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Crimean-Congo Hemorrhagic Fever

Kırım Kongo Kanamalı Ateşi

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic infectious disease caused by the CCHF virus belonging to the genus *Nairovirus* of the *Bunyaviridae* family. Transmission occurs mainly as a result of *Hyalomma m. marginatum* (from *Ixodidae* family) tick bite. Nosocomial, laboratory-related transmission and travel-related cases have also been reported. Contact with the blood and infected products of viremic animals is another mode of transmission. Crimean-Congo hemorrhagic fever was first described in 1944 in the former Soviet Union on the peninsula of Crimea. In Turkey, the disease was recognized in 2002 and the first laboratory-confirmed case was reported in 2003. Crimean-Congo hemorrhagic fever has been reported in more than 30 countries in Asia, the Middle East, Europe, and Africa since it was first described. It is characterized by fever, muscle and joint pain, thrombocytopenia, elevation of liver and muscle enzymes, bleeding, and shock in serious cases. Although the case-fatality rate has been reported between 5-80%, this rate is 5% on average for Turkey. There is currently no effective treatment or safe vaccine specific to CCHF. With its wide geographical distribution and mortality, CCHF continues to be an important health problem in endemic regions such as our country.
Keywords: Crimean-Congo hemorrhagic fever, viral hemorrhagic fever, tick-borne disease, ribavirin, hyperimmunoserum

Öz

Kırım Kongo kanamalı ateşi (KKKA) *Bunyaviridae* ailesi, *Nairovirus* cinsine mensup KKKA virüsü tarafından oluşturulan kene kaynaklı zoonotik bir enfeksiyon hastalığıdır. Bulaş, başlıca *Ixodidae* ailesinden *Hyalomma marginatum* başta olmak üzere *Hyalomma* cinsi kenelerle temas sonucu oluşur. Bunun yanında nozokomiyal, laboratuvar kaynaklı bulaş ve seyahat ilişkili olgular da bildirilmiştir. Viremik dönemdeki hayvanların kan ve enfekte ürünleri ile temas diğer bulaş yollarındandır. Kırım Kongo kanamalı ateşi ilk olarak 1944 yılında eski Sovyetler Birliği'nde Kırım yarımadasında tanımlanmıştır. Türkiye'de ise 2002 yılında hastalığın farkına varılmış ve 2003 yılında laboratuvar konfirme ilk olgu bildirilmiştir. Kırım Kongo kanamalı ateşi görüldüğü tarihten günümüze kadar Asya, Orta Doğu, Avrupa ve Afrika'da 30'dan fazla ülkede bildirilmiştir. Hastalık ateş, kas ve eklem ağrısı, trombositopeni, karaciğer ve kas enzimlerinde yükselme, ciddi olgularda kanama ve şok ile karakterizedir. Olgu-fatalite oranı %5-80 arasında bildirilmiş olmakla birlikte Türkiye için bu oran ortalama olarak %5'tir. Günümüzde hastalığa özgü etkin bir tedavi ve güvenli bir aşı bulunmamaktadır. Geniş coğrafik dağılımı ve ölümcül olabilmesi nedeni ile KKKA, ülkemizin de içerisinde bulunduğu endemik bölgelerde önemli bir sağlık sorunu olmaya devam etmektedir.
Anahtar Kelimeler: Kırım Kongo kanamalı ateşi, viral hemorajik ateş, kene kaynaklı virüs, ribavirin, hiperimmün serum

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a life-threatening hemorrhagic fever endemic in Africa, Asia, Eastern Europe, and the Middle East. The virus that causes CCHF belongs to the genus *Nairovirus* in the *Bunyaviridae* family, and is transmitted via the bite of various tick species in the genus *Hyalomma*, particularly *Hyalomma m. marginatum*, or through contact with blood and body fluids of infected patients[1]. The main symptoms are high fever, headache, fatigue, nausea, vomiting, and diarrhea. Common laboratory findings are leukopenia, thrombocytopenia, elevated liver enzymes, elevated levels of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), and disrupted hemostasis parameters. Although most cases display a mild febrile illness, some patients develop severe hemorrhagic disease[2]. Crimean-

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Congo hemorrhagic fever presents an important health problem due to its wide geographical distribution, mortality risk, lack of specific treatment, and challenges in prevention and control.

The disease was first described in Turkey in 2002 and the number of cases has increased in subsequent years. Crimean-Congo hemorrhagic fever continues to be a serious public health concern in Turkey each year especially in the spring and summer months[2]. Although mortality rates of approximately 30% are reported globally, the average rate is only about 5% in Turkey[3]. Symptomatic treatment is important due to the lack of a specific treatment. For this reason, early detection and timely management of severe cases with high mortality risk has a positive impact on prognosis[4]. In this review, we discuss CCHF, a disease which poses an important health problem in our geographic region, in light of recent data.

History and Epidemiology

The first known CCHF outbreak occurred in the summer of 1944, during World War II, when 200 military personnel in the Crimean Peninsula developed an acute febrile illness with hemorrhage and shock. Investigators determined that the disease had developed following tick exposure, and the condition was named "Crimean hemorrhagic fever". The Crimean hemorrhagic fever virus was isolated in 1967. Later, this virus was renamed CCHF virus (CCHFV) in light of its antigenic similarity to the virus isolated in the Congo[2].

Crimean-Congo hemorrhagic fever was first recognized in Turkey when patients presented with clinical signs of hemorrhagic fever in the province of Tokat in the spring and summer of 2002, and a definitive diagnosis was confirmed with laboratory tests in 2003[3,5]. However, the disease is believed to have been present in Turkey much earlier due to our proximity to the Crimean region and the characteristics of the virus circulating in our country[2].

Crimean-Congo hemorrhagic fever virus is a tick-borne virus, and is the most common of the medically significant arboviruses after dengue fever virus[6]. After the virus was first described in 1967, cases were reported from the former Soviet Union (Crimea, Astrakhan, Rostov, Uzbekistan, Tajikistan, and Kazakhstan) and Bulgaria[7]. In the following years, outbreaks were seen in some parts of Africa (Congo, Uganda, and Mauritania). A number of cases have also been reported from Middle Eastern countries such as Iraq and Saudi Arabia[8,9]. Most cases reported in the last decade have been from Pakistan, Iran, Bulgaria, India, and Turkey[3,5,10-12].

