Increased efficacy of whole lung lavage treatment in alveolar proteinosis using a new modified lavage technique

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Abstract

Background.

Autoimmune pulmonary alveolar proteinosis (aPAP) is an ultra-rare pulmonary disease. Due to heterogeneity and small patient cohorts, no standardized treatment protocol exists. Whole lung lavage (WLL) is considered the gold standard therapy and aims to remove the highest protein amount by flushing the lung with the lowest possible instilled volume. We report a new protocol for a new modified lavage technique (nMLT) in which controlled repetitive manual hyperinflation (MH) and intermittent chest percussion are used to enhance WLL efficacy.

Methodology.

We included all patients with aPAP treated with nMLT between 2013 and 2018. nMLT consisted of repetitive MH with intermittent chest percussion every third wash cycle. We reported: instilled and recovered volume, protein concentration, and optical density (OD) using spectrophotometry. Pulmonary function (FVC %predicted and DLCO %predicted) and serum biomarkers (LDH, CA 15 − 3, SP-D, and YKL-40) two months prior and post nMLT, and one year after nMLT treatment were evaluated. Data are displayed as mean (± SD) or median [IQR]. Comparisons were made using Student t-test and Wilcoxon test.

Results.

We included 11 patients (64% male) in whom a total of 65 nMLTs were performed. One nMLT consisted of 15 [12–18] wash cycles. Protein removal was 9.80 [7.52–12.66] grams per nMLT. After 3–4, 6–7, and 9-10L of instilled and recovered volume 56 [49–61] %, 81 [77–84] % and 91 [88–94] % of protein was removed respectively. OD was measured 116 times and increased from 1.13 (± 0.52) to 1.31 (± 0.52) after MH ($p < 0.001$). Our study also showed that pulmonary function improves after WLL with nMLT. Improvement of biomarkers was only reflected by CA 15 − 3. No adverse events related to the procedure were observed.

Conclusion.

Efficacy of WLL seems to be enhanced by using manual hyperinflation and applying this every third cycle. Our technique of WLL with nMLT could be used to increase the amount of protein recruited while instilling the lung with the smallest volume of fluid as possible. The duration of anesthesia and the risk of complications is thereby reduced.

Background

Pulmonary alveolar proteinosis (PAP) is an ultra-rare lung disease with a prevalence of 3.7 to 40 cases per million people (1,2). Autoimmune PAP (aPAP) accounts for 90% of cases (2). aPAP emerges due to autoantibodies against Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) neutralizing the biologic activity resulting in impaired macrophages-mediated surfactant clearance (3–5). Cellular and intracellular accumulation of surfactant in the alveoli and distal airways leads to distortion of oxygen absorbance while the interstitial lung architecture remains normal (2). The clinical course of aPAP is variable, ranging from hypoxemic respiratory failure in severe cases to spontaneous resolution in mild manifestations (6,7). There are several treatment therapies for patients with aPAP including whole lung lavage (WLL) and GM-CSF inhalation therapy (7,8). WLL is considered gold standard of therapy for severe PAP (7), however no standardized protocol for WLL exists. The multiple available technical descriptions are based on the report by Ramirez and colleagues in 1963 (7,9–11). The main goal of WLL is to remove the highest amount of excessive protein material from the alveoli by flushing the lung with the lowest possible instilled volume (7,12). The technique used in our hospital is based on a technique described by Bonella and colleagues in 2012 named the modified lavage technique (MLT) (12). In this study we will provide a protocol for a new modified lavage technique (nMLT) in which repetitive manual hyperinflation (MH) and intermittent chest percussions are used to further enhance WLL efficacy.

Methods

Study population

We retrospectively included all patients with aPAP who underwent WLL using the nMLT at the St. Antonius Hospital in the Netherlands between September 2013 and July 2018. Patients were excluded from lung function and biomarker follow-up if they received simultaneous treatment with GM-CSF therapy. Data were extracted from the hospital's digital information systems. The Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital approved this study (R05-08A) and written informed consent was obtained from all participants.

