

WHO Blood Regulators Network (BRN)

Position Paper on Collection and Use of Convalescent Plasma or Serum as an Element in Filovirus Outbreak Response*

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1. Consideration of the use of convalescent plasma as an element in filovirus outbreak response

1.1 Overview

The periodicity and extent of filovirus outbreaks in Africa have increased significantly since the initial identification of these viruses in the mid-1960s. Addressing the threat of filovirus outbreaks has become an urgent global public health priority. Vaccines and antiviral therapies are under development but currently they are not approved by regulatory authorities. Recent work has shown that immune therapies based on anti-filovirus glycoprotein (GP) monoclonal antibodies (mAbs) and convalescent monkey immunoglobulin preparations are effective in the filovirus rodent and monkey lethal challenge models. Cocktails of anti-filovirus GP mAbs against different species of filoviruses produced in plants are currently under development. The efficacy of the mAbs and convalescent monkey immunoglobulin preparations in non-human primate models suggest that human convalescent plasma or sera could be used to treat and prevent filovirus infection and severe clinical complications. Furthermore, there is anecdotal evidence (small number of patients and uncontrolled studies) suggesting that human convalescent plasma might be effective in filovirus patients during outbreaks. Although mortality from filovirus infections can reach up to 90% in humans, thousands of survivors from past outbreaks in Africa who developed long-lasting anti-filovirus antibodies could be recruited as a source of convalescent human plasma. However, the efficacy of human convalescent plasma or sera has not been determined in the monkey lethal challenge model, which closely resembles filovirus infection in humans. Because of the significant variation in anti-filovirus antibody responses observed in vaccinated or infected monkeys and patients, units of convalescent plasma or serum may not contain sufficient amounts of neutralizing antibodies. The question whether human convalescent plasma or serum should be used as a practical and cost-effective approach to treat and prevent infections during filovirus outbreaks remains open. Because not all neutralizing anti-GP mAbs are protective in NHPs and there is some controversial evidence of antibody-dependent enhancement (ADE) of filovirus infection (1-6), the safety and efficacy of human convalescent plasma or serum should be demonstrated in controlled pilot studies in patients and could be further explored in the NHP challenge model. Testing of total and neutralizing anti-GP ebola antibodies by assays that could be performed in a conventional laboratory environment would be optimal to assure that high levels of protective antibodies were being transfused to patients. Development of fractionated immunoglobulins from convalescent human plasma or sera should also be considered to increase the safety and efficacy of the anti-filovirus immunotherapy.

1.2 Background

The *Filoviridae* is a family of filamentous, negative-strand RNA, enveloped viruses consisting of three genera: *Ebolavirus* and *Marburgvirus*, which cause severe

hemorrhagic fever in humans and nonhuman primates (NHPs) with high morbidity and mortality rates up to 90% (7, 8), and *Cuevavirus*, which is pathogenic in bats and was recently discovered in Spain (9). Filoviruses are BSL-4 pathogens classified as “Category A” bioterrorism agents, and currently there are no licensed therapeutics or vaccines to prevent or treat filovirus infections. The filovirus non-segmented negative-strand RNA genome of approximately 19 kb contains 7 genes: nucleoprotein (NP), VP35, VP40, glycoprotein (GP), VP30, VP24, and the polymerase (L) (10). The GP is a type-I transmembrane glycoprotein that is cleaved into disulfide-linked GP1 and GP2 subunits. The mature GP forms homotrimers that are presented as spikes on the surface of infected cells and virions, and is responsible for receptor binding, viral entry, and immunity (11, 12). Immunization with GP is sufficient to protect animals against ebolavirus lethal challenge in the mouse, guinea pig, and NHP models. Several GP-based vaccine candidates are currently under development such as virus-like particles and virus-vectored vaccines, which confer protection from lethal challenge in animal models including NHPs (13-22).

