

## Efficacy of sputum induction from lower respiratory tract in children

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### Abstract

**Background** Although sputum is a good specimen for various examinations, such as cytology and microbiological culture, sputum induction (SI) is not a routine procedure in children.

**Objective** To identify the efficacy of SI to obtain specimen from lower respiratory tract in children, identify side effects of SI, and the results of microbiological examination.

**Methods** A cross sectional study was performed in children (aged 1 month to 18 years) who underwent SI. Sputum induction was performed by inhalation with hypertonic solution, consisted of salbutamol for 15 minutes continued with NaCl 3% solution for another 15 minutes. Sputum specimens were examined for number of alveolar macrophage cell, surfactant protein A (SP-A) concentration, also acid-fast bacili smear, and *M. tuberculosis* culture, or aerobic microbial culture.

**Results** Forty subjects with lower respiratory tract infection participated in this study, and SI was successfully performed in all subjects. Youngest subject was 2 month old, the eldest was 16 year 7 month old. Median duration of SI was 45 minutes, and majority of volume was 3 or 4 mL. Side effects were nosebleeds (40%) and vomiting (2.5%). Macrophage alveolar more than 5 cells in one specimen was found in 97.5% subjects. Surfactant protein A examination was performed in 30 specimens, and SP-A was detected in all specimens (median concentration 264.528 pg/mL). Culture for *M. tuberculosis* was positive in 1 of 27 subjects, while acid fast bacili smear was negative in all examined subjects. Aerobic microbial culture was positive in 5 of 13 subjects.

**Conclusions** Sputum induction has good efficacy in obtaining lower respiratory tract specimen and it is safe to perform in children. Specimen from sputum induction yields good positive result for aerobic microbial cultures. [*Paediatr Indones.* 2015;55:101-8].

**Keywords:** *sputum induction, macrophage alveolar cell, side effects, culture*

Specimen from respiratory tract is important to understand pathological condition in respiratory tract in various illness, either to diagnose the disease or to evaluate the disease by cytology, histology, or imunohistology. Specimen collection from lower respiratory tract requires more complicated and invasive techniques than from upper respiratory tract.<sup>1</sup>

Sputum is easy to be collected in adults and it has good success rate.<sup>2</sup> Despite its advantages, sputum collection is not a standard procedure in babies and children. Babies and children tend to swallow the sputum thus good sputum specimen is difficult to be obtained.<sup>3-6</sup> Due to its benefits as a safe, easy to perform, well tolerated, and semi-invasive method, sputum induction is sometimes more preferable in obtaining specimen from lower respiratory tract than other invasive methods, for example bronchoalveolar lavage.<sup>6-18</sup> Nonetheless, not many studies describe success rate of sputum induction to obtain lower

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respiratory tract specimen in children. Numerous parameter can be used to evaluate whether the collected sputum specimen is representative for lower respiratory tract, among others are alveolar macrophage cell and lung surfactant.

This study aimed to identify the efficacy of sputum induction (SI) to obtain specimen from lower respiratory tract in children with lower respiratory tract infection. In addition, this study also aimed to acknowledge the safety of sputum induction procedure, and results of microbiological culture of the collected sputum specimen.

## Methods

A cross sectional study was performed in Pediatric Respiriology Clinic or Emergency Room at Dr. Cipto Mangunkusumo Hospital, Jakarta, from March to May 2014, to identify the proportion of sputum specimen obtained by induction which was representative for lower respiratory tract as described as containing a minimum of 5 alveolar macrophage cells, and/or surfactant protein A (SP-A). The collected sputum specimen was also cultured for aerobic microbiological or *M. tuberculosis*, and an acid fast bacili smear.

Inclusion criteria were children aged 1 month to 18 years, with lower respiratory tract infection and had not been treated with antibiotic or anti-tuberculosis drugs, also were allowed by parents to participate in this study. Exclusion criteria were oxygen saturation below 92%, in emergency condition, had history of moderate or severe asthma attack, history of recurrent convulsion, history of recurrent nosebleed, and face deformity.

The diagnoses of lower respiratory tract infection were made based on clinical symptoms (fever,

cough, dyspnoe/tachypnoe, wheezing, and/or rales found in chest examination), supported with chest x-ray accordingly, meanwhile tuberculosis (TB) was diagnosed based on TB scoring system.<sup>19</sup>

Subjects underwent sputum induction procedure, consisted of inhalation of 2.5 mg salbutamol (diluted in 2 mL of NaCl 0.9%) for 15 minutes, continued with NaCl 3% solution for another 15 minutes. Subjects who were 5 year old or older were guided to expectorate sputum by productive coughing. If subjects were younger than 5 years, sputum was collected by suctioning through mouth and nose, using different mucous extractors (no 8 Fr). Sputum specimen from one subject was divided into three preparations. One preparation was processed to make 2 slides of sputum specimens for examination of alveolar macrophage cells, one preparation was placed in a container for microbiological examination (aerobic bacterial culture, or *M. tuberculosis* culture, and an acid fast bacili smear), and other preparation was placed in a container for SP-A examination by ELISA method.

