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Increased Efficacy of Whole Lung Lavage Treatment in Alveolar Proteinosis Using a New Modified Lavage Technique

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Background: **Autoimmune pulmonary alveolar proteinosis is an ultra-rare pulmonary disease. Whole lung lavage (WLL) is considered the gold standard therapy. We report a 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.**

Methods: **We included all subjects with autoimmune pulmonary alveolar proteinosis treated with nMLT between 2013 and 2018. nMLT consisted of repetitive MH with intermittent chest percussion every third wash. We reported: instilled volume, protein concentration, and optical density using spectrophotometry. Pulmonary function (FVC %predicted and DLCO % predicted) at start of nMLT was recorded. Data are displayed as mean (±SD), median [interquartile range], or number (%). Comparisons within individuals were made using Students *t* test.**

Results: **We included 11 subjects (64% male) in whom a total of 67 nMLTs were performed. One nMLT consisted of 15 [12-18] washes. Protein removal was 9.80 [7.52-12.66] g per nMLT. After the first, second, and third cycle of 3 washes, 56% [49% to 61%], 81% [77% to 84%], and 91% [88% to 94%] of the final protein yield was removed, respectively. Optical density was measured 116 times and increased from 1.13 (±0.52) to 1.31 (±0.52) after MH ($P < 0.001$).**

Conclusion: **Efficacy of WLL seems to be enhanced by applying MH every 3 washes. 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.**

Key Words: autoimmune pulmonary alveolar proteinosis, interventional pulmonology, modified technique, optical density, whole lung lavage

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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 pulmonary alveolar proteinosis (aPAP) accounts for 90% of cases.[2] aPAP emerges due to autoantibodies against granulocyte macrophage-colony stimulating factor (GM-CSF) which neutralize the biological 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 mild disease or even spontaneous resolution.[6,7] There are several treatment options for patients with aPAP including whole lung lavage (WLL) and off label 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 goals of WLL are: (1) to remove the highest amount of excessive protein material from the alveoli, (2) flushing the lung with the lowest possible instilled volume, and (3) minimizing complications by reducing anesthesia time and postprocedural hospitalization.[7,12] The technique used in our hospital is based on a technique described by Bonella et al in 2012 named the modified lavage technique (MLT).[12] In this study,

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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.

PATIENTS AND METHODS

Study Population

We retrospectively included all subjects with aPAP who underwent WLL using the nMLT at our hospital between September 2013 and July 2018. No follow-up of lung function was possible since several subjects received simultaneous treatment with GM-CSF therapy. Data were extracted from the hospital's digital information systems. The Medical research Ethics Committees United of our 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, detection of GM-CSF autoantibodies (AE/mL) for diagnosis and smoking habits. Pulmonary function was recorded at start of WLL treatment with nMLT. Pulmonary function included the percentage of the predicted value of FVC (%predicted) and of diffusion capacity of the lungs for carbon monoxide (DLCO %predicted). IgG anti-GM-CSF was measured with radioimmunoassay.

Decisions on starting or stopping WLL and the frequency of WLLs with nMLT were made in a multidisciplinary team of experts and depended on several factors including: complaints and discomfort reported by the subject, disease progression, pulmonary function tests and Disease Severity Score (DSS). DSS was based on the presence of symptoms and PaO_2.[13] Adverse events related to the procedure were retrieved from the electronic health record and included; pneumothorax, hemodynamic instability, and aspiration of the ventilated lung due to fluid spill over during the procedure. To roughly assess the possibility of fluid spill over, auscultation of the ventilated lung was performed after every 3 washes. Post lavage, patients were admitted to the hospital ward for 1 day. They received oxygen support and underwent x-ray investigation to assess the occurrence of a pneumothorax.

nMLT

The first WLL procedure in our center was performed in 2004. Until 2013, the classical lavage technique based on Ramirez and colleagues was used.[10–12] During these procedures, clear fluid recovery indicated the end of the procedure. In 2013, we started to implement our nMLT based on a protocol provided by Bonella et al in 2012.[12] Introduction of optical density (OD) measurement now made it possible to compare procedures between subjects. WLL is performed in separate sessions on each lung for a given subject.

