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Treatment of Argentine hemorrhagic fever

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Abstract

Argentine hemorrhagic fever (AHF) is a rodent-borne illness caused by the arenavirus Junin that is endemic to the humid pampas of Argentina. AHF has had significant morbidity since its emergence in the 1950s, with a case-fatality rate of the illness without treatment between 15% and 30%. The use of a live attenuated vaccine has markedly reduced the incidence of AHF. Present specific therapy involves the transfusion of immune plasma in defined doses of neutralizing antibodies during the prodromal phase of illness. However, alternative forms of treatment are called for due to current difficulties in early detection of AHF, related to its decrease in incidence, troubles in maintaining adequate stocks of immune plasma, and the absence of effective therapies for severely ill patients that progress to a neurologic–hemorrhagic phase. Ribavirin might be a substitute for immune plasma, provided that the supply is guaranteed. Immune immunoglobulin or monoclonal antibodies should also be considered. New therapeutic options such as those being developed for systemic inflammatory syndromes should also be valuated in severe forms of AHF.

Keywords: Argentine hemorrhagic fever; Viral hemorrhagic fever; Arenavirus; Junin virus

1. Introduction

Argentine hemorrhagic fever (AHF) is a severe viral hemorrhagic fever endemic to the fertile farming plain of central Argentina, the "humid pampas" (Fig. 1) (Maiztegui, 1975). Junin virus (family Arenaviridae), the etiologic agent of AHF, is a rodent-borne virus. *Calomys musculinus* has been identified as its principal reservoir. Human exposure to Junin virus is believed to occur through inhalation of aerosolized body fluids or excretions of infected rodents, typically during agricultural work.

The emergence of AHF in the 1950s is hypothesized to have resulted from human alterations of the habitat in relation to agricultural practices. Such changes in the environment are reported to have favored the population growth of *C. musculinus*. Since the recognition of the illness, annual outbreaks have been registered without interruption, with number of cases between 300 and 1000, approximately. With the availability of an effective live attenuated Junin virus vaccine, a consistent reduction in the incidence of AHF was achieved in the 1990s (Enria and Barrera Oro, 2002; Enria et al., 2004). The objective of this article is to review knowledge acquired on the treatment of this illness and to discuss future expectations.

2. Clinical disease in AHF

The incubation period is usually from 6 to 14 days. Most infections with Junin virus (80%) result in clinical disease. Three phases are recognized in the illness: prodromal, neurological–hemorrhagic, and convalescence (Enria et al., 2004).

Prodromal phase: This phase lasts for the first week from onset of symptoms. The onset is insidious, with chills, malaise, anorexia, headache, myalgia centered particularly over the lower back, and moderate hyperthermia (38–39 °C). Other common symptoms include retro-orbital pain, nausea or vomiting, epigastric pain, photophobia, dizziness, constipation or mild diarrhea. Physical examination reveals flushing of the face, neck and upper chest; conjunctival congestion and periorbital edema. The gums look congested and may bleed spontaneously or under slight pressure. Over the soft palate, an enanthem represented by petechiae and small vesicles is almost constantly found. Typically, the patients have cutaneous petechiae in the axillary

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Fig. 1. AHF endemic area and last geographic extension.

regions, upper chest and arms. Lymph nodes in the laterocervical regions are enlarged. Generally, no signs of pulmonary abnormalities are detected. Relative bradycardia and orthostatic hypotension are frequently found. Hepatomegaly, splenomegaly and jaundice are very rare. At the end of this phase the patient may be irritable, lethargic, and with a fine tremor of the hand and tongue. Moderate ataxia, cutaneous hyperesthesia, and a decrease in deep tendon reflexes and muscular tonicity are present. In females, the presence of metrorrhagia is characteristic. Superimposed oral candidiasis is frequently found at the end of this phase.

Neurologic–hemorrhagic phase: Around 20–30% of the cases with AHF between 8 and 12 days after onset of symptoms enter in this phase, presenting severe hemorrhagic or neurologic manifestations, shock and superimposed bacterial infections. Hemorrhagic signs include hematemesis, melena, hemoptysis, epistaxis, hematomas, metrorrhagia and hematuria. Neurological involvement begins with mental confusion, marked ataxia, increased irritability and tremors that are followed by delirium, generalized convulsions and coma. Superimposed bacterial infections, presenting as pneumonia and septicemia may complicate the disease during this period. Acute renal failure is uncommon, but may appear in this phase in terminal cases, usually after prolonged periods of shock, as a consequence of an acute tubular necrosis.

