STUDY REPORT-2

‘A Systematic Evaluation of Risks and Mitigation Strategies for Convalescent Plasma Therapy for COVID-19’

TFORD-COVID19 INDIA

Project Executed by:

Entrepreneurship Development Center, CSIR-NCL

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DSIR

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

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Acknowledgement

The authors are grateful to the Department of Scientific & Industrial Research (DSIR) for funding the work under A2K+ Scheme of DSIR. The authors are also thankful to the advisory group of the Task Force for Repurposing of Drugs (TFORD) for COVID-19, under the aegis of the Science and Technology core group.

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Executive Summary

Plasma therapy has the potential of being a life-saving effective therapy for patients, especially those diagnosed with COVID-19. Long being used as a treatment for acute symptoms following infectious diseases, this therapy has some limitations and risks. One of the first patients treated with plasma therapy in India died of anaphylactic shock. The principal scientific advisor to government of India along with the Council of Scientific and Industrial Research (CSIR) had set up a task force for systematic evaluation of repurposed drugs (TFORD). Experts from diverse fields and across the country meet regularly and share information. Clinical trials for plasma therapy have been coordinated by the Indian Council of Medical Research (ICMR) through a centralized approach. To understand and prioritize the risks associated with this therapy, the task force organized a symposium on various aspects of plasma therapy.

Introduction

The World Health Organization (WHO) announced the COVID-19 pandemic on March 11, 2020, and more than 100 million infected, and more than 2.2 million dead, are reported as of Jan. 30, 2021. A multitude of mechanisms of pathogenesis mediated by viral, immunological, and host factors contribute to the symptoms (1-–3). The initial infection results in viremia when subjects may be largely asymptomatic. In the second week, some patients develop a violent immune response that contributes to the rapid progression of disease. With a plethora of therapies in clinical trials and some being approved, and more than 100 vaccines in development and a few now approved (4–6), treatment of patients is dependent on several conditions, each of which is associated with risks (7). As of late 2020, the use of convalescent sera from donors exposed to the virus has a high potential of ameliorating disease progression (8). This treatment is particularly important for older patients and those with compromised immune systems. With reinfection or recrudescence of symptoms and with immunity purportedly being short-lived, passive immunity (plasma and antibodies) and active immunization boosters continue to be high priority for research and clinical use. There have been several reports on approvals of use of plasma therapy for emergency use by regulatory agencies and announcements on the risks, which range from adverse events to futility. The authors have systematically reviewed the risks of plasma therapy in this report.

Task force for repurposing of drugs for COVID-19

In March 2020, the principal scientific advisor to the government of India had constituted a Science and Technology Core Group on COVID-19. Under the aegis of this group on COVID-19, a Task Force has been formed focused on Repurposing of Drugs for COVID-19 (TFORD-COVID19) operating a project called Speeding up the Lab to Market Journey: Repurposing Drugs for COVID-19. The TFORD-COVID19 has a multidisciplinary advisory group that reviews data and literature on the subject facilitated by a nerve center located at Venture Center, Pune on the campus of CSIR–National Chemical Laboratory. The purpose of the TFORD is to track, compile, analyze, and disseminate information to support decisions on repurposing of drugs for COVID-19 amongst other objectives. The advisory group reviews data, helps prioritize leads, identifies barriers, and provides opinions.

While reviewing the data available on repurposing of drugs, the TFORD-COVID19 has also looked at therapeutic strategies broadly. A subgroup of the TFORD-COVID19 also deliberated on plasma therapy as a therapeutic option and also organized a symposium on the topic. This report summaries discussions of this sub-group exploring potential risks of plasma therapy systematically while also suggesting areas of research required.

COVID-19: from infection to symptoms

SARS Coronavirus 2 (SARS-Cov-2) infects cells through a multi-step process of binding of spike protein to the ACE2 receptor, proteolytic cleavage by membrane-associated proteolytic enzymes, such as TMPRSS2, and fusion with the cell membrane (1, 2, 9, 10). The virus escapes the endo-lysosome through a currently undefined mechanism. The positive RNA strand translates approximately 26 viral proteins. These structural and non-structural proteins have diverse functions including promoting entry-fusion, replication-assembly, blocking intra-cellular protein-protein interactions, and interference with transcriptional and enzymatic processes. The ubiquitous expression of ACE2 receptor, high cytopathicity, and shedding of this virus through mucosal secretions makes one of the most infectious pathogens in humans.