Data collection

Demographic data included: gender, age at time of diagnosis, date of diagnosis, method used for diagnosis (detection of GM-CSF autoantibodies (AE/mL), or positive Periodic acid–Schiff (PAS) staining in bronchoalveolar lavage fluid), and smoking habits. Pulmonary function and biomarkers were recorded at three time points: two months prior or after WLL with nMLT and one year after WLL with nMLT treatment had stopped. Pulmonary function included the percentage of the predicted value of forced vital capacity (FVC %predicted) and the diffusion capacity of the lungs for carbon monoxide (DLCO %predicted). Serum biomarkers included: lactate dehydrogenase (LDH, U/L), cancer antigen 15 − 3 (CA 15 − 3, kU/L), Surfactant protein-D (SP-D, ng/mL), chitinase-3-like protein 1 (YKL-40, ng/mL), and chemokine ligand 18 (CCL18, ng/mL). In our clinic CA 15 − 3 was routinely measured instead of Krebs von den Lungen-6 (KL-6, U/mL). It has been demonstrated previously that CA 15 − 3 is correlated with KL-6 and can be used as alternative (13). IgG anti-GM-CSF was measured with radioimmunoassay. LDH and CA15-

3 were measured on a Cobas e601 and c601 analyzer respectively (Roche diagnostics Ltd, Rotkreuz, Switzerland). YKL-40 (Quidel corporation, San Diego, CA, USA), SP-D and CCL18 (both R&D systems, Minneapolis, MN, USA) were measured with enzyme-linked immunosorbent assay.

Decisions on starting WLL as well as the frequency of WLLs with nMLT were made in a multidisciplinary team of experts and was dependent on several factors including: complaints and discomfort reported by the patient, disease progression, pulmonary function tests and Disease Severity Score (DSS). DSS was based on the presence of symptoms and PaO_2 as previously described by Inoue and colleagues (14). Adverse events related to the procedure were retrieved from the electronic health record.

New modified lavage technique

The first WLL procedure in our center was performed in 2004. WLL is performed in separate sessions on each lung for a given patient. Until 2013, the classical lavage technique based on Ramirez and colleagues was used (10–12). In 2013 we started to implement the MLT described by Bonella and colleagues in 2012 (12) with a modification, however, on three major points. First, during the infusion-recovery procedure intermittent chest percussion using a large-surface vibrator with 3-dimensional vibration was performed (Senator type Professional 3D, Offenbach, Germany). Intermittent chest percussion was performed in two phases: during instillation of the first 500 mL of saline and during the phase of recovery of the last 500 mL of saline. Second, manual hyperinflation (MH) was applied after every third cycle of infusion recovery instead of applying this technique only at the end of the procedure when the optical density (OD) is below 0.4 (12). During manual hyperinflation the flushed lung is manually inspired using a low positive inspiratory flow to a volume with maximum ventilation pressure up to 40 cm H_2O. After a pause of three seconds an unobstructed expiration (via open valve) was initiated together with manual chest compression on the hemithorax of the flushed lung. Thirdly, controlled MH was started directly after the last infusion-recovery cycle without first instilling 500 mL of saline based on the fact that after three cycles a residue of approximately 500 mL already is present in the lung. Based on these important changes we designated our nMLT protocol.

OD of the lavage fluid was measured in duplicate at a wavelength of 405 nm (i2 visible Spectrophotometer, Hanon Instruments, Jinan, China). The recovery fluid was centrifuged at 1720xg for 10 minutes. In the supernatant, the protein concentration was measured on a cobas c501 analyzer (Roche diagnostics ltd, Rot Kreuz, Switzerland). The total protein recovery was calculated by multiplying protein concentration in the fluid (g/mL) by volume (mL).

Data analysis

Quantile-quantile plots were used to determine data distribution. Normally distributed data were presented as mean and standard deviation (±SD). Non-normally distributed data were presented as median and inter quartile range [Q1-Q3, IQR]. Categorical data were presented as number and percentage. For comparisons within individuals, paired Students t-test was used for normally distributed parameters and Wilcoxon test for non-normally distributed parameters. Spearman correlation was calculated for non-

normally distributed variables to assess bivariate correlation between instilled and recovered volume and amount of removed protein. SPSS version 23.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Patient characteristics

Between September 2013 and July 2018, 11 patients with aPAP underwent treatment with WLL using the nMLT in the St. Antonius Hospital. Two patients were excluded from analysis of total lung function and biomarker follow-up and three patients were excluded from analysis of pulmonary function and biomarkers follow-up one year after WLL with nMLT treatment had stopped. Patient characteristics are displayed in Table 1. Median age at time of diagnosis was 48 [31–56] years. A male predominance of 64% was seen. Ten patients (91%) tested positive for GM-CSF autoantibodies. In one patient, the diagnosis was based on positive PAS staining on bronchoalveolar lavage fluid. Nine (64%) patients were smokers or former smokers (18% never, 55% former, 27% current). Of (former) smokers 24%, 38%, and 38% had respectively < 10, 10–20, > 20 pack years. Time between diagnosis and start of WLL with nMLT was 2 [0–19] months. Of patients 46% was assigned to DSS-2, 27% to DSS-3, and 27% to DSS-4. No patients were assigned to category DSS-1 or DSS-5.

