

Comparative study between different methods of aliquots suction during bronchoalveolar lavage

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Background Bronchoalveolar lavage (BAL) is a widely performed diagnostic and research procedure.

Objectives The aim of the present study was to standardize the method of retrieving BAL in our institution through comparing three methods of BAL retrieval regarding efficacy and safety.

Methods A total of 60 adult patients were randomly divided according to the method for retrieving BAL infusate into three groups, of 20 patients each. These are by using gentle hand suction into sterile syringe (Group I), using gentle syringe suction into a fluid trap (Group II), or using gentle suction by aspirator, collecting the lavage specimen into a collection trap (Group III).

Results No statistical difference was noted between groups regarding age, sex, presenting symptoms, anesthesia, patient position, introduction site, postprocedural complications, and total cell count in the retrieved fluid. The volume of the recovered fluid using the method in group III was significantly higher than that of the method used in group II ($P=0.001$). Although the volume of the recovered fluid by the method in group III was apparently higher than that of the

method in group I, and that for the method in group I was apparently higher than that in group II, both lacked significance ($P=0.188$ and 0.066 , respectively).

Conclusion All studied methods of retrieving BAL infusate are safe. Using an aspirator into a fluid trap is superior to using syringe suction into a fluid trap in retrieving more voluminous BAL infusate.

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Introduction

Bronchoalveolar lavage (BAL), performed during flexible fiberoptic bronchoscopy, has gained widespread acceptance as a minimally invasive method that provides important information about immunologic, inflammatory, and infectious processes taking place at the alveolar level [1]. It allows the recovery of both cellular and noncellular components of the alveolar lining fluid and epithelial surface of the lower respiratory tract and alveoli [2,3].

The technical aspects of the lavage procedure are very important. Some of the controversy about interpreting lavage results can be traced to different centers having different results because of variations in the technique; thus, as in any test, the details are important. It became apparent that the difference in technique led to differences in results [4].

There have been attempts to obtain some standardization, mainly by way of expert consensus [1,4–8]. The optimal technique used to instil and recover bronchoalveolar lavage fluid (BALF) is still a matter of debate [6]. The aim of this study was to standardize the method of retrieving BAL in our institution through comparing three methods of BAL retrieval regarding efficacy and safety.

Patients

This was a randomized, prospective comparative clinical study. It included 60 adult patients of both sexes, admitted in or referred to Alexandria Main University Hospital and for whom bronchoscopy was recommended as part of their diagnostic work-up. Patients were excluded from the study if they had any contraindication to bronchoscopy [9–11] or refused to give an informed consent.

All patients were randomly categorized into three groups (20 patients each) as follows:

Group I [direct syringe suction (DSS) group], for which BAL retrieval was carried out using direct hand suction into sterile syringe.

Group II [syringe suction with trap (SST) group], for which BAL retrieval was done using gentle syringe suction into a fluid trap.

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Group III [aspirator suction with trap (AST) group], for which BAL retrieval was done using gentle suction by portable aspirator, collecting the lavage specimen in a collection trap.

Methods

The study was approved by the institutional ethics committee. Informed consent was taken from all patients as regards commitment to this research work, disadvantages and then benefits of the procedure, possible complications, and alternative options. All patients in the present study were subjected to history-taking, physical examination (pulse, blood pressure, respiratory rate, temperature, arterial oxygen saturation, local chest examination, and general examination), routine laboratory investigations, and radiological (plain chest radiography and CT chest) evaluation before the procedure. Two bronchoscopes [EB-1575 K videobronchoscope (5.2 mm in diameter; Pentax Medical, Montvale, New Jersey, USA) and FB-18 V fiber bronchoscope (5.9 mm in diameter; Pentax medical)], with different diameters of working channel (2.0 and 2.8 mm, respectively), were randomly used.

Patients' preparation

All patients received nothing by mouth 6 h before bronchoscopy. The procedure was explained to the patient before the bronchoscopy. Intravenous access was established.