Neutralizing antibodies and plasma therapy for COVID-19

Several reports have indicated that the majority of the subjects infected with SARS-CoV-2 generate antibodies to the virus surface and structural proteins (i.e., spike, membrane, envelope, hemagglutinin esterase and nucleocapsid) (11–14). These antibodies directed to the receptor binding domain (RBD) of spike protein have been demonstrated to neutralize virus infection *in vitro*. Clinical studies have demonstrated that infusion of plasma containing neutralizing antibodies can curb the progression of disease in patients (9). The methods for passive infusion of neutralizing antibodies to block viral entry and replication that are being developed utilize three approaches: convalescent plasma therapy, purified antibodies from plasma of infected-and-recovered subjects, and recombinant antibodies generated from the CDR3 sequences of spike-antibody-specific B cells clones from infected-and-recovered subjects.

Convalescent plasma. The advantage of plasma therapy from previously infected individuals is that they have developed a high titer neutralizing antibody to the SARS-Cov2 virus (13). This adoptive transfer therapy has been used successfully for a multitude of infectious diseases including the 1918 influenza pandemic, H1N1 influenza, and SARS-CoV-1 (15–17). Several controlled clinical trials have been initiated to evaluate the safety and efficacy of plasma therapy (8). The scalability of this process is limited by the availability of serum from patients.

Purified neutralizing antibodies from plasma. To develop a scalable process for passive neutralizing antibody therapy, spike-specific IgG antibodies are purified using immuno-affinity chromatography purification. The process involves several steps such as ammonium sulphate precipitation to obtain the serum protein; protein-A affinity chromatography to purify IgG antibodies; spike protein-affinity purification to purify spike-specific antibodies; and viral inactivation. The process to purify the antibody is laborious, time consuming, and incurs significant costs. The scalability of this process is limited by the availability of serum sample from patients.

Recombinant anti-spike neutralizing IgG antibodies. To generate recombinant antibodies, memory B cell clones, which secrete neutralizing antibodies to RBD domains of spike protein, can be isolated from patients exposed to the virus. The heavy and light variable region of these antibodies can be cloned onto the gene for IgG framework sequences and expressed as recombinant forms of the corresponding antibodies. In this respect, four of the antibodies produced in these studies (31B5, 32D4, P2C-2F6, and P2C-1F11) (18–20)showed high neutralizing potential *in vitro*, and all inhibited ACE2/RBD binding (**Table I**). The generation of a hybridoma producing a monoclonal neutralizing against SARS-CoV-2 provides the potential for a therapeutic Ab that can be directly administered to patients to block ongoing infection and potentially even as a prophylactic. The process development of recombinant antibodies requires molecular engineering technologies, fermentation, and purification processes. Once developed, these antibodies can be scalable and be cost-effective by economies-of-scale. While plasma therapy is the route to be followed in the short term, recombinant specific antibodies are essential in the long run.

Table I: SARS-Cov2-specific neutralizing human monoclonal antibodies.

Several clinical trials of recombinant antibodies are in progress. Regeneron is testing a cocktail of three antibodies in an outpatient and prevention trial. Recombinant antibody therapy LY-CoV555 (Eli Lilly and AbCellera Biologics) will enroll 2400 hospital and nursing home staff with recent COVID-19 infection in a Phase III trial (BLAZE-3). Another combination antibody trial is being conducted by the US National Institute of Allergy and Infectious Diseases in outpatient and non-severe patients. The results of these studies are awaited.

Risk identification, evaluation, and assessment

The authors have performed a retrospective risk analysis and mitigation strategy for plasma therapy for COVID-19 patients using the failure mode and effects analysis (FMEA) methodology. The method entails capturing the potential risks of plasma therapy by comprehensively identifying all potential causes, ranking multiple effects, and planning actions to mitigate the failure chains. Residual risks that remain are quantitated and documents in a systematic tree-like workflow (**Figure 1**).

All figures courtesy of the authors.

In order to systematically identify, evaluate, analyze risks, and define mitigation strategies, the FMEA approach has a defined process that initiates with definitions of purposes of plasma therapy (**Figures 2-6**).

Figure 2. FMEA approach.

The World Health Organization (WHO) announced the COVID-19 pandemic on March 11, 2020, and more than 100 million infected, and more than 2.2 million dead, are reported as of Jan. 30, 2021. A multitude of mechanisms of pathogenesis mediated by viral, immunological, and host factors contribute to the symptoms (1-–3). The initial infection results in viremia when subjects may be largely asymptomatic. In the second week, some patients develop a violent immune response that contributes to the rapid progression of disease. With a plethora of therapies in clinical trials and some being approved, and more than 100 vaccines in

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Ab Source	Clone	Type of antibody	Reference
Derived from COVID-19 patients	31B5 32D	Human monoclonal	(20)
Derived from SARSCoV-1 or MERS-CoV-1 animal models	47D11	Recombinant human monoclonal derived from hybridomas of immunized transgenic H2L2 mice	(18)
Human monoclonal single domain antibody isolated by phage display	N3130	Human monoclonal	(29)

