Aetiology of PCR negative suspected Crimean-Congo hemorrhagic fever cases in an endemic area

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Crimean–Congo hemorrhagic fever (CCHF) is a potentially fatal tick-borne viral infection that is widely distributed worldwide. The diagnosis is frequently missed due to the non-specific initial symptoms and the differential diagnosis included many infectious and non-infectious causes. This retrospective study describes the clinical features and final diagnoses of 116 suspect CCHF cases that were admitted to a tertiary CCHF center in Turkey, and were CCHF IgM and PCR negative.

Keywords: Crimean–Congo hemorrhagic fever, Misdiagnosis, Endemic area, Viral hemorrhagic fever

Introduction

Crimean–Congo hemorrhagic fever (CCHF) is a potentially fatal viral disease, seen in Africa, Asia, the Middle East, and Eastern Europe. The causative agent is a virus from the Nairovirus group of the Bunyaviridae family, and a history of tick bite is reported in 69% of cases.[1] It emerged in Turkey in 2002, and until 2014, 9069 cases of CCHF have been reported, with a case fatality rate of 4.8%. The disease is mainly endemic to the north-eastern Anatolia region of Turkey (Tokat, Amasya, Sivas, Gumushane, Yozgat, and Corum) and has seasonal variation with most cases presenting during the spring to summer months (May–September).[1–3]

Fever and bleeding are the major clinical features of CCHF, but the diagnosis is frequently missed due to the non-specific initial symptoms (headache, myalgia, weakness, nausea, vomiting, abdominal pain, and diarrhea).[1–4] In severe cases, this can lead to nosocomial infection of HCWs in both endemic settings and from exported cases.[5] In Turkey, a well-established surveillance and referral system supported by regional molecular reference laboratories have resulted in good community engagement, rapid diagnosis, and case isolation.

During spring to summer period, 50% of cases that are admitted with suspected CCHF are found to be polymerase chain reaction (PCR) or IgM positive, but the etiology of the negative cases is unclear.[3] They share similar early clinical features, and the differential diagnosis includes infectious causes such as conventional community-acquired infections and other rarer presentations such as brucellosis, leptospirosis, ricketsiosis, viral hepatitis, Q fever, or potentially other viral hemorrhagic fevers (VHFs). Non-infectious causes include hematological diseases, malignancies, cirrhosis, and collagen tissue disorders.[6–8] The aim of the study is to identify the diagnoses in suspected CCHF cases that are PCR /IgM negative, and report their frequencies and clinical features.

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Materials and methods

Ondokuz Mayıs University Hospital is a tertiary healthcare facility with a 1150 bed capacity, which also serves as a regional referral center for CCHF patients. A total of 420 patients, all 18 years or above, were referred to the Infectious Diseases service between 2005 and 2014 with a suspected diagnosis of CCHF. Amongst these 420 patients, 116 patients who, following laboratory examination, were not diagnosed with CCHF were included in the present study.

Demographics, clinical features, laboratory results, and clinical progress were evaluated retrospectively from case records and the Ministry of Health Database. Serum samples were obtained from patients both at the time of admission and time of discharge, and sent to Refik Saydam Hygiene Institute Virology Reference and Research Laboratory. All the samples were stored and transferred in accordance with cold chain regulations. The serum samples were analyzed using enzyme-linked immunosorbent assay (ELISA) to detect CCHFV antibodies, and reverse transcription polymerase chain reaction (RT-PCR) to detect CCHFV RNA. Patients were evaluated according to criteria published in national guidance developed by the Turkish Republic's Ministry of Health[9,10] (Table 1).