Table 1. Patient characteristics

Characteristics	Cohort (n = 11)
Gender, n (%)	
Male	7 (64%)
Age, median years [IQR]	48 [31-56]
Diagnosis based on, n (%)	
GM-CSF autoantibodies	10 (91)
Positive PAS-staining in BAL	1 (9)
Smoking habits, n (%)	
Never	2 (18)
Previous	6 (55)
Current	3 (27)
Disease Severity, n (%)	
DSS-1	0 (0)
DSS-2	5 (46)
DSS-3	3 (27)
DSS-4	3 (27)
DSS-5	0 (0)

Abbreviations: n, number of patients; IQR, interquartile range; PAS, Periodic acid–Schiff; BAL, bronchoalveolar lavage; DSS, disease severity score

Efficacy of WLL with nMLT

Between September 2013 and July 2018 data of 65 WLL procedures using nMLTs were recorded. No adverse events related to the procedure were observed in all patients. MH did not affect hemodynamics. Also, there was no spill-over to the ventilated lung after the procedure of MH.

The median amount of WLL with nMLT procedures per patient was 5 [2–8], one patient with severe treatment refractory aPAP received up to 23 lavages.

Median amount of removed protein in grams during one WLL with nMLT was 9.80 [7.52–12.66]. The median volume instilled and recovered saline during one WLL with nMLT was 15 [12–18] L. Minimal volume instilled and recovered saline was 4L, and volume never exceeded 23L. Correlation coefficients of instilled and recovered volume with amount of removed protein was 0.426 ($p < 0.001$). After the first 3 to 4 washing cycles a median of 56 [49–61] % of the final protein yield was already removed. After 6 to 7

cycles a median of 81 [77–84] % and after 9 to 10 cycles up to 91 [88–94] % of the final protein yield was removed.

The effect on protein wash out using the nMLT was objectified in five patients. OD measurements were done in 31 WLL procedures using nMLT. A total of 116 OD measurements were recorded. OD course during nMLT is shown in Fig. 1. A significant increase of OD was found when comparing OD measurements before and after MH (1.13 (± 0.52) to 1.31 (± 0.52), respectively ($p < 0.001$), measurements are displayed in Fig. 2.

Pulmonary function and biomarkers

Median pulmonary function and biomarker measurements before, after, and one year after stop of WLL with nMLT are displayed in Table 2. Median FVC %predicted at start of WLL with nMLT was 76 [61–87] % and did not change after the procedure ($p = 0.361$). One year after WLL with nMLT treatment FVC %predicted increased significant to 89 [77–96] % ($p = 0.046$). DLCO %predicted increased significant after WLL with nMLT from 45 [41–51] % to 49 [37–54] % and after one year to 60 [49–73] % ($p = 0.021$ and $p = 0.138$, respectively). Biomarker CA 15 – 3 decreased significant after WLL with nMLT from 99 [55–136] kU/L to 76 [43–94] kU/L and after one year to 34 [19–134] kU/L ($p = 0.021$ and $p = 0.043$, respectively). Biomarkers LDH, YKL-40, SP-D, and CCL18 did not change significant during follow up after WLL with nMLT.

Table 2. Pulmonary function and biomarkers during and after WLL treatment with nMLT

	Before WLL with nMLT	After WLL with nMLT	*p-value*[1]	One-year stop	*p-value*[2]
Pulmonary function					
FVC (%pred)	76 [61-87], n=18	77 [56-93], n=18	0.361	89 [77-96], n=6	**0.046**
DLCO (%pred)	45 [41-51], n =16	49 [37-54], n=16	**0.021**	60 [49-73], n=5	0.138
Biomarkers					
LDH (U/L)	353 [278-467], n=30	324 [271-393], n=21	0.795	246 [221-270], n=4	0.068
CA 15-3 (kU/L)	99 [55-136], n=30	76 [43-94], n=21	**0.021**	34 [19-134], n=5	**0.043**
SP-D (ng/mL)	54 [42-80], n=30	44 [34-64], n=21	0.307	40 [18-59], n=5	0.176
YKL-40 (ng/mL)	149 [90-207], n=30	128 [98-171], n=21	0.687	111 [74-190], n=5	0.104
CCL18 (ng/mL)	82 [55-148], n=30	85 [58-130], n=21	0.722	98 [73-145], n=5	0.080

Values are presented as median [IQR]. Comparison of data was done with Wilcoxon test. [1]Comparison between before WLL with nMLT and after WLL with nMLT. [2]Comparison between before WLL with nMLT and one year after stop of WLL with nMLT.

