HUMAN ARENAVIRAL INFECTIONS

ABSTRACT

Arenaviruses are RNA enveloped viruses, that are distinguished by the presence of cellular ribosomes inside the virions. They produce chronic persistent infection in rodents that are their natural reservoirs.

There are currently 19 recognized arenaviruses, and six of them have been associated with human illnesses: Lymphocytic Choriomeningitis, the prototype member of the family, that produces a disease characterized by a febrile syndrome, sometimes with central nervous system compromise; Junin virus (Argentine Haemorrhagic Fever); Machupo virus (Bolivian Haemorrhagic Fever), Guanarito virus (Venezuelan Haemorrhagic Fever); Sabia virus (hemorrhagic fever in Brazil), and Lassa virus (Lassa Fever). The transmission of the infection from rodent to humans occurs mainly by inhalation of aerosols from the excreta of infected rodents. The arenavirus genome consists of two RNA segments, and has an original strategy of genomic codification named “ambisense”. The S-segment encodes for the principal structural components: the nucleoprotein (N or NP), and a precursor glycoprotein (GPC), which by post-transcriptional cleavage gives origin to the two glycoproteins of the viral envelope (G1 or GP1, and G2 or GP2). The L segment codifies for a RNA-dependent polymerase and for a small protein (Z), with potential capacity for combining with metals.

A common characteristic of arenavirus infection, and particularly of the hemorrhagic fevers, is the induction of alterations on the cellular function without prominent morphologic changes. In human infections, the role of the monocyte-macrophage lineage is crucial in the outcome. For AHF, the specific treatment is immune plasma, and an effective live attenuated Junin virus vaccine is available. For the other arenaviral hemorrhagic fevers, intravenous ribavirin is the suggested therapy.

INTRODUCTION

The family Arenaviridae comprises several RNA spherical to pleomorphic viruses, including various important agents of hemorrhagic fevers in Africa and America. The prototype virus of this family, Lymphocytic Choriomeningitis (LCM) has a probably worldwide distribution in relation to the wide-
spread presence of its reservoir, *Mus musculus*, and produces diseases in humans characterized by a febrile syndrome, sometimes with central nervous system (CNS) compromise. There are currently at least 19 arenaviruses recognized, and only six have been associated with human illness; in South America, Junin virus (Argentine Hemorrhagic Fever- AHF), Machupo virus (Bolivian Hemorrhagic Fever- BHF), Guanarito virus (Venezuelan Hemorrhagic Fever- VHF) and Sabia virus (Hemorrhagic Fever in Brazil); in Africa, Lassa virus (Lassa Fever), and the above mentioned LCM (Table 1, Figure 1) (Clegg et al. 2000).

Table 1: The Arenaviridae Family

<table>
<thead>
<tr>
<th>Old World Arenaviruses</th>
<th>Reservoir</th>
<th>Geographic Distribution</th>
<th>Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytic choriomeningitis (LCM)</td>
<td><em>Mus musculus</em></td>
<td>Europe and America</td>
<td>Febrile syndrome, Aseptic meningitis</td>
</tr>
<tr>
<td>Lassa</td>
<td><em>Mastomys sp.</em></td>
<td>West Africa</td>
<td>Lassa Fever</td>
</tr>
<tr>
<td>Mopeia</td>
<td><em>Mastomys sp.</em></td>
<td>South Africa</td>
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<tr>
<td>Mobala</td>
<td><em>Praomys sp.</em></td>
<td>Central Africa</td>
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<tr>
<td>Ippy</td>
<td><em>Arvicomys sp.</em></td>
<td>Central Africa</td>
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<thead>
<tr>
<th>New World Arenaviruses or Tacaribe Complex</th>
<th>Reservoir</th>
<th>Geographic Distribution</th>
<th>Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Junin</td>
<td><em>Calomys musculinus</em></td>
<td>Argentina</td>
<td>Argentine Hemorrhagic Fever</td>
</tr>
<tr>
<td>Machupo</td>
<td><em>Calomys callosus</em></td>
<td>Bolivia</td>
<td>Bolivian</td>
</tr>
<tr>
<td>Guanarito</td>
<td><em>Zygadontomyys breviceoda</em></td>
<td>Venezuela</td>
<td>Venezuelan Hemorrhagic Fever</td>
</tr>
<tr>
<td>Tacaribe</td>
<td><em>Artiodactyla sp.</em> (bat)</td>
<td>Trinidad</td>
<td>Hemorrhagic Fever</td>
</tr>
<tr>
<td>Amapari</td>
<td><em>Necromys guianae</em></td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Flexal</td>
<td><em>Oryzomys bicolor</em></td>
<td>Brazil</td>
<td></td>
</tr>
<tr>
<td>Pichindé</td>
<td><em>Oryzomys albigranis</em></td>
<td>Colombia</td>
<td></td>
</tr>
<tr>
<td>Latino</td>
<td><em>Calomys callosus</em></td>
<td>Bolivia</td>
<td></td>
</tr>
<tr>
<td>Paraná</td>
<td><em>Oryzomis biancatus</em></td>
<td>Paraguay</td>
<td></td>
</tr>
<tr>
<td>Tamiami</td>
<td><em>Sigmoidon hispidus</em></td>
<td>United States</td>
<td></td>
</tr>
<tr>
<td>Oliveros</td>
<td><em>Necromys benefactus</em></td>
<td>Argentina</td>
<td></td>
</tr>
<tr>
<td>Pirital</td>
<td><em>Sigmoidon alstoni</em></td>
<td>Venezuela</td>
<td></td>
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<tr>
<td>Whitewater Arroyo</td>
<td><em>Bootomia albignula</em></td>
<td>United States</td>
<td></td>
</tr>
<tr>
<td>Sabía</td>
<td><em>Host unknown</em></td>
<td>Brazil</td>
<td>Hemorrhagic Fever</td>
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</table>
The Viruses.

