Airway Secretion Clearance by Mechanical Exsufflation for Post-Poliomyelitis Ventilator-Assisted Individuals

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- Pulmonary complications from impaired airway secretion clearance mechanisms are major causes of morbidity and mortality for post-polio-myelitis individuals. The purpose of this study was to review the long-term use of manually assisted coughing and mechanical insufflation-exsufflation (MI-E) by post-polio-myelitis ventilator-assisted individuals (PVAIs) and to compare the peak cough expiratory flows (PCEF) created during unassisted and assisted coughing. Twenty-four PVAIs who have used noninvasive methods of ventilatory support for an average of 27 years, relied on methods of manually assisted coughing and/or MI-E without complications during intercurrent respiratory tract infections (RTIs). Nine of the 24 individuals were studied for PCEF. They had a mean forced vital capacity (FVC) of 0.54 ± 0.47L and a mean maximum insufflation capacity achieved by air stacking of ventilator insufflations and glossopharyngeal breathing of 1.7L. The PCEF were as follows: unassisted, 1.78 ± 1.16L/sec; following a maximum assisted insufflation, 3.75 ± 0.73L/sec; with manual assistance by abdominal compression following a maximum assisted insufflation, 4.64 ± 1.42L/sec; and with MI-E, 6.97 ± 0.89L/sec. We conclude that manually assisted coughing and MI-E are effective and safe methods of airway secretion clearance for PVAIs with impaired expiratory muscle function who would otherwise be managed by endotracheal suctioning. Severely decreased maximum insufflation capacity but not vital capacity indicate need for a tracheostomy.

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For physically intact adults, peak expiratory flows normally reach 7 to 12L/sec depending on sex, height, and age. Peak cough expiratory flows (PCEF) are usually 20% to 30% less. For individuals with bulbar and/or respiratory muscle weakness or severe pulmonary disease, coughing may not generate sufficient intrathoracic and abdominal pressures for the PCEF necessary to effectively clear airway secretions. This renders the individual susceptible to airway secretion retention, particularly during respiratory tract infections (RTIs), from which mucopurulent exudates can obstruct smaller bronchi and cause atelectasis, pneumonia, and acute respiratory failure. For example, traumatic quadriplegic patients' mean PCEF generally do not exceed 4lt/sec. For the typical elderly nursing facility resident with increased risk of pulmonary morbidity, mean forced vital capacity (FVC) may be diminished to 40% of predicted normal and PCEF not exceed 3 to 4L/sec. Individuals with skeletal or progressive neuromuscular conditions and restrictive pulmonary syndromes are also likely to have severely reduced PCEF. This is particularly true for post-polio-myelitis ventilator users with or without impaired bulbar muscle function and chronic alveolar hypoventilation.

When PCEF are inadequate, clinicians turn to techniques of chest percussion and postural drainage and endotracheal intubation for aspiration of proximal secretions. The former are commonly used but are often of questionable efficacy in the prevention or treatment of atelectasis, pneumonia, and mucus plugging. These chest physical therapy techniques are not more effective than vigorous expulsion of airway secretions alone, whether by coughing or otherwise. Positioning an acutely ill or severely disabled individual for postural drainage and chest percussion can also be difficult, time consuming, and at times impossible. Likewise, tracheal suctioning may also be inadequate because this method generally removes only tracheal and right main stem bronchus secretions leaving patients particularly susceptible to left lower lobe pneumonias and other pulmonary complications. Numerous complications of using endotracheal intubation or indwelling tracheostomy for long-term intermittent positive pressure ventilation (IPPV) and tracheal suctioning have also been described. Manually assisted coughing techniques may be used for individuals whose coughing mechanisms are inadequate to generate effective unassisted PCEF. For adults with less than about 1.5L of FVC, manually assisted PCEF may be significantly increased by giving the individual the greatest volume of air that he can hold with a closed glottis (maximum insufflation capacity) by some combination of venti-
lator-assisted insufflations and glossopharyngeal breathing (GPB). Indeed, manually assisted coughing from the individual's maximum insufflation capacity may be more effective at removing airway secretions than tracheal suctioning. Unfortunately, these techniques are generally not effective in the presence of severe scoliosis and the presence of osteoporosis of the rib cage can restrict the use of certain types of manually assisted coughing methods. Good caregiver-patient coordination is also necessary to deliver the types of manually assisted coughing methods. Good caregiver-patient coordination is also necessary to deliver the manually applied thrusts precisely as the patient initiates the cough expiratory flow. The frequent and around-the-clock need for assisted coughing for acutely ill patients is also labor intensive and may excessively strain the caregiver's wrists and shoulders. Access to an acutely ill patient's chest and/or abdomen to apply these techniques may also be inadequate.

