

Convalescent plasma: new evidence for an old therapeutic tool?

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Abstract

Passive immunisation for the prevention and treatment of human infectious diseases can be traced back to the 20th century. The recent Ebola virus outbreak in West Africa has turned the spotlight onto the possible use of convalescent whole blood and convalescent plasma in the treatment of infectious diseases because they are the only therapeutic strategy available in some cases, given the unavailability of vaccines, drugs or other specific treatments. Convalescent blood products could be a valid option in the treatment/prophylaxis of several infectious diseases both in association with other drugs/preventive measures and as the only therapy when a specific treatment is not available. However, there are still some issues to consider in determining the advisability of implementing a large-scale convalescent plasma transfusion programme.

Keywords: passive immunisation, emergent pathogens, transfusion safety, convalescent plasma, Ebola virus.

Introduction

Passive immunisation (PI) for the prevention and treatment of human infectious diseases and its related concept of artificially acquired passive immunity can be traced back to the 20th century, when specific antibodies were sought from serum of stimulated animals (especially rabbits and horses). Human blood was also identified as a source of antibodies^{1,2}. PI is a technique to achieve immediate short-term immunisation against infectious agents by administering pathogen-specific antibodies. Since its introduction, it has proven to be lifesaving for many acute infections and, more recently, has also shown possible applications in cancer therapy^{1,3}. Although antibiotics have largely supplanted the use of PI in bacterial infections, it remains an important tool in the treatment of many viral infections when vaccines or other specific treatments are not available.

Convalescent blood products (CBP), obtained by collecting whole blood or plasma from a patient who has survived a previous infection and developed humoral immunity against the pathogen responsible for the

disease in question, are a possible source of specific antibodies of human origin⁴. The transfusion of CBP is able to neutralise the pathogen and eventually leads to its eradication from the blood circulation. Different CBP have been used to achieve artificially acquired passive immunity¹⁻⁴: (i) convalescent whole blood (CWB), convalescent plasma (CP) or convalescent serum (CS); (ii) pooled human immunoglobulin (Ig) for intravenous or intramuscular administration; (iii) high-titre human Ig; and (iv) polyclonal or monoclonal antibodies.

CP has been the subject of increasing attention, especially in the wake of large-scale epidemics⁵. Apheresis plasma is currently the preferred therapeutic tool for several reasons: larger volumes collected per session, the possibility of more frequent donations, and the absence of impact on the donor's haemoglobin thanks to the reinfusion of his or her red blood cells. The recruitment of donors living in areas in which an epidemic has broken out can offer the added value of providing specific, artificially acquired passive immunity against the local infectious agent while CBP supplied from other regions may be less effective due to (possible) strain variation of the pathogen in question^{6,7}. Nevertheless, the identification, selection, and recruitment of potential donors can be difficult, as convalescent subjects must also meet donor selection criteria, in compliance with national policies and routine procedures. However, because of the potential importance of the treatment some donor selection criteria, although designed to protect the donor's health, may be relaxed, as also suggested by the World Health Organization (WHO)⁸. Notably, the use of pathogen inactivation could guarantee additional safety thus supporting less strict selection criteria.

Ebola virus (EBOV) is the most recent in a long series of infectious agents for which the WHO has proposed a PI-based approach⁸.

The aim of this review article is to provide an overview on the use of CBP focusing on CP.

Historical background

From the 1880s to the antibiotic era, CBP were used to prevent and treat many bacterial and viral infections in

humans and in animal models⁹. In 1890, the first rational approach exploited by the physiologists von Behring and Kitasato¹⁰ to treat diphtheria was blood serum; initially, it was produced from immunised animals but soon whole blood or serum from recovered donors with a specific humoral immunity were identified as a possible source of specific antibodies of human origin. There are several examples of the use of CBP for the prophylaxis or treatment of bacterial infectious diseases such as scarlet fever in the 1920-40s and pertussis until the 1970s¹¹.