Based on the serologic properties, genetic data and geographic distribution, arenaviruses have been divided in two main groups: Old World, and New World, or Tacaribe complex (Rowe et al. 1970, Casals et al. 1975, Wulff et al. 1978).

The viruses belonging to New World group are closely related, according to complement fixation and immunofluorescence tests, but the cross-reactions with Old World viruses are slight.

The molecular phylogenetic analyses have also shown that the Old World and the New World arenaviruses occupy two different clades. The New World arenaviruses comprise 3 lineages of evolution, named A, B, C. The lineage A includes Flexal, Paraná, Pichinde, Pirital and Tamiami viruses. The lineage B includes the four agents of South American Hemorrhagic Fevers (Junin, Mapucho, Guanarito and Sabia), as well as Tacaribe and Tamiami viruses.

Lineage C includes Latino and Oliveros virus (Bowen et al. 1996). In a recent report, White Water Arroyo virus is recognized as a product of genetic recombination between lineage A and lineage B virus (Charrel et al. 2002). Among the Old World arenaviruses, LCM is the most closely related to New World viruses.

Morphology.

Virions are spherical or pleomorphic particles between of 50 and 300 nm in diameter, with a lipoprotein envelope, from which club-shaped projections of 8 to 10 nm are projected (Pedersen 1979). The virions are relatively unstable and can be rapidly inactivated by ultraviolet or gamma irradiation, heating at 56°C or through exposition to pH outside the 5.0-8.5 range. On the other hand, the presence of a lipid envelope makes them susceptible to the inactivation by solvents or detergents (Buchmeier et al. 1980, Peters 1994).
isolated cases can occur. This classical epidemiological pattern has been modified since 1991 through the selective vaccination of adults at higher risks with a live attenuated Junin virus vaccine (Enria et al. 1998) (Figure 3).

BHF was an important public health problem during the 1960's. The explosive outbreaks originated effective and intensive rodents control actions and the community outbreaks were interrupted. There were no reported BHF since middle 1970's, although it is believed that sporadic cases still occurred. In 1993, and after 20 years of silence, the reemergence of the illness was reported. This re-emergence included in 1994 an intrafamilial outbreak in which 6 out of the 7 affected members died (Kilgore et al. 1995).
For VHF, the alternation of periods of low and high incidence has been described (de Manzione et al. 1998).

Only one case of natural infection, and two laboratorial infections with Sabia virus has been reported (Colmbrana et al. 1994, Vasconcelos et al. 1994, Barry et al. 1995).

Lassa Fever appears to be a very frequent disease in the endemic region of Occidental Africa. In the most affected area of Sierra Leona, Lassa fever could be responsible of a quarter of the hospital admissions and deaths (McCormick et al. 1987).

The annual incidence of acute LCM cases is not known because diagnostic testing is not commonly performed. In Argentina, all AHF notified cases are also studied for LCM infection, and every year between one and two new acute cases are recognized in the humid pampa.

**Seasonal distribution and risk factors.**

Although AHF cases can occur during the whole year, annual outbreaks are registered during autumn and winter, with a peak in the month of May. The classical epidemiological pattern of AHF is of a disease four times more frequent in men than in women, and is also more frequent (90%) among rural than in urban inhabitants. The children of less than 14 years comprise around 10% of the annual cases (Enria et al. 1998).

BHF has also a seasonal distribution, with the majority of cases presenting during the dry season, coincident with the peak in agricultural activities. Most sporadic BHF cases have been male rural workers, while the familial and community outbreaks have comprised both genera and all age groups, and have been related to the rodents invasion of towns (Johnson et al. 1967).