The majority of cases in Turkey (about two-thirds) occur in Kelkit valley and the surrounding areas (Tokat, Sivas, Yozgat, Çorum, and Erzurum). This is attributed to climate, geographical factors, the presence of livestock, and increased risk of tick contact. Two out of three patients are farmers residing in rural areas[3,13]. Since 2002, growing awareness of the disease among physicians and the general population of Turkey has been accompanied by a considerable increase in the number of cases documented. Over 9000 cases have been reported in Turkey to date. The number of cases peaked in 2008-2009 and gradually decreased in the following years[5,14] (Figure 1). This may be explained by immunity within the community due to persistent immunity in those who have had the disease previously, or increased use of protection

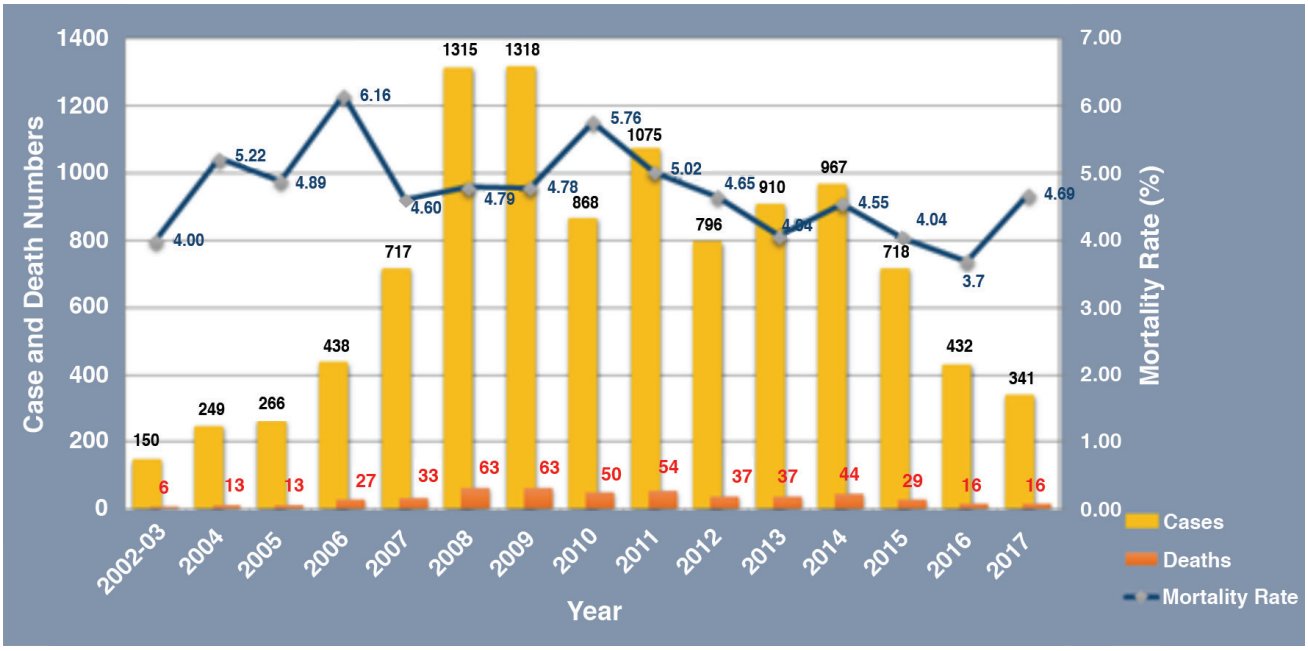

Figure 1. Reported cases of Crimean-Congo hemorrhagic fever, number of deaths, and mortality rates in Turkey between 2002 and 2017 (Ministry of Health data)

measures. Crimean-Congo hemorrhagic fever is seen between March and October in Turkey, being the most common in June and July. The disease affects men and women equally, and is more common in young adults (20-30 years)[2]. In 2009, a seroprevalence study utilizing "Enzyme-linked immunosorbent assay" (ELISA) to examine immunoglobulin G (IgG) in serum samples from 3,557 individuals living in endemic regions of Turkey determined a Crimean-Congo hemorrhagic fever virus seropositivity rate of 10%. This rate was found to increase with age, with 20.2% seropositivity among individuals 60-70 years old and 19.9% in those over 70. This finding is important because it shows that the disease has been present in Turkey from a much earlier point in time. In the same study, comparison of the population presumed to have encountered the disease based on the seropositivity rates (15156 people) with the number of diagnosed cases according to Ministry of Health data (1806 patients) led the authors to conclude that CCHF infection is asymptomatic in the vast majority of cases, and manifests as a symptomatic infection in about 10% of patients[15].

Virus Features

Crimean-Congo hemorrhagic fever virus is a member of the genus *Nairovirus* of the *Bunyaviridae* family, which also includes the genera *Orthobunyavirus*, *Hantavirus*, *Phlebovirus*, and *Tospovirus*. Nairoviruses are tick-borne viruses and are distinguished from other bunyaviruses by large L segments. Because it is an enveloped virus, CCHFV is not resistant to physical and chemical agents and cannot survive outside a host. The envelope glycoproteins (Gn and Gc) play a role in the attachment of the virion to host cell receptors. Gn and Gc are responsible for the fusion of infected cells, hemagglutination, and induction of neutralizing antibodies[16]. The virus genome is a negative-sense, single-stranded RNA with three segments called small (S), medium (M), and large (L). The S segment encodes the viral nucleocapsid protein, M encodes the Gn and Gc glycoproteins, and the L segment encodes the RNA-dependent RNA polymerase[17]. Crimean-Congo hemorrhagic fever virus shows the most genetic variation of all the arboviruses. Analysis of viral isolates has demonstrated 20% variation in segment S, 31% in segment M, and 22% in segment L[2].

