

*Immune and Physiological Human
Responses to CCHF Infections &
Factors Influencing the Outcome in
Patients*

Dr. Zülal ÖZKURT, MD

*Atatürk University, Medical Faculty, Department of
Clinical Bacteriology and Infectious Diseases,
Erzurum, Turkey*

Overview

- Immune response
- Physiological response to CCHF infections
- Factors influencing outcome & clinical progress

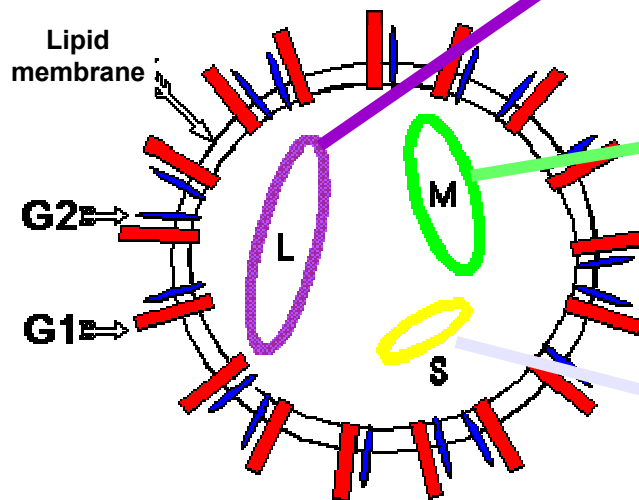


IMMUNE RESPONSE

- Antigens and antibodies
- Cytokines
- CD4⁺ - CD8⁺ T cells
- Interferons

Antigens and Antibodies

Virus includes three RNA regions



Bunyaviridae

L (Large)
Viral RNA polymerase

M (Medium)
Structural proteins
(G1=G_N and G2=G_C)

S (Small)
Nucleocapsid
protein (N)

Antigens

Major viral antigens:

- N protein is highly antigenic protein
- NSs gene is an important virulence factor for Bunyaviridea family
- NSs protein is an interferon antagonist and has a critical function of suppressing the production of interferon in virus infected-cells

Antigens

- G1 and G2 envelop proteins
 - They have a role in the induction of immunity
 - They are responsible for viral neutralization, fusion of infected cells, and hemagglutination
 - Features effecting pathogenesis related to G1 and G2 envelop proteins
 - Tropism to tissue, type of host and virulence of the virus
- GP38, GP85 and G160 glycoproteins have also been described

Antibodies (1)

- Relatively little is known about the mechanisms by which Bunyaviruses enter cells or how infection can be prevented by neutralizing antibodies and MAbs that block CCHFV infection have not been described
 - Monoclonal antibodies (MAbs) to G1 or G2 or both, but not to N proteins, can neutralize viral infectivity in vitro
 - Passive transfer neutralizing, but not nonneutralizing, MAbs or monospecific polyclonal sera to G1 or G2 or both, protect animals from challenge with homologous viruses
 - Immunization of animal with recombinant viruses expressing G1 or G2 (or both) results in protective immunity

These data show that a humoral immune response occurring against one or two envelop proteins (G1 and G2) may be sufficient for protection from infection with viruses in the family

Antibodies (2)

- A novel study showed that:
 - Anti-G_C MAbs did not neutralize CCHFV infection in human cell culture but provide partial protection to mice 24 hours before viral challenge
 - When these MAbs were administered 24 hours after virus challenge, protection was usually not observed
 - It was claimed that those MAbs do possess some neutralizing activity that was not detected in that in vitro assay or that other antibody-based effector mechanisms, such as antibody-dependent cell mediated toxicity or complement-mediated cell lysis, function in this context

Antibodies (3)

- Likewise, many of the anti-G_N MAbs conferred significant protection to CCHFV challenge, even when applied 24 hours after virus challenge and even though they did not prevent virus infection of human cell culture
- These data show that there is an imperfect relationship between in vitro neutralization and in vivo protective ability, and that the ability of an antibody to neutralize CCHFV may depend in part on host factors

Bertolotti-Ciarlet A et al. J Virol, 2005

Antibodies (4)