Cases of VHF are reported during the whole year, but the outbreaks occurred during the major agricultural activities, with a peak between November and January (de Manzione et al. 1998).

Lassa Fever is a common illness of adults and children in occidental Africa. This pattern of similar distribution in men, women, and children has been considered evidence that the peridomestic exposition to the virus is probably very important. On the other hand, the lack of biosafety measures in the care of the patients has played an important in the inter-human transmission (McCormick & Fisher-Hoch 2002).
The incidence of LCM human disease peaks in the fall and winter, presumably reflecting the seasonal invasion of homes by mice, and is associated with the regional prevalence of mouse infection.

Factors in the Emergence of Arenaviral Hemorrhagic Fevers.

Many of the factors linked to the emergence of these "new" illnesses caused by "old" viruses are unknown. Several hypotheses have been suggested, but the great majority has not been proved in the field, given the difficulties and the financial costs of the long-term studies required.

The model Junin virus-AHF has been the best studied. It has been suggested that the emergence of AHF in the 1950's was the result of the alterations produced in the ecosystem as a result of the agricultural practices. These changes have benefited the growth of Calomys musculinus populations. The humid pampas of Argentina are a patchwork of intensively cultivated areas, bordered by roads, railways, wire fence, etc. Six species of small rodents coexist in the region, and 5 of them belong to the Sigmodontinae subfamily: Calomys musculinus, C. laucha, Akodon azarae, Necromys bairdii and Oligoryzomys flavescens. The Murinae subfamily is represented by the introduced species Mus musculus. It is considered that the rodent communities of the pre-agricultural pampas were dominated by Akodon, Bolomys and Oligoryzomys (Crespo 1944, Sabattini & Maiztegui 1970, Maiztegui & Sabattini 1977, de Villafañé et al. 1977, Sabattini & Contigiani 1982). Both Bolomys and Akodon live almost exclusively in linear habitats and are dominant over Calomys species. C. musculinus can easily invade modified habitats. They are more frequently captured in linear habitats, but they are also found in cultivated fields, before and after harvesting. They are rarely captured in peri-domestic habits (Mills et al. 1991, Mills et al. 1992, Ellis et al. 1997).

A long series of environmental variables has been related to the fluctuating rodent populations, although the issue is still controversial. Climate is one of the factors that more probably influence the rodent population densities and, therefore, is very likely to contribute to cycles in the incidence of South American hemorrhagic fevers. In the Argentine humid pampas, it has been suggested that the cold and humid winters as well as the hot and dry summers would result in a decrease, while the warm and dry winters and the chilly and rainy summers would contribute to the increase in the rodent's densities. Other factors that have been considered important are the increment or decreases in the harvest, and the type of crop; the burning or the burning and cut of the linear habitats, that give refuge to the rodents, and the intensity in the use of herbicides and insecticides (Sabattini & Maiztegui 1970, Maiztegui & Sabattini 1977, de Villafañé et al. 1977).

On the other hand, the description of the geographic limits of Junin virus activity is based mainly on the distribution of the disease, and not of the virus. The principal reservoir of Junin virus is found in most central and northwestern Argentina, and its distribution exceeds widely the known AHF endemic area. A gradient of infection with Junin virus has been described in C. musculinus within the endemic region. The prevalence of Junin virus infection in C. musculinus is more higher in the newly involved areas, and has been reported as low or absent outside the endemic area (Mills et al. 1992). However, Junin virus has been isolated from rodents trapped in areas without human cases in the last 10 years and in areas where the disease has not been recognized yet, suggesting the possibility of new extensions of the endemic region and of the reemergence in areas currently considered historic (García et al. 2000). Junin virus infection of C. musculinus has a focal distribution. The reasons for this particular pattern are not cleared. The complete elucidation of the factors responsible for AHF emergence and progressive extension deserves further investigation.

The reservoir of Machupo virus, Calomys callosus, is found preferably in the areas where prairies are connected with the forest. C. callosus can also live in urban areas, and has the capacity of invading houses, especially in the flood areas, and looking for lands at higher altitudes. It is considered that the outbreaks affecting towns and cities would arise during epizootics, especially when the densities of rodents reach unusual levels and invade urban areas (Kuns 1965, Johnson et al. 1967, Musser & Carleton 1993).

For VHF, the deforestation of some areas for agriculture use has been the factor linked to the emergence. Both Zygodontomys brevicauda (reservoir of Guanarito virus), and Sigmodon alstoni (reservoir of Pirital virus) have been captured in the cultivated field, in their borders and in those of the roads. Only exceptionally there are found in domestic habitats. VHF outbreaks have been associated
with the increase in the densities of *Z. brevicauda* that occur when densities of *S. albomuri* decreased. This suggests that interspecies competence plays a role in the emergence of the illness (Fulhorst et al. 1999).

