Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Odds ratio of prognosis associated with 1 μg/ml increment in D-dimer level on admission.

Table SII. Odds ratio of prognosis associated with 1 μg/ml increment in D-dimer level.

Table SIII. Differences in D-dimer level among patients with and without cardiovascular disease and survivors and non-survivors with cardiovascular disease in the poor group.

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Reflection on passive immunotherapy in those who need most: some novel strategic arguments for obtaining safer therapeutic plasma or autologous antibodies from recovered COVID-19 infected patients

The COVID-19 pandemic is an emerging new human disease, for which no vaccines, or monoclonal antibodies (mAbs) or drugs, are currently available for therapy. Active vaccination requires the induction of an immune response against a given agent in a susceptible individual for the purpose of preventing or treating an infectious disease and this usually takes time to develop. Thus, the use of existing autologous Ab administration, obtainable from recovered COVID-19 patients two weeks after recovery, is the best and the most practical strategy for providing immediate passive immunity to susceptible recipients in need. Recently, the use of convalescent blood-derived products was proposed by one of the authors of this paper (JS) as an early option for treating patients with Ebola virus disease.[1,2] Therefore, human convalescent plasma, obtainable by plasmapheresis of plasma or immunoglobulin-containing fractions donated by volunteers recovered from a COVID-19 attack, has been proposed and implemented with success in COVID-19 cases.[2–6] Specific requirements and standards for preparation, qualification, storage and distribution of these blood preparations need to be fully explored. Administration of volumes ranging from 200 to 600 ml of immune plasma (8–10 ml/kg) once per day and for up to 3–7 consecutive days is generally recommended to be safe. However, some technical issues still need to be solved such as the optimal threshold for the serum titer of specific neutralizing antibodies [>160 or >320 by the enzyme immunoassay (EIA) method] in the preparation and the real utility of performing a pathogen (viral) inactivation treatment of such products. In fact, critically ill COVID-19 patients as well as those in early phases of the disease might be excellent candidates for passive immunotherapy, and for further randomized clinical trials for addressing its clinical usefulness in various patient subcategories. The immunocompromised status associated with haematological malignancies may enhance the risk of COVID-19 infections. Based on this consideration, it might be postulated that either the preventive or the therapeutic use of convalescent plasma may be beneficial in chemotherapy-treated cancer patients, possibly mitigating the impact of COVID-19.[7] However, the incidence and potential predictive parameters of mortality of COVID-19 in patients with haematological malignancies is still matter of investigation.[5] Moreover, gene response, and

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differences in the kinds of immunity, underlying conditions or life style behaviours in males and females, and race (e.g. African having four times more fatal outcomes than other populations) could expose to an increased risk for COVID-19-related complications.

In this mini-report we propose three potential additional options for sources of such autologous Ab and provide some operational and evidence-based arguments to support the urgent implementation of such strategic approaches to saving the lives of those in need: (i) the use of hyperimmune immunoglobulin concentrates, which are derived from the plasma of physiologically immunized donors. It is debatable that this method may be even more effective than plasmapheresis since it uses a smaller dose of about 200 ml, which causes higher donor variability compared to the product of plasmapheresis which provides 600 ml that can be used as a triplet of satellite bags for three recipients. Moreover, the derived cryo-supernatant from well-established plasmapheresis donors is a better policy, as known plasmapheresis donors are virally a safer population and modern apheresis procedures provide a cleaner product in terms of consistency in leucodepletion and containing limited cellular contaminations and their fragments as compared to the variable routine conventional fresh frozen plasma (FFP) preparations. (ii) Abs may be obtained from cadaveric body fluid upon early consent or from washed solution of severely damaged lungs of patients with COVID-19, as a new optional source. Interestingly, a washed solution of the lung, even by using an oxygenated saline or even better plasmalyte, represents an excellent solution of choice for clinical use as previously reported by our group.[8] The collected washed solution can be sterilized later in special blood packages obtainable from a biotech company, which can be used in some well-established procedures for sterilization and pathogen reduction using ultraviolet C (UVC). This is a well-known procedure in use in some hospital blood centres for the sterilization of platelet concentrates. This protocol may allow a comparison of male *versus* female donors or other cohorts showing some difference in mortality rates as recently observed in Asian and African groups and in those who recovered *versus* those who passed away. (iii) It is possible to isolate such material from volunteer critically ill patients via haemapheresis or from bronchoalveolar lavage fluid as described above or any plasma-derived products that contain the appropriate Abs with neutralizing potential that are physiologically produced and circulating in the patient's blood. In the former option some online adsorption affinity column can be incorporated in the system to remove directly the required antibody that can then be used in the recipient, subsequent to UVC irradiation to remove traces of the circulating antigen and possibly its circulating complexes, or be desorbed as new source on immunoglobulin if required for further characterization.[5] Such a purer and cleaner sterilized antibody can be injected intramuscularly or intravenously in specific effective doses as required. Of course, such an affinity column procedure can be performed off line with both types of source product, plasma-derived bioproducts or lung-wash solutions.