Studies conducted during the Spanish influenza pandemic of 1918 to 1920 suggested that the use of CBP might be effective¹²⁻¹⁷ and for the first time CP was identified as a potential therapy for a number of viral infections¹⁸. In the following decades, possible therapeutic efficacy was claimed for the management of measles, Argentine haemorrhagic fever, influenza, chickenpox, infections by cytomegalovirus, parvovirus B19 and, more recently, Middle East respiratory syndrome coronavirus (MERS-CoV), H1N1 and H5N1 avian flu, and severe acute respiratory infections (SARI) viruses¹⁹⁻²⁹. Furthermore, animal models of influenza pneumonia have shown the benefit of CS (protection against H1 and H3 challenge), equine hyperimmune F(ab')₂ globulin (protection against H5N1 challenge), and monoclonal antibodies (against H1, H3, and H5N1 challenge)³⁰⁻³². Interestingly, hospitalised patients with Lassa fever were also reported to have an apparently better outcome after CP administration³³. Furthermore, a meta-analysis on Spanish influenza-CBP (involving 8 suitable studies for a total of 1,703 patients) showed a significantly reduced mortality risk in the treated patients and suggested that CBP could be evaluated in the treatment of H5N1-related diseases³⁴.

A 2015 systematic review and exploratory post-hoc meta-analysis by Mair-Jenkins *et al.* on the effectiveness of CP and hyperimmune Ig for the treatment of SARI of viral aetiology reported a statistically significant reduction (75%) in the odds of mortality among SARI-affected patients who were treated in comparison to those who received a placebo or no therapy³⁵.

Narrative analyses showed "consistent evidence for a reduction in mortality", especially with early CP administration. However, as studies were "commonly of low or very low quality, lacked control groups, and at moderate or high risk of bias", the authors claimed that "this therapy should be studied within the context of a well-designed clinical trial or other formal evaluation", including the treatment of MERS-CoV infection³⁵.

As far as concerns CBP in the treatment of haemorrhagic fevers, in 1976 CP was used for a young woman infected with EBOV in the Democratic Republic of Congo. The woman was treated, without benefits, with plasma from a person who had survived an infection

with the closely related Marburg virus³⁶. During the same outbreak, 201 units of CP containing anti-EBOV antibodies (titre of at least 1:64) were obtained and frozen. Two units were transfused to an infected laboratory worker and the subject's recovery suggested the possible therapeutic effect of CP for EBOV patients³⁷.

CP was also used to treat patients with Argentine haemorrhagic fever caused by the Junin virus³⁸⁻⁴¹. In a double-blind trial carried out in 1979, patients treated with CP had a lower mortality rate compared to subjects treated with "normal plasma". An analysis of 23 consecutive annual epidemics of Argentine haemorrhagic fever in a group of 4,433 patients, observed from 1959 to 1983, showed a significant difference in overall mortality between patients managed with conventional treatment or CP (42.85% vs 3.29%)⁴². Immunotherapy was also attempted through the passive transfer of immunity with CP from patients who had recovered from Crimean Congo haemorrhagic fever, but the efficacy of this treatment for this disease is still not clear⁴³.

Since the first EBOV outbreak in Congo, passive immunisation in infected animals (e.g. monkeys) has been obtained with the administration of IgG preparations from horses hyper-vaccinated with EBOV thus suggesting a potential use in humans⁴⁴⁻⁴⁷. In a 1995 outbreak in Kikwit, Zaire, eight patients received 150-400 mL of CWB and seven survived, for a mortality rate of 12.5% in comparison to 80% in untreated patients⁴⁸. However, given the small number of treated patients and the lack of control subjects, the authors recognised the high risk of their work not being representative and involving confounding issues. In 2007, Oswald and colleagues reported a failure of passive transfer to protect macaques against challenge with EBOV⁴⁹. These negative findings contrasted with the above mentioned claimed results in the treatment of EBOV infection and highlighted the need for better comprehension not only of the characteristics and titre of antibodies able to affect the course of diseases but also of the role of the recipients' immune response⁴⁹. In 2012, Dye and colleagues⁵⁰ reported that passively transferred species-matched polyclonal IgG were able to provide total protection in Filovirus-challenged non-human primates as well as the maintenance of sufficiently high levels of IgG after multiple administrations until the host's adaptive immune responses could be recruited to clear the viral infection. In the same year, Olinger *et al.*⁵¹ and Qiu *et al.*⁵² reported that neutralising anti-EBOV glycoprotein monoclonal antibodies protected monkeys before and after lethal virus challenge.

