Online supplement

Hydrogen/oxygen mixed gas inhalation improves disease severity and dyspnea in patients with Coronavirus disease 2019 in a recent multicenter, open-label clinical trial

Methods

Participating sites*:*

Shanghai Public Health Clinical Center
Henan Provincial People's Hospital
Shenzhen Third People's Hospital
The First People's Hospital of Yunnan
Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine
Shanghai 6th People's Hospital
Guangdong Provincial Hospital of Traditional Chinese Medicine

Approval:

Obtained from the Institutional Review Board of each participating site.

Informed consent:

Obtained from all patients in this study.

***Inclusion Criteria*:**

All subjects participating in this clinical study must meet all of the following criteria:

The diagnosis was made according to the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 5).

Patients were aged between 18 years and 85 years, had the modified Medical Research Council dyspnea scale of 1 or greater on admission and at enrollment, and had a laboratory-confirmed diagnosis of COVID-19. Patients should also have a proper understanding of the trial procedures and have signed the informed consent form.

Exclusion Criteria:

Having other respiratory or systemic diseases other than COVID-19 -- a disease that, according to the investigator's judgment, might increase the risk of developing adverse outcomes or affect the outcome measures after participation in the study.

Women who were pregnant or breastfeeding or planned to be pregnant during the study.

Subjects with one of the following respiratory diseases:

** Asthma: Subjects diagnosed as having asthma based on the investigator's judgment.

** Subjects in critical or unstable conditions, wherein, critical conditions refer to any of the following: 1. Respiratory failure needing mechanical ventilation; 2. Shock; 3. Other organ failure necessitating ICU admission.

** Having the risk factors for pneumonia: immunosuppressive diseases, severe neurological disease affecting upper respiratory tract control, or other risk factors that the investigator believes can cause a significant risk of pneumonia in subjects.

** Subjects with serious heart, liver, kidney, hematopoietic system and other important organs or

systems.

** Subjects with mental disorders and cognitive impairment.

** Patients with mental retardation, poor motivation, drug abuse (including drugs and alcohol) or other disease history restricting the effectiveness of informed consent in this study.

** Use of antioxidants, including large doses of vitamin C and vitamin E.

** Subjects unsuitable for participation in this study in the judgment of the investigators.

Outcome measures

During the study, the peripheral blood oxygen saturation was continuously monitored. The oxygen saturation and respiratory rate were measured at the resting state every day during the treatment – namely, after the end of daily treatment and 30 minutes after the inhalation of oxygen and hydrogen/oxygen.

Panel 1. A five-category ordinal scale for evaluating the severity of Covid-19 as proposed by the Chinese National Health Commission

Score	Criteria
4	Critical illness: Respiratory failure needing invasive mechanical ventilation; shock; organ failure needing admission to intensive care unit
3	Severe illness: Fulfilling any of the following criteria: 1) Tachypnea -- respiratory rate being 30 per minute or greater; 2) resting oxygen saturation being 93% or lower; 3) Oxygenation index being 300 mmHg or lower
2	General illness: Having fever and respiratory symptoms, having signs of pneumonia on chest radiology
1	Mild illness: Mild symptoms without pneumonia on chest radiology
0	Discharge from hospital, or meeting all of the criteria for discharge from hospital: 1) normalization of temperature for more than 3 days; 2) significant recovery of respiratory symptoms; 3) marked improvement in chest radiology; 4) conversion of sputum or nasopharyngeal swab viral assay findings during two consecutive measurements, at least 24 hours apart

Reference: Diagnosis and Treatment Protocol for COVID-19 (Trial Version 7)

RESULTS

Table E1. Baseline demographic and clinical characteristics at enrollment

Variables	Treatment group N=44	Control group N=46	*P* value
Age (yrs, $\bar{x} \pm s$)	58.8±11.6	59.8±13.2	0.682
Males (No., %)	26 (59.1)	23 (50.0)	0.513
Disease severity scale ($\bar{x} \pm s$)	2.5±0.8	2.5±0.7	0.141

Table E2. Symptoms of Covid-19 at enrollment

Variables	Treatment group N=44	Control group N=46	*P* value
Dyspnea scale ($\bar{x} \pm s$)	2.0±0.9	1.5±0.7	0.005
Fever (No., %)	8 (18.2)	13 (28.3)	0.378
Coughing scale ($\bar{x} \pm s$)	1.8±1.2	1.5±0.8	0.176
Chest distress scale ($\bar{x} \pm s$)	1.9±1.1	1.5±1.0	0.008
Chest pain scale ($\bar{x} \pm s$)	0.9±1.0	0.3±0.6	<0.001