The substantial differences in the antigenic structures of CCHFV in different geographical regions have been revealed by nucleotide and amino acid sequence data. Six different CCHFV genotypes have been identified through nucleic acid sequence analyses of the virus genome based on the S segment (Table 1). The data from S segment analysis suggest that the virus migrated from Africa to Europe, then to the Middle East, and finally to Asia. Evaluation of the L segment showed that the West African and Greek viruses were the oldest strains[2]. Knowing the different viral genotypes enables interpretation of the epidemiological data and may allow us to determine the source of outbreaks and spread of the disease. Furthermore, significant antigenic variations among the viral subgroups must be considered to determine the target of new vaccines[1].

Table 1. Distribution of Crimean-Congo hemorrhagic fever virus subgroups according to geographical region[2,16]

Group	Geographic area
1	Iran, South Africa, Senegal, Mauritania
2	South Africa, Nambia, Uganda, Congo
3	South Africa, Nambia, Senegal, Nigeria, Sudan
4	Iran, Pakistan, Iraq, China, Uzbekistan, Kazakhstan, Tajikistan, Oman
5	Iran, Turkey, Europe, Russia
6	Greece and Turkey

The CCHFV strains in Turkey are closely related to those found in Russia and Kosovo. They are 97-98% similar in nucleotide sequence and 100% at the protein level, and differ from the strain that caused an outbreak in Iran in 2002[5,18-20]. However, another study determined that the strains in Turkey were phylogenetically similar to some Iranian strains[21]. Ticks carried by migrating birds are thought to play a role as the vector of CCHF outbreaks. In a study examining 188 ticks collected from migratory birds in Turkey, the CCHFV genome was detected by polymerase chain reaction (PCR) in only 2 ticks of *Hyalomma* and *Ixodes* spp. It should be kept in mind that future epidemics may occur in other areas along the migration routes of migratory birds[22].

Transmission

Transmission of CCHFV to humans occurs primarily through salivary secretions passed from an infected tick as it feeds on the host. The incidence of CCHF is higher in the spring and summer months when *Hyalomma m. marginatum* and other vector species require blood meals in order to mature to their adult stages. In Turkey, the majority of patients live in rural areas and tick exposure is reported in about two out of three cases. The virus can also be transmitted by direct contact with body fluids (e.g., blood, placenta) of viremic animals or touching an infected tick with bare hands (e.g., extracting or crushing)[3].

Another route is nosocomial transmission via exposure to blood and body fluids of an infected patient. A study evaluating nine Turkish healthcare centers during 2002-2014 determined that 51 CCHF-related injuries were reported, 25 of which had CCHF infections confirmed by laboratory tests. In those 25 patients, percutaneous injury was reported as the main route of transmission. Since the disease was first identified in Turkey, 5 of the 25 patients infected by

nosocomial transmission died[13,23,24]. In the literature, there is also a report of nosocomial transmission from a single patient to 8 healthcare workers, believed to have occurred due to aerosol inhalation[25].

There is also evidence that the virus may be sexually transmitted. One study reported that three CCHF patients with a definite diagnosis were believed to have been infected through sexual contact during the incubation period or the early disease stages when symptoms were mild[26]. Although not recommended in the literature, it may be important to advise patients with active disease and those in recovery to practice safe sex.

Crimean-Congo hemorrhagic fever is also encountered as a travel-related disease. Twenty-one cases of travel-related CCHF have been documented in the literature, 12 of which were fatal. Infection of 4 healthcare workers by secondary transmission from 2 of those patients was reported in the same study. Travel was within Asia to Asia in 9 of those cases, within Africa in 5 cases, from Africa to Europe in 3 cases, from Asia to Europe in 2 cases, and within Europe in 2 cases. To date, there have been no reported case of transmission from travelers in Turkey[27].

Horizontal transmission of CCHFV from mother to baby has also been documented[28]. In a study evaluating two breastfeeding women with CCHF, virus was detected in the mother's milk by PCR, but the disease was not detected in the infants[29].

Pathogenesis

Although CCHF is a widespread disease, its pathogenesis is still unclear. The common pathogenic feature of all hemorrhagic fever viruses is their ability to disable the host immune response by disrupting the cells that initiate the antiviral response[1].

Endothelial and immune system cells are responsible for the pathogenesis. The epithelial cells are the first barrier the virus faces when it enters the body. After passing the epithelial cells, it enters the systemic circulation through the basolateral membrane of endothelial cells. Some viral particles remain in the endothelial cells, causing local inflammation[16,30]. After entering the circulatory system, the virus first replicates in the blood, liver, and spleen. It proliferates in tissue macrophages and dendritic cells and spreads to the local lymph nodes, liver, and spleen. It later reaches and proliferates in the lungs, kidneys, and brain. The brain is one of the organs infected in the late stage of the disease[31,32].

The primary mechanism implicated in the pathogenesis of CCHF is endothelial damage[33]. This damage may arise due to the direct effects of the virus or through indirect pathways[34]. A positive correlation has been observed between adhesion molecules and cytokine levels, which are indirect pathogenetic factors in CCHF, and endothelial damage. There is evidence that vascular endothelial growth factor receptor (sVEGFR) and vascular activation markers (sICAM-1 and sVCAM-1), which are indicators of endothelial damage, are elevated in serum and significantly higher in fatal cases[35-37]. Crimean-Congo hemorrhagic fever-infected endothelial cells were found to cause upregulation of sICAM-1 and sVCAM-1 and secretion of interleukins (IL-6 and IL-8)[32]. Serum levels of transforming growth factor-β (TGF-β), which is responsible for repairing endothelial damage, were shown to be lower in clinically severe cases exhibiting hemorrhage[38]. Numerous studies have shown that proinflammatory cytokine response is important in the pathogenesis of CCHF. Elevated tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), IL-6, and IL-10 levels have been observed in CCHF patients with severe clinical course[39-41]. Increased TNF-α synthesis leads to destabilization of the microtubules, resulting in increased vascular permeability and subsequent hypotension, organ failure, and shock. In another study, IFN-inducible protein 10, monocyte chemoattractant protein 1, and viral load were found to be associated with disease course[42]. Reactive hemophagocytic lymphohistiocytosis leading to pancytopenia has been observed in some patients and documented by bone marrow aspiration[43,44]. Adenosine deaminase, xanthine oxidase, neopterin, and chitotriosidase enzyme levels have been shown to be associated with pathogenesis[45-47]. Levels of plasma signal peptide-CUB-EGF domain-containing protein and C-type natriuretic peptide, which are new biomarkers that indicate vascular tone, were found to be higher in patients with severe disease[48,49].