The role of peridomestic exposition to Lassa is considered important, with the densities of rodents being much higher inside the houses than in agricultural and forest areas. Human infections would be increased by the common practice of hunting and eating rodents and by the preservation of food in open places (McCormick & Fisher-Hoch 2002).

**Clinical Description.**

The hemorrhagic fevers caused by the viruses Junin, Guanarito and Mapucho (and probably also Sabia) produce similar clinical pictures, while Lassa human infection has some differences. AHF will be used as a model of this description.

**Argentine Hemorrhagic Fever.** - AHF is characterized by hematological, cardiovascular, renal, neurological and immunologic alterations (Malztergui 1975).

The incubation period is between 6 and 14 days, with a range of 4 to 21 days.

The disease begins with a prodromal phase, characterized by the insidious onset of unspecific symptoms such as malaise, headaches, and moderate fever (38 to 39 °C). During the following days, myalgias, low backache, arthralgias, retro-orbital pain, epigastric pain, dizziness, nausea and vomiting may appear. Constipation or diarrhea may be also present. During this phase, hemorrhages are limited to discrete bleeding of the gums, epistaxis or metrorrhagia in women. The almost constant absence of productive cough, sore throat, or nasal congestion is very useful for distinguishing the initial symptoms of AHF from those of respiratory infections.

During the first week, on the physical examination there is an erythematous exanthema in the face, neck and upper part of the trunk. In the skin of the axillary regions or in the internal superior part of the arms, there are generally isolated petequiae. Conjunctival congestion and periorbital edema may also be present. The oropharyngeal membranes are congested, and there is congestion of the vessels bordering the gums, which may bleed spontaneously or under a slight pressure. Over the soft palate, there is an enanthem characterized by the presence of petequiae and small vesicles. In the laterocervical regions, there may be enlarged lymph nodes that are not painful. Signs of pulmonary abnormalities are generally absent, although some patients can refer cough. Relative bradycardia and orthostatic hypotension are frequently found. Generally, there is no hepatomegaly or splenomegaly, and jaundice is rare. At the end of the first week of illness, different degrees of dehydration may occur.

During the second week of illness, 70% to 80% of the cases begin to improve. The remaining 20% to 30% enters in a hemorrhagic/neurological phase, characterized by severe hemorrhages or neurological manifestations, shock and superimposed bacterial infections that appear between 8 to 12 days after the onset of symptoms. Profuse bleeding may occur in the form of hematemesis, melena, hemoptysis, epistaxis, hematomas, metrorrhagia or hematuria. Acute renal failure is uncommon, but may arise in terminal cases or after prolonged periods of shock, and is secondary to acute tubular necrosis. Superimposed bacterial infections such as pneumonia and septicemia can also complicate the clinical course, usually appearing after 8 days from onset of symptoms.

Patients destined to survive begin to improve by the third week of illness, and the convalescence may last up to 3 months. Temporary hair loss is common. Many patients have asthenia, irritability and memory changes. Approximately 10% of cases treated with immune plasma develop in convalescence a late neurological syndrome (Enria et al. 1996).

**Differential findings with the other South American arenaviral hemorrhagic fevers.** - In a nosocomial outbreak of BHF of high lethality occurring in a high altitude area, jaundice was found in some cases (Kins 1965, Johnson et al. 1967, Peters 1994). Cases with VH refer sore throat frequently among initial symptoms (de Manzione 1998). In the unique natural infection with Sabia virus seen so far, and extensive hepatic necrosis was found (Coimbra et al. 1994).

**Lassa fever.** - Lassa fever is prevalent in both males and females and all age groups. The disease begins after 3-21 days of incubation (mean 10 days) with the insidious onset of fever, generalized weakness, severe headaches, generally frontal, and malaise (McCormick & Fisher-Hoch 2002). Other common symptoms include aching in the large joints, low backache, non-productive cough, sore throat, retrosternal chest pain, abdominal pain and a variety of other generalized symptoms. Vomiting and
diarrhea are also referred. On physical examination, conjunctivitis with or without conjunctival hemorrhage, facial and neck swelling in florid cases, pharyngitis or diffuse inflamed and swollen posterior pharynx and tonsils, sometimes with exudates but exceptionally with petechiae have been described. In half of the patients, tenderness of the abdomen is found (Peters et al. 1997).

The bleeding is often mild, and is seen as nose, mouth, and genitourinary or gastrointestinal tract. Severe bleeding has been described in only 15-20% of the patients, with vaginal bleeding in pregnant women been potentially hemodynamically significant. No characteristic rash has been described in Lassa fever, although in white-skinned patients maculo-papular or petechial rashes have been observed.

