease, the hemostatic system can become dysregulated, leading to bleeding and thrombosis.

The Coagulation System

The coagulation system contains procoagulant factors that are required for the formation of fibrin and anticoagulant factors that regulate different points of the coagulation cascade. The coagulation cascade can be divided into the extrinsic, intrinsic, and common pathways. The extrinsic pathway (TF [tissue factor]/F [factor] VIIa complex) primarily initiates blood coagulation. Under normal conditions, TF is expressed by cells underlying the vascular endothelium.[48] Damage to a blood vessel leads to the activation of the coagulation protease cascade by the TF/FVIIa complex.[48] The intrinsic pathway is initiated by the activation of FXII.[49] FXIIa activates FXI to FXIa, which then activates FIX to FIXa. FIXa in association with its cofactor FVIIIa forms the intrinsic Xase complex. The common pathway includes the prothrombinase complex (FXa/FVa) that converts prothrombin to thrombin. Thrombin is the central protease of the coagulation cascade, cleaving fibrinogen to form fibrin monomers and activating

Downloaded from http://ahajournals.org by on January 27, 2026

the transglutaminase FXIII, the cofactors FV and FVIII, as well as platelets.[49] It should be noted that FXIIa can also activate prekallikrein to kallikrein, which cleaves high molecular weight kininogen, resulting in the liberation of bradykinin, which increases vascular permeability.[49]

Fibrinogen is comprised of 2 copies each of 3 chains: Aα, Bβ, and γ.[50] Fibrinogen is assembled in hepatocytes and secreted as a soluble protein.[50] Upon cleavage by thrombin, fibrinogen is converted to fibrin monomers. These monomers oligomerize and eventually form protofibrils. These protofibrils aggregate to form an insoluble fibrin mesh. The stability of the fibrin mesh is further enhanced through cross-linking by FXIIIa.[50]

Platelets

Platelets are small, anucleate blood cells produced from megakaryocytes in the bone marrow.[51] They are rapidly recruited to sites of vessel injury. Initial platelet tethering to the injured vessel is mainly mediated by the platelet receptor GPIb-V-IX binding vWF (von Willebrand Factor) that is deposited from the plasma or released from activated endothelial cells (ECs). Firm adhesion requires the interaction of various platelet integrins with components in the extracellular matrix. Next, binding of soluble agonists, such as thrombin, ADP, or thromboxane A2, to platelet G protein–coupled receptors activates the platelets. Finally, binding of fibrinogen to activated $\alpha_{IIb}\beta_3$ on platelets mediates platelet aggregation.[51]

The Endothelium

The vascular endothelium is an antithrombotic surface that inhibits the activation of platelets and coagulation.[52] For example, ECs inhibit platelet activation by expressing CD39, which degrades ATP, and by releasing nitric oxide and prostacyclin. The endothelium also expresses anticoagulant proteins, such as TF pathway inhibitor and components of the protein C system (eg, thrombomodulin and endothelial protein C receptor), as well as molecules (eg, heparin sulfates) that bind antithrombin. In addition, ECs release tPA (tissue-type plasminogen activator), which promotes fibrinolysis.[52] The integrity of the EC barrier is maintained by tight junctions and adherens junctions between adjacent ECs and the EC glycocalyx.[53,54] Under pathological conditions, however, the endothelium is converted into a prothrombotic surface by the release of vWF and P-selectin from Weibel-Palade bodies, downregulation or shedding of anticoagulant proteins (eg, thrombomodulin), and induction of TF expression.[52]

The Fibrinolytic System

The fibrinolytic system generates plasmin that degrades cross-linked fibrin. tPA and uPA (urokinase-type plasminogen activator) activate plasminogen to plasmin.[55] tPA and plasminogen bind to fibrin, which enhances tPA activation of plasminogen. uPA binds to the uPA receptor on cells to activate plasminogen. Upon activation, plasmin cleaves cross-linked fibrin to generate fibrin degradation products (FDPs), such as D-dimer.[55] tPA and uPA are inhibited by PAI (plasminogen activator inhibitor)-1 and PAI-2. PAI-1 has a higher affinity for tPA, while PAI-2 has a higher affinity for uPA. Plasmin is inhibited by α2-antiplasmin. Thrombin also activates thrombin-activatable fibrinolysis inhibitor, which cleaves plasminogen binding sites in fibrin.[55]

MECHANISMS OF HEMOSTATIC DERANGEMENT IN VHF

In this section, possible mechanisms leading to bleeding during VHF are discussed. Many different biomarkers have been measured in both humans and nonhuman primates (NHPs) during VHF. Unfortunately, most human data can be thought of as a single snapshot that may or may not accurately represent the total infection course. In addition, most timepoints from humans are based on symptom onset. Because many of the initial symptoms of VHF can be mild and nonspecific, pinpointing the exact timepoint during infection when a sample was acquired from an infected individual can be difficult and are estimates at best. Thus, data from NHP models have been key to exploring and elucidating the mechanisms underlying bleeding during VHF. Tables 3 and 4 summarize various hemostatic-related parameters and biomarkers in both humans and NHPs infected with the different HFVs, respectively.

Bleeding can arise from either the consumption of coagulation factors and platelets or inadequate synthesis of coagulation factors and platelet production. Coagulation activation is part of the host response to viral infection and is thought to limit pathogen dissemination and prevent plasma leakage resulting from damage due to infection.[56] However, viral infection can lead to disseminated intravascular coagulation that results in the consumption of coagulation factors (consumptive coagulopathy) and platelets (thrombocytopenia), leading to bleeding. The International Society on Thrombosis and Hemostasis recently updated the scoring criteria for the clinical diagnosis of disseminated intravascular coagulation (Table 5).[57] An increase in the prothrombin time and a decrease in fibrinogen indicate the consumption of coagulation factors. A low platelet count constitutes thrombocytopenia, and D-dimer is a marker of both coagulation activation and fibrinolysis. Severe coagulopathies, including disseminated intravascular coagulation, are life threatening.[57]

The Coagulation System Is Activated During VHF

Coagulation activation is a feature common to nearly all HFV infections. TF is a transmembrane protein that

Downloaded from http://ahajournals.org by on January 27, 2026

Table 3. Hemostatic-Related Parameters and Biomarkers in Humans Infected With Different Viruses Causing Viral Hemorrhagic Fever

Parameter	EBOV	MARV	LASV	RVFV	CCHFV	HTNV	SNV	YFV	DENV
PT	Increased[197]		Increased*†[73,198–200]	Increased[74]	Increased*[75,76,133,201,202]	Normal‡[77,78]	Increased[8,15]	Increased[79,80]	Normal[100,183,184]
aPTT	Increased[197]	Increased[81,203,204]	Increased†[200]	Increased[74]	Increased*[75,76,82,83,133,201,202]	Increased‡[77,185]	Increased[8,15]	Increased[80]	Increased[100,183,184]
TAT			Normal[68]				Increased‡[70]		Increased[100,186]
D-dimer/FDP	Increased‡[161,162,187]	Increased[81]	Increased[188]	Increased[69,176]	Increased*[75,76,133,189,190]	Increased‡[77,185]	Increased[15,70]	Increased[80,84]	Increased[100,183,186]
Thrombocytopenia	Yes[85,197]	Yes[81,203,204]	No*[86,198–200]	Yes[69,74,87,88]	Yes[75,82,83,133,189,190,202]	Yes‡[77,78,185]	Yes‡[15,205]	Yes[79,80]	Yes[89,100,184]
TF	Increased‡[60]		Increased[68]				Increased[70]		Increased[64–66]
tPA	Increased[161]		Increased[188]	Increased†[176]			Increased[191]		Increased[100,184]
PAI-1			Increased[68,188]	Normal†[176]			Increased[191,194]		Increased[100,184]
Elevated EC activation or EC glycocalyx disruption markers	s-TM s-ICAM-1 s-P-selectin[162]		s-TM s-ICAM-1 s-VCAM-1 s-vWF[68]	s-vWF s-VEGF[176]	s-ICAM-1[173,174] s-vWF[173]		s-VEGF[144]	Syndecan-1[165]	Syndecan-1[166]

aPTT indicates activated partial thromboplastin time; CCHFV, Crimean-Congo hemorrhagic fever virus; DENV, dengue virus; EBOV, Ebola virus; EC, endothelial cell; FDP, fibrin degradation product; HTNV, Hantaan virus; ICAM-1, intercellular adhesion molecule-1; LASV, Lassa fever virus; MARV, Marburg virus; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; RVFV, Rift Valley fever virus; s, soluble; SNV, Sin Nombre virus; TAT, thrombin antithrombin; TF, tissue factor; TM, thrombomodulin; tPA, tissue-type plasminogen activator; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; vWF, von Willebrand Factor; and YFV, yellow fever virus.

*Minority of studies show normal values or the opposite result.

†Limited patient number.

‡Not all separated by viral strain/serotype.

localizes FVII/FVIIa to the surface of cells and extracellular vesicles (EVs). The TF/FVIIa complex is the primary activator of coagulation in vivo, and TF expression is increased in many diseases.[48] However, measuring TF in plasma is difficult due to its low abundance.[58,59]

A landmark study demonstrated that EBOV increases TF expression in peripheral blood mononuclear cells (notably the monocyte) in vitro and in vivo using an NHP model.[60] Whether other cell types also upregulate TF following EBOV infection is unknown. In addition, a recent study showed that there is increased EV-TF activity in plasma from EBOV-infected NHPs.[61] TF expression is central to EBOV pathogenesis, as administration of the TF/FVIIa inhibitor NapC2 (nematode anticoagulant protein c2) reduced disease (eg, delayed appearance of characteristic rash and lower D-dimer) and prolonged and increased survival in EBOV-infected NHPs.[62]

Infections with MARV in an NHP model also led to increased TF expression in peripheral blood mononuclear

Table 4. Hemostatic-Related Parameters and Biomarkers in Nonhuman Primates Infected With Different Viruses Causing Viral Hemorrhagic Fever

Parameter	EBOV	MARV	LASV	RVFV	CCHFV	HTNV	SNV	YFV	DENV*
PT	Increased[101]	Increased[2]	Increased[102,127]	Increased†[192,206,207]			Increased[90]	Increased[84,208]	
aPTT	Increased[101,136]	Increased[2]	Increased[102,127]	Increased[192,206,207]	Increased[209]		Increased[90]	Increased[208]	
TAT									
D-dimer/FDP	Increased[60,62,136,193]	Increased[2,63]	Increased[102,103,127]	Increased[192]			Normal[90]	Increased[84,208]	
Thrombocytopenia	Yes[60,101,136,193,195]	No†[2,63,210]	No†[102,103,127,211,212]	Yes[192,207]	Yes[209,213]		No[90]	Yes[208]	
TF	Increased[60–62]	Increased[63]							
tPA	Increased[60,195]								
PAI-1	Increased[195]								

aPTT indicates activated partial thromboplastin time; CCHFV, Crimean-Congo hemorrhagic fever virus; DENV, dengue virus; EBOV, Ebola virus; FDP, fibrin degradation product; HTNV, Hantaan virus; LASV, Lassa fever virus; MARV, Marburg virus; PAI-1, plasminogen activator inhibitor-1; PT, prothrombin time; RVFV, Rift Valley fever virus; SNV, Sin Nombre virus; TAT, thrombin antithrombin; TF, tissue factor; tPA, tissue-type plasminogen activator; and YFV, yellow fever virus.

*Does not cause overt clinical manifestations in nonhuman primates.

†Consensus of the majority of studies.

Downloaded from http://ahajournals.org by on January 27, 2026

Table 5. Disseminated Intravascular Coagulation Scoring Criteria According to the International Society on Thrombosis and Hemostasis

Parameter	Values	Score
Platelet count ($\times 10^9$/L)	<50	2
	50–100	1
Prothrombin time, s	Increased ≥6	2
	Increased 3–6	1
Fibrinogen, mg/dL	<100	1
D-dimer	>7× upper normal limit	3
	>3× upper normal limit	2

cells compared with controls.[63] However, the administration of NapC2 was not protective in a MARV NHP model.[63] The authors noted that the virus isolate used was more virulent than other strains previously used in the same NHP model.[63] While inhibition of the TF/FVIIa complex was not effective in preventing severe disease in this MARV model, it does not preclude the possibility that TF/FVIIa inhibition may be effective for less virulent MARV strains.