Table E3. Treatment effects in terms of primary and secondary endpoints at different time points expressed as the continuous variables

Variables	Day 2					Day 3					End-of-treatment				
	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI
n*	44	44	-	-	-	44	44	-	-	-	44	44	-	-	-
Mean improvement in five-scale disease severity (%)	0.20	0.02	0.008	0.2	(0.1, 0.3)	0.41	0.11	0.010	0.3	(0.1, 0.5)	1.07	0.27	<0.001	0.8	(0.5, 1.1)
n*	44	46	-	-	-	44	46	-	-	-	44	46	-	-	-
Mean improvement in dyspnea scale (%)	0.70	0.24	0.003	0.5	(0.2, 0.8)	1.14	0.54	0.001	0.6	(0.2, 1.0)	1.89	1.20	<0.001	0.7	(0.3, 1.1)
n*	43	46	-	-	-	43	46	-	-	-	43	46	-	-	-
Mean improvement in chest distress scale (%)	0.80	0.20	0.001	0.6	(0.3, 1.0)	1.20	0.40	<0.001	0.8	(0.5, 1.2)	1.70	1.00	0.001	0.7	(0.3, 1.3)
n*	44	46	-	-	-	44	46	-	-	-	44	46	-	-	-
Mean improvement in chest pain scale (%)	0.50	0	<0.001	0.5	(0.3, 0.8)	0.70	0	<0.001	0.7	(0.4, 0.9)	0.90	0.10	<0.001	0.6	(0.5, 1.2)
n*	44	46	-	-	-	44	46	-	-	-	44	46	-	-	-
Mean improvement in cough scale (%)	0.50	0.10	<0.001	0.4	(0.2, 0.7)	1.00	0.30	<0.001	0.7	(0.4, 1.1)	1.60	0.80	<0.001	0.8	(0.4, 1.2)
n*	34	34	-	-	-	34	34	-	-	-	34	34	-	-	-
Mean improvement in resting oxygen saturation (%)	1.60	0.50	0.003	1.1	(0.4, 1.8)	2.60	0.80	<0.001	1.8	(0.9, 2.7)	4.10	2.00	0.001	2.1	(0.9, 3.3)

RR: relative risk; 95% CI: 95% confidence interval.

Count (percentage) was adopted for summarizing categorical variables, and compared with Chi-square tests or Fisher's exact test. The relative risk (RR) and 95% confidence interval (95% CI) were calculated. Continuous variables were presented with mean ± standard deviation, and compared with independent *t*-test or Wilcoxon rank-sum test. *, denotes number of patients evaluated.

The median duration of hydrogen inhalation was 64 (interquartile range, 24-175) h in treatment group, corresponding to 7.7 (interquartile range, 6.0-18.3) h per day. Oxygen therapy in control group lasted for a median of 24 (interquartile range, 22.6-24.0) h per day.

Table E4. Treatment effects of dyspnea scale at different time points when stratified by the baseline disease severity

Variables	Day 2					Day 3					End-of-treatment				
	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI
Mild to general illness															
n*	22	15	-	-	-	22	15	-	-	-	22	15	-	-	-
Improvement in dyspnea scale	0.70	0.10	0.024	0.6	(0.1, 1.0)	1.00	0.30	0.014	0.7	(0.2, 1.3)	1.70	1.10	0.024	0.6	(0.1, 1.0)
Severe to critical illness															
n*	22	29	-	-	-	22	29	-	-	-	22	29	-	-	-
Improvement in dyspnea scale	0.70	0.30	0.028	0.4	(0.1, 0.9)	1.20	0.70	0.021	0.5	(0.1, 1.0)	2.10	1.20	0.005	0.9	(0.3, 1.4)

RR: relative risk; 95% CI: 95% confidence interval

Count (percentage) was adopted for summarizing categorical variables, and compared with Chi-square tests or Fisher's exact test. The relative risk (RR) along with 95% confidence interval (95% CI) were calculated. Continuous variables were presented with mean ± standard deviation, and compared with independent *t*-test or Wilcoxon rank-sum test. *, denotes number of patients evaluated.

The median duration of hydrogen inhalation was 64 (interquartile range, 24-175) h in treatment group, corresponding to 7.7 (interquartile range, 6.0-18.3) h per day. Oxygen therapy in control group lasted for a median of 24 (interquartile range, 22.6-24.0) h per day.