Recent experience and future perspectives

In order to promote urgent research in response to the ongoing Ebola crisis, the European Commission's

Directorate-General for Research and Innovation, in collaboration with the WHO and the European Medicines Agency, has mobilised about € 25 million from the European Research and Innovation Programme Horizon 2020⁵³, launched in 2013 with funding of nearly € 80 billion available over 7 years. The European Union's framework programme for research and support has mainly been focused on the development of a safe and effective vaccine^{54,55} and CBP therapies⁸.

At the moment, considering the absence of licensed therapeutic and diagnostic tools to limit the EBOV outbreaks in West African countries, the WHO has prioritised a number of products for further investigation through human testing. These include two candidate vaccines, a short list of antiviral drugs, EBOV diagnostics, and CWB and CP^{5,8}. The evaluation of the possible role of CP and CWB as therapeutic tools is being conducted through controlled clinical studies that, it is to be hoped, will provide evidence-based data to evaluate their safety and efficacy^{5,56}. According to the WHO's criteria, only clinically asymptomatic survivors, 28 days after being discharged and who have twice tested negative for EBOV RNA by molecular techniques, should be considered as potential CBP donors⁸. Moreover, the discharge records of recovered patients should be reviewed before considering them as potential donors in compliance with national donor selection criteria. However, given the life-saving potential of CBP donated by survivors of this fatal disease, the WHO has suggested reviewing and possibly relaxing the donor selection criteria used in the country in question. Potential donors who meet the WHO criteria of recovery from EBOV disease and who also meet the donor selection criteria and have given informed consent should then be subjected to pre-donation testing to assess their final suitability for donation, according to national policy and routine procedures. The creation of a register or database of patients recovered from EBOV disease as potential CBP donors is strongly encouraged. A recently published WHO guideline provides further technical information on the collection and preparation of CBP, which should be performed by trained staff operating under standard operating procedures in accordance with international guidelines⁸. The WHO also dealt with key considerations enabling effective information, education, and engagement of patients recovered from EBOV disease and the communities in which they live, to consider donations of CBP for use in the treatment of EBOV disease and for use in clinical trials in the affected countries⁵⁷. A WHO additional guidance document also deals with the ethics of using CBP during EBOV epidemics⁵⁸.

CWB donated by patients who have recovered from an EBOV infection has been administered in Sierra Leone in a trial run by the government since late 2014⁵⁹.

A phase I/II pilot clinical trial of CP began in Liberia at the same time and is currently recruiting patients under the auspices of Clinical Research Management Inc. (a clinical research organisation) with the USA government and the Bill and Melinda Gates Foundation. The study, in which it is intended to treat 70 EBOV-infected patients with 90-110 mL of CP from two ABO-compatible donors, will evaluate the efficacy and safety of CP and could provide important results as well as the answers to several questions regarding this still incompletely understood therapeutic tool^{60,61}.

Guinea is also currently running a plasma trial through a partnership with institutes in Belgium, the UK, France, and Médecins Sans Frontières. So far, about 100 patients have been transfused in Sierra Leone and Guinea. Although very recently, Gutfraind and Meyers suggested that CP-based therapy is not only safer but also more efficient than CWB therapy in reducing mortality⁶², available data from the above mentioned trials are currently still being analysed for definitive evidence of efficacy and standards are being developed.

In addition, at the end of December 2014, the European Blood Alliance launched a new protocol, developed and managed by the UK National Health Service Blood and Transplant (NHSBT), for the coordination of European stocks of EBOV CP⁶³. Availability of CP from survivors in the European Union/European Economic Area is monitored by the European Blood Alliance but is currently scarce and a treatment protocol is under development⁶⁴. The German Federal Government has responded to the Ebola outbreak with a catalogue of various measures such as a study dedicated to the use of hyperimmune plasma undertaken by the Paul-Ehrlich Institute (Langen, Germany)⁸.

