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Research article

Reduction in the COVID-19 pneumonia case fatality rate by silver nanoparticles: A randomized case study

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has devastated mankind. To date, no approved treatment is available to completely combat this disease. Although many studies reported the potential of silver nanoparticles' (AgNPs) action mechanism and effect against SARS-CoV-2, this is the first clinical trial that aimed to prove this effect. This open-label, randomized, parallel-group, investigator-initiated study (IIS) was conducted in India from 2021 to 2022 and included 40 patients diagnosed with moderately-severe to severe COVID-19 pneumonia. This study proved a significantly higher survival rates ($p < 0.05$) and significantly lower number of days until supplemental oxygenation was required ($p < 0.0001$) for patients receiving intravenous AgNPs in form of AgSept® in addition to the standard COVID-19 treatment. This study highlights the importance of intravenous AgNPs administration in the treatment of virus-induced pneumonia.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) has devastated mankind. First detected in China in November 2019, over 620 million cases and 6.5 million deaths were reported worldwide by September 2022 [1]. Studies have demonstrated that all countries, including India, have been affected with many cases [2]. As a highly transmissible disease, spread of the virus can occur via the release of aerosols during sneezing and coughing from an affected individual [3]. COVID-19 can cause a wide range of symptoms such as cough, fever, cold, sore throat, headache, conjunctivitis, or loss of appetite [4,5]. In severe cases it can lead to severe respiratory distress with ground glass opacity of the lungs and multiorgan failure [4,5]. Different comorbidities, such as chronic obstructive pulmonary disease, chronic kidney and liver disease, diabetes, and hypertension as well as older age and male sex can lead to increased mortality rates [6,7]. Therefore, despite the different treatment protocols applied, a wide range of case fatality rates of 11%–62% was observed worldwide [7]. A prospective analysis in India reported older age as a high-risk factor for mortality, with increased mortality rates of 56.5% after 30 days [8].

Currently, prophylactic vaccination against SARS-CoV-2 is considered the only effective way to prevent the progression of COVID-19 to severe disease [9]. Oxygen therapy, corticosteroids and antithrombotic medicines are the only drugs proven to be effective for

Abbreviations: AgNPs, silver nanoparticles.

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COVID-19 infection [10]. Nevertheless, only low-dose steroid treatment proved to be effective by decreasing the death rate by one-third among ventilated severe COVID-19 patients [11]. Although the broad-spectrum antiviral remdesivir has been approved for emergency use in COVID-19 patients, it showed mixed results [12] and was subsequently discarded.

Recent studies [13–15] have identified nanotechnology products (nanomaterials) as possible therapeutic agents against SARS-CoV-2 due to their extensive broad antiviral activity. Nanomaterials are defined as structures approximately 1–100 nm (nm) in size and are used in many parts of our lives, such as agriculture, electronics, imaging and medical purposes. Most recent applications are in the fields of medical implants, imaging and detection systems, cancer treatment, and vaccines. To date, the Federal Drug Agency (FDA) and the European Medicines Agency (EMA) have approved approximately 58 nanoparticle-based therapies and imaging agents [13–15].

In comparison to their corresponding chemical elements at higher scales, nanoparticles possess a unique profile of physical, chemical, and biological properties, which is based on their higher surface-to-volume ratio [16,17]. Silver nanoparticles (AgNPs) have well-established antimicrobial and antifungal effects [18]. The antiviral activity of AgNPs against different types of viruses, including human immunodeficiency virus, influenza virus, hepatitis B virus, monkey-pox virus, herpes simplex virus, tacaribe virus, and other respiratory viruses, has been investigated [19,20]. The antiviral effects of AgNPs may be due to the binding of AgNPs to the surface glycoproteins of RNA viruses preventing the fusion of the virus to host cells [21]. By binding to those surface proteins, receptors on the viral surface are blocked and the membrane potential is altered leading to a decrease in virus penetration [22]. In addition to interference with the viral attachment process and penetration into host cells, AgNPs can interfere with the viral genome and thus, block the viral replication inside the host cell [23]. This action is either directly or by the production of reactive oxygen species (ROS), which can interact with biomolecules [24]. Moreover, several studies have indicated the effect of AgNPs on hemagglutinin and neuraminidase proteins of viruses, which are the main factors in pathogenicity [25,26]. In an in vitro study on a Vero cell line infected with SARS-CoV-2, AgNPs 10 nm in size were found to inhibit viral replication with minimal toxicity [27]. In the A549 epithelial cell line, AgNPs with a 10–12 nm size distribution at a dose of 50 μg/ml have shown maximum antiviral properties without toxicity [28].

In addition to antiviral activity, the anti-inflammatory, antiplatelet, angiogenesis, and anticancer properties of AgNPs have been widely described [19]. A recent study indicated that AgNPs administration in mice resulted in a significant reduction in proinflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), chemokine ligand 5 (CCL5), and interferons (IFNs) [29]. In a preclinical study, BALB/c mice were inoculated with AgNPs and respiratory syncytial virus (RSV), and significant

