Advances in Rheumatology

RESEARCH

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Safety and immunogenicity of influenza A(H3N2) component vaccine in juvenile systemic lupus erythematosus

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Abstract

Introduction Seasonal influenza A (H3N2) virus is an important cause of morbidity and mortality in the last 50 years in population that is greater than the impact of H1N1. Data assessing immunogenicity and safety of this virus component in juvenile systemic lupus erythematosus (JSLE) is lacking in the literature.

Objective To evaluate short-term immunogenicity and safety of influenza A/Singapore (H3N2) vaccine in JSLE.

Methods 24 consecutive JSLE patients and 29 healthy controls (HC) were vaccinated with influenza A/Singapore/INFIMH-16-0019/2016(H3N2)-like virus. Influenza A (H3N2) seroprotection (SP), seroconversion (SC), geometric mean titers (GMT), factor increase in GMT (FI-GMT) titers were assessed before and 4 weeks post-vaccination. Disease activity, therapies and adverse events (AE) were also evaluated.

Results JSLE patients and controls were comparable in current age [14.5 (10.1–18.3) vs. 14 (9–18.4) years, $p=0.448$] and female sex [21 (87.5%) vs. 19 (65.5%), $p=0.108$]. Before vaccination, JSLE and HC had comparable SP rates [22 (91.7%) vs. 25 (86.2%), $p=0.678$] and GMT titers [102.3 (95% CI 75.0–139.4) vs. 109.6 (95% CI 68.2–176.2), $p=0.231$]. At D30, JSLE and HC had similar immune response, since no differences were observed in SP [24 (100%) vs. 28 (96.6%), $p=1.000$)], SC [4 (16.7%) vs. 9 (31.0%), $p=0.338$), GMT [162.3 (132.9–198.3) vs. 208.1 (150.5–287.8), $p=0.143$] and factor increase in GMT [1.6 (1.2–2.1) vs. 1.9 (1.4–2.5), $p=0.574$]. SLEDAI-2K scores [2 (0–17) vs. 2 (0–17), $p=0.765$] and therapies remained stable throughout the study. Further analysis of possible factors influencing vaccine immune response among JSLE patients demonstrated similar GMT between patients with SLEDAI <4 compared to SLEDAI ≥ 4 ($p=0.713$), as well as between patients with and without current use of prednisone ($p=0.420$), azathioprine ($p=1.0$), mycophenolate mofetil ($p=0.185$), and methotrexate ($p=0.095$). No serious AE were reported in both groups and most of them were asymptomatic (58.3% vs. 44.8%, $p=0.958$). Local and systemic AE were alike in both groups ($p>0.05$).

Conclusion This is the first study that identified adequate immune protection against H3N2-influenza strain with additional vaccine-induced increment of immune response and an adequate safety profile in JSLE. (www.clinicaltrials.gov, NCT03540823).

Keywords Systemic lupus erythematosus, Influenza, H3N2, Vaccine, Safety, Immunogenicity

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Introduction

Influenza is a significant seasonal respiratory infection since it affects 5 to 15% worldwide annually [1]. The attack rates of these viruses are highest in the pediatric population with frequent hospitalizations for severe forms of the infection [2]. Immunocompromised children and adolescents are at additional greater risk of severe influenza-associated illnesses [3–6].

In light of these data, vaccination is considered an effective preventive measure in reducing the risk of several infections including influenza viruses in patients with adult autoimmune rheumatic diseases (ARD) [7, 8] and juvenile patients [3–6, 9]. In previous studies with juvenile SLE (JSLE) patients, it has been demonstrated that seasonal influenza vaccine is well tolerated and no severe adverse events (AE) were detected [10–12].

It should be highlighted that influenza vaccine is composed by three or four components that are changed almost every new season and the influenza A(H3N2) subtype has become of greater importance since during influenza season (between 2015 and 2019), immunosuppressed patients had a higher risk for influenza despite vaccination [13]. Moreover, worldwide number of hospitalization due to influenza A(H3N2) was identified to be two times greater than influenza A(H1N1) infection in the last 50 years [13]. According to antigenic analysis of circulating strains, the influenza A/Singapore/INFIMH-16-0019/2016(H3N2) component was recently integrated to influenza A vaccine worldwide in 2018 in the Southern Hemisphere and in 2018–2019 in the Northern Hemisphere [14]. It is important to notice that in Brazil, the circulation of influenza A virus in 2017 was almost exclusively from the H3N2 subtype, whereas in 2018 we observed a mixed H3N2-H1N1 [15].