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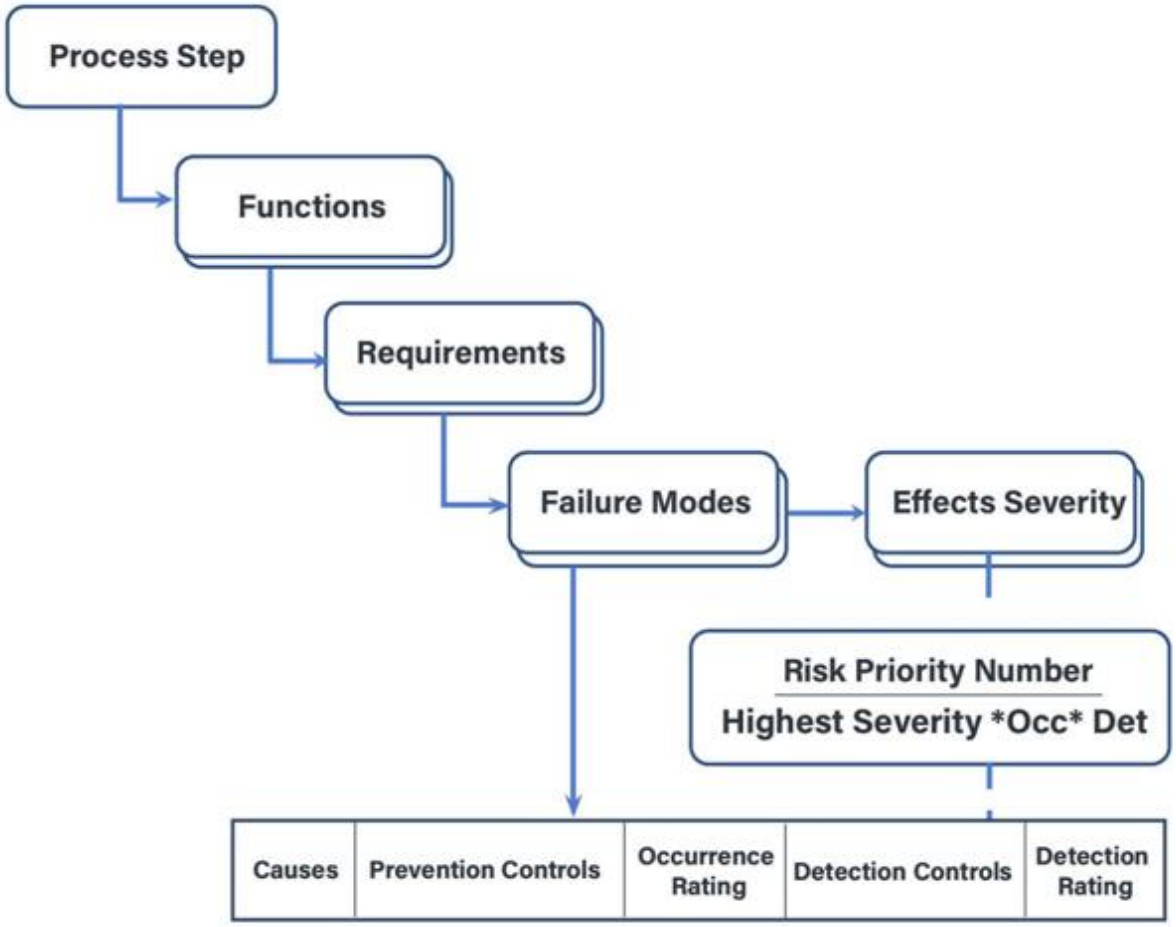

Figure 1. Diagram showing workflow for residual risks.

All figures courtesy of the authors.

In order to systematically identify, evaluate, analyze risks, and define mitigation strategies, the FMEA approach has a defined process that initiates with definitions of purposes of plasma therapy (**Figures 2-6**).