Several studies have measured plasma levels of TF antigen in DENV-infected individuals. It is difficult to compare these studies because they use different assays to measure TF and samples from patients with varied disease severities. One study found increased plasma TF in severe DF compared with DF.[64] Interestingly, the healthy control group and the DF group did not differ significantly. This could be due to the timing of sample collection, as samples were collected 1 to 15 days post-symptom onset, which, on the extreme end, may be past the window of induction.[64] A study examining TF expression in the plasma of children with severe DF at different times during disease observed that children with severe DF had high levels of plasma TF at hospital admission that decreased over time.[65] In addition, peripheral blood mononuclear cells from individuals with severe DF had increased TF protein expression compared with those with DF or healthy controls.[66] A fourth study found a trend towards increased EV-TF activity in individuals with DF compared with healthy controls.[67] However, the point during the disease at which the samples were collected is unclear.[67]

Individuals with LASV had higher levels of plasma TF protein compared with non-LASV febrile controls.[68] In fatal cases of RVFV, plasma TF antigen is also increased.[69] An increase in plasma EV-TF activity was also observed in individuals infected with SNV.[70] However, the cellular source of TF during LASV, RVFV, and SNV infections remains to be explored. An increase in EV-TF activity in the plasma of individuals infected with Puumala virus, a close relative of HTNV, has also been noted and was associated with intravascular coagulation.[71]

TF levels during other HFV infections have not been measured. In addition, because all the HFVs examined in this review are enveloped, the new virions derived from TF-positive host cells may incorporate TF into the viral envelope as has been shown for several herpesviruses.[72] However, this remains to be established for HFVs. In summary, increased TF on peripheral blood mononuclear cells (likely monocytes), other cell types, and EVs is likely the primary activator of the coagulation cascade observed in VHF (Figure 3). Understanding the triggers that activate coagulation in VHF may identify new therapeutic targets to prevent coagulation activation during VHF.

VHF Is Associated With Liver Damage

VHF is associated with liver damage in humans and NHP models, as evidenced by increased aspartate aminotransferase and alanine aminotransferase and liver pathology.[68,69,73–99] The liver is the primary site for synthesis of most coagulation factors, including prothrombin and fibrinogen. Hepatocytes are also the primary source of the anticoagulant protein C. Indeed, the level of protein C decreased in humans and NHPs infected with HFVs (EBOV, MARV, LASV, YFV, and DENV).[2,60,62,63,80,100–103] Several of the HFVs (EBOV, MARV, LASV, RVFV, CCHFV, and YFV) are hepatotropic, meaning that they directly infect hepatocytes (Table 1). Some of these viruses are also cytopathic in cultured hepatocytes (EBOV, LASV, CCHFV, and YFV), while others are not (MARV).[104–108] From examination of pathological specimens, hepatocytes stain positive for viral antigen and show signs of apoptosis and necrosis. However, whether this is due to viral replication or immune-mediated mechanisms cannot be determined. In addition to directly killing hepatocytes, these viruses may also lead to generalized hepatocyte dysfunction upon infection, which is further increased by severe inflammation. For the nonhepatotropic viruses (HTNV, SNV, and DENV), general liver dysfunction or pathology likely arises through immune-mediated mechanisms. Ultimately, both processes lead to a reduction in coagulation factor and protein C synthesis (Figure 3).

Thrombocytopenia and Platelet Dysfunction During VHF

Thrombocytopenia can be due to decreased platelet production, increased platelet activation and consumption, or a combination thereof. The role of platelets during HFV infection is best characterized for DENV.[109] Both decreased platelet production and increased activation and consumption contribute to thrombocytopenia during DENV infection. Transient bone marrow suppression and infection of megakaryocytes may contribute to decreased platelet production early during infection.[110–112]

During the toxic phase of DENV infection in which thrombocytopenia is a key feature, the bone marrow is normal, suggesting that increased platelet clearance and

Downloaded from http://ahajournals.org by on January 27, 2026

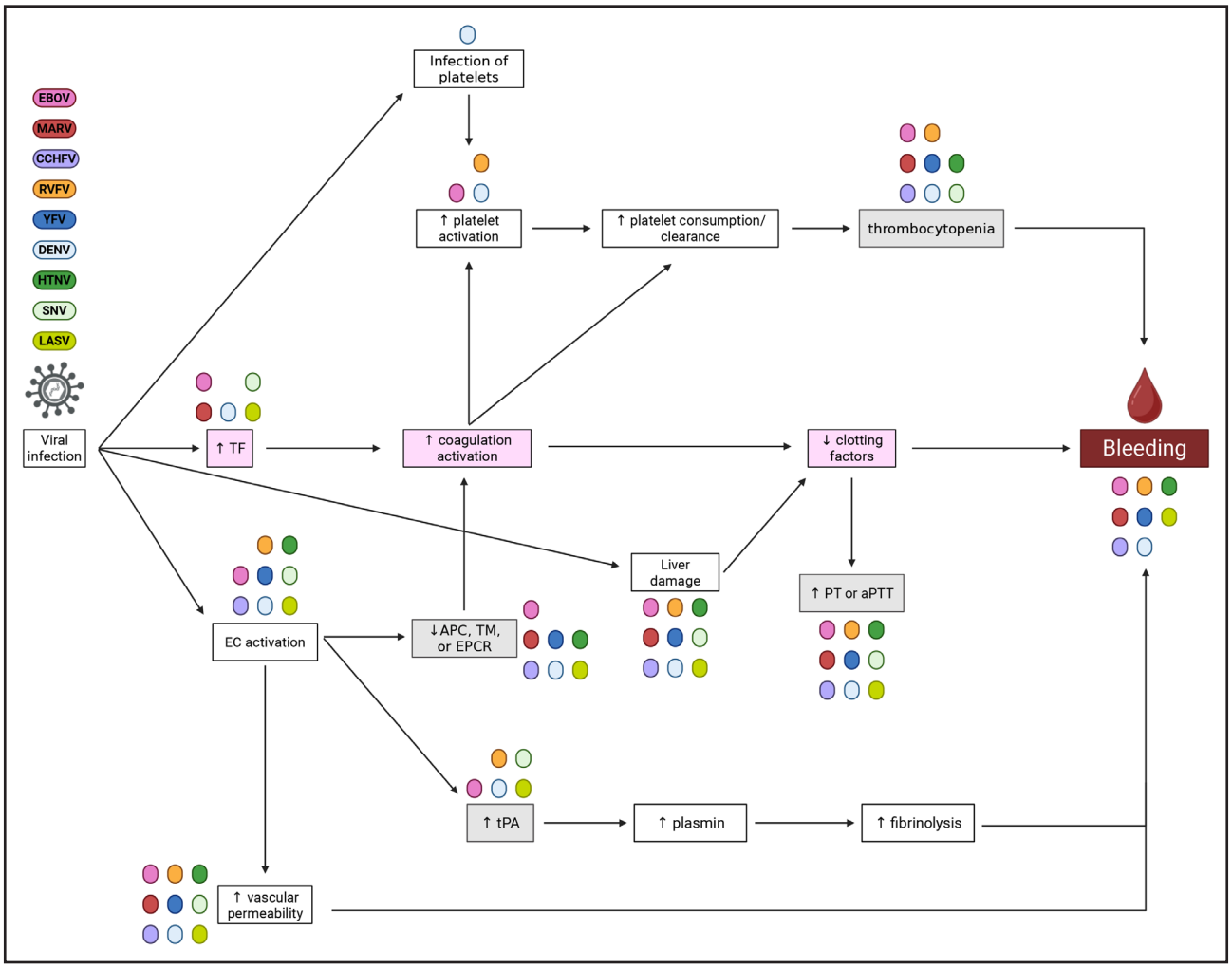

Figure 3. Potential mechanisms underlying bleeding during viral hemorrhagic fever.
Viral infection induces TF (tissue factor) expression in host cells, resulting in coagulation activation and consumption of clotting factors. Liver infection leads to decreased production of clotting factors. Coagulation activation and viral infection lead to platelet activation and clearance, resulting in thrombocytopenia. Viral infection also activates endothelial cells (ECs), leading to increased vascular permeability and decreased protein C anticoagulant activity. Activated ECs also release tPA (tissue-type plasminogen activator), resulting in increased plasmin and enhanced fibrinolysis. Cumulatively, these mechanisms result in bleeding during viral hemorrhagic fever. For each potential mechanism, a colored dot corresponding to each virus that has evidence supportive of that mechanism is shown: Ebola virus (EBOV; pink), Marburg virus (MARV; red), Crimean-Congo hemorrhagic fever virus (CCHFV; purple), Rift Valley fever virus (RVFV; orange), yellow fever virus (YFV; dark blue), dengue virus (DENV; light blue), Hantaan virus (HTNV; dark green), Sin Nombre virus (SNV; light green), and Lassa fever virus (LASV; yellow green). The figure was made with BioRender.com. APC indicates activated protein C; aPTT, activated partial thromboplastin time; EPCR, endothelial protein C receptor; PT, prothrombin time; and TM, thrombomodulin.

consumption are underlying thrombocytopenia. Platelets isolated from individuals infected with DENV are activated, exhibiting increased surface expression of active $\alpha_{IIb}\beta_3$, P-selectin, and phosphatidylserine.[113–116] Platelets from individuals with DENV are also less responsive to in vitro agonist stimulation compared with those from convalescent individuals or healthy controls. This suggests that platelets from infected individuals may be exhausted and have reduced function, and as such, cannot adequately respond to stimuli.[115] In addition, some platelets isolated from individuals with DENV are apoptotic.[113,114]

Platelet activation likely occurs through several mechanisms during DENV infection. Both viral RNA and antigen have been detected in isolated human platelets.[116,117] This observation in combination with human platelets supporting DENV replication in vitro suggests that DENV may replicate to some extent in platelets in vivo.[116–118] However, whether platelets produce new virions in vitro or in vivo is hotly debated, and platelets likely do not contribute to viremia.[119] The viral protein NS1 (nonstructural protein 1), which is released into the circulation during infection, can also activate platelets directly.[120,121] The levels of NS1 are positively correlated to disease severity and the development of thrombocytopenia.[121] Indeed, in vitro NS1-stimulated platelets have increased levels of phosphatidylserine and P-selectin

Downloaded from http://ahajournals.org by on January 27, 2026

surface expression, as well as prolonged aggregation following in vitro agonist stimulation.[122] In a DENV mouse model that develops thrombocytopenia, genetic deletion of NS1 prevented thrombocytopenia.[122] Activated platelets may also bind to activated ECs.[122] EC-bound platelets are removed from the circulation and cannot be measured during routine blood counts, ultimately contributing to the observed thrombocytopenia.

Activated platelets express various receptors that mediate their clearance.[123] Both increased phosphatidylserine exposure and the coating of platelets with antibodies or complement enhance engulfment by phagocytes. Both antiplatelet antibodies and antibody- and complement-coated platelets have been isolated from individuals with DENV.[116,124] Moreover, increased platelet-phagocyte aggregates have been isolated from individuals with DENV; additionally, phagocytosis of platelets isolated from individuals with DENV compared with healthy controls was increased in vitro, and this correlated with disease severity.[113] Exposure of platelets to NS1 also led to increased phagocytosis in vitro, suggesting a mechanism by which phagocytosis may be stimulated in vivo.[122] Platelets may also be removed by phagocytes through opsonization or lysed via complement activation and subsequent formation of the membrane attack complex.[124,125]

Platelets can also contribute to inflammation during DENV infection via the release of various cytokines, which can activate other cell types and increase vascular permeability.[109] DENV-infected platelets may also be an important source of secreted NS1, which directly contributes to increased vascular permeability in addition to its role in platelet activation.[126]

Thrombocytopenia occurs mainly during severe LASV infection and rarely in nonsevere infection. In NHPs, platelet survival time is normal. However, platelets from both humans and NHPs with severe LASV infection exhibit aggregation defects in response to in vitro agonist stimulation.[68,127,128] In a follow-up study, human plasma from severe LASV cases inhibited aggregation of platelets collected from healthy donors. Platelet aggregation activity was restored following washing of the platelets, suggesting the presence of a platelet inhibitor in the plasma of individuals with severe LASV.[129] Aggregation curves also show that platelets from individuals with LASV disaggregate, which may be suggestive of defects in granule release, which is known to sustain platelet aggregation over time.[68,128] Improved aggregation responses also coincided with clinical improvement, suggesting that platelet inhibition contributes to the pathology of LASV.[128]

Pathogenic *Orthohantaviruses* (eg, HTNV and SNV) bind the inactive form of β_3 integrins.[130] This may lead to defective platelet aggregation and adhesion responses. Indeed, regardless of illness severity, platelets from individuals with HFRS exhibit aggregation and granule release defects in response to in vitro agonist stimulation that improve as illness resolves.[131] However, the defects in severely ill individuals were worse compared with moderately and mildly ill individuals. These properties seem to be intrinsic to the platelets themselves, rather than due to their environment (eg, plasma), as mixing normal platelets with HFRS plasma led to normal aggregation. In addition, quiescent platelets bind infected ECs via cell-surface displayed viral GPs (glycoproteins) in vitro, which may lead to reduced circulating platelet numbers.[130]

Individuals with CCHFV have an increased immature platelet fraction and mean platelet volume compared with healthy individuals.[132] This suggests that the bone marrow is likely not suppressed, and thrombocytopenia during CCHFV infection is due to increased activation and consumption rather than decreased production. However, studies on bone marrow specimens show conflicting results, with some showing a normal marrow with respect to megakaryocyte numbers and platelet production and others showing decreased megakaryocyte numbers.[133–135] It remains unclear if platelets are activated or if they exhibit defective aggregation responses during CCHFV infection.