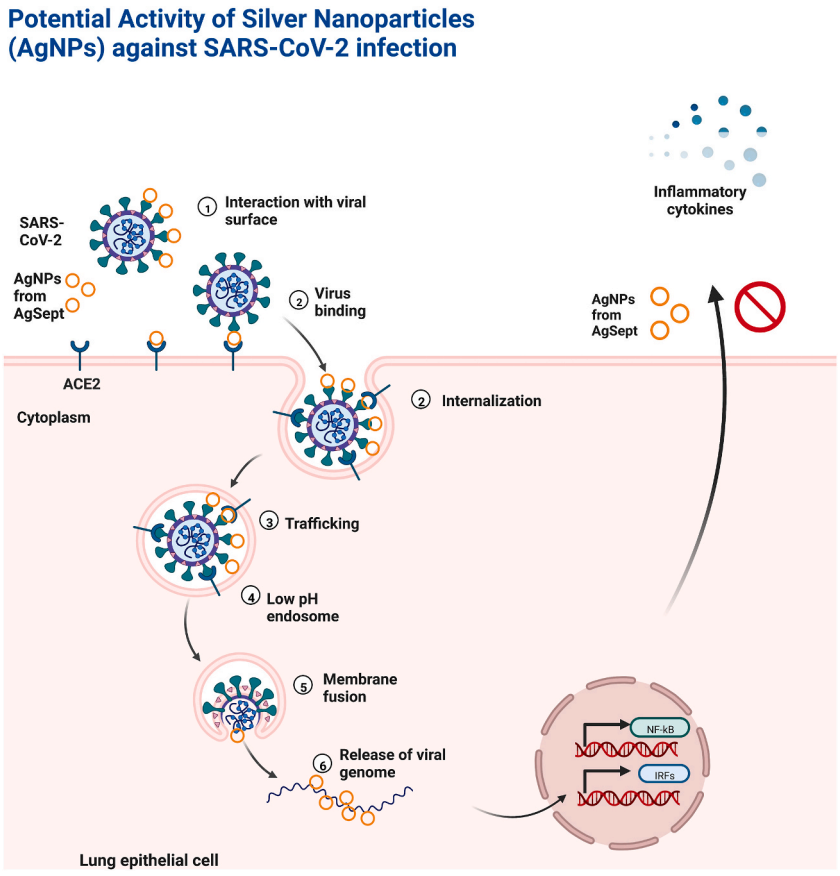

Fig. 1. Potential activity of silver nanoparticles (AgNPs) against SARS-CoV-2 infection. Points of mode of action are depicted. Created with BioRender.com.

antiviral and immunomodulatory effects were observed. AgNPs up to a dose of 4 mg/kg of body weight were used without any significant toxicity [30]. Remarkably, the application of AgNPs as adjuvants in vaccines against respiratory viruses has been reported [31]. In this mouse trial, AgNPs led to bronchus-associated lymphoid tissue neogenesis with increased antigen specific immunoglobin A production [31].

Many researchers have summarized the potential of AgNPs in COVID-19 treatment [13,19,20,27,32,33]. Fig. 1 schematically represents the hypothesized function of AgNPs as an additional treatment for COVID-19. The antiviral activity, ranging from interaction with the viral surface and interference in viral binding to trafficking and binding to the released viral genome, is depicted. Moreover, the capability of AgNPs to abrogate inflammatory cytokines terminating inflammation and fibrosis in COVID-19 (reviewed in Ref. [24]) is summarized (Fig. 1).

This current investigator-initiated study (IIS) is the first clinical trial that aimed to use AgNPs administered intravenously, as adjuvant treatment, in addition to the standard of care, to treat moderate-severe to severe COVID-19-induced pneumonia. We hypothesize that AgNPs might reduce mortality among COVID-19 patients due to their combined antiviral, anti-inflammatory, anticoagulant and antimicrobial properties.

2. Methods

2.1. Study design

This was an open, randomized, parallel-group, active-controlled, investigator-initiated study (IIS) that took place in India and recruited patients with moderately severe and severe COVID-19 pneumonia in June 2021 and January 2022, during the Delta variant (B.1.617.2), and Omicron variant (B.1.1.529) peak waves of the pandemic. This study was designed to determine the efficacy and safety of investigational product (IP) AgNPs (AgSept®) used as an additional treatment to the standard of care for COVID-19 patients. This study was conducted in the general COVID wards of the College of Medicine and Sagore Dutta Hospital, Kolkata, India. All patients received standard therapy for moderately severe or severe COVID-19 pneumonia, which included oxygen treatment, corticosteroids, antibiotics, low-molecular-weight heparin or other forms of anticoagulation, and medications for concomitant diseases. This study included 40 patients with moderately severe or severe COVID-19 pneumonia, who met the protocol criteria. The patients were randomized at a 1:1 ratio, using the block randomization method. Half of the patients (20 patients) received additional treatment with AgNPs, while the other half did not receive any additional treatment. Each treatment group consisted of 10 patients diagnosed with the Delta variant and 10 patients with the Omicron variant of SARS-CoV-2. This sample size was chosen for the pilot study to identify

Physiological Parameters	3	2	1	0	1	2	3
Respiration Rate (BPM)	≤8		9-11	12-20		21-24	≥25
Oxygen Saturations (%)	≤91	92-93	94-95	≥96			
Any Supplemental Oxygen		Yes		No			
Temperature (°C)	≤35		35.1-36.0	35.1-38.0	38.1-39.0	≥39.1	
Systolic Blood Pressure (mmHg)	≤90	91-100	101-110	111-219			≥220
Heart Rate (BPM)	≤40		41-50	51-90	91-110	111-130	≥131
Level of Consciousness				A			V, P or U
Age*	≥65*				≤65*		

Fig. 2. Comparison between the Indian and the national EWS (NEWS2)s system. Parameters included in score system are depicted. Abbreviations: P: responsive to pain stimuli, U: unconscious; V: responsive to verbal stimuli. Asterisk(*) indicates that this value is only included in the Indian version of the EWS.

preliminary results.

