Correlation between the Expression of Melanoma-Associated Antigen-A3 and Cytology Results on Bronchoalveolar Lavage on Nsclc

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Abstract

Background: Lung cancer is still considered an alarming health problem, causing a major cause of death in malignant disease. The incidence rate is likely to increase and most of it comes with an advanced stage of diagnosis. To improve the healing rate and life expectancy, the detection of lung cancer should be done early, when it is still small and localized. Bronchoalveolar lavage (BAL) cytology is a method of examination that can be used in lung cancer. The expression of Melanoma-Associated Antigen (MAGE) becomes the marker that is sensitive to certain types of cancer, including lung cancer.

Objective: To analyze the correlation between expression MAGE-A3 and cytology of BAL specimen on NSCLC.

Method: Bronchoscopy was performed on patients with NSCLC lung tumor at pulmonology unit of Dr. Soetomo Teaching Hospital Surabaya Indonesia to collect the BAL. Histologic examination for BAL and MAGE-A3 examination were conducted on patients. Statistical analysis used to determine the correlation between the expression of MAGE A3 and cytology of BAL specimens on NSCLC was fisher's exact test.

Result: We found that there were 7.14% of subjects with BAL cytology had cancer cells (*adenocarcinoma*). MAGE-A3 expression was positive only in 28.60% of subjects diagnosed with NSCLC. The result of MAGE A3 expression analysis with cytology of BAL specimen showed p = 0.286.

Conclusion: There was no significant correlation between MAGE-A3 expression and histopathology type. The results of MAGE A3 RT PCR fluid expression examination showed that most of them were in the negative category.

***Keywords**: MAGE-A3, BAL, NSCLC*

Introduction

Lung cancer is still an alarming health problem in the world and is considered the leading cause of death in malignant disease. Its incidence is likely to rise almost worldwide, in developed countries like the United States and in developing countries, including Indonesia. The increasing trend of this case is not only in male but also in female. Diagnosis of lung cancer is 14% of all types of cancer in the world, ranked second after prostate cancer in male and breast cancer in female. Lung cancer in Indonesia ranks the fourth most common of all cancers [1].

Lung cancer type non-small cell carcinoma (NSCLC) is the most common type of lung cancer, with the incidence of 75-80% of all lung cancer. To improve the healing rate and life expectancy, the detection of lung cancer should be done early, when it is still small

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and localized. A lot number of research has been done to find the tool for early lung cancer detection; low-dose frequency computed tomography (CT) is able to detect lung cancer that is smaller (less than 1 cm) compared to chest x-ray with is 0.5 cm. However, this procedure has the disadvantage of providing a non-specific mass picture, causing a large false positive rate and increased deaths from unnecessary surgery. In addition, CT scan is costly and repeated CT evaluation scan might increase the risk of adverse side effects of radiation in the patients[2]

Developments in the field of biomolecular provide an alternative to find an early detection tool for cancer that is not invasive, one of them is tumor antigen. Tumor antigen is one of the tumor makers/biomarkers that provide useful information in patients with developing cancer. Tumor antigens has been widely developed for early detection of lung cancer, being the target of therapy (immunotherapy) and a promising field in the future. However, the specificity and sensitivity of tumor antigens in the diagnosis of cancer might vary [3].

The gene of *melanoma associated antigen* (MAGE) belongs to an important group of CT antigens and is grouped according to the expressing tissue and gene structure. It is suspected that the expression of the MAGE gene family can be a marker that is sensitive to certain types of cancer including lung cancer. Overexpression of MAGE gene can be used not only for early diagnosis and screening but also as a target of adjuvant treatment in lung cancer. A study suggests that there is a significant correlation between MAGE expression and histopathology of lung cancer as well as the stage of lung cancer [3].

Currently, there are already several modalities of molecular detection in determining genetic alterations. One of them is *polymerase chain reaction* (PCR) that is capable of detecting 10_3-10_4 copies of gene mutation [4]. In addition, PCR helps determine histopathologic classification and detection of specific tumor antigens despite the very few amount of sample. Compared to other molecular modalities, PCR has several advantages that are objective, fast, versatile and cost-effective when applied to small tissue samples. In the context of detecting tumor antigens, the CT antigen of the MAGE family can be detected by RTPCR [5].

Bronchoscopy is a medical procedure of inserting a pipe into the airway through the nose or mouth. Some types of specimens that can be obtained with bronchoscopy are sweeps, rinses, Bronchoalveolar lavage (BAL), forceps biopsy *and transbronchial needle aspiration* (TBNA). *Fiber optic bronchoscopy* (FOB) with BAL is a fairly practical action with moderate risk and is fast and reliable. BAL diagnostic results show a value of more than 50% and it is better than transbronchial biopsy for diagnosis of peripheral lesions that can not be seen through bronchoscopy. In the patients with *bronchoalveolar cell carcinoma*, the BAL examination has a high diagnostic value [6].