- A typical humoral immune response and neutralizing antibody inhibit the viral replication
- **IgM**
 - IgM antibody is detectable in the sera after the 6th day of disease,
 - maximum titers were usually attained in the 2 to 3 weeks after the disease
 - declines to undetectable levels by the 4th month after infection
- **IgG**
 - Titers of Ig G increased markedly between 2 and 4 months after onset of disease
 - remained readily demonstrable 5 years after infection

Antibodies (5)

- Complement-fixation (CF) antibodies
 - slowly increase after CCHF infection and reach maximum levels in the sera in 6 weeks
 - Half-life of CF antibodies is 2-3 years
 - Especially in old patients, these antibodies (CF) could not be detectable
- Hemagglutination-inhibition antibodies increase fast in the first week of the disease and later decrease to some extent, but stay life-long

Cytokines (1)

A). Proinflammatory cytokines

■ IL-6

- found at high levels both in severe and mild cases
- positive correlation between DIC scores and IL-6 levels
- has an association with mortality

-Ergonul O. et al, J Infect Dis, 2006
-Papa A. et al, J Clin Virol, 2006

Cytokines (2)

■ TNF- α

- TNF- α was found at high levels and associated with the severity of the disease
- positive correlation between DIC scores and TNF- α
- significant association with mortality, too.

-Ergonul O. et al, J Infect Dis, 2006

-Papa A. et al, J Clin Virol, 2006

Cytokines (3)

B). Antiinflammatory cytokines

■ IL-10

- a negative correlation between IL-10 and DIC scores
- not associated with mortality

Ergonul O. et al, J Infect Dis, 2006

- detected in only one fatal case

Papa A et al, J Clin Virol, 2006

Interferon

- Interferone- α probably have an important role in immune response to the disease
- The type I interferon system, that includes protein kinase R, 2-5 oligoadenylate syntase/RNase L and Mx proteins, has been shown to be important for antiviral activity

Andersson I et al. J Med Virol, 2006

- Delayed interferon induction in response to infection appears to lead to unchecked viremia resulting in severe hepatic injury in Rift Valley fever infection (The virus is an other Bunyaviridea member)
- An early interferon response dampens viremia, reduces the degree of hepatic injury, and result in transient illness with full recovery

Morrill JC et al. Arch Virol, 1990

CD4⁺-CD8⁺ T Cells (1)

In our study conducted in 22 patients revealed that:

- CD4 values were found slightly increased in only 2 patients
- CD8 values increased in 1 and decreased in 1 patient of 22 patients
- There is no significant difference between CD4 and CD8 values and CD4/CD8 ratio.
 - in the patient and healthy control groups
 - at different stages (early, acute and recovery periods of the disease)

CD4-CD8 (2)

- CD4/CD8 ratios were reversed in 4 patients (19%) at early and acute stage of disease, and spontaneously returned to normal levels at recovery period.
- There was no association between clinical and laboratory response with CD4/CD8 ratio and influence on clinical outcome
- To determine the exact role of CD4 and CD8 in CCHF, further studies are needed.

Immunity

- Immunity after CCHF infection probably stays life-long

Us D. Arboviruses, Basic and Clinical Microbiology (in Turkish), Ankara, 1999



PHYSIOPATHOLOGICAL RESPONSE to CCHF INFECTION

- Pathological changes
- Clinical changes
- Laboratory changes

Pathological Changes (1)

- The histopathologic features of CCHF resemble those of other viral hemorrhagic fevers (VHF)
- VHF viruses have an ability to disable the host immune response by attacking and manipulating the cells that initiate the antiviral response
- This damage is characterised by marked replication of the virus together with dysregulation of the vascular system and lymphoid organs

-Burt FJ et al. Arch Patol Lab Med 1997

-Chen JP, Cosgriff TM. Blood Coagul Fibrinolysis, 2000

Pathological Changes (2)

- Main targets of CCHFV :
 - Endothelial cells
 - Hepatocytes
 - Mononuclear phagocytes

Endothelium

- Viral antigens are found in endothelium and liver
- Infection of endothelium has an important role in CCHF pathogenesis
- The endothelium can be targeted in two ways indirectly by viral factors or virus mediated host-derived soluble factors that cause endothelial activations and dysfunction, and/or directly by virus infection and replication in endothelial cells
- Endothelial damage contributes to haemostatic failure by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade

Pathological Changes (3)