The state of platelets during EBOV and MARV infections is unclear. Excessive coagulation activation was thought to be the primary driver of thrombocytopenia during EVD and MARV disease for many years. This was because little data exists on platelet function during EVD and MARV disease in humans or NHPs. One study using an NHP model of EBOV infection showed platelet aggregation defects in response to in vitro agonist stimulation that coincided with increased levels of plasma platelet factor-4 in vivo.[136] These results suggest increased platelet activation and degranulation during infection. Effects of EBOV on the bone marrow are conflicting, with a singular report from an unspecified number of patients suggesting normal bone marrow cellularity, including megakaryocyte number; in contrast, a more recent and thorough investigation on the bone marrow from NHPs infected with EBOV suggests that EBOV can infect megakaryocytes and induce bone marrow suppression.[91,137]

Mechanisms underlying thrombocytopenia in YFV and RVFV infections remain to be elucidated.

Infection With HFVs Induces Endothelial Cell Activation and Increased Vascular Permeability

HFV infection leads to EC activation, which is broadly characterized by a loss in antithrombotic properties, expression of procoagulant, proadhesive, and proinflammatory molecules, and increased vascular permeability. This can occur directly through viral infection, by the action of specific viral proteins, or through inflammatory mediators released in response to viral infection. These

Downloaded from http://ahajournals.org by on January 27, 2026

mechanisms likely co-occur for many of the HFVs and can lead to disrupted barrier function (ie, the loosening of junctions between cells), increased vascular permeability (the loss of EC barrier function in vivo), and increased vascular leak (the leakage of cells and proteins from the blood into the tissue).

Regardless of whether ECs are direct targets or sustain any outward damage, infection with HFVs leads to EC activation and disruption, marked by increases in ≥1 of the following proteins in the circulation: vWF, soluble (s)-ICAM-1 (intercellular adhesion molecule-1), s-VCAM-1 (vascular cell adhesion molecule-1), and s-P-selectin. Viral antigens from most of the HFVs have also been found in ECs from different tissues, and all of them can replicate in cultured ECs. However, only some of the viruses have evidence of replication in ECs in vivo (eg, EBOV, MARV, CCHFV, HTNV, SNV, YFV, and DENV), and even fewer (eg, MARV, RVFV, and DENV) are directly cytopathic in ECs in vitro.[8,138–141] This suggests there are alternative mechanisms leading to the observed increases in vascular permeability during VHF.

How each virus alters ECs ultimately shapes the observed pathology. For example, severe HTNV infection manifests as HFRS, and concordantly, the strongest viral antigen staining is observed in the kidney, particularly renal capillary ECs.[142] In contrast, SNV infection presents as HCPS, and the ECs lining the lung capillaries and other small vessels stain the strongest for viral antigen.[8] Indeed, pathogenic *Orthohantaviruses* use β_3 integrins in the inactive conformation as entry receptors, including $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, expressed by ECs and platelets.[143] Importantly, neither HTNV nor SNV kills ECs directly but drastically increase vascular permeability.[8,142] Markers of EC activation (s-E-selectin, s-ICAM-1, s-VCAM-1, and VEGF [vascular endothelial growth factor]) and a marker of EC glycocalyx degradation (syndecan-1) are elevated in the circulation in individuals with HFRS or HCPS (Table 3).[144,145] In vitro work suggested several possible mechanisms by which HTNV and SNV may increase vascular permeability. The first mechanism is that the virus binds to the surface of the endothelium and inhibits the binding of host proteins to β_3 integrins. β_3 integrin inhibition then leads to increased VEGF-induced vascular permeability in ECs.[146,147] The second mechanism builds on the first and posits that increased VEGF combined with hantaviral inhibition of the integrin $\alpha_V\beta_3$ leads to internalization of VE-cadherin (vascular endothelial cadherin) and a redistribution of the junctional protein ZO-1 (zonula occluden-1), resulting in decreased barrier function.[15,147–152] However, a later study using a more sophisticated in vitro capillary system did not observe changes in VE-cadherin or VEGF levels.[153] Rather, in the third proposed mechanism, increased permeability was the result of increased FXII binding and autoactivation and increased kallikrein, which, together, led to increased bradykinin and increased vascular permeability.[153] Whether 1 or any of these mechanisms occurs in vivo remains unclear. However, all ultimately would lead to loosened EC junctions, resulting in decreased barrier function in vitro and increased vascular permeability in vivo without cell death.

For other viruses, such as EBOV, and likely MARV, ECs are targets late in infection but seem to have minimal damage in vivo.[91,154] The viral protein GP is known to induce EC activation and changes in EC barrier function.[155–158] These changes are further enhanced by the presence of TNFα (tumor necrosis factor alpha), which is present in the blood at high levels during EBOV and MARV infections.[2,159,160] There are increased levels of various markers of EC activation in the circulation, including s-thrombomodulin, s-P-selectin, s-PECAM-1 (platelet and endothelial cell adhesion molecule 1), and s-ICAM-1, with some being associated with severe disease (s-thrombomodulin, s-P-selectin, s-PECAM-1, and s-ICAM-1), hemorrhage (s-ICAM-1 and s-thrombomodulin), or death (s-thrombomodulin; Table 3).[161,162]

There is evidence for multiple mechanisms of decreased EC barrier function and increased vascular permeability and leak in DENV and YFV infections. DENV NS1, a viral protein, directly induces decreased EC barrier function.[163] While DENV NS1 reduced the barrier function of ECs of various origins in vitro, YFV NS1 decreased the barrier function of only liver ECs.[164] Data suggest that both DENV and YFV NS1 also induce glycocalyx disruption in vitro.[163,164] Indeed, sydecan-1 is elevated in individuals with severe DENV and YFV infection (Table 3).[165,166] There is also evidence that DENV may replicate in ECs. However, similar evidence for YFV is lacking.[138] Flaviviruses NS2 and NS4 have also been suggested to have viroporin-like activities, which may contribute further to increased permeability, but this remains to be established.[167] Both DENV and YFV infections are associated with increased levels of various cytokines, which can further enhance the vascular permeability induced by viral proteins and viral replication.[168,169]

For CCHFV and LASV, there is histopathologic evidence of outward EC damage, but it remains unclear if this is virus- or immune-mediated.[13,170] CCHFV activates ECs via infection in vitro in a dose-dependent manner, and the viral protein GP38 decreased EC barrier function in vitro by disrupting the EC glycocalyx.[171,172] GP38 also increased vascular permeability and leak in vivo in a CCHFV mouse model.[172] During CCHFV infection, plasma levels of vWF and s-ICAM-1 are significantly elevated compared with healthy controls (Table 3).[173] In a separate cohort, s-ICAM-1, s-VCAM-1, and VEGF were higher in individuals who died from CCHFV infection compared with those who survived (Table 3).[173,174] LASV infection is also associated with increases in EC activation markers (eg, vWF, s-ICAM-1, and s-VCAM-1) in the plasma compared with non-LASV febrile controls or healthy controls (Table 3).[68] In addition, in vitro data suggest that LASV is not cytopathic to ECs.[68,175]

Downloaded from http://ahajournals.org by on January 27, 2026

Markers of EC activation (VEGF, s-PECAM-1, and s-E-selectin) are moderately elevated in the circulation of individuals infected with RVFV compared with healthy individuals (Table 3).[176] In RVFV infection, no staining for viral antigen is observed in ECs from infected domesticated ruminant or mouse tissues, which suggests an immune-mediated mechanism of EC activation.[177,178]

While increased vascular permeability and leak contribute to the shock and multiorgan failure observed in end-stage VHF, increased vascular permeability also causes cells underlying the vascular endothelium to become exposed to the circulation. Importantly, some of these cells are a source of high levels of TF. When exposed to the circulation at the scale occurring during VHF, this likely contributes to coagulation activation. In addition, activation of ECs leads to downregulation of anticoagulant proteins, exacerbating the hypercoagulable state.

Phosphatidylserine Exposure and Viral Mimicry

Phosphatidylserine is normally located on the inner leaflet of the cell membrane. During damage or cellular death, phosphatidylserine is flipped to the outer membrane. Normally, this serves as an eat me signal to phagocytes and results in clearance of the damaged cells.[179] In addition to dying cells, activated platelets have increased phosphatidylserine exposure.[179] Phosphatidylserine also contributes to coagulation as phosphatidylserine binding to TF leads to a conformational change in TF that enhances the procoagulant activity of the TF/FVIIa complex.[180] In addition, phosphatidylserine provides a surface for the assembly and function of coagulation proteases/cofactor complexes.[48]

During viral infection, both dying cells and activated platelets are important sources of phosphatidylserine. In addition, phosphatidylserine may be incorporated into both the viral envelope of new virions and into EVs released from phosphatidylserine-positive cells. Indeed, EBOV, MARV, LASV, YFV, and DENV have all been shown to incorporate phosphatidylserine into their viral envelopes, facilitating viral entry into host cells using receptors that bind phosphatidylserine (eg, T-cell immunoglobulin and mucin receptor family protein and TAM [TYRO3-AXL-MER] family proteins).[181] This mechanism of entry not only increases the ability of the virus to enter phagocytes, a preferred target of infection for many HFVs, but may also promote coagulation. However, coagulation activation directly by viral particles remains to be established for HFVs as has been done for other viruses (cytomegalovirus and herpes simplex virus-1 and -2).[72,182]

Fibrinolysis During VHF

Dysregulation of the fibrinolytic system may contribute to bleeding in VHF. Indeed, D-dimer is elevated to some extent in all VHFs considered herein. In addition, tPA is elevated in humans or NHPs infected with EBOV, LASV, RVFV, SNV, and DENV, and levels of plasmin antiplasmin complexes are elevated in individuals with severe DF (Tables 3 and 4)[2,15,60,62,63,69,70,75–77,80,81,84,100,102,103,127,133,136,161,162,176,183–193] However, PAI-1 is also increased in humans with the same viruses, which may limit the activation of the fibrinolytic system by tPA (Table 3).[68,100,161,176,184,188,191,194]

To better understand the kinetics of when these elevations occur, animal models are essential. In an NHP model of EBOV infection, D-dimer begins to rise at 1 day post-infection and continues to increase over the course of infection. Both tPA and uPA increased by day 4 post-infection.[60] Unfortunately, PAI-1 could not be measured in this model because the available assays were incompatible with NHP samples.[60] In 2 other studies using transcriptomics and a multiplex bead-based assay on whole blood from NHPs infected with a different EBOV strain, PAI-1 and tPA both increased.[195,196]

Few studies have examined biomarkers of fibrinolysis other than D-dimer or total PAI-1. While these biomarkers can suggest increased fibrinolysis, other biomarkers such as plasmin antiplasmin complexes or tPA/PAI-1 complexes may be better metrics of fibrinolytic activity. As such, it is presently unknown whether fibrinolytic dysregulation contributes directly to bleeding in VHF. Thus, more studies on the fibrinolytic system in both humans and NHPs are needed.