The severity of COVID-19 was assessed with early warning scores (EWS). Several studies have highlighted the impact of monitoring the prognosis of severity of an COVID-19 infection by the national early warning score and its modification national early warning score-2 (NEWS2) [34–37]. It was demonstrated that one of the best performing models to predict ICU admittance for COVID-19 patients is the NEWS2 score [37]. With a threshold NEWS2 score ≥5, the sensitivity and specificity of 84.7% (95% CI 78.9%–89.4%) and 44.3 (95% CI 41.5%–47.0%), respectively were calculated regarding prediction of critical illness within 24 h after presentation. A NEWS2 of 5 or more at admission can prognosticate poor outcomes [35]. The recruiting site used the data collected by the Indian EWS system, which were subsequentially converted into a NEWS2 for statistical purposes. The difference between the Indian EWS and the NEWS2 consisted of the "age" parameter, which is found only in the Indian EWS (Fig. 2).

This study included adult patients of both sexes with a positive real-time reverse transcription polymerase chain reaction (RT-PCR) test for qualitative detection of nucleic acids from SARS-CoV-2 who were clinically diagnosed with moderately-severe or severe COVID-19 pneumonia and had an Indian EWS ≥5. All patients included in this study signed the approved inform consent form. If patients were unconscious, relatives with respective decree were obtained for consent.

This study included the following visits: screening visit including hospital admission and the start of standard COVID-19 treatment; randomization visit at the start of the study-specific treatment with AgNPs; and follow-up visits (on days 1, 3, 5 and 30 after randomization). A schematic representation of the recruiting process and the trial design is depicted in Fig. 3.

All patients received standard treatment for moderately-severe or severe COVID-19 pneumonia, according to the judgment of the investigator and in line with the national guidelines or best standard of care. In addition to this standard treatment, after randomization, half of the patients received AgNPs (1.8 mg dissolved in 500 ml of normal saline solution) delivered intravenously within 30 min infusion, for three consecutive days, taking all aseptic precautions. The total quantity of AgNPs administered in the study was 5.4 mg/patient, which represented 30% of the human equivalent dose (HED).

To define the HED of the AgNPs, preclinical studies were used to determine no adverse effect level (NOAEL). Morris et al. defined an intravenous dosage of 4 mg/kg of body weight in BALB/c mice [29].Using this value, the systemic dose of AgNPs for the human model was calculated based on the following formula [38].

The HED was calculated considering the reference body weight of a mouse of 0.02 kg, reference body weight of a human as 60.0 kg and a NOAEL of 4 mg/kg.

$$HED\left[\frac{mg}{kg}\right] = animal\ NOAEL\left[\frac{mg}{kg}\right] x \frac{weight_{animal}\,[kg]^{(1-0.67)}}{weight_{human}\,[kg]} \quad (1)$$

Equation (1). Calculation of the human equivalent dose (HED).

Using Equation (1), the HED was calculated as 0.3 mg/kg, whereas considering a 60 kg patient, the HED would be 18 mg. The systemic intravenous route of delivery was chosen due to the patients' status, and the AgNPs dose for this study was calculated as 30% of the HED to prevent any potential toxicity.

AgNPs were supplied by BHS Medical Solutions GmbH, Germany in the form of the AgSept® product used for research purposes. AgNPs were 99.99% pure, with a size distribution of 10 nm, spherical shape and good water solubility. The concentration of AgNPs was 1000 parts per million (ppm) in AgSept®.

All patients were clinically monitored for consciousness level, blood pressure (BP), heart rate (HR), oxygen saturation (SpO_2), respiratory rate, body temperature, status at the AgNPs infusion site and routine laboratory tests. Patients whose health status improved and were hemodynamically stable were discharged according to the hospital guidelines. All patients were observed until

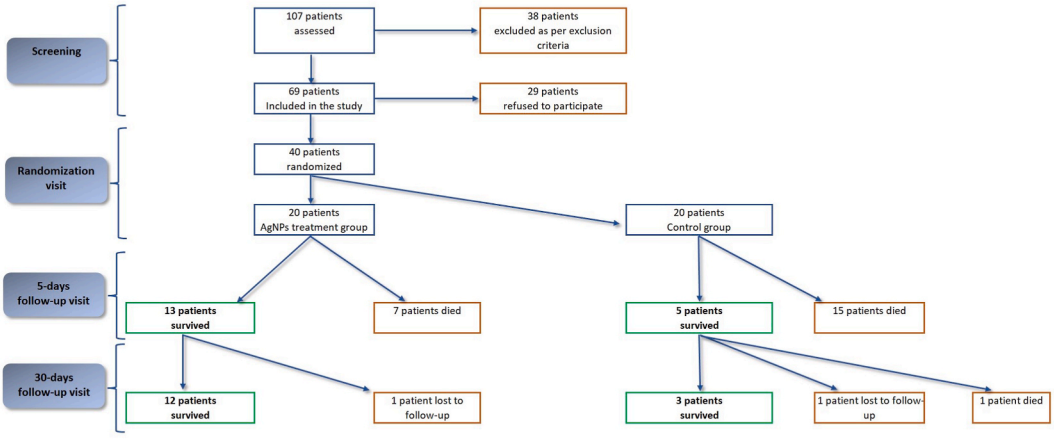

Fig. 3. Clinical trial design and patient recruitment process.

discharge and followed up for 30 days.

The primary study objective was to determine the efficacy of the given AgNPs in addition to standard therapy for treating moderately-severe to severe COVID-19 pneumonia patients with or without comorbidities, in terms of hemodynamic stability, oxygen requirement, duration of hospital stay and mortality prevention. The secondary objective was to determine the profile of intravenous use of AgNPs on changes in clinical findings (pulse rate, BP, oxygen saturation, and respiratory rate), as well as the blood parameters and collection of safety data.

