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Original Research

Non-bronchoscopic Bronchoalveolar Lavage as a Refinement for Safely Obtaining High-quality Samples from Macaques

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Nonbronchoscopic bronchoalveolar lavage (NB-BAL) is a minimally invasive diagnostic and research tool used to sample the cells of lower airways and alveoli without using a bronchoscope. Our study compared NB-BAL and bronchoscopic bronchoalveolar lavage (B-BAL) in terms of costs, cell yields, and the number of post-procedural complications in macaques. We also analyzed procedure times, BAL fluid volume yields, and vital signs in a subset of animals that underwent NB-BAL. Compared with the B-BAL technique, NB-BAL was less expensive to perform, with fewer complications, fewer animals requiring temporary or permanent cessation of BALs, and higher cell yields per mL of recovered saline. The average procedure time for NB-BAL was 6.8 ± 1.6 min, and the average NB-BAL lavage volume yield was 76 ± 9%. We found no significant differences in respiration rate before, during, or after NB-BAL but did find significant differences in heart rate and oxygen saturation (SpO_2). This study demonstrates that NB-BAL is a simple, cost-effective, and safe alternative to B-BAL that results in higher cell yields per mL, improved animal welfare, and fewer missed time points, and thus constitutes a refinement over the B-BAL in macaques.

Abbreviations: BAL, Bronchoalveolar lavage; B-BAL, bronchoscopic BAL; BAL (NB-BAL), non-bronchoscopic; ET, endotracheal, ONPRC, Oregon National Primate Research Center; IDR, Infectious Disease Resource; rhCMV, rhesus cytomegalovirus; SpO_2, peripheral oxygen saturation; $PaCO_2$, partial pressure of carbon dioxide; TW, transtracheal washing; MA, manual aspiration; SPA, suction pump aspiration

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Bronchoalveolar lavage (BAL) is a minimally invasive diagnostic and research tool used to retrieve cells, microbes, and biomarkers from the lower airways and alveoli. The technique is used in both human and veterinary medicine to aid in diagnosing respiratory tract diseases such as lower airway infections, neoplasia, pulmonary hemorrhage, hypereosinophilic syndromes, hypersensitivity pneumonitis, and environmental lung diseases. BAL is also used in research to investigate the immunologic response of the lower airways to induced or spontaneous diseases. BAL fluid can be analyzed in terms of flow cytometry, gene expression, antibody titers, inflammatory mediators, and the confirmation or monitoring of experimental infections via culture or polymerase chain reaction.

BAL may be performed with or without the use of a bronchoscope. Bronchoscopic BAL (B-BAL) has several advantages, including visualization of the upper and lower airways and the capacity to select particular lung lobes for fluid instillation and retrieval. B-BAL is also cited as having a better ability to "wedge" the scope into a bronchus, creating a tight seal that may improve volume yield.[7] However, B-BAL has several disadvantages, including high equipment cost, the need for trained bronchoscopists, and the difficulty of effective and efficient instrument sterilization. Similarly, B-BAL may not be possible in small human or veterinary patients, and visualization of airways and selection of a particular lung lobe may not be necessary for research purposes.

Nonbronchoscopic BAL (NB-BAL) is typically performed by passing a small suction catheter through an endotracheal (ET) tube into the lower airways. This technique has been used in infants and domestic cats requiring small diameter ET tubes because until recently the smallest available bronchoscope with a lavage channel would obstruct the ET tube lumen in these subjects.[6] Although advancements in endoscope technology have made fine diameter broncho-fiberscopes with lavage channels available, NB-BAL has several advantages over B-BAL, including lower cost, minimal needs for technical skill, and no need for bronchoscope equipment. In addition, the use of sterile, single-use catheters reduces the potential for cross-contamination between patients and samples.[9]

Our group, the Oregon National Primate Research Center (ONPRC) Infectious Disease Resource (IDR), provides technical support to facilitate nonhuman primate (NHP) studies in areas such as immunology, infectious disease pathogenesis, and