To the best of our knowledge, at least 24 cases of EBOV infection have been treated in Europe and the USA. Many of these cases were healthcare workers who contracted EBOV in West Africa and were transported back to their home countries for treatment. Most of them received CP in association with multiple other experimental treatments and advanced supportive care. For American patients, investigational new drug and compassionate use investigational device exemption (for pathogen-reduced plasma) has been used as a mechanism to permit collection and clinical use of CP⁶⁵.

The Cerus Corporation (Concord, CA, USA) has recently submitted a clinical protocol to the USA Food and Drug Administration to allow the use of the INTERCEPT Blood System[®] for the treatment of CP collected from EBOV disease survivors as an additional safety measure to reduce the risk of any transfusion-transmitted infection through plasma used as passive immune therapy in patients with serious or life-threatening EBOV disease⁶⁶. The same technology, for the same reason, is being used

in African trials. In fact, after collection, every plasma unit from the above donor source should undergo pathogen inactivation by an approved method to enhance the safety margin of transfused units⁶⁷. In this regard, a newly developed process of solvent/detergent inactivation for use on single-donor plasma or mini-pools of plasma in blood establishments in developing countries⁶⁸, could also be exploited as an additional safety tool⁶⁹.

Unfortunately, the waning of the epidemic in West Africa will probably have the effect of significantly reducing the ability to obtain useful and valid evidence-based efficacy data from any of the trials planned or in progress⁷⁰.

Scientific uncertainties and limitations regarding the use of convalescent plasma

Although its efficacy and safety have not been fully proven yet, CP treatment could be a valid option in the treatment/prophylaxis of several infectious diseases both in association with other drugs/preventive measures and as the only therapy when a specific treatment is not available. However, there are still some issues to consider in determining the advisability of implementing a large-scale CP transfusion programme⁷¹: (i) the lack of high-quality studies (i.e. randomised clinical trials); (ii) the risk of transmitting infections to transfusion service personnel⁷² (e.g. when handling laboratory specimens from infected recipients for pre-transfusion testing); (iii) the need for adequate selection of donors with high neutralising antibody titres; (iv) suitable risk assessment when considering relaxing the selection criteria against the risk impact of excluding donors; (v) case-fatality rates in CP trials will be influenced not only by patients' risk factors but also by the specific supportive care offered by clinical centres; (vi) immunotherapy using monoclonal antibodies could be more effective; (vii) many healthcare workers transferred to Europe or the USA received CP and survived but also benefited from experimental therapies and optimal supportive treatment, which are rarely available in developing countries; and (viii) in endemic areas, the risk of other transfusion-transmitted infections (e.g. human immunodeficiency virus, hepatitis B virus, hepatitis C virus and syphilis) cannot be excluded and pathogen reduction technologies should play a key role in guaranteeing safe CP transfusion.

Conclusions

The recent EBOV outbreak in West Africa has turned the spotlight onto the possible use of CBP in the treatment of infectious diseases because, in some cases, due to the unavailability of vaccines, drugs or other specific treatments, such blood products are the only therapeutic strategy available. Although many studies have reported the efficacy and safety of CP infusion in the

treatment of various infections (especially those caused by viruses), due to the lack of large-scale, randomised, well-designed clinical trials, we tend to consider CP an "empirical" therapy. Since most of the studies conducted so far are case series, they can only provide us with low-quality scientific evidence that may or may not be representative of the target populations. Furthermore, it is worth noting that none of the studies on therapeutic CBP usage seems to have taken into consideration the possibility that such treatment could be harmful. Dengue is a perhaps not applicable, but certainly sobering, example of an infection for which immune enhancement of pathogenicity is considered possible⁷³⁻⁷⁵. In fact, this disease "provides the most abundant example in human medicine and the greatest human illness burden caused by the phenomenon of intrinsic antibody-dependent infection enhancement" which, through the infection of monocytes or macrophages with infectious immune complexes, suppresses innate antiviral systems thus allowing logarithmic intracellular growth of the virus⁷⁶. This mechanism through which viruses take advantage of anti-viral humoral immune responses to infect host target cells is not limited to dengue⁷⁷⁻⁷⁹.