SUMMARY AND PERSPECTIVES

VHF is a catch-all term, encompassing several different diseases caused by viruses from distinct families. Bleeding is the symptom for which the term VHF was coined, but bleeding is not necessarily a feature in all cases. Regardless, bleeding is a severe manifestation of the spectrum of diseases caused by HFVs. Herein, we propose some potential common mechanisms underlying bleeding in VHF (Figure 3). Viral infection activates coagulation by inducing increased TF expression on infected cells. The activity of the TF/FVIIa complex is further enhanced by increased phosphatidylserine exposure, resulting in even more coagulation activation. It is possible that the intrinsic pathway also contributes to the activation of coagulation in VHF. In parallel, there is decreased coagulation factor synthesis by the liver due to damage during infection. Activation of platelets and decreased platelet production lead to thrombocytopenia. In addition, platelet dysfunction occurs in VHF. Viral infection also activates ECs, causing decreased activity of the activated protein C anticoagulant pathway, conversion to a prothrombotic surface, and increased vascular permeability. EC activation also leads to increased tPA release, which ultimately results in increased plasmin activity and

Downloaded from http://ahajournals.org by on January 27, 2026

enhanced fibrinolysis. We propose that a combination of these pathways leads to bleeding during VHF.

The pathways highlighted above are based on data primarily from individual viruses, as a complete picture for any singular virus is missing. Thus, it still remains unclear if the mechanisms discussed herein extend to all the HFVs. Some pathways, such as fibrinolysis, also remain largely unexplored. More studies are needed to better understand the complete picture of the hemostatic dysregulation during VHF caused by different viruses. While human biomarker data have been helpful in opening avenues of investigation, human data alone at a singular point after an undefined amount of time after infection cannot provide the full picture. In vitro data also cannot capture the complex interplay of the coagulation and immune systems. Thus, animal models have been and will continue to be essential in advancing our understanding of mechanisms underlying bleeding in VHF. A better understanding may open new therapeutic avenues to treat and prevent bleeding during VHF.

ARTICLE INFORMATION

Affiliations

Department of Microbiology and Immunology (M.V.P.), UNC Blood Research Center, Department of Medicine (M.V.P., N.M.), and Division of Hematology, Department of Medicine (N.M.), University of North Carolina at Chapel Hill.

Sources of Funding

This work was supported by funding from the National Institutes of Health, National Heart, Lung, and Blood Institute grant R35HL155657 (to N. Mackman), the John C. Parker professorship (to N. Mackman), and the American Heart Association grant 25PRE1375748 (to M.V. Perkins).

Disclosures

None.

REFERENCES

1. Baseler L, Chertow DS, Johnson KM, Feldmann H, Morens DM. The pathogenesis of Ebola virus disease. *Annu Rev Pathol*. 2017;12:387–418. doi: 10.1146/annurev-pathol-052016-100506
2. Hensley LE, Alves DA, Geisbert JB, Fritz EA, Reed C, Larsen T, Geisbert TW. Pathogenesis of Marburg hemorrhagic fever in cynomolgus macaques. *J Infect Dis*. 2011;204:S1021–S1031. doi: 10.1093/infdis/jir339
3. Geisbert TW. Marburg and Ebola hemorrhagic fevers (Filoviruses). *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 2015;1995–1999. doi: 10.1016/B978-1-4557-4801-3.00166-1
4. Murphy HL, Ly H. Pathogenicity and virulence mechanisms of Lassa virus and its animal modeling, diagnostic, prophylactic, and therapeutic developments. *Virulence*. 2021;12:2989–3014. doi: 10.1080/21505594.2021.2000290
5. Ganaie SS, Schwarz MM, McMillen CM, Price DA, Feng AX, Albe JR, Wang W, Miersch S, Orvedahl A, Cole AR, et al. Lrp1 is a host entry factor for Rift Valley fever virus. *Cell*. 2021;184:5163–5178.e24. doi: 10.1016/j.cell.2021.09.001
6. Hawman DW, Feldmann H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol*. 2023;21:463–477. doi: 10.1038/s41579-023-00871-9
7. Avšič-Županc T, Saksida A, Korva M. Hantavirus infections. *Clin Microbiol Infect*. 2019;21S:e6–e16. doi: 10.1111/1469-0691.12291
8. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. *Am J Pathol*. 1995;146:552–579.
9. Gardner CL, Ryman KD. Yellow fever: a reemerging threat. *Clin Lab Med*. 2010;30:237–260. doi: 10.1016/j.cll.2010.01.001
10. Douam F, Ploss A. Yellow fever virus: knowledge gaps impeding the fight against an old foe. *Trends Microbiol*. 2018;26:913–928. doi: 10.1016/j.tim.2018.05.012
11. Schaefer TJ, Panda PK, Wolford RW. *Dengue fever*. 2025. StatPearls Publishing LLC.
12. Yun NE, Walker DH. Pathogenesis of Lassa fever. *Viruses*. 2012;4:2031–2048. doi: 10.3390/v4102031
13. Shieh WJ, Demby A, Jones T, Goldsmith CS, Rollin PE, Ksiazek TG, Peters CJ, Zaki SR. Pathology and pathogenesis of lassa fever: novel immunohistochemical findings in fatal cases and clinico-pathologic correlation. *Clin Infect Dis*. 2022;74:1821–1830. doi: 10.1093/cid/ciab719
14. McMillen CM, Hartman AL. Rift Valley fever in animals and humans: current perspectives. *Antiviral Res*. 2018;156:29–37. doi: 10.1016/j.antiviral.2018.05.009
15. Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, Merlin TL, McFeeley PJ, Umland ET, Zumwalt RE. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. *Hum Pathol*. 1995;26:110–120. doi: 10.1016/0046-8177(95)90123-x
16. Paz-Bailey G, Adams LE, Deen J, Anderson KB, Katzelnick LC. Dengue. *Lancet*. 2024;403:667–682. doi: 10.1016/S0140-6736(23)02576-X
17. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. 2023;21:87–96. doi: 10.1038/s41579-022-00789-8
18. Dar O, McIntyre S, Hogarth S, Heymann D. Rift Valley fever and a new paradigm of research and development for zoonotic disease control. *Emerg Infect Dis*. 2013;19:189–193. doi: 10.3201/eid1902.120941
19. Hartman A. Rift Valley fever. *Clin Lab Med*. 2017;37:285–301. doi: 10.1016/j.cll.2017.01.004
20. Vial PA, Ferrés M, Vial C, Klingström J, Ahlm C, López R, Le Corre N, Mertz GJ. Hantavirus in humans: a review of clinical aspects and management. *Lancet Infect Dis*. 2023;23:e371–e382. doi: 10.1016/S1473-3099(23)00128-7
21. Ikegami T, Makino S. The pathogenesis of Rift Valley fever. *Viruses*. 2011;3:493–519. doi: 10.3390/v3050493
22. Weaver SC, Barrett AD. Transmission cycles, host range, evolution and emergence of arboviral disease. *Nat Rev Microbiol*. 2004;2:789–801. doi: 10.1038/nrmicro1006
23. Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, Swanepoel R, Paddock CD, Balinandi S, Khristova ML, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog*. 2009;5:e1000536. doi: 10.1371/journal.ppat.1000536
24. Izudi J, Bajunirwe F. Case fatality rate for Ebola disease, 1976-2022: a meta-analysis of global data. *J Infect Public Health*. 2024;17:25–34. doi: 10.1016/j.jiph.2023.10.020
25. Sibomana O, Hakayuwa CM, Munyantore J. Marburg virus reaches Rwanda: How close are we to a vaccine solution? *Int J Infect Dis*. 2025;153:107371. doi: 10.1016/j.ijid.2024.107371
26. WHO. Introduction to Crimean-Congo haemorrhagic fever [Web]. who.int: WHO; 2018. Accessed August 2025. https://cdn.who.int/media/docs/default-source/documents/health-topics/crimean-congo-haemorrhaigc-fever/introduction-to-crimean-congo-haemorrhagic-fever.pdf
27. Zhang S, Wang S, Yin W, Liang M, Li J, Zhang Q, Feng Z, Li D. Epidemic characteristics of hemorrhagic fever with renal syndrome in China, 2006-2012. *BMC Infect Dis*. 2014;14:384. doi: 10.1186/1471-2334-14-384
28. WHO. Marburg virus disease who.int: WHO; 2024. Accessed August 2025. https://www.who.int/news-room/fact-sheets/detail/marburg-virus-disease#:~:text=Key%20facts,to%2088%25%20in%20past%20outbreaks
29. WHO. Lassa Fever: afro.who.int; 2023. Accessed August 2025. https://www.afro.who.int/health-topics/lassa-fever
30. WHO. Rift Valley fever: who.int; 2018. Accessed August 2025. https://www.who.int/news-room/fact-sheets/detail/rift-valley-fever
31. Ergönül O. Crimean-Congo haemorrhagic fever. *Lancet Infect Dis*. 2006;6:203–214. doi: 10.1016/S1473-3099(06)70435-2
32. Kelly JD, Barrie MB, Mesman AW, Karku S, Quiwa K, Drasher M, Schlough GW, Dierberg K, Koedoyoma S, Lindan CP, et al. Anatomy of a hotspot: chain and seroepidemiology of Ebola virus transmission, Sukudu, Sierra Leone, 2015-16. *J Infect Dis*. 2018;217:1214–1221. doi: 10.1093/infdis/jiy004
33. Frank MG, Weaver G, Raabe V; State of the Clinical Science Working Group of the National Emerging Pathogens Training. Crimean-Congo hemorrhagic fever virus for clinicians-epidemiology, clinical manifestations, and prevention. *Emerg Infect Dis*. 2024;30:854–863. doi: 10.3201/eid3005.231647
34. Lee HW. Hemorrhagic fever with renal syndrome in Korea. *Rev Infect Dis*. 1989;11:S864–S876.