However, there are few studies that addressed the influenza A(H3N2) virus vaccine component in SLE population [14, 16–20]. Recently, Formiga et al. demonstrated a high immune protection and a good safety profile of vaccination against the influenza A(H3N2)/Singapore in the adult SLE, but no study evaluated the safety and immunogenicity of this influenza component vaccination in JSLE patients [21].

Therefore, this prospective study aimed evaluate for the first time the short-term immunogenicity and safety of influenza A(H3N2)/Singapore vaccine in JSLE patients.

Patients and methods

Population

Twenty-four consecutive juvenile systemic lupus erythematosus (JSLE) patients routinely followed at the Pediatric Rheumatology Unit of a tertiary hospital were included. All patients fulfilled the international American College (ACR) of Rheumatology classification criteria for SLE [22]. A healthy group of 29 subjects composed by patients' siblings and schoolmates was consecutively included during the same time period as the JSLE patients. Inclusion criteria of both groups were current age ≥ 9 and ≤ 18 years old. Exclusion criteria were anaphylactic response to vaccine components or to egg, previous vaccination with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before the entry; influenza vaccination within 6 months; acute infection with fever over 37.8ºC at the time of vaccination; blood transfusion or immunoglobulin within 3 months; Guillain-Barré syndrome or demyelinating syndromes; and any clinical condition that required hospitalization.

This protocol was approved by the Local institutional ethical committee and an informed consent was obtained from all participants and their legal guardians. The study was registered with clinicaltrials.gov under the number #NCT03540823.

Study design

This prospective open study was performed during Influenza Vaccine Campaign from May to July 2018. Patients and healthy controls were vaccinated with one dose of the inactivated and fragmented influenza vaccine (A/Michigan/45/2015 (H1N1) pdm09-like virus, A/Singapore /INFIMH-16-0019/2016 (H3N2)-like virus; B/Phuket/3073/2013-like virus] at the immunization center of our hospital.

Blood samples were collected at study entry (D0) and after 30–45 days (D30–45) and were stored at −70 °C for further analysis of immunogenicity assays. jSLE patients were assessed for complete clinical and laboratorial evaluation (complete blood count, anti-dsDNA, C3, C4, urine I, and protein/creatinine ratio). Leukopenia and lymphopenia were defined as bellow <4000 and $<1500 \times 10^3$, respectively. Disease activity was defined according to SLE Disease Activity Index 2000 (SLEDAI-2K) score [23] that were calculated at entry (D0) and D30–45.

Vaccine

JSLE patients and healthy controls were vaccinated with one intramuscular dose of 0.5 mL of the inactivated and fragmented influenza vaccine (A/Michigan/45/2015 (H1N1) pdm09-like virus, A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus; B/Phuket/3073/2013-like virus produced by Instituto Butantan (Brazil).

Immunogenicity

Antibody levels against A/Singapore/INFIMH-16-0019/2016 (H3N2) virus were evaluated using the hemagglutination inhibition assay (HIA) at the Laboratory of Respiratory Viruses and Measles of

FIOCRUZ [24]. Sera were tested in duplicate at an initial dilution of 1:10, and at a final dilution of 1:1280.

Immunogenicity endpoints included seroprotection (SP) rates (HIA titer ≥ 1:40), seroconversion (SC) rates (prevaccination HIA titer < 1:10 and postvaccination HIA titer ≥ 1:40 or prevaccination titer ≥ 1:10 and ≥ fourfold increase in post-vaccination titer), geometric mean titers (GMT), and factor increase (FI) in GMT (FI–GMT).

Safety

At study entry, in the day of immunization, JSLE patients and healthy controls received a diary of safety surveillance including local (pain, redness, swelling, and itching) and systemic adverse events (fever, malaise, nausea, vomit, diarrhea, vertigo, tremor, headache, myalgia, muscle wellness, arthralgia, cough, coryza, sore throat, and conjunctivitis). Severe AE were defined as hospitalization or death.

Statistical analysis

Categorical variables were presented as number (percentage) and compared using McNemar's test, Fisher's exact test or Pearson chi-square test. Continuous variables were presented as mean ± standard deviation or median (range) and compared using two-sided Student's t-test or Mann–Whitney U-test, respectively. HI antibodies titers (GMT) were analyzed in a log-normal distribution. Significance was set at a p value < 0.05.