Marburgvirus is antigenically stable and present in a single species, marburgvirus (MARV), whereas *Ebolavirus* consists of five species, Ebola (formerly) Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), Reston ebolavirus (RESTV), and Bundibugyo ebolavirus (BDBV) (9). The zoonotic nature of filovirus infection became apparent soon after the emergence of MARV in 1967 and Ebolavirus (EBOV) in 1976. The first recognized MARV outbreak in humans was linked to infected monkeys imported from Uganda that infected laboratory workers from the European cities of Marburg and Belgrade. The initially identified ebolavirus (EBOV) outbreak occurred in workers from a single cotton factory in Sudan. Most human filovirus outbreaks have been linked to infected non-human primates or bats in cotton factories, caves, and mines (for a review, see (23)). MARV infects bats and these animals are currently believed to be the natural host for all filoviruses, which transmit the virus directly to humans or indirectly via infected NHPs or other animals. Although anti-EBOV antibodies have been detected in bats during field surveillance studies, EBOV so far has not been isolated from infected bats in nature. However, experimentally-infected bats survive EBOV infection, develop limited signs of disease, the virus replicates in lung endothelial cells, and the animals develop a viremic phase shedding the virus in the feces (24). Infected bats could spread filoviruses directly to NHPs and humans via aerosol and excretions. In NHPs and humans, filovirus infection results in severe hemorrhagic fever with high levels of morbidity and mortality. Animal mortality has been shown to precede human filovirus outbreaks in Gabon and the Republic of Congo, which have been linked to contact with dead monkeys, gorillas, chimpanzees, and duikers (25). Human contact with infected primates and duikers or their carcasses has been linked to transmission of filoviruses to the human population. Close contact with infected individuals is responsible for outbreaks in the human population and perhaps NHPs colonies. The ecology of filoviruses is poorly understood, and it is possible that other animals are also involved in the transmission of this virus. For instance, dogs that ate infected carcasses and were in contact with human cases developed asymptomatic infection and seroconverted, but it is unknown whether dogs can transmit EBOV to humans (26). All the species of filoviruses identified in Africa, i.e. EBOV, SUDV, BDBV, TAFV and MARV are pathogenic for humans and NHPs and cause different degrees of morbidity and mortality. However, RESTV, the only species of ebolavirus identified in Asia, is pathogenic in NHPs but not in humans, who seroconvert after exposure from RESTV-infected NHPs but do not develop disease. Interestingly, RESTV has been

detected in the Philippines in domestic pigs co-infected with circovirus type 2 having respiratory disease, which was associated with the seroconversion of animal caretakers (27). Pigs were also experimentally infected with EBOV by mucosal exposure and developed lung pathology, produced high titers of virus in the respiratory tract, shed virus from the oronasal mucosa, and transmitted the disease to cohabiting pigs (28). These data suggests that pigs could also be naturally infected with EBOV strains that are pathogenic to humans. In summary, natural EBOV infections result in very different diseases depending on the host and virus strain and ranging from inapparent disease in bats and dogs, respiratory or subclinical disease in pigs (29), to ebolavirus disease (EVD) in humans and NHPs with high levels of morbidity and mortality. The currently ongoing EBOV outbreak is primarily due to an unprecedented scale of human-to-human transmission presumably after a limited introduction from animals or environmental sources in Guinea.

Filovirus infection in humans elicits cellular and humoral immune responses (for a review, see (30)) that are early and vigorous in survivors. Fatal cases are associated with immune dysregulation and high viremia (31, 32). Most vaccine candidates including vesicular stomatitis virus (VSV) and adenovirus vectored-vaccines induce moderate to high levels of anti-GP antibodies in NHPs (for a review, see (33)), which correlate with protection against lethal challenge in the rodent and NHP models (34-36). Some filovirus vaccine candidates, including parainfluenza and Newcastle virus vectored-vaccines (37) and virus-like particles (VLPs) (14), induce significant levels of anti-GP neutralizing antibodies in NHPs. Because neutralizing antibodies are generated during filovirus infection in humans (38) and passive transfer of neutralizing mAbs (39, 40) and monkey convalescent immunoglobulin preparations (41) protected NHPs against filovirus lethal challenge, it is possible that human convalescent antibody preparations could be an effective immunotherapy in filovirus outbreaks.

Serum therapies were successfully used to treat many infectious diseases (anthrax, plague, scarlet fever, measles, tularemia, diphtheria, dysentery, meningococcal meningitis, rabies, pneumococcal pneumonia) for half a century after Emil von Behring first demonstrated their effective use as a therapeutic in diphtheria. Their general use fell into disfavour after the advent of antibiotic therapies and in consideration of the problems of adverse reactions to animal derived sera and whole serum. However, human and animal derived immunoglobulins remain important therapies for a variety of conditions (parvovirus, CMV, hepatitis B, rabies, hepatitis A, botulism, envenoming, etc.) Additionally, there is precedent in the modern era for effective management of Argentine Hemorrhagic Fever (Junin Virus) with immune plasma as part of a nationally organized response (42). By analogy, although there are many uncertainties, the possibility exists that convalescent plasma could play a role in the urgent response to filovirus outbreaks in settings where vaccination and/or effective antiviral chemotherapy is lacking.