Convalescence phase: Surviving cases experience a prolonged, protracted convalescence that lasts from 1 to 3 months. Patients experience asthenia, irritability, memory changes and hair loss. Around 10% of the cases treated with immune plasma develop a late neurological syndrome (LNS). This LNS begins after a period free of symptoms, and is characterized by febrile symptoms, cerebellar signs and cranial nerve palsies (Enria et al., 2004; Enria, 2005). No cases of LNS have been registered among AHF patients who have recovered without specific treatment (Maiztegui et al., 1979; Enria et al., 1985). A single case has been observed in a patient who was treated late in the course of the illness with intravenous ribavirin (Enria et al., 1987).

Clinical laboratory studies: During the first week of the illness, there is a progressive leucopenia and thrombocytopenia, with counts around 1000–2000 white cell and 50,000–100,000 platelets per mm^3. The sedimentation rate is normal or decreased. There is proteinuria, and urinary sediment containing hyaline-granular casts and red blood cells. Elevations in aspartate transaminase (AST), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) are common, but mild. Serum creatinine and urea are generally normal, but are increased in severe cases in proportion to dehydration and shock.

During the acute illness, cerebrospinal fluid (CSF) is normal, even in patients with a severe neurological form. However, the CSF in patients with LNS showed a moderate increase in the number of cells, with normal sugar; normal to moderate increase in the number of cells and the presence of antibodies against Junin virus in titers that exceed the 1:40 ratio compared with those in the serum (Enria et al., 2004; Enria, 2005).

3. Pathogenesis and immunology

As noted, most cases of AHF are believed to result from inhalation of virus-containing material from infected rodents. Viral replication is thought to occur at the initial site of infection, generally the lungs, with subsequent dissemination to other parenchymal tissues. A wide variety of organs may be affected, including vascular endothelium, myocardium, kidneys and the central nervous system (Buchmeier et al., 2006). Gross pathologic changes are generalized but non-specific (Maiztegui, 1975).

The bleeding seen in AHF is considered the result of thrombocytopenia, abnormal platelet function induced by a plasma component, and alterations in blood coagulation with fibrinolysis activation (Marta et al., 2000). Haemostatic abnormalities include prolongation of activated partial thromboplastin time (APTT), low levels of factors VIII, and IX; increased values of factor V, von Willebrand factor, and fibrinogen; decreases in antithrombin III and plasminogen. Endothelial cell involvement is shown by increased levels of von Willebrand factor (Heller et al., 1995).

In AHF, viremia is present throughout the acute febrile period. Very high titers of endogenous interferon-α (IFN-α) have been demonstrated accompanying viremia (Levis et al., 1984, 1985). The levels of IFN-α decreased after the transfusion of immune plasma. Tumor necrosis factor-α (TNF-α) titers are also increased (Heller et al., 1992).

During the prodromal and the neurological–hemorrhagic phases, there is an acute transitory immunodeficiency. This

is demonstrated by a delay in the humoral response, and a depressed cell-mediated immunity, that return to normal values in early convalescence (Enria et al., 1983, 1986; Vallejos et al., 1989).

4. Animal models useful for preclinical studies

Laboratory-bred mice, rats, guinea pigs, and non-human primates infected experimentally with Junin virus have given useful data for the understanding of the pathogenic process of AHF (Boxaca et al., 1961; Green et al., 1987; Kenyon et al., 1988; McKee et al., 1985, 1987). Guinea pigs reproduce most of the human lesions, with increasing viraemia from day 7 post-infection until death around day 13. Infected guinea pigs developed leucopenia and thrombocytopenia, reproduced the hemorrhagic manifestations of AHF, and died without detectable antibodies. In this model, immune serum treatment decreased the mortality when administrated 1 day before or 5 days after the infection. In animals treated after day 9 of infection, the mortality was not modified, but all died without the classic hemorrhagic manifestations of the illness (Weissenbacher et al., 1968). In the same animal model infected with Junin virus and treated with pooled, homologous immune serum, with titers of antibodies measured in therapeutic units of immune sera, the sera prevented all signs of illness when administered 24 h after infection, and also prevented illness and death as late as 6 days after infection if the amount of therapeutic units were increased (Kenyon et al., 1990). Some surviving animals developed a late neurological syndrome with prominent hind-limb paralysis (Kenyon et al., 1986b). Treatment of guinea pigs with ribavirin or tributylribavirin did not reduce mortality, although viral replication was delayed and the mean time of death prolonged (Kenyon et al., 1986a).