Results

Twenty-four JSLE patients and 29 healthy controls were included in the study. Both groups were comparable regarding median current age [14.5 (10.1–18.3) vs. 14 (9–18.4) years, $p = 0.448$] and female sex [21 (87.5%) vs. 19 (65.5%), $p = 0.108$]. Frequencies of Caucasian race [14 (58.3%) vs. 17 (58.6%), $p = 1.000$] and median body mass index (BMI) were similar among these studied groups [21.6 (14.8–31.8) vs. 19.8 (15.2–33.2) Kg/m^2, $p = 0.318$]. JSLE patients and healthy controls had also comparable frequencies of comorbidities: arterial hypertension [1 (4%) vs. 0 (0%), $p = 0.453$], diabetes mellitus [0 (0%) vs. 0 (0%), $p = 1.000$], dyslipidemia [0 (0%) vs. 0 (0%), $p = 1.000$], coronary artery disease [0 (0%) vs. 0 (0%), $p = 1.000$], hypothyroidism [0 (0%) vs. 0 (0%), $p = 1.000$], and peptic disease [1 (4%) vs. 1 (3%), $p = 1.000$]. At study entry, 24 (100%) of JSLE were under hydroxychloroquine, 15 (62.5%) prednisone [median dose of 15 (2.5–30) mg/day] and 20 (83%) were currently treated with immunosuppressive drug, including 12 (50%) mycophenolate mofetil, 5 (21%) azathioprine and 4 (17%) methotrexate.

Immunogenicity

Before immunization SP rates were comparable in JSLE patients and healthy controls [22 (91.7%) vs. 25 (86.2%), $p = 0.678$], as well as GMT [102.3 (95%CI 75.0–139.4) vs. 109.6 (95%CI 68.2–176.2), $p = 0.231$] (Table 1).

At D30, immune response parameters maintained comparable in JSLE and healthy controls including SP [24 (100%) vs. 28 (96.6%), $p = 1.000$)], SC rates [4 (16.7%) vs. 9 (31.0%), $p = 0.338$), GMT [162.3 (132.9–198.3) vs. 208.1 (150.5–287.8), $p = 0.143$], and factor increase in GMT [1.6 (1.2–2.1) vs. 1.9 (1.4–2.5), $p = 0.574$] (Table 1). A significant increment in GMT was observed from D0 to D30 in JSLE patients ($p < 0.001$) as well as in the control group ($p < 0.001$).

The comparison of responders (n = 4) and non-responders (n = 20) JSLE patients based on the SC demonstrated similar current age [14.5 (13–18) vs. 14.6 (10.1–18.3), $p = 0.892$], female sex [3 (75%) vs. 18 (90%), $p = 0.437$], Caucasian race [1 (25%) vs. 13 (65%), $p = 0.272$], baseline SLEDAI [2 (2–4) vs. 2 (0–17), $p = 0.846$], prednisone use [3 (75%) vs. 12 (60%), $p = 1.0$], prednisone dose [12.5 (10–15) vs. 15 (2.5–30), $p = 0.746$] and immunosuppressive drugs, including mycophenolate mofetil [1 (25%) vs. 11 (55%), $p = 0.590$], azathioprine [1 (25%) vs. 4 (20%), $p = 1.0$] and methotrexate [1 (25%) vs. 3 (15%), $p = 0.544$].

Further analysis of possible factors influencing vaccine immune response among JSLE patients demonstrated similar GMT of anti-H3N2 antibodies between patients with SLEDAI < 4 compared to SLEDAI ≥ 4 [167.1 (95%CI 130—214.8) vs. 153.2 (100.4–233.7), $p = 0.713$], as well as

Table 1 Immunogenicity of Influenza H3N2 vaccine in juvenile systemic erythematosus (JSLE) patients and healthy controls

	JSLE (n = 24)	Healthy controls (n = 29)	*P*
Before immunization			
Seroprotection rate, n (%)	22 (91.7)	25 (86.2)	0.678
GMT, value (95% CI)	102.3 (75.0–139.4)	109.6 (68.2–176.2)	0.231
After immunization			
Seroprotection rate, n (%)	24 (100)	28 (96.6)	1.000
Seroconversion rate, n (%)	4 (16.7)	9 (31.0)	0.338
GMT, value (95% CI)	162.3* (132.9–198.3)	208.1* (150.5–287.8)	0.143
Factor increase in GMT	1.6 (1.2–2.1)	1.9 (1.4–2.5)	0.574