Data that will be available in the near future may also enhance knowledge of EBOV and the characteristics and immune response of hosts. Information about the EBOV proteins targeted by the immune system (especially by T cells) during natural infections should be useful in producing effective vaccines⁵⁵ and rapid progress in this field could make the use of obsolete, not advantageous CP. There may be major organisational and technological challenges in complying with standard operating procedures for the collection⁸⁰, production, and use of CBP in developing African countries. In this regard, it is also worth mentioning the ethical and practical difficulties of designing randomised clinical trials on the use of CBP, particularly with respect to the selection of control group patients.

Nonetheless, considering the possible seriousness and the high mortality rate of EBOV infection, it is important to provide as many people as possible a chance to receive safe and effective products that could save their lives. Collective efforts should be focused not only on the comprehensive evaluation of the feasibility of plasma treatment for infectious diseases but also on facilitating access to widespread and affordable treatments, especially in developing countries. Last but not least, it is important to ensure that the production and use of CBP take place according to precise ethical and controlled conditions and clinical trials are completed to produce the evidence base for a possible role of these products of human origin in preparedness plans for future outbreaks of emergent or re-emergent pathogens.

The Authors declare no conflicts of interest.

References

- 1) Rajam G, Sampson J, Carlone GM, Ades EW. An augmented passive immune therapy to treat fulminant bacterial infections. *Recent Pat Antiinfect Drug Discov* 2010; **5**: 157-67.
- 2) Keller MA, Stiehm ER. Passive immunity in prevention and treatment of infectious diseases. *Clin Microbiol Rev* 2000; **13**: 602-14.
- 3) Viridi V, Depicker A. Role of plant expression systems in antibody production for passive immunization. *Int J Dev Biol* 2013; **57**: 587-93.
- 4) Burnouf T, Seghatchian J. Ebola virus convalescent blood products: where we are now and where we may need to go. *Transfus Apher Sci* 2014; **51**: 120-5.
- 5) World Health Organization. Position Paper on collection and use of convalescent plasma or serum as an element in filovirus outbreak response. WHO Blood Regulators Network (BRN). 14 August 2014. Geneva: World Health Organization; 2014.
- 6) Dodd RY. Emerging pathogens and their implications for the blood supply and transfusion transmitted infections. *Br J Haematol* 2012; **159**: 135-42.
- 7) Lai KY, Ng WY, Cheng FF. Human Ebola virus infection in West Africa: a review of available therapeutic agents that target different steps of the life cycle of Ebola virus. *Infect Dis Pov* 2014; **3**: 43.
- 8) World Health Organization. Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks. Interim guidance for national health authorities and blood transfusion services. Geneva: World Health Organization; September 2014. Available at: http://apps.who.int/iris/bitstream/10665/135591/1/WHO_HIS_SDS_2014.8_eng.pdf?ua=1. Accessed on 21/05/2015.
- 9) Simon J. Emil Behring's medical culture: from disinfection to serotherapy. *Med Hist* 2007; **51**: 201-18.
- 10) Behring E, Kitasato S. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei thieren. *Deutsche medizinische Wochenschrift* 1890; **16**: 1113-4. In: *Milestones in Microbiology: 1556 to 1940*. Brock TD, editor. Washington: ASM Press; 1998. p. 138.
- 11) Luke TC, Casadevall A, Watowich SJ, et al. Hark back: passive immunotherapy for influenza and other serious infections. *Crit Care Med* 2010; **38**: e66-73.
- 12) Francis FD, Hall MW, Gaines AR. Early use of convalescent serum in influenza. *Mil Surg* 1920; **47**: 177-9.
- 13) Redden WR. Treatment of influenza-pneumonia by use of convalescent human serum. *Boston Med Surg J* 1919; **161**: 688-91.
- 14) Jacobaeus. Treatment of influenza pneumonia with serum from convalescents. *Svenska Lakartidnin* 1920; **18**: 385-99.
- 15) Bogardus FB. Influenza pneumonia treated by blood transfusions. *NY Med J* 1919; **109**: 765.
- 16) Miller OO, McConnell WT. Report of influenza treated with serum from recovered cases. *Ky Med J* 1919; **17**: 218-9.