Junin virus infection of rhesus monkeys and marmosets produced lesions similar to those reported in human cases of AHF (McKee et al., 1985). Common findings include hemorrhage, bone marrow necrosis, mild hepatocellular necrosis, poliencephalomyelitis and autonomic glanglioneuritis. *Callithrix jacchus* infected with Junin virus developed acute hematological and neurological manifestations with anemia, leucopenia and thrombocytopenia and died within 17–24 days after inoculation without demonstrable anti-Junin antibodies. Immune serum treatment of Junin virus-infected marmosets was found to reduce mortality from 100% to 25%. Viraemias and viral titers in organs were lowered and late neurological signs appeared in 30% of treated survivors (Weissenbacher et al., 1986a,b; Avila et al., 1987). The treatment of Junin virus infected *C. jacchus* with the antiviral ribavirin resulted in an increased in survival rates and in a delay of the mean day of death. Rhesus macaques infected with different strains of Junin virus obtained from humans with different clinical forms of AHF developed anorexia, lassitude, gastrointestinal disturbances and vascular phenomena during the second week after inoculation. The late disease patterns induced by the strains were analogous to those seen in humans from whom they were derived (McKee et al., 1987; Green et al., 1987). Prophylactic or therapeutic treatment of Junin virus infected rhesus macaques with ribavirin proved useful in modifying the initial course of illness, although survivors developed a late-onset central nervous system illness, with some deaths (McKee et al., 1988).

5. Human studies

5.1. Immune plasma

Historical aspects: At the end of the 1950s, several viral human infections were treated either with immune sera or with gamma globulins obtained from them. They were used based on the beneficial effect obtained either preventing the development of the illness, decreasing the severity of the disease or preventing the development of complications, according to the time at which they were administered (Gross et al., 1959; Rinaldo, 2005). In AHF, almost since the discovery of Junin virus, plasma coming from persons who had AHF on clinical grounds was used as a therapeutic measure. This empirical use was based on the advantageous results that had been reported in hepatitis, rubella, poliomyelitis, mumps and measles. The results obtained were reported to be satisfactory, but there had been no attempts to measure its efficacy. By the 1970s the beneficial effects of this form of treatment were considered inconclusive and for this reason, a placebo-controlled trial was designed (Ruggiero et al., 1964, 1977).

Double blind trial with immune plasma: Between 1974 and 1978, a double blind placebo-controlled study was performed among patients with a clinical diagnosis of AHF hospitalized in Pergamino with less than 8 days from onset of symptoms. The patients were randomly allocated to receive intravenously either 500 ml of immune plasma or normal plasma. Ultimately, 188 cases with a laboratory confirmation of infection with Junin virus entered in the trial. The case-fatality rate among cases treated with normal plasma was 16.5% while the rate in those patients treated with immune plasma was 1.1% (Maiztegui et al., 1979).

Public health actions to reduce AFH case-fatality rate: After the demonstration of the efficacy of immune plasma, a National Program for the control of AHF was developed. The program endorsed the early use of immune plasma in patients with a clinical diagnosis of AHF as the standard specific treatment of the illness. Immune plasma banks with available units of certified quality were established around the AHF endemic area. As the treatment is only effective when transfused during the first week of illness (Enria and Maiztegui, 1994), an intensive training program among physician and nurses of the endemic area was developed in order to accomplish the early clinical diagnosis required. This was done in conjunction with a comprehensive activity of education in health in the affected communities with the objective of promoting the immediate medical consultancy under the presence of febrile sign and symptoms. The activity was also addressed to encourage persons who had survived AHF to donate plasma.

