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Review article

## The pulmonary route as a way to drug repositioning in COVID-19 therapy

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### ABSTRACT

**Introduction:** The outbreak of the disease caused by the new coronavirus (COVID-19) has been affecting society's routine and its patterns of interaction worldwide, in addition to the impact on the global economy. To date, there is still no clinically effective treatment for this comorbidity, and drug repositioning might be a good strategy considering the established clinical safety profile. In this context, since COVID-19 affects the respiratory tract, a promising approach would be the pulmonary drug delivery.

**Objective:** Identify repurposing drug candidates for the treatment of COVID-19 based on the data of ongoing clinical trials and in silico studies and also assess their potential to be applied in formulations for pulmonary administration.

**Method:** A integrative literature review was conducted between June and July 2020, by extracting the results from Clinical Trials, PubMed, Web of Science and Science Direct databases.

**Results:** By crossing the results obtained from diverse sources, 21 common drugs were found, from which only 4 drugs presented studies of pulmonary release formulations, demonstrating the need for greater investment and incentive in this field.

**Conclusion:** Even though the lung is a target that facilitates viral infection and replication, formulations for pulmonary delivery of suitable drugs are still lacking for COVID-19 treatment. However, it is indisputable that the pandemic constitutes a concrete demand, with a profound impact on public health, and that, with the appropriate investments, it will give the pharmaceutical industry an opportunity to reinforce the pulmonary delivery field.

### 1. Introduction

Drug repositioning is a practice widely used by the pharmaceutical industry due to the significant cost and time reduction in the development of new medicines [1–3]. The concept refers to new uses for drugs already known, whether of marketed, discontinued, in experimentation or archived molecules, what means drugs developed for some disease being applied to another one. In this context, approximately one third of the new approvals by the Food and Drug Administration (FDA) in the last decade referred to repositioned drugs, reaching about 25% of the

revenue for some companies [1,3].

An alternative route in the repositioning of drugs is based on the development of new delivery systems or changes in administration routes. There are many reasons to choose this approach: inadequate pharmacokinetic, problematic toxicology, low water solubility, among others [4]. Several cases have already been reported of drugs that have undergone this process, some of them having reached clinical use, such as tobramycin and aztreonam, originally designed for intravenous or intramuscular administration and repositioned for administration by nebulization [5].

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The recent emergence of the disease caused by the new coronavirus (COVID-19) has raised a huge challenge for the entire health systems, on a global scale [6]. The severe pandemic status induces research institutions and industrial sector to search for problem resolutions. Along with vaccines, new therapies are being evaluated in several centers across many countries. While a definitive drug therapy is not available, the clinical protocols based on the repositioning of approved drugs [7]. However, its pharmacokinetic aspects sometimes might not be adequate [8].

The SARS-CoV-2 virus causes COVID-19, which induces the severe acute respiratory syndrome that mainly affects the airways [9]. An interesting alternative for COVID-19 treatment could be the identification of a suitable repositioned drug to be administered via pulmonary route. Since, this pathway has shown very positive results in the treatment of other lung diseases, which may be related to its rapid onset, low metabolic activity at the site, lower risk of adverse effects, among others [10–12].

Thus, this article aims to present possible drugs that are candidates for repositioning via a pulmonary formulation for the treatment of COVID-19, the scientific basis for its use, as well as the related technological and regulatory aspects. It is expected to stimulate the debate and, mainly, to present a preliminary evaluation model for the selection of drugs with a view to repositioning in a pandemic situation and the need for emergency actions to attendant public health demands.

## 2. Methods

The present study constitutes a literature integrative review [13], which aimed to identify repositioning drugs candidates for pulmonary administration for the COVID-19 treatment.

### 2.1. Clinical trials drug selection

Searches were conducted in the international registry of clinical trials ([ClinicalTrials.gov](http://ClinicalTrials.gov)) to identify drugs currently investigated to treat COVID-19. These searches were carried out on June 19th, 2020, using the following strategy: situation (“All studies”); condition or disease (“COVID-19”); other terms (no selection); countries (no selection). After obtaining the list of clinical trials, a filter by type of intervention was performed, selecting only the options that included “drugs” and “product”. The following exclusion criteria were used: all non-medicated treatments, such as food supplements, convalescent plasma, cells, gases, among others; tests with new molecules; and trials that were not intended for the COVID-19 treatment. After excluding trials that did not meet the established criteria the proposed drugs were listed.

### 2.2. In silico studies criteria

Afterward, for identification of promising drugs for the COVID-19 treatment from articles that performed in silico studies, searches were conducted in the Web of Science, Science Direct, and PubMed databases, using the following search strategy: (coronavirus OR COVID-19) AND (repositioning OR repurposing) AND (in silico OR computational simulation). The terms were identified in the title, abstract or keywords, and exclusion criteria were review articles, articles that were not completely available with electronic access, and articles that were outside of this review’s scope. The searches were carried out on June 22nd, 2020.

The articles involving in silico methods have been refined according to criteria on the methods they employed. First, the preparation of the receptor and the ligand used was verified, this involves analysis of protonation (referring to the optimum pH of action of the enzyme) and minimization of the 3D structure. The second criterion is based on whether the article used any software to analyze the chemical interactions between the receptor and the ligands, in order to verify the crucial interactions for the binding to the active site of the enzyme (types

pi-alkyl, hydrogen bond, pi-pi etc.). The next criterion was whether the work used binding affinity prediction methods based mainly on the methods defined by Poisson Boltzman or Generalized Born equation - MM/PB (GB) SA. And finally, if the article uses any molecular dynamics technique to simulate the interaction between enzyme-ligand in biological systems.

### 2.3. Selected molecules and pulmonary delivery

Subsequently, the drugs reported into clinical trials and in silico studies were compared in order to seek for drugs that appeared in both research types. An investigation based that list was carried out to identify if there were studies with pulmonary formulations for these selected drugs. The search was realized in the Web of Science, Science Direct, and PubMed databases, using the following search strategy: (name of the drug) AND (“pulmonary release”) OR (“pulmonary drug delivery”) OR (“pulmonary delivery”). Documents other than scientific articles, such as book chapters, conference abstracts, indexes, among others, were excluded. In addition, papers that were not entirely available online and the ones with no relevant subjects to this review were eliminated. Furthermore, a hand searching in the reference list of the selected papers was carried out, in order to incorporate eligible articles into this review that was not covered by the adopted search strategy. The searches were performed between July 2nd and 17th, 2020. After applying the exclusion criteria, all texts were skimmed, and those considered relevant were carefully read. The articles that presented pulmonary formulations were included and will be discussed in this work. Fig. 1 illustrates a representative scheme of the rationale described for the article’s selection process.