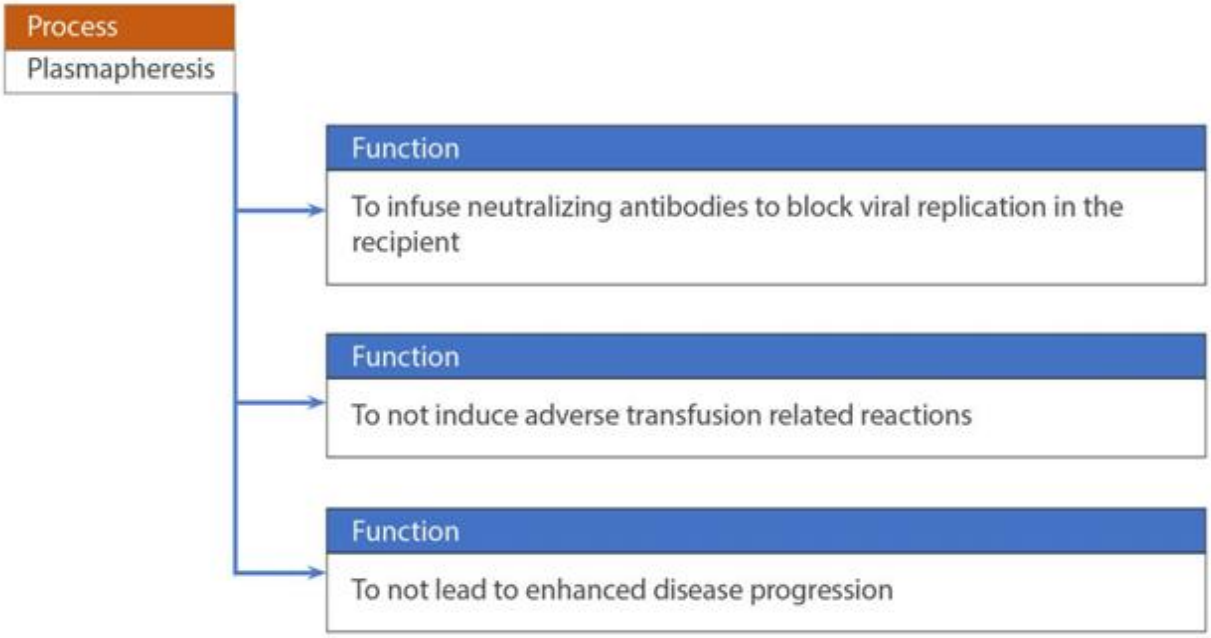

Figure 2. FMEA approach.

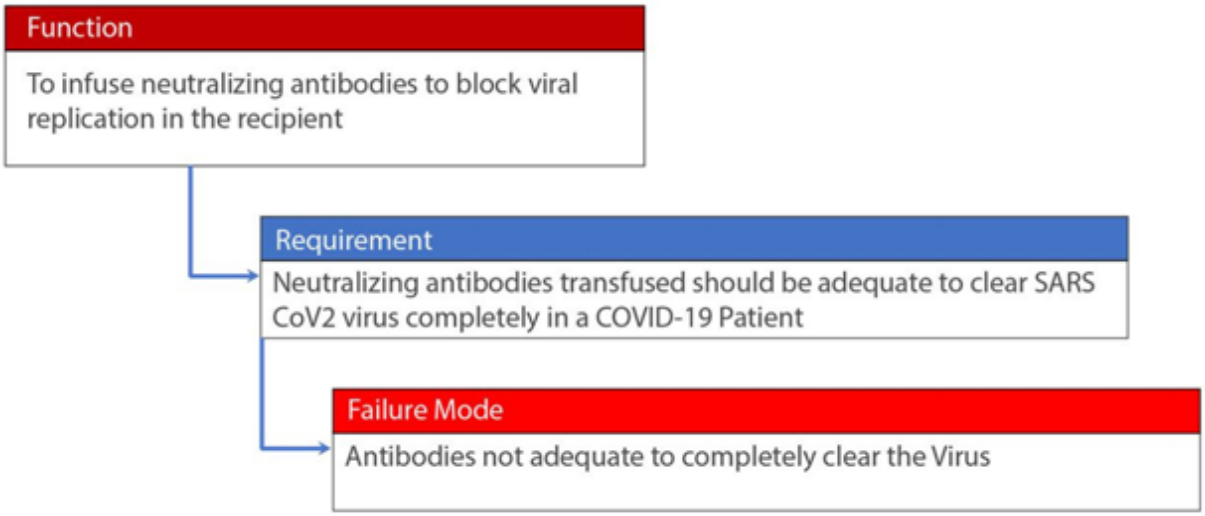

Figure 3. FMEA approach.