## Results

During study period, 40 subjects were recruited, with the youngest was 2 month old and the oldest was 16 year 7 month old. Majority of subjects were boy (22 subjects), and from age group older than 5 years (19 subjects). The most common diagnosis of respiratory tract infection was tuberculosis (27 subjects), followed by pneumonia (11 subjects), and bronchiolitis (2 subjects).

Sputum induction was successfully performed in all subjects (100%). Sputum expectoration was performed in 14 from 19 subjects who were 5 year

**Table 1.** Aerobic microbial culture results

Diagnosis	Age (months)	Sputum volume (mL)	Bacterial results
Bronchiolitis	2	4	<i>Klebsiella pneumonia</i>
Pneumonia	4	4	<i>Klebsiella pneumonia</i> <i>Staphylococcus aureus</i>
Pneumonia	19	4	<i>Citrobacter youngae</i>
Pneumonia	3	3	<i>Streptococcus pneumonia</i>
Pneumonia	3	4	<i>Klebsiella pneumonia</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>

old or older. Three subjects with tuberculosis (aged 5, 8, and 7 years) were unable to expectorate sputum because they were uncooperative when guided to perform productive cough. A 12 year old boy diagnosed with tuberculosis peritonitis refused to perform productive cough due to abdominal pain, and an 8 year old girl with pneumonia was also developed encephalopathy as measles complication, therefore after induction, sputum was suctioned. Twenty one subjects who were younger than 5 years also underwent sputum suctioning.

The lowest collected sputum volume was 1 mL, while the highest was 10 mL. The lowest volume was collected from a 6 year old boy with pneumonia, hyper-IgE syndrome, and multiple lung bullae, who refused to use mucous extractor. While the highest volume was suctioned from a 7 year old girl with tuberculosis and HIV stage 4 because she was not cooperative to perform productive cough and expectorate sputum. Most subjects (12 subjects) had 3 mL of sputum, and 12 other subjects had 4 mL of sputum. Median duration of sputum induction (from inhalation with salbutamol until sputum was collected) was 45 minutes (the fastest was 37 minutes and the longest was 60 minutes).

Hypoxemia due to bronchospasm after inhalation of hypertonic solution was not found in all subjects. All 14 subjects who expectorate sputum did not experience sore throat after a sputum induction procedure. Four subjects from age group 5 year old or older who underwent sputum suctioning with mucous extractor also reported not experiencing sore throat after sputum induction procedure was done.

In this present study, reported side effects were nosebleed and vomiting. Nosebleed was reported in 16 subjects (40%) who entirely underwent sputum suctioning. Nosebleed was described as blood streak in suction tube while suctioning, that did not flow from nostrils and ceased after the procedure was stopped. Vomiting was found in a 3 year old girl (2.5% of subjects) after sputum suctioning.

Sputum from lower respiratory tract were represented by at least 5 alveolar macrophage cells and/or containing SP-A. Five or more alveolar macrophage cells were found in sputum specimen from 39 subjects. Only an 11 year 5 month old girl with tuberculosis had a sputum specimen consisting less than 5 alveolar macrophage cells. This child

was uncooperative to perform productive cough and expectorate sputum, also refused to be suctioned, therefore this subject only produced saliva rather than sputum. The ELISA testing for SP-A was only performed in 30 sputum specimens, while 10 specimens had low volume after centrifugation thus could not be tested. From 30 specimens, SP-A was detected in all specimens (median 264.528 pg/mL). Lowest SP-A concentration was 63.903 pg/mL, and the highest was 1,286.723 pg/mL. Sputum specimen from an 11 year 5 month old subject who had less than 5 alveolar macrophage cells, had SP-A level of 569.308 pg/mL.

According to diagnosis, in 2 subjects with bronchiolitis, median SP-A level was 303.291 (range 86.174 to 520.408) pg/mL. In subjects with pneumonia, SP-A level was detected in 6 subjects from 11 diagnosed subjects (aged range 8 months to 1 year 5 months), with median 200.565 (range 63.903 to 750.112) pg/mL. In subjects with tuberculosis, SP-A was detected in 22 subjects from 27 diagnosed subjects (aged 11 months to 15 years), with median 288.538 (range 102.505 to 1,286.723) pg/mL.