Endothelial damage stimulates platelet coagulation and degranulation, thus activating the intrinsic coagulation cascade and contributing to hemostatic disruption[16]. Coagulation parameters become imbalanced and disseminated intravascular coagulation (DIC) may develop in severe cases. Insufficient synthesis of coagulation factors due to liver dysfunction, thrombocytopenia, and DIC are factors that lead to hemorrhage. Thrombocytopenia is a common finding in CCHF infection resulting from bone marrow hypoplasia and increased consumption due to DIC (Figure 2)[2,33].

Inadequate immune response is another important pathogenetic mechanism in CCHF. It has been shown that the antibody response is inadequate in deceased patients[33]. The CCHFV weakens the innate (natural) immune response and retards the adaptive (acquired) immune response, resulting in uncontrolled viral replication and dissemination throughout the body. Partial activation of macrophages and dendritic cells, delayed induction of IFN synthesis, weak antibody response, hemophagocytosis, and reduced natural killer (NK) cells and lymphocyte counts are all factors contributing to a weak immune response[31,32,50].

Although CCHFV is sensitive to IFN, it suppresses the antiviral effect by delaying IFN production. In IFN-receptor blocked mice, even very small viral doses caused rapidly progressive and fatal CCHF, showing that IFN has an important role in preventing liver damage and controlling viral spread[51]. IFN-α administration before infection of human endothelial cells was found to inhibit CCHFV replication, but did not decrease virus titers when given after infection[52].

The role of host genetics in the pathogenesis and prognosis of CCHF is not well understood. Evidence in humans suggests that human leukocyte antigens (HLA) molecules, which are also called the major histocompatibility complex (MHC) and are known to play a role in the host immune response and in susceptibility to various diseases (cancer, autoimmune diseases, infectious diseases, etc.), may have a role in the immunosuppression seen in CCHF. In one study, a relationship was established between HLA molecules and disease severity, with the *HLA-A*02* gene implicated in susceptibility to the disease and the *HLA-B*27* gene reported to have a protective role[53]. In a large-scale study conducted in South Africa, no association was found between viral genotype and pathogenicity[54].

Histopathological Findings

There have been few studies evaluating histopathologic abnormalities in CCHF due to difficulties in autopsy procedures[2]. Findings in livers obtained from autopsied patients vary broadly from mild necrosis characterized by Kupffer cell hyperplasia and Councilman bodies (eosinophilic necrotic hepatocytes), to extensive destruction of the hepatic lobules. Despite lack of cellular inflammatory response in the lobules, minimal infiltration of periportal lymphocytes and histiocytes has been observed. Lymphocyte necrosis and generalized lymphoid depletion were detected in the spleen. It was concluded that these hepatic and splenic findings were similar to those seen with other hemorrhagic fever viruses such as Lassa, Marburg, and Ebola[34,55].

Macroscopic imaging of the visceral organs of mice infected with the CCHFV shows serosal petechial rash and intestinal hyperemia, while microscopic examination reveals extensive hepatocellular necrosis and marked lymphocyte depletion and apoptotic debris in the spleen[31].

Pulmonary pathologies such as interstitial pneumonia, diffuse alveolar damage, and intraalveolar and interstitial hemorrhage have been reported in CCHF[34]. Postmortem histopathological examination of the kidneys of deceased CCHF patients showed extensive hemorrhage, primarily in the tubules and to a lesser extent in the glomeruli and interstitial tissue, but no cellular inflammatory response was observed[55].

Clinical and Laboratory Findings

Although the infection is asymptomatic in many other vertebrate hosts, CCHF can manifest as a severe hemorrhagic disease in humans[2].