Mechanically assisted coughing was described in the early 1950s. MI-E involves the delivery of an insufflation by a positive pressure blower followed immediately by an expulsive decompression or exsufflation in which high expiratory flow rates are coupled with a high expiratory pressure gradient between the mouth and the alveoli. Typically, a pressure drop of 65 to 90 cm H2O is created in less than 0.1 seconds. Insufflation and exsufflation pressures are independently adjusted for efficacy and comfort.

This study was designed to compare the PCEF of PVAls created during unassisted coughing, coughing following a maximal insufflation provided by an air stacking maneuver and/or GPB, during manually assisted coughing following a maximal insufflation, and with the use of newly constructed mechanical exsufflators. Peak pulmonary volumes and expiratory flow rates prior to and immediately following the use of MI-E were evaluated for the possibility of exsufflation-induced airway collapse. The efficiency of air stacking by ventilator use and by GPB was examined as was the long-term individual experience with the use of assisted coughing techniques and MI-E. The use of the maximum insufflation capacity rather than the FVC will be discussed as an indication of the need to resort to tracheostomy for ventilatory assistance.

**PATIENTS AND METHODS**

Post-polioymielitis individuals who have been supported by noninvasive methods of ventilatory support and who have routinely used both manually assisted coughing and MI-E during intercurrent RTIs were candidates for this study. Twenty-four candidates (14 females, ten males) were identified. Nine of the 24 candidates (five females, four males) agreed to have their PCEF and pulmonary function studied.

Pulmonary volumes and expiratory flows (except during MI-E) were measured from the maximum FVC observed in five to eight attempts on the Respiradyne Pulmonary Function Monitor. The measured pulmonary volumes included the following: the patients' FVC; maximum insufflation assisted FVC achieved by air stacking of two or more ventilator insufflations to the maximum volume that could be held with a closed glottis; maximum insufflation assisted FVC by using glossopharyngeal breathing to the maximum single breath capacity (GPmaxSBC) achieved by taking a deep breath then adding to it by maximum depth GPB; and maximum insufflation-assisted FVC achieved by using air stacking followed by a maximum depth GPB. The FVC, ratio of forced expiratory volume in one second (FEV1) to FVC, peak expiratory flow (PEF), and forced expiratory flow during the middle-half of the FVC (FEF25 to 75%) were measured both before and immediately following MI-E.

PCEF were measured during unassisted coughing, during coughing following a maximum insufflation by air stacking or maximum depth GPB which ever was greater, during a manually assisted cough following a maximum insufflation, and during MI-E. Seven of the nine individuals were skilled at GPB and routinely used it to assist in coughing. Manually assisted coughing was performed by applying a thrust timed to the initiation of the patient's cough following the maximum insufflation or GPB maneuver. It was administered by the individuals' trained home care attendants, family members, or individuals who provided this service for the individual during RTIs. MI-E was applied to the patient via an anesthesia mask. The optimal insufflation and exsufflation pressures were identified by independently adjusting and gradually increasing them until the individual indicated the maximum comfortable levels used during RTIs. The frequency and duration of the delivered insufflations and exsufflations were also adjusted for patient comfort. Insufflations were generally sustained for one second and exsufflations for one to three seconds. Approximately four to six consecutive insufflation-exsufflations were delivered followed by a period of unassisted or routine ventilator-assisted breathing to prevent hyperventilation.