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Table 1 CCHF case definitions according to Ministry of Health and Laboratory Criteria for diagnosis

Suspected case	Sudden onset with at least two of the following findings: fever, headache, diffuse body pain, arthralgia, fatigue, diarrhea, and bleeding with individuals who reside in endemic area or who has visited the endemic region or with tick bite or contact with tissue or blood/body fluids of infected animals within the previous two weeks
Probable case	Suspected cases who had thrombocytopenia, leukopenia, elevated AST and ALT levels
Confirmed case	CCHF IgM of PCR positivity in the blood or body fluids of the patient

Patients who tested negative for CCHF ELISA IgM and/or PCR CCHFV RNA were considered not to have CCHF. Ethical approval was obtained to investigate the archived files (This study was approved by Ondokuz Mayis University Ethical Committee (OMU KAEK 2013/488).

Results

Between 2005 and 2014, 116 of 420 patients (28%), who were referred with a diagnosis of suspected CCHF tested negative for CCHF IgM and CCHF RNA. The mean age of patients was 43.4 years and 66/116 (56.9%) were male. 51/116 patients (44%) had a history of living in an endemic region and/or a history of travel to an endemic region in the previous two weeks.

The most common complaint at the time of referral was fever (81/116, 70%). Other common complaints included weakness (46/116, 40%), headache (35/116, 30%), nausea (32/116, 28%), and myalgia (25/116, 22%). Three patients (2.5%) reported a history of bleeding, two with melena, and one with vaginal bleeding. Eighty-three patients (72%) reported a history of tick bite. Seventy-six of the suspect cases had an alternative diagnosis (Table 2), whereas the remaining patients ($n = 30$) only complained of a history of tick bite.

Amongst the 76 patients with a non-CCHF diagnosis, 45 patients (59.2%) had infectious diseases, whilst 31 patients (40.8%) had non-infectious diseases. The majority of infectious diseases was non-specific community infections (49%), whereas hematological diseases (58%) were the most common non-infectious diseases diagnosed. Twelve patients had documented cellulitis ($n = 12$) occurring around the tick bite site with localized edema, erythema, and warmth present with associated fever and/or leucocytosis. Patients diagnosed with respiratory tract infections ($n = 12$) reported a history of dyspnoea, cough and sputum production, had signs of auscultaion, and associated changes in chest radiography. Both groups received empiric antibiotic therapy.

In the six patients diagnosed with urinary tract infection, all had signs and symptoms consistent with either upper or lower tract infection and four had positive urine culture for *Escherichia coli* and empirical antibiotic therapy was started. The two culture-negative patients had already received empiric antibiotic therapy at the referring hospital prior to sample collection. Three patients with non-CCHF infectious diseases (respiratory tract infections, intraabdominal infection, and infective endocarditis) died during their hospital admission. All had previously been managed in the outpatient setting with suspected CCHF, with potenetial delays to definitive diagnosis and therapy.

The most common laboratory findings in the 76 patients, who had a non-CCHF diagnosis were elevated liver transaminases. The other most common laboratory findings were thrombocytopenia and leukopenia (Table 3). Of the 30 patients who were admitted to hospital with the only complaint of tick bite, 21/30 had low-grade fever and 13/30 had mild thrombocytopenia (110.000–140.000/mm^3). All were CCHF RT-PCR negative.

Discussion

In this study, we report the clinical features and diagnoses of a cohort of patients who were initially suspected of having CCHF, but tested negative and were later diagnosed with other diseases. The most common diagnoses were community-acquired infectious diseases and hematological disorders.

Table 2 Distribution of patients diagnosed other than CCHF

Diagnosis	*N* (%)	Subgroup diagnosis	*N*
Infectious diseases	45 (59.2)	Cellulitis	12
		Respiratory tract infection	12
		Urinary tract infection	6
		Intra-abdominal infection	4
		Sepsis unknown origin	3
		Leptospirosis	2
		Infective endocarditis	2
		Brucellosis	1
Non-infectious diseases	31 (40.8)	Isolated thrombocytopenia	13
		Toxic hepatitis	4
		Cryptogenic liver cirrhosis	4
		Myelofibrosis	3
		Megaloblastic anemia	2
		Myositis	2
		Colon cancer	1
		FMF	1
		Behcet's disease	1