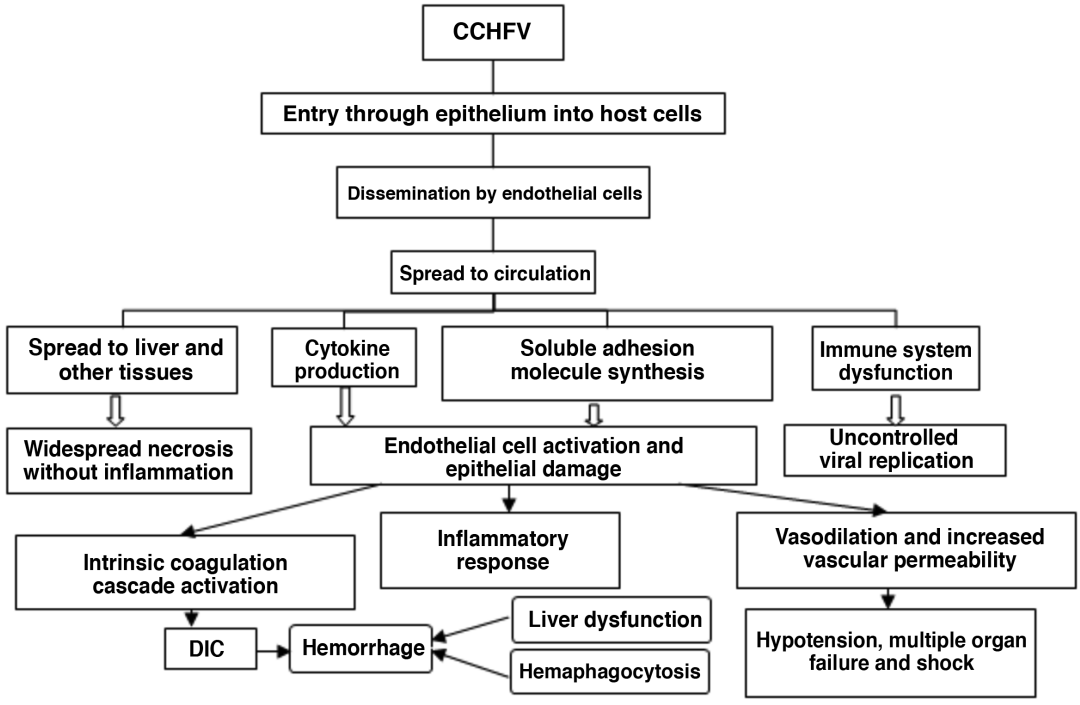

Figure 2. Pathogenesis of Crimean-Congo hemorrhagic fever[2,33]
CCHFV: Crimean-Congo hemorrhagic fever virus, DIC: Disseminated intravascular coagulation

The incubation period varies from 1 to 7 days (maximum two weeks), depending on how the virus is transmitted. Initial symptoms are nonspecific, such as fever, malaise, and headache, and appear suddenly[2]. The fever can rise upto 39-41 °C and remain high for 5-12 days. It can also show a biphasic course. Common symptoms in this stage are photophobia, back and abdominal pain, nausea, vomiting, diarrhea, generalized muscle pain, and sore throat[2,7]. Petechial and maculopapular rash and ecchymosis may be seen on the skin, conjunctiva, and mucous membranes[2]. Hemorrhage usually occurs 3-5 days after onset and may manifest as hematemesis, melena, epistaxis, hematuria, gingival bleeding, vaginal bleeding, and lung and brain hemorrhages[16]. In the following days, patients with severe hemorrhage may develop pulmonary parenchymal hemorrhage appearing as hemoptysis, dyspnea, signs of chest pain, and infiltration on chest X-ray[56]. Some cases result in death due to hemorrhage, multiple organ failure, DIC, and shock[16]. Hepatosplenomegaly is another common finding[5]. Clinical hepatitis develops with jaundice, hepatomegaly, and elevated serum transaminase levels[5,16]. Some cases also exhibit lymphadenopathy[16]. In some patients, abdominal ultrasound images reveal findings such as gallbladder wall edema and thickening, pericholecystic fluid, and hepatosplenomegaly[57]. Pulmonary findings are present in all stages of CCHF. Acute respiratory distress syndrome (ARDS) and diffuse alveolar hemorrhage associated with systemic inflammatory response have been reported while hemorrhagic findings are present. In a study evaluating 200 patients, cough and shortness of breath were reported as common symptoms, and patients with hemoptysis had a higher rate of ARDS[58].

In the first week of the disease, leukopenia appears first, followed by thrombocytopenia. Generally, neutropenia is transient and it is not necessary to treat patients for febrile neutropenia. Leukocytosis is an important finding in severe cases[59]. Abnormalities in coagulation parameters are also observed in the disease. Possible hemostatic changes include prolonged prothrombin time (PT) and active partial thromboplastin time (aPTT), decreased fibrinogen, and increased fibrin degradation products and D-dimer levels[16]. Patients may have elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) due to progressive hepatic involvement, and CPK and LDH levels are increased (Table 2)[2,16].

Prognostic Factors, Fatality, and Discharge Criteria

Mortality usually occurs in the second week of illness. Liver, kidney, heart, and lung failure are seen in fatal cases[62]. Patients with mild to moderate clinical course recover within about 9-10 days, with full recovery over a period of 2-6 weeks[16,40]. Although surviving patients do not typically suffer sequelae, symptoms such as prolonged fatigue, headache, sweating, and nausea may occur in the convalescence period[7,16].

Various studies have demonstrated a significant association between high viral load (>10^8 copies/mL) and clinical and laboratory findings such as deep thrombocytopenia, elevated AST and ALT, prolonged PT and aPTT, low fibrinogen, high ferritin level, hematemesis, melena, diarrhea, confusion, and somnolence[5,33,62-67]. In one study, high aPTT, thrombocytopenia, somnolence, and melena were identified as independent risk

Table 2. Clinical and laboratory findings of Crimean-Congo hemorrhagic fever[3,5,41,57,60,61]

Clinical findings	Prevalence (%)	Laboratory findings	Prevalence (%)
Fever	75-89.4	Leukopenia	78-90
Generalized muscle soreness	65.6-100	Thrombocytopenia	93.2-100
Headache	41.9-70.9	Elevated ALT and AST	85.9-100
Facial rash	32.2	Elevated CK	65.9-90
Nausea/vomiting	63-77.4	Elevated LDH	75.8-96.6
Abdominal pain	17.2-32.9	Prolonged aPTT/INR	66.6/16.6
Diarrhea	24.8-51.6		
Skin rash	10.8-51.6		
Petechiae, purpura, ecchymosis	25.5		
Lymphadenopathy	13-40		
Hepatomegaly, splenomegaly	12.9-43.3		
Splenomegaly	14-23.3		
Hyperemic conjunctiva	11.6		
Hemorrhage	23-48		
Change in consciousness	3.2-14		