Table E5. Treatment effects of dyspnea scale at different time points when stratified by the duration of hydrogen inhalation

Variables	Day 2					Day 3					End-of-treatment				
	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI	Treatment group	Control group	P value	Mean difference	95% CI
n*	24	44	-	-	-	24	44	-	-	-	24	44	-	-	-
Patients with improvement in five-scale disease severity (%)	**0.33**	**0.02**	**0.005**	**0.3**	**(0.1, 0.5)**	**0.71**	**0.11**	**0.001**	**0.6**	**(0.3, 0.9)**	**1.04**	**0.27**	**<0.001**	**0.7**	**(0.4, 1.1)**
n*	24	46	-	-	-	24	46	-	-	-	24	46	-	-	-
Patients with improvement in dyspnea scale (%)	**1.00**	**0.24**	**0.002**	**0.8**	**(0.3, 1.2)**	**1.33**	**0.54**	**0.002**	**0.8**	**(0.3, 1.3)**	**1.79**	**1.20**	**0.026**	**0.6**	**(0.1, 1.1)**
n*	21	34	-	-	-	21	34	-	-	-	21	34	-	-	-
Mean improvement in resting oxygen saturation (%)	**1.8**	**0.5**	**0.023**	**1.3**	**(0.2, 2.3)**	**3.1**	**0.8**	**0.001**	**2.3**	**(1.0, 3.5)**	**4.1**	**2.0**	**0.008**	**2.1**	**(0.6, 3.7)**

Treatment group as demonstrated herein only consisted of the patients who have collectively inhaled hydrogen/oxygen mixed gas for less than 64 hours (the median level among all patients in the treatment cohort).

Data in bold indicated the comparisons with statistical significance; RR: relative risk; 95% CI: 95% confidence interval.

Count (percentage) was adopted for summarizing categorical variables, and compared with Chi-square tests or Fisher's exact test. The relative risk (RR) along with 95% confidence interval (95% CI) were calculated. Continuous variables were presented with mean ± standard deviation, and compared with independent *t*-test or Wilcoxon rank-sum test. *, denotes number of patients evaluated.

The median duration of hydrogen inhalation was 64 (interquartile range, 24-175) h in treatment group, corresponding to 7.7 (interquartile range, 6.0-18.3) h per day. Oxygen therapy in control group lasted for a median of 24 (interquartile range, 22.6-24.0) h per day.

Table E6. Comparison of the adverse events in the full analysis set

Adverse events	Treatment group	Control group	*P* value
Total	8 (18.2%)	14 (30.4%)	0.223
Worsening of dyspnea	2 (4.5%)	3 (6.5%)	>0.999
Worsening of chest distress	1 (2.3%)	10 (21.7%)	0.008
Worsening of chest pain	1 (2.3%)	1 (2.2%)	>0.999
Worsening of cough	3 (6.8%)	4 (8.7%)	>0.999
Abnormal blood routine test	0	2 (4.3%)	0.495
Abnormal liver function	0	2 (4.3%)	0.495
Abnormal renal function	0	1 (2.2%)	>0.999
Abnormal blood biochemical findings	1 (2.3%)	1 (2.2%)	>0.999

Figures

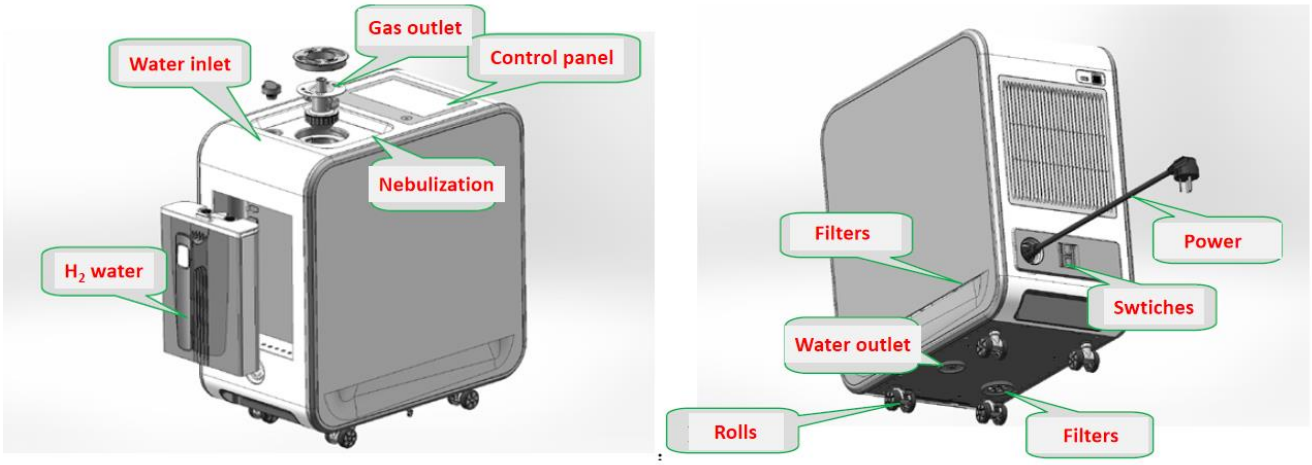

Figure E1. A schematic diagram of the Hydrogen/Oxygen Generator (model AMS-H-03). Courtesy of Shanghai Asclepius Meditec Co., Ltd. (Shanghai, China).