Subjects underwent double-lumen intubation in supine position. Ventilation with 100% oxygen using a volume-controlled ventilator (Servo; Siemens, Danvers, MA) was started. The tube was tested for leaks by single-lung ventilation. Furthermore, a flexible fibreoptic bronchoscope was used to ascertain the proper tube position initially and during the procedure. The lung to be washed was clamped for 5 minutes to allow oxygen absorption. Saline solution at body temperature was instilled into the nonventilated lung with a tidal washing volume of 1000 mL during each wash. If total lung capacity was below 2.5 L, the washing volume was decreased to 750 mL. The operating bed was set to the lowest position during instillation of saline while during recovery of the fluid the bed was set at the highest position.

The differences between our nMLT and the previously described MLT from Bonella are based on different use of 2 known techniques: intermittent chest percussion and MH. 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 2 phases: during instillation of the first 500 mL of saline and during the phase of recovery of the last 500 mL of saline. Second, MH was applied after every 3 washes of infusion recovery instead of applying this technique only at the end of the WLL when OD is below 0.4 as described in the MLT. During MH 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 3 seconds an unobstructed expiration (via open valve) was initiated together with manual chest compression on the hemi thorax of the flushed lung. Controlled MH was started directly after the last infusion-recovery wash without first instilling 500 mL of saline based on the fact that after 3 washes a residue already is present in the lung. The exact residue after each wash was calculated by extracting recovered from instilled volume.

Recovery of the opaque fluid was done before the next cycle of 3 washes began. 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 1720*g* for 10 minutes.[12] 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). OD measurement of <0.4 indicated the end of the procedure.

Data Analysis

Quantile-quantile plots were used to determine data distribution. Normally distributed data were presented as mean and SD. Non-normally distributed data were presented as median and interquartile range [Q1-Q3]. Categorical data were presented as number and percentage. Comparisons within individuals was made using Paired Students *t*-test for normally distributed parameters. Spearman correlation was calculated for non-normally distributed variables to assess bivariate correlation between instilled volume and amount of removed protein. SPSS version 23.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

RESULTS

Subject Characteristics

Between September 2013 and July 2018, 11 subjects with aPAP underwent treatment with WLL using the nMLT in our hospital. Subject characteristics at diagnosis and at start of WLL with nMLT are displayed in Table 1. The median age at time of diagnosis was 48 [31 to 56] years. A male predominance of 64% was seen. All subjects tested positive for GM-CSF autoantibodies. At time of diagnosis 18% of subjects never smoked, 55% of subjects were former smokers, and 27% of subjects were current smokers. Of former smokers and smokers combined: 24%, 38%, and 38% had respectively <10, 10 to 20, >20 pack years at diagnosis. Time between diagnosis and start of WLL with nMLT was 2 [0 to 19] months. Of subjects, 46% were assigned to DSS-2, 27% to DSS-3, and 27% to DSS-4. No subjects were assigned to category DSS-1 or DSS-5. At start of WLL with nMLT median FVC %predicted was 74 [57 to 88], DLCO %predicted was 44 [37 to 49]. Due to simultaneous treatment with GM-CSF therapy no follow-up analysis of pulmonary function was done.

TABLE 1. Subject Characteristics at Diagnosis and at Start of WLL With nMLT

Characteristics	Cohort (n = 11)
Gender, n (%)	
Male	7 (64)
Age at diagnosis, median years [IQR]	48 [31-56]
Smoking habits at diagnosis, n (%)	
Never	2 (18)
Previous	6 (55)
Current	3 (27)
Disease severity at start of WLL with nMLT, n (%)	
DSS-1	0 (0)
DSS-2	5 (46)
DSS-3	3 (27)
DSS-4	3 (27)
DSS-5	0 (0)
Pulmonary function at start of WLL with nMLT	
FVC (%pred)	74 [57-88], n = 9
DLCO (%pred)	44 [37-49], n = 9

DLCO %pred indicates diffusion capacity of the lungs for carbon monoxide in percentage of predicted; DSS, disease severity score; IQR, interquartile range; n, number of subjects; nMLT, new modified lavage technique; WLL, whole lung lavage.

Efficacy of WLL With nMLT

Between September 2013 and July 2018, 11 subjects with aPAP underwent treatment with a total of 67 unilateral (31 left and 36 right lung) WLL procedures using the nMLT in our hospital. The median amount of WLL with nMLT procedures per subject was 4 [2 to 8], 1 subject with severe treatment refractory aPAP received up to 23 lavages. One pneumothorax was observed as adverse event related to the procedure. MH did not affect hemodynamics. Based on auscultation of the ventilated lung, although being a rather coarse technique of measurement, we did not detect fluid spillover to the ventilated lung during the procedure.