During the second week of illness, up to a third of Lassa fever cases progress to a severe illness, with the frequent presence of persisting vomiting and diarrhea. Hypotension and tachycardia are observed. Elevated respiratory rates are also frequent, with pleural effusion being common. Pericardial effusions are only occasionally seen in the terminal stages. These pleural and pericardial effusions have been linked to the severe retrosternal or epigastric pain referred by many patients.

**LCM illnesses.** - The great majority of LCM infections are subclinical; most of the clinical infections are characterized by a undetermined febrile syndrome, while other consist of an initial febrile phase, followed by a short afebrile remission that precedes the CNS diseases. The neurologic sign and symptoms are those of an aseptic meningitis.

**Clinical Laboratory Studies.**

Clinical laboratory studies are very useful to establish an early diagnosis in South American Hemorrhagic Fevers. During the acute phase, there are progressive low white cell and platelet counts ranging from 50,000 and 100,000 mm³. The sedimentation rate is normal or decreased. Almost constantly, there is protein in the urine, with alteration in the sediment, with presence of hyaline-granular casts, red blood cells and round cells with cytoplasmatic inclusions. Serum creatinine and urea are normal or increased in proportion to dehydration and shock in the severely ill patient. In AHF, aspartate aminotransferase (AST), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) are commonly slightly increased, but hyperbilirubinemia or hyperamylasemia is rare (Enria et al. 1998). During the acute phase, the CSF is normal, even in the severe neurological forms of the disease.

In Lassa fever, thrombocytopenia is mild to moderate (usually less than 100,000 mm³). The while cell counts are generally normal, although a mild diminution with lymphopenia may occur. Severely ill patients are more likely to be thrombocytopenic, are usually lymphopenic, and may have an elevated WBC count with neutrophilia. In these patients, a moderate hemocencentration and proteinuria could also be seen. The AST is often elevated and higher values are predictive of a poor prognosis. A similar association is found with the level of viremia. DIC is not a common finding (Fisher-Hoch et al. 1988).

In LCM infections, leukopenia with lymphopenia, thrombocytopenia, and elevation of LDH and AST have been described in the prodromal phase. During the neurologic phase, the CSF is characterized by a normal or low glucose, with a moderate increase in cell counts, predominantly lymphocytes.

**Differential Diagnosis.**

During the prodromal phase, the clinical manifestations of South American hemorrhagic fevers are non-specific and the differential diagnosis includes dengue, DHF, typhoid fever, hepatitis, infectious mononucleosis, leptospirosis, hantavirus infections, and rickettsioses. Malaria and yellow fever should also be considered in endemic areas. Diseases presenting with hematological or neurological alterations, such as intoxications, rheumatic diseases, and blood dyscrasias may also be taken into consideration. In the respective endemic areas or in patients with a history of travel to the specific geographic regions, a febrile syndrome with leukopenia and thrombocytopenia is suspicious of a South American hemorrhagic fever (Figure 4).

At the initial stages, the nonspecific presentation of Lassa Fever needs to be differentiated of most febrile illnesses found in West Africa, including malaria, typhoid and bacillary dysentery.

**Etiologic Diagnosis.**

Viremia is present throughout the acute febrile period in all the arenaviral hemorrhagic fevers and the viruses can be isolated from blood and tissues (particularly lymphoid tissues) of fatal cases. Isolation is usually performed in Vero cells, but other cellular lines or host, such as suckling mice or guinea pigs for AHF or suckling hamsters for BHF can also be used (Peters et al. 1972, Peters et al. 1987). In
Febrile syndrome of undetermined etiology in a patient living or visiting the endemic area during the 3 previous weeks

Fever, headaches, intense myalgias, metrorrhagia in women

Enanthem, axillary petechiae, ataxia, decreased tendon reflex

Clinical laboratory studies (white cell and platelet counts, sedimentation rate, urine analysis)

- White cells ≥ 4,000/mm³
  Platelets ≥ 120,000/mm³
  Repeat control daily during first week
- White cells < 4,000/mm³
  Platelets < 100,000/mm³
  Suspicious case
  Consider treatment with immune plasma
- White cells < 2,500/mm³
  Platelets < 100,000/mm³
  Probable case

Figure 4: Algorithm for the detection of Argentine hemorrhagic fever (AHF). The AHF algorithm could also be effective for early detection of the other South American hemorrhagic fevers. In suspicious and probable cases, specific virologic studies should be performed for confirmation of disease origins.

AHF, co-cultivation of peripheral blood mononuclear cells improves the sensitivity of virus recovery (Ambrosio et al. 1986). The presence of virus can also be determined by reverse transcription-polymerase chain reaction (RT-PCR). In AHF, RT-PCR has been successfully applied and is playing an important role in establishing the etiologic diagnosis before the appearance of the specific antibodies, particularly in fatal cases. For Lassa fever, a sensitive assay exists, but the strain variation and the problems linked to cross-contamination pose practical problems. Fatal cases can also be diagnosed by immunohistochemistry on fixed tissues (Zaki & Peters 1997).