- 17) Carlyle PM. Injection of whole blood in influenza. *Br Med J* 1919; **1**: 698.
- 18) Lesne E, Brodin P, Saint-Girons F. Plasma therapy in influenza. *Presse Med* 1919; **27**: 181-2.
- 19) Hemming VG. Use of intravenous immunoglobulins for prophylaxis or treatment of infectious diseases. *Clin Diagn Lab Immunol* 2001; **8**: 859-63.
- 20) Nour B, Green M, Michaels M, et al. Parvovirus B19 infection in pediatric transplant patients. *Transplantation* 1993; **56**: 835-8.
- 21) World Health Organization Mers-Cov Research Group. State of knowledge and data gaps of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in humans. *PLoS Curr* 2013; **12**: 5.
- 22) Zingher A, Mortimer P. Convalescent whole blood, plasma and serum in the prophylaxis of measles: *JAMA* 12 April, 1926; 1180-1187. *Rev Med Virol* 2005; **15**: 407-21.
- 23) Young MK, Nimmo GR, Cripps AW, Jones MA. Post-exposure passive immunisation for preventing measles. *Cochrane Database Syst Rev* 2014; **4**: CD010056.
- 24) Wong VW, Dai D, Wu AK, Sung JJ. Treatment of severe acute respiratory syndrome with convalescent plasma. *Hong Kong Med J* 2003; **9**: 199-201.
- 25) Kong LK, Zhou BP. Successful treatment of avian influenza with convalescent plasma. *Hong Kong Med J* 2006; **12**: 489.
- 26) Beigel JH, Luke TC. A study in scarlet - convalescent plasma for severe influenza. *Crit Care Med* 2012; **40**: 1027-28.
- 27) Leider JP, Bruncker PA, Ness PM. Convalescent transfusion for pandemic influenza: preparing blood banks for a new plasma product? *Transfusion* 2010; **50**: 1384-98.
- 28) Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. *N Engl J Med* 2007; **357**: 1450-1.
- 29) Hui DS, Lee N. Adjunctive therapies and immunomodulating agents for severe influenza. *Influenza Other Resp Viruses* 2013; **7** (Suppl 3): 52-9.
- 30) Prince GA, Hemming VG, Horswood RL, et al. Effectiveness of topically administered neutralizing antibodies in experimental immunotherapy of respiratory syncytial virus infection in cotton rats. *J Virol* 1987; **61**: 1851-4.
- 31) Mozdzanowska K, Furchner M, Washko G, et al. A pulmonary influenza virus infection in SCID mice can be cured by treatment with hemagglutinin-specific antibodies that display very low virus-neutralizing activity in vitro. *J Virol* 1997; **71**: 4347-55.
- 32) Simmons CP, Bernasconi NL, Suguitan AL, et al. Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza. *PLoS Med* 2007; **4**: e178.
- 33) Jahrling PB, Frame JD, Rhoderick JB, Monson MH. Endemic Lassa fever in Liberia. Selection of optimally effective plasma for treatment by passive immunization. *Trans R Soc Trop Med Hyg* 1985; **79**: 380-4.
- 34) Luke TC, Kilbane EM, Jackson JL, Hoffman SL. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med* 2006; **145**: 599-609.
- 35) Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al.; Convalescent Plasma Study Group. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis* 2015; **211**: 80-90.
- 36) World Health Organization. Experimental therapies: growing interest in the use of whole blood or plasma from recovered Ebola patients (convalescent therapies). Ebola situation assessment - 26 September 2014. Available at: <http://www.who.int/mediacentre/news/ebola/26-september-2014/en/>. Accessed on 21/05/2015.
- 37) Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978; **56**: 271-93.
- 38) Enria DA, Briggiler AM, Fernández NJ, et al. Importance of dose of neutralizing antibodies in treatment of Argentine hemorrhagic fever with immune plasma. *Lancet* 1984; **2**: 255-6.
- 39) Maiztegui JI, Fernández NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine hemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet* 1979; **2**: 1216-7.
- 40) Enria DA, Maiztegui JI. Antiviral treatment of Argentine hemorrhagic fever. *Antivir Res* 1994; **23**: 23-31.
- 41) Enria DA, Briggiler AM, Sánchez Z. Treatment of Argentine hemorrhagic fever. *Antivir Res* 2008; **78**: 132-9.
- 42) Ruggiero HA, Pérez Isquierdo F, Milani HA, et al. [Treatment of Argentine hemorrhagic fever with convalescent's plasma. 4433 cases]. *Presse Med* 1986; **15**: 2239-42. [In French.]
- 43) Bajpai S, Nadkar MY. Crimean Congo hemorrhagic fever: requires vigilance and not panic. *JAPI* 2011; **59**: 164-7.
- 44) Jahrling PB, Geisbert J, Swearingen JR, et al. Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. *Arch Virol Suppl* 1996; **11**: 135-40.