Abbreviations; n, number of patients; WLL, whole lung lavage; nMLT; new modified lavage technique

Discussion

In this study we report a new variant of the modified lavage technique for WLL that aims to remove the highest amount of excessive protein material from the alveoli by flushing the lung with the lowest possible instilled volume. Our nMLT was shown to be effective as repetitive MH every third wash cycle and intermittent chest percussions significantly increased protein removal in the consecutive cycle. Furthermore, our study supports flushing with the lowest possible instilled volume as a volume of 9 to 10L was sufficient to remove up to 91% of the total protein amount removed during the WLL procedure.

Recruitment of additional protein from the alveoli by repetitive manual ventilation and chest percussions was already objectified by Bonella and colleagues. They reported an increase of protein removal in the wash cycle after manual ventilation was applied (12). However, they only applied this technique at the end of the WLL procedure when the OD was below 0.4, indicating that a low amount of extra protein was recruited. Based on our data we suggest applying this technique with MH every third cycle in order to enhance WLL efficiency. In our patients the median total amount of removed protein was 9.80 grams. This amount does not differ from reported averages of 2–33 grams in the literature (12,15,16). The amount of removed protein per WLL in our cohort was influenced by the instilled volume ($r = 0.426$, $p < 0.001$). We used an average instilled and recovered volume of 15L and did not exceed a volume of 23L. Compared to the literature, Bonella and colleagues used up to 71L with their MLT, significantly increasing total protein wash out compared to the classic technique (12). However, no difference in protein removal between the classic lavage technique and their MLT was seen when adjusting for volume (12). Therefore, our repetitive MH and intermittent chest percussions do seem to increase direct protein wash out per volume, however total protein recovery is not increased.

Although increased volume leads to more protein wash out, our results make clear that after 9 to 10 cycles of nMLT up to 91% of the final protein yield is already recruited. This phenomenon is in concordance with results of Bonella and colleagues (12). At the moment, lavage duration is based on OD measurement of the recovered fluid. OD measurement after 9 to 10 cycles does not yet reach the target value of 0.4. However, based on our data one could debate on the additional effect of using more than 10 cycles. On average, less than 18L total instilled and recovered volume is used among global centers (7). The importance of using the lowest possible instilled volume is emphasized by the fact that WLL is an invasive procedure. Although it is determined to be safe, procedure-related morbidity is reduced by decreasing risk of hypoxemia, fluid leakage, and pleural effusion (7). Also, prolonged duration of anesthesia and intubation is unfavorable (17,18). In our cohort no adverse events were documented. A global survey by Campo and colleagues suggested that instilled and recovered volume was not correlated to clinical outcome (7). Highlighting again, in our opinion, that ideally the smallest instilled volume should be used in order to reduce the duration of anesthesia and thereby the risk of complications. Our technique supports this.

Our study also shows that pulmonary function improves after WLL with nMLT. Significant improvement of biomarkers was only reflected by CA 15 – 3. Although other aPAP related biomarkers such as YKL-40, SP-D and CCL18 were elevated in our patients with aPAP, they did not correlate with functional improvement after WLL. This is in concordance with previous studies addressing biomarkers in diagnosis

and prognosis of PAP (13,19–23). It is important to state that CA 15 – 3 is correlated with KL-6 and can be used as alternative as described previously (13). Unfortunately, within our cohort KL-6 was not available.

We are aware that there are several limitations to our study. First, the number of patients was small, making conclusions less robust. Second, no randomization for WLL with nMLT was done. This is however balanced by the fact that OD was measured before and after MH in a remarkable sample size.

Conclusions

Efficacy of WLL seems to be enhanced by using manual hyperinflation instead of manual ventilation and applying this every third cycle instead of at the end of the procedure. Our technique of WLL with nMLT could be used to increase the amount of protein recruited while instilling the lung with the smallest volume of fluid as possible. The duration of anesthesia and the risk of complications is thereby reduced.