Initial passive immunity studies in animal models showed that human convalescent sera (43) or equine, ovine, or caprine neutralizing immunoglobulin preparations protected rodents but not NHPs (44-48) against filovirus lethal challenge. Similarly, a human mAb molecularly cloned from a survivor also protected rodents against lethal challenge (49) but failed to protect NHPs (50). Treatment of patients with convalescent human blood has been used in limited occasions in uncontrolled studies that yielded controversial outcomes (51-53) but further studies with larger

numbers of patients will be required to evaluate the immunoprophylaxis potential of convalescent plasma or sera. Although blood transfusion from convalescent individuals was associated with survival in seven out of eight treated patients in an uncontrolled study (53), it is unclear whether this association is due to the enhanced care that these patients received, the positive effect of blood components, the presence of memory immune cells in blood, the presence of anti-ebolavirus antibodies, or a combination of these factors. Controlled studies would be needed to evaluate whether a blood transfusion or the anti-ebolavirus immune response factors present in the blood of the convalescent donors might enhance survival in transfused patients. However, recent work using cocktails of mouse neutralizing mAbs (39, 40) or convalescent monkey immunoglobulin preparations (41) protected NHPs against filovirus lethal challenge showing that anti-ebolavirus neutralizing antibodies are protective and suggesting that they could be used as therapeutics in humans. Taken together, these data indicated that a high dose of a mixture of anti-GP mAbs or purified IgG preparations from convalescent monkeys is highly effective in preventing (pre-exposure) and treating (1-3 days post-exposure) filovirus infection in NHPs and suggested that a passive immunity strategy using immunoglobulin preparations from human convalescent plasma or sera could also be effective during filovirus outbreaks. Because of the current lack of infrastructure in West Africa to produce immunoglobulin preparations from convalescent plasma, the magnitude of the current ebolavirus outbreak, and the unavailability of other treatment alternatives, transfusion of plasma or serum from convalescent patients could be considered as an investigational therapy.

2. Collection and use of Convalescent Plasma or Serum

2.1 Role of Regulatory Agencies

Though convalescent plasma may potentially be effective in treating filovirus-infected individuals, at this time there are limited data in animal models and only anecdotal experience in humans regarding the efficacy of this treatment. Regulatory agencies should first consider the ethical¹, scientific, and logistic resource issues that need to be addressed in order to evaluate and implement this therapy in the context of the available infrastructure in the affected countries. Although filovirus convalescent human plasma or serum could be and has been prepared and used locally as a medical practice (51-53), an organized effort to establish safe use and to determine true efficacy of this therapy has to be established by applicants and well controlled by regulatory authorities. Such an approach is needed especially in consideration of the likelihood that convalescent plasma or serum will be used empirically without defined standards in urgent conditions when effective vaccines, antiviral drugs, and antimicrobial agents are unavailable. In conjunction with public health agencies, regulatory authorities could play a role in identifying the need for a scientific evaluation in this area. The availability of well characterized filovirus NHP lethal challenge models that closely resemble the disease in humans currently being used in Canada, UK, and the US under BSL-4 facilities could facilitate testing of candidate plasma pools and immunoglobulin preparations from convalescent patients in preclinical proof of concept studies to determine the efficacy of such preparations. Tests to screen blood and/or plasma donors for anti-filovirus total and neutralizing

¹ <http://who.int/mediacentre/news/statements/2014/ebola-ethical-review-summary/en/>

antibodies under BSL-2 conditions are currently available [for a review, see (54)] and could be transferred and implemented at blood establishments. Moreover, design of clinical trials for proof of concept should be regulated by national authorities. For example, it is suggested that efforts could be directed to carrying out trials using filovirus convalescent plasma, serum or immunoglobulin preparations. Because previous efforts during the mid-1970s showed that many patients who survived filovirus infection also suffered other endemic diseases (51), it is also suggested that the current effort focus on immunoglobulin preparations produced using pathogen inactivation and reduction technologies. Furthermore, neutralizing anti-filovirus titers in blood of surviving patients are not very high so immunoglobulin preparations containing concentrated neutralizing antibodies are likely to be more effective than plasma or sera containing lower antibody titers.