Standardization of doses of immune plasma: The therapeutic dose of immune plasma of 500 ml was originally established

empirically. The beneficial effect of this treatment was attributed mainly to the specific action of neutralizing antibodies against Junin virus. The viremia was shown to be reduced in AHF patients after the transfusion of immune plasma (Montardit et al., 1979). Till the beginning of the 1980s, complement fixation and indirect immunofluorescence were the methods used for the serological diagnosis of AHF, and for the selection of immune plasma donors. When it became possible to standardize a plaque neutralization assay in cell culture, it was found that titers varied widely in people who had AHF. On the other hand, there was no correlation among the titers of complement fixation, immunofluorescence and neutralizing antibodies in an individual's sera. It was then decided to determine whether a low dose of neutralizing antibodies was associated with a higher mortality and to define the therapeutic dose of immune plasma on the basis of the quantity of neutralizing antibodies to be given. The "therapeutic units" of neutralizing antibodies were calculated from the body weight of the patient and the weight and titer of neutralizing antibodies in each unit of immune plasma given:

$$\text{TU (kg)} = \frac{\sum(\text{weight} \times \text{titer of each unit})}{\text{body weight}}$$

First, in a retrospective study, it was shown that a lower dose of neutralizing antibodies was associated with a higher mortality. Then, in a prospective study the dose of immune plasma was established in 3500 TU/kg of neutralizing antibodies per kg body weight (Enria et al., 1984, 1986).

Effectiveness of immune plasma in the treatment of AHF: The impact of the institution of immune plasma as the specific AHF treatment is shown in the decrease of the case-fatality rate. However, coincident with a marked decrease in the incidence of the illness due to vaccination with Candid #1 vaccine, an increase in the case-fatality rate of AHF is being registered (Figs. 2 and 3). This is attributable to a failure in timely recognition of the early signs of the illness among the population and by health care workers due to the decline in the morbidity.

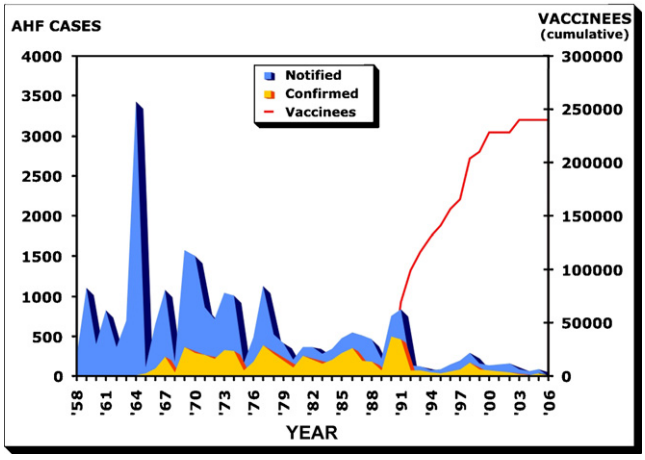

Fig. 2. AHF notified and confirmed cases (1958–2006).

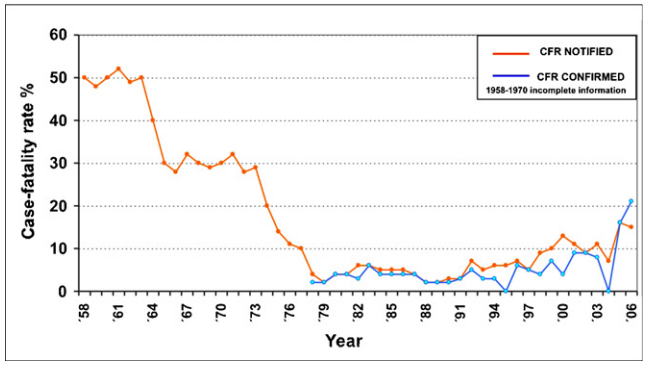

Fig. 3. Case-fatality rate in AHF.