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safety and efficacy testing of therapeutics and vaccines. BAL fluid contains abundant effector memory $CD8^+$ T cells derived from an easily accessible mucosal site[4] and is commonly requested by our investigators, with requests of 10 to 50 samples per workday. We initially used the ONPRC Surgical Services Unit (SSU) to obtain samples using B-BAL; this approach was expensive, frequently required medical intervention (supplemental oxygen, terbutaline and/or furosemide), and caused many animals to miss multiple BAL time points due to periprocedural complications. In addition, due to high procedure volumes and a limited number of bronchoscopes, equipment was commonly cleaned but not sterilized between animals. Therefore, we sought an inexpensive, simple BAL technique that promoted equipment sterility between animals, provided comparable results for investigators, and resulted in better clinical outcomes for study animals. We hypothesized that NB-BAL would provide comparable research results (cell yields) to B-BAL at a lower cost to our researchers. We also hypothesized that using the NB-BAL method would minimize complications as compared with the B-BAL method. Finally, we analyzed procedure times, BAL fluid volume yields, and vital signs in a subset of animals that underwent NB-BAL.

Materials and Methods

Animal information. From May 14, 2018, to November 7, 2019, 3,206 NB-BALs were performed for research purposes at the ONPRC. During this time, 523 rhesus (*Macaca mulatta)* and cynomolgus (*Macaca fascicularis)* macaques (202 females and 321 males, age range 0.9 to 21.3 y, weight range 2.7 to 13.6 kg) underwent the procedure. From January 1, 2018 to May 14, 2018, 2,084 B-BALs were performed for research purposes at the ONPRC. During this time, 421 rhesus and cynomolgus macaques (177 females and 244 males, age range 0.8 to 21.0 y, weight range 2.6 to 10 kg) underwent the procedure.

All animals were housed and cared for in compliance with the ONPRC animal care program, which is accredited by AAALAC International and is based on the laws, regulations, and guidelines determined by the United States Department of Agriculture (USDA) and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. All animal research was approved by the ONPRC Institutional Animal Care and Use Committee (IACUC).

Nonbronchoscopic bronchoalveloar lavage procedure. Animals were fasted overnight and sedated with an intramuscular injection of 8 to 12 mg/kg ketamine HCl (Ketathesia, Henry Schein Animal Health) and 0.015 mg/kg dexmeditomidine hydrochloride (Dexmedesed, Dechra, Overland Park, KS). Once anesthetized, animals were intubated with a sterile endotracheal tube to the approximate level of the thoracic inlet. Lidocaine 1% for injection (approximately 0.1 mL) was applied to the laryngeal folds of some animals to prevent laryngospasm and facilitate intubation. Once intubated, animals were placed in left lateral recumbency, with the head and neck extended. 100% O_2 (1 to 3 L/min) was administered to all animals immediately before and during the procedure via a nasal cannula attached to the endotracheal tube connector (Figure 1). Heart rate and peripheral oxygen saturation were continuously monitored via pulse oximetry. Depending on endotracheal tube size, an 8 or 10 french sterile, single-use pediatric suction catheter (Airlife Tri-Flo Suction Catheter with Control Port, Carefusion, Yorba Linda, CA) was inserted through the endotracheal tube connector and blindly passed through the trachea and into a mainstem bronchi (Table 1). The catheter was passed until resistance was felt, indicating the catheter had wedged into a distal bronchus.

Figure 1. Rhesus macaque positioned for nonbronchoscopic bronchoalveolar lavage. Animals were intubated and placed in left lateral recumbency, with a nasal cannula attached to the endotracheal tube connector supplying 100% O_2. Peripheral oxygen saturation and heart rate were monitored continuously via pulse oximetry.

Table 1. Endotracheal tube sizes (mm internal diameter) with corresponding suction catheter sizes (French) used for macaque nonbronchoscopic bronchoalveolar lavage (NB-BAL) at our institution.

Endotracheal tube size (mm)	Corresponding suction catheter size (Fr)
3.5	8
4.0	8
5.0	10

Airlife Tri-Flo suction catheters were used for all NB-BALs.