Table 3 Laboratory findings at admission in patients with non-CCHF diagnosis

Laboratory findings	Number of cases	%
Elevated AST	49	57
Elevated ALT	43	50
Thrombocytopenia	34	40
Leukopenia	22	26

The clinical presentation of VHFs can be non-specific including fever, weakness, myalgia, nausea, and vomiting, and can be confused with various infectious and non-infectious causes. Improved understanding of the different VHF clinical syndromes has highlighted important differences that can aid diagnosis, such as the high prevalence of gastrointestinal disturbance in patients during the 2014–2016 West African Ebola outbreak,[11] whereas hemorrhagic features are less common, but frequently seen in CCHF.[1,2,7] These differences can only fully be evaluated through prospective longitudinal observation studies, including in the outbreak setting.

CCHF has a wide geographical distribution, including in Africa, the Middle East, Russia, and Eastern Europe. Whilst assessing patients for CCHF, it is also important to consider other VHFs (Lassa, Ebola, Marburg, Yellow Fever) and arboviral infections with similar modes of transmission.[6] It is also key not to overlook more common life-threatening infectious diseases that require immediate treatment and to also consider a broad range of non-infectious causes.

Of the 116 patients in our series that were referred with suspected CCHF and subsequently tested negative, 30 only had a history of tick bite, and as a result not all were admitted. A previous study has evaluated 251 patients who were admitted to emergency department with tick bite in a CCHF endemic region, and found that 82 patients (36%) were hospitalized with suspected CCHF, but CCHF PCR and/or IgM positivity was present in only 25.1% of the 251 patients.[12] It is, however, important to ensure the follow-up of patients with tick bites from CCHF endemic areas, particularly those with mild laboratory abnormalities at baseline. Those developing either fever or other non-specific clinical features of CCHF, or laboratory features such as thrombocytopenia, leucopenia, or elevated liver enzymes require admission, assessment, and CCHF testing. However patients who have only had potential exposure to CCHFV through a tick bite in an endemic area should not be considered suspect cases or routinely referred for CCHFV testing. The number of patients with tick bite only that were referred as suspect CCHF cases in our study reflects a previous lack of adherence to national guidance, and is now a rare occurrence as education programs for healthcare workers and improved understanding of CCHF epidemiology and disease has developed.[13]

In this study, fever was the most common complaint (70%) in suspected CCHF cases that were CCHF PCR and IgM negative. Other common complaints included weakness, headache, nausea, and myalgia. Amongst the 45 patients with non-CCHF infectious diseases, all patients were febrile, except some four patients with cellulitis in the bitten region, two patients with acute hepatitis, a patient with a respiratory tract infection, and a patient with sepsis. Fever, headache, myalgia, vertigo, nausea, vomiting, and diarrhea are all seen during pre-hemorrhagic period of CCHF, and these symptoms can be confused with the early stages of respiratory tract infections, influenza, brucellosis, leptospirosis, Q fever, ricketsiosis, Hanta virus infection, viral hepatitis, malaria, and sepsis. It is important that healthcare workers in endemic settings maintain a balance of the necessary heightened awareness for CCHF, to prevent delayed diagnosis with potential nosocomial implications, with the realization that up to 50% of suspect cases have an alternative diagnosis that may require immediate treatment.

Epidemiological features are important components of this risk assessment and clinical evaluation for CCHF. The differential diagnosis of CCHF also requires knowledge of the frequency of other infectious diseases in a given region and an understanding of the non-infectious causes that can mimic its presentation. In accordance with previous reports, we detected a range of infections, including at common sites such as of the respiratory and urinary tract, but also noted cases of leptospirosis, brucellosis, and infective endocarditis in suspected CCHF. Zoonotic infections are common in Turkey and brucellosis has previously been confirmed in a 13-year-old boy initially suspected to have CCHF due to a history of tick bite, epistaxis, anaemia, and thrombocytopenia.[14] Another patient that was evaluated for CCHF with tick bite, hemoptysis, and thrombocytopenia was later diagnosed with acute Q fever.[15] Co-infections can also rarely occur as reported in fatal case from Bulgaria with malaria co-infection,[16] and with secondary bacteraemia as reported from Turkey.[3]