Downloaded from http://ahajournals.org by on January 27, 2026

35. MacNeil A, Ksiazek TG, Rollin PE. Hantavirus pulmonary syndrome, United States, 1993-2009. *Emerg Infect Dis*. 2011;17:1195–1201. doi: 10.3201/eid1707.101306
36. Staples JE, O'Laughlin K. Yellow fever. CDC Yellow Book: Health Information for International Travel. 2025. https://www.cdc.gov/yellow-book/hcp/travel-associated-infections-diseases/yellow-fever.html
37. Feldmann H, Sprecher A, Geisbert TW. Ebola. *N Engl J Med*. 2020;382:1832–1842. doi: 10.1056/NEJMra1901594
38. Colebunders R, Tshomba A, Van Kerkhove MD, Bausch DG, Campbell P, Libande M, Pirard P, Tshioko F, Mardel S, Mulangu S, et al; International Scientific and Technical Committee "DRC Watsa/Durba 1999 Marburg Outbreak Investigation Group." Marburg hemorrhagic fever in Durba and Watsa, Democratic Republic of the Congo: clinical documentation, features of illness, and treatment. *J Infect Dis*. 2007;196:S148–S153. doi: 10.1086/520543
39. Noh JY, Cheong HJ, Song JY, Kim WJ, Song KJ, Klein TA, Lee SH, Yanagihara R, Song J-W. Clinical and molecular epidemiological features of hemorrhagic fever with renal syndrome in Korea over a 10-year period. *J Clin Virol*. 2013;58:11–17. doi: 10.1016/j.jcv.2013.06.027
40. Servadio JL, Muñoz-Zanzi C, Convertino M. Estimating case fatality risk of severe Yellow Fever cases: systematic literature review and meta-analysis. *BMC Infect Dis*. 2021;21:819. doi: 10.1186/s12879-021-06535-4
41. Marzi A, Feldmann H. Filovirus vaccines as a response paradigm for emerging infectious diseases. *NPJ Vaccines*. 2024;9:186. doi: 10.1038/s41541-024-00985-y
42. Carey BD, Yu S, Geiger J, Ye C, Huzella LM, Reeder RJ, Mehta M, Hirsch S, Bernbaum R, Cubitt B, et al. A Lassa virus live attenuated vaccine candidate that is safe and efficacious in guinea pigs. *NPJ Vaccines*. 2024;9:220. doi: 10.1038/s41541-024-01012-w
43. de Silva A, White L. Immunogenicity of a live dengue vaccine (TAK-003). *J Infect Dis*. 2022;227:163–164. doi: 10.1093/infdis/jiac424
44. Jacob ST, Crozier I, Fischer WA 2nd, Hewlett A, Kraft CS, Vega MA, Soka MJ, Wahl V, Griffiths A, Bollinger L, et al. Ebola virus disease. *Nat Rev Dis Primers*. 2020;6:13. doi: 10.1038/s41572-020-0147-3
45. Saka SA, Lawal QO, Otaigbe O, Blackie FF, Ighodaro O, Odafen PI, Okogbenin S. Lassa fever survivors: long-term health effects and chronic sequelae - a scoping review. *BMC Infect Dis*. 2025;25:823. doi: 10.1186/s12879-025-11262-1
46. Lambert AJ, Hughes HR. Clinically important phleboviruses and their detection in human samples. *Viruses*. 2021;13:1500. doi: 10.3390/v13081500
47. WHO. *Dengue: guidelines for diagnosis, treatment, prevention and control. Dengue: guidelines for diagnosis, treatment, prevention and control: New Edition*. Geneva: World Health Organization. 2009.
48. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38:709–725. doi: 10.1161/ATVBAHA.117.309846
49. Grover SP, Mackman N. Intrinsic pathway of coagulation and thrombosis. *Arterioscler Thromb Vasc Biol*. 2019;39:331–338. doi: 10.1161/ATVBAHA.118.312130
50. Wolberg AS. Fibrinogen and fibrin: synthesis, structure, and function in health and disease. *J Thromb Haemost*. 2023;21:3005–3015. doi: 10.1016/j.jtha.2023.08.014
51. van der Meijden PEJ, Heemskerk JWM. Platelet biology and functions: new concepts and clinical perspectives. *Nat Rev Cardiol*. 2019;16:166–179. doi: 10.1038/s41569-018-0110-0
52. Neubauer K, Zieger B. Endothelial cells and coagulation. *Cell Tissue Res*. 2022;387:391–398. doi: 10.1007/s00441-021-03471-2
53. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch*. 2007;454:345–359. doi: 10.1007/s00424-007-0212-8
54. Cerutti C, Ridley AJ. Endothelial cell-cell adhesion and signaling. *Exp Cell Res*. 2017;358:31–38. doi: 10.1016/j.yexcr.2017.06.003
55. Longstaff C, Kolev K. Basic mechanisms and regulation of fibrinolysis. *J Thromb Haemost*. 2015;13:S98–105. doi: 10.1111/jth.12935
56. Antoniak S. The coagulation system in host defense. *Res Pract Thromb Haemost*. 2018;2:549–557. doi: 10.1002/rth2.12109
57. Iba T, Levy JH, Maier CL, Helms J, Umemura Y, Moore H, Othman M, Thachil J, Connors JM, Levi M, et al. Updated definition and scoring of disseminated intravascular coagulation in 2025: communication from the ISTH SSC Subcommittee on Disseminated Intravascular Coagulation. *J Thromb Haemost*. 2025;23:2356–2362. doi: 10.1016/j.jtha.2025.03.038
58. Mackman N, Sachetto ATA. Challenges with measuring tissue factor antigen and activity in human plasma. *Blood Vessel Thromb Hemost*. 2024;1:100022. doi: 10.1016/j.bvth.2024.100022
59. Bonifay A, Mackman N, Hisada Y, Sachetto ATA, Hau C, Gray E, Hogwood J, Aharon A, Badimon L, Barile L, et al. Comparison of assays measuring extracellular vesicle tissue factor in plasma samples: communication from the ISTH SSC Subcommittee on Vascular Biology. *J Thromb Haemost*. 2024;22:2910–2921. doi: 10.1016/j.jtha.2024.05.037
60. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis*. 2003;188:1618–1629. doi: 10.1086/379724
61. Greenberg A, Huber BR, Liu DX, Logue JP, Hischak AMW, Hart RJ, Abbott M, Isic N, Hisada YM, Mackman N, et al. Quantification of viral and host biomarkers in the liver of rhesus macaques: a longitudinal study of Zaire Ebolavirus Strain Kikwit (EBOV/Kik). *Am J Pathol*. 2020;190:1449–1460. doi: 10.1016/j.ajpath.2020.03.003
62. Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, Young HA, Fredeking TM, Rote WE, Vlasuk GP. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet*. 2003;362:1953–1958. doi: 10.1016/S0140-6736(03)15012-X
63. Geisbert TW, Daddario-DiCaprio KM, Geisbert JB, Young HA, Formenty P, Fritz EA, Larsen T, Hensley LE. Marburg virus Angola infection of rhesus macaques: pathogenesis and treatment with recombinant nematode anticoagulant protein c2. *J Infect Dis*. 2007;196:S372–S381. doi: 10.1086/520608
64. de Azeredo EL, Solórzano VE, de Oliveira DB, Marinho CF, de Souza LJ, da Cunha RV, et al. Increased circulating procoagulant and anticoagulant factors as TF and TFPI according to severity or infecting serotypes in human dengue infection. *Microbes Infect*. 2017;19:62–68. doi: 10.1016/j.micinf.2016.08.005
65. Wills BA, Oragui EE, Stephens AC, Daramola OA, Dung NM, Loan HT, Chau NV, Chambers M, Stepniewska K, Farrar JJ, et al. Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with Dengue shock syndrome. *Clin Infect Dis*. 2002;35:277–285. doi: 10.1086/341410
66. de Azeredo EL, Kubelka CF, Alburquerque LM, Barbosa LS, Damasco PV, Avila CA, Motta-Castro ARC, da Cunha RV, Monteiro RQ. Tissue factor expression on monocytes from patients with severe dengue fever. *Blood Cells Mol Dis*. 2010;45:334–335. doi: 10.1016/j.bcmd.2010.08.004
67. Hamali HA, Mobarki AA, Akhter MS, Saboor M, Madkhali AM, Halwani AJ, et al. Elevated levels of procoagulant microvesicles in patients with dengue fever. *Future Virol*. 2020;15:701–706. doi: 10.2217/fvl-2020-0202
68. Horton LE, Cross RW, Hartnett JN, Engel EJ, Sakabe S, Goba A, Momoh M, Sandi JD, Geisbert TW, Garry RF, et al. Endotheliopathy and platelet dysfunction as hallmarks of fatal Lassa fever. *Emerg Infect Dis*. 2020;26:2625–2637. doi: 10.3201/eid2611.191694
69. McElroy AK, Harmon JR, Flietstra T, Nichol ST, Spiropoulou CF. Human biomarkers of outcome following Rift Valley fever virus infection. *J Infect Dis*. 2018;218:1847–1851. doi: 10.1093/infdis/jiy393
70. Tatsumi K, Hisada Y, Connolly AF, Buranda T, Mackman N. Patients with severe orthohantavirus cardiopulmonary syndrome due to Sin Nombre virus infection have increased circulating extracellular vesicle tissue factor and an activated coagulation system. *Thromb Res*. 2019;179:31–33. doi: 10.1016/j.thromres.2019.04.032
71. Schmedes CM, Grover SP, Hisada YM, Goeijenbier M, Hultdin J, Nilsson S, Thunberg T, Ahlm C, Mackman N, Fors Connolly A-M. Circulating extracellular vesicle tissue factor activity during orthohantavirus infection is associated with intravascular coagulation. *J Infect Dis*. 2020;222:1392–1399. doi: 10.1093/infdis/jiz597
72. Sutherland MR, Raynor CM, Leenknegt H, Wright JF, Pryzdial EL. Coagulation initiated on herpesviruses. *Proc Natl Acad Sci USA*. 1997;94:13510–13514. doi: 10.1073/pnas.94.25.13510
73. White HA. Lassa fever. A study of 23 hospital cases. *Trans R Soc Trop Med Hyg*. 1972;66:390–401. doi: 10.1016/0035-9203(72)90269-6
74. McElroy AK, Nichol ST. Rift Valley fever virus inhibits a pro-inflammatory response in experimentally infected human monocyte derived macrophages and a pro-inflammatory cytokine response may be associated with patient survival during natural infection. *Virology*. 2012;422:6–12. doi: 10.1016/j.virol.2011.09.023
75. Sonmez M, Aydin K, Durmus A, Sucu N, Yilmaz M, Akdogan E, Koksal I, Ovali E, Omay SB. Plasma activity of thrombin activatable fibrinolysis inhibitor in Crimean-Congo hemorrhagic fever. *J Infect*. 2007;55:184–187. doi: 10.1016/j.jinf.2007.02.010
76. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis*. 1989;11:S794–S800. doi: 10.1093/clinids/11.supplement_4.s794
77. Chen WJ, Du H, Hu HF, Lian JQ, Jiang H, Li J, Chen Y-P, Zhang Y, Wang P-Z. Levels of peripheral blood routine, biochemical and coagulation