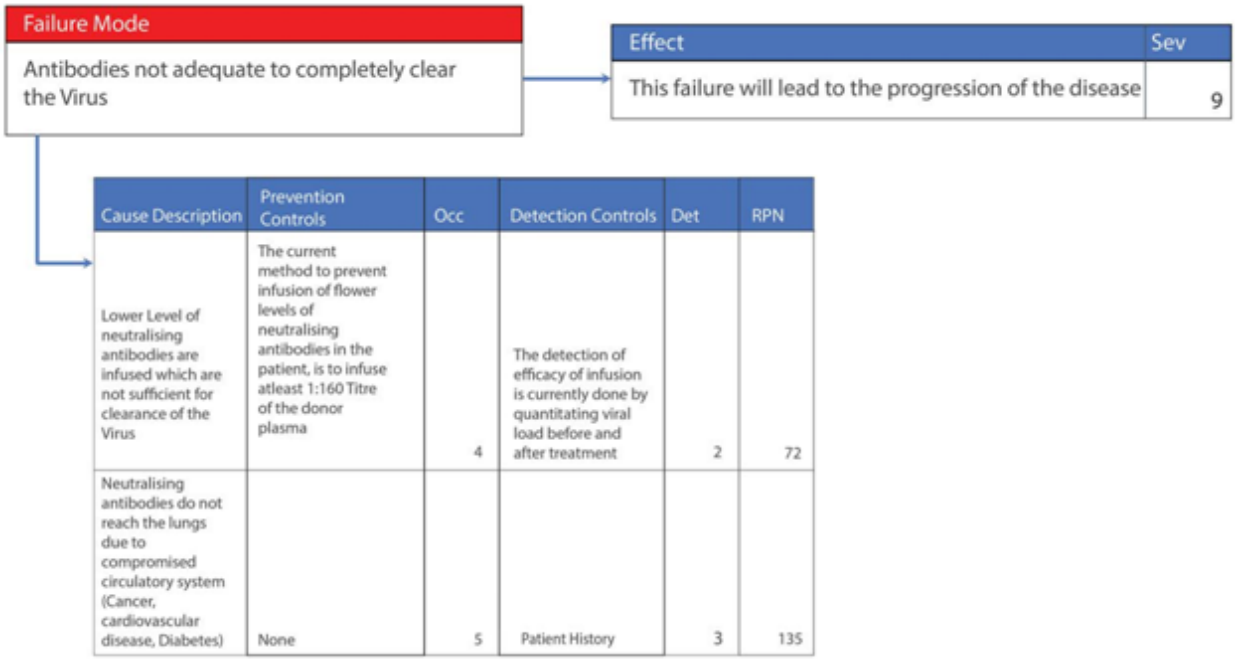

Figure 4. FMEA approach.

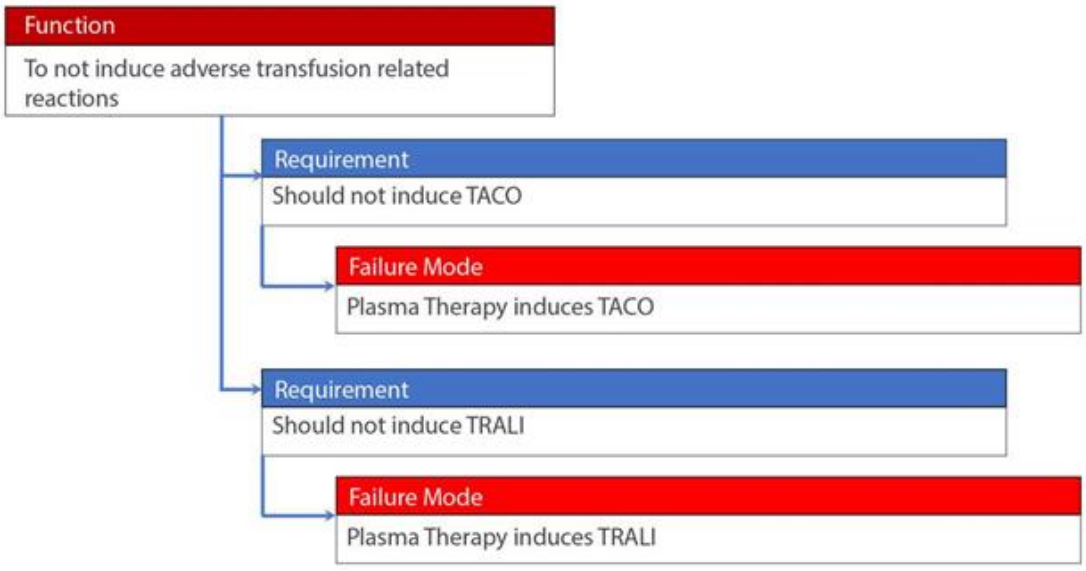

Figure 5. FMEA approach.