In this study, sputum specimens from 27 subjects were examined for acid fast bacilli smears and culture for *M. tuberculosis*. From 27 specimens, acid fast bacilli smear was negative in all subjects, and only 1 specimen had positive culture for *M. tuberculosis*.

Aerobic microbiological cultures performed in 13 subjects (11 subjects with pneumonia and 2 subjects with bronchiolitis), positive cultures were found in 5/13 subjects (Table 1). *Klebsiella pneumonia* was found in specimens from 3 subjects (as single colony in a subject with bronchiolitis, and co-infection in 2 subjects with pneumonia). *Staphylococcus aureus* was positive in 2 subjects as co-infection. Moreover, other cultures were positive for *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, and *Citrobacter youngae*.

## Discussion

Sputum induction is a routine procedure in adults, not in children.<sup>3</sup> In adults, sputum induction aims to obtain specimen from lower respiratory tract in patients who are not able to expectorate sputum spontaneously.<sup>2,13,17,18</sup> Inhalation with hypertonic solution induces secretory production from respiratory

tract by increasing osmolarity. Increased osmolarity of lung and respiratory tract results in increased production of submucosal glands and bronchial vascular permeability which are modulated by capsaicin.<sup>18</sup>

In Indonesia, sputum induction is not a chosen procedure in children to obtain secretion from lower respiratory tract because its doubtful success rate. In addition to their habit to swallow sputum, children are also more difficult to follow the guidance to perform productive cough and expectorate sputum. Nevertheless, this study shows that sputum induction was able to be performed in all participated subjects, with high success rate in obtaining sputum specimen representative for lower respiratory tract.

Sputum induction took 37 to 60 minutes (median 45 minutes) to be performed. Inhalation took most of the time, consisting of inhalation of salbutamol and NaCl 3% solution which took 15 minutes each. Duration of sputum collection was depending on subjects' cooperation when they were guided to perform productive cough and expectorate sputum, or suctioned. The fastest procedure duration was 37 minutes, in a 7 year old boy who expectorated 7 mL sputum. Most subjects were cooperative and able to perform good productive cough, therefore the procedure could end shortly. The longest duration was 60 minutes in a 12 year old boy with peritonitis. Initially, this patient was guided to expectorate sputum but only small volume of sputum was collected. He agreed to be suctioned, thus sputum suctioning was performed and a total of 3 mL sputum was collected.

Duration of inhalation may influence collected specimen composition. Many sputum induction consensus suggest 15-20 minutes for inhalation, because that duration results in optimal surfactant level and cytology in sputum.<sup>18</sup> A study in adult asthma patients concludes that the optimal duration for inhalation was 12 minutes according to the level of inflammation cells and SP-A level.<sup>20</sup> This current study used 15 minutes as the duration for inhalation because not only it was the optimal time based on the previous study on sputum microbiological composition, but also children can tolerate this length of time used for inhalation.

In sputum induction procedure, cough is an effective action to expectorate secretion from lower

respiratory tract.<sup>21</sup> In order to obtain good quality sputum with sufficient volume, ability to cough is compulsory for sputum collection, either with expectoration or suction. In this study, suction was performed when sputum was already collected in oropharynx after subjects were coughing and expelling the sputum from lower respiratory tract to oropharynx. Suction had to be performed immediately before subjects swallowed the sputum. Cough appeared after subjects were inhaled with hypertonic solution. In subjects who were 5 year old or older, and cooperative, sputum expectoration required subjects' ability to follow guidance in performing productive cough, expectorate sputum properly, and not to swallow the sputum after accumulated in oropharynx.

Cough is an important part of a sputum induction procedure. Cough should be performed properly, following these steps. Subjects were instructed to take deep breaths, exhale rapidly and powerfully.<sup>11</sup> Cough can be stimulated either by inhalation of hypertonic saline solution<sup>21</sup> or voluntarily. With deep breathing followed by powerful exhalation, sputum was forcefully brought from lower to upper respiratory tract. Then, sputum accumulated in upper respiratory tract (oropharynx) was able to be expectorated or suctioned.<sup>11,22</sup> The challenge was to educate and remind subjects to not swallow the sputum. This process was repeated many times because subjects kept on swallowing sputum, instead of expectorating it.