During unassisted and manually assisted coughing, PCEF were measured using a peak flow meter (model 710). Because we could find no commercially available flow monitors with sufficiently low internal resistance to permit accurate measurement of the high flows generated during MI-E, we adapted a previously described technique for this purpose. A tapped differential pressure tube was used to measure the differential pressure generated across a slightly restricted orifice (fig 1). To further enhance the differential output pressure of this device the high side tap was angled at 90° to face the direct impact of the flow. The differential pressure thus developed was fed into a differen-
tial pressure transmitter (model 603-4°) to obtain an electrical signal that was measured and converted onto a strip chart (strip chart recorder type 720f) (fig 2). In order to calibrate the peak output of the curves thus obtained, a set up was designed using a large, flaccid collecting balloon. A steady flow of air was obtained through a speed-controlled blower. The speed was set to achieve the maximum excursions of the chart needle. Once the excursion had been set, the air flow was directed into the collection balloon for a measured period of time, ie, three seconds. The collecting balloon was then pinched off and connected to the intake side of a two-bottle passive water siphon transfer system with a total volume of approximately ten gallons. The water system was placed above a collecting container on a weighing scale (fig 3). The air content of the balloon was then opened to the intake side of the siphon and the siphon water allowed to flow into the collecting container of known weight until all the air in the collecting balloon was transferred out and the balloon collapsed. This automatically shut off the siphon. The total volume of water collected in the container was weighed and converted into liters. This volume was then divided by time. The flow of air in liters per second was indicated by the height of the strip chart curve. The total MI-E volume was depicted by the total area under the curve (fig 4).

RESULTS

The 24 PVAIs with a mean age of 57.7 ± 7.3 years have been ventilator assisted for a mean of 27 ± 10.8 years. They reported having used MI-E during acute RTIs. Several used it on 30 or more occasions without complications. They all also reported having relied heavily on the use of manually assisted coughing during intercurrent RTIs. These 24 PVAIs reported a total of 25 hospitalizations for treatment of respiratory conditions including seven hospitalizations for episodes of pneumonia over a mean period of 1.2 years prior to beginning ventilatory support definitively. While using overnight noninvasive ventilatory support, these 24 PVAIs reported having had a total of seven pneumonias and 29 hospitalizations over a cumulative period of 93.7 years (one hospitalization every 3.23 years) mostly for the management of intercurrent RTIs. The 18 PVAIs who either used 24 hour noninvasive ventilatory support since the onset of poliomyelitis or who have progressed to 16 to 24 hours per day of noninvasive ventilatory support reported a total of 13 pneumonias and 85 hospitalizations over a cumulative period of 489.1 years (one hospitalization every 5.8 years).

Seven of the PVAIs had been tracheostomized either

Fig 2—The differential pressure developed during mechanical exsufflation was fed into a differential pressure transmitter to obtain an electrical signal that was measured and converted onto a strip chart.

Fig 3—The water displaced by the airflow was weighed thus determining air flow volume.

Fig 4—During mechanical exsufflation, inspiratory airflow correlates with the amplitude below the isobaric line and expiratory airflow above the isobaric line. Each vertical box is the equivalent of 355ml/sec. The total inspiration-expiration volume was found by integrating the area above and below the isobaric line with each small box the equivalent of 14.2mL.
prior to switching to noninvasive methods of ventilatory support or for surgical management of intercurrent non-pulmonary conditions. All seven, six of whom were skilled at GPB, switched back to noninvasive methods of ventilatory support and had their tracheostomy sites closed. Two other PVAIs were ultimately tracheostomized during a RTI and continue to use IPPV via tracheostomy after 36 and 29 years of 24 hour noninvasive ventilatory support, respectively. Both were skilled at GPB. They reported one intercurrent episode of pneumonia and one hospitalization over a period of 8.3 years. One has expressed the desire to return to noninvasive methods of ventilatory support.

The nine individuals who agreed to be studied had a mean age of 55.6 ± 7.25 years and had used ventilatory support for 30.9 ± 11.9 years. Seven of the nine individuals used ventilatory support 24 hours a day. The other two used support overnight, for up to several daytime hours, and up to 24 hours a day during RTIs. Four of the nine PVAIs were ventilator supported 24 hours a day since onset of poliomyelitis. The other five PVAIs developed chronic alveolar hypoventilation that led to hospitalizations for acute respiratory failure and ultimately introduction of definitive ventilatory assistance. Two of these five individuals were hospitalized for respiratory failure on many occasions before definitively beginning daily ventilatory assistance. During their 278 patient-years of noninvasive ventilatory support these nine PVAIs experienced a total of 67 hospitalizations (one hospitalization every 4.15 years) for management of intercurrent RTIs and only one PVAI reported having had an episode of pneumonia over this period of time.