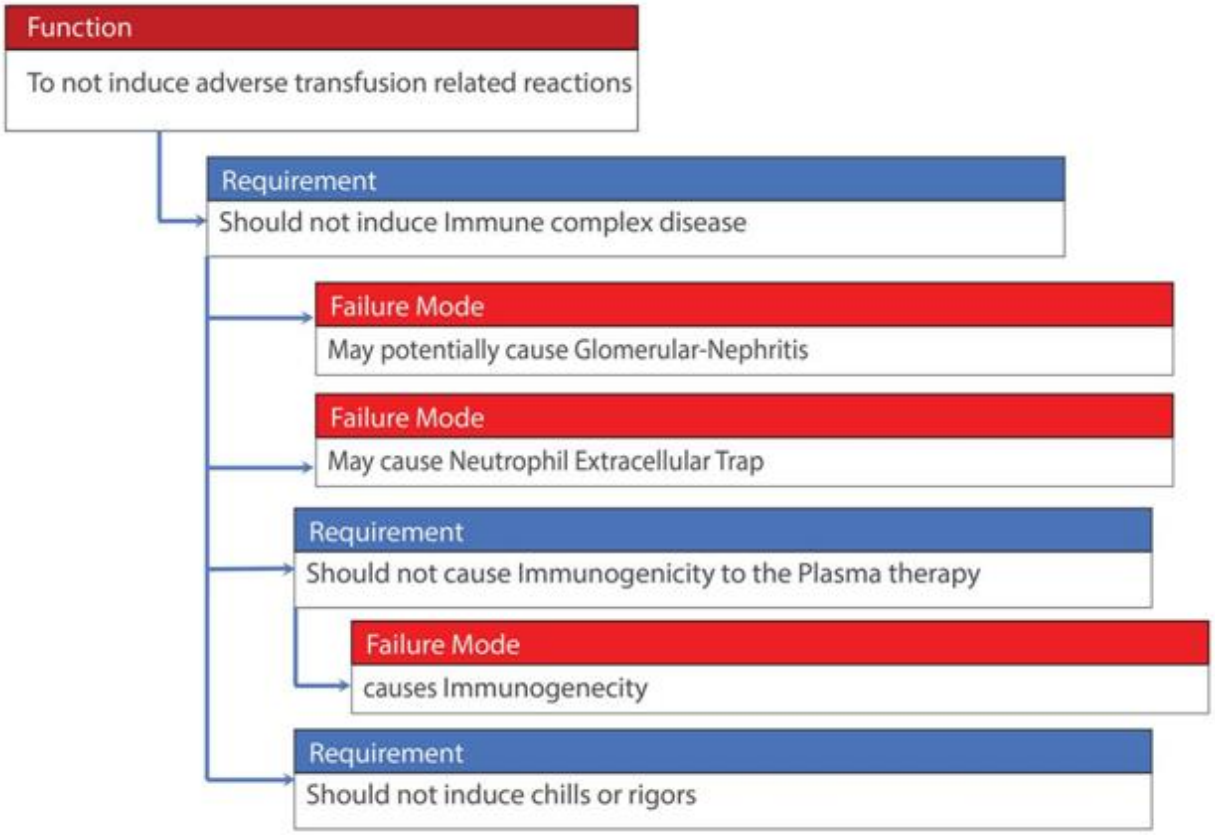

Figure 6. FMEA approach.

There are three major purposes of plasma therapy:

- The therapy should infuse neutralizing antibodies that can block viral replication in the recipient.
- The therapy should not induce transfusion related severe adverse reactions, or transfusion related infection.
- The therapy should not lead to enhanced disease progression.

Analysis of risks of plasma therapy using FMEA

Neutralizing antibodies

The first function of plasma therapy is to infuse neutralizing antibodies that can block viral replication in the recipient. There is one requirementto this function: neutralizing antibodies

transfused should be adequate to clear the SARS-CoV-2 virus completely in a COVID-19 patient. This requirement will not be met if the antibodies do not completely clear the virus. The effect of this failure will be the progression of the disease. The potential causes for this failure are the following:

- Lower levels of neutralizing antibodies are infused which are not sufficient for the clearance of the virus. The current method to prevent infusion of lower levels of neutralizing antibodies in the patient is to infuse at least 1:160 titer of donor plasma. The detection for efficacy of the infusion is currently done by quantitative viral load before and after treatment.
- Neutralizing antibodies do not reach lungs in sufficient concentrations due to compromised circulatory systems (e.g., patients with cancer, cardiovascular disease, and diabetes). The control for detection of the presence of cause is diagnostics tests for comorbid conditions, prior to treatment, reflected through patient history.

Avoid adverse events

The second function of plasma therapy is that it should not induce any transfusion-related adverse events in the recipient. There are six requirements and associated failures identified for this function.