- (1) Premedication with sedatives [propofol (3 mg/kg)] as indicated by the patients' condition, was done before and/or during the procedure.
- (2) Local lidocaine 10% was sprayed transnasally and transorally before the procedure. Topical anesthesia at the larynx was completed using lidocaine 2%; usually a total of 4–6 ml is used. Additional 2 ml aliquots of 2% lidocaine was instilled at the carina, at the division of the right lower lobe and right middle lobe entrance.
- (3) BAL was carried out before any other bronchoscopic interventions or to decrease the risk of contamination or bleeding. The site in which BAL was carried out was determined according to the patient's CT chest. The method of suction was randomly chosen using the sealed-envelope method. These three methods are shown in Figs 1–3.

The procedure for lavage

The bronchoscope was positioned into a subsegmental bronchus. Good positioning was indicated through the

wink test [12] by a bronchoscope that could be fully maintained in position by the bronchoscopist and an airway that did not fully close immediately on gentle suction.

- (1) A 20 ml syringe was prefilled with normal saline (at room temperature), 20 ml for each aliquot in six successive syringes. When the patient and bronchoscopist were ready, the first syringe of saline was instilled by the assistant while the bronchoscopist maintained the position in the subsegmental bronchus.
- (2) The volume retrieved during BAL was collected in sterile syringe or fluid trap according to the method previously chosen. Dwell time was kept to a minimum.
- (3) The retrieved BALF was expelled gently into a labeled sterile container; the volume of BALF was recorded and then sent to a lab within 1 h for further assessment.
- (4) Any complications during the procedure were noticed and recorded with emphasis on increased dyspnea, drop of saturation, fever, change in blood pressure, change in respiratory rate, chest pain, and bleeding.
- (5) The participants were sent to recover for 2–4 h in a ward.
- (6) During this time, they were allowed to rest and then only eat and drink at least 60 min after the procedure (when their swallow was assessed as safe) to reduce the risk for aspiration.
- (7) Postprocedural observations were monitored and recorded. A clinical examination was carried out before discharge.

Laboratory analysis of bronchoalveolar lavage specimens

The analysis was performed in the routine laboratory of the Clinical Pathology Department, Faculty of Medicine, University of Alexandria, Egypt. The BAL was examined. White blood cell (WBC) counts, as well as differential WBC count, were determined using the Neubauer hemocytometer (VWR Scientifics, West Chester, PA) [13–15].

Total WBCs count

A drop (10 μ l) of the whole undiluted BALF was applied on each quadrant of the Neubauer hemocytometer and the WBCs were identified on the basis of their morphology and were counted. The number of WBCs was calculated per cubic millimeter (n/cm^3) on the basis of the standard cell-counting procedure using the Neubauer hemocytometer.

Figure 1



Direct syringe suction through the working channel of the flexible bronchoscope. Group I (DSS group).

Figure 2



Syringe suction with trap. Group II (SST group).

Figure 3



Aspirator suction with trap. Group III (AST group).

Furthermore, an aliquot of BALF was mixed with 3% WBCs solution (3% acetic acid solution in distilled water with few drops of Leishman stain). According to the degree of clarity of the fluid and the presence or absence of reddish tinge indicating hemorrhagic fluid, the dilution of the fluid was adjusted accordingly; the cells were counted using the Neubauer hemocytometer and the counted cells were calculated considering the dilution factor. The cells were classified to either mononuclear cells or polymorphnuclear cells according to nuclear segmentation.

Differential WBC count

For the sake of classification of the cells in the BAL, a stained thick film was created. The BALF was centrifuged, the supernatant was discarded, and the

deposit was resuspended gently, mixed with equal volume of the patient serum (or serum albumin) and the mixture centrifuged once more. Finally, 10 ml from the deposit were spread in a circular manner at the center of a microscopic slide, the preparation was left to dry, then stained with Leishman stain, and later examined under the microscope for differential cell count. WBCs were classified as macrophages, neutrophils, lymphocytes, eosinophils, mast cells, or other cells according to the standard morphological criteria [14].

Statistical analysis of the data

Data were fed to the computer and analyzed using SPSS software package (IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Armonk, NY). Qualitative data were described as number and percentage. Quantitative data were described as range (minimum and maximum), mean, SD, and median. Comparison between different groups regarding categorical variables was carried out using the χ^2 -test. When more than 20% of the cells had expected count less than 5, correction for χ^2 was conducted using the Monte Carlo correction. The distributions of quantitative variables were tested for normality. If it revealed normal data distribution, parametric tests were applied. If the data were abnormally distributed, nonparametric tests were applied. For normally distributed data, comparison between more than two independent populations was carried out using the F -test (analysis of variance) and the post-hoc test (least significant difference). For abnormally distributed data, comparison between more than two independent population was carried out using the Kruskal–Wallis test and pair wise comparison was

assessed using the Mann–Whitney test. Significance of the obtained results was judged at the 5% level.