ALT: Alanine aminotransferases, AST: Aspartate aminotransferases, CK: Creatine kinase, LDH: Lactate dehydrogenase, aPTT/INR: Active partial thromboplastin time/International normalized ratio

factors for mortality[64]. Laboratory follow-up in surviving patients showed improvement in leukopenia, hemostasis parameters, and renal function test results[57,60]. It was reported that thrombocytopenia persisted in patients who died; while surviving patients had normal platelet counts on day 11 of disease. According to the same study, deceased patients showed two peaks in ALT level, on days 5 and 9 of the disease, which were associated with hepatocyte damage[41]. In animal experiments, NK, T and B lymphocyte counts were elevated on the first day of infection and started to fall substantially by the third day. This finding can be explained as an initial increase in cells due to the effect of cytokines, followed by a subsequent reduction in numbers in the blood and spleen due to apoptosis and development of lymphopenia[31]. Leukopenia is a very common hematological finding in CCHF. A study of leukocyte subgroup values on days 1 and 3 showed that increased neutrophil count and decreased lymphocyte and monocyte counts were indicators of poor prognosis[58]. In another study, although there was no significant difference between T and B lymphocyte counts in severe cases, NK cell count was found to be significantly higher, which is thought to be a result of high NK proliferative response to excessive cytokine synthesis[68]. Akıncı et al. reported no difference between deceased and surviving patients in terms of NK cells; cytotoxic T cells were increased and correlated with viral load in deceased patients[69]. Bakir et al.[70] found that plasma cell-independent DNA was associated with viral load and reported it as a prognostic biomarker. They determined that serum plasma cell-independent DNA levels were higher in surviving patients. It has been shown that immune and antibody responses are weak in fatal cases, and that adhesion molecules and cytokine levels are associated with mortality[37,40,67,71]. Some studies have identified a relationship between human genotype and disease severity[53].

Though many parameters have been studied to determine prognosis in CCHF and some of them have emerged as important prognostic indicators, the main prognostic factor is high viral load (>10^8 copies/mL). In fact, some investigations have shown correlation between viral load and other parameters, with high viral load influencing these other parameters[39,41,63,72].

Taking previous CCHF prognostic studies into consideration, Bakir et al.[73] developed a severity scoring system. The score included age, presence of hemorrhage, organ failure, transaminase level, and DIC parameters. Using this system, they determined that all patients in the low-risk group (score <5) survived and the moderate-risk group had a mortality rate of 10%, while high-risk group had a mortality rate of 67%.

Crimean-Congo hemorrhagic fever is rare in pregnancy, but the course is severe when it does occur. In a study of three pregnant women with CCHF in Turkey, both the mother and fetus died in one case, and the babies of the two surviving patients died after birth. The authors emphasized that CCHF can cause miscarriage, neonatal complications, and death in pregnant patients, and concluded that outcomes were related to the gestation period (trimester) and the disease severity[74]. In a case report of a patient infected with CCHF in week 30 of pregnancy, the baby was born at 37 weeks completely healthy with no signs or symptoms related to the disease[75]. In another report, two pregnant patients in Turkey survived and gave birth to healthy babies by vaginal delivery[76]. A review of pregnant patients reported that maternal mortality was 34% (14/41) and fetal/neonatal mortality was 58.5% (24/41), and the presence of hemorrhage was associated with maternal and fetal mortality[77].

The Turkish Ministry of Health developed an algorithm for healthcare workers in order to standardize the approach to tick-exposure and CCHF cases[78]. This algorithm can help avoid potential problems in the diagnosis and treatment of CCHF, which is considered to be endemic in Turkey. Serious cases that meet the specified severity criteria are referred to the tertiary/referral hospitals. Discharge from hospital can be considered

Table 3. Biochemical and clinical risk factors considered to affect survival*[57,59,63]

Variables	Odds/Hazard ratio	95% CI	p value
aPTT ≥60 s	9.67	2.40-56.27	0.002
Platelet count ≤20x10^9/L	9.67	1.16-80.68	0.036
Increased urea level	1.236	1.066-1.433	0.005
White blood cell count >2950/µL	8.3	1.55-50.62	0.014
ALT >119.5 U/L	7.26	1.12-47.27	0.038
LDH >967.5 U/L	8.23	1.45-46.56	0.017
Diarrhea	6.875	1.600-29.538	0.010
Melena	6.39	1.64-24.93	0.008
Somnolence	6.30	1.80-22.09	0.004
Time from symptom onset to presentation	1.453	1.058-1.995	0.021

*Multivariate logistic regression analysis results, aPTT: Active partial thromboplastin time, CI: Confidence interval, ALT: Alanine aminotransferases, LDH: Lactate dehydrogenase

for patients who are clinically stable (fever resolved, no signs of hemorrhage) and whose laboratory values have normalized (platelet count >100.000/mm^3, normal hemostasis values, liver function tests less than x5 normal levels)[14]. Studies have shown that viral DNA is present in the urine and saliva of recovering patients, so they should be informed of the risk of transmission after discharge[79].

Diagnosis

Epidemiological data provide guidance in diagnosis. Diagnostic tests should be performed for patients with history of tick exposure, who live or have travelled to endemic areas, and have clinical and laboratory findings consistent with CCHF. Laboratory methods used to establish a definitive diagnosis include direct or indirect tests such as viral isolation and viral genome or specific antibody detection.

Serological assays and molecular methods are the most commonly used tests in routine practice. A study evaluating ELISA, immunofluorescence assay (IFA), quantitative reverse transcriptase (RT)-PCR and low-density macroarray assays in the diagnosis of CCHF reported test sensitivity of 87.8-93.9% for immunoglobulin M (IgM) serology, 80.4-86.1% for IgG serology, and 79.6-83.3% for viral genome detection. All tests had very high specificity, ranging from 95.5 to 100%. In the same study, IgM and IgG serology sensitivities were found to be 88% and 80% for ELISA and 93.9% and 86.1% for IFA, respectively[80].

Serological Assays

immunoglobulin M antibody response can be detected in the serum by 5-7 days and IgG antibodies also appear by 7-10 days after disease onset. Specific IgM level peaks at 2-3 weeks and falls to an undetectable level 4 months after infection. IgG levels can be detected for at least 5 years. Due to lack of reinfection in this disease, it is reported that IgG antibodies provide lifelong protection[16]. immunoglobulin M and IgG antibodies can be detected by ELISA and indirect IFA. ELISA has much higher specificity and sensitivity than IFA. Recently, recombinant nucleoprotein-based IgG ELISA methods have also been developed[81,82].