## 3. Results and discussion

### 3.1. Drug selection

The clinical trials identified 801 studies that, after applied the exclusion criteria, returned an amount of 291 drugs. On the other hand, 25 studies were found out using in silico methodologies and, after eligibility criteria, cited 98 different drugs. After matching both research, 22 drugs were identified with potential use for COVID-19 based on clinical trials and in silico studies (Fig. 2).

### 3.2. In silico criteria

The 17 articles selected by search criteria used different molecular targets: Mpro [8,14–19], RdRp [20–23], Spike protein [24], PLpro [25], NTD n-protein [26], EndoU or Nsp15 [27], TMPRSS2 [8], and RBD [15]. An amount of 12 of the 17 articles analyzed used programs to structure optimization/energy minimization and protonation, in addition to evaluating the chemical interactions observed between the molecular targets and ligands analyzed. However, only 6 of these articles performed the binding affinity prediction (MM/PB (GB) SA) and molecular dynamics simulation, an important criterion for the prediction of the molecular interactions between two molecules. The six articles that passed the criteria established in silico can be seen in Table 1.

The evaluated studies show different programs to perform molecular docking and score calculation, and the work of Chandra and coworkers [27] was the only that used more than one program and compared different metrics of docking score in its results. Among these are DK scoring (Smina Tools) and Idock score (Idock), both programs adopt scoring functions similar to those of Vina. The DK scoring shows a better correlation between the calculation and the binding energy obtained experimentally, considering the solvation effects of AutoDock 4.2 and the hydrogen interactions of Vina [28]. The Idock score implements C++, data structures, numerical models, and Monte Carlo algorithms in a different way from Vina, which reflects in better CPU utilization [29].

The work from Al-Khafaji and coworkers [17] used two score

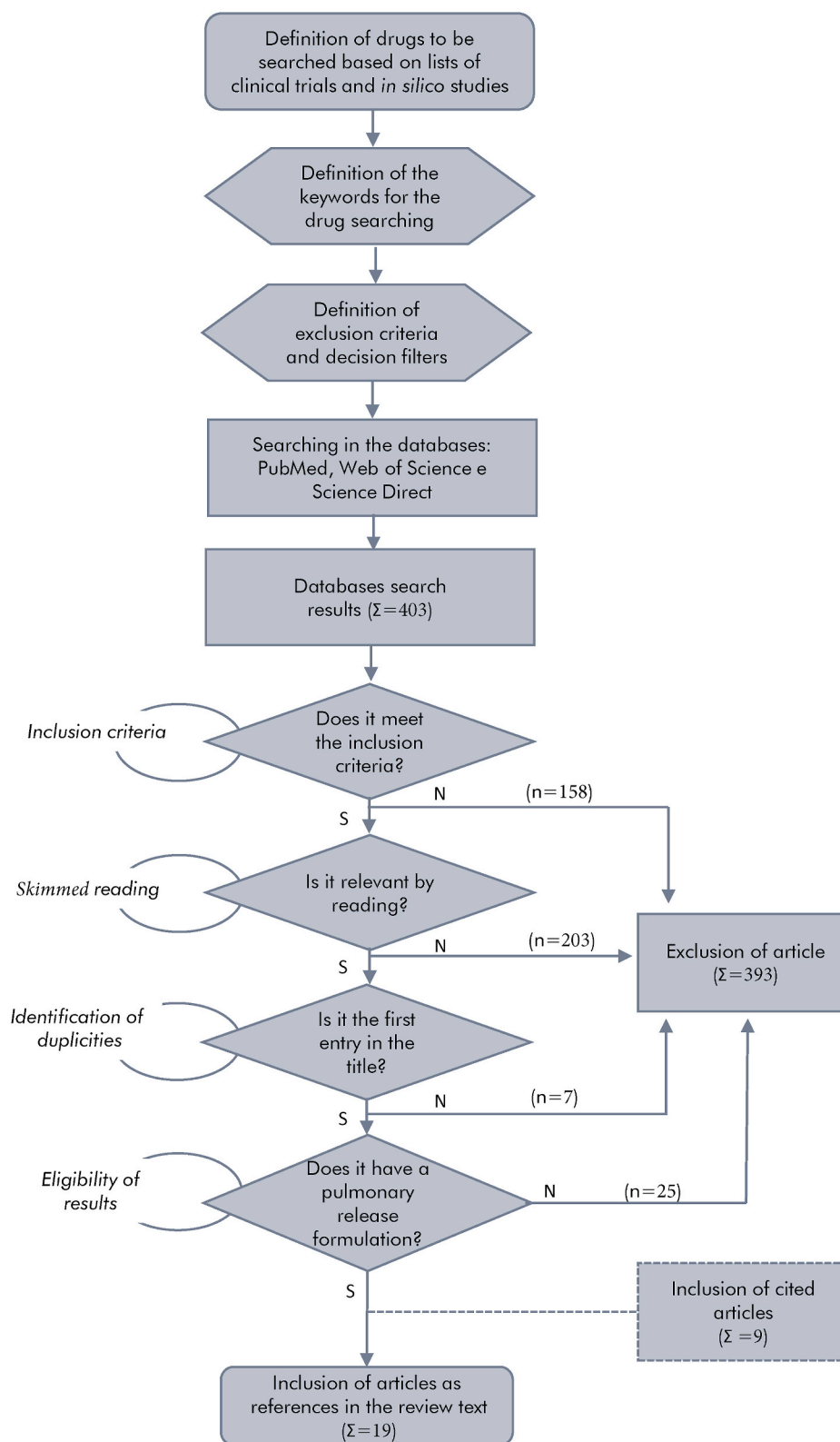


Fig. 1. Flowchart of the proposed methodology.

calculations: docking score and glide score (generated by Glide), they differ only in the penalties implementation. The docking score additionally uses another program for calculation, the Epik, which generates a set of states that are more appropriate for connections involving metals. The interactions between metals evaluated by Epik will have penalties that will reflect in the final score [30].