During the hospitalizations the PVAIs received aggressive assisted coughing and were, for the most part, temporarily ventilated by combining the use of the iron lung with mouth IPPV (see fig 5). More recently, however, the PVAIs are being managed at home with oximetry monitoring and with the use of 24 hour mouth or nasal IPPV, manually assisted coughing, and MI-E. None of the nine PVAIs has been hospitalized for respiratory impairment since 1987.

The FVCs, maximum insufflation assisted FVC’s and the MI-E volumes are listed in table 1. All assisted pulmonary volumes including the FVC achieved by using a maximum depth GPB were significantly greater than the unassisted FVC (p < 0.001). There was no significant difference between the maximum insufflation-assisted FVC and the FVC following maximum depth GPB. However, the combination of ventilator assisted air stacking followed by maximum depth GPB provided a significantly greater maximum insufflation capacity than the use of either technique alone (p < 0.01). The MI-E volumes were greater than the maximum insufflation assisted FVC; however, the difference did not reach statistical significance (p = 0.161).

The pulmonary flow measurements taken just before and immediately following MI-E are listed in table 2. There were no significant differences between the pulmonary flows preceding and those following the use of MI-E.

The PCEF generated during unassisted coughing, unassisted coughing following a maximum insufflation by air stacking or GPB, manually assisted coughing following a maximum insufflation, and MI-E are presented in table 3.

The PCEF achieved during MI-E were significantly greater than the PCEF achieved by any other method including those achieved by manually assisted coughing following a maximum insufflation (p = 0.008). The PCEFs measured during unassisted coughing were significantly less than those achieved by every other method including those achieved during unassisted coughing following a maximum insufflation (p = 0.002).

DISCUSSION

Coughing usually begins as a brief inspiration greater than normal resting tidal volume. Brief closure of the glottis, then generation of 50 to 100mm Hg abdominal, pleural, and alveolar pressures during expiratory effort. Within 30 to 50msec the PCEF occurs and can exceed 12L/sec. A minimum PCEF of five to six liters per second appears to be necessary for individuals with profuse airway secretions to avoid a markedly increased risk of serious pulmonary complications. In addition to flows exceeding five liters per second, normal adults expel about 2,200mL of air per cough. For PVAIs the air flows generated by MI-E more closely matched the flow volumes and velocities of normal coughing than did manually assisted or unassisted coughing. MI-E also accomplishes this with less than one-third of the abdominal and intrathoracic pressures generated during the unassisted coughing of physically intact adults. This aspect makes it especially applicable for use by patients following chest or abdominal surgery.

Following instillation of a mucin-thorium dioxide suspension into the lungs of anesthetized dogs, bronchograms revealed virtually complete elimination of the suspension...
Table 1: Maximum Assisted and Unassisted Pulmonary Volumes

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Aid*</th>
<th>GPmaxSBC (mL)</th>
<th>IC (mL)</th>
<th>MIC (mL)</th>
<th>MI-E (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>F</td>
<td>40</td>
<td>0</td>
<td>1,250</td>
<td>1,050</td>
<td>1,370</td>
</tr>
<tr>
<td>53</td>
<td>M</td>
<td>40</td>
<td>900</td>
<td>2,070</td>
<td>1,820</td>
<td>2,340</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>37</td>
<td>620</td>
<td>2,840</td>
<td>3,020</td>
<td>3,020</td>
</tr>
<tr>
<td>67</td>
<td>F</td>
<td>37</td>
<td>525</td>
<td>1,260</td>
<td>1,640</td>
<td>1,640</td>
</tr>
<tr>
<td>64</td>
<td>M</td>
<td>36</td>
<td>220</td>
<td>2,250</td>
<td>2,250</td>
<td>2,100</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>36</td>
<td>0</td>
<td>1,850</td>
<td>1,550</td>
<td>2,050</td>
</tr>
<tr>
<td>58</td>
<td>M</td>
<td>11†</td>
<td>1,490</td>
<td></td>
<td>1,760</td>
<td>1,760</td>
</tr>
<tr>
<td>44</td>
<td>F</td>
<td>10‡</td>
<td>710</td>
<td>1,090</td>
<td>1,080</td>
<td>1,160</td>
</tr>
</tbody>
</table>

Mean ± SD

30.9 ± 11.8 540 ± 470 1,660 ± 630 1.640 ± 720 1,880 ± 600 2.030 ± 370

Abbreviations: VC, forced vital capacity; GPmaxSBC, glossopharyngeal maximum single breath capacity; IC, insufflation capacity produced by air stacking of ventilator assisted breaths; MIC, maximum insufflation capacity produced by air stacking followed by deep glossopharyngeal breathing (GPB) when possible; MI-E, Mechanical insufflation-exsufflation volume.