For animals weighing 3 kg and above, 3 to 4 lavages of 11 mL each of sterile 0.9% saline were performed (33 to 44 mL total), depending on the volume requested by the investigator. A total of 30 to 40 mL actually entered the lungs due to the volume of the tube (approximately 1 mL) so analysis was performed based on this volume, as the volume remaining in the tube did not yield cells. For animals weighing less than 3 kg, 3 lavages of 6 mL each were performed (18 mL total). To perform the lavage, a 20 mL syringe containing the lavage fluid was attached to the end of the catheter and infused over 1 to 2 s. Immediately after the infusion, the aliquot was manually aspirated into the syringe using gentle pulsating suction. If needed, the catheter was moved slightly (up to 5 mm) during aspiration to maximize fluid return. This process was repeated for 2 to 3 more aliquots, depending on the volume requested. Lavage fluid was immediately placed into 50 mL conical tubes containing R10 media. After the procedure, anesthesia was reversed with atipamazole hydrochloride (Antisedan, Zoetis, Kalamazoo, MI), and all animals were extubated and allowed to recover.

Bronchoscopic bronchoalveolar lavage procedure. Animals were fasted overnight and sedated with an intramuscular injection of 10 to 20 mg/kg ketamine HCl (Ketathesia, Henry Schein Animal Health). Once anesthetized, animals were placed in dorsal recumbency. 100% O_2 (1.5 L/min) was administered to all animals immediately before and during the procedure via a nasal cannula. Heart rate and peripheral oxygen saturation were continuously monitored via pulse oximetry. A laryngoscope was used to allow the insertion of a bronchoscope into the trachea. The bronchoscope was positioned slightly cranial to the carina, and a 1 mL bolus of 1% lidocaine was introduced

through the working channel. The bronchoscope tip was inserted into the right main stem bronchus and slowly advanced 3 to 5 bronchi branchings past the carina. Once bronchoscope placement was satisfactory, 3 aliquots of 10 mL of sterile 0.9% saline were infused followed by air to ensure that the entire volume entered the lungs. After each infusion, aspiration of the infusate was performed under continuous vacuum at approximately 80 to 100 mm Hg. Lavage fluid was immediately placed into 50 mL conical tubes containing R10 media. The bronchoscope was withdrawn to the carina and reinserted into the left main stem bronchus, repeating the procedure for the left lung. After the procedure, the bronchoscope was withdrawn, and the animals recovered on the table.

Complication rates: NB-BAL compared with B-BAL. Complications were assessed for all NB-BAL performed from May 14, 2018, to November 7, 2019. Complications were also assessed for all B-BAL procedures performed from January 1, 2018, to May 14, 2018. The following were considered clinical complications: oxygen desaturation (peripheral oxygen saturation, SpO2, less than 90%) requiring greater than 5 min of post-procedure oxygen support, medical intervention (e.g., administration of terbutaline and/or furosemide) with or without extended post-procedure oxygen support, and medical intervention with or without extended oxygen support after the procedure, and notes of low (less than 20 mL return) or hemorrhagic BAL fluid return (ranged from pink tinge to frank blood).

Analysis of bronchoalveolar lavage fluid. Lymphocyte and monocyte yields in BAL fluid were compared for a subset of 48 rhesus macaques (24 NB-BAL, which had received 30 mL of infusate, and 24 B-BAL, which had each received 60 mL). Both groups of animals had been vaccinated with rhesus cytomegalovirus (rhCMV) as part of their experimental protocol, underwent BALs to evaluate cellular responses to vaccination, and were at comparable time points in their studies.