5.2. *Rationale for the evaluation of alternative forms of treatment for AHF*

Some arguments give the rationale for the evaluation of alternative forms of treatment in AHF:

- The lack of efficacy of the immune plasma in patients with more than 8 days of evolution (Enria and Maiztegui, 1994).
- The risk of transfusion-borne diseases (Saavedra et al., 1997).
- The presentation of a late neurological syndrome in around 10% of the treated AHF survivors (Maiztegui et al., 1979; Enria et al., 1985; Enria, 2005).
- The difficulties with the maintenance of an adequate stock of immune plasma.

5.3. *Ribavirin*

Ribavirin (1.β.D. ribofuranosyl.1.2.4. triazole-3-carboxamide) was considered a suitable antiviral to be tested in AHF cases, according to *in vitro* tests and preclinical studies (Huggins et al., 1984; Rodríguez et al., 1986). Considering that there was already an effective treatment, clinical trials in human volunteers were originally performed in AHF cases with more than 8 days from onset of symptoms (Enria et al., 1987; Enria and Maiztegui, 1994). These studies were done in two phases: first, an open study in which 7 patients received ribavirin, and second, a double blind trial in 18 cases in which 8 received ribavirin and the other 10 placebo. Ribavirin (Viratek, Costa Mesa, California) was given intravenously according to the following schedule: 34 mg/kg as a loading dose, followed by 17 mg/kg every 6 h for 4 days, and by 8 mg/kg every 8 h for the following 6 days. Ultimately, 14 patients in whom the diagnosis of AHF was confirmed received ribavirin, of whom 4 died (case-fatality rate 28.57%) (Table 1).

Although ribavirin treatment was not able to demonstrate a significant reduction in the mortality, some results indicate that the drug had an antiviral effect in these advanced cases of AHF. These include the clearance of viraemias observed in all cases 4 days after the initiation of treatment, a drop in endogenous IFN titers observed 2 days from the first dose of the drug, and a delay in the time of death in the 4 patients who died. The only adverse effect observed in the ribavirin-treated AHF patients was the

Table 1
Case-fatality rate in AHF cases treated with ribavirin

Study	Died	Survived	%
Open trial	3	3	50
Double blind trial	1	7	12.5
Total	4	10	28.57

development of anemia, an event previously documented among the secondary reactions to this drug (Shulman, 1984). On the other hand, 1 of the 10 survivors treated with ribavirin developed during early convalescence a febrile syndrome with alterations in the CSF, and abnormalities in the evoked responses similar to the ones described in AHF patients treated with immune plasma with a LNS (Cristiano et al., 1985; Enria, 2005). This finding suggests that treatment with ribavirin in AHF might be expected to result in some cases in an untoward effect similar to that produced by immune plasma treatment.

The results obtained in these clinical trials argued in favor of a possible beneficial effect of ribavirin in AHF. It was considered that the lack of efficacy observed in the trials could be attributable to the late initiation of the treatment. For this reason, a protocol for early use of ribavirin in AHF cases was developed. In a preliminary phase, cases with less than 5 days of evolution would be included. The patients would receive ribavirin for 2 days, and upon finishing this treatment would also receive a transfusion of immune plasma early enough to be effective, given that all cases would have received it within 8 days from onset of symptoms. Although the protocol was approved, no patients were included due to problems with obtaining an adequate continuous supply of the drug. Another point considered was the high cost of the treatment.

6. New antiviral drugs

Junin virus as well as all South American pathogenic arenaviruses are included by the National Institute of Allergy and Infectious Diseases (NIAID) among Category A agents that could be weaponized by bioterrorists. For this reason, hemorrhagic fevers of arenaviral origin are considered priority for the biodefense programs. Some projects for the development of new antiviral drugs for potential inclusion in a strategic stockpile were initiated and resulted in products that might deserve an evaluation in the treatment of AHF.

IFN alfacon-1, an unnaturally occurring bioengineered IFN-α approved for the treatment of chronic hepatitis C, is active against Pichinde and Tacaribe viruses in cell cultures. In the hamster model of Pichinde virus infection, interferon alfacon-1 treatment significantly protected animals from death, prolonged the survival of those that died, reduced virus titers, and limited the liver damage. Furthermore, interferon alfacon-1 also showed some efficacy when the initiation of treatment was delayed up to 2 days after infection (Gowen et al., 2005). Combined therapy of ribavirin with IFN alfacon-1 for the treatment of Pichinde infection in hamsters resulted in a synergistic activity. This combination therapy reduced the effective dose of ribavirin, which would serve to limit its toxicity (Gowen et al., 2006).