2.2 General Regulatory Considerations

A regulatory approach rather than solely a medical practice approach to empirical use of convalescent plasma or serum in the treatment and prevention of filovirus infection would have advantages in promoting patient safeguards and in the collection of useful scientific information. The following issues warrant consideration by blood regulatory authorities:

- Efficacy of human convalescent immunoglobulin preparations in protection against disease

There is only anecdotal evidence of the therapeutic efficacy of human plasma in filovirus infected patients (mainly the study by Mupapa et al., 1999, in which 7 out of 8 patients who received whole blood from convalescent patients survived ebolavirus infection). Also, immunoglobulin preparations from EBOV and MARV convalescent NHPs protected NHPs against lethal challenge (41). Therefore, it is possible that human convalescent plasma could be efficacious in preventing and treating filovirus infection in humans. The efficacy of immunoglobulin preparations from convalescent human plasma or sera has not been established and should be demonstrated in controlled pilot studies in patients and could be further explored in the NHP challenge model.

- Qualification of donors and donations

Donors should be qualified based on their general health, negative test results for EBOV RNA and with high total and neutralizing anti-GP antibodies. However, the current local capabilities for Ebola testing may be limited, in which case donors may be qualified based on clinical criteria and blood samples collected for delayed testing as part of a scientific assessment. Additionally, selection of donations that are negative for HBV, HCV, HIV, syphilis or other locally transmitted infections is necessary to minimize risks.

- Clinical use of convalescent plasma or serum should be regarded as investigational

Because the safety and efficacy of convalescent plasma or serum are unproven, clinical use of this product should be managed as an experimental therapy consistent with ethical safeguards (informed consent, institutional approval,

special labeling) and a commitment to gather and report outcome data independently of the outcome of the study (to prevent publication bias).

- Standards for product manufacturing should maximize safety of donors and recipients

Collection and preparation should be performed by trained staff operating under standard operating procedures in accordance with international guidelines (5). Segregation of filovirus convalescent donations from the routine blood inventory should be considered. Selection criteria for donors should include all established safeguards for prevention of transfusion transmitted diseases (see “WHO Blood Regulators Network (BRN): Donor selection in case of pandemic situations” at <http://www.who.int/bloodproducts/brn>). There is no evidence that filoviruses establish persistent infections in humans. In patients who survive filovirus infection, viral antigens are not detected and virus cannot be isolated after approximately 3 weeks from the onset of symptoms (55, 56). PCR analysis showed that viral nucleic acids cannot be detected in a significant number of patients 90 days after infection and in most patients approximately two years after (55, 56). It has been shown that high titers of anti-filovirus antibodies remain for several years after infection (55, 56), and immunity against filoviruses can be detected more than a decade after infection (57). Consequently, most patients who survived filovirus infection could be used as a safe source of plasma to produce immunoglobulin preparations after the outbreak subsides and patients become PCR negative. Furthermore, filoviruses are enveloped negative-strand RNA viruses that are highly likely to be inactivated with solvent-detergent treatment, and any residual genome material is unlikely to be infectious. Filovirus infection survivors constitute a safe source of convalescent plasma to produce plasma for transfusion and potentially immunoglobulin concentrates, and blood donations of qualified donors could be performed in areas where filovirus outbreaks have already subsided (a current list of ebolavirus outbreaks can be found at <http://www.who.int/csr/don/archive/disease/ebola/en/>, and marburgvirus virus can be found at http://www.who.int/csr/don/archive/disease/marburg_virus_disease/en/). Where feasible, pathogen inactivation of plasma is desirable. Care should be taken to minimize disruption to the collection and processing of blood and components for other patient needs.