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Institutional Ethics Committee, College of Medicine and Sagore Dutta Hospital, India. This study was registered in the Clinical Trial Registry of India (CTRI) with the number CTRI/2021/09/036781.

2.2. Statistical information

Statistical analyses were performed using the Statistical Analysis System (SAS) statistical software package, version 9.4 (SAS Institute Inc., Cary, NC, USA). The baseline variables were compared by the independent sample Wilcoxon-test for continuous variables and the chi-square test (Fisher's exact test was applied if the cell size was smaller than 5) for nominal variables. Kaplan-Meier analysis was performed, and groups were compared by using the log rank test. Two Cox regression models were used to compare treatment groups, including the baseline national and Indian EWSs as explanatory variables. Mean values for laboratory tests (Day 1 and Day 3) were compared between treatment groups by two-sample t tests. The survival rates were analyzed by Fisher's exact test. The P values were determined as indicated in each figure legend. P values < 0.05 were considered significant.

3. Results

3.1. Baseline results

This IIS included 40 patients diagnosed with moderately severe or severe COVID-19 pneumonia, predominantly males (75% of participants) with a mean age of 69.5 ± 13.5 years. There were no significant differences between the study groups with regard to the severity of COVID-19-induced pneumonia, consciousness status, concomitant diseases and vital signs, except for concomitant pulmonary disease and body temperature at inclusion (Table 1).

3.2. Efficacy results

The efficacy of the treatment was calculated using the 5-day and 30-day survival rates, health status improvement, period of time

Table 1
Baseline characteristics.

	Female		Male		Total		p value[a]
	AgNPs treatment group	Control group	AgNPs treatment group	Control group	AgNPs treatment group	Control group	
Consciousness N (%)							
Conscious	3 (60%)	2 (40%)	5 (33.33%)	10 (66.67%)	8 (40%)	12 (60%)	0.2059
Unconscious	2 (40%)	3 (60%)	10 (66.67%)	5 (33.33%)	12 (60%)	8 (40%)	
Concomitant diseases N (%)	5 (100%)	5 (100%)	14 (93.33%)	13 (86.67%)	19 (95%)	18 (90%)	1.0000
Diabetes	4 (80%)	5 (100%)	11 (73.33%)	11 (73.33%)	15 (75%)	16 (80%)	1.0000
Hypertension	4 (80%)	2 (40%)	11 (73.33%	8 (53.33%)	15 (75%)	10 (50%)	0.1025
Coronary artery disease	1 (20%)	0 (0%)	3 (21.43%)	0 (0%)	4 (21.05%)	0 (0%)	0.1050
Pulmonary disease	1 (20%)	0 (0%)	3 (21.43%)	0 (0%)	4 (21.05%)	0 (0%)	0.0471
Vital signs, Mean ± SD							
Temperature	37.3 ± 0.8	37.6 (1.4)	36.7 (0.4)	37.7 (1.0)	36.9 (0.6)	37.7(1.1)	0.0374
Respiratory rate	21.2 ± 3	19.2 (1.1)	20.4 (2.7)	20.3 (2)	20.6 (2.7)	20.1 (1.8)	0.4745
SpO2	89.2 ± 6.7	93 (6.6)	91.6 (3.9)	93.3 (2.7)	91 (4.6)	93.3 (3.8)	0.0635
SBP (mm/Hg)	128.4 ± 34.7	123 ± 11.1	120.3 ± 18.5	121.7 ± 17.2	122.4 ± 22.7	122 ± 15.7	0.9035
DBP (mm/Hg)	76.8 ± 19.4	74 ± 4.9	71.1 ± 16.6	74.1 ± 7.9	72.6 ± 17	74.1 ± 7.1	0.2971
HR beats/min	98.2 (13.6)	85.2 (9.4)	91.9 (14.3)	87 (16.1)	93.5 (14.1)	86.6 (14.5)	0.0771
EWS at inclusion (national)	8.4 ± 4.1	6.2 ± 1.9	7.9 ± 2.2	6.9 ± 2.7	8.0 ± 2.7	6.8 ± 2.5	0.1299
EWS at inclusion (Indian)	9.6 ± 3.3	9.2 ± 1.9	10.3 ± 1.7	9.1 ± 2.4	10.1 ± 2.1	9.2 ± 2.2	0.1911

Abbreviations: AgNPs: silver nanoparticles; EWS: early warning score; DBP: diastolic blood pressure; HR: heart rate; SpO2: oxygen saturation; SBP: systolic blood pressure.

[a] The P values were determined using the Wilcoxon test for continuous variables and chi-square or Fisher's exact test for frequencies.

until intensive care unit (ICU) discharge, need and duration of O_2 supplementation, and evolution of the laboratory results.

When analyzed as stand-alone values, for both, the 5-day and 30-day survival rates, there were significant differences between the groups, in favor of the AgNPs treatment group ($p < 0.05$). Within the AgNPs treatment group, 13 patients were alive on Day-5, while in the control group, only 5 patients survived. By Day-30, two patients were lost to follow-up (one patient in each study group), and 12 patients in the AgNPs treatment group; and 3 patients in the control group were alive (Table 2).

When severity EWSs were added as an explanatory factor, for both, national (NEWS2) and Indian EWSs, the 5-day survival rate showed a significant difference in favor of the AgNPs treatment group ($p < 0.05$) (Table 3).

We analyzed the mortality rates by Day-5, based on the national EWS (NEWS2) ranking and observed that they were lower within the AgNPs treatment group (Fig. 4).