In a high-throughput screening for identification of small molecule inhibitors, 400,000 small molecule compounds were screened in a Tacaribe virus-induced cytopathic effect assay. A compound named ST-294 was chosen for drug development. ST-294 demonstrated protective antiviral efficacy in a Tacaribe mouse challenge model (Bolken et al., 2006).

A thepyrazine derivate, T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) also showed efficacy for treating Pichinde infection in hamsters (Gowen et al., 2007). The administration of the decay thiaptamer, XBY-52, to Pichinde-infected guinea pigs resulted in a significant reduction in mortality and enhanced the guinea pigs macrophages (Fennewald et al., 2007). Among 15 antiretroviral Zn-finger active compounds screened *in vitro* against Junin virus, three showed antiviral activity. The aromatic disulphide NSC20625 was considered a very potent virucidal agent (García et al., 2000).

7. Other therapeutic approaches

7.1. Immune globulins from human immune plasma

The Instituto de Hemoderivados de Córdoba, belonging to the Universidad Nacional de Córdoba, Argentina, produced in the 1980s immune immunoglobulin for intramuscular use that was derived from immune plasma obtained at INEVH. This was never used in a clinical trial, considering that for a therapeutic effect the immunoglobulin should be given intravenously. On the other hand, intramuscular injections are contraindicated in the viral hemorrhagic fevers. A project to produce immune immunoglobulin for intravenous use was developed. The available methodology for other immunoglobulin departs from volumes of plasma that are not possible to obtain in the case of AHF, due to the low incidence of the disease and the restricted endemic geographic area. For this reason, some modifications and new developments were required. The product would be an orphan one, without commercial revenue for which, a special funding was required to initiate the activities. No products have been available so far for preclinical studies.

7.2. Monoclonal antibodies

With the objective of developing monoclonal antibodies for therapeutic use from substrates such as peripheral lymphocytes or bone narrow specimens from AHF convalescents selected on the basis of their titers of neutralizing antibodies against Junin virus, different projects have been initiated. So far, these have not yielded a product to initiate animal studies.

8. Feasibility of a clinical trial of new drugs

Although for AHF we already have an effective treatment that can reduce the mortality and an effective vaccine that can reduce the morbidity, there would be still the need for better therapeutic options. It is expected that even with adequate vaccine coverage, AHF cases could occur (between 0 and 15 cases per year, distributed in three main sites). As recognition of the illness by physicians is now worse than before, it is also expected that

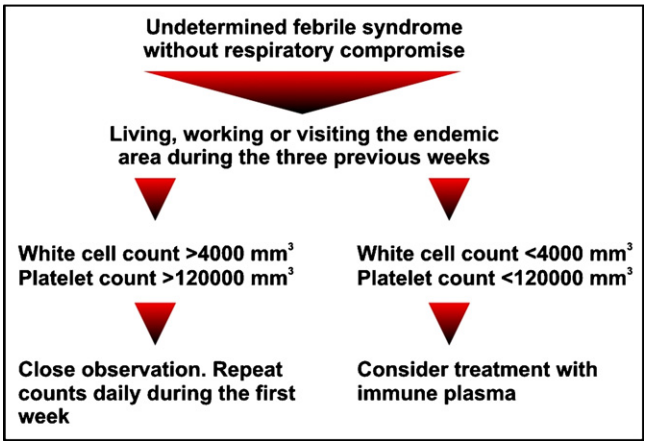

Fig. 4. AHF algorithm for the detection of patients during the prodromal phase.

we would more frequently see severe forms. The availability of drugs with a broad antiviral spectrum would be welcome, as it can be evaluated simultaneously for other agents for which there is not an effective therapy that are among the differential diagnosis of AHF. The same rationale can be applied to drugs that are being developed for the treatment of systemic inflammatory disorders.