Liver

- Necrosis:
 - Focal
 - Spread along lobules
 - Necrotic areas:
 - marked by **hemorrhage** and **cell loss**
 - always associated with **eosinophilic change** of hepatocytes and prominent **Councilman bodies**
 - Inflammatory mononuclear infiltrates in necrotic areas are either absent or mild, and are not related to extent of hepatocellular damage
 - The lack of significant inflammatory response in association with infected and damaged hepatocytes suggest that cellular damage is mediated by a direct viral cytopathic effect
 - Severe hepatic necrosis correlates with fatal outcome and with low or inefficient immune response
- Fatty change
- Kupffer cell hyperplasia

Pathological Changes (4)

Immune system

- Exhaustion of lymphoid cells and infection of mononuclear phagocytes may result in:
 - immune inactivation
 - escape of the virus from immune system
 - systemic dissemination of the virus

Spleen

- Lymphoid depletion
- Focal necrosis
- Scattered lymphoblasts in periarterial sheaths

Pathological Changes (5)

■ Myocardium

- Congestion
- Slight interstitial edema

■ Lung

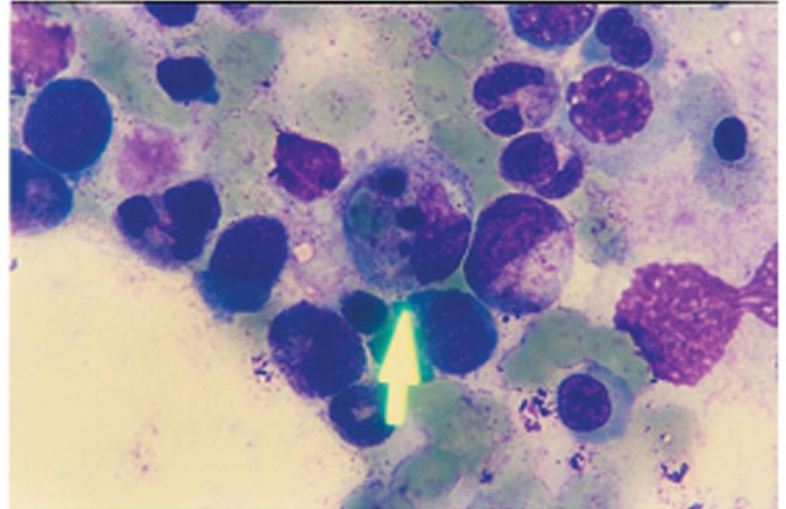
- Diffuse alveolar damage
- Intra-alveolar hemorrhage
- Hyaline membrane formation
- Mononuclear interstitial pneumonitis

-Burt FJ et al. Arch Patol Lab Med 1997

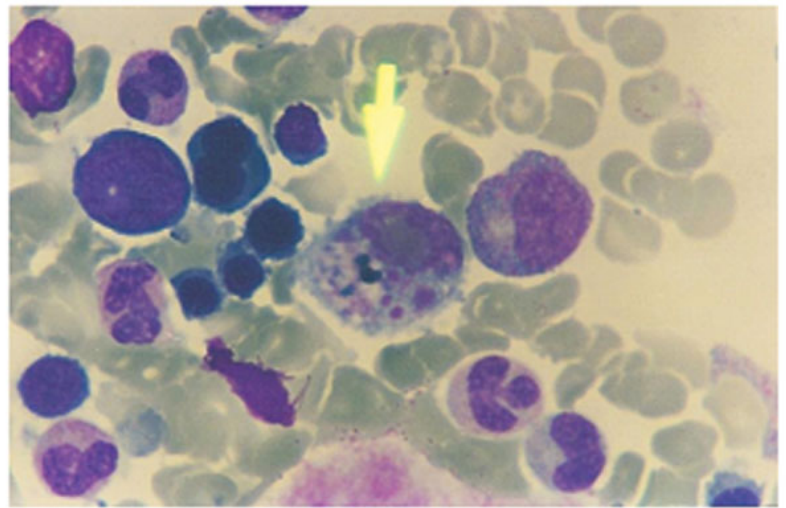
-Chen JP, Cosgriff TM. Blood Coagul Fibrinolysis, 2000