Results

Demographic data

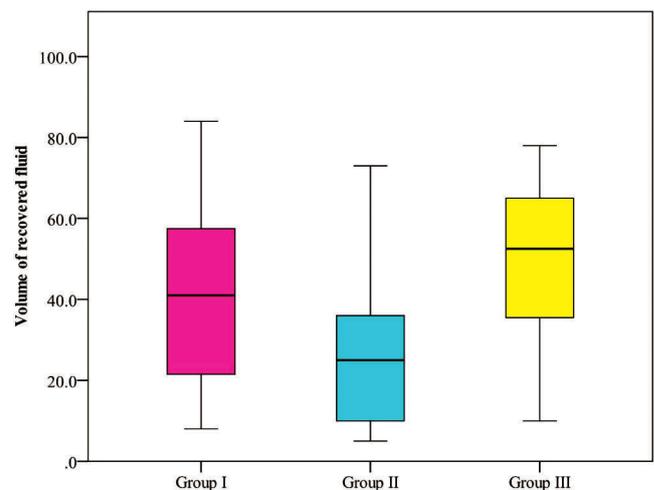
This study included 43 (72%) men and 17 (28%) women patients. Their mean age was 55.32 ± 13.13 years. A total of 42 (70%) of those patients were smokers. Distribution of demographic data among groups is shown in Table 1. There was no statistical significant difference between groups regarding age, sex, and smoking status ($P=0.921$, 0.354 , 0.490 , respectively).

Most of the patients presented with cough and dyspnea, as shown in Table 1. No statistical significant difference was found between groups regarding patients' presenting symptoms. Indication of bronchoscopy varied between patients in each group, as shown in Table 1. In group I (DSS group), the most common indication was haemoptysis, followed by lung mass, whereas in the other groups, bronchoscopy was carried out most commonly in suspected lung cancers. Despite randomization, this difference showed statistical difference. Periprocedural data, including anesthesia, patient's position, introduction route, and site of lavage, are listed in Table 2.

Median volume of recovered fluid was 41, 25, and 52.50 ml in groups I (DSS group), II (SST), and III

(AST), respectively. The volume of recovered fluid using the method in group III (AST group) was significantly higher than that of the method used in group II (SST group) ($P=0.001$). Although the volume of recovered fluid using the method in group III (AST group) was apparently higher than that of the method used in group I (DSS group) and that of the method used for group I (DSS group) was apparently higher than that in group II (SST), both lacked significance ($P=0.188$ and 0.066 , respectively) (Fig. 4 and Table 3).

Figure 4



Comparison between the three groups according to the median recovered volume of bronchoalveolar lavage (ml).

Table 1 Comparison between the three studied groups according to demographics, presenting symptoms and indication of bronchoscopy

	Group I (N=20)	Group II (N=20)	Group III (N=20)	P
Sex				
Male	14 (70.0)	15 (75.0)	14 (70.0)	0.921
Female	6 (30.0)	5 (25.0)	6 (30.0)	
Age (years)	51.90 ± 15.04	55.55 ± 14.44	58.50 ± 8.79	0.354
Smoking				
Yes	12 (60.0)	15 (75.0)	15 (75.0)	0.490
No	8 (40.0)	5 (25.0)	5 (25.0)	
Presenting symptoms				
Cough	20 (100.0)	20 (100.0)	20 (100.0)	
Hemoptysis	1 (5.0)	0 (0.0)	2 (10.0)	0.772
Dyspnea	19 (95.0)	19 (95.0)	16 (80.0)	0.343
Chest pain	1 (5.0)	1 (5.0)	1 (5.0)	1.000
Fever	0 (0.0)	0 (0.0)	3 (15.0)	0.099
Indication of bronchoscopy				
Lung mass	4 (20.0)	17 (85.0)	18 (90.0)	<0.001*
Multiple consolidations	3 (15.0)	0 (0.0)	1 (5.0)	0.312
Hemoptysis	7 (35.0)	0 (0.0)	1 (5.0)	0.004*
Mediastinal lymphadenopathy	1 (5.0)	0 (0.0)	0 (0.0)	1.000
Lobar consolidation	2 (10.0)	0 (0.0)	0 (0.0)	0.324
Interstitial lung disease	3 (15.0)	3 (15.0)	0 (0.0)	0.321

Abnormally quantitative data expressed in median (interquartile range) and was compared using Kruskal–Wallis test.