Molecular Methods

Molecular diagnosis is based on demonstration of viral nucleic acids by RT-PCR method. Because CCHF viremia lasts about two weeks, serum analysis must be done during the active phase of the disease. RT-PCR is preferable when a rapid diagnosis is needed, as the method is extremely specific, sensitive, and rapid. The newly developed automated "real time assay" method is even more sensitive and specific. This method minimizes the risk of contamination. Yapar et al.[83] first applied the real-time RT-PCR technique in Turkey in 2005. They stated that the real-time RT-PCR technique allows faster diagnosis and also provides data on the virus load in the samples. Genetic sequencing of CCHFV strains isolated as infectious agents in Turkish cases in 2006, 2007, and 2008 revealed phylogenetic similarities with CCHFV strains isolated in other regions[84].

Viral Isolation

Although it is not required for CCHF diagnosis, the viral isolation procedure should be done in a biosafety level 4 laboratory. Cell cultures such as Vero E6, BHK-21, SW 13, LLC-MK2, and CER may be used. Viral isolation from cell culture is simpler and faster than intracranial or intraperitoneal inoculation of neonatal mice, but is less sensitive. Blood sampling is recommended during the first week of viremia for culture[84].

Differential Diagnosis

In patients with clinical and laboratory findings consistent with CCHF, rickettsia, brucellosis, leptospirosis, viral hepatitis, typhoid fever, Q fever, borreliosis and sepsis must be considered, depending on the geographic area. Other infections with hemorrhagic clinical presentations should also be considered, including meningococcal infections, hantavirus, and depending on their endemic areas, malaria, yellow fever, dengue fever, Omsk hemorrhagic fever, and Kyasanur forest disease. In Africa, the differential diagnosis also includes Lassa fever, filovirus infections, and Ebola and Marburg infections[16]. Noninfectious causes of hemorrhagic diathesis such as acute leukemia, hemolytic uremic syndrome, idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, DIC, collagen vascular diseases, and poisoning should also be included in the differential diagnosis.

Treatment

The disease shows a broad clinical spectrum ranging from mild to severe or deadly presentations. Follow-up and treatment is fairly straightforward in patients with mild to moderate clinical course. Patients with severe prognostic markers should be followed at tertiary hospitals by a multidisciplinary team comprising infectious disease specialists and other branches such as hematologists and intensive care specialists. Supportive therapy is the main treatment for CCHF. Vital signs should be closely monitored and supported[6]. Any fluid imbalance should be corrected and fluid therapy should be provided until normal oral intake resumes. Fluid and blood

products (platelet suspension, erythrocyte suspension, fresh frozen plasma) should be administered in case of hemorrhage or hypovolemic shock, and vasopressors and positive inotropic drugs should be used when necessary. For patients with hemorrhage, random donor platelet concentrate at a dose of 1 unit per 15 kg, or a single unit of apheresis platelet suspension is recommended as replacement therapy. Platelet suspension is given to non-hemorrhagic patients with fever or hemostatic imbalance when platelet count falls below 20,000/mm^3; for patients with no hemorrhage or fever and normal coagulation parameters, platelet replacement is recommended when the platelet count is below 10,000/mm^3. Fresh frozen plasma is recommended at 10-15 mL/kg/day for patients with hemostatic imbalance[4]. Hemodialysis should be considered in case of renal insufficiency. Severe cases may develop a clinical presentation similar to ARDS or intrapulmonary hemorrhage. Such patients should be monitored accordingly and respiratory support should be provided as needed, with mechanical ventilation if necessary[4].

Aspirin and similar drugs that are toxic to platelets or impair their function, nonsteroidal anti-inflammatory drugs, anticoagulant therapy, and intramuscular injections are contraindicated. Invasive procedures that increase the risk of bleeding should be avoided unless necessary. In patients with a high risk of bleeding, oral nutrition should be interrupted and gastroprotective drugs such as H2 receptor blockers or proton pump inhibitors should be given[4].

Patients who die of CCHF have either absent or diminished IgG responses compared to survivors. It was also shown that deceased patients had significantly higher viral load compared to survivors[63,72]. These data suggest that administering an immunoserum, or antibody preparation, early in the disease course may be beneficial in facilitating viral clearance. In a Turkish study in which immunoserum prepared from patient sera was administered to 26 patients, it was reported that 13 (86.6%) of 15 high-risk patients with a high viral load (>10^8) survived and 2 patients died despite receiving hyperimmunoserum[85].

There is insufficient data on the use of plasmapheresis in CCHF. The use of double-filtration plasmapheresis was reported in one case and resulted in clinical improvement[86].

The only antiviral agent tested in CCHF treatment is ribavirin. An *in vitro* study showed that ribavirin inhibited viral activity and some strains of CCHFV were more susceptible than others; however, the efficacy of ribavirin in CCHF treatment has not been proven. Although some retrospective clinical studies have reported that ribavirin affects prognosis positively, there are also studies with contradictory findings[61,87-92]. Results of a single randomized controlled study conducted in Turkey suggested that ribavirin was ineffective[91]. In a meta-analysis of this topic, it was determined that ribavirin did not decrease the mortality rate[92]. The use of ribavirin has decreased since the early years of CCHF in Turkey, but the incidence and mortality rates have remained about the same. The Turkish Ministry of Health removed ribavirin from the treatment guidelines in 2008 due to the lack of proven efficacy[14]. There is no consensus regarding this issue in Turkey; the medical centers treating CCHF patients create their own treatment protocols. Our previous studies have shown that ribavirin treatment is not beneficial. Therefore, ribavirin is not included in our treatment protocol.

A study using a mouse model showed that ribavirin and arbidolon did not affect survival, while favipiravir was effective[93].