BAL fluid was collected in 50 mL conical tubes, and samples were centrifuged at 300 x *g* for 10 min using a Sorvall Legend XT centrifuge to pellet the cells. After the cells were pelleted, liquid was removed by vacuum aspiration, and pellets were resuspended in 5 mL of 0.9% saline. Cells were enumerated by removing 0.1 mL and counting on a Horiba ABX Penta 60C+ DIFF analyzer. Lymphocyte number was calculated using the lymphocyte count/mL value generated by the Horiba analyzer and multiplied by the 5 ml volume that samples had been resuspended in ([Lymphocyte Count/mL] * [Volume] = [Total Lymphocyte Yield]). Lymphocyte per mL was calculated by dividing the total lymphocyte yield by the volume initially used for collecting the BAL (30 mL for NB- BAL and 60 mL for B-BAL). Monocyte numbers were calculated in the same manner.

NB-BAL procedure time, volume yield, and vital sign analysis. Procedure times, BAL fluid volume yields, and vital signs were recorded in a subset of rhesus macaques ($n = 34$) that underwent NB-BAL. For this subset of macaques, 3 lavages were performed per animal. Procedure time was considered as from the time of intubation to the time of removal of the suction catheter from the ET tube (immediately after collecting the last aliquot). BAL fluid aspirate volume (mL) was recorded, and a percent fluid yield was calculated. Animals were maintained on 100% O_2 starting before and continuing until after completion of the procedure. Vital signs (respiration rate, heart rate, and SpO_2) were recorded before the procedure, during the procedure (immediately after aspiration of the 2nd aliquot), 60s after the procedure, and 60 s after cessation of O_2 administration. The animals received the reversal agent atipamezole after vital sign recordings were completed.

Statistical analysis. All results were evaluated and plotted using GraphPad Prism (version 8.4.2). Statistical tests included X^2 unpaired Mann–Whitney *t* test, and Friedman test. Dunn–Bonferroni posthoc tests were conducted when Friedman tests showed significant differences between the 2 groups. All data are shown as standard deviation (SD) of the mean. Results were considered statistically significant when the *P* value was less than 0.05.

Results

Cost analysis. During the fiscal year of May 1, 2019, to April 30, 2020, the charge for B-BAL at the ONPRC was $79.40 per procedure (calculated based on staff time, equipment maintenance, and disposables). As of November 7, 2019, the comparable charge for NB-BAL (calculated based on staff time, equipment maintenance, and disposables) was $10.25 per procedure. This difference is equivalent to a cost savings of 87% per procedure for the investigators. This charge does not include the cost of training staff to perform the procedures, which is considerably lower for the NB-BAL technique.

Complication rates: NB-BAL compared with B-BAL. Complication rates for NB-BAL and B-BAL are shown in Table 2. A X2 test of homogeneity was performed to compare the total complication rates of animals undergoing B-BAL ($n = 421$) and NB-BAL ($n = 523$). Overall, the NB-BAL technique had a significantly (X^2 (1, $n = 5290$) = 252.6, $P = 0.00001$)) lower complication rate (0.2%) than did the B-BAL technique (8.5%). Animals undergoing NB-BAL required less O_2 support after the procedure and less medical intervention than animals undergoing B-BAL. Only one of the 523 animals (0.2%) undergoing NB-BAL required cessation of sampling due to periprocedural complications. In contrast, 23 (5.5%) of the 421 animals undergoing B-BAL had one or more missed time points due to poor recovery and/or bronchial mucosa friability/bleeding. This difference constituted a significant reduction in the need to forgo BAL sampling (X^2 (1, $n = 944$) = 26.2, $P = 0.00001$)) with the NB-BAL technique.

Flow cytometric analysis of bronchoalveolar lavage fluid. Lymphocyte and monocyte yields from a subset of animals that had NB-BAL ($n = 24$) or B-BAL ($n = 24$) were compared. Mean lymphocyte yield for NB-BAL was $6.29 \times 10^6 \pm 3.80 \times 10^6$ cells and for B-BAL was $5.69 \times 10^6 \pm 2.61 \times 10^6$ cells. These yields were not significantly different (unpaired Mann–Whitney *t* test, $P = 0.53$). Mean lymphocyte yield per mL for NB-BAL was $0.19 \times 10^6 \pm 0.12 \times 10^6$ cells and for B-BAL was $0.09 \times 10^6 \pm 0.04 \times 10^6$ cells (Figure 2). The NB-BAL technique resulted in significantly higher lymphocyte yields per mL as compared with the B-BAL technique (unpaired Mann-Whitney *t* test, $P = 0.00067$).