Pant and coworkers [16] used, in addition to the docking score generated by Glide, the Glide emodel score as a metric. This score is a combination of the Glide score, energy grid score and (for flexible docking) the internal strain energy for the model potential used to direct the conformational-search algorithm [30].

Different software was used for the chemical interactions analysis. In

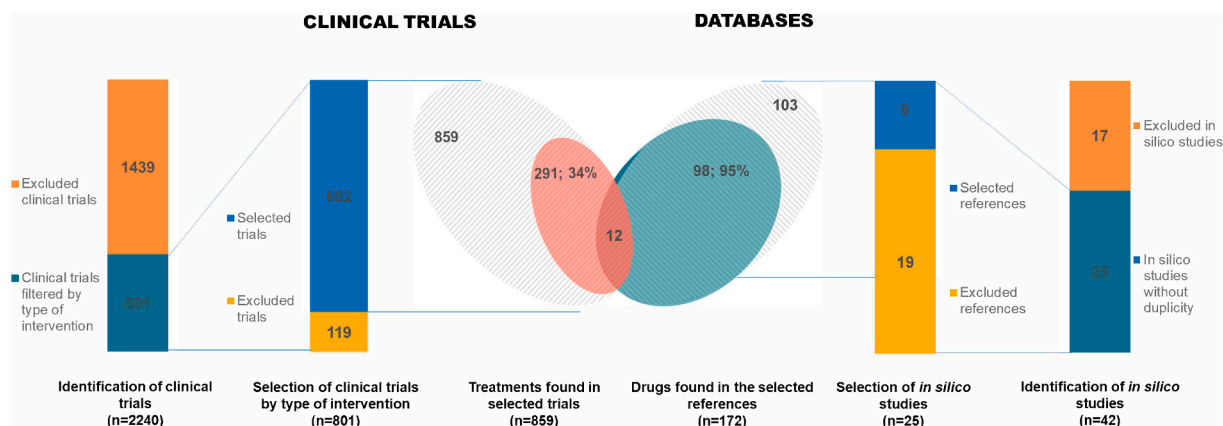


Fig. 2. Representative scheme of the results obtained from clinical trials and research databases.

Table 1

List of molecular targets and programs used in the 6 evaluated articles.

Molecular Targets	Softwares				Scoring calculations	Ref
	Chemical interactions	Dynamics simulations	MM/PB(GB)SA	Docking molecular		
Mpro and TMPRSS2	BIOVIA Discovery Studio	NAMD	Calculation of Free Energy (CaFE)	Vina	Binding score	[8]
EndoU	UCSF Chimera	GROMACS	Prime MM/GBSA module Schrödinger	Vina Idock Smina tools Glide	Binding score Idock score DK scoring Docking score	[27]
Mpro	SiteMap Mestro Glide	Desmond	Gromacs 4.6.5		Docking score	[14]
Mpro and RBD	BIOVIA Discovery Studio	NAMD	Calculation of Free Energy (CaFE) 1.0 VMD plugin	Glide	Glide score	[15]
Mpro	Glide	Desmond	Prime MM/GBSA module Schrödinger	Glide	Docking score Glide Emodel score	[16]
Mpro	Covdock - Glide	GROMACS	Prime MM/GBSA module Schrödinger	Glide	Docking score Glide score	[17]

this step, the data described in the analyzed articles about the interactions according to each molecular target were analyzed and what is already known in the literature was described.

The main residues of the Mpro enzyme active site are Pro168, Ala191, Thr190, Gln189, Ser46, His41, Cys145, Asn142, Leu141, Glu166 [31,32]. All 5 articles analyzed showed the regions of interaction and chemical interactions that occurred in the simulations with these amino acid residues. The TMPRSS2 protein has its active site composed of residues Gly462, Asp435, Ser441, Ser460, Ile356, Asp345 and His296 [33,34]. Elmezayen and coworkers [8] evaluated interactions between these residues and the ligands analyzed. The work of Tariq and coworkers [15] had RBD as one of the targets and interaction with 2 of the 5 amino acid residues of the active site already described has been reported (Gly496 and Try505), showing no interactions of the ligands analyzed with the Ans487, Glu37, Arg393 residues [35,36].

The article by Chandra and coworkers [27], 2020 target the EndoU protein and reported interactions with all the amino acid residues that are part of the active protein site (His235, Thr341, Tyr343, His250, Ser294 and Lys290) [37,38].

In addition to the mentioned criteria, the 3D structures used for in silico tests were evaluated, as a non-excluding criterion, comparing them with the 3D conformation that performs the biological function. The Mpro enzyme has biological activity in its dimeric form [32], however only the work published by Elmezayen and coworkers [8] performed the in silico simulations using the native form of Mpro. The other evaluated articles just cited the use of the enzyme in its monomeric form. Likewise, the enzyme EndoU performs biological function in its hexameric form [38], however Chandra and coworkers [27] used its

dimeric form (PDBid: 6W01) to perform the simulations. Tariq and coworkers [15] uses RBD as a molecular target in monomeric form, its biologically active form [39] (PDBid: 6Y84).

### 3.3. Selected molecules and COVID-19

Drug repositioning approaches using in silico tools, such as molecular docking studies, virtual screening, and dynamic simulation accelerate the identification of promising drugs to treat specific pathologies [12,13]. However, analyzing the results obtained in this work is possible to realize that this strategy does not seem to be sufficiently explored by the pharmaceutical industry. Among 291 drugs currently evaluated in clinical trials, only 12 were also indicated by in silico studies: argatroban, artemisinin, atovaquone, bicalutamide, chloroquine, deferoxamine, doxycycline, estradiol, mefloquine, montelukast, oseltamivir and remdesivir (Fig. 2). This demonstrates that applying the results from academy to the clinical practice is still a challenging process.