- 45) Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferonalpha2b for treatment of experimental Ebola virus infections. *J Infect Dis* 1999; **179**: S224-34.
- 46) Kudoyarova-Zubavichene NM, Sergeev NN, Chepurnov AA, Netesov SV. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. *J Infect Dis* 1999; **179**: S218-23.
- 47) Parren PW, Geisbert TW, Maruyama T, et al. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. *J Virol* 2002; **76**: 6408-12.
- 48) Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis* 1999; **179**: S18-23.
- 49) Oswald WB, Geisbert TW, Davis KJ, et al. Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathog* 2007; **3**: e9.
- 50) Dye JM, Herbert AS, Kuehne AI, et al. Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. *PNAS* 2012; **13**: 5034-9.
- 51) Olinger GG, Pettitt J, Kim D, et al. Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. *Proc Natl Acad Sci USA* 2012; **109**: 18030-5.
- 52) Qiu X, Audet J, Wong G, et al. Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies. *Sci Transl Med* 2012; **4**: 138ra81.
- 53) European Commission. HORIZON 2020 WORK PROGRAMME 2014-2015 . European Commission Decision C (2014) 4995 of 22 July 2014. Available at: http://ec.europa.eu/research/participants/portal/doc/call/h2020/common/1617601-part_1_introduction_v2.0_en.pdf. Accessed on 21/05/2015.
- 54) Cohen J. Ebola vaccine: little and late. *Science* 2014; **345**: 1441-42.
- 55) McElroy AK, Akondy RS, Davis CW, et al. Human Ebola virus infection results in substantial immune activation. *Proc Natl Acad Sci USA* 2015; **112**: 4719-24.
- 56) Gulland A. First Ebola treatment is approved by WHO *Br Med J* 2014; **349**: 5539.
- 57) World Health Organization. Community engagement and education, recruitment and retention of people recovered from Ebola as potential donors for convalescent whole blood and plasma. Interim guidance for National Health Authorities, Blood Transfusion Services and researchers. April 2015. Available at: http://www.who.int/bloodsafety/interim-guidance_CWB-CP.pdf. Accessed on 21/05/2015.
- 58) World Health Organization. Ethics of using convalescent whole blood and convalescent plasma during the Ebola epidemic. Interim guidance for ethics review committees, researchers, national health authorities and blood transfusion services. 2015. Available at: http://apps.who.int/iris/bitstream/10665/161912/1/WHO_HIS_KER_GHE_15.1_eng.pdf?ua=1&ua=1. Accessed on 21/05/2015.
- 59) World Health Organization. Essential medicines and health products. Available at: http://www.who.int/medicines/ebola-treatment/emp Ebola_therapies/en/. Accessed on 21/05/2015.
- 60) Clinical Research Management, Inc. Clinical trial to evaluate the efficacy and safety of convalescent plasma for Ebola treatment (EVD001). ClinicalTrials.gov Identifier: NCT02333578. Available at: <https://clinicaltrials.gov/ct2/show/NCT02333578>. Accessed on 21/05/2015.
- 61) European Centre for Disease Prevention and Control. Overview of Ebola research – March 2015 ECDC. Treatment and vaccine development. Last updated 5 March 2015 Available at: http://ecdc.europa.eu/en/healthtopics/ebola_marburg_fevers/Pages/treatment-vaccines.aspx. Accessed on 21/05/2015.
- 62) Gutfraind A, Meyers LA. Evaluating large-scale blood transfusion therapy for the current Ebola epidemic in Liberia. *J Infect Dis* 2015; **211**: 1262-7.