Supplemental oxygenation with a low-flow system via nasal cannula was required for all patients starting at inclusion. On the first day of treatment (Day-1) all patients received supplemental oxygenation, while only one patient in the control group needed assisted ventilation. A significant difference between the treatment groups was observed with regard to the number of days until supplemental oxygenation was required ($p < 0.0001$). Supplemental oxygenation need was analyzed among surviving patients on Day-5. At this timepoint, supplemental oxygenation was needed by 3 out of 5 surviving patients (60%) in the control group, while none of the 13 surviving patients from the AgNPs treatment group required support ($p = 0.0020$) (Table 4). Interestingly, in the AgNPs treatment group the need for supplemental oxygenation decreased from Day-1 to Day-5 (Fig. 5).

All patients who survived the 5 day period showed improvement on Day-5, which was also confirmed by Day-30 follow-up survival data. It should be mentioned that despite COVID-19 pneumonia severity, due to the limited bed capacity in the ICU during the peak outbreak period in India, none of the patient were hospitalized in the ICU on Day 1, so the ICU length of stay parameter was not calculated.

The evolution in the laboratory results showed only minor differences between the groups during the hospital stay, with no statistical significance except for the creatinine results (Day-3, $p < 0.05$), and direct bilirubin values (Day-3, $p < 0.05$) (Table 5).

3.3. Safety results

Safety data were collected during the hospital stay and at the telephonic follow-up visit on Day-30. There were no reported adverse events related to AgNPs administration during the observation period.

4. Discussion

The current IIS is the first clinical trial examining the efficacy and safety of intravenous administration of AgNPs in the treatment of adult patients diagnosed with moderately severe and severe COVID-19 pneumonia that collected survival data for at least 30 days after the first signs of the disease and confirmatory positive PCR tests. The treatment agent was selected according to available preclinical and in vitro studies on efficacy and safety data of AgNPs use for different viral infections, including coronaviruses [27]. The use of AgNPs in the medical field has recently gained interest, and various researchers have addressed their potential as additional drugs in the treatment of COVID-19 [14,19,39].

This study included elderly patients with severe COVID-19 pneumonia (mean age 69.5 ± 13.5 years), who were predominantly male (75.0%) and unconscious (50.0% of the total group; 60.0% in the AgNPs treatment group, respectively 40.0% in the control group), with mean NEWS2s of 8.0 ± 2.7 for the AgNPs treatment group, and 6.8 ± 2.5 for the control group, and multiple comorbidities [diabetes (77.5%), and HBP (62.5%)] as aggravating factors for COVID-19 pneumonia evolution. All patients included received treatment as per national COVID-19 treatment guidelines [36]; nevertheless, despite the severity of the COVID-19 pneumonia at presentation, due to the limited ICU bed capacity, none of the included patients were treated in the ICU. Additionally, at inclusion, there were no significant differences observed between the AgNPs treatment group and the control group.

There are multiple lines of evidences that elderly patients, due to age-dependent decline in immunity corroborated with multiple comorbidities, are commonly affected by severe forms of COVID-19 pneumonia and old age is a significant predictor of mortality from

Table 2
The 5 -day and 30-day survival rates.

	Statistics	AgNPs treatment group	Control group	Total	P[a]
		(N = 20)	(N = 20)	(N = 40)	
Patient alive on Day 5 N (%)	**Yes**	13 (65%)	5 (25%)	18 (45%)	0.0110
	No	7 (35%)	15 (75%)	22 (55%)	
		AgNPs treatment group (N = 19)[c]	**Control group (N= 19)**[c]	**Total (N= 38)**	**P[b]**
Patient alive on Day 30 N (%)	**Yes**	12 (63.2%)	3 (15.8%)	15 (39.5%)	0.0069
	No	7 (36.8%)	16 (84.2%)	23 (60.5%)	

Abbreviations: AgNPs: silver nanoparticles.
[a] The P values were determined using the chi-square test.
[b] The P values were determined using Fisher's exact test.
[c] At the 30-day evaluation, two patients were lost to follow-up, one in each group.

Table 3
Survival rates (Day-5).

Statistics	AgNPs treatment group	Control	P[a] value using the national EWS		P[a] value using the Indian EWS	
	N = 20	N = 20	Treatment allocation and NEWS2 score	Baseline NEWS2 score	Treatment allocation and Indian EWS score	Baseline Indian EWS
No. of surviving patients on Day 5, N (%)	13 (65%)	5 (25%)	0.0097	0.0028	0.0211	0.0036

Abbreviations: AgNPs: silver nanoparticles; EWS: early warning score, NEWS2: national EWS modification 2.
[a] The p values were determined using two Cox regression models, that included the baseline National, and Indian EWSs as explanatory variables.