It was also observed that different strategies were used to select drugs in the two cases evaluated herein. While in silico trials consider the particularities of the virus and possible drug-receptor interactions to deduce which drugs would be suitable for the treatment of COVID-19, the drugs on clinical trials focus on symptomatic treatment of the disease. Thus, in silico studies mainly indicate antivirals, capable of inhibiting virus replication, while clinical trials explore the most diverse pharmacological among classes, such as antibiotics and anticoagulants, others.

A combination of the aforementioned strategies is probably more recommended, since antivirals could be particularly viable in moderate



cases, preventing the worsening of the disease and reducing overcrowding in health care systems [40,41]. However, for patients in severe conditions, it is necessary another strategy treatment due to the associated immune dysregulation [42].

After searching for the names of each drug previously mentioned combined with keywords that relate them with pulmonary drug release, no results were considered relevant for the following drugs: argatroban, artemisinin, atovaquone, bicalutamide, chloroquine, deferoxamine, mefloquine, and remdesivir. For doxycycline (DOX), estradiol, montelukast (MLK), and oseltamivir the search returns the results described in Fig. 3, identifying 278 research articles. After applying the eligibility and exclusion criteria, 19 papers were included in this review.

The four selected drugs belong to two main pharmacology groups: drugs with a systemic target, with an antibiotic or anti-inflammatory action, and drugs with a specific viral molecular target. The drugs in the first groups are: doxycycline (DOX), estradiol and montelukast (MLK). The only drug in the second group is oseltamivir.

DOX is a broad-spectrum antibacterial drug commonly associated with an anti-inflammatory effect. Its ability to reduce the inflammatory processes associated with cystic fibrosis and chronic lung diseases has been documented [43,44]. Regarding COVID-19, its ability to interact with the viral SARS-CoV-2 was evaluated *in silico* through molecular docking studies, showing that this drug could interact with specific sites of both main viral replication protease (Mpro) and S1 protein (Table 1) [15].

For some decades, estradiol (17 $\beta$ -estradiol), has been evaluated as a safer alternative to the use of artificial hormones [45]. Concerning COVID-19, Pant and coworkers [15], through computational methods, proposed that estradiol has a potential for interaction and inhibition of the main protease of SARS-CoV-2 (Table 1).

MLK, a cysteinyl-leukotriene type 1 (CysLT1) receptor antagonist,

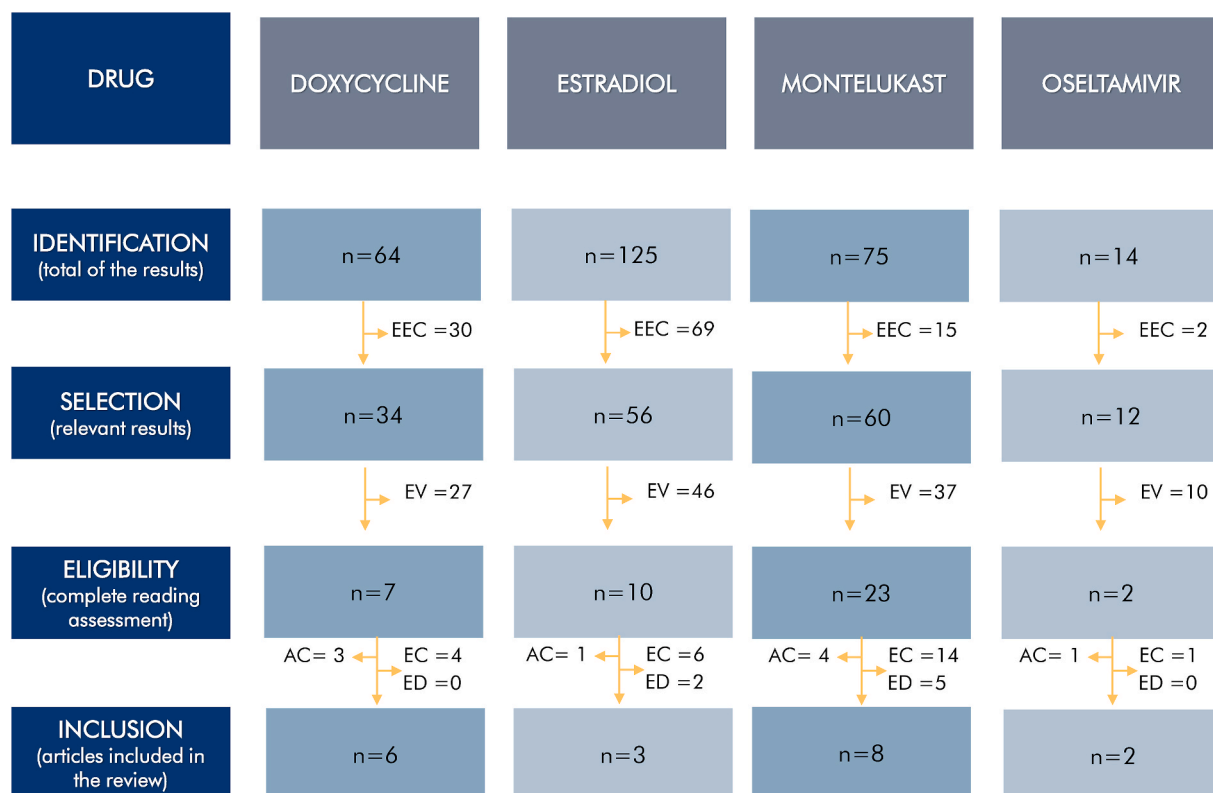
inhibits the inflammation process, especially the ones caused by leukotriene D4 [46,47]. It is used, not only in asthma treatment, but also to prevent wheezing, chest tightness, cough, and exercise-induced bronchospasm in adults and children [47,48]. Besides that, it could be used to treat other neutrophil activation diseases, such as seasonal allergies [49]. Virtual screening and molecular dynamics studies identified that MLK could function as a protease inhibitor of SARS-CoV-2 (Table 1) [14].

Oseltamivir phosphate is a prodrug that is converted into its carboxylate derivative, recommended by the World Health Organization as the first-line treatment against influenza virus. It is available for oral administration and was first registered as Tamiflu® by Hoffmann-La Roche Ltd [50]. Al-Khafaji and coworkers evaluate the covalent docking of several antiviral drugs and oseltamivir showed a good ability to interact with SARS-CoV-2 Mpro (Table 1) [17].