Downloaded from http://ahajournals.org by on January 27, 2026

parameters in patients with hemorrhagic fever with renal syndrome and their relationship with prognosis: an observational cohort study. *BMC Infect Dis*. 2024;24:75. doi: 10.1186/s12879-023-08777-w
78. Liu R, Ma R, Liu Z, Hu H, Shu J, Hu P, Kang J, Zhang Y, Han M, Zhang X, et al. HTNV infection of CD8(+) T cells is associated with disease progression in HFRS patients. *Commun Biol*. 2021;4:652. doi: 10.1038/s42003-021-02182-2
79. Kallas EG, D'Elia Zanella L, Moreira CHV, Buccheri R, Diniz GBF, Castiñeiras ACP, et al. Predictors of mortality in patients with yellow fever: an observational cohort study. *Lancet Infect Dis*. 2019;19:750–758. doi: 10.1016/S1473-3099(19)30125-2
80. Jardim LL, Franco MB, de Oliveira NR, de Carvalho BN, Basques F, Ribeiro DD, Lisman T, Pereira LS, Rezende SM. Hypocoagulability in severe yellow fever infection is associated with bleeding: results from a cohort study. *Res Pract Thromb Haemost*. 2024;8:102427. doi: 10.1016/j.rpth.2024.102427
81. Gear JS, Cassel GA, Gear AJ, Trappler B, Clausen L, Meyers AM, Kew MC, Bothwell TH, Sher R, Miller GB, et al. Outbreake of Marburg virus disease in Johannesburg. *Br Med J*. 1975;4:489–493. doi: 10.1136/bmj.4.5995.489
82. Cevik MA, Erbay A, Bodur H, Gülderen E, Başţuğ A, Kubar A, Akinci E. Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. *Int J Infect Dis*. 2008;12:374–379. doi: 10.1016/j.ijid.2007.09.010
83. Saksida A, Duh D, Wraber B, Dedushaj I, Ahmeti S, Avsic-Zupanc T. Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean-Congo hemorrhagic fever. *Clin Vaccine Immunol*. 2010;17:1086–1093. doi: 10.1128/CVI.00530-09
84. Bailey AL, Kang LI, de Assis Barros D'Elia Zanella LGF, Silveira CGT, Ho YL, Foquet L, Bial G, McCune BT, Duarte-Neto AN, Thomas A, et al. Consumptive coagulopathy of severe yellow fever occurs independently of hepatocellular tropism and massive hepatic injury. *Proc Natl Acad Sci USA*. 2020;117:32648–32656. doi: 10.1073/pnas.2014096117
85. Hunt L, Gupta-Wright A, Simms V, Tamba F, Knott V, Tamba K, Heisenberg-Mansaray S, Tamba E, Sheriff A, Conteh S, et al. Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study. *Lancet Infect Dis*. 2015;15:1292–1299. doi: 10.1016/S1473-3099(15)00144-9
86. Duvignaud A, Jaspard M, Etafo IC, Gabillard D, Serra B, Abejegah C, le Gal C, Abidoye AT, Doutchi M, Owhin S, et al; LASCOPE Study Group. Lassa fever outcomes and prognostic factors in Nigeria (LASCOPE): a prospective cohort study. *Lancet Glob Health*. 2021;9:e469–e478. doi: 10.1016/S2214-109X(20)30518-0
87. Al-Hazmi M, Ayoola EA, Abdurahman M, Banzal S, Ashraf J, El-Bushra A, Hazmi A, Abdullah M, Abbo H, Elamin A, et al. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. *Clin Infect Dis*. 2003;36:245–252. doi: 10.1086/345671
88. Adam AA, Karsany MS, Adam I. Manifestations of severe Rift Valley fever in Sudan. *Int J Infect Dis*. 2010;14:e179–e180. doi: 10.1016/j.ijid.2009.03.029
89. Malavige GN, Gomes L, Alles L, Chang T, Salimi M, Fernando S, Nanayakkara KD, Jayaratne S, Ogg GS. Serum IL-10 as a marker of severe dengue infection. *BMC Infect Dis*. 2013;13:341. doi: 10.1186/1471-2334-13-341
90. Safronetz D, Prescott J, Feldmann F, Haddock E, Rosenke R, Okumura A, Brining D, Dahlstrom E, Porcella SF, Ebihara H, et al. Pathophysiology of hantavirus pulmonary syndrome in rhesus macaques. *Proc Natl Acad Sci USA*. 2014;111:7114–7119. doi: 10.1073/pnas.1401998111
91. Martines RB, Ng DL, Greer PW, Rollin PE, Zaki SR. Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. *J Pathol*. 2015;235:153–174. doi: 10.1002/path.4456
92. Al-Tikriti SK, Al-Ani F, Jurji FJ, Tantawi H, Al-Moslih M, Al-Janabi N, Mahmud MI, Al-Bana A, Habib H, Al-Munthri H, et al. Congo/Crimean haemorrhagic fever in Iraq. *Bull World Health Organ*. 1981;59:85–90.
93. Quaresma JA, Barros VL, Fernandes ER, Pagliari C, Takakura C, da Costa Vasconcelos PF, de Andrade HF, Duarte MIS. Reconsideration of histopathology and ultrastructural aspects of the human liver in yellow fever. *Acta Trop*. 2005;94:116–127. doi: 10.1016/j.actatropica.2005.03.003
94. Lyon GM, Mehta AK, Varkey JB, Brantly K, Plyler L, McElroy AK, Kraft CS, Towner JS, Spiropoulou C, Ströher U, et al; Emory Serious Communicable Diseases Unit. Clinical care of two patients with Ebola virus disease in the United States. *N Engl J Med*. 2014;371:2402–2409. doi: 10.1056/NEJMoa1409838
95. Centers for Disease Control and Prevention (CDC). Imported case of Marburg hemorrhagic fever - Colorado, 2008. *MMWR Morb Mortal Wkly Rep*. 2009;58:1377–1381. doi: 10.1097/inf.0b013e3181d467bc
96. Foberg U, Frydén A, Isaksson B, Jahrling P, Johnson A, McKee K, Niklasson B, Normann B, Peters C, Bengtsson M. Viral haemorrhagic fever in Sweden: experiences from management of a case. *Scand J Infect Dis*. 1991;23:143–151. doi: 10.3109/00365549109023392
97. McCormick JB, King IJ, Webb PA, Johnson KM, O'Sullivan R, Smith ES, Trippel S, Tong TC. A case-control study of the clinical diagnosis and course of Lassa fever. *J Infect Dis*. 1987;155:445–455. doi: 10.1093/infdis/155.3.445
98. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R. Lassa fever. Effective therapy with ribavirin. *N Engl J Med*. 1986;314:20–26. doi: 10.1056/NEJM198601023140104
99. de Souza LJ, Nogueira RM, Soares LC, Soares CE, Ribas BF, Alves FP, Vieira FR, Pessanha FEB. The impact of dengue on liver function as evaluated by aminotransferase levels. *Braz J Infect Dis*. 2007;11:407–410. doi: 10.1590/s1413-86702007000400007
100. Van Gorp ECM, Setiati TE, Mairuhu ATA, Suharti C, Cate Ht Ht, Dolmans WMV, Van Der Meer JWM, Hack CE, Brandjes DPM. Impaired fibrinolysis in the pathogenesis of dengue hemorrhagic fever. *J Med Virol*. 2002;67:549–554. doi: 10.1002/jmv.10137
101. Ebihara H, Rockx B, Marzi A, Feldmann F, Haddock E, Brining D, LaCasse RA, Gardner D, Feldmann H. Host response dynamics following lethal infection of rhesus macaques with Zaire ebolavirus. *J Infect Dis*. 2011;204:S991–S999. doi: 10.1093/infdis/jir336
102. Safronetz D, Strong JE, Feldmann F, Haddock E, Sogoba N, Brining D, Geisbert TW, Scott DP, Feldmann H. A recently isolated Lassa virus from Mali demonstrates atypical clinical disease manifestations and decreased virulence in cynomolgus macaques. *J Infect Dis*. 2013;207:1316–1327. doi: 10.1093/infdis/jit004
103. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. 2011;8:205. doi: 10.1186/1743-422X-8-205
104. Scoon WA, Mancio-Silva L, Suder EL, Villacorta-Martin C, Lindstrom-Vautrin J, Bernbaum JG, Mazur S, Johnson RF, Olejnik J, Flores EY, et al. Ebola virus infection induces a delayed type I IFN response in bystander cells and the shutdown of key liver genes in human iPSC-derived hepatocytes. *Stem Cell Rep*. 2022;17:2286–2302. doi: 10.1016/j.stemcr.2022.08.003
105. Kash JC, Mühlberger E, Carter V, Grosch M, Perwitasari O, Proll SC, Thomas MJ, Weber F, Klenk H-D, Katze MG. Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence. *J Virol*. 2006;80:3009–3020. doi: 10.1128/JVI.80.6.3009-3020.2006
106. Prescott JB, Liu KJ, Lander A, Pek NMQ, Jha SK, Bokelmann M, Begur M, Koh PW, Yang H, Lim B, et al. Metabolically purified human stem cell-derived hepatocytes reveal distinct effects of Ebola and Lassa viruses. *bioRxiv*. Preprint posted online February 21, 2025. doi: 10.1101/2025.02.17.638665
107. Rodrigues R, Paranhos-Baccalà G, Vernet G, Peyrefitte CN. Crimean-Congo hemorrhagic fever virus-infected hepatocytes induce ER-stress and apoptosis crosstalk. *PLoS One*. 2012;7:e29712. doi: 10.1371/journal.pone.0029712
108. Woodson SE, Holbrook MR. Infection of hepatocytes with 17-D vaccine-strain yellow fever virus induces a strong pro-inflammatory host response. *J Gen Virol*. 2011;92:2262–2271. doi: 10.1099/vir.0.031617-0
109. Quirino-Teixeira AC, Andrade FB, Pinheiro MBM, Rozini SV, Hottz ED. Platelets in dengue infection: more than a numbers game. *Platelets*. 2022;33:176–183. doi: 10.1080/09537104.2021.1921722
110. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol*. 1995;8:249–270. doi: 10.1016/s0950-3536(05)80240-9
111. Vogt MB, Lahon A, Arya RP, Spencer Clinton JL, Rico-Hesse R. Dengue viruses infect human megakaryocytes, with probable clinical consequences. *PLoS Negl Trop Dis*. 2019;13:e0007837. doi: 10.1371/journal.pntd.0007837
112. Noisakran S, Onlamoon N, Hsiao HM, Clark KB, Villinger F, Ansari AA, Perng GC. Infection of bone marrow cells by dengue virus in vivo. *Exp Hematol*. 2012;40:250–259.e4. doi: 10.1016/j.exphem.2011.11.011
113. Alonzo MT, Lacuesta TL, Dimaano EM, Kurosu T, Suarez LA, Mapua CA, Akeda Y, Matias RR, Kuter DJ, Nagata S, et al. Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus infections. *J Infect Dis*. 2012;205:1321–1329. doi: 10.1093/infdis/jis180
114. Hottz ED, Oliveira MF, Nunes PC, Nogueira RM, Valls-de-Souza R, Da Poian AT, Weyrich AS, Zimmerman GA, Bozza PT, Bozza FA. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost*. 2013;11:951–962. doi: 10.1111/jth.12178
115. Michels M, Alisjahbana B, De Groot PG, Indrati AR, Fijnheer R, Puspita M, Dewi IMW, van de Wijer L, de Boer EMS, Roest M, et al. Platelet function