Majority of sputum volume obtained were 3 and 4 mL. Therefore if a sputum induction is chosen to obtain specimen from lower respiratory tract, volume should be a consideration as many laboratory examinations required minimum amount of volume. A study succeed in performing sputum induction in a 1 month old subject,<sup>13</sup> while other in a 3 month old subject.<sup>14</sup> Our study has similar result, the youngest subject which sputum induction was successfully performed aged 2 months. This result shows that sputum induction is safe to perform since early age if it is needed in management of respiratory tract diseases.

A study reported that sputum induction followed by expectoration can be performed as early as 6 year old.<sup>7</sup> Other study reported that sputum induction and expectoration was able to be performed in children as youngest as 5 year old,<sup>23</sup> while another study reported in 4 year old children.<sup>14</sup> This present study initially set

5 years old as age limit to perform expectoration after sputum induction. However during study period, this study showed that subjects were cooperative enough to follow instructions to perform productive cough and expectorate sputum since age 6 years.

In this study, hypoxemia, manifested in decreased oxygen saturation, and sore throat were not found in any subjects. Nosebleeds were found in 16 subjects who entirely had undergone sputum suctioning. Nosebleed was probably caused by traumatic action of suctioning and only manifested as blood streak in suction tube. Blood was not oozing from nostrils after suctioning was stopped. Vomiting was found in a subject who underwent suctioning eventhough subject had been fasting for 3 hours prior to procedure. Risk of vomiting in sputum induction can be reduced by fasting several hours before the procedure. However in this subject, vomiting was induced by suctioning to oropharynx, also because subject was constantly crying. This present study also showed that from 14 subjects who expectorated sputum, no subject had reported side effects. Therefore, sputum induction is a safe procedure to be performed in children, especially when sputum collection is done by expectoration.

There are several obstacles that can occur while performing sputum induction, namely long duration of procedure, lack of medical officers' skill to perform sputum induction, limited space, and concern of nosocomial infection.<sup>14</sup> In this study, limited space and concern of nosocomial infection were the main obstacles. Therefore, a special room with good ventilation is important when performing sputum induction.

In order to identify whether the collected sputum was representative for lower respiratory tract, there are several markers that can be applied, for example alveolar macrophage cells.<sup>1,2,14,24</sup> More alveolar macrophage cells found in a sputum specimen indicated that specimen was more adequate in representing lower respiratory tract.<sup>2,24</sup> Macrophage alveolar cells was attached in alveolar walls, digesting cellular debris and foreign material, then transferred those digested material to bronchus or lymphatic system in terminal bronchiolus.<sup>1</sup> This study defined sputum specimen which was representative for lower respiratory tract as containing of at least 5 alveolar macrophage cells. From 40 subjects, specimen from 39 subjects (97.5%) had at least 5 alveolar macrophage

cells, and only 1 specimen from a 11 year 5 month old subject who were not cooperative to expectorate sputum had less than 5 cells. This suggests sputum induction is a good method to obtain specimen from lower respiratory tract, if the procedure is performed properly.

In addition to alveolar macrophage cells, lung surfactant is a lipoprotein complex synthesized by type II pneumocyte and Clara cells in lower respiratory tract.<sup>10,25,26</sup> Surfactant proteins are consisted of SP-A, SP-B, SP-C, SP-D, also SP-G and SP-H that were discovered recently.<sup>26,27</sup> Among others, SP-A is the major protein surfactant, also consists of 2 expression which are SP-A1 and SP-A2. In human lung, SP-A is composed of 2 SP-A1 molecules and 1 SP-A2 molecule, forming a trimer.<sup>25,26</sup> Initially known to be specific for lower respiratory tract, SP-A is recently found in various organs, such as jejunum, colon, prostate, thymus, spleen, mesothelial, synovium, saliva, and nasal epithelial.<sup>26-28</sup> Eventhough, not many studies have learned about SP-A level in organs other than lung, also many studies regarding SP-A level in organs besides lung are based on immunohistochemistry and PCR examination.

Studies on SP-A level show various results on concentration and unit that was used. Those are probably resulted since there are still various method in examining SP-A and various specimens for the examination.<sup>20,24,30-32</sup> Various SP-A levels can also be caused by different antibody used in antigen-antibody based examination.<sup>33-35</sup>