### 3.4. Pulmonary route

COVID-19 is a disease that affects the respiratory tract. Evidence suggests that the angiotensin-converting enzyme receptor 2 (ACE2) may facilitate SARS-CoV-2 entry cells [51], and as a healthy lung tissue has alveolar epithelial cells expressing ACE2 [52], it is highlighted that the lungs can be considered a primary target to promote viral invasion and replication. Therefore, the use of pulmonary drug delivery could be considered a promising alternative to fight against this disease and considering the main findings of academia that were discussed herein, the technology developed could be rapidly transferred as to enable the clinical treatment of COVID-19.

It is quite evident that the pulmonary route is an extremely important target for distinguishing diseases, likewise asthma and DPOC. Clearly, not only COVID-19 motivates investments in this segment, but also



The results described were identified in PubMed, Web of Science and Science Direct databases, using the search strategy: (*drug name*) AND ("pulmonary release") OR ("pulmonary delivery") OR ("pulmonary drug delivery").

Abbreviations used: EEC: excluded by the exclusion criteria; EV: excluded after vertical reading due to the relevance of the theme; AC: added by citation; EC: excluded after complete reading; ED: excluded due to duplication.

Fig. 3. Relevant results obtained from the search for pulmonary formulations of the drugs.

could be an acceleration force for new research and even a redirection of several companies' portfolio. At this moment, pharmaceutical laboratories are facing a concrete demand, with a profound impact on public health. Investment in research and development of formulations for pulmonary release could bring to these institutions a character of innovation that has not been verified for a long time.

Drug delivery systems prove to be a highly favorable path in incremental innovation [53], essential for the development and registration of numerous drugs currently available clinically. Faced with an international scenario in which the pharmaceutical sector has been envisaging serious difficulties to launch innovative drugs, delivery systems can be an excellent opportunity both to attribute new pharmacokinetic profiles to existing drugs and to lead them to new uses.

A successfully application of drug repurposing as pulmonary route is the asthma disease treatment. The review published by Anderson brings several examples of drugs that were administered by oral or intravenous route and became available as inhaled in order to reduce side-effects, dose, and allow a prophylactic treatment [54]. A classic example was budesonide, where the oral and aerosol formulations were compared and only the inhaled form showed antiasthmatic effect, proving that its local action in the airways was better than systemic activity after absorption [55]. The repurpose of iloprost (Ilomedin®) was also interesting. This drug is a prostacyclin analogue used for severe forms of peripheral arterial disease that was repurposed for aerosol therapy of pulmonary hypertension (PAH). Nowadays, iloprost is recommended as class I monotherapy in patients with PAH [56]. In addition, the agencies contribution with documents such as the Food and Drugs and Cosmetic Act 505(b)(2) facilitates the regulatory approval of drugs with changes in dosage form, strength, formulation, dosing regimen, or route of administration [57]. Thus, reducing costs and time to new formulations market launching. As numerous cases, COVID-19 can be a lever in the innovation process in this area, as can be concluded by everything previously exposed in the article.

In this context, considering the potential benefits of pulmonary route for COVID-19, a literature search was conducted to find possible pulmonary release formulations with the drugs previously selected. The relevant information of the 17 analyzed papers of pulmonary delivery of these drugs are summarized in Table 2. For DOX, dry powder inhaler (DPI) formulations are the most reported in the literature, being the spray drying (SD) the most applied technique to produce this powder. It was used for producing microparticles [58,59] and also, as a drying process combined with other methodologies [60,61], to provide the powder for the DPI. In addition to solid formulations, DOX nebulizer solutions have also been reported [62] (Table 1).

SD was the most used technique also for estradiol formulations and the most used excipients to formulate this drug were albumin, lactose, and dipalmitoylphosphatidylcholine (DPPC) [63] (Table 1). This last one is the major phospholipid present in pulmonary surfactant, which is essential for mammalian breath because its ability of solubilize poorly water-soluble molecules and reduce the surface tension in a water solution [64,65]. Due to this, the role of lung surfactant to optimize pulmonary drug delivery and distribute poorly water-soluble drugs over the respiratory air-liquid interface has been reported for other drugs and has been applied in pulmonary formulation of estradiol [64].

Due to the lower expression of cytochrome P450 3A4 enzyme in the lungs [66], when MLK is administered via pulmonary route its metabolism is reduced compared to the liver [67]. So, the pulmonary route can improve its bioavailability [68] in comparison with the oral route, for which is approximately 64%, due to the first pass effect [46,69,70]. As MLK is used for asthma treatment, there are plenty of investigations for its pulmonary administration. Probably, because of that, 6 of 8 studies with MLK presented in vivo experimental data (Table 2). The majority uses DPI, as microparticles, but it was also possible to find one study with pressurized metered-dose inhaler (pMDI) (Table 2) [68]. The SD technique was widely used and several strategies were applied to increase drug release and reach the deep lung, such as porous particles

[67,71]. Distinguish works evaluate the mucoadhesiveness characteristic of the obtained particles, since this property could increase the formulation retention time in the pulmonary region [44]. It is relevant to point it out that some of the strategy applied for this drug could be applied for other ones accelerating the investigative process.

On the other hand, only two oseltamivir pulmonary formulations are proposed in the literature, and they are presented as DPI or nebulizer solution. Both present an increase in AUC when compared to other administration routes (Table 2).

### 3.5. Critical evaluation of technical issues regarding pulmonary delivery systems

From Tables 2 and 3, it is possible to observe some peculiarities of the studies with the selected drugs. The first one concerns the type of device most commonly used, in up to 78.9% of the studies, which is DPI [46,50, 58–61,63,69,71–74,76–79]. Its preponderance is superlative compared to 10.5% of solutions/suspensions for nebulization [62,80]. This is an excellent indication of how a given technology is more promising in terms of therapeutic application. This data, in a way, agrees with the literature that, in fact, places DPI as one of the most viable approaches for pulmonary drug administration [83]. The technology for obtaining the release systems, in turn, accounts for 57.9% of cases using the spray drying technique. This approach is already foreseen, since it is a type of processing that is commonly used for obtaining spherical shaped particles, which are very useful for pulmonary administration [84]. In addition, it presents a high scalability, and it is already a traditional process in the pharmaceutical industry [85].