- 63) European Blood Alliance. Newsletter 2015-1. Available at: http://www.sweba.se/sites/default/files/EBA%20Newsletter%202015_1.pdf. Accessed on 21/05/2015.
- 64) European Centre for Disease Prevention and Control. Treatment and vaccine development. Last updated 5 March 2015. Available at: http://ecdc.europa.eu/en/healthtopics/ebola_marburg_fevers/Pages/treatment-vaccines.aspx. Accessed on 21/05/2015.
- 65) The New York Times. How many Ebola patients have been treated outside of Africa? Updated Jan. 26, 2015 Available at: http://www.nytimes.com/interactive/2014/07/31/world/africa/ebola-virus-outbreak-qa.html?_r=0. Accessed on 21/05/2015.
- 66) Cerus Corporation. Press Release Details, 10/20/2014. Cerus Submits Compassionate Use IDE Supplement for INTERCEPT Blood System Treatment of Ebola Convalescent Plasma. Available at: <http://www.cerus.com/Investors/Press-Releases/Press-Release-Details/2014/Cerus-Submits-Compassionate-Use-IDE-Supplement-for-INTERCEPT-Blood-System-Treatment-of-Ebola-Convalescent-Plasma/>. Accessed on 21/05/2015.
- 67) Picker SM. Current methods for the reduction of blood-borne pathogens: a comprehensive literature review. *Blood Transfus* 2013; **11**: 343-8.
- 68) Burnouf T, Goubran HA, Radosevich M, et al. A process for solvent/detergent treatment of plasma for transfusion at blood centers that use a disposable bag system. *Transfusion* 2006; **46**: 2100-8.
- 69) Liumbruno GM, Franchini M. Solvent/detergent plasma: pharmaceutical characteristics and clinical experience. *J Thromb Thrombolysis* 2015; **39**: 118-28.
- 70) Vetter P, Schibler M, Dayer JA, Kaiser L. Ebola: the unexpected emerging disease. *Rev Med Suisse* 2015; **11**: 877-83.
- 71) Colebunders RL, Cannon RO. Large-scale convalescent blood and plasma transfusion therapy for Ebola virus disease. *J Infect Dis* 2015; **211**: 1208-10.
- 72) Katz LM, Tobian AAR. Ebola virus disease, transmission risk to laboratory personnel, and pretransfusion testing. *Transfusion* 2014; **54**: 3247-51.
- 73) Ubol S, Halstead SB. How innate immune mechanisms contribute to antibody-enhanced viral infections. *Clin Vaccine Immunol* 2010; **17**: 1829-35.
- 74) Boonnak K, Slike BM, Burgess TH, et al. Role of dendritic cells in antibody-dependent enhancement of dengue virus infection. *J Virol* 2008; **82**: 939-51.
- 75) Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. *Arch Virol* 2013; **158**: 1445-59.
- 76) Halstead SB. Dengue antibody-dependent enhancement: knowns and unknowns. *Microbiol Spectr* 2014; **2**: AID-0022-2014.
- 77) Wang SF, Tseng SP, Yen CH, et al. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. *Biochem Biophys Res Commun* 2014; **451**: 208-14.
- 78) Tirado SM, Yoon KJ. Antibody-dependent enhancement of virus infection and disease. *Viral Immunol* 2003; **16**: 69-86.
- 79) Olsen CW, Corapi WV, Ngichabe CK, et al. Monoclonal antibodies to the spike protein of feline infectious peritonitis virus mediate antibody-dependent enhancement of infection of feline macrophages. *J Virol* 1992; **66**: 956-65.
- 80) Wong HK, Lee CK, Hung IF, et al. Practical limitations of convalescent plasma collection: a case scenario in pandemic preparation for influenza A (H1N1) infection *Transfusion* 2010; **50**: 1967-71.

Arrived: 22 May 2015 - Revision accepted: 23 July 2015

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