The median amount of removed protein in grams during 1 WLL with nMLT was 9.80 [7.52 to 12.66]. The median volume instilled saline during 1 WLL with nMLT was 15 [11 to 18] L. Minimal volume instilled saline was 4 L, and volume never exceeded 23 L. The median residue after the first cycle of 3 washes was 623 [372 to 853] mL. At the end of the procedure (OD < 0.4) the median residue was 1233 [863 to 1605] mL. Correlation coefficients of instilled volume with amount of removed protein was 0.426 ($P < 0.001$). After the first cycle of 3 washes, a median of 56% [49% to 61%] of the final protein yield was removed. After the second cycle of 3 washes a median of 81% [77% to 84%] and after the third cycle of 3 washes 91% [88% to 94%] of the final protein yield was removed (Fig. 1).

The effect on protein wash out using the nMLT was objectified in 31 WLL procedures using nMLT. A total of 116 OD measurements were recorded. OD course during nMLT is shown in Figure 2. 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 ($P < 0.001$). The course of OD during WLL with nMLT recorded in 1 representative subject is displayed in Figure 3.

DISCUSSION

In this study we report a new variant of the MLT 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 3 washes and chest percussions during every wash significantly increased protein removal in the consecutive cycle. Furthermore, our study supports flushing with the lowest possible instilled volume as after the third cycle of 3 washes, up to 91% of the total protein amount was already removed.

The use of manual ventilation was first performed by Bingisser et al in 1988.[14] Also Bonella et al[12] objectified recruitment of additional protein from the alveoli by repetitive manual ventilation and chest percussions in 2012. They reported an increase of protein removal in the wash cycle after manual ventilation was applied. Although intermittent chest percussion is not a novel technique, we provide a technical description to improve protein wash out. Our results make clear that the pressure build up due to MH is essential in additional protein recruitment. It is important to state, however, that compared with our protocol Bonella only applies 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. On the basis of our data we suggest applying this technique with MH after every 3 washes in order to enhance WLL efficiency. In our subjects, the median total amount of removed protein was 9.80 g. This amount does not differ from reported averages of 2 to 33 g in the literature.[12,15,16] However, variation in centrifugation techniques per center have led to pellet varying amounts of protein, making comparison of results with previous studies difficult.[17] The amount of removed protein per WLL in our cohort was influenced by the instilled volume. We used an average instilled volume of 15 L and did not exceed a volume of 23 L. Compared with the literature, Bonella et al[12] used up to 71 L with their MLT, significantly increasing total protein wash out compared with the classic technique. 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.

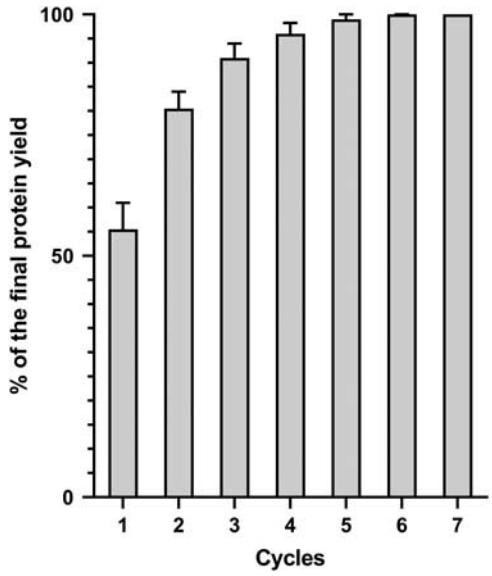

FIGURE 1. Percentage of the final protein yield per cycle. One cycle consisted of 3 consecutive washes. Per cycle the lung was flushed with approximately 3 L of saline. Data are displayed as median and interquartile range.