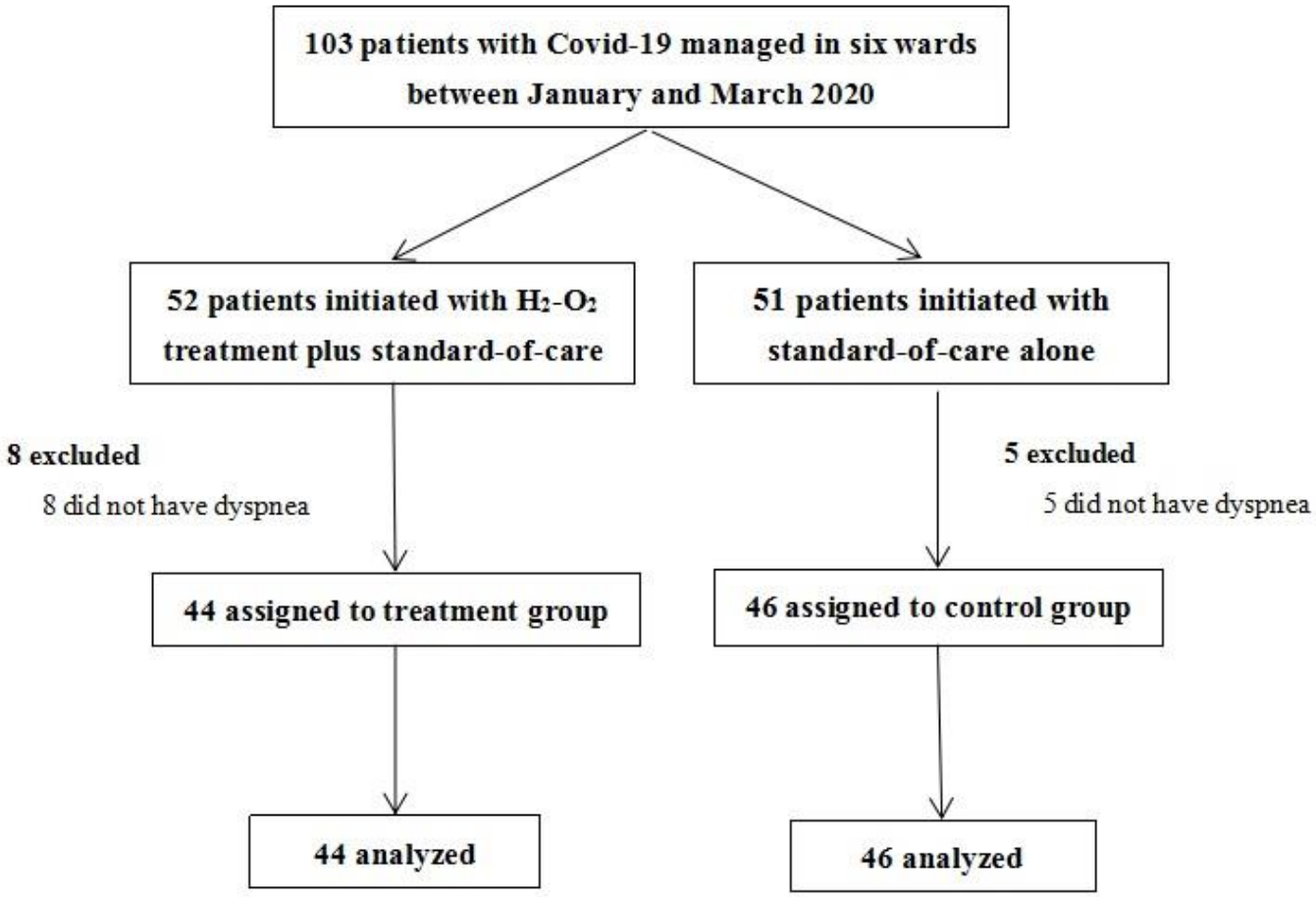

Figure E2. Study flow chart.