MAGE-related researchers are still not widely exposed and a previous research reported MAGE expression in lung cancer of 30-50%. In Dr. Soetomo General Hospital Surabaya, Indonesia, there has never been any research or data publication showing the benefits of MAGE expression especially MAGE A3. This study aims to analyze the correlation between the expression of MAGE A3 andthe cytology results of BAL specimen on *NSCLC.*

Method

The subjects of the study were lung cancer patients who underwent treatment at Dr. Soetomo General Hospital Surabaya, Indonesia that met the inclusion and exclusion criteria. The inclusion criteria include patients aged 20-70 years with histopathologic results, including NSCLC. Subject exclusion criteria exclude patients with primary tumor in other organs and metastatic lung cancer from cancer of other organs. Subjects who were willing to be involved in this study filled the informed consent in advance [7].

This study used cross-sectional design and consecutive sampling method to obtain 14 subjects. This study was conducted in the pulmonology unit room of Dr. Soetomo General Hospital Surabaya, Indonesia. Tools and materials used include patients' medical records, questionnaires containing patient baseline data, CT thoracic scan results, bronchoscopy device, MAGE reagents, real-time PCR, and BAL dosage tubes. Before conducting the research, the we initially underwent ethical test (445/Panke, KKE/VII/2017) at Dr. Soetomo General Hospital Surabaya, Indonesia [8].

The research procedure includes the diagnosis of lung cancer based on thoracic CT-scan with contrast and histopathology results including NSCLC (adeno carcinoma, squamous cell carcinoma and large cell carcinoma). We performed identification based on subject criteria, followed by examination of MAGE-A3

examination which was the result of BAL liquid sediment examination using primary MMRP-3 & MAGE-A3 with*Reverse Transcription*(RT) technique of PCR detected in 569 *base pairs*in the shape of intron ribbon F: 5'-GAAGCCGGCCCAGGCTCG- 3'. The examination performed was BAL fluid cytology as the result of bronchial tumor epithelial cells examination contained in the liquid BAL in the form of*adeno carcinoma cells*, *squamous cell carcinoma* and *large cell carcinoma.* The data used in this study were primary data from thoracic CT scan examination, histopathology result, cytology and MAGE expression examination result from BAL preparation [9].

The data of measurement results were processed using SPSS software version 20.0 (SPSS, Inc., Chicago, IL). The data were analyzed and presented in tables and descriptions. The data were grouped into two types: numerical and categorical data. The numerical data were presented as mean ± standard deviation and the categorical type data were presented in percentage form [10]. The data on the correlation between MAGE-A3 correlation and cytology results of BAL specimen on NSCLC were analyzed usingchi-square test ($p < 0.05$) if the requirements were met. Otherwise,chi-square test was to be replaced by fisher's exact test ($p < 0.05$).

Result

Subjects' Characteristics

Table 1. Subjects' Characteristics

variable	Category	%	Mean ± SD
age (years)			60.07 ± 10.42
sex	Male Female	50.00 50.00	
occupation	housewives farmer retired teacher entrepreneur welding worker	28.61 35.70 14.30 7.13 7.13 7.13	
the status of smokers	Smoker non-smokers	35.70 64.30	
smoking duration	20 years 30 year 50 year	40.00 40.00 20.00	
histopathological results	pulmonary adeno ca pulmonary squamous cell ca large cell	85.74 7.13 7.13	
type of cytology	a malignant cell no malignant cells	7.13 92.87	
MAGE-A3 expression	positive negative	28.60 71.40	

Table above shows BAL preparation was found only in 1 subject with malignant cell result (7.13%). The majority of subjects had BAL preparations in the category of no malignant cells (92.87%). There were BAL preparations with positive MAGE-A3 expression (28.60%) in all study subjects. Most subjects had MAGE-A3 expression in the negative category (71.40%;). From the results of the examination, we found 7.14% of subjects who had MAGE-A3 expression with positive category and BAL cytology in the category of malignant cells. Most subjects had MAGE-A3 expression with negative category and BAL cytology in the category of no malignant cells (71.43%).