1. Plasma therapy should not induce TACO. This requirement fails to be met when TACO is induced (21).

2. Plasma therapy should not induce TRALI. This requirement fails to be met when TRALI is induced (21).

3. Plasma therapy should not induce immune complex-mediated hypersensitivity. There are at least two ways in which this requirement may not be met:

- The immune complexes may be formed during plasma therapy. The effect of formation of immune complexes is the potential for Glomerular-Nephritis. In order to prevent this adverse event, the presence of immune complexes could be detected using diagnostic tests. Treatment generally comprises of immune suppressive regimens of corticosteroids. In order to prevent this, developing innovative method of detecting and suppressing immune complexes can be the recommended action of this risk.

- Another reason for failure to this requirement is formation of Neutrophil Extracellular Traps (NET) which are induced by hyperactivation of neutrophils (22). The NET in turn can have an effect of dysfunction of lungs. There are two potential causes for formation of NET during plasma therapy. First, immune complexes can induce Neutrophil activation through Fc receptor interactions, which results in secretion of NET and the patient shows symptoms-lung function failure. Second, immune complexes can induce Neutrophil activation, which results in secretion of NET and the patient does not show symptoms.

The detection of levels of the elevated levels of cell-free DNA, myeloperoxidase (MPO-DNA), citrullinated histone H3 level should be measured. The treatment options of patients with lung inflammation includes corticosteroids.

4. Plasma therapy should not cause immunogenicity. This requirement will fail to be met with if the therapy potentially leads to immunogenicity. The therapy will fail to meet the requirement if anti-drug antibodies are developed by the donor. There are five effects of this failure mode:

- Hypersensitivity Reactions I (IgE-mediated reactions)
- Hypersensitivity Reactions II (Complement mediated reactions)
- Hypersensitivity Reactions III (Immune complex mediated reactions)
- Hypersensitivity Reactions IV (Delayed-type Hypersensitivity)
- Contributes to Cytokine storm (Inflammation).

Each effect will have a different mode of action (i.e., treatment option) (see **Table II**).

Risk	Effects with highest severity	Severity ranking	Prevention (Action)	Treatment (Action)	RPN	Reference
Anaphylactic hypersensitivity reaction	Death with warning	9		Antihistamines; Epinephrine, hemodynamic stabilization and airway management	441	(14)
Cytokine storm	Death if untreated	8		Tocilizumab treatment	189	(18)
TRALI	Hypersensitivity reactions	8	Ensuring the quality of the plasma sample prior to the plasma therapy	Discontinue transfusion, contact blood bank Supportive management: Maintain sufficient ventilation/oxygen supply Control hemodynamic parameters Anti-inflammatory therapy with IV steroids (only in specific circumstances)	32	(16)
NET-Induced lung inflammation	Hypersensitivity reactions	8			320	(17)
Immune complex mediated glomerular nephritis	Hypersensitivity reactions	7		Corticosteroids treatment	63	(15)
TACO	Hypersensitivity reactions	7		Discontinue transfusion Fluid mobilization with diuretics. Supplementary oxygen, and Assisted ventilation if indicated. NIPPV if ineffective, intubation may be required	98	(16)
Transmission of infections	Death if untreated	8		Screening of donor samples		

Table II. Risks and Mitigation identified through the FMEA process. RPN is risk priority number. TRALI is transfusion-related acute lung injury; TACO is transfusion-associated circulatory overload.

Precautions with RPN

As seen in **Table II**, the FMEA technique helps identifying and prioritizing actions to mitigate risks. A metric for priority is the risk priority number (RPN), which is the multiplication of the severity, occurrence and detection ratings. Risk priority number, when used in isolation or without accompanying descriptive context of causes and effects, can obfuscate the nature of risk. For example, Severity=10, Occurrence=5 and detection=2 can lead to a RPN of 100. Severity=4, Occurrence=5 and Detection=5 also will result in the same RPN of 100. In the first case, the severity of effects is extreme. A severity rating of 10 is reserved for potentially fatal consequences occurring without warning or an opportunity to react. The second instance is not so serious. RPN used without context poses these problems.

There are two possible causes of this failure: the induction of antibodies to the antibodies in the plasma to which the detection method is by checking Anti–drug (Plasma) – Antibodies and major histocompatibility complex (MHC) background of the Host which can be *detected* by doing the MHC gene sequencing.

5. Plasma therapy should not induce chills and rigors.

6. Plasma therapy should not cause other infectious diseases.

Prevent disease progression

The third function is that the therapy should not lead to enhanced disease progression. The treatment options for each of the hypersensitivity-related adverse reactions are listed in **Table II**. In summary, the detailed process of risk assessment of plasma therapy has outlined several direct effects, sequential effects, and sequential effects.