Results are expressed in median (95% CI) and n (%)

GMT geometric mean titer

*$p < 0.001$—comparison of GMT at D0 versus D30

between patients with and without current use of prednisone [171.5 (130.5–225.4) vs. 148.1 (104.7–209.5), $p=0.420$], azathioprine [160.0 (64.2–398.6) vs. 162.9 (133.9–198.4), $p=1.0$], mycophenolate mofetil [184.9 (145.6–234.7) vs. 142.5 (101.2–200.8), $p=0.185$] and methotrexate [113.1 (59.9–213.9) vs. 174.5 (140.6–216.6), $p=0.095$].

Safety

Disease activity in JSLE measured by SLEDAI-2K score remained stable before and after immunization [2 (0–17) vs. 2 (0–17), $p=0.765$] (Table 2). Importantly, no significant changes were observed in the frequencies of positive anti-dsDNA [9 (38) vs. 9 (38), $p=0.480$] and hypocomplementemia [4 (17) vs. 3 (13), $p=1.000$] among groups (Table 2).

During the study, no significant changes were identified in the median leukocytes [5.09 (1.39–13.7) vs. 4.9 (1.76–11.11) $\times 10^3$, $p=0.989$] as well as lymphocytes [1.52 (0.17–3.15) vs. 1.5 (0.65–2.78) $\times 10^3$, $p=0.648$] (Table 2). Importantly, no significant changes were observed in the frequencies of leukopenia and lymphopenia (Table 2).

The comparison of therapy between entry and at the end of the study revealed no significant changes in most of the JSLE patients (Table 2). In fact, all were receiving antimalarials [24 (100%) vs. 24 (100%), $p=1.000$], oral prednisone [15 (62.5%) vs. 15 (62.5%), $p=1.000$] with a median dosage of 15 (2.5–30) mg/day during the period, mycophenolate mofetil [12 (50%) vs. 12 (50%), $p=1.000$], and methotrexate [4 (17%) vs. 4(17%), $p=1.000$] with a median dosage of 17.5 (7.5–25) mg/week during all the study (Table 2). Only one patient started azathioprine [5 (21%) vs. 6 (25%), $p=1.000$] but this increase was not significant. Importantly, none of the JSLE patients were taking IVCYC or Rituximab during the period of the study (Table 2).

No serious AE were reported in both groups. Most of the JSLE patients and healthy controls were asymptomatic (58.3% vs. 44.8, $p=0.958$) (Table 3). Local and systemic AE were alike in JSLE and healthy controls groups. Local pain (29% and 28%) and headache (25% and 17%) were the most frequent observed AE but their frequencies were alike in both studied groups (Table 3). The apparent higher frequency of coryza in healthy controls [5 (17%) vs. 0 (0), $p=0.056$] did not reach statistical significance (Table 3).

Table 2 SLEDAI-2K, laboratorial characteristics and treatment of juvenile systemic lupus erythematosus (JSLE) patients before (D0) and after vaccination (D30)

	D0 (n = 24)	D30 (n = 24)	*p*
Disease parameters			
SLEDAI-2K score	2 (0–17)	2 (0–17)	0.765
Positive Anti-dsDNA	9 (38)	9 (38)	0.480
Hypocomplementemia	4 (17)	3 (13)	1.000
Leukocytes, $\times 10^3$	5.09 (1.39–13.7)	4.9 (1.76–11.11)	0.989
Leukopenia % < 4000	6 (25)	7 (29.1)	1.000
Lymphocytes, $\times 10^3$	1.52 (0.17–3.15)	1.5 (0.65–2.78)	0.648
Lymphopenia % < 1500	11 (45.8)	12 (50)	1.000
Treatment			
Hydroxychloroquine	24 (100)	24 (100)	1.000
Prednisone	15 (62.5)	15 (62.5)	1.000
Current dose, mg/day	15 (2.5–30)	15 (2.5–30)	1.000
Azathioprine	5 (21)	6 (25)	1.000
Mycophenolate mofetil	12 (50)	12 (50)	1.000
IVCYC	0 (0)	0 (0)	1.000
Methotrexate	4 (17)	4 (17)	1.000
Current dose, mg/week	17.5 (7.5–25)	17.5 (7.5–25)	1.000
Rituximab	0 (0)	0 (0)	1.000