9. Discussion

Immune plasma is the specific therapy that has been successfully used in AHF. This success is strongly linked to the fact that epidemics involving 300–1000 cases were registered every year in a restricted endemic area, mostly rural. With the availability of the effective live attenuated Junin virus vaccine Candid #1, a marked reduction in the incidence of AHF was achieved (Figs. 1 and 2). On the other hand, a new geographic extension of the endemic area was registered in the last decades involving the surroundings of a big city (Rosario), which has more than 1,000,000 inhabitants. In this new situation, the early recognition of AHF according to the algorithm proposed by the National Program for AHF control is not as operative as before (Fig. 4). AHF diagnosis is established more frequently late in the course of the illness in a smaller number of patients, resulting in an increase in the case-fatality rate (Fig. 3). The reduction in disease incidence also heralds future problems with the availability of plasma donors. Although plasma from Candid #1 vaccinees can be used as an alternative source, the neutralizing antibody titers in vaccinees are much lowered than those in convalescents of the illness (Enria and Barrera Oro, 2002).

One of the most puzzling secondary events associated with the treatment of immune plasma is the LNS. From the very first description of AHF it was recognized that some patients could present a neurological disease after the acute phase of the illness. The disease was known among inhabitants of the endemic area as “relapse,” but this entity was then named Late Neurological Syndrome of AHF. Several physiopathogenic mechanisms have been suggested to explain LNS. The humoral immune response is different in patients with LNS; they present a delayed primary response, as in all cases treated with immune plasma, but achieve higher titers of neutralizing antibodies against Junin virus than other surviving cases, even those that have not received immune plasma. The ratio between antibody titers in paired serum and CSF samples may suggest local synthesis of antibodies (Enria et al., 1985, 1986).

The search for Junin virus by isolation attempts, even by cocultivation, and by RT-PCR from blood, lymphoid tissues and CSF has been consistently negative. The higher titers of neutralizing antibodies and the CSF-serum antibody ratio in LNS cases suggest the possibility of a more prolonged antigenic stimulation, probably through a longer persistence of virus or antigens in the central nervous system.

One case of LNS was observed among AHF cases treated with ribavirin. As neither ribavirin nor immune plasma crosses the blood–brain barrier, it can be argued that LNS is produced by virus already established in the central nervous system. Although the interval free of symptoms had led to the consideration of the LNS as a post-infectious encephalomyelitis, the absence of lesions in the white matter, as shown by magnetic resonance imaging, has ruled out this possibility (Cristiano et al., 1985; Enria, 2005).

If AHF cases occurred outside of Argentina, either as a result of a traveler being hospitalized outside the country or as a consequence of a bioterror attack, AHF immune plasma would probably not be readily available. Furthermore, in the USA or in some European countries there is the possibility that this therapy would find resistance because of safety, regulatory and/or medical legal concerns. In such cases, ribavirin would probably be used empirically.

Many considerations argue for the exploration of substitute treatments. In patients with more than 8 days of evolution, in whom anti-inflammatory responses and a failure of adaptive immunity are considered to lead to a terminal state of immune paralysis (Bracco et al., 1978; Molinas and Maiztegui, 1981; Enria et al., 1983, 1986; Levis et al., 1984; Vallejos et al., 1989; Marta et al., 1998, 1999), the elucidation of common pathogenetic mechanisms with infectious systemic inflammatory syndromes might lead to new treatment possibilities (Bray, 2005). There are already suitable animal models to perform preclinical studies. There would be also possibilities to perform clinical studies, as AHF cannot be eradicated, and even with good vaccine coverage, isolated cases and small outbreaks are expected to continue. However, recruitment of volunteers would not be as easy as it was when continuous prominent outbreaks allowed the performance of clinical trials.

10. Conclusion

- Current specific treatment for AHF consists of the early transfusion of immune plasma in standardized doses of neutralizing antibodies.
- Alternative forms of treatment are required, given the difficulties in maintaining a sufficient stock of immune plasma and possible adverse events. On the other hand, new treat-

ments for severe cases that evolve to a hemorrhagic and/or neurological form of the illness are also needed.

- There are suitable animal models and there would be some possibilities for the performance of clinical trials if new products are developed.
- Ribavirin may prove useful in AHF treatment provided that the drug is initiated early in the course of the illness. There should be a guarantee of continuous supply of the drug and a reduction in its cost to consider its evaluation in a clinical trial.

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