* Years of ventilatory support, currently 24 hour ventilator use.
† Mean for the seven individuals capable of effective GPB
‡ Mean for the seven individuals capable of effective GPB was 1530 ± 780 mL.
§ Mean for the seven individuals capable of effective GPB was 1840 ± 670 mL.

after six minutes of MI-E.17 The technique was shown to be equally effective in expulsing bronchoscopically inserted foreign bodies.17 The use of MI-E through an indwelling tracheostomy tube was demonstrated to be effective in reversing the acute atelectasis associated with productive airway secretions during an RTI for a poliomyelitis patient in 1954.25 Barach and Beck18 demonstrated clinical and radiographic improvement in 92 of 103 acutely ill bronchopulmonary and neuromuscular patients with RTIs with the use of MI-E.18 This included 72 patients with bronchopulmonary disease and 27 with skeletal or neuromuscular conditions including poliomyelitis;25 however, it was more effective for the latter than for the former.18 The authors noted that the lack of benefit observed for nine of the 103 patients was due, at least in part, to lack of cooperation and to inadequate experience in the correct application of MI-E in the early part of the study. No significant complications were described for either patient population and the technique did not have to be discontinued for any patient.

Colebatch19 observed that applying negative pressure of 40 to 50 mm Hg is unlikely to have any deleterious effects on pulmonary tissues.20 He noted that because the negative pressure applied to the Airways is analogous to positive pressure on the surface of the lungs during a normal cough, it is improbable that this negative pressure can be more detrimental to the lungs than the normal pressure gradient generated during unassisted coughing. Bickerman21 stated that after careful examination of the lungs of animals treated with MI-E, no evidence of parenchymal damage, including hemorrhage, alveolar tears, or emphysematous blebs was observed. Barach and Beck18 reported no serious complications in the 103 patients they treated. No reports of damaging side effects have been disclosed in more than 2,000 treatments with MI-E.20 Consistent with this is the fact that over the period of 650 patient-years and hundreds of applications of MI-E by our 24 PVAIs, no episodes of pneumothorax, aspiration of gastric contents, or blood-streaking of sputum were observed. Borborygmus and abdominal distention were infrequent and eliminated by decreasing insufflation pressures. The absence of any decrease in forced expiratory flows in the immediate post-exsufflation period indicated that no air trapping occurred as a result of MI-E.

In addition to the clearing of pulmonary infiltrates and atelectasis, an increase in FVC of 15% to 42% was noted immediately following treatment in 67 patients with obstructive dyspnea. A 55% increase in FVC was noted following MI-E in patients with neuromuscular conditions.18 We have subsequently observed 15% to 50% improvement in FVC and normalization of oxyhemoglobin saturation when MI-E is used to remove mucus plugs for acutely ill ventilator-assisted neuromuscular patients.

Despite the remarkable success and convenience of MI-E in secretion mobilization and evacuation, the technique

Table 2: Pulmonary Flow Measurements Just Preceding and Following Mechanical Exsufflation

<table>
<thead>
<tr>
<th>FVC (before/after)</th>
<th>FEV1/FVC (% before/after)</th>
<th>PEF (before/after)</th>
<th>FEF25%-75% (before/after)</th>
</tr>
</thead>
<tbody>
<tr>
<td>540 ± 470/550 ± 470</td>
<td>88.4 ± 14.8%/90.8 ± 10.9%</td>
<td>1,590 ± 1,010/1,600 ± 1,030</td>
<td>680 ± 560/750 ± 660</td>
</tr>
</tbody>
</table>