Phagocytosis of erythrocytes

A



B



Phagocytosis of thrombocytes

Hematologic changes

- Hypoplasia of bone marrow
- Hemophagocytosis (50%)
- Reduced megacaryocytes in bone marrow
- Low platelet count
 - Increased consumption due to DIC
 - Decreased production

Karti S et al. Emerg Infect Dis 2000

Burt FJ et al. Arch Patol Lab Med 1997

Clinical Changes

Symptoms

■ In the early stage (first 5 days) of the disease, these symptoms are observed

	<u>%</u>
■ Malasia and fatigue	94-100
■ Myalgia and arthralgia	62-100
■ Fever	75-91
■ Nausea and vomiting	73-90
■ Headache	76-85
■ Diarrhea	30-38
■ Cough	29-30
■ Abdominal pain	28
■ Confusion	8-14
■ Mood alterations	

Symptoms

- After 5 days of the disease %
 - Bleeding 46-48
 - Epistaxis 17-52
 - Hematemesis 7-34
 - Melena 1-14
 - Hemoptysis 9
 - Hematuria 8-19
 - Other sites
 - Vagina 11
 - Subcutaneous 30
 - Gingiva 8
 - Ear 1
 - Intraabdominal 2
 - Brain 1
 - Multiple sites 3-25
- Bleeding was seen in 90% of cases in Iran

-Elaldi N. KLİMİK CCHF Train Meet, 2004, Ankara
-Ozkurt Z et al. J Infect 2006

-Bakır M et al. J Med Microbiol, 2005
-Ergonul O et al. Clin Microbiol Infect, 2006

-Alavi-Naini R et al. J Infect, 2006

Signs

	<u>%</u>
■ Fever	43-85
■ Facial hyperemia	50
■ Bleeding	29-48
■ Hepatomegaly	30-43
■ Lymphadenopathy	13-40
■ Skin eruption	32-39
■ Maculopapular rash	29-57
■ Petechia and ecchymosis	30-46
■ Lung involvement	4-28
■ Splenomegaly	14-23
■ Peritoneal irritation	12-21
■ Conjunctivitis	11-50
■ Cardiac involvement	1-11
■ Neck stiffness	11
■ Jaundice	1-12

Laboratory Changes (1)

Increased

	<u>%</u>
■ Lactate dehydrogenase (LDH)	98-100
■ Aspartate aminotransferase (AST)	91-100
■ Alanine aminotransferase (ALT)	73-100
■ Creatine phosphokinase (CPK)	24-90
■ Blood urea nitrogen (BUN)	22
■ Creatinine	19

levels

■ Thrombocytopenia	98-100
■ Leukopenia	75-90
■ Anemia	11-53
■ Proteinuria	42
■ Hematuria	32

Laboratory Changes (2)

Prolonged

	<u>%</u>
■ Prothrombin time (PT)	21
■ Active prothrombin time (aPTT)	24-66
■ International normalized ratio (INR)	16

-Elaldi N. KLİMİK CCHF Train Meet, 2004, Ankara
-Ozkurt Z et al. J Infect 2006
-Bakır M et al. J Med Microbiol, 2005
-Ergonul O et al. Clin Microbiol Infect, 2006
-Alavi-Naini R et al. J Infect, 2006

Table 4 Laboratory results of patients with CCHF

	Confirmed cases (<i>n</i> =26), <i>n</i> (%)	Suspected cases (<i>n</i> =34), <i>n</i> (%)	Total (<i>n</i> =60), <i>n</i> (%)	Mean	Range
Elevated ALT ^a	26 (100)	34 (100)	60 (100)	804 U/l	55-19 580 U/l
Elevated AST	26 (100)	34 (100)	60 (100)	620 U/l	78-5923 U/l
Thrombocytopenia	25 (96.1)	34 (100)	59 (98.3)	42 589/ μ l	3000-153 000 \times 10 ³ / μ l
Elevated LDH	24 (92.3)	34 (100)	58 (96.6)	2037 U/l	381-25 380 U/l
Leucopenia	25 (96.1)	29 (85.2)	54 (90.0)	2200/ μ l	1100-8500 μ l
Elevated CK	26 (100)	28 (82.3)	54 (90.0)	788 U/l	40-3888 U/l
Long PTT	19 (73.0)	21 (61.7)	40 (66.6)	42.6 s	24-113 s
High ESR	12 (46.1)	25 (73.5)	37 (61.6)	27.5 mm/h	4-70 mm/h
Anemia	12 (46.1)	20 (58.8)	32 (53.3)	11.9 g/dl	4.9-11.6 g/dl
Proteinuria	12 (46.1)	13 (38.2)	25 (41.6)	150 mg/dl	100->300 mg/dl
Hematuria	8 (30.7)	11 32.3)	19 (31.6)		2-Many
High INR	6 (23.0)	4 (11.7)	10 (16.6)	1.25	0.87-6.0
High CRP	3 (11.5)	7 (20.5)	10 (16.6)	1.8 mg/dl	0.1-12
Pyuria	3 (11.5)	6 (17.6)	9 (15.0)	3-5	2-Leucocyte groups