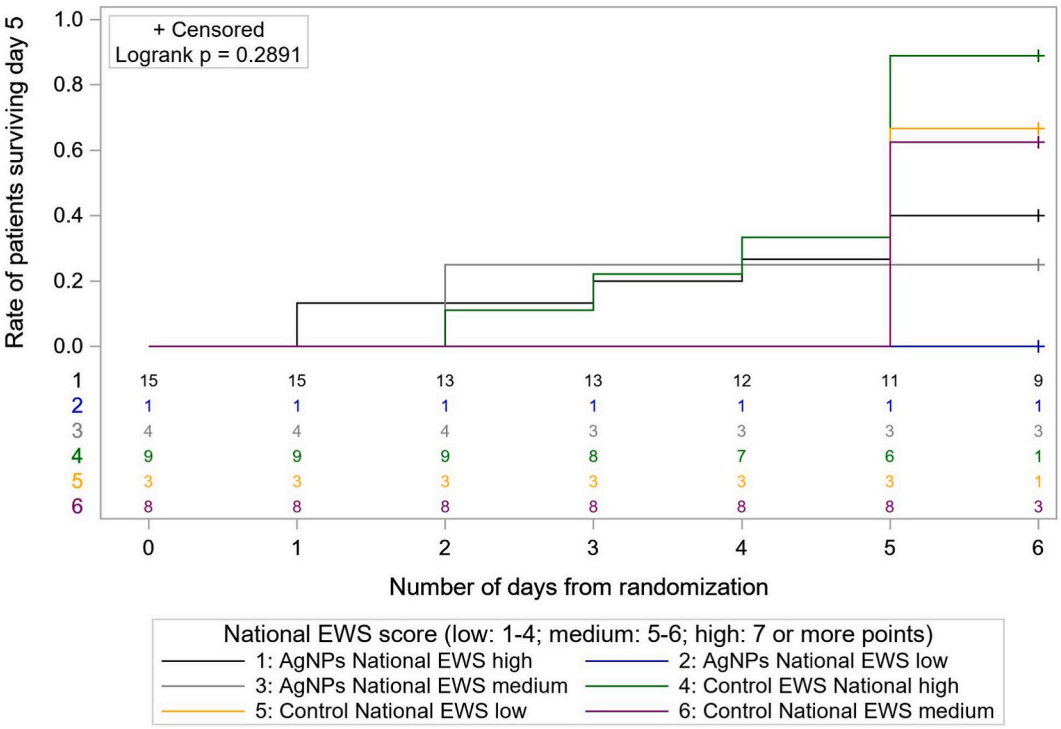

Fig. 4. 5 -day survival and national EWS score ranges for case group (AgNPs, lines 1 to 3) and control group (line 4 to 6).

COVID-19 pneumonia [8,37–39].

In this study, it was noticed that on day 5, survival was significantly higher in the group receiving AgNPs in addition to the standard of care for COVID-19 (65.0% of patients survived), than in the control group, which received only standard treatment for COVID-19 (25.0% of patients survived) ($p < 0.05$). The same trend was maintained at the 30-day follow-up, when in the AgNPs treatment group, 63.2% of patients survived compared to 15.8% in the control group ($p < 0.05$).

The 30-day mortality rates observed for the total group were 60.5%, higher than those mentioned in other larger studies from India, in which mortality rates were 56.6% among severe COVID-19 cases [8]. This difference might be explained by the fact that in the current study, none of the patients had access to the ICU due to limited bed capacity, and the included population was older overall and had more comorbidities. Nevertheless, when 30-day mortality was evaluated only for patients receiving AgNPs in addition to standard treatment for COVID-19, survival rates were higher (84.2%) than those mentioned in the medical literature, suggesting an adjuvant effect of AgNPs when used in addition to standard treatment for COVID-19.

There were significant differences between the groups in regard to the need and the length of supplemental oxygenation ($p < 0.05$), in favor of the AgNPs treatment group, in which the mean duration of supplemental oxygenation was 3.2 ± 1.09 days and none of the patients required supplemental oxygenation on Day-5. In contrast, in the control group 3 out of the 5 surviving patients required supplemental oxygenation on Day-5.

Laboratory test evolution during hospitalization identified very few significant differences between the groups, namely in the mean values for creatinine, total bilirubin, direct bilirubin, and hemoglobin ($p < 0.05$).

The effects observed for the intravenous use of AgNPs might be explained by their already known extensive broad antiviral, anti-inflammatory, antiplatelet and antimicrobial activities [19].

Table 4
Supplemental oxygenation.

	Supplemental oxygenation Total group		Supplemental oxygenation Patients alive on Day 5	
	Results of the Kaplan-Meier analysis			
Statistics	**AgNPs treatment group N=20**	**Control N=20**	**AgNPs treatment group N=13**	**Control group N=5**
Number of patients who stopped requiring supplemental oxygenation during the study	14	8	13	2
Number of patients who never stopped requiring supplemental oxygenation during the study	6	12	0	3
Time supplemental oxygenation was required (number of days after randomization)				
Mean (SD)	3.2 (1.05)	5.0 (0.00)	3.2 (1.09)	5.0 (0.00)
Median	3	.	3	.
(95% confidence interval)	(3, 5)	(5, NC)	(2, 3)	(5, NC)
25th–75th percentile (Q1-Q3)	3–5	5 - NA	3–3	5 - NA
Minimum–Maximum	2–5	5–5	2–5	5–5
	P-value[a] <0.0001		P-value[a] 0.0033	

[a] The P values were determined using the Wilcoxon test.