Downloaded from http://ahajournals.org by on January 27, 2026

alterations in dengue are associated with plasma leakage. *Thromb Haemost.* 2014;112:352–362. doi: 10.1160/TH14-01-0056
116. Ojha A, Nandi D, Batra H, Singhal R, Annarapu GK, Bhattacharyya S, Seth T, Dar L, Medigeshi GR, Vrati S, et al. Platelet activation determines the severity of thrombocytopenia in dengue infection. *Sci Rep.* 2017;7:41697. doi: 10.1038/srep41697
117. Noisakran S, Gibbons RV, Songprakhon P, Jairungsri A, Ajariyakhajorn C, Nisalak A, Jarman RG, Malasit P, Chokephaibulkit K, Perng GC. Detection of dengue virus in platelets isolated from dengue patients. *Southeast Asian J Trop Med Public Health.* 2009;40:253–262.
118. Simon AY, Sutherland MR, Pryzdial EL. Dengue virus binding and replication by platelets. *Blood.* 2015;126:378–385. doi: 10.1182/blood-2014-09-598029
119. Kar M, Singla M, Chandele A, Kabra SK, Lodha R, Medigeshi GR. Dengue virus entry and replication does not lead to productive infection in platelets. *Open Forum Infect Dis.* 2017;4:ofx051. doi: 10.1093/ofid/ofx051
120. Alcon S, Talarmin A, Debruyne M, Falconar A, Deubel V, Flamand M. Enzyme-linked immunosorbent assay specific to Dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. *J Clin Microbiol.* 2002;40:376–381. doi: 10.1128/JCM.40.02.376-381.2002
121. Erra EO, Korhonen EM, Voutilainen L, Huhtamo E, Vapalahti O, Kantele A. Dengue in travelers: kinetics of viremia and NS1 antigenemia and their associations with clinical parameters. *PLoS One.* 2013;8:e65900. doi: 10.1371/journal.pone.0065900
122. Chao CH, Wu WC, Lai YC, Tsai PJ, Perng GC, Lin YS, Yeh T-M. Dengue virus nonstructural protein 1 activates platelets via toll-like receptor 4, leading to thrombocytopenia and hemorrhage. *PLoS Pathog.* 2019;15:e1007625. doi: 10.1371/journal.ppat.1007625
123. Quach ME, Chen W, Li R. Mechanisms of platelet clearance and translation to improve platelet storage. *Blood.* 2018;131:1512–1521. doi: 10.1182/blood-2017-08-743229
124. Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, Yang TI, Sheu FC, Kuo CF, Lin YS. Generation of IgM anti-platelet autoantibody in dengue patients. *J Med Virol.* 2001;63:143–149. doi: 10.1002/1096-9071(20000201)
125. Mastellos DC, Hajishengallis G, Lambris JD. A guide to complement biology, pathology and therapeutic opportunity. *Nat Rev Immunol.* 2024;24:118–141. doi: 10.1038/s41577-023-00926-1
126. Quirino-Teixeira AC, Rozini SV, Barbosa-Lima G, Coelho DR, Carneiro PH, Mohana-Borges R, Bozza PT, Hottz ED. Inflammatory signaling in dengue-infected platelets requires translation and secretion of nonstructural protein 1. *Blood Adv.* 2020;4:2018–2031. doi: 10.1182/bloodadvances.2019001169
127. Fisher-Hoch SP, Mitchell SW, Sasso DR, Lange JV, Ramsey R, McCormick JB. Physiological and immunologic disturbances associated with shock in a primate model of Lassa fever. *J Infect Dis.* 1987;155:465–474. doi: 10.1093/infdis/155.3.465
128. Fisher-Hoch S, McCormick JB, Sasso D, Craven RB. Hematologic dysfunction in Lassa fever. *J Med Virol.* 1988;26:127–135. doi: 10.1002/jmv.1890260204
129. Cummins D, Fisher-Hoch SP, Walshe KJ, Mackie IJ, McCormick JB, Bennett D, Perez G, Farrar B, Machin SJ. A plasma inhibitor of platelet aggregation in patients with Lassa fever. *Br J Haematol.* 1989;72:543–548. doi: 10.1111/j.1365-2141.1989.tb04321.x
130. Gavrilovskaya IN, Gorbunova EE, Mackow ER. Pathogenic hantaviruses direct the adherence of quiescent platelets to infected endothelial cells. *J Virol.* 2010;84:4832–4839. doi: 10.1128/JVI.02405-09
131. Cosgriff TM, Lee HW, See AF, Parrish DB, Moon JS, Kim DJ, Lewis RM. Platelet dysfunction contributes to the haemostatic defect in haemorrhagic fever with renal syndrome. *Trans R Soc Trop Med Hyg.* 1991;85:660–663. doi: 10.1016/0035-9203(91)90386-d
132. Ozmen ZC, Deveci K, Coskun USS, Ozmen Z, Aydogan L, Barut HS. Immature platelet fraction as a useful marker in Crimean-Congo hemorrhagic fever. *Trop Biomed.* 2023;40:101–107. doi: 10.47665/tb.40.1.016
133. Joubert JR, King JB, Rossouw DJ, Cooper R. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. *S Afr Med J.* 1985;68:722–728.
134. Suleiman MN, Muscat-Baron JM, Harries JR, Satti AG, Platt GS, Bowen ET, Simpson DI. Congo/Crimean haemorrhagic fever in Dubai. An outbreak at the Rashid Hospital. *Lancet.* 1980;2:939–941. doi: 10.1016/S0140-6736(80)92103-0
135. Negredo A, de la Calle-Prieto F, Palencia-Herrejón E, Mora-Rillo M, Astray-Mochales J, Sánchez-Seco MP, Bermejo Lopez E, Menárguez J, Fernández-Cruz A, Sánchez-Artola B, et al; Crimean Congo Hemorrhagic Fever@Madrid Working Group. Autochthonous Crimean-Congo hemorrhagic fever in Spain. *N Engl J Med.* 2017;377:154–161. doi: 10.1056/NEJMoa1615162
136. Fisher-Hoch SP, Platt GS, Neild GH, Southee T, Baskerville A, Raymond RT, Lloyd G, Simpson DI. Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). *J Infect Dis.* 1985;152:887–894. doi: 10.1093/infdis/152.5.887
137. Liu DX, Pahar B, Perry DL, Xu H, Cooper TK, Huzella LM, Hart RJ, Hischak AMW, Bernbaum J, St Claire M, et al. Depletion of Bone marrow hematopoietic cells in Ebolavirus-infected rhesus macaques: a possible cause of hematologic abnormalities in Ebolavirus disease. *Am J Pathol.* 2023;193:2031–2046. doi: 10.1016/j.ajpath.2023.08.010
138. Póvoa TF, Alves AM, Oliveira CA, Nuovo GJ, Chagas VL, Paes MV. The pathology of severe dengue in multiple organs of human fatal cases: histopathology, ultrastructure and virus replication. *PLoS One.* 2014;9:e83386. doi: 10.1371/journal.pone.0083386
139. Schnittler HJ, Mahner F, Drenckhahn D, Klenk HD, Feldmann H. Replication of Marburg virus in human endothelial cells. A possible mechanism for the development of viral hemorrhagic disease. *J Clin Invest.* 1993;91:1301–1309. doi: 10.1172/JCI116329
140. Quellec J, Piro-Megy C, Cannac M, Nisole S, Marty FH, Gosselet F, Shimizu F, Kanda T, Cêtre-Sossah C, Salinas S. Rift Valley fever virus is able to cross the human blood-brain barrier in vitro by direct infection with no deleterious effects. *J Virol.* 2024;98:e0126724. doi: 10.1128/jvi.01267-24
141. Avirutnan P, Malasit P, Seliger B, Bhakdi S, Husmann M. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J Immunol.* 1998;161:6338–6346. doi: 10.1128/jvi.01630-10
142. Kim S, Kang ET, Kim YG, Han JS, Lee JS, Kim YI, Hall WC, Dalrymple JM, Peters CJ. Localization of Hantaan viral envelope glycoproteins by monoclonal antibodies in renal tissues from patients with Korean hemorrhagic fever H. *Am J Clin Pathol.* 1993;100:398–403. doi: 10.1093/ajcp/100.4.398
143. Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER. beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. *Proc Natl Acad Sci USA.* 1998;95:7074–7079. doi: 10.1073/pnas.95.12.7074
144. Gavrilovskaya I, Gorbunova E, Koster F, Mackow E. Elevated VEGF levels in pulmonary edema fluid and PBMCs from patients with acute hantavirus pulmonary syndrome. *Adv Virol.* 2012;2012:674360. doi: 10.1155/2012/674360
145. Connolly-Andersen AM, Thunberg T, Ahlm C. Endothelial activation and repair during hantavirus infection: association with disease outcome. *Open Forum Infect Dis.* 2014;1:ofu027. doi: 10.1093/ofid/ofu027
146. Robinson SD, Reynolds LE, Wyder L, Hicklin DJ, Hodivala-Dilke KM. Beta3-integrin regulates vascular endothelial growth factor-A-dependent permeability. *Arterioscler Thromb Vasc Biol.* 2004;24:2108–2114. doi: 10.1161/01.ATV.0000143857.27408.de
147. Gavrilovskaya IN, Gorbunova EE, Mackow ER. Hypoxia induces permeability and giant cell responses of Andes virus-infected pulmonary endothelial cells by activating the mTOR-S6K signaling pathway. *J Virol.* 2013;87:12999–13008. doi: 10.1128/JVI.02103-13
148. Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Ksiazek TG, Rollin PE, Nichol S, Umland ET. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. The Hantavirus Study Group. *N Engl J Med.* 1994;330:949–955. doi: 10.1056/NEJM199404073301401
149. Gavrilovskaya IN, Gorbunova EE, Mackow NA, Mackow ER. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine 1-phosphate inhibit hantavirus-directed permeability. *J Virol.* 2008;82:5797–5806. doi: 10.1128/JVI.02397-07
150. Gavrilovskaya IN, Peresleni T, Geimonen E, Mackow ER. Pathogenic hantaviruses selectively inhibit beta3 integrin directed endothelial cell migration. *Arch Virol.* 2002;147:1913–1931. doi: 10.1007/s00705-002-0852-0
151. Gorbunova E, Gavrilovskaya IN, Mackow ER. Pathogenic hantaviruses Andes virus and Hantaan virus induce adherens junction disassembly by directing vascular endothelial cadherin internalization in human endothelial cells. *J Virol.* 2010;84:7405–7411. doi: 10.1128/JVI.00576-10
152. Krautkrämer E, Grouls S, Stein N, Reiser J, Zeier M. Pathogenic Old World hantaviruses infect renal glomerular and tubular cells and induce disassembling of cell-to-cell contacts. *J Virol.* 2011;85:9811–9823. doi: 10.1128/JVI.00568-11

Downloaded from http://ahajournals.org by on January 27, 2026

153. Taylor SL, Wahl-Jensen V, Copeland AM, Jahrling PB, Schmaljohn CS. Endothelial cell permeability during hantavirus infection involves factor XII-dependent increased activation of the kallikrein-kinin system. *PLoS Pathog*. 2013;9:e1003470. doi: 10.1371/journal.ppat.1003470
154. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Larsen T, Kagan E, Hensley LE. Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *Am J Pathol*. 2003;163:2371–2382. doi: 10.1016/S0002-9440(10)63592-4
155. Escudero-PérezB,VolchkovaVA,DolnikO,LawrenceP,VolchkovVE.ShedGP of Ebola virus triggers immune activation and increased vascular permeability. *PLoS Pathog*. 2014;10:e1004509. doi: 10.1371/journal.ppat.1004509
156. He J, Melnik LI, Komin A, Wiedman G, Fuselier T, Morris CF, Starr CG, Searson PC, Gallaher WR, Hristova K, et al. Ebola virus delta peptide is a viroporin. *J Virol*. 2017;91:e00438-17. doi: 10.1128/jvi.00438-17
157. Yang ZY, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med*. 2000;6:886–889. doi: 10.1038/78645
158. Wahl-Jensen VM, Afanasieva TA, Seebach J, Ströher U, Feldmann H, Schnittler HJ. Effects of Ebola virus glycoproteins on endothelial cell activation and barrier function. *J Virol*. 2005;79:10442–10450. doi: 10.1128/JVI.79.16.10442-10450.2005
159. Baize S, Leroy EM, Georges AJ, Georges-Courbot MC, Capron M, Bedjabaga I, Lansoud-Soukate J, Mavoungou E. Inflammatory responses in Ebola virus-infected patients. *Clin Exp Immunol*. 2002;128:163–168. doi: 10.1046/j.1365-2249.2002.01800.x
160. Reynard S, Journeaux A, Gloaguen E, Schaeffer J, Varet H, Pietrosemoli N, Mateo M, Baillet N, Laouenan C, Raoul H, et al. Immune parameters and outcomes during Ebola virus disease. *JCI Insight*. 2019;4:e125106. doi: 10.1172/jci.insight.125106
161. McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, Spiropoulou CF. Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. *J Infect Dis*. 2014;210:558–566. doi: 10.1093/infdis/jiu088
162. McElroy AK, Harmon JR, Flietstra TD, Campbell S, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Kratochvil CJ, et al. Kinetic analysis of biomarkers in a cohort of US patients with Ebola virus disease. *Clin Infect Dis*. 2016;63:460–467. doi: 10.1093/cid/ciw334
163. Puerta-Guardo H, Glasner DR, Harris E. Dengue virus NS1 disrupts the endothelial glycocalyx, leading to hyperpermeability. *PLoS Pathog*. 2016;12:e1005738. doi: 10.1371/journal.ppat.1005738
164. Puerta-Guardo H, Glasner DR, Espinosa DA, Biering SB, Patana M, Ratnasiri K, Wang C, Beatty PR, Harris E. Flavivirus NS1 triggers tissue-specific vascular endothelial dysfunction reflecting disease tropism. *Cell Rep*. 2019;26:1598–1613.e8. doi: 10.1016/j.celrep.2019.01.036
165. de Sousa FTG, Warnes CM, Manuli ER, Tjang LV, Carneiro PH, Maria de Oliveira Pinto L, Ng A, Bhat S, Zambrana JV, D'Elia Zanella LGFAB, et al. Yellow fever disease severity and endothelial dysfunction are associated with elevated serum levels of viral NS1 protein and syndecan-1. *EBioMedicine*. 2024;109:105409. doi: 10.1016/j.ebiom.2024.105409
166. Suwarto S, Sasmono RT, Sinto R, Ibrahim E, Suryamin M. Association of endothelial glycocalyx and tight and adherens junctions with severity of plasma leakage in dengue infection. *J Infect Dis*. 2017;215:992–999. doi: 10.1093/infdis/jix041
167. Gopala Reddy SB, Chin WX, Shivananju NS. Dengue virus NS2 and NS4: minor proteins, mammoth roles. *Biochem Pharmacol*. 2018;154:54–63. doi: 10.1016/j.bcp.2018.04.008
168. ter Meulen J, Sakho M, Koulemou K, Magassouba N, Bah A, Preiser W, Daffis S, Klewitz C, Bae H-G, Niedrig M, et al. Activation of the cytokine network and unfavorable outcome in patients with yellow fever. *J Infect Dis*. 2004;190:1821–1827. doi: 10.1086/425016
169. Srikiatkhachorn A, Mathew A, Rothman AL. Immune-mediated cytokine storm and its role in severe dengue. *Semin Immunopathol*. 2017;39:563–574. doi: 10.1007/s00281-017-0625-1
170. van Eeden PJ, van Eeden SF, Joubert JR, King JB, van de Wal BW, Michell WL. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part II. Management of patients. *S Afr Med J*. 1985;68:718–721.
171. Connolly-Andersen AM, Moll G, Andersson C, Akerström S, Karlberg H, Douagi I, Mirazimi A. Crimean-Congo hemorrhagic fever virus activates endothelial cells. *J Virol*. 2011;85:7766–7774. doi: 10.1128/JVI.02469-10
172. Pahmeier F, Monticelli SR, Feng X, Hjorth CK, Wang A, Kuehne AI, Bakken RR, Batchelor TG, Lee SE, Middlecamp M, et al. Antibodies targeting Crimean-Congo hemorrhagic fever virus GP38 limit vascular leak and viral spread. *Sci Transl Med*. 2025;17:eadq5928. doi: 10.1126/scitranslmed.adq5928
173. Arslan M, Yilmaz G, Mentese A, Yilmaz H, Karahan SC, Koksal I. Importance of endothelial dysfunction biomarkers in patients with Crimean-Congo hemorrhagic fever. *J Med Virol*. 2017;89:2084–2091. doi: 10.1002/jmv.24881
174. Ozturk B, Kuscu F, Tutuncu E, Sencan I, Gurbuz Y, Tuzun H. Evaluation of the association of serum levels of hyaluronic acid, sICAM-1, sVCAM-1, and VEGF-A with mortality and prognosis in patients with Crimean-Congo hemorrhagic fever. *J Clin Virol*. 2010;47:115–119. doi: 10.1016/j.jcv.2009.10.015
175. Lukashevich IS, Maryankova R, Vladyko AS, Nashkevich N, Koleda S, Djavani M, Horejsh D, Voitenok NN, Salvato MS. Lassa and Mopeia virus replication in human monocytes/macrophages and in endothelial cells: different effects on IL-8 and TNF-alpha gene expression. *J Med Virol*. 1999;59:552–560. doi: 10.1002/(SICI)1096-9071(199912)
176. de St Maurice A, Harmon J, Nyakarahuka L, Balinandi S, Tumusiime A, Kyondo J, Mulei S, Namutebi A, Knust B, Shoemaker T, et al. Rift Valley fever viral load correlates with the human inflammatory response and coagulation pathway abnormalities in humans with hemorrhagic manifestations. *PLoS NeglTrop Dis*. 2018;12:e0006460. doi: 10.1371/journal.pntd.0006460
177. Van der Lugt JJ, Coetzer JA, Smit MM. Distribution of viral antigen in tissues of new-born lambs infected with Rift Valley fever virus. *Onderstepoort J Vet Res*. 1996;63:341–347.
178. Smith DR, Steele KE, Shamblin J, Honko A, Johnson J, Reed C, Kennedy M, Chapman JL, Hensley LE. The pathogenesis of Rift Valley fever virus in the mouse model. *Virology*. 2010;407:256–267. doi: 10.1016/j.virol.2010.08.016
179. Nagata S, Suzuki J, Segawa K, Fujii T. Exposure of phosphatidylserine on the cell surface. *Cell Death Differ*. 2016;23:952–961. doi: 10.1038/cdd.2016.7
180. Ansari SA, Pendurthi UR, Rao LVM. Role of cell surface lipids and thiol-disulphide exchange pathways in regulating the encryption and decryption of tissue factor. *Thromb Haemost*. 2019;119:860–870. doi: 10.1055/s-0039-1681102
181. Amara A, Mercer J. Viral apoptotic mimicry. *Nat Rev Microbiol*. 2015;13:461–469. doi: 10.1038/nrmicro3469
182. Pryzdial EL, Wright JF. Prothrombinase assembly on an enveloped virus: evidence that the cytomegalovirus surface contains procoagulant phospholipid. *Blood*. 1994;84:3749–3757. doi: 10.1182/blood.V84.11.3749
183. Avila-Aguero ML, Avila-Aguero CR, Um SL, Soriano-Fallas A, Cañas-Coto A, Yan SB. Systemic host inflammatory and coagulation response in the Dengue virus primo-infection. *Cytokine*. 2004;27:173–179. doi: 10.1016/j.cyto.2004.05.007
184. Huang YH, Liu CC, Wang ST, Lei HY, Liu HL, Lin YS, Wu HL, Yeh TM. Activation of coagulation and fibrinolysis during dengue virus infection. *J Med Virol*. 2001;63:247–251. doi: 10.1002/1096-9071(200103)63:3<247::aid-jmv1008>3.0.co;2-f
185. Tang K, Zhang Y, Zhang C, Hu H, Zhuang R, Jin B, Zhang Y, Ma Y. Hantaan virus-induced elevation of plasma osteoprotegerin and its clinical implications in hemorrhagic fever with renal syndrome. *Int J Infect Dis*. 2023;126:14–21. doi: 10.1016/j.ijid.2022.11.005
186. Chagan-Yasutan H, Lacuesta TL, Ndhlovu LC, Oguma S, Leano PS, Telan EF, Kubo T, Morita K, Uede T, Dimaano EM, et al. Elevated levels of full-length and thrombin-cleaved osteopontin during acute dengue virus infection are associated with coagulation abnormalities. *Thromb Res*. 2014;134:449–454. doi: 10.1016/j.thromres.2014.05.003
187. Rollin PE, Bausch DG, Sanchez A. Blood chemistry measurements and D-Dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus. *J Infect Dis*. 2007;196:S364–S371. doi: 10.1086/520613
188. Strampe J, Asogun DA, Speranza E, Pahlmann M, Soucy A, Bockholt S, Pallasch E, Becker-Ziaja B, Duraffour S, Bhadelia N, et al. Factors associated with progression to death in patients with Lassa fever in Nigeria: an observational study. *Lancet Infect Dis*. 2021;21:876–886. doi: 10.1016/S1473-3099(20)30737-4
189. Onguru P, Dagdas S, Bodur H, Yilmaz M, Akinci E, Eren S, Ozet G. Coagulopathy parameters in patients with Crimean-Congo hemorrhagic fever and its relation with mortality. *J Clin Lab Anal*. 2010;24:163–166. doi: 10.1002/jcla.20383
190. Hasbek M, Kıymaz Y, Büyüktuna SA, Yavuz H. Prognostic value of ratios of inflammatory markers in the prognosis of Crimean-Congo hemorrhagic fever. *Trop Med Infect Dis*. 2025;10:99. doi: 10.3390/tropicalmed10040099