From 30 specimens, SP-A was detected in all specimens with 63.903 pg/mL as the lowest and 1,286.723 pg/mL as the highest (median 264.528 pg/mL) levels. Compared to the previous studies, this present study resulted in lower SP-A level.<sup>29-32</sup> Based on diagnosis, median SP-A level in subjects with pneumonia and tuberculosis (200.565 pg/mL and 288.538 pg/mL, respectively) was lower than in subjects with bronchiolitis (303.291 pg/mL). Nevertheless, different number of subjects in each diagnosis and absence of level in healthy control cause difficulties in interpreting difference in SP-A level between various respiratory infections. Until now, no cut off point for SP-A level in sputum indicates that sputum specimen is representative for lower respiratory tract. Level of SP-A in extra-pulmonary organs has not been studied yet, and the



available studies do not report level of SP-A due to immunohistochemistry based studies. Because lung is the main organ for SP-A synthesis, if SP-A is found in a sputum specimen and the specimen has at least 5 alveolar macrophage cells, it can be concluded that the sputum specimen is representative for lower respiratory tract. Therefore, SP-A level is additional examination to support the notion that sputum is representative for lower respiratory tract although it is not specific. According to alveolar macrophage and SP-A, this study shows that sputum induction in children successfully obtains specimen from lower respiratory tract.

In this study, *M. tuberculosis* microbiology examination resulted in one positive culture and no positive acid fast bacilli was found on smears from 27 subjects diagnosed with tuberculosis. Tuberculosis in children is difficult to diagnose due to difficulty in microbiological diagnosis. In adults, *M. tuberculosis* culture is positive in 10-19% cases.<sup>14</sup> Other studies showed that in children with tuberculosis who underwent sputum induction had low positive *M. tuberculosis* culture and acid fast bacilli smear. Low positive result in our study is probably caused by small sample size. Co-infection with HIV can also complicate diagnosis of tuberculosis due to increased paucibacillary condition.<sup>37</sup> In our study, there were 3 subjects with tuberculosis coinfecting with HIV and results for culture and acid fast bacilli smear were all negative.

About 27.6% mortality in neonates and 22.8% mortality in children under 5 year old are due to respiratory tract infection, particularly pneumonia.<sup>38</sup> Etiology of pneumonia in children is difficult to identify because specimen from lung tissue is not easy to obtain. In this study, sputum specimens from 11 subjects with pneumonia and 2 subjects with bronchiolitis were examined for aerobic microbiological culture. Positive results were found in 5 subjects for *K. pneumoniae*, *S. pneumoniae*, *Citrobacter youngae*, *P. aeruginosa*, and *S. aureus*. Microbiological etiology for pneumonia in this study was similar with results reported by other studies that stated that *S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* were the common etiology for pneumonia in children.<sup>13,39,40</sup>

Sputum suctioning procedure through nasal cavity and mouth is possible to contaminate the

specimen with upper respiratory tract bacteria. Normal flora in nasal cavity and mouth are *Corynebacterium sp.*, *S. aureus*, *S. epidermis*, *S. viridans*, *Neisseria sp.*, *Bacteroides sp.*, *Fusobacterium sp.*, and *Lactobacillus sp.*<sup>41</sup> A study comparing bacterial etiology of pneumonia and bacteria found in nasopharyngeal aspirate reported that bacterial profiles between the two sites were different, therefore positive results from sputum induction specimen were possible coming from lower respiratory tract.<sup>13</sup> Our study shows good results in aerobic microbiological culture from sputum induction specimen (5 of 13 subjects), also it has different bacterial profiles with normal flora from upper respiratory tract. Therefore, bacterial results from this study are possible as etiology of pneumonia or bronchiolitis. However, *S. aureus* found in this study should be considered as contaminant because it was found positive with other bacteria in one specimen. This study suggests that sputum induction is a good method to obtain specimen for aerobic microbiological culture in children with lower respiratory tract infection.

There are two limitations in this study, namely: (1) Sputum induction was only performed in children with lower respiratory tract infection. There was no comparison or control of specimen from healthy children, or specimen from respiratory tract obtained by other methods; (2) Absence of SP-A cut off point complicated interpretation for SP-A results. Nevertheless, this study shows that sputum induction in children was able to obtain specimen from lower respiratory tract, also the procedure was safe to be performed in children.

We conclude that sputum induction is successfully performed and obtaining specimen in all subjects. From the collected sputum, a minimum of 5 alveolar macrophage cells are found in sputum specimen from 97.5% subjects and SP-A is detected in all examined specimen with median level 264.528 (range 63.903 to 1,286.723) pg/mL. Sputum induction is a safe procedure to be performed in children. Side effects are minor, which are nosebleed (40%) and vomiting (2,5%). Microbiological examination from sputum specimen shows negative acid fast bacilli smear results and one positive *M. tuberculosis* culture from 27 subjects with tuberculosis. Aerobic microbiological cultures are positive in 5 of 13 subjects with pneumonia or bronchiolitis. Bacteria isolates are *K. pneumoniae*, *S. pneumoniae*, *P. aeruginosa*, *C. youngae*, dan *S. aureus*.

## Conflict of interest

None declared

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