Prevention

There is currently no vaccine against CCHFV that meets modern standards. A CCHF vaccine prepared from formalin-inactivated mouse brain was developed in the Soviet Union and was used in 1970[12]. In Bulgaria, a similar vaccine is administered to soldiers, healthcare workers, and high-risk populations in endemic regions[94]. The V42/81 vaccine strain described in 1970 and used in Bulgaria is similar to strains isolated in Bulgaria, Kosovo, and Turkey[95]. A reduction in the number of cases has been noted in endemic regions where the vaccine is used. A total of 1,105 cases were reported in the years 1953-1974, whereas only 279 cases were reported in the 20 years period after the vaccine was introduced[96]. The vaccine was shown to boost cellular and humoral responses, with a 2- to 4-fold higher neutralizing antibody response in individuals who received the 4-dose vaccination[97]. Recently, a DNA vaccine against the M genome segment of CCHFV has been developed, but reports from experimental animal models indicate that it is not highly immunogenic[98]. Despite insufficient neutralizing antibody titers in mice immunized with DNA plasmids developed against virus capsid and envelope proteins, Th1 response was reported to be important in protection[99]. A vaccine developed in Turkey against the Turkey-Kelkit-06 CCHFV strain resulted in measurable levels of neutralizing antibody in the serum of infected mice after a second vaccination[100]. In another study, a vaccine expressing CCHFV glycoproteins was shown to improve cellular and humoral immunity in mice, but did not prevent death[101,102].

Since a protective vaccine has not yet been developed, preventative measures include avoiding or minimizing exposure to the virus. The main risk group comprises individuals living in endemic regions where tick exposure is likely (e.g. farmers and livestock farmers)[20]. Persons in contact with infected animals (veterinarians, butchers, soldiers, campers), healthcare personnel,

laboratory workers, researchers, and patients' relatives are also included in the risk group[103].

Fighting ticks is very important in prevention. Although tick behavior varies regionally and by species, the most common vector for CCHF, *Hyalomma m. marginatum*, is generally active in Turkey in April-October. Therefore, outbreaks typically occur in these months. The highest priority is preventing host exposure to ticks. People living in endemic areas should avoid tick-dense areas, frequently check themselves for ticks, and wear full-coverage clothing. Those working with livestock should avoid contact with infected tissues and blood and should not attempt to remove or crush ticks found on animals with bare hands. Another key precaution is to treat pets and other domesticated animals with appropriate acaricides. Making mandatory campaigns increases their effectiveness especially in endemic areas. Animal shelters should be constructed in such a way that prevents ticks from inhabiting them, and any cracks and crevices should be repaired and whitewashed. Appropriate acaricides should be applied to animal shelters where ticks are found. Insect repellents may be used to protect both humans and animals from tick infestations[103].

Because transmission can occur via the blood and body fluids of patients during acute illness, the healthcare personnel who care for these patients should follow contact and droplet isolation precautions. A surgical mask is enough for those entering and exiting a patient's room. However, N95/FFP3 masks must be used in situations where aerosol inhalation may occur (e.g., resuscitation, surgical interventions, intubation, aspiration, bronchoscopy) or blood and other body fluids may splash or be inhaled (e.g., centrifuge). Patients should be isolated or followed as a cohort[103].

In case of injury with a contaminated sharp instrument, the wound should be thoroughly washed with soap and water and wiped with 70% alcohol as an antiseptic. If broken skin or mucosa is exposed to infected body fluids, the area should be washed immediately with soap and water. In such cases, the patient should report the incident to the infection control team or a physician and be monitored for infection. It is recommended to monitor body temperature and complete blood count values for 14 days after exposure, and patients with fever or laboratory findings such as leukopenia or thrombocytopenia should be followed for suspected CCHF[1]. There is conflicting data regarding the efficacy of ribavirin used as post-exposure prophylaxis[94,104]. However, in percutaneous injuries such as needle-sticks, in which the risk of contamination is high, the best approach is to discuss and make prophylactic decisions with the injured healthcare worker. The World Health Organization recommends prophylactic ribavirin at a dose of 500 mg, 4 times daily for 7 days[105].

Travelers to endemic areas should be informed about risky activities and protective measures. Wearing light-colored clothing and applying permetrin to clothing and DEET solution to the skin is recommended. Patients who develop a fever after travel should seek immediate medical attention[27,103].

Crimean-Congo Hemorrhagic Fever in the Future

Certain studies have provided data suggesting that CCHFV vector ticks will spread to new areas. A more realistic approach predicts that the illness may be seen in countries such as Spain and Italy where *Hyalomma* ticks are present but the disease is not reported. In fact, cases have been reported following tick exposure in Spain[106]. It is also thought that CCHF may be seen in a more widespread geographical area as a result of passage of the virus to other vectors such as *Ixodes*, which cause tickborne encephalitis in Europe and Russia[2].

Conclusion

Crimean-Congo hemorrhagic fever is an important infectious disease due to its wide geographical distribution and mortality risk. It should be considered in the differential diagnosis of individuals with epidemiological risk factors and precautions should be taken to avoid secondary infection. Although some patients have history of tick exposure, the disease can also be encountered in patients with no tick contact. Therefore, the diagnosis should not be excluded in patients with consistent clinical signs, even those without a history of tick contact. Epidemiology of the disease does not change in terms of age or gender but shows seasonality. The most common complaints are nonspecific symptoms such as fatigue, fever, headache, nausea, vomiting, and diarrhea. These characteristics make it possible to overlook the diagnosis if epidemiological factors are not taken into consideration. For early intervention, patients should be carefully assessed using clinical and laboratory values, and early diagnosis is important because the initial stage is critical in terms of disease course.

Since CCHF was first described, numerous studies have investigated pathogenesis and prognosis of the disease. As cranial pathologies such as altered consciousness and intracranial hemorrhage may occur in the course of the disease, there is a need for studies in which patients are evaluated based on cranial imaging and electroencephalogram findings. Previous studies have not reported any association between viral genotypes and pathogenicity; nevertheless, it is important to evaluate whether there is a relationship between the virus genotypes found in Turkey and pathogenicity.

Although studies regarding treatment and vaccines are ongoing, an effective treatment or vaccine for the disease has not been

developed. There is no consensus, either globally or within Turkey, on the use of ribavirin in treatment. In light of previous studies, randomized controlled studies are needed in order to make progress in prevention and treatment.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.K., E.A., H.B., Design: S.K., E.A., H.B., Data Collection or Processing: S.K., E.A., H.B., Analysis or Interpretation: S.K., E.A., H.B., Literature Search: S.K., E.A., H.B., Writing: S.K., E.A., H.B.

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