Although increased volume leads to more protein wash out, our results make clear that after the third cycle of 3 washes, up to 91% of the final protein yield is already recruited. This phenomenon is in concordance with results of Bonella et al.[12] At the moment, lavage duration is based on OD measurement of the recovered fluid. OD measurement after the third cycle of 3 washes does not yet reach the target value of 0.4. However, based on our data one could debate on the additional effect of using >18 L. The procedure of repetitive MH can be performed in ~5 minutes, indicating a minimal impact on the total duration of the WLL. The most important finding from our study in light of the duration of the WLL is the fact that almost no extra protein can be removed after using 18 L of flushing. This seems relevant since in a global survey it was found that 35% of experienced centers use between 18 and 40 L of flushing during the WLL.[7] Based on our data, one could debate whether in general it is still necessary to use >18 L of volume during the procedure of a WLL.

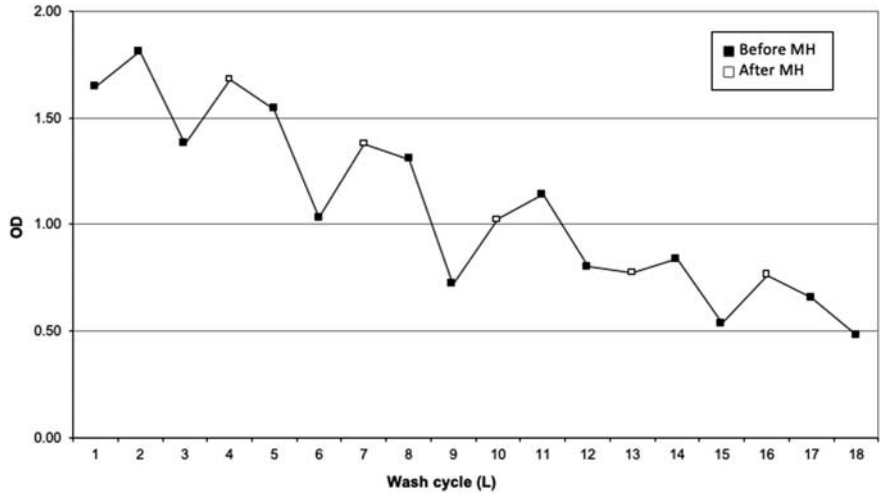

FIGURE 2. The course of OD during WLL with nMLT recorded in 1 representative subject. MH indicates manual hyperinflation; nMLT, new modified lavage technique; OD indicates optical density; WLL, whole lung lavage.

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.[18,19] In our cohort, 1 pneumothorax was observed as adverse event related to the procedure. This is higher than the known complication rate of pneumothorax related to WLL of 0.8%.[7] This could mean that nMLT has a higher complication rate. However, as undergoing postlavage x-ray investigation is standard of care at our institution, the rate of incidental pneumothoraxes due to this standard practice is probably higher. Therefore, we suggest nMLT is generally applicable for aPAP patients in contrast to Bingisser who reserves manual ventilation to severely impaired patients. A global survey by Campo et al[7] suggested that instilled volume was not correlated to clinical outcome. 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.

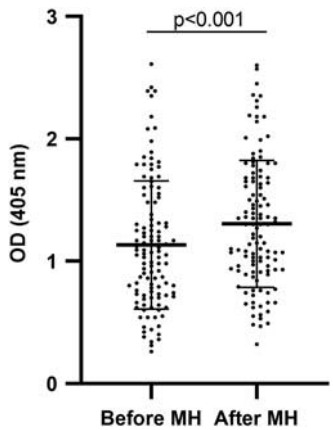

FIGURE 3. Significant increase of OD when comparing before and after MH. Paired Students *t* test was used to compare a total of 116 OD measurements recorded during 31 WLL treatments with nMLT in 5 subjects. Values are presented as mean (± SD). MH indicates manual hyperinflation; OD, optical density.

We are aware that there are several limitations to our study. First, the number of subjects was small, making conclusions less robust. Unfortunately, no comparison with our previous experience could be made as before 2013 no OD measurement was done. 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 for an ultra-rare disease. Third, our study could not demonstrate the effect of WLL with nMLT on pulmonary function since subjects received simultaneous treatment with GM-CSF therapy. Previous studies debate the positive effect of WLL on lung function in diagnosis and prognosis of PAP.[4,9,20,21]

In conclusion, efficacy of WLL seems to be enhanced by applying MH every 3 washes. 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. In general, our data suggest that using 18 L during a WLL adds little to increase protein yield.

Study data were collected and managed using Research Electronic Data Capture (REDCap)

electronic data capture tools hosted at our hospital.[22,23] The data that support the findings of this study are available from the corresponding author upon reasonable request.

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