Results are expressed in median (range) and n (%)

SLEDAI-2K Systemic Lupus Erythematosus Disease Activity Index 2000, *IVCYC* intravenous cyclophosphamide

Table 3 Adverse events of influenza vaccination in juvenile systemic lupus erythematosus (JSLE) and healthy controls

	JSLE (n = 24)	Healthy controls (n = 29)	*P*
Asymptomatic	14 (58.3%)	13 (44.8%)	0.958
Local reactions			
Pain	7 (29)	8 (28)	1.000
Redness	2 (8)	0 (0)	0.20
Swelling	2 (8)	1 (3)	0.584
Itching	0 (0)	2 (7)	0.495
Systemic reactions			
Fever	2 (8)	2 (7)	1.000
Malaise	0 (0)	1 (3)	1.000
Nausea	1 (4)	1 (3)	1.000
Vomit	1 (4)	0 (0)	0.453
Diarrhea	1 (4)	0 (0)	0.453
Vertigo	1 (4)	0 (0)	0.453
Tremor	0 (0)	0 (0)	1.00
Headache	6 (25)	5 (17)	0.518
Myalgia	0 (0)	2 (7)	0.495
Muscle weakness	0 (0)	2 (7)	0.495
Arthralgia	0 (0)	1 (3)	1.000
Cough	1 (4)	5 (17)	0.204
Coryza	0 (0)	5 (17)	0.056
Sore throat	2 (8)	4 (14)	0.678
Conjunctivitis	0 (0)	0 (0)	1.000

Results are expressed in n (%)

Discussion

This is the first prospective study that describe the immunogenicity and safety of influenza A/Singapore/INFIMH-16–0019/2016(H3N2)-like virus in JSLE patients after this influenza vaccine component. A striking pre-vaccination anti-H3N2 immune protection and antibody titer was observed in our JSLE patients under immunosuppressive therapy with an incremental of humoral response after vaccination.

The great advantage of our study was the prospective design with a rigorous schedule for JSLE and controls which allowed a precise longitudinal assessment of the immunogenicity of influenza A/Singapore (H3N2) vaccine and also its safety. Another significant strength of the present study was the age- and sex-balancing with healthy controls since these parameters could interfere in the vaccine immune response [25, 26]. In fact, older age has been associated with higher immune responses to H3N2 variant in the pediatric population [27]. We also prospectively evaluated disease safety of influenza A/Singapore (H3N2) vaccine, assessing validated lupus activity parameter, as well as therapies since they may influence immunogenicity [26, 28]. Indeed, a previous study from our group identified a reduced response to pandemic influenza A H1N1/2009 vaccine in lupus patients under immunosuppressive therapy [12, 29]. A limitation of the present study was the lack of assessment of vaccine effectiveness based on post-vaccination influenza infection rates, and limited number of JSLE patients. Furthermore, the short follow-up time did not allow for the assessment of the long-term immunogenicity and safety of the vaccine in the evaluated population.

Importantly, nonadjuvanted preparation was used in order to exclude possible confounding variables in the evaluation of disease activity since there are some intriguing data about adjuvant-induced autoimmunity in both experimental models and humans [29, 30]. However, the literature related to this issue is very controversial, and recent vaccine studies in ARD patients did not the causal trigger relation with adjuvants and autoimmunity [31].

In the present study, JSLE and healthy controls had similar and high SP rates as well as comparable GMT titers at entry. These findings could be explained by previous influenza vaccination of both groups suggesting that they were effective. Moreover, cross-reaction between vaccine-elicited antibodies and contemporary H3N2 influenza viruses were previously described in children, suggesting that both natural infection and vaccination may add to the immune responses against H3N2 strain [27]. Importantly, after immunization JSLE patients had an effective immune response since SP rate was identified in all these patients and this rate was comparable to heathy controls. This finding strongly suggests that the component influenza A/Singapore/INFIMH-16–0019/2016 (H3N2) that was incorporated to influenza A vaccine [14] indeed improved immunogenicity of this vaccine. This assumption is also reinforced by the high GMT and factor increase in GMT that were observed after vaccination in both studied groups. A study from our group members assessing vaccine immunogenicity in health care workers also found that the A/Singapore/INFIMH-16-0019/2016 (H3N2) induced high SP and GMT levels, although the GMT to Singapore virus induced by vaccination was lower than the GMT to the 2017 vaccine component A/Hong Kong/4801/2014 [15].