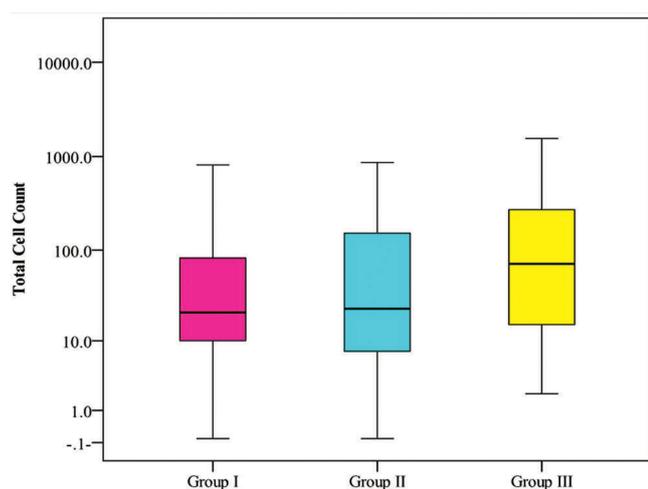
Qualitative data was expressed using number and percentage and was compared using χ^2 .

*Statistically significant at $P \leq 0.05$.

Median total cell count in the retrieved fluid was 21.0, 24.50, and 71.0 cells/cm³ for the DSS, SST, and AST groups, respectively. Although there was an apparent difference between total cell count in group III (AST group) compared with group I (DSS group) and group II (SST group), there was no statistically significant difference among the three studied groups ($P=0.208$). Furthermore, there was no statistical significant difference between groups regarding differential cell count in retrieved BALF (Table 4 and Fig. 5).

Generally, postprocedural complications were few. Bleeding was recorded in only one patient in group II (SST group), whereas increased dyspnea was experienced in two patients (one in DSS group and another one in SST group). Important to mention, a single patient in group I (DSS group) died 48 h after the procedure. It is unknown whether the death was related or not to the procedure. There was no

Figure 5



Comparison between the three groups according to total cell count (cell/cm³).

significant difference between complications (bleeding and increased dyspnea) encountered in the three studied groups ($\chi^2=1.851$, $^{MC}P=1.000$; $\chi^2=1.276$, $^{MC}P=1.000$; respectively).

Discussion

BAL is a useful and widely implemented diagnostic and research tool [5]. Despite its undoubted value, the interpretation of BAL findings is still hindered because the procedure cannot be precisely standardized. Certain factors such as the amount of fluid instilled, the number of aliquots used, or the technique for applying suction can vary greatly from center to center [7].

There have been attempts to obtain some standardization, mainly by way of expert consensus [1,4–8]. The optimal technique used to instil and recover BALF is still a matter of debate.

The aim of this study was to compare between three methods of BAL retrieval – DSS, SST, and AST – regarding efficacy and safety.

Comparing the three groups, there was an increase of 11.5 ml in the volume of recovered BALF in the AST group compared with the DSS group ($P=0.188$). Similarly, the former group showed an increase in the BALF volume by 27.5 ml compared with the SST group ($P=0.001$). The latter difference was statistically significant.

Task forces from ERS and ATS described more than one technique of BALF aspiration. These are hand suction into a syringe, allowing retrieved fluid to flow out by gravity into a container, or machine suction into a fluid trap [4,7,16].