The serologic diagnosis can be done by complement fixation (CF), immunofluorescence, ELISA and neutralization tests. CF has been widely employed in the past, but have low sensitivity. ELISA tests for detection of IgG antibodies in paired serum samples (one from acute period and the other from convalescence) is the method of choice for the diagnosis of AHF. Neutralization tests are very important to confirm the specificity of the reactions.

For BHF, antigen detection ELISA and IgM ELISA have been successfully applied (Peters 2002). Acute Lassa fever is best diagnosed in early phases by combining ELISA for IgM antibody and
antigen. IgG ELISA antibodies takes longer to develop (approximately 3 weeks), but appear earlier than the neutralizing antibodies, that are often detected several weeks after disease resolves; the final response is low in titer (McCormick & Fisher-Hoch 2002).

Pathology and Pathophysiology.

Pathologic descriptions of arenavirus infections in humans are limited to a few necropsy series. Gross and microscopic pathologic findings in these human cases are generally similar among different arenavirus infections. Common gross findings at postmortem examinations include ecchymoses and petechial hemorrhages involving skin, conjunctivae, mucous membranes, and internal organs. The degree of hemorrhage varies, and sometimes can be minimal or absent. Conjunctival suffusion, pleural effusion, pericardial effusion, and ascites are frequently present (Child et al. 1967, Elsner et al. 1973, Walker et al. 1982, Walker & Murphy 1987).

Microscopically, congestion and variable degrees of necrosis are usually observed in all organ systems. Necrosis is most prominent in the liver and spleen, but is also frequently found in adrenal gland, kidney, and gastrointestinal mucosa. The most consistent histopathologic feature is seen in the liver and consists of multifocal hepatocellular necrosis with cytoplasmic eosinophilia, Councilman body formation, nuclear pyknosis, cytolytic, and fatty metamorphosis. Inflammatory cell infiltrates in necrotic areas are usually mild and, when present, consist of a mixture of mononuclear cells and neutrophils. The spleen usually shows depletion of follicles and focal necrosis. Other pathologic features that may be present in arenavirus infections include mild interstitial pneumonia, diffuse alveolar damage, myocarditis, and acute renal tubular necrosis.

Because none of these pathologic features are specific, a confirmatory test such as immunohistochemistry is essential to a definitive tissue diagnosis, and such studies have also demonstrated a wide spectrum of tissue tropism. In Lassa fever, the localization of viral antigens can be summarized as the following: (1) hepatocytes, especially those in the areas of necrosis; (2) cells of the mononuclear phagocytic system such as Kupffer cells in hepatic sinusoids, alveolar macrophages, and endothelial cells; (3) mesothelial cells lining pericardium, pleura, peritoneum, and serosal surfaces of other organs; and (4) specialized cells involved in hormone secretion, including those in adrenal gland, ovary, uterus, placenta, and breast (Zaki & Peters 1997, Shieh et al. 1997).

In AHF, ultrastructural and immunohistochemical studies revealed characteristic intracellular inclusions that were more prominent in lymphatic tissues, coincident with the presence of Junin virus antigens. In the kidney, a large number of virus-like intracytoplasmic particles are found in distal and collecting tubules coincident with severe tissue necrosis and large quantities of Junin virus antigen as demonstrated by immunofluorescence. Morphologic studies of the bone marrow indicate that an acute and transient arrest of hemopoiesis occurs, with bone marrow hypocellularity, but without permanent hematological sequelae in survivors (Cossio et al. 1975, Malztegul et al. 1975, Ponziibbio et al. 1979, Gonzalez et al. 1980).

During the progression to severe illness, there are clinical evidences of capillary leakage, that seems to be more prominent in Lassa fever cases, in which there are effusions in the peritoneum, pleura and pericardia, progressing to facial (but not lower extremity) edema, pulmonary edema with adult respiratory distress syndrome (ARDS), and hypovolemic shock. ARDS is a frequent cause of death in Lassa infection, and have been reported late in the course of fatal AHF cases. Encephalopathy, but not encephalitis, is prominent in severe neurological AHF patients and in some with Lassa Fever. There is little or no virus found in CNS, and no evidence of parenchymal damage in the brain. Leaky capillaries and edema may well explain this process (McCormick & Fisher-Hoch 2002).

A common characteristic of arenavirus infections, and particularly of the hemorrhagic fevers, is the lack of histological lesions to explain the altered organ function and death. This is the origin of the concept that arenaviruses induce an alteration of the cellular functions without morphologic changes.

In the South American hemorrhagic fevers, particularly in AHF, the pathophysiology seems to be the result of direct viral action, in contrast to the mouse models of LCM virus infection in which immunopathologic cell action causes encephalitis and B-cell products result in chronic immune complex disease.