Abbreviations

aPAP = autoimmune pulmonary alveolar proteinosis

GM-CSF = Granulocyte-Macrophage Colony-Stimulating Factor

WLL = whole lung lavage

MLT = modified lavage technique

nMLT = new modified lavage technique

MH = manual hyperinflation

DLCO %predicted = diffusion capacity of the lungs for carbon monoxide in percentage of predicted

FVC %predicted = forced vital capacity in percentage of predicted

LDH = lactate dehydrogenase

CA 15-3 = cancer antigen15-3

CCL18 = chemokine ligand 18

SP-D = surfactant protein-D

YKL-40 = chitinase-3-like protein 1

OD = optical density

DSS = disease severity score

PAS = Periodic acid–Schiff staining

Declarations

Ethics approval and consent to participate: The Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital approved this study (R05-08A) and written informed consent was obtained from all participants.

Consent for publication: not applicable.

Availability of data and materials: Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at the St. Antonius Hospital (24,25).

Competing interests: The authors declare that they have no conflicting interests.

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Authors' contributions: Each author have made substantial contributions to the study. Study design was made by LG and MV. Data collection was done by LG and ES. Clinical chemistry data were collected by HR and JvdV. Data analysis and interpretation was done by LG and MV. LG, ES, MV, CC, and JvdV contributed to writing the manuscript. Critical revision of the content of the manuscript was done by MV, EvD, and HR. Language editing was done by ES. All authors read and approved the final manuscript. All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Figures

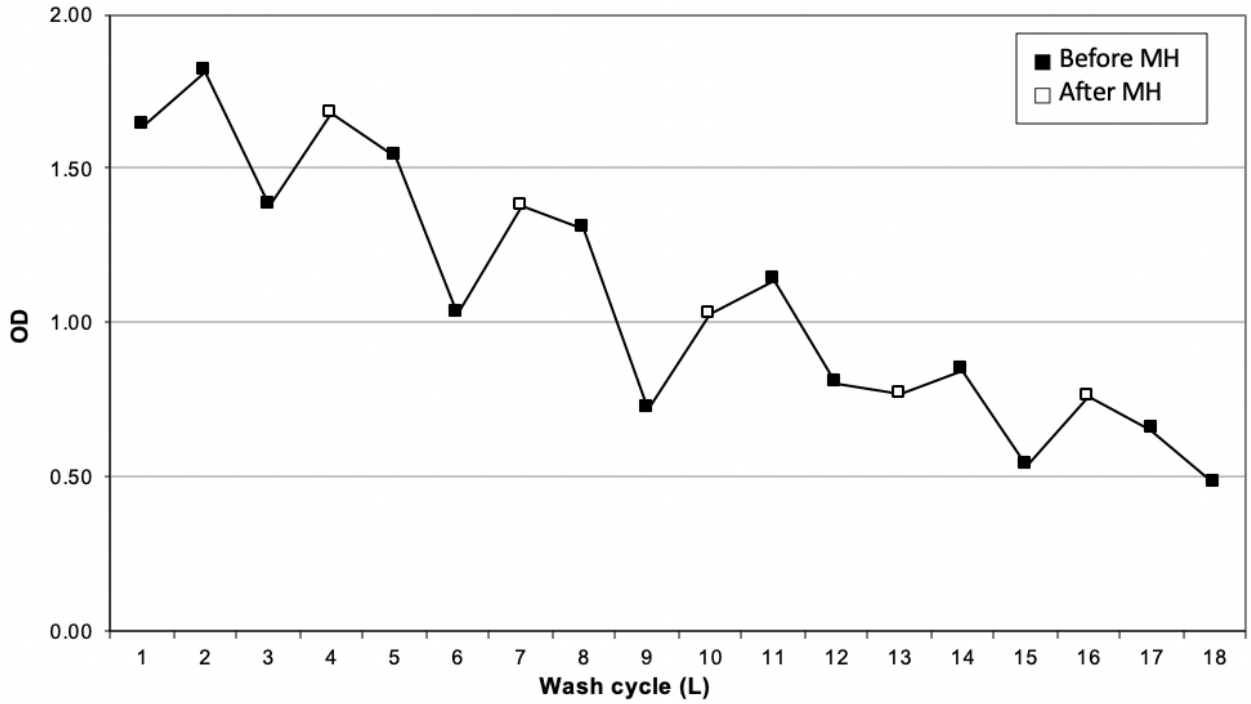

Figure 1

The course of OD during one representative WLL with nMLT. Abbreviations: OD, optical density; MH, manual hyperinflation; WLL, whole lung lavage; nMLT, new modified lavage technique