It is conceivable that pooling multiple sera from several patients will also provide multifunctional Abs against various infectious diseases. Furthermore, a combined validated physical sonication combined with UVC irradiation methodology could be applied for the preparation of safer immunoglobulin products for therapeutic purposes.

There are several potential clinical applications related to the use of these novel and innovative proposed strategies. It is well known that COVID-19 patients may develop an acute respiratory distress syndrome and/or multiple organ failure.[9] One of the most important mechanisms underlying the deterioration of the disease leading to organ injury is the cytokine storm. At present, some therapies such as interleukin-6 antibody blocker, stem cell therapy, and transfusion of convalescent plasma have been applied to counteract the cytokine storm with considerable success. Importantly, it has been demonstrated that this novel COVID-19 uses the same cell entry receptor, ACE2, as SARS-CoV-2. Hence the above mentioned therapeutic modality provides a unique interventional substitution programme, currently under consideration by our group.[10]

To conclude, apart from the benefit of the proposed operations of collecting autologous human Abs from different sources for either prevention of or treatment for COVID-19, it is timely that we do not restrain from putting all these innovative inspiring proposals into action. In fact, a practical way of inactivating pathogens plays an important role, especially in the light of newly discovered or unknown viruses, which cannot be safely excluded via prior testing. Hence building upon the information gained on UVC and ultrasonic methods for optimally inactivating the pathogens in human plasma products prior to transfusion as an additional safety measure, is a step forward in achieving the highest level of viral inactivation. If applied immediately after harvesting, these procedures leave blood products unaltered, which makes them especially suitable for use in third-world countries where virus contamination of blood preparations poses a major problem.

In summary, in countries without access to advanced blood-processing technologies, the choice may initially be restricted to convalescent whole blood or plasma. In technologically advanced countries, additional passive-mode immunotherapy options may be taken into account. However, in western countries the use of convalescent plasma and related strategies may become a reality provided that our hospitals will be recommended to obtain informed consent from recovered COVID-infected patients to collect and store their fresh-frozen plasma (FFP) and the derived bioproducts. In the UK the use of plasmapheresis for the preparation of FFP from some family members of patients who experienced infection with SARS-CoV-2 was started in May 2020 and so far, 150 units have been collected. The establishment of a comprehensive database including clinical and laboratory

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data from COVID patients/donors may be helpful in planning subsequent steps including convalescent plasma collection and much safer experimental and therapeutic interventions as the main goal of precision transfusion. Our numerous plausible and evidence-based methodological interventions are one step forward in this direction. This will be helpful in designing future clinical trials, since the optimal use of convalescent plasma at a global level is highly demanding in COVID-19-infected patients.

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The association between severe COVID-19 and low platelet count: evidence from 31 observational studies involving 7613 participants

A novel coronavirus disease broke out in 2019 (COVID-19). This disease was found to be a result of infection from the 2019 novel coronavirus (2019-nCoV).[1] The severity of COVID-19 disease ranges from asymptomatic to critically severe; clinical research reported that 10–15% of the patients were in the severe category and required a great deal of medical treatment and nursing care.[2] Indicators are needed to evaluate and predict the severity of the disease. We conducted the present meta-analysis to clarify whether platelet count might be a potential indicator to evaluate and predict the severity of COVID-19 in patients.

Relevant studies published in English up to 30 April 2020 were searched through PubMed Scopus, EMBASE, Web of Science and Cochrane Library. Keywords included "COVID-19", "platelet count", "severe" and "thrombocytopenia". To be included in the analysis, the studies had to report the mean and (±standard deviation, SD) of the platelet count in both severe and non-severe COVID-19 patients, or report the median and interquartile range (IQR) or median and range of platelet count, from which we could extract information about the mean (±SD).[3,4] The studies that reported the proportions of patients with thrombocytopenia in both severe and non-severe COVID-19 were also included. Studies were excluded when the participants might have a large overlap with other included studies. Study quality was evaluated by a checklist from the Agency for Healthcare Research and Quality. The pooled standardized mean difference (SMD) and odds ratio (OR) with 95% confidence interval (CI) were worked out by STATA 12.0 software (StataCorp LCC, College Station Texas, USA), and shown in the forms of forest plots figures. The detailed statement of materials and methods is shown in Data S1.

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