- Criteria for patients to be treated

Development of a case definition for confirmation of disease in a candidate patient might prevent delay of therapy in settings where specific diagnostic testing is impractical. Also, it may be useful to establish priorities for clinical use. Studies using immunoglobulin preparations from convalescent monkeys showed that post-exposure treatment at 48 hours after challenge was highly effective in the filovirus NHP lethal challenge model (41), suggesting that human immunoglobulin preparations could be effective for prophylaxis and treatment of filovirus infection. Clinical trials would be needed to evaluate the treatment window during which human convalescent plasma or immunoglobulin concentrates made from convalescent patients might be effective. Protective monoclonal antibodies and vaccines are under development but may not be available soon in the outbreak areas. Supportive care including administration of

saline and antibiotics to prevent secondary infections is currently the only effective treatment and should be prioritize if limited resources do not allow blood, plasma, or sera transfusions. Passive immune therapy is generally more effective when given earlier in the course of disease, and may be accomplished with lower doses than those needed for treatment in established disease. Because filoviruses result in high morbidity and mortality rates within a week of the onset of symptoms, patients in outbreak areas should be treated as soon as possible.

- General considerations for plasma products are applicable

As with other plasma therapies, attention should be given to ABO compatibility. As feasible, preference for male sourced plasma or serum (and/or testing of female donors for antibodies to HLA and anti-granulocyte antibodies) may minimize risk of TRALI². Dosing guidelines should be provided and consideration should be given for use of units from at least two different donors in recognition of biologic variations in the immune response. ABO compatibility would not be an issue for an immunoglobulin product.

- Outcome monitoring should be oriented towards determination of product safety and efficacy and the rapid communication of best practices

Patient outcome monitoring and reporting should include indicators of safety and efficacy. Electronically available, fillable Case Report Forms would facilitate capture of essential data. Additionally, specimen collection from both donors and recipients (pre- and post-treatment) should be performed to permit retrospective determination of the characteristics of an effective product and dosage regimen. Mechanisms for rapid aggregation of clinical experience and dissemination of information to clinicians should be established in advance.

- Multiple filovirus species that do not confer cross-protection co-circulate in endemic regions

Ebolavirus and *Marburgvirus* are the *Filoviridae* genera that cause disease in humans. In addition to EBOV, BDBV, TAFV, and SUDV, MARV also co-circulates in sub-Saharan Africa. There is no cross-protection in the NHP challenge models between EBOV, SUDV, and MARV. Cross-protection against BDBV has been shown in NHPs immunized with some EBOV vaccines. Therefore, a comprehensive approach to prevent and treat filovirus infection should include convalescent plasma or sera from individuals infected with EBOV, SUDV, and MARV. Immunoglobulin preparations from each of these filoviruses could be produced from convalescent blood of patients that survived filovirus infection and could be shared by countries in the endemic region.

- Feasibility of large scale production including manufacture of immunoglobulins

Filovirus outbreaks in sub-Saharan Africa have been increasing in periodicity and magnitude. Thousands of survivors have been documented, who could be tapped as a source of blood/plasma donations for the manufacturing of convalescent

² Transfusion related acute lung injury (TRALI)

immunoglobulin preparations. Consideration could be given to industrial scale production of a specific human plasma derived immunoglobulin based on the outcome of studies on the effectiveness of convalescent serum or plasma. Any such product should be made only under GMP in a well-established and legally regulated facility.

3. Summary

Under the current crisis situation of this ebolavirus outbreak in West Africa where there are significant resource and logistic constraints, local considerations are needed regarding prioritization of this avenue of therapeutic development vis-à-vis other measures such as improving supportive care or the experimental use of candidate antiviral drugs, monoclonal antibody mixtures, vaccines or a combination of such therapies. However, if possible, the WHO Blood Regulators Network recommends that scientific studies on the feasibility and medical effectiveness for collection and use of convalescent plasma or serum be explored through clinical trials. In particular, an opportunity exists to study the feasibility, safety and effectiveness of convalescent plasma or serum and possibly other passive immunotherapies in filovirus infection. Well characterized filovirus NHP lethal challenge models, assays to diagnose infection, and tests to detect and quantitate total and neutralizing antibodies are currently available and could be used to support the immunotherapy approach. Acting within their mandates, regulatory agencies can play an essential role to enable progress in this area. Countries which want to engage in this type of practice should take all necessary steps to establish appropriate regulatory conditions for the collection of convalescent plasma or serum, the conduct of clinical studies and the monitoring and reporting of patient outcomes. Programs conducted at the national level should ensure the use only of convalescent plasma or serum collections that meet the safety, quality and efficacy criteria consistent with established regulatory standards. The feasibility of production on a large scale, possibly including a specific immunoglobulin, should be considered for the longer term, based on the outcome of studies, the course of the epidemic, and the available infrastructure for manufacturing under GMP.

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