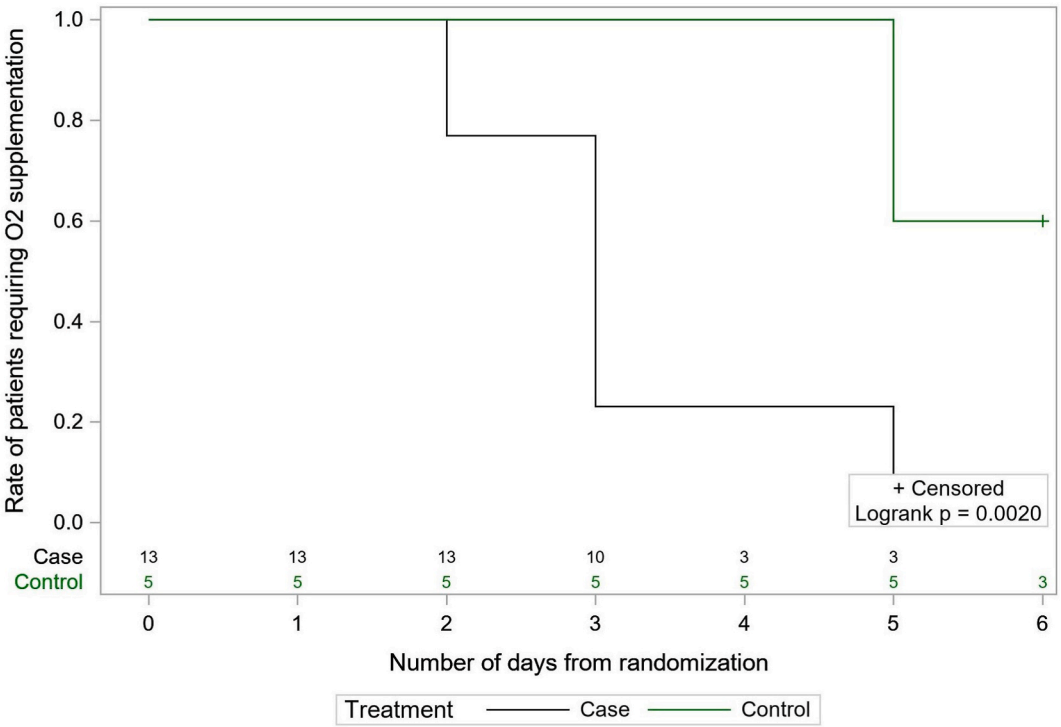

Fig. 5. Supplemental oxygenation needs during hospitalization for surviving patients on Day 5.

There were no adverse events observed in this study, which is in line with other study data in which commercially available oral doses of AgNPs were used with daily ingestion rates of 100 μg/day for 10 ppm of AgNPs and 480 μg/day for 32 ppm of AgNPs, resulting in no significant clinical changes in physical findings, morphology or metabolic, hematologic, or urine profiles [40]. Additionally, the AgNPs dose used for the current study, was selected based on data from in vivo studies in which AgNPs with a 10–12 nm size distribution at a dose of 50 μg/ml showed maximum antiviral properties without toxicity [28]. In addition, the AgNPs dose was calculated as only 30% of the HED (3 doses of 1.8 mg/day = 5.4 mg/patient) for minimal toxicity risks, and the AgNPs were 99.99% pure at a concentration of 1000 ppm, and a size distribution of 10 nm, spherical shape, with good water solubility.

Table 5
Laboratory parameters assessed during the study.

Parameter (Mean ± SD)		Assessment time		
		Screening	Day 1	Day 3
Creatinine (mg/dl)	AgNPs group	1.2 ± 1	1.3 ± 1	0.9 ± 0.3
	Control group	1.1 ± 0.8	1.2 ± 1	1.4 ± 0.9
	P[a] value	0.6245	0.8157	0.0330
SGOT (U/L)	AgNPs group	56.4 ± 38.1	51.6 ± 26	46.7 ± 19.8
	Control group	51.3 ± 32.1	52.7 ± 27.1	56.1 ± 18.8
	P[a] value	0.6524	0.9046	0.1645
SGPT (U/L)	AgNPs group	54.9 ± 39.3	51.9 ± 38.5	45.6 ± 18.4
	Control group	47.7 ± 28.8	49.6 ± 24.8	54.3 ± 19.6
	P[a] value	0.5104	0.8198	0.1899
Alkaline phosphatase (U/L)	AgNPs group	70.3 ± 22.3	69.3 ± 24.1	59.4 ± 16.3
	Control group	65.7 ± 27.8	64.5 ± 25.9	67 ± 13.6
	P[a] value	0.5642	0.5503	0.1531
Total bilirubin (mg/dl)	AgNPs group	1.1 ± 0.7	0.9 ± 0.4	0.9 ± 0.4
	Control group	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.4
	P[a] value	0.9098	0.0982	0.0455
Direct bilirubin (mg/dl)	AgNPs group	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
	Control group	0.4 ± 0.1	0.5 ± 0.3	0.6 ± 0.3
	P[a] value	0.7995	0.0762	0.0098
Total protein (g/dl)	AgNPs group	6.3 ± 0.8	6.2 ± 0.8	6.2 ± 0.7
	Control group	5.9 ± 0.8	6 ± 0.6	5.9 ± 0.7
	P[a] value	0.0569	0.2460	0.2509
Albumin (g/dl)	AgNPs group	3.2 ± 0.8	3 ± 0.6	3 ± 0.5
	Control group	3 ± 0.7	2.9 ± 0.7	2.7 ± 0.7
	P[a] value	0.5575	0.8742	0.1821
Sodium (mEq/L)	AgNPs group	140.8 ± 6.7	140.8 ± 6.6	138.1 ± 7.5
	Control group	136.9 ± 7.9	135 ± 5.8	135.1 ± 4.6
	P[a] value	0.1018	0.0064	0.1737
Potassium (mEq/L)	AgNPs group	4.2 ± 0.8	4.2 ± 0.7	4.1 ± 0.6
	Control group	4.1 ± 0.8	4.2 ± 0.8	4.3 ± 1.1
	P[a] value	0.6935	0.9387	0.5152
Glucose (mg/dl)	AgNPs group	240.5 ± 105.6	204.1 ± 95.9	162.7 ± 33.3
	Control group	241.4 ± 105.2	187.6 ± 72.3	186.3 ± 79.1
	P[a] value	0.9779	0.5495	0.2617
WBC	AgNPs group	13 ± 3.8	12.5 ± 4.4	11.3 ± 2.7
10^3/ul	Control group	11.7 ± 3.6	11.6 ± 2.8	12.2 ± 2.5
	P[a] value	0.2775	0.4659	0.3043
HGB (g/dl)	AgNPs group	12.2 ± 2	12.1 ± 1.9	11.4 ± 1.6
	Control group	11.3 ± 1.6	10.9 ± 1.5	10.3 ± 1.3
	P[a] value	0.1235	0.0417	0.0453
Neutrophiles (10 [3]/ul)	AgNPs group	10.9 ± 3.5	10.1 ± 3.7	7.5 ± 2.9
	Control group	9.6 ± 3.2	8.7 ± 2.6	9 ± 2.4
	P[a] value	0.2307	0.1733	0.1285
Lymphocytes (10 [3]/ul)	AgNPs group	1.1 ± 0.9	1.5 ± 1.3	2.2 ± 1.1
	Control group	1.5 ± 1.3	1.9 ± 0.9	2.9 ± 0.9
	P[a] value	0.3109	0.3040	0.0656