Mean monocyte yield for NB-BAL was $1.59 \times 10^6 \pm 1.36 \times 10^6$ cells and for B-BAL was $1.21 \times 10^6 \pm 5.28 \times 10^5$ cells (Figure 3). These yields were not significantly different (unpaired Mann–Whitney *t* test, $P = 0.20$). Mean monocyte yield per mL for NB-BAL was $5.31 \times 10^4 \pm 4.54 \times 10^4$ cells and for B-BAL was $2.01 \times 10^4 \pm 8.81 \times 10^3$ cells. The NB-BAL technique resulted in significantly higher monocyte yields per mL as compared with the B-BAL technique (unpaired Mann–Whitney *t* test, $P = 0.0010$).

NB-BAL procedure time, volume yield, and vital sign analysis. The average procedure time for NB-BAL was 6.8 ± 1.6 min, and the mean lavage volume yield for NB-BAL was 76 ± 9%. Mean heart rate, respiration rate, and SpO_2 before, during, and after NB-BAL (with and without O_2 support) from a subset of animals ($n = 34$) were compared and are shown in Table 3. No significant differences in the respiration rate were detected at any time point (Friedman test, $P = 0.008$; Dunn–Bonferroni posthoc test, $P > 0.05$ for all time points). Heart rate showed significant

Table 2. Complication rates of nonbronchoscopic bronchoalveolar lavage (n = 3,206) and bronchoscopic bronchoalveolar lavage (n = 2,084).

Complication type	Nonbronchoscopic BAL	Bronchoscopic BAL
Extended O_2 support only (without medication)	0.09%	4.17%
Medical intervention (with or without extended O_2 support)	0.06%	2.40%
Medical intervention (with or without extended O_2 support; with notes of low and/or bloody return)	0.03%	1.97%
Total Complication Rate	**0.18%**	**8.54%**

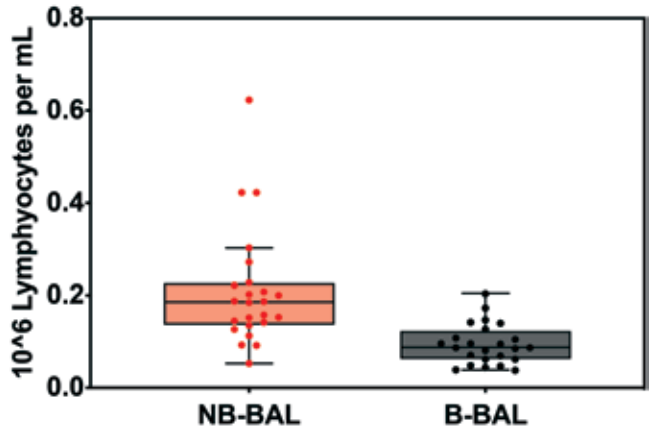

Figure 2. Bronchoalveolar lavage (BAL) was performed on 48 rhesus macaques, with 24 animals lavaged using the nonbronchoscopic BAL (NB-BAL) technique and 24 animals using the bronchoscopic BAL (B-BAL) technique. Boxplots compare the number of lymphocytes (10^6) per mL between the 2 techniques, with each animal being represented by a dot on the plot. Plots show jittered points, a box comprising the second and third quartiles (IQR), a line at the median, and whiskers extending to the farthest data point within 1.5*IQR above and below the box.