On the other hand, a parameter that is imperative for the proper understanding of the systems is the particle size. It was expected that this parameter would be controlled in all studies, including seeking a more direct correlation with the effects and results obtained. The absence of aerodynamic size determination in 40% of the articles is worrying since the guides of FDA (Guide with recommendations for the development of DPIs and metered-dose inhalers (MDIs), European Medicines Agency (EMA) (Guidance on pharmaceutical quality of inhaled and nasal products) and ANVISA (Normative Instruction No. 33, on April 17, 2019), which is the Brazilian Health Regulatory Agency, recommend evaluating aerodynamic particle size distribution by cascade impactor test for formulations intended for pulmonary release [86–88]. In addition, in many of the articles in which this trial was conducted, the method was not adequately described or properly justified. Anyway, when the particle size results are mentioned, they are below 12  $\mu\text{m}$ , except for one article, which showed 26.3  $\mu\text{m}$  in size [60]. These values are in accordance with the general literature on pulmonary delivery systems [89,90].

Regarding the excipients use in pulmonary formulations, EMA guide emphasizes the importance of using excipients with well-established use for pulmonary release and, if this is not possible, safety studies for such application must be carried out [86]. The FDA provides a list of the excipients used in the approved for various administration routes, which can facilitate the development or repositioning of drugs via the pulmonary route [91]. Among them, lactose [73,78,79], mannitol [46,76], and lecithin [50,69] stand out. However, most of the articles found in this research used excipients not recommended by the FDA (Table 2) and that could bring some hurdles in terms of registration, especially in the case of the development of a new drug product. It was possible to find, for example, papers that used albumin as an excipient [63,69,79], which is commonly used to induce asthma in animal models [92], so is an dangerous excipient for pulmonary delivery. Albumin was also employed (Table 1) and is not on the list of excipients approved by the FDA for the inhalation route [91].

Concerning in vitro and in vivo evaluations, there is also insufficient data, as might be expected. In both cases, only about 50% of the studies make some evaluation in this regard. This means that half of the reports develop formulations, but do not assess them properly to confirm the

**Table 2**  
Main characteristics of the pulmonary formulations proposed for the described drugs.

Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	<i>In vitro</i> release	<i>In vivo</i> assays	Dosage (mg/kg/day)	Main results	Ref.
Doxycycline	DPI	MP obtained by SD DOX and ciprofloxacin MPs obtained by SD	Dispersion in water, no excipient addition	<10	N/I	4 3,7	31,5 33,6	N/I	N/I	N/I	N/I	The evaluation of the deposition of the formulation showed homogeneity in the process, allowing the delivery of two combined antibiotics to the lower respiratory tract. However, due to the limitation of the device used, multiple doses would be necessary to achieve the desired concentration.	[72]
	DPI	Polymeric MP obtained by SD DOX and ciprofloxacin polymeric MPs obtained by SD	PVA	N/I	N/I	2,15 2,21	24,5 ~26	N/I	<50% in 6 h, showing a prolonged release	N/I	N/I	The proposed formulation combined the antibacterial effect with the sustained release. Despite releasing lower doses of the drug, the system is able to do this locally, in theory increasing residence time and, consequently, therapeutic efficacy.	[58]
	DPI	Polymeric MP obtained by SD	Microcrystalline cellulose, leucine, and ethanol	3,1	4,9	N/I	49,3	N/I	~72% in 24 h, showing a prolonged release	N/I	N/I	The increase in the concentration of hydrophilic polymers forms larger and denser particles. The use of leucine generated less cohesive particles, with good yield and flow. The experimental design led to an optimized formulation of 14% microcrystalline cellulose, 33% leucine and 36% ethanol.	[59]
	N/I	MP obtained by solvent evaporation, with UV radiation and water/oil/water emulsification technique	Photoinitiator DMPA, Poly(ester-anhydride) microspheres and PVA	26,3	10,9	N/I	N/I	N/I	N/I	N/I	N/I	The variation in the concentration of photoinitiator to obtain microspheres led to porous particles, with good drug retention capacity and good flow properties, allowing their use for delivery of drugs via the lung.	[60]
	DPI	MP produced by SD using ionotropic gelification technique	Chitosan, sodium alginate, MCC, carbopol, PVP, mannitol, sodium triphosphate	N/I	2,8	2,67	72,6	N/I	~100% in 12 h	Nine times increase on Cmax of the drug at the bronchoalveolar fluid in relation to intravenous administration.	15	Microparticles were obtained combining DOX and levofloxacin with good aerodynamic properties, prolonged release, low cytotoxicity, and good fraction of the drugs reaching the lower respiratory tract in vivo after inhalation.	[61]
	Solution for nebulization	Dispersion of the drug and therapeutical adjuvant in water	Polyamino-Isoprenyl	N/I	<4,42	2,31	54	N/I	N/I	N/I	N/I	N/I	Evaluation of the proposed formulation in three different nebulizers. The PARI LC Sprint type of nebulizer showed

(continued on next page)



Table 2 (continued)

Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	In vitro release	In vivo assays	Dosage (mg/kg/day)	Main results	Ref.
												better drug distribution homogeneity and greater nebulization flow. These properties reduce administration time and generate greater comfort to the patient.	
Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	In vitro release	In vivo assays	Dosage (mg/kg/day)	Main results	Ref.
Montelukast	DPI	Microspheres by SD	Chitosan, mannitol, lecithin e glutaraldehyde	7–12	N/I	N/I	N/I	N/I	50–60% in 30 min and sustained release for up to 8 h	Increase in mucoadhesiveness at the in vitro rat intestine model along with the drug to polymer ratio increase.	N/I	It was possible to produce particles of ideal size using the spray drying technique with sustained release.	[46]
	DPI	Polymeric MP by SD	Lactose and xyloglucan	0,9-6	2,53	1,74	43,8	89,86	N/I	No difference was observed between the bioavailability of the proposed formulation and the pure drug.	1	It is expected that the formulation will show prolonged release, reducing the number of doses administered. Complementary studies are needed.	[73]
	DPI	MP by SD	NH <sub>4</sub> HCO <sub>3</sub> and ethanol	N/I	3,63	1,86	48,3	N/I	N/I	N/I	N/I	Ammonium bicarbonate showed no improvement in the formulations causing an increase in the roughness of the particles, decreasing the process yield, and also worsening aerodynamic parameters.	[74]
	DPI	MP of inhalable soft agglomerates	Mannitol, leucine, lecithin, and ovalbumin	4,28	1,68	N/I	~50	68,11	95% in 5 min	Reduction in the number of macrophages, neutrophils, and leukocytes in the bronchoalveolar lavage. Histopathology showing decreased alveoli congestion.	1	Similar responses were found in the reduction of leukotrienes and cell infiltrate in the lung with a 10-fold lower dose of the proposed formulation compared to the commercial oral formulation. The sample was able to de-agglomerate at low pressure, being compatible for aerosolization.	[69]
	DPI	Nanostructured lipid carriers obtained through fusion-emulsification-ultrasound technique	Precirol ATO-5, Capryol-90, surfactant (CAE) and mannitol	0,18	2,8	N/I	90,2	N/I	Sustained release for 24 h	The DPI presented better pharmacokinetics characteristics in relation to aqueous solution of the drug via IT	0,05	In the in vitro cytotoxicity test, the nanostructures proved to be biocompatible and non-toxic. The proposed formulation showed a greater amount of drug retained in the lung for a longer time, confirming the sustained release of the drug.	[76]
	DPI	MP produced by double emulsion followed by solvent evaporation and lyophilization	PEI, PLA, PVA	7,7	2,51	N/I	N/I	N/I	Fast release at the first 30 min and 31% after 4 days	Decrease of total proteins and proinflammatory enzymes at the bronchoalveolar lavage with a 61% decrease of the cellular infiltrate.	0,5	The produced particle showed good powder flow. Even with a 20x lower dose via the lung than the commercial oral dose, the particles were more	[77]

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Table 2 (continued)

Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	<i>In vitro release</i>	<i>In vivo assays</i>	Dosage (mg/kg/day)	Main results	Ref.
										Improvement at the histopathology and growth of the respiratory flow with 68% resistance to methacholine.		effective in treating air model animals.	
Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	<i>In vitro release</i>	<i>In vivo assays</i>	Dosage (mg/kg/day)	Main results	Ref.
Montelukast	DPI	MP produced by double emulsion followed by solvent evaporation and lyophilization	PEI, PLA or PLGA, PVA	7,5	2,5	N/I	N/I	N/I	7% at the first 30 min and 30% after 4 days	The particles formed by PLA and PEI showed higher distribution and retention on the pulmonary tissue.	N/I	The presence of PEI increases the particle size, but also the porosity and, thereby, improves the flow and aerodynamic size. Due to their larger size, these particles are also not recognized by macrophages. Finally, the particles formed with PEI had a greater release of the drug. Even with a 20x lower dose via the lung than the commercial oral dose, the particles were more effective in treating air model animals.	[71]
	pMDI	Solubilization into cosolvent and placement into propellant gas	PEG-400, ethanol and HFA-134A	N/I	1–5	1–2,18	48–58,8	80–90	N/I	N/I	N/I	The study did not present results of effectiveness of the use of the formulation in the treatment. however, it shows that the formulation did not present toxicity to macrophages in the pulmonary airways of rats or in human lung cells.	[68]
Estradiol	DPI	Porous MP by SD	Albumin, lactose and DPPC	N/I	4,1	10,1	32,87	–	N/I	In Wistar rats, 600 µg dosages showed a bioavailability of 86% and high drug systemic concentration for 5 days.	0,6	The high values of average geometric diameter and the low mass density of the formulations favoured the efficient entry of particles into the lungs and the prolonged release of the drug into the systemic circulation. Thus, the porous particles performed better compared to the conventional aerosol.	[78]
	DPI	Porous MP by SD	Albumin, lactose and DPPC	N/I	1 a 3	3 a 15	N/I	49 a 92	N/I	N/I	N/I	By varying the concentration of excipients and drying parameters, it was possible to obtain the appropriate physical properties for the DPI formulation.	[79]
	DPI	MP obtained by SD	Leucine and ethanol	2,5	2,38	0,8	73,4	85	N/I	N/I	N/I	The formulation containing 30% v/v ethanol and 6% w/w leucine showed the highest FPF value, highlighting the excellent aerodynamic	[63]

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Table 2 (continued)

Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	<i>In vitro</i> release	<i>In vivo</i> assays	Dosage (mg/kg/day)	Main results	Ref.
												properties of the obtained powder. The authors also emphasize the importance of evaluating the viscosity of the formulation before drying, since it has been shown to influence not only the size, but also the density of the dry particles obtained.	
Drug	Type of formulation	Methodology	Employed excipients	Size* (µm)	MMAD (µm)	GSD (µm)	FPF (%)	FR (%)	<i>In vitro</i> release	<i>In vivo</i> assays	Dosage (mg/kg/day)	Main results	Ref.
Oseltamivir	DPI	Film dispersion followed by SD	Lactose, L-leucine and mannitol.	3,5	N/I	N/I	35,4	N/I	Sustained release for 20 h	Marked improvement of 1,14 to 1,22 times at the ASC and Cmax in comparison to the orally administrated solution with the same dosage.	12	The proposed formulation was able to reach the lung with a lower dose and less adverse effects compared to the oral solution.	[50]
	Solution for nebulization	N/I	N/I	N/I	N/I	N/I	N/I	N/I	N/I	Increment of 842 times at the ASC in comparison to intravenous administration of the drug.	6	ASC of epithelial lining fluids was 842 times higher by nebulization than plasma ASC by intravenous administration, indicating a great biopharmaceutical advantage in pulmonary administration.	[80]

DPI: dry powder inhaler; pMDI: pressurized metered-dose inhaler MP: microparticles; SD: spray-dryer; MMAD: median mass aerodynamic diameter; GSD: median geometric diameter; FPF: fine particles fraction; FR: relevant respirable fraction; DOX: doxycycline; UV: ultraviolet; PVA: poly (vinyl alcohol); MCC: microcrystalline cellulose; PVP: polyvinylpyrrolidone; PLA: poly (lactic acid) PLGA: poly(lactic-co-glycolic acid); PEI: polyetherimide; AUC: area under curve; C<sub>max</sub>: maximum plasma concentration; DPPC: dipalmitoyl phosphatidylcholine; IT: intratracheal; PEG-400: polyethylene glycol-400; N/I: not informed.