Clinical and laboratory findings of hematological diseases, such as febrile neutropenia and acute leukemia can also be confused with CCHF in those from endemic settings. Gastrointestinal (GI), genito-urinary, and respiratory tract bleeding are seen during the hemorrhagic period of CCHF,[6] yet malignancies, peptic ulcer disease, and connective tissue diseases can also have similar presentations. In our study, two patients had GI bleeding, and one patient had vaginal bleeding due to a spontaneous abortion. Given the risk of CCHFV human-to-human transmission, and well-reported nosocomial outbreaks, any invasive intervention in patients with bleeding must be carefully considered with adequate protective measures in place.[17–19] As a result, healthcare professionals may delay interventions in patients with suspected CCHF, prolonging the diagnosis and treatment of the underlying disease. In our series, delayed diagnosis may have contributed to the deaths of three elderly patients initially suspected of CCHF but later diagnosed with a respiratory tract infection, intra-abdominal infection, and infective endocarditis. Contrary to the

standard treatment of other VHFs such as Ebola virus disease, patients with CCHF do not receive empiric antibiotic treatment, unless a bacterial infection is suspected or confirmed.

The most common laboratory findings in CCHF are low thrombocyte and white blood cell counts; elevated AST, ALT, LDH, CPK; and prolonged PT and aPTT.[4,12] Previous studies have reported that thrombocytopenia and leukopenia are the most common laboratory findings in Turkish CCHF patients.[1,20] In the present study, an elevated liver function test was the most common laboratory finding in the 76 patients who had a non-CCHF diagnosis (elevated AST in 49 patients; elevated ALT in 43 patients). Other common findings were thrombocytopenia (n = 34) and leukopenia (n = 22). These findings contributed to patients being initially suspected of CCHF. Subsequently, these patients were mainly diagnosed with isolated thrombocytopenia, myelofibrosis, and cirrhosis. Many of the other acute infections such as severe respiratory tract and intra-abdominal infections, brucellosis, and leptospirosis also have similar laboratory findings.

CCHF can also be overlooked due to the non-specific symptoms in the early stages. Fisgin et al. reported that 95 of 140 patients with a confirmed CCHF diagnosis (68%) were misdiagnosed initially, and 23% of these patients received unnecessary antibiotics.[21] The diagnosis of CCHF is confirmed by the detection of IgM antibodies and/or PCR for CCHFV RNA. On the other hand, these tests are only carried out in reference laboratories, prolonging the confirmation of a diagnosis. At the same time, given that the minimum time for IgM antibody positivity is five days,[22] it can be negative during early stage disease and presentation. Thus, the development of practical and rapid CCHF point-of-care tests will be a useful aid to routine practice and facilitate early diagnosis.

Potential limitations in our study reflect the sample population, based on our unit being a tertiary referral center for CCHF in Turkey. As such we are more likely to manage severe/complicated CCHF altering the range of diseases seen in the CCHF negative suspect cases. In secondary hospitals where mild CCHF is managed, a higher proportion of CCHF negative suspect cases is seen, often with a mild febrile disease post tick-bite without a diagnosis being confirmed. This may well reflect rickettsial disease or Q fever, and is the subject of ongoing prospective research.

CCHF must be considered in all febrile cases in endemic regions, particularly during the spring and summer months, to enable early diagnosis and disease management. Key tenets of early CCHF diagnosis are education and awareness of healthcare workers and endemic communities, supported by thorough clinical assessment and rapid access to molecular diagnosis. As we have discussed, a broad differential diagnosis must also be considered, to avoid delay in the diagnosis and treatment of other important infectious and non-infectious diseases.

Conflict of interest

The authors have no conflict of interest.

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