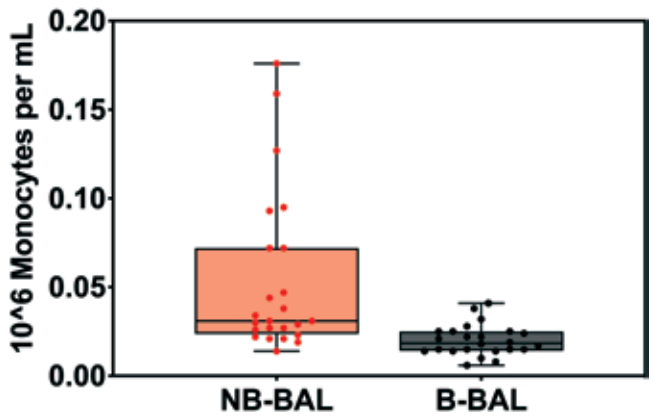

Figure 3. Bronchoalveolar lavage (BAL) was performed on 48 rhesus macaques, with 24 animals lavaged using the nonbronchoscopic BAL (NB-BAL) technique and 24 animals using the bronchoscopic BAL (B-BAL) technique. Boxplots compare the number of monocytes (10^6) per mL between the 2 techniques, with each animal being represented by a dot on the plot. Plots show jittered points, a box comprising the second and third quartiles (IQR), a line at the median, and whiskers extending to the farthest data point within 1.5*IQR above and below the box.

differences among time points (Friedman test, P = 0.000). Heart rate was significantly lower after NB-BAL, both with and without O_2 support, as compared with pre-NB-BAL (Dunn–Bonferroni posthoc test, $P < 0.05$ for both time points). Heart rate was also significantly lower after NB-BAL (with O_2 support), as compared with during NB-BAL (Dunn–Bonferroni posthoc test, $P < 0.05$). Heart rate showed no significant differences among other time points (Dunn–Bonferroni posthoc test, $P > 0.05$). Finally, significant differences in SpO_2 were detected among time points (Friedman test, P = 0.000). SpO_2 was significantly lower after NB-BAL without O_2 support as compared with during NB-BAL and after NB-BAL with O_2 support (Dunn–Bonferroni posthoc test, $P < 0.05$ for both time points). Significant differences in SpO_2 were not detected among other time points (Dunn–Bonferroni posthoc test, $P > 0.05$).

Discussion

In May 2018, our group switched from performing B-BAL to NB-BAL to collect BAL samples for investigators. We found that NB-BAL was less costly to perform than B-BAL, resulting in a cost savings to investigators of 87% per procedure at our institution. NB-BAL also had a significantly lower overall periprocedural complication rate requiring intervention (0.2%) compared with B-BAL (8.5%). Although we found no significant differences in respiration rate before, during, or after NB-BAL, we found significant differences in heart rate and SpO_2. We found no significant difference in total lymphocyte and monocyte yields from NB-BAL fluid as compared with B-BAL fluid, despite the use of lower lavage volumes for NB-BAL. Lymphocyte and monocyte yields from NB-BAL fluid were significantly higher on a per mL basis compared with B-BAL fluid. Finally, the average procedure time for NB-BAL was 6.8 ± 1.6 min, and the average NB-BAL lavage volume yield was 76 ± 9%. Although we did not compare procedure times and lavage volume yields from B-BAL and NB-BAL in this study, we provided this information to prospective investigators seeking to use this technique. Overall, the biggest benefits of the NB-BAL technique were improvements in animal welfare and the near-total elimination of missing data points, which previously occurred on a more regular basis (5.5% of animals) due to complications associated with the B-BAL technique. In summary, we found many benefits of the NB-BAL over the B-BAL technique, including reduced cost and expertise requirements, improved equipment sterility, lower complication rates, higher cell yields per mL, and avoidance of missed time points.

The entry cost to perform NB-BAL is low because bronchoscopes, imaging towers, and sterile processing equipment are not required. Ongoing expenses are also lower, as few supplies are needed to perform the procedure, most supplies are disposable, and required equipment has minimal maintenance cost. In addition to intubation supplies and sedatives, supply requirements for NB-BAL include a pulse oximeter, an oxygen supply, suction catheters, syringes, and sterile saline. Finally, with adequate training, NB-BAL is relatively easy to perform. In our hands, research technicians with no prior experience could be trained to intubate animals and perform NB-BAL with as little as 1 h of didactic and 2 h of hands-on training time.