**Table 3**  
Quantitative evaluation of the formulation parameters.

Drug	Number of studies	Formulation		Spray-drying	Microparticles	Parameters					Ref.	
		DPI	Solution/suspension for nebulization			Particle size	FPF	FR	In vitro assays	In vivo assays		Dosage
Doxycycline	6	4	1	4	5	3	5	0	3	1	1	[58–62,72]
Estradiol	3	3	0	3	3	1	2	2	0	1	0	[63,78,81]
Montelukast	8	7	0	3	7	6	5	3	5	6	4	[46,68,71,69,73,74,76,77]
Oseltamivir	2	1	1	1	1	1	1	0	1	2	2	[50,80]
Total	19	78,9%	10,5%	57,9%	84,2%	57,9%	68,4%	26,3%	47,4%	52,6%	36,8%	

DPI: dry powder inhaler; MP: microparticles; SD: spray-dryer; FPF: fine particles fraction; FR: relevant respirable fraction.

expected effects. In addition, even fewer are the articles that performed both in vitro and in vivo tests (Table 3), a sign that the majority did not seek a correlation between them. With this gap, it appears that the correlation between the physicochemical characterization of formulations and their biological impact is not a standard goal in academic work. It should also be noted that in the case of in vitro tests, there is no standardization between them, either in terms of the type of study, or in relation to the parameters used in the measurement (Table 2).

It is important to stand out that the dissolution test, which is extensively used for oral administration drugs, does not have a standard pharmacopeial methodology or aerosols' regulatory requirements. One of the main difficulties in standardizing an effective dissolution method for inhaled formulations is that the drug dissolution process in the lung has many peculiarities, such as the extremely low volume of pulmonary fluid (10–30 mL), the variable amount of particles reaching the lung, which is not equivalent to the total amount of particles inhaled, the pulmonary fluids, which are composed of several surfactants, among others [93,94]. Therefore, an ideal method should perform, before the dissolution itself, the collection of the relevant dosage respirable fraction (RF), in addition to using the appropriate components to simulate the lung environment in vivo [94].

However, among studies evaluated in this paper, less than 30% investigated RF, and for both doxycycline and oseltamivir, none of the proposed formulations were evaluated regarding this parameter (Table 3). On the other hand, the fine particles fraction (FPF) was assessed in more than 65% of the studies (Table 3). This parameter deals with the percentage of particles smaller than a certain size, in micrometers, according to the type of impactor used for the test [94]. Beside de FPF has been measured in 65% of the articles, the use of these information for in vitro and in vivo study are not sufficient, since RF refers to the ratio between FPF and the recovered dose [94]. So ideally, it is necessary to calculate both parameters. According to the FDA, the isolated assessment of MMAD, GSD or FPF values cannot be considered appropriate, so, the acceptance criteria should be based on the amount of drug retained in each one of all the stages of the cascade impactor [87].

It is important to highlight that advances in academic and scientific scenarios do not always follow the regulatory scope and vice versa. There are many studies showing promising results but that do not detail important aspects that would be necessary in the case of registering a drug product. On the other hand, the legislation already available on this subject does not always present a more detailed framework in the light of scientific advances in the area. These divergences are usually more common in developing countries, whose regulatory scenario is still in consolidation [95].

Thus, at least in the universe of drugs defined in the present study, scientific articles do not always consider the current regulatory guidelines, that standardized tests in pharmacopoeias are not followed and that there is a considerable range of articles with insufficient results to prove their assumptions regarding the benefits of the formulations developed. It can be concluded, therefore, that although there has been

some progress in the development of formulations for the pulmonary route with potential impact in the treatment of COVID-19, there is still a lot to be done, especially regarding projects that reflect the regulatory scope and that aim industrial application of the results.

#### 4. Conclusion

The pulmonary administration of drugs is a route that presents quite satisfactory results for respiratory diseases and, since that region is the gateway to the new coronavirus, the repositioning of drugs for administration throughout this route is an interesting strategy.

However, the present study showed that pulmonary formulations containing promising drugs for the treatment of COVID-19 are still scarce and almost all of them are not always based on current regulatory guidelines. In addition, many of the articles found did not perform a complete evaluation of their effectiveness, lacking results that prove the benefits of the formulations in question.

It is important to highlight that based on emergency of COVID-19 pandemic, which needs some fast solution, this paper focused on molecules who were already being evaluated in clinical trials. However, in the future it is necessary also evaluate molecules with potential for specific anti-coronavirus molecular actions that are not necessarily in ongoing clinical trials.

Despite this uninspiring scenario, it is believed that the pandemic represents an opportunity for investment in research into alternative routes of release, stimulating the research and the investment by the pharmaceutical industry. Finally, it is emphasized the need for the development effort to be accompanied by greater attention to the regulatory scope, in order to facilitate the registration of such products and their earlier market release.

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#### Author contributions

**Michelle Alvares Sarcinelli:** Conceptualization, Methodology, Investigation, Writing- Original draft preparation, Writing - Review & Editing, Visualization, Supervision **Thalita Martins Silva:** Conceptualization, Methodology, Investigation, Writing- Original draft preparation, Writing - Review & Editing, Visualization **Andressa Daniele Artico Silva:** Conceptualization, Methodology, Investigation, Writing-Original draft preparation, Writing - Review & Editing, Visualization **Beatriz Ferreira de Carvalho Patricio:** Conceptualization, Methodology, Investigation, Writing- Original draft preparation, Writing - Review & Editing, Visualization **Flávia Costa Mendes de Paiva:** Methodology, Investigation, Writing- Original draft preparation **Raissa Santos de Lima:** Methodology, Investigation, Writing - Review & Editing **Manuela Leal da Silva:** Methodology, Investigation, Writing - Review & Editing

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#### Declaration of competing interest

None.

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