Moreover, in the present study immunogenicity of the influenza A/Singapore (H3N2) vaccine was not affected by disease activity, since most our JSLE patients had inactive or low active disease. This is an important issue to be considered since a previous study of our group identified that the immune response after vaccination could be reduced by disease activity [12]. Another concern in the vaccine effectiveness is the use of steroids and its daily dose [32] as well as other under immunosuppressive therapy [29]. The influence of these therapies also did not promote a significant reduction of immunogenicity of our JSLE patients since most of them were using prednisone daily dose under 20 mg [32]. Despite the high frequency of immunosuppressive drugs use, it should also be highlighted that all of them were under antimalarials that seems to increase the reduced response to influenza A vaccine in lupus patients even under immunosuppressive therapy [29]. The role of both conditions did not influence the influenza A/Singapore (H3N2) vaccine effectiveness.

Concomitantly to their fundamental role in the pathogenesis of chronic inflammatory immune-mediated diseases, Th17 cells and their cytokines (IL-17A, IL-17F, IL-21, and IL-22) play a crucial role in host defense against various infections, re-infections, and colonization. IL-17 and IL-22 work together to enhance antimicrobial proteins in skin keratinocytes and induce the expression of host-defense genes in bronchial epithelial cells, strengthening the epithelial barrier function. Additionally, the use adjuvants may also increase IL-17 levels, favoring a specific Th-17 response, which could induce autoimmune disease flare [33, 34]. Of note, the present study also demonstrated that the component influenza A/Singapore/INFIMH-16-0019/2016(H3N2) of influenza A vaccine is safe since no severe AE were observed. Our data reinforce previous studies with JSLE patients, it has been demonstrated that seasonal influenza vaccine is well tolerated and no severe AE were detected [10–12]. Our group also demonstrated that two doses of influenza A

H1N1/2009 vaccine in ARD patients promoted an effective antibody response without significant AE reinforcing the importance of this vaccination [35].

In conclusion, this prospective study evaluated for the first time that influenza A/Singapore (H3N2) vaccine has an adequate short-term immunogenicity and safety in JSLE patients.

Acknowledgements
We are grateful to all study participants for their kind cooperation. We thank Margarete Borges Galhardo Vendramini and Nicole Fontoura from Laboratório de Investigação Médica 17 (LIM17); Juliana Yamashiro MD from Department of Infectious and Parasitic Diseases HCFMUSP; Marilda Agudo Mendonça Teixeira de Siqueira MD PhD and Milene Dias Miranda MD PhD from Laboratory of Respiratory Virus and Measles, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro; CEAC group and Divisão de Laboratório Central (DLC)—Hospital das Clinicas HCFMUSP, Faculdade Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil for their support and Instituto Todos pela Saúde (ITPS 01/2021, C1313 to CAS, SGP, NEA and EB).

Author contributions
NEA: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. EFB: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. VAB: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. LMAC: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. KTK: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. MDM: Analysis and interpretation of data. Draft and design of the work. Revision of the work. ASVC: Analysis and interpretation of data. Draft and design of the work. Revision of the work. ACTP: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. EPL: Analysis and interpretation of data. Draft and design of the work. Revision of the work. AJSD: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. MHL: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. CAS: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. EB: Design and conception of the study. Interpretation and analysis of data. Draft and design of the work. Revision of the work. All authors have read and approved the manuscript.

Funding
This study was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP #2018/16162-3 to EFB, #2015/03756-4 to CAS, SGP, NEA and EB, #2010/10749-0 to EB and, #2017/14352-7 to TNP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #303378/2022-0 to EFB, #304984/2020-5 to CAS, and #305242/2019-9 to EB).

Availability of data and materials
The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Ethical Committee (Comissão de Ética para Análise de Projetos de Pesquisa—CAPPesq) of Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Brazil (number 2.593.404). The study was registered with clinicaltrials.gov under the number #NCT03540823.

Consent for publication
All participants provided a written informed consent.

Competing interests
The authors declare that they have no competing interests.

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Received: 15 August 2023 Accepted: 18 November 2023
Published online: 28 November 2023

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