Table 2 Comparison between the three studied groups according to periprocedural data

Anesthesia	Group I (N=20)	Group II (N=20)	Group III (N=20)	P
Local	20 (100.0)	20 (100.0)	19 (95.0)	1.00
General	0 (0.0)	0 (0.0)	1 (5.0)	
Patient position				
Supine	20 (100.0)	20 (100.0)	19 (95.0)	1.000
Sitting	0 (0.0)	0 (0.0)	1 (5.0)	
Introduction site				1.000
Nasal	20 (100.0)	20 (100.0)	19 (95.0)	1.000
Oral	0 (0.0)	0 (0.0)	1 (5.0)	
Site of lavage				
Right lung (RML)	20 (100.0)	16 (80.0)	16 (80.0)	0.109
Left lung (lingula)	0 (0.0)	4 (20.0)	4 (20.0)	

Abnormally quantitative data expressed in median (interquartile range) and was compared using Kruskal–Wallis test. Qualitative data was expressed using number and percentage and was compared using χ^2 .

RML, right middle lobe.

Table 3 Comparison among the three groups according the volume of recovered fluid

Volume of recovered fluid (ml)	DSS (N=20)	SST (N=20)	AST (N=20)
Median (IQR)	41.0 (19.75–58.75)	25.0 (10.0–36.50)	52.50 (35.25–67.50)
95% CI	29.39–50.61	18.11–35.39	39.05–58.65
Significance between groups	$P_1=0.066$	$P_3=0.001^*$	$P_2=0.188$

Abnormally quantitative data expressed in median (IQR) and was compared using Kruskal–Wallis test.

AST, aspirator suction with trap group (III); CI, confidence interval; DSS, direct syringe suction group (I); IQR, interquartile range; LSD, least significant difference; SST, syringe suction with trap group (II).

P_1 : P value for post-hoc test (LSD) for comparing between DSS group and SST group.

P_2 : P value for post-hoc test (LSD) for comparing between DSS group and AST group.

P_3 : P value for post-hoc test (LSD) for comparing between SST group and AST group.

*Statistically significant at $P \leq 0.05$.

Table 4 Comparison among the three groups according to total and differential cell count of retrieved fluid

	DSS (N=20)	SST (N=20)	AST (N=20)	$KW \chi^2$	P
Total cell count (n/cm ³)					
Minimum–maximum	0.0–811.0	0.0–860.0	2.0–1550.0	3.142	0.208
Median	21.0	24.50	71.0		
Macrophages					
Minimum–maximum	0.0–0.88	0.0–0.76	0.0–1.0	2.637	0.267
Median	0.33	0.18	0.28		
Neutrophils					
Minimum–maximum	0.0–1.0	0.0–0.97	0.0–0.86	0.225	0.894
Median	0.53	0.48	0.34		
Lymphocytes					
Minimum–maximum	0.0–0.55	0.0–0.50	0.0–0.77	0.675	0.714
Median	0.16	0.09	0.16		
Plasma cells					
Minimum–maximum	0.0–0.0	0.0–0.01	0.0–0.36	3.790	0.150
Median	0.0	0.0	0.0		
Epithelial cells					
Minimum–maximum	0.0–0.02	0.0–0.18	0.0–0.0	2.104	0.349
Median	0.0	0.0	0.0		
Monocytes					
Minimum–maximum	0.0–0.02	0.0–0.02	0.0–0.08	0.002	0.999
Median	0.0	0.0	0.0		
Eosinophils					
Minimum–maximum	0.0–0.0	0.0–0.0	0.0–0.02	2.000	0.368
Median	0.0	0.0	0.0		
Other					
Minimum–maximum	0.0–0.06	0.0–0.0	0.0–0.0	2.000	0.368
Median	0.0	0.0	0.0		

Abnormally quantitative data expressed in median (interquartile range) and was compared using Kruskal–Wallis test ($KW \chi^2$).

AST, aspirator suction with trap group (III); DSS, direct syringe suction group (I); SST, syringe suction with trap group (II).

In agreement with the current study, the methods of aspiration in question propose the least possible pressure in order not to cause collapse of the distal airways or trauma to the airway mucosa, which either reduces the volume of recovered fluid or contaminates the sample with blood [16].

Different syringe sizes may be used while performing BAL. The ATS statement [16] recommended a 50 ml syringe to be used during the procedure. In their multicenter study, Rosell *et al.* [17] recommended connecting a 50 ml syringe via a plastic tube (the

rubber portion of the intravenous administration set) to the working channel to increase the volume of recovered BALF as well as to have better control over the negative pressure applied.