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatin phosphokinase; PTT, partial prothromboplastine time; INR, international normalized ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

^a ALT, alanine aminotransferase.



FACTORS INFLUENCING OUTCOME

- Clinical factors
- Laboratory factors
- Immunologic factors
- Viral factors

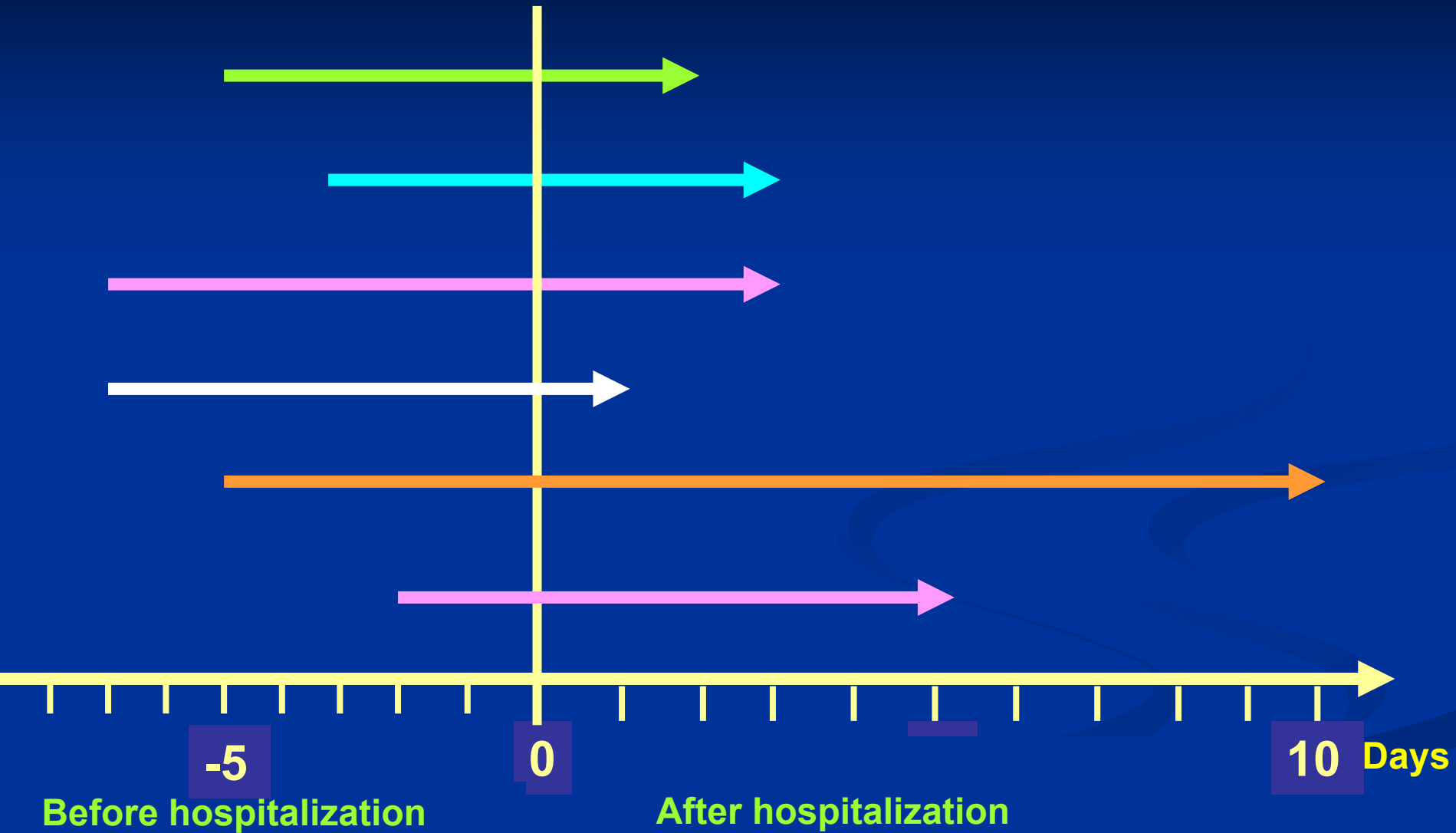
Clinical Outcome

- Mild cases usually improve at days 9-10
- Fatality rates range from 5 to 80 % (In Turkey: 5-10 %)
- Death occurs usually at the second week of the disease
- No sequela is seen in survivors

Clinical Outcome

- Convalescent period last long about 2-3 weeks after onset of the disease
 - Prolonged and generalized weakness
 - Weak pulse
 - Hair loss
 - Sweating
 - Polyneuritis
 - Headache
 - Dizziness
 - Nausea
 - Poor appetite
 - Labored breathing
 - Poor vision
 - Memory loss

Clinical Outcome of Fatal Cases



Clinical Factors Influencing Outcome (1)

Clinical predictors of poor prognosis

- Cerebral hemorrhage
- Massive liver necrosis
- Severe anemia
- Severe dehydration
- Shock-associated prolonged diarrhea
- Myocardial infarction
- Lung edema
- Pleural effusion
- Multiple organ failure
 - Brain
 - Liver
 - Kidney
 - Heart
 - Lung

Clinical Factors Influencing Outcome (2)

Clinical predictors of fatal outcome

- Confusion
- Neck stiffness
- Bleeding from multiple sites
- Fever during hospitalization

Ozkurt Z et al. J Infect 2006

- Impaired consciousness
- Splenomegaly

Bakır M et al. J Med Microbiol, 2005

- Somnolence
- Hematemesis
- Melena

Ergonul O et al. Clin Microbiol Infect, 2006

- High-grade fever
- DIC
- Renal failure

Jamil B et al, Trans Roy Soc Trop Med Hygiene 2005

Laboratory Predictors on Severity of CCHF Infection (1)

- Many of the laboratory changes were evident at an early stage of the disease and had a highly predictive value for fatal outcome of infection
- Changes were present but less marked in nonfatal cases

Swanepoel R, et al. Rev Infect Dis 1989

Laboratory Predictors on Severity of CCHF Infection (2)

Severity criteria of the disease

- At the early stage (first 5 days) of the disease:

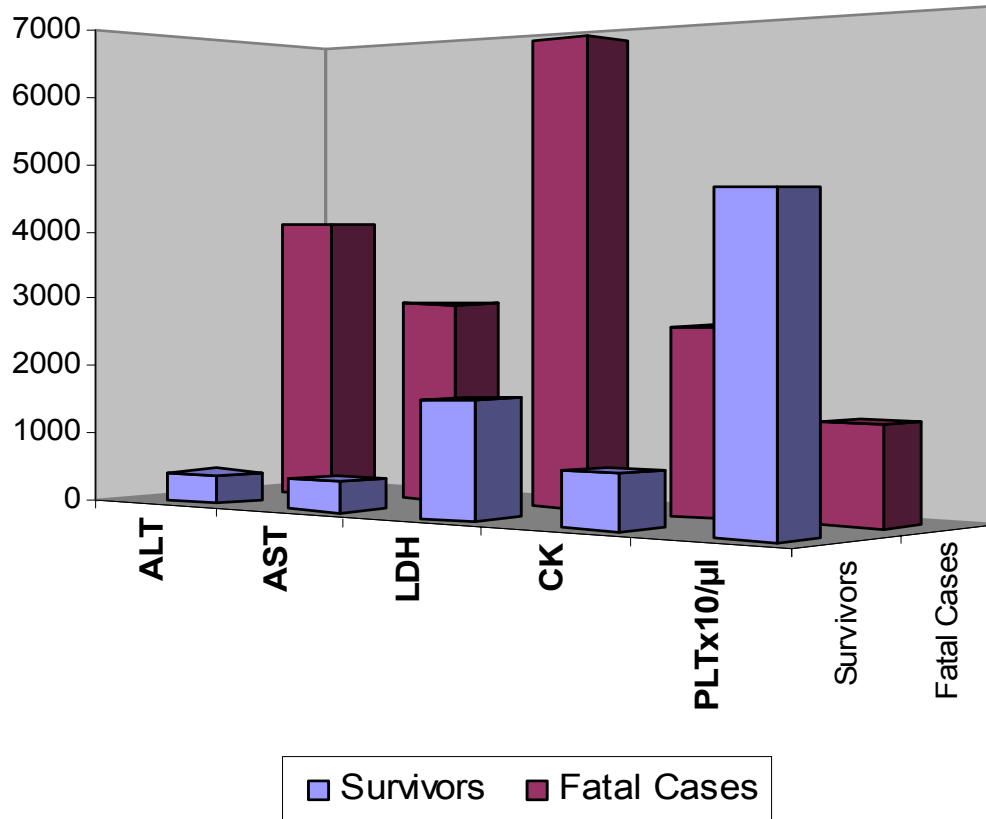
- WBC $\geq 10 \times 10^9$ cells /L
- Platelet $\leq 20 \times 10^9$ /L
- AST ≥ 200 U/L
- ALT ≥ 150 U/L
- aPTT ≥ 60 sn