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Risk mitigation

The potential risks of treatment of COVID-19 patients with plasma therapy should be considered during treatment. Potential adverse events that could be associated with plasma therapy are outlined in this article. Each event (symptom) should be carefully observed for treatment of the patient. With clinical trials under way, there will be additional experiences of plasma therapy, which will be important for future treatment protocols. To reduce the risk of the use of plasma, approaches to purify the neutralizing antibody and develop recombinant neutralizing antibodies are under development. These processes for developing these technologies will require time and are in progress. For this urgent time, plasma therapy may be able to provide treatment options for COVID-19 patients that have progressive disease.

Clinical trial summaries

Plasma therapy is being used across the world both for emergency use, as well as in clinical trials (23). Currently more than 204 trials are ongoing for convalescent plasma treatment based on regulatory requirements (24). Some studies published trial results, and the findings are summarized in **Table III**. Among four published studies, one study from Spain shows promise; however, a trial in The Netherlands was halted when it was observed that patients already had high antibody titers; trials in China and India did not prove to be effective (25–26). Plasma therapy was found to be effective in two small trials conducted in China (26–27). The Mayo Clinic reported statistically significant benefit by decreased mortality rates in severe cases; patients who received plasma therapy within three days of diagnosis had a seven-day death rate of 8.7%, while those who got plasma four days or later had an 11.9% rate (28).

Clinical Trial Number	NCT04342182	ChiCTR2000029757	NCT04345523	CTRI/2020/04/024775	ChiCTR2000030046	NCT04338360	ChiCTR2000033056	NCT04343261
Study	A. Gharbharan, et al	Ling Li, et al	Cristina Avendano-Sola, et al	A. Agarwal, et al	Kai Duan, et al	Michael Joyner, et al	Can Jin, et al	Danyal Ibrahim, et al
Location	Netherlands	China	Spain	India	China	USA	China	USA
Study type	Multicenter open label, Randomized	Randomized	Randomized	Open-label, parallel-arm, phase II, multicentre, randomized	Single arm, Interventional	Expanded access	Retrospective	single-arm open-label Phase II
Sample size	86	103	81	464	10	5000	06	38
Stage	Severe	Severe	Severe	Moderately ill	Severe	Severe	Severe	Severe, Critical
Age group (median)	61 years	70 years	59 years	52 years	52.5 years	62.3 years	60 years	63 years
Primary endpoint	Day-60 mortality	Time to clinical improvement within 28 days	Proportion of patients in categories 5, 6 or 7 of the COVID-19 ordinal scale at day 15	Composite measure of progress to severe disease or all-cause mortality at 28 days	Safety of CP transfusion	Safety of transfusion of COVID-19 convalescent plasma	Time to negative SARS-CoV-2 nucleic acid test	Rate of adverse events and hospital mortality
Outcome	No effect	No effect	Positive	No effect	Positive	Positive*	Positive	Positive
Authors' conclusion	Most COVID-19 patients already have high neutralizing antibody titers at hospital admission.	Among patients with severe or life-threatening COVID_19, convalescent plasma therapy added to standard treatment, compared with standard treatment alone, did not result in a statistically significant improvement in time to clinical improvement within 28 days.	Convalescent plasma could be superior to standard of care in avoiding progression to mechanical ventilation or death in hospitalized patients with COVID-19.	CP was not associated with reduction in mortality or progression to severe COVID-19.	This study showed CP therapy was well tolerated and could potentially improve the clinical outcomes through neutralizing viremia in severe COVID-19 cases.	Given the deadly nature of COVID-19 and the large population of critically ill patients included in these analyses, the mortality rate does not appear excessive. These early indicators suggest that transfusion of convalescent plasma is safe in hospitalized patients with COVID-19.	Convalescent plasma treatment of COVID-19 is beneficial for those patients with recurrent COVID and critical cases.	Convalescent plasma is safe and has the potential for positive impact on clinical outcomes including recovery and survival if given to patients early in the course of COVID-19 disease.

*Evaluated for safety and not efficacy.

Table III. Key data from published clinical trials for convalescent plasma therapy in COVID 19.

In summary, although the efficacy is yet to be proved with results from a large, randomized trial, the safety aspect has been addressed by expanded access trials (6). Results from well-structured trials would help the understanding the efficacy and safety of plasma therapy.

Summary

This article describes a detailed risk identification and evaluation of plasma therapy for the treatment of COVID-19 patients using FMEA. This process ensures a thorough systematic approach of ensuring comprehensive risk assessment. These risks can be either a direct result of plasma therapy, or indirect: multiple, sequential, or cascading. By understanding all the risks involved in plasma therapy, health care providers can be aware of mitigation strategies prior to severe safety events and ensure safe treatment of COVID-19 patients.

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