Downloaded from http://ahajournals.org by on January 27, 2026

191. Bondu V, Bitting C, Poland VL, Hanson JA, Harkins MS, Lathrop S, Nolte KB, Lawrence DA, Buranda T. Upregulation of P2Y(2)R, Active uPA, and PAI-1 are essential components of hantavirus cardiopulmonary syndrome. *Front Cell Infect Microbiol*. 2018;8:169. doi: 10.3389/fcimb.2018.00169
192. Cosgriff TM, Morrill JC, Jennings GB, Hodgson LA, Slayter MV, Gibbs PH, Peters CJ. Hemostatic derangement produced by Rift Valley fever virus in rhesus monkeys. *Rev Infect Dis*. 1989;11:S807–S814. doi: 10.1093/clinids/11.supplement_4.s807
193. Geisbert TW, Hensley LE, Larsen T, Young HA, Reed DS, Geisbert JB, Scott DP, Kagan E, Jahrling PB, Davis KJ. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am J Pathol*. 2003;163:2347–2370. doi: 10.1016/S0002-9440(10)63591-2
194. Bondu V, Schrader R, Gawinowicz MA, McGuire P, Lawrence DA, Hjelle B, Buranda T. Elevated cytokines, thrombin and PAI-1 in severe HCPS patients due to Sin Nombre virus. *Viruses*. 2015;7:559–589. doi: 10.3390/v7020559
195. Cross RW, Speranza E, Borisevich V, Widen SG, Wood TG, Shim RS, Adams RD, Gerhardt DM, Bennett RS, Honko AN, et al. Comparative transcriptomics in Ebola makona-infected ferrets, nonhuman primates, and humans. *J Infect Dis*. 2018;218:S486–S495. doi: 10.1093/infdis/jiy455
196. Woolsey C, Cross RW, Chu VC, Prasad AN, Agans KN, Borisevich V, Deer DJ, Harrison MB, Martinez JK, Dobias NS, et al. The oral drug obeldesivir protects nonhuman primates against lethal Ebola virus infection. *Sci Adv*. 2025;11:eadw0659. doi: 10.1126/sciadv.adw0659
197. Rees PS, Lamb LE, Nicholson-Roberts TC, Ardley CN, Bailey MS, Hinsley DE, Fletcher TE, Dickson SJ. Safety and feasibility of a strategy of early central venous catheter insertion in a deployed UK military Ebola virus disease treatment unit. *Intensive Care Med*. 2015;41:735–743. doi: 10.1007/s00134-015-3736-y
198. Choi MJ, Worku S, Knust B, Vang A, Lynfield R, Mount MR, Objio T, Brown S, Griffith J, Hulbert D, et al. A case of Lassa fever diagnosed at a community hospital-Minnesota 2014. *Open Forum Infect Dis*. 2018;5:ofy131. doi: 10.1093/ofid/ofy131
199. Kulkarni PA, Chew D, Youssef-Bessler M, Hamdi HA, Montoya LA, Cervantes KB, Mazur NL, Lucas D, Wells JW, Cennimo D, et al. Case report: imported case of Lassa fever - New Jersey, May 2015. *Am J Trop Med Hyg*. 2018;99:1062–1065. doi: 10.4269/ajtmh.17-0316
200. Amorosa V, MacNeil A, McConnell R, Patel A, Dillon KE, Hamilton K, Erickson BR, Campbell S, Knust B, Cannon D, et al. Imported Lassa fever, Pennsylvania, USA, 2010. *Emerg Infect Dis*. 2010;16:1598–1600. doi: 10.3201/eid1610.100774
201. Ozturk B, Tutuncu E, Kuscu F, Gurbuz Y, Sencan I, Tuzun H. Evaluation of factors predictive of the prognosis in Crimean-Congo hemorrhagic fever: new suggestions. *Int J Infect Dis*. 2012;16:e89–e93. doi: 10.1016/j.ijid.2011.06.005
202. Fletcher TE, Leblebicioglu H, Bozkurt I, Sunbul M, Bilek H, Asik Z, Barut S, Gunes F, Gemici U, Hewson R, et al. Rotational thromboelastometry alongside conventional coagulation testing in patients with Crimean-Congo haemorrhagic fever: an observational cohort study. *Lancet Infect Dis*. 2019;19:862–871. doi: 10.1016/S1473-3099(19)30112-4
203. Martini GA. Marburg agent disease: in man. *Trans R Soc Trop Med Hyg*. 1969;63:295–302. doi: 10.1016/0035-9203(69)90001-7
204. Ristanović ES, Kokoškov NS, Crozier I, Kuhn JH, Gligić AS. A forgotten episode of Marburg virus disease: Belgrade, Yugoslavia, 1967. *Microbiol Mol Biol Rev*. 2020;84:e00095–e00019. doi: 10.1128/MMBR.00095-19
205. Koster F, Foucar K, Hjelle B, Scott A, Chong YY, Larson R, McCabe M. Rapid presumptive diagnosis of hantavirus cardiopulmonary syndrome by peripheral blood smear review. *Am J Clin Pathol*. 2001;116:665–672. doi: 10.1309/CNWF-DC72-QYMR-M8DA
206. Hartman AL, Powell DS, Bethel LM, Caroline AL, Schmid RJ, Oury T, Reed DS. Aerosolized Rift Valley fever virus causes fatal encephalitis in African green monkeys and common marmosets. *J Virol*. 2014;88:2235–2245. doi: 10.1128/JVI.02341-13
207. Peters CJ, Jones D, Trotter R, Donaldson J, White J, Stephen E, Slone TW. Experimental Rift Valley fever in rhesus macaques. *Arch Virol*. 1988;99:31–44. doi: 10.1007/BF01311021
208. Dennis LH, Reisberg BE, Crosbie J, Crozier D, Conrad ME. The original haemorrhagic fever: yellow fever. *Br J Haematol*. 1969;17:455–462. doi: 10.1111/j.1365-2141.1969.tb01393.x
209. Haddock E, Feldmann F, Hawman DW, Zivcec M, Hanley PW, Saturday G, Scott DP, Thomas T, Korva M, Avšič-Županc T, et al. A cynomolgus macaque model for Crimean-Congo haemorrhagic fever. *Nat Microbiol*. 2018;3:556–562. doi: 10.1038/s41564-018-0141-7
210. Daddario-DiCaprio KM, Geisbert TW, Geisbert JB, Ströher U, Hensley LE, Grolla A, Fritz EA, Feldmann F, Feldmann H, Jones SM. Cross-protection against Marburg virus strains by using a live, attenuated recombinant vaccine. *J Virol*. 2006;80:9659–9666. doi: 10.1128/JVI.00959-06
211. Jahrling PB, Hesse RA, Eddy GA, Johnson KM, Callis RT, Stephen EL. Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *J Infect Dis*. 1980;141:580–589. doi: 10.1093/infdis/141.5.580
212. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. 2017;23:1146–1149. doi: 10.1038/nm.4396
213. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. 2020;14:e0008637. doi: 10.1371/journal.pntd.0008637

Downloaded from http://ahajournals.org by on January 27, 2026