In a comparative study by De Blasio *et al.* [18] it was found that compared with a 50 ml syringe, using a 20 ml syringe was associated with more voluminous BALF as well as fewer complications. Moreover, we believe that using a 20 ml syringe is far easier, needs less effort, and allows better control over the syringe while applying negative pressure.

In the current study, the fluid was instilled with syringe through the biopsy channel of the bronchoscope using a standard number of input aliquots of 20 ml each (commonly six aliquots were recommended), constituting a total volume of 120 ml.

Similarly, the ATS statement [16] recommended a volume of BAL infusion of 100–150 ml. Moreover, some studies [12,19] recommended up to 250 ml of instilled fluid. We followed the same volume recommended by most task forces and studies [16,18,20]. The idea was that a larger volume instilled would gain a larger retrieved volume and that smaller instilled volumes (100 ml or less) would increase the likelihood of contamination by the bronchial spaces, including inflammatory cells derived from the larger airways, which may skew the differential cell counts.

Furthermore, we preferred using larger number of aliquots as we believe that the more the number of aliquots the higher the likelihood the retrieved fluid is alveolar in origin [19].

The site of BAL may result in variable volumes of retrieved fluid. Anterior segments of the lung (right upper lung, left upper lung, lingual, and middle lobe) were preferred, as gravity could have increased the volume of retrieved BALF [1,4,12].

In the current study, the lavaged segment was selected according to CT findings or during exploration by fiberoptic bronchoscopy. In all patients, the procedure was carried out either in right middle lobe or lingular subsegmental bronchi. We preferred the predescribed bronchi as they are more easily accessible and likely to allow good return of lavage fluid.

The most popular method of suction of instilled fluid used in most medical centers is the direct hand-held syringe aspiration. This is also recommended by most task forces and studies [12,17,21].

A modified direct syringe suction (connected to a plastic tube) is approved as well in some studies [17,22,23]. Suction by a machine is less likely recommended for fear that a higher pressure may cause alveolar collapse.

On the contrary, Wood *et al.* [23,24] in two experimental studies recommended machine suction with a fluid trap over both direct hand-held syringe and modified syringe suction. They even stated that it was preferred in larger segments if the bronchoscope was not well fitted.

The median recovered fluid volume in our patients was the highest using machine suction (AST group) (52.5 ml) compared with direct syringe suction with or without trap (25 ml and 41 ml; $P=0.001$ and 0.188 , respectively). This observation was difficult to interpret, but this may be attributed to the familiarity of the whole medical staff with the former technique as it is the most popular technique of BAL suction locally used in our institution.

The reported complications of BAL procedure mainly comprise a transient decrease in lung function parameters, alveolar infiltrates, fever, bronchial hyperactivity, and bronchospasm [25].

Rosell *et al.* [17] in their study showed a clear difference in the complication rates between groups (8.3% without tubing vs. 1.4% with tubing), which seems to be related to the mean volume of saline retained [19].

The reported complications in the current study were few. These were transient bleeding and/or increased dyspnea, and respiratory distress. A single case of death 48 h after the procedure was reported; we exactly do not know whether or not it was related to the BAL procedure.

The ideal method of collecting BAL sample is debateful. Some studies [17,19,22] recommended discarding the first aliquot, as the authors believed that it was bronchial in origin, containing either cells or secretions, and was better sent for microbiological analysis.

In contrast, we included all the samples to be sent for microbiological and microscopic evaluation, as there was insufficient data to support one approach over the other. Moreover, we believed that whether to pool the BAL sample or not depended in the first place on the indication of BAL procedure.

To the best of our knowledge, this is the first randomized, direct prospective comparative study that compared between different methods of BAL suction. Furthermore, we included a new method of suction – that is, direct syringe suction with trap.

Limitations of this study included the relatively small number of patients and that it was carried out in a single center rather than in multicenters. Moreover, BAL procedure was generally carried out when indicated rather than in a specific disease entity.

The current study was an attempt to reach the ideal method of suction of the instilled BALF as a single

item in a protocol aiming at standardization of the procedure in our institution.

Conclusion

All the studied methods of retrieving BAL infusate are safe. Using an aspirator into a fluid trap is superior to using syringe suction into a fluid trap in retrieving more voluminous BAL infusate.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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