In AHF, several studies have demonstrated that immune complexes, complement activation or
DIC are not relevant pathogenic mechanisms (de Bracco 1978). Mediators released or activated as a result of the virus-cell interactions, such as lymphokines, vasoactive mediators, and proteolytic enzymes may explain some of the observed alterations. Hemorrhagic manifestations are considered to be the result of the thrombocytopenia, abnormal platelet function induced by a plasma component, and alterations of the blood coagulation with fibrinolysis activation. The haemostatic alteration found include prolongation of activated partial thromboplastin time (APTT), low levels of factors VIII, IX and XI, increased values of factor V, von Willebrand factor, and fibrinogen; and mild decreases in antithrombin III and plasminogen. Endothelial cell involvement is also suspected, based on the fact that Junin virus replicates in cultured endothelial cells and that in human disease there are increased levels of von Willebrand factor (Molina et al. 1981, Molinas & Maiztegui 1981, Heller et al. 1995).

AHF is also characterized by an acute transitory immunodeficiency. There is a lag in the humoral immune response, with antibodies appearing during the second week of illness, coincident with the recovery of patients. During the acute phase, cell mediated immunity is also depressed, as it has been shown by tests of delayed-type hypersensitivity to non-viral recall antigens and lymphocyte proliferation stimulated by mitogens. During this acute period, there are also changes in the T-cell subpopulations, that return to normal values in convalescence (Enria et al. 1986, Vallejos et al. 1989).

Also during the initial phase of AHF, very high titers of endogenous interferon α (IFNα) have been demonstrated circulating in the serum of the patients. The titers of IFNα are significantly higher in cases developing severe forms of the illness, and are considered a marker link to fatal evolution. Tumor necrosis factor-α (TNFα) levels are also elevated, particularly in fatal cases. Another study suggested the association between histocompatibility antigens and the severity of the disease (Levis et al. 1985, Saavedra et al. 1985).

Lassa fever has a similar pathogenesis as South American hemorrhagic fevers. Although platelet counts do not fall to the low levels found in the other arenaviral hemorrhagic fevers, an extensive inhibition of platelet aggregation mediated by soluble serum factors is found (Fisher-Hoch & McCormick 1988, Zaki & Peters 1997). There is a quick B-cell response to Lassa virus, with a classic IgG and IgM antibody response that often coexist with high viremia in both humans and primates. Neutralizing antibodies appear late in the course of illness, and their levels are low. Thus, the clearance of Lassa virus and the recovery of infection are not mediated by antibody, and presumably depend on CMI response. In experimental models, a lack of protection with passive transfer of antibodies has been shown, and spleen cell transfer was linked with clearance of viremia and survival. On the other hand, the sensorineural deafness during convalescence has been suggested to be the result of an immune-mediated injury (McCormick & Fisher-Hoch 2002).

**Treatment.**

**Specific treatment.** - For AHF, a specific treatment is available and consists of the transfusion of immune plasma upon a standardized amount of neutralizing antibodies to Junin virus. Immune plasma reduces the case-fatality rate from 15-30% to less than 1% provided that it is transfused during the first 8 days from onset of symptoms, but is of no benefit to patients when they are treated after the first week of illness (Maiztegui et al. 1979, Enria & Maiztegui 1994).

For the other South American hemorrhagic fevers, the specific treatment suggested is intravenous ribavirin. This may also prove useful in the treatment of AHF patients (Enria & Maiztegui 1994, Kilgore et al. 1997). For Lassa fever, also the specific treatment is the nucleoside analogue ribavirin. Patients with poor prognostic indicators (high AST or viremia levels) should be treated intravenously, and the suggested dose is 30 mg/kg initially, 15 mg/kg every 6 hours for 4 days, and 7.5 mg/kg every 8 hours for 6 more days. Oral ribavirin in less severe forms has also shown efficacy. There are evidences indicating that is more effective when given early in the course of illness (McCormick et al. 1986, Fisher-Hoch et al. 1992).

**General supportive measures.** - Supportive treatment consists of adequate hydration, symptomatic measures, and proper management of the neurological alterations, blood losses, shock, and superimposed infections. There is no specific indication for the use of steroids. Medication should be given by the oral or intravenous route. Intramuscular and subcutaneous injections are contraindicated because of the risk of hematomas.

In the AHF endemic area, several observations have been made: Pneumonia is the most
common secondary bacterial infection and is often accompanied by radiographic changes and an increase in fever, but not by leukocytosis; it usually responds to ampicillin. Other common secondary infection is oral candidosis. Platelet transfusions have been used, but the complex nature of the coagulopathy and clinical experience suggest they are not useful. Transfusions are needed occasionally, but most of the severe forms are neurological. It is useful to sedate agitated patients with diphenhydramine or diazepam; diazepam also gives some protection against seizures. Seizures are generally treated with diphenhydantoin. Cerebral edema may require both steroids and mannitol.