Although we did not study contamination rates of NB-BAL and B-BAL samples, we believe the NB-BAL technique is

Table 3. Vital signs (respiration rate, heart rate and peripheral oxygen saturation) before, during, and after nonbronchoscopic BAL (NB-BAL; $n = 34$). Respiration rate expressed in breaths per minute. Heart rate expressed in beats per minute. Peripheral oxygen saturation expressed as percentage. Data are shown as standard deviation (SD).

	Before	During	Post (O_2 Support)	Post (no O_2 support)
Respiration rate	29.8 ± 7.2	29.1 ± 6.5	33.5 ± 7.8	32.0 ± 8.7
Heart rate	82.7 ± 10.5	82.0 ± 1.5	78.1 ± 1.9	76.6 ± 15.6
Peripheral oxygenation saturation	98.7 ± 1.5	99.0 ± 1.5	98.8 ± 1.9	98.0 ± 2.3

superior to the B-BAL technique in terms of potential for cross-contamination between samples and animals. At our institution, we routinely conduct up to 50 BALs per day, which precludes bronchoscope sterilization between animals due to time and resource limitations. Instead, bronchoscopes are cleaned between animals by flushing channels with tap water and saline and wiping external surfaces with 70% alcohol. However, even when using sterile processing equipment, endoscope sterilization can be difficult to achieve. Ineffective sterilization of bronchoscope equipment could result in cross-contamination of BAL samples and transmission of naturally and experimentally acquired infections between animals, negatively affecting study quality and compromising animal health. With the NB-BAL technique, sterilized ET tubes and single-use sterile catheters are used for all animals, which reduces the risk of transmission of infectious agents and sample cross-contamination.

Overall, we had good BAL fluid retrieval using the NB-BAL technique and found that NB-BAL resulted in better cell yields, despite lower administered lavage volumes. NB-BAL fluid retrievals in human and veterinary medicine are not frequently reported and vary among studies. However, we had higher retrieval volumes (76 ± 9%) than are reported by others,[2,6] perhaps due to the type of catheter used. One study in infants had fluid yield of 59 ± 6%,[6] and another reported retrieval rates of greater than 50% for the diagnosis of ventilator-associated pneumonia in infants.[2] Unfortunately, we did not collect data on how NB-BAL compares to B-BAL fluid retrieval, but other studies indicate comparable volume retrieval for B-BAL and NB-BAL.[8,9] In addition, we found no difference in cell yields between NB-BAL and B-BAL samples, despite lower lavage volumes for NB-BAL (30 mL/BAL) compared with B-BAL (60 mL/BAL). However, our NB-BAL samples had higher cell yields per mL than did our B-BAL samples. This finding contradicts other reports of higher cell yields using the B-BAL technique.[9] Other differences in BAL technique could significantly impact fluid and cell yields,[10] which may also explain these results. For example, the method of BAL fluid retrieval (suction pump or manual) could significantly affect fluid and cell yields. We used manual aspiration for NB-BAL and suction pump aspiration for B-BAL. A study in dogs showed significantly higher BAL fluid retrieval with suction pump aspiration (SPA) compared with manual aspiration (MA), but found no significant differences in total nucleated cell counts per microliter.[12] Conversely, a study in humans had higher volume retrievals using MA as compared with SPA.[10] Clearly, fluid mechanics during BAL are complex, and many factors such as operator experience, equipment, and technique differences, and patient health status may affect BAL fluid and cellular yields.

Although we found no significant differences in respiration rate before, during, or after NB-BAL, we found significant differences in heart rate and SpO_2. Heart rate was generally lower after NB-BAL, which was likely a side effect of dexmedetomidine sedation. Bradycardia is a common side effect of dexmedetomidine, which likely became more pronounced the longer the animal was sedated. SpO_2 was significantly lower after NB-BAL without O_2 support (98% ± 2%), as compared with during NB-BAL (99% ± 1%) and after NB-BAL with O_2 support (99% ± 2%). However, despite this decrease, lower SpO_2 levels were still within normal reference range and caused no observable clinical effects in the macaques.