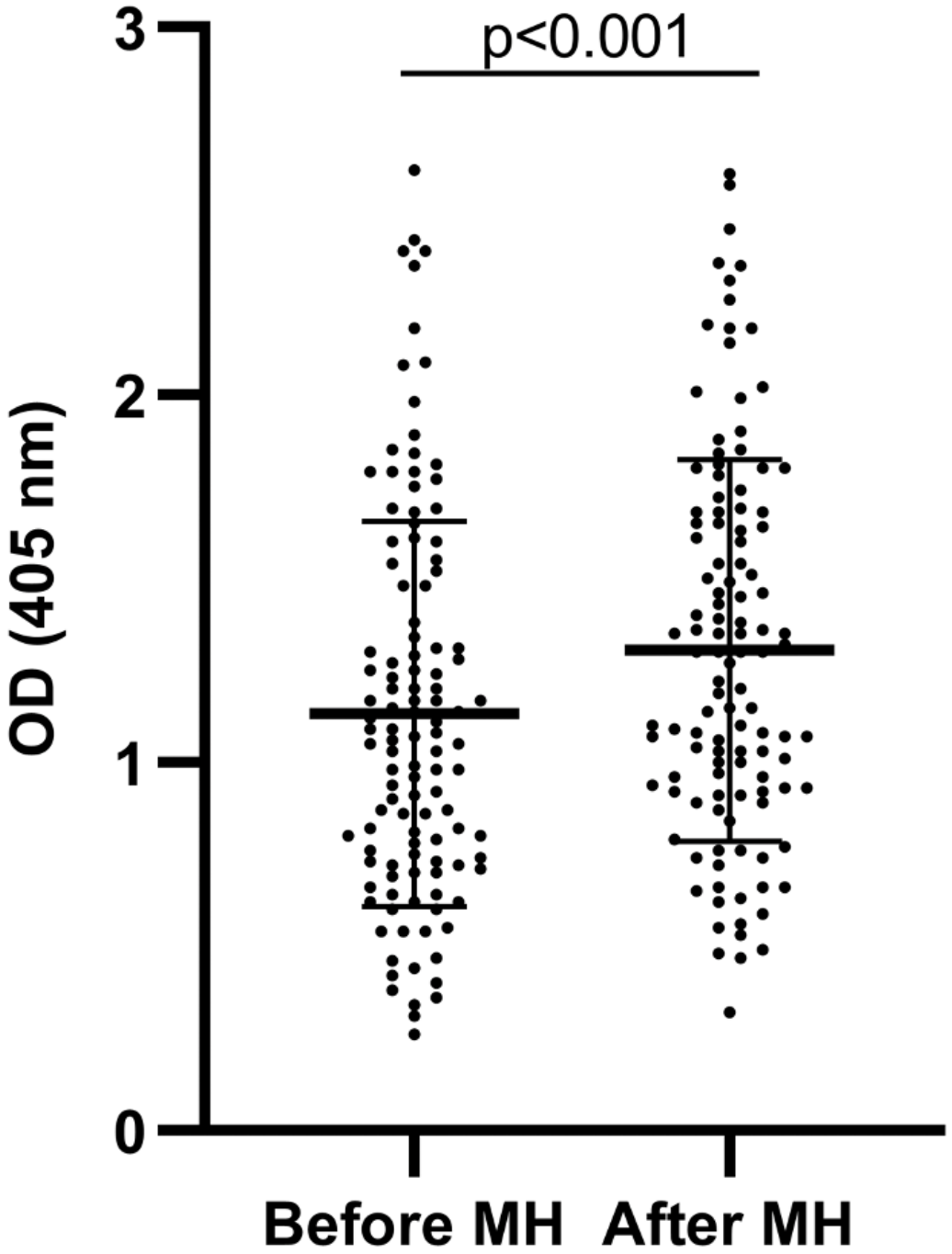

Figure 2

OD measured before and after MH. Values are presented as mean (±SD). Abbreviations: MH, manual hyperinflation; OD, optical density; SD, standard deviation