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COVID-19 recurrence associated with the virus storage in the Spleen

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INTRODUCTION

The spleen is the main fast defense organ due to its capacity of removing foreign particles, old cells, parasites, bacteria, fungi, viruses, and other antigens from the bloodstream without opsonization[1,2]. In general, the particles removed from the blood flow are destroyed and metabolized. However, parasites such as *Plasmodium* and *Leishmania*, viruses such as HIV, and bacteria, after being removed from the bloodstream by the spleen, may not be destroyed and remain within this organ for unlimited time period. Eventually, part of these parasites and viruses leave the spleen and cause transient disease recurrence[1,3-7]. Several studies have shown that after splenectomy, despite reducing the body's defense, the adverse events of these diseases disappear or reduce their intensity[1,5,6]. Most people who acquire COVID-19 persist asymptomatic, indicating that human defenses are able to control this virus. However, some people who had manifested this disease, and despite having been vaccinated, may present new episodes of COVID-19, which are ascribed to a new contamination[8,9].

According to these findings, our point of view is that SARS-CoV-2 can be removed from the bloodstream by the spleen and stored without being destroyed. Eventually, this virus recirculates and reactivates the disease without a new contagion. This possibility can be experimentally assessed in several mammals and also in human clinical trial.

METHODS

To verify the reinfection by coronavirus stored in the spleen, experimental and clinical studies may be performed as follows.

Experimental study

This proposal must be submitted to an ethics committee for research in animals and started only after its approval.

The Coronaviridae family has been investigated in many animal models, such as normal and genetically modified mice, rats, hamsters, rabbits, and pigs, among others[10-16]. Due to the high number of animals needed in this study, rabbits seem to be the most appropriate[10,11,13]. A non-lethal coronavirus (CoV) associated with mild disease is required to preserve the animals alive during all these experiments. According to literature data, the MERS (*Middle East respiratory syndrome*) beta-CoV seems to be the most adequate for this infection[10,13,14].

A total of 60 rabbits will be contaminated with intranasal inoculation of 10^3 $TCID_{50}$ (median tissue culture infectious dose) of EMC/2012 MERS-CoV strain[10,13,14]. These animals will be distributed in six groups (n=10) with equal number of males and females (n=5), according to the follow-up period as follows: group 1 (7 days), group 2 (14 days), group 3 (21 days), group 4 (30 days), group 5 (60 days), and group 6 (90 days).

At the end of the follow-up period, under general anesthesia (ketamine 10 mg/kg and propofol 2 mg/kg intravenous injection), the spleen will be removed after the ligature of its vascular pedicle through a median laparotomy. Spleen samples will be processed in the routine pathological analysis and stained with hematoxylin and eosin for microscopic study of histopathological findings caused by the CoV. Other spleen specimens will be processed in the real-time quantitative *polymerase chain reaction (RT-qPCR)* assays for the detection of the MERS-CoV by using appropriate primers kits[10,13,14]. The results of all groups will be

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statistically compared to verify the intensity and length of the spleen damage. The presence of the MERS-CoV in splenic parenchyma will be assessed as well, with emphasis on its intensity and period of its occurrence.

CLINICAL STUDY

This project must be submitted to an ethics committee for research in humans and only started after its approval.

This study will be a multicenter trial and carried out using electronic means of sections linked to the registry of patients who have been diagnosed with COVID-19. All patients who had recorded recurrence of their disease regardless of age and sex will be included and investigated. These patients will be contacted by telephone, social network, email, or personally. The researchers will introduce themselves and explain the purpose of this study. They will then request the consent of patients to be included in this work.

After signing the consent, each volunteer will be identified by age, gender, occupation, and ethnicity. They will be asked if they have undergone a surgical procedure, highlighting the splenectomy and the disorder that indicated this procedure. The date of splenectomy and the date of infections by COVID-19 and of its recurrences will be recorded. These patients will be divided into two groups, with and without spleen, to statistically compare the incidence of COVID-19 recurrence in both the groups.

DISCUSSION

The animal study may verify the presence of the CoV stored inside the spleen even after the animals be considered healthy and without signs of any disorder. Even being apparently cured, the alive virus can be present in a spleen that was not able to destroy it. Thus, the virus may multiply in the spleen and return to the bloodstream as a recurrence of the disease without a new contagion[1,4].

The multicenter clinical trial may assess the recurrence of COVID-19 in splenectomized volunteers. If the hypothesis of this article is correct and no recurrence will be found in splenectomized patients, further studies will be necessary to characterize whether the recurrence is due to a new contagion or due to the persistence of live COVID-19 in the spleen. This knowledge will be useful to better understand the infection caused by COVID-19 and to recommend more specific propedeutics and therapeutic strategies. Despite the possible presence of COVID-19 stored in the spleen, it is worth emphasizing the importance of keeping this organ due to its multiple relevant functions, including the vaccine efficacy[1].

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