Consistent with other studies, we found that NB-BAL complication rates were lower than those for B-BAL. Complications of BAL include hypoxemia, bronchospasm, trauma, cardiac arrhythmias, bradycardia, increased intracranial pressure, and increased blood pressure.[6,9] One study showed that NB-BAL caused less marked hemodynamic and ventilatory disturbance than did B-BAL.[9] In that study, patients undergoing B-BAL had increased heart rate, higher systolic blood pressure, more pronounced ST-segment depression, higher PaCO2, lower oxygen saturation, and higher sedative requirements than did those undergoing NB-BAL. Although we do not monitor many of these parameters in macaques undergoing B-BAL or NB-BAL, we saw reduced use of rescue drugs (terbutaline, furosemide) and decreased need for prolonged oxygen administration in animals undergoing NB-BAL, indicating improved clinical outcomes overall. In addition, before switching to the NB-BAL technique, significantly more animals experienced repeated B-BAL complications necessitating the use of rescue drugs and prolonged oxygen support, typically associated with lower airway friability, mucosal bleeding, and oxygen desaturation. Macaques that experienced complications were placed on "BAL rest" for periods of weeks to months, which prevented sampling at key study time points. Since we instituted the NB-BAL technique, only 1 macaque has required "BAL rest" during the subsequent 1.5-y period. This benefit has improved investigator satisfaction, study quality, and animal welfare.

Although we did not evaluate why our animals experienced fewer complications with NB-BAL compared with B-BAL, several explanations are plausible. First, less lavage fluid is needed to acquire comparable cell yields with the NB-BAL technique compared with the B-BAL technique, and fluid is only administered into one lung field, leaving a nonimpacted lung for gas exchange. Second, the tracheal suction catheter is less rigid than a flexible bronchoscope, with multiple holes to prevent tissue trauma while suctioning, resulting in less trauma and irritation of the lower airways than the bronchoscope and preventing bronchoconstriction and airway mucosal damage. Third, animals undergoing NB-BAL received dexmedetomidine in addition to ketamine, and thus were more deeply sedated than those that underwent the B-BAL technique, so animal coughing and movement may have contributed to the higher rate of complications associated with B-BAL. Fourth, differences in positioning during B-BAL (dorsal recumbency) compared with NB-BAL (lateral recumbency) may affect ventilation and lung perfusion, and thus may be an important determinant of gas exchange.[7] However, the adverse effects, as well as the need for rescue drugs and prolonged oxygen support, lasted well into the recovery period, when B-BAL and NB-BAL animals were positioned identically. Lastly, MA using a syringe during NB-BAL may result in less suction and potential for airway trauma than

SPA used with B-BAL. This may also result in a lower chance of airway collapse and subsequent oxygen desaturation.[10,12] As a final note, these groups underwent the procedures at different times, so other variables such as respiratory disease acquisition could have influenced these outcomes. However, no significant differences in environment, study, or background clinical disease were noted in the medical histories of these macaques.

Other unguided methods of sampling secretions from the lungs and airways exist, including transtracheal washing (TW) and blind protected BAL (BP-BAL)[1,3,5,11] The benefits of these methods are similar to those of NB-BAL in terms of cost, training requirements, and equipment needs and can be used if investigators do not require visualization of airways or sampling of a particular lung lobe. Protected techniques, such as BP-BAL, can obtain completely uncontaminated samples of secretions from the lower airway.[1,3] This approach may be preferable for investigators who wish to collect samples from the lower airway.

In conclusion, our results demonstrate that for experimental protocols where visualization of the airways or selection of a specific lung lobe are not essential, the NB-BAL is a refinement over the B-BAL technique and provides superior results in terms of animal wellbeing, avoidance of missed time points, procedure sterility, costs, and cell yield. This technique is also easily mastered by technicians in just a few training sessions, and the low rates of peri-procedural complications make it safe for junior technicians to perform.

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