and/ or
Fibrinogen ≥ 110 mg/dl

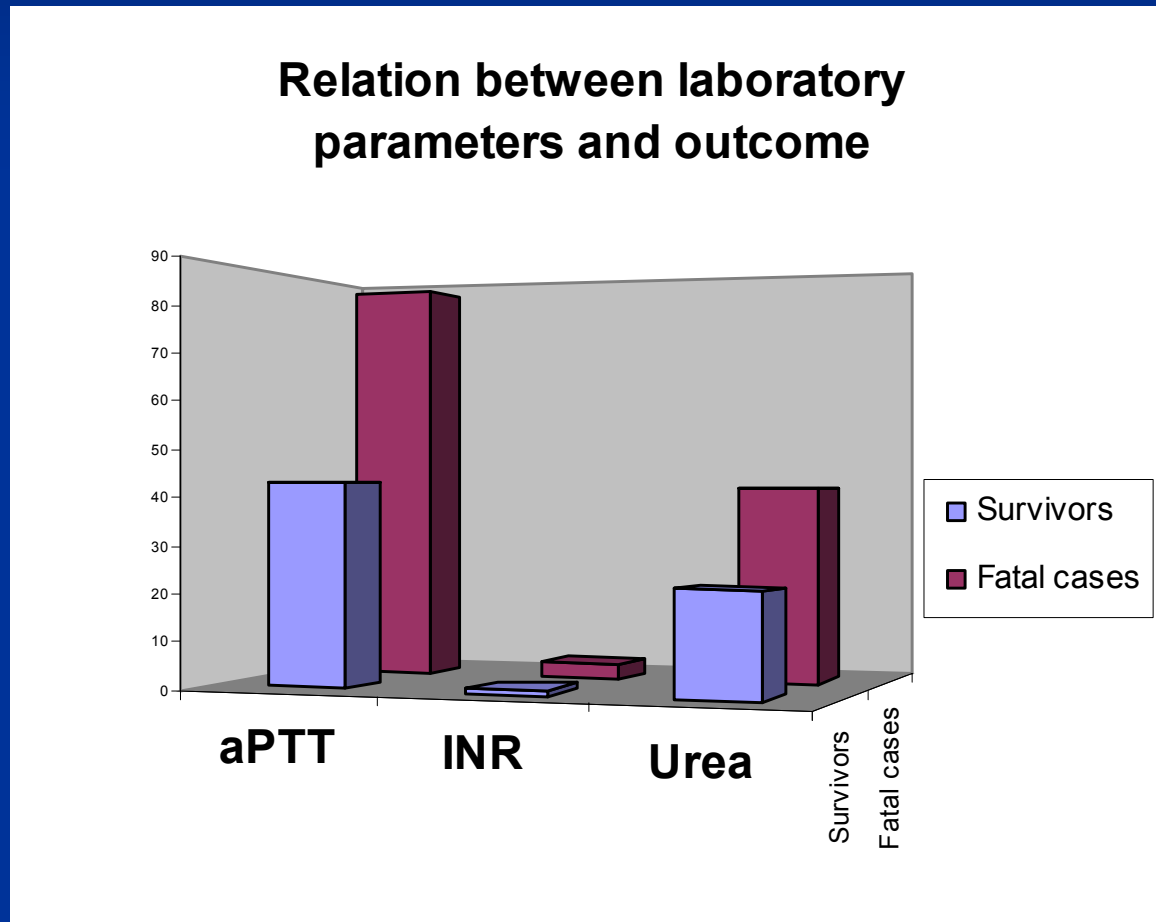
predict fatal cases

Laboratory Predictors on Severity of CCHF Infection (3)

Relation between laboratory parameters and outcome



Laboratory Predictors on Severity of CCHF Infection (4)



Laboratory Predictors on Severity of CCHF Infection (5)

- INR
- AST
- LDH
- CPK values are significantly higher in patients died

Bakır M et al. J Med Microbiol, 2005

- ALT, AST and fibrinogen levels were found higher
- PLT count lower
- PT and aPTT were longer in fatal cases
- Higher levels of AST (> 700 U/L) and ALT (>900 U/L) were suggested for the use of severity criteria

Ergonul O et al. Clin Microbiol Infect, 2006

Immunologic Predictors on Severity of CCHF Infection

- Antibodies
- Cytokines

Association between antibodies and severity of the disease

- Most patients develop relatively low levels of neutralizing antibodies
- Fatal cases do not usually develop a measurable antibody response
- Antibody response is rarely detectable in fatal cases
- It is possible that virus replication in fatal cases is of such intensity that endogenous antibody is bound in immune complex to circulating virus or noninfectious antigen and hence cannot be detected
- Endogenous antibody response was demonstrated in only 2 of 15 patients died

-Shepherd AJ et al. Rev Infect Dis, 1989
-Shepherd AJ et al. J Clin Microbiol, 1988
-Swanepol R. Exotic Viral Infection, Kass Handbook of Infectious diseases, London, 1995

Table1. Association between antibodies and severity of the disease

	Fatal Cases	Survivors	References
	n (%)	n (%)	
IgM positivity	1/4 (25)	47/50 (94)	Ergonul O. et al CMI, 2006
	4/10 (40)	54/90 (60)	Ozkurt Z. et al (unpublished data)