Abbreviations: HGB: Hemoglobin; SGOT: serum glutamic-oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; WBCs: white blood cells.

[a] The P values were determined using a two-sample *t*-test.

The current study findings are in line with the published literature regarding AgNPs as ideal candidates for developing nanotherapeutics against different viral infections [25]. Recent in vitro studies demonstrated that various surface coatings and particle sizes of AgNPs have different virucidal activities on SARS-CoV-2, of which 50-nm branched polyethyleneimine (BPEI) showed the strongest antiviral effect. In addition, the AgNPs efficacy was positively correlated with the corresponding zeta potential. Preclinical data on the effectiveness of AgNPs against other coronaviruses (e.g., avian) already exist, which further strengthens the hypothesis on their application as a potential treatment.

The effects observed for the intravenous use of AgNPs might be explained by their extensive broad antiviral, anti-inflammatory, antiplatelet and antimicrobial activities, as well as their preference for binding to thiol groups, which can be predominantly found at the cysteine residues of SARS-CoV-2 spike glycoproteins [19].

A possible mechanism of action of intravenous administration of AgNPs for COVID-19 infection might consist of direct binding of SARS-COV-2 viruses over the respiratory epithelium, while systemic use might reduce the fatal viremia in the bloodstream. As the viral load is reduced in the respiratory epithelium, as well as in other body fluids, there will be less chance of spread from infected persons to healthy ones. It is well known that the main source of spread of COVID-19 infection is by coughing or sneezing with expulsion of virus-loaded droplets [3], which can be decreased by treatment using AgNPs. Studies have demonstrated that Ag^+ ions leach out from the

nanoparticles, which inhibit virus binding with respiratory epithelium. The Ag^{+} ions released from the AgNPs result in alteration of the pH of the respiratory epithelium. This shift in pH value to the alkaline region, prevents acid-dependent activation of the virus, and the environment will be hostile for the viruses to replicate and survive. Experimental evidence suggests that there is direct low pH-dependent fusion activation of SARS-CoV-2 during entry into host cells. If the virus has already entered to the host cell and introduces a replication cycle, then the AgNPs can bind to viral RNA and proteins, inhibiting viral replication and further spreading of the virus [24,40]. The disruption of the immune system in COVID-19-related pneumonia leads to excess production of proinflammatory cytokines. AgNPs may decrease the production of proinflammatory cytokines and increase the production of anti-inflammatory cytokines leading to decreased mortality. AgNPs have a long half-life and high antibacterial activity against various gram-negative and gram-positive bacteria, preventing secondary bacterial infections, as commonly seen in severe cases of COVID-19 pneumonia.

Our current study findings are in line with recently published data summarizing the antiviral activity of AgNPs (size of 2–15 nm) against SARS-CoV-2, their immunomodulatory action due to their ability to inhibit cytokine storms, and their anti-inflammatory, anti-fibrosis activities, and secondary prevention by potent antimicrobial effects [24]. A recent study including health care workers in high-risk areas, demonstrated that the application of AgNPs (mouthwash and nose-rinse solution containing 60 ppm) can prevent incidence of SARS-CoV-2 infection with an 85% efficacy.

4.1. Limitations of this study

In the current study, due to the small sample size, we could not make definitive statements regarding the causal relationship of the observed findings within our study. In addition, some variables were missing due to chance. Another important aspect that should be mentioned is that none of the patients had access to the ICU due to limited bed capacity when study recruitment took place during the peak times of the COVID-19 outbreaks.

5. Conclusion

We conclude, that this is the first study performed on moderate-severe and severe COVID-19 pneumonia patients using intravenous administration of AgNPs. Intravenous use of AgNPs proved to be safe and effective and could represent an affordable and accessible additional treatment for severe COVID-19 pneumonia cases by reducing mortality and supplemental oxygen need, regardless of SARS-COV-2 variants. AgNPs might also be a solution for other respiratory infections, given the increasing acquired resistance of pathogens against established anti-infective medicines.

Declarations

Author contribution statement

Laura Wieler: conceived and designed the experiments; contributed reagents, materials, analysis tools or data; wrote the paper. Oana Vittos: conceived and designed the experiments; analyzed and interpreted the data; wrote the paper. Nirmalya Mukherjee: conceived and designed the experiments Subhasish Sarkar: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare the following conflict of interests. Financial competing interest: The author LW is employed at the company BHS Medical Solutions GmbH that sponsored the material AgSept® in the clinical trial. The author LW declares that she was not involved neither in the clinical trial, nor the statistical analysis of the data. The company BHS Medical Solution has filed an application for a patent correlated to this issue.

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