The samples in this study were all patients with NSCLC lung tumor who underwent bronchoscopy according to inclusion and exclusion criteria in Dr. Soetomo General Hospital Surabaya. The average age of the subjects undergoing bronchoscopy examination to collect BAL preparation for cytological examination and MAGE-A3 RT PCR was60.07 ± 10.42 years old. The number did not significantly differ between male and female. The subjects had diverse occupations, but most of them were farmers (35.70%), followed by housewives (28.61%). Most subjects were not active smokers and those who smoke had been smokers for 20-50 years. Most histopathologic results are adenocarcinoma lung (85.74%), squamous cell carcinoma lung (7.13%) and large cell ca (7.13%).

Table 2. Correlation between MAGE-A3 expression and BAL cytology

MAGE-A3	BAL (n = 14)		p*
	a malignant cell	no malignant cells	
positive	7.14	21.43	0.286
negative	0.00	71.43	

* fisher exact test, unit percentage

The result of statistical analysis shows no significant correlation between MAGE-A3 expression with cytology of BAL (p = 0.286)

Discussion

BAL is a standard diagnostic procedure in patients including lung cancer patients, especially in peripheral tumor with a diagnostic value ranging from 33-69%. Cytology preparation with BAL is based on exfoliation cells of malignant lesions of the bronchial epithelium. This BAL fluid component shows inflammatory conditions as well as immune status of the lower airway and alveoli. Adequate cytological dose conditions depend on several factors: the rate of difference in cancer growth, cytological morphology preservation, and operator ability in fluid retention lavage of the bronchi [11]. The results of BAL cytology in this study shows preparation with malignant cells, *adenocarcinoma*. There was no malignant cells found in the other thirteen preparations. This might be due to the number of cancer cells that are expected to collapse with the liquid *bronchoalveolar lavage* in a few number or none [12].

PCR RT examination is one of the methods used to distinguish different MAGE proteins. With this technique, transcripts of MAGE mRNA activation, expression and aberration are found in many types of cancer, one of which is lung cancer. Some literature mentioned that MAGE expression in lung cancer is 30-50%. The results of RT PCR examination for liquid *bronchoalveolar lavage* (BAL) shows 4 preparations (28.60%) expressed by positive MAGE-A3 of the 14 examined preparations. This may be caused by the number of cancer cells that fall along with the fluid of *bronchoalveolar lavage,*in a little amount or none, as well as other MAGE-A types that were not examined in this study. Other MAGE-A which are also expressed in primary lung cancer are MAGE A1, A4, A6 and A10.
The type of cancer cells can also be a consideration on the cause of MAGE-A3 expression in this study from adenocarcinoma, large cell ca, squamous cell ca and some adenocarcinoma which indicate a positive MAGE-A3 expression. MAGE-A3 is more often expressed in

NSCLC, especially squamous cell ca. In the preparation study with squamous cell ca there is a preparation with negative MAGE A3 expression results [13]. However, the result can not be considered inappropriate since there was only 1 preparation with squamous cell ca. More number of subjects withsquamous cell ca is required to find out if MAGE A3 expressed more in the cell type of squamous cell ca compared to adenocarcinoma [14].

The correlation between MAGE-A3 expression and BAL cytology result showed no significant result. This may be due to the positively expressed MAGE-A3 along with BAL cytology where there was only 1 preparation with malignant cells, whereas the other BAL cytology in the preparation with positive MAGE-A3 expression showed no malignant cells. [15]. Detection of peripheral lung cancer with MAGE A1-6 on bronchial rinse preparations shows that MAGE A1-6 had higher peripheral lung cancer detection capability than conventional cytological examination. MAGE detection on sputum and BAL of lung cancer patients showed that from 23 patients with lung cancer examined by their BAL liquid there were 18 patients (78%) with positive MAGE expression, whereas the cytology result from 23 lung cancer patients showed only 8 patients (35%). BAL fluid cytology preparation and BAL fluid preparation for RT PCR were examined, but on cytological preparation there was only 1 preparation indicating cancer cell, i.e. *adenocarcinoma* [2].

Examination of MAGE expression on the case of lung malignancy was able to detect such malignancy. 70-85% of MAGE expressed in lung cancer tissue, tumor tissue preparations during surgery of patients with early-stage lung cancer. MAGE expressed positively in 59% cytology sputum and 70% in BAL fluid. Percutaneous needle aspiration biopsy was performed on patients suspected of lung cancer and we found MAGE sensitivity of 83% and specificity of 58%.

Conclusion

The results of BAL cytology examination showed that no malignant cells the majority of subjects were in the category of no malignant cells. The results of MAGE A3 RT PCR fluid expression examination showed that most of them were in the negative category. In this study there was no significant correlation between MAGE-A3 expression and BAL cytology result.

Ethical Clearance: This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The authors have not found any conflict of interest related to this research so far.

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