IgG positivity	0/4 (0)	31/50 (62)	Ergonul O. et al CMI, 2006
	0/10 (0)	35/76 (46)	Ozkurt Z. et al (unpublished data)

Cytokines and Outcome

■ IL-6 and TNF- α

- positive correlation between DIC scores and severity of the disease
- have an association with mortality

Ergonul O. et al, J Infect Dis, 2006

Papa A. et al, J Clin Virol, 2006

■ IL-10

- a negative correlation with DIC scores
- not associated with mortality

Ergonul O. et al, J Infect Dis, 20

Viral Factors (1)

- Severity of the disease is related to:
 - **Virulence of the virus**
 - Virulence changes while transfer between different hosts
 - The last host seems to have a major effect on virulence
 - It has been predicted that the last host regulates virulence by phenotypic changes

-Gonzales JP et al. Res Virol, 1995

Viral Factors (2)

- **Viremia, antigenemia and viral load**
 - Viremia is intense and prolonged in CCHF, especially in fatal cases
 - Persistence of viremia and antigenemia were detected in fatal cases
 - In patients who survived, neither antigenemia nor viremia was demonstrated later than day 9 after the onset of the disease
 - But at day 11 all specimens from which virus was isolated and which antigen was demonstrated were from patients who subsequently died

-Shepherd AJ et al. J Clin Microbiol, 1988
-Us D. Arboviruses, Basic and Clinical Microbiology, 1999
-unpublished data from multicenter study in Turkey

Conclusion

- Immunopathogenesis of the disease is not clear enough, yet
- There is a need for studies especially to show that either it is limited to cellular immunity or to humoral immune response, or both
- In the future, the results of such studies will enlighten the therapy of disease and the immunization