Abbreviations: FVC, forced vital capacity; FEV1/FVC, forced expiratory volume in one second divided by FVC; PEF, peak expiratory flow; FEF25%-75%, forced expiratory flow during expiration of the middle half of the VC during the measurement of FVC.
ventilatory support, including IPPV provided by oral, nasal, and oral-nasal interfaces have been described. These techniques, whether used alone or in combination with body ventilators, safely and conveniently permit the use of up to 24-hour long-term ventilatory support without a tracheostomy for individuals who have adequate oropharyngeal muscle function and who satisfy other criteria. However, in the presence of intercurrent RTIs, and especially for patients who have conditions, such as severe kyphoscoliosis, which hamper the use of manually assisted coughing, the discomfort and ineffectiveness of suctioning of the upper airway via the mouth or nose and the undesirability of endotracheal suctioning indicate the need for the use of MI-E if tracheal intubation and tracheostomy are to be avoided. The success of these assisted coughing techniques is reinforced by the fact that these patients with little or no FVC have managed to survive so long and with so few hospitalizations or pulmonary complications without indwelling tracheostomies.

Various criteria based on FVC have been cited to justify when to intubate a patient for ventilatory support. FVCs of 600 to 800mL have been cited as well as FVCs of 15mL/kg of body weight, and 33% of predicted normal. There are, however, many exceptions to this with some individuals free of ventilatory support with FVCs of 320mL or less, and others who may require at least nocturnal support with sitting FVCs approaching 2,000mL. Further, numerous patients have been described with little or no measurable FVC who have received ventilatory support for up to 40 years by noninvasive methods without the presence of an indwelling tracheostomy tube. All of these ventilator-assisted individuals, however, had maximum insufflation capacities greater than 500mL with the great majority exceeding 1,000mL or they had ready access to MI-E. The maximum insufflation capacity is a function of oropharyngeal muscle strength. The weaker the oropharyngeal musculature, the lower the maximum insufflation capacity, the greater the difficulty in clearing airway secretions, and the more likely the need for tracheostomy. All of the patients who were studied have maximum insufflation capacities exceeding 1,100mL and could, therefore, be successfully managed during periods of increased airway secretion by using manually assisted coughing techniques with only occasional resort to MI-E.

The avoidance of tracheostomy permits many patients with little or no FVC to learn to use GPB to increase their ventilatory support, including IPPV provided by nasal, and oral-nasal interfaces have been described. These techniques, whether used alone or in combination with body ventilators, safely and conveniently permit the use of up to 24-hour long-term ventilatory support without a tracheostomy for individuals who have adequate oropharyngeal muscle function and who satisfy other criteria. However, in the presence of intercurrent RTIs, and especially for patients who have conditions, such as severe kyphoscoliosis, which hamper the use of manually assisted coughing, the discomfort and ineffectiveness of suctioning of the upper airway via the mouth or nose and the undesirability of endotracheal suctioning indicate the need for the use of MI-E if tracheal intubation and tracheostomy are to be avoided. The success of these assisted coughing techniques is reinforced by the fact that these patients with little or no FVC have managed to survive so long and with so few hospitalizations or pulmonary complications without indwelling tracheostomies.

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Thus, GPB should also be taught to all neuromuscular ventilator-assisted individuals without completely incompetent oropharyngeal musculature unless they are being ventilated by tracheotomy without a one-way valve to prevent air flow out of the tracheotomy site.

**CONCLUSION**

Post-poliomyelitis individuals using noninvasive methods of ventilatory support have greater PCEF produced by manually assisted coughing and by MI-E than can be produced by unassisted coughing. Thus, if intubation and tracheostomy arc to be avoided, it becomes crucial for these individuals and their caregivers to learn and have access to these methods, particularly during RTIs when expiratory muscle weakness is exacerbated. These principles may also apply to individuals with severe expiratory muscle weakness due to other neuromuscular conditions. We believe that it is largely because of the lack of general knowledge of and access to MI-E that other centers are more likely to resort to intubation and tracheostomy for patients with advanced neuromuscular disease and RTIs. We conclude that loss of FVC in itself does not mandate the need for intubation or tracheostomy for ventilatory support. A maximum insufflation capacity below 500mL may be a better indicator. MI-E should be further explored as a convenient and probably more effective alternative to endotracheal suctioning for ventilator-supported neuromuscular individuals.

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**References**


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