Biosafety Measures.
The patients with arenoviral hemorrhagic fevers are viremic during the acute period and the virus can be transmitted by parental inoculation. The South American hemorrhagic fevers are not considered highly contagious in the endemic areas where they occur. Nosocomial transmission has not been described for AHF, but was observed at least in one outbreak for BHF. Nevertheless, unusual patients capable of disseminating both Junin and Machupo viruses to hospital staff and families have been described. Nosocomial transmission of Lassa fever has been also reported. The absence of parental precautions has caused serious epidemics in African hospitals. Moreover, transmission to hospital staff or other patients was related to close contact with infected secretions, blood or tissues from hospitalized patients with Lassa fever. There is no credible epidemiological evidence of airborne transmission (Helmick et al. 1986, Peters et al. 1996).

Outside the endemic area where they occur, it is suggested to use small-particle aerosol precautions to treat these patients, but emphasizing that the most important measures would be those preventing parenteral and droplet exposure to blood and body fluids.

During convalescence, patients are not generally contagious. Hemorrhagic fevers patients have transmitted virus to spouses sometimes during convalescence. Thus, intimate contact should be cautious and condoms should be used during sex for at least one month. Lassa virus has been isolated from urine for several weeks and the use of disinfectant in the toilet bowl before voiding is advised.

For the manipulation of potentially infected samples in the laboratory, biosafety-2 measures are adequate. However, in all procedures capable of aerosol production, the use of biosafety cabinet is indicated. Virus cultures should be handle under biosafety level 3 and outside the respective endemic areas, under biosafety level 4.

All persons working in relation to Junin virus should be immune. Candid # 1 live attenuated Junin virus vaccine is available for seronegative personnel. The same consideration should be considered for personnel working with Machupo virus.

Prevention and Control.
Rodent control has been successful in the control of BHF outbreaks occurring in towns, but sporadic cases still occur after rural exposure or contact with a case (Mercado 1975).

The control of the rodent reservoir of Junin virus is impractical given the large geographic zones involved and the difficulties of intervening in local agricultural economies. Control of human contact with rodents is also not feasible. For this reason, almost since the discovery of the disease, all efforts for prevention have been directed towards the development of a vaccine (Guerrero 1977).

The remarkable progress achieved with the discovery of the XJ Clone 3 vaccine candidate in the 1960s, prompted the development of a live attenuated Junin virus vaccine through the effort of an international collaborative project initiated in the late 1970's. Candid # 1 vaccine was the final result of this project. Candid # 1, the first vaccine against an arenavirus, was shown to be safe, highly immunogenic and effective for the prevention of AHF. Moreover, Candid # 1 showed promise in preventing two natural arenoviral hemorrhagic fevers and thus potentially diminishing the medical significance of Junin and Machupo viruses as bioterrorism agents (Enria & Barrera Oro 2002).

Vaccination of high-risk population corroborated the results obtained in phases II, III trials, and I and indicated that the vaccine will also be immunogenic under field conditions. Studies of antibody persistence showed stability of antibody positivity for at least 10 years after immunization. The long-term protection afforded by only one dose of Candid # 1 argues in favor of its excellence for the control of AHF.

The observed changes in the current compositions of AHF cases, that are attributable to the im-
pact of selected vaccination of high risk people, is indicative that a broader strategy of vaccination to protect the whole population at risk should be evaluated. This objective would be accomplished with a full operation of vaccine production facilities at the INEWH, in Pergamino, Argentina. With sufficient supplies of Candid # 1 vaccine, the definitive control of AHF may be envisioned. However, the disease cannot be eradicated because Junin virus reservoirs are rodents, and even with good vaccine coverage, small outbreaks and isolated AHF cases may be expected (Enria & Barrera Oro 2002).

The widespread control of the Mastomys species reservoir of Lassa virus in West Africa is also unfeasible as a broad approach for controlling the illness. The improvement of housing, and the educational campaigns to avoid rodents as food source, and for proper food storage might reduce the domestic rodent population. Rodent trapping in villages with a high transmission rate may have their impact. Barrier nursing in African hospital should be emphasized as well as adequate burial procedures of fatal cases. The control of Lassa fever in Africa would only by achieved with the availability of an effective vaccine. The studies on potential vaccines to Lassa fever began in the 1980s, through the development of a recombinant vaccine candidate using vaccinia vector. This approach gave promising results in experimental models, but the use of inactivated candidates has not been successful. Available evidence suggests that a successful vaccine against Lassa fever should be able to induce cellular immune response (Auperin 1993, Whitton 2002).

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