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Melanoma-associated antigen A1 and A3 as new candidate of diagnostic for non-small cell lung cancer

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ABSTRACT

The early diagnosis of lung cancer has long been an interesting field for early treatment of the disease. Many diagnostic methods use specific tumor antigens for the diagnosis of lung cancer with low to medium specificity and sensitivity. Recently, melanoma-associated antigen (MAGE) A1 and A3 detection has been emerged as a new tool for lung cancer diagnosis and can be a promising tool in the future. However, it still needs to be investigated to measure the sensitivity and specificity. This research is an analytic observational study conducted at Dr. Soetomo Hospital Surabaya. The population of the study was all patients diagnosed with suspected lung cancer. The research sample was 100 patients' biopsy samples of lung cancer patients who underwent a core biopsy (CB), bronchoalveolar lavage (BAL), and forceps biopsy (FB) (31 core biopsy, 37 BAL, and 32 forceps biopsy). The sample was taken by histopathology and the expression of MAGE A1 and A2 was measured. The results were analyzed using the Chi-Square Test using SPSS for Mac Version 20.00. Histopathologic results on CB, BAL, and FB showed that 37 patients were positive for carcinoma (47.7%), with the majority of adenocarcinoma (31.6%) and the results of the PA CB, the sensitivity value was 30.43% and the specificity value was 75.00%. In conclusion, the examination of the tumor antigen MAGE A1 and A3 can be used as a new candidate for lung cancer diagnosis in the future.

Keywords: lung cancer, Non-small cell carcinoma, Tumor antigen, MAGE

Introduction

In patients with suspected lung cancer, routine invasive procedures namely taking biopsy samples are required for diagnosis [1, 2]. These biopsy samples can be taken from outside the chest cavity (transthoracic) such as Fine Needle Aspiration Biopsy (FNAB) and core biopsy (CB) with either ultrasound or CT-scan guidance, as well as from invasive examinations through the airway (transbronchial) from

bronchoscopy (aspiration biopsy, forceps biopsy, brushing, and bronchoalveolar lavage) [3, 4]. But the sensitivity is still relatively low, especially in the early stages (63%) [5]. The development of knowledge in the biomolecular field can provide an alternative space in finding a more sensitive and non-invasive early detection tool for lung cancer, one of which is the tumor antigen. This tumor antigen has been expressed since the onset of carcinogenesis, however, its expression value is still low so that it cannot be used routinely in everyday clinical practice and still requires research to strengthen the evidence. Melanoma-associated antigen (MAGE) is a proto-oncogene belonging to the cancer testicular antigen (CTA) family whose biological function is not well understood [6, 7]. It is known to play a role in oncogenesis and inhibition of apoptosis by blocking the caspase cycle [8]. The MAGE protein is generally classified into 2 subgroups based on differences in gene structure and specific expression in tissues, namely MAGE I and II [9]. MAGE I expression has been detected in several tumors, such as lung cancer [10, 11]. The expression of MAGE depends

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on the methylation of the CpG site in the promoter region [12]. In pulmonary malignancies, MAGE expression is more prevalent in squamous cell carcinoma than adenocarcinoma, which may be explained that MAGE originates from skin melanoma. In another study, it was found that MAGE subtypes A1, A2, A3, A4, and A6 were expressed in 20-50% of lung cancers and MAGE A3 was at the highest frequency. When combined, this figure can reach 64.3-83.3%. The mean detection rate of sputum obtained varies depending on the type of tumor histopathology. The RT-PCR of MAGE-A1 and A3 shows high sensitivity in respiratory samples and shows promising results for early detection of lung cancer [13]. However, it still needs to be further investigated how the sensitivity is when compared to conventional cytology examinations [14].

Although the expression of MAGE A1 and A3 in biopsy tissue has been widely used for early diagnosis of NSCLC lung cancer, no study has yet provided a comparison of the method of evaluating MAGE A1 and A3 expression in NSCLC lung cancer biopsy samples with histopathological and cytopathological methods, which have become a gold standard.

Materials and Methods

This study was an analytical observational study that aimed to determine the expression of MAGE-A1 and A3 from patients with lung tumors as biomaterials for the development of lung carcinoma diagnosis, compared with cytopathological and histopathological examinations of biopsy samples as the gold standard. The research type was experimental in vitro. Subjects to be the research sample signed informed consent. The research was approved by the Ethical Committee of Dr. Soetomo Surabaya letter no 445/Panke.KKE/VII/2017. Samples were collected in the Lun Intervention Room at Diagnostic Center Building dr. Soetomo Hospital Surabaya. Cytopathology and histopathology examinations were carried out at the Pathology Anatomy Installation of dr. Soetomo Surabaya. RT-PCR examination and MAGE A1 and A3 sequencing were carried out at the Tropical Disease Center, Airlangga University, Surabaya. The research sample was a biopsy sample of a patient with suspected lung cancer who underwent invasive diagnostic procedures (CB, FB, and BAL) in the Lung Intervention Room, Diagnostic Center Building, Dr. Soetomo Surabaya. Samples were taken by consecutive sampling, patients who met the inclusion and exclusion criteria were included in this study until the number of samples was met. The equipment that will be used in the study are as follows: Fiber optic bronchoscopy with the Olympus brand and its accessories (forceps, aspiration biopsy, brushing, BAL holding tube), Corazor CB, DNA sequencer, patient medical records, questionnaire containing patient baseline data, Chest CT-scan, ultrasound device, a local anesthetic (2% Lidocaine), 10cc syringe, sterile hole duk, sterile gauze, Povidone-iodine, CB device, alcohol cotton, wound plaster, MAGE reagent, Real time-PCR. Some of the samples were sent to the anatomical

pathology installation of Dr. Soetomo Hospital for histopathological examination and the rest were stored in the transport medium and then taken to the PCR-ITD laboratory for processing according to RT-PCR examination procedures and DNA sequencing. For cytopathology, BAL was centrifuged and then made a slide and diagnosed by a PA specialist. For histopathology, CB samples, forceps, and aspiration biopsy from bronchoscopy examination performed by making slides, diagnosis by a PA expert. The tissue obtained from BAL was centrifuged first to obtain cells. Cells were used for mRNA extraction with the RellaPrep RNA Tissue Miniprep kit, then used for RT-PCR with the GoTag (R) Green Master Mix kit then electrophoresed and analyzed. Using the easy Plus Mini Kit RNA (Qiagen, Germany), and extracted RNA from testicular and lung tissue from CB, BAL, and FB samples. The procedure is carried out according to the protocol instructions. Total RNA was stored at -20°C for further use. Then, Reverse Transcription PCR (RT-PCR) was performed using ReverTraAce® qPCR RT Master mixed with DNA remover (Toyobo, Japan). A total volume of 50µl reaction mixture containing 25µl of the template RNA was stored on freezer at -65°C for 5 minutes for RNA to be denatured; 12 µl 4x DN master mix (with genomic DNA remover) was then added along with 3 µl random primer and incubated at 37°C for 5 minutes to remove DNA. Finally, 10 µl 5x RT master mix II was added for cDNA synthesis. The reaction mixture was incubated at 37°C for 15 minutes and at 50°C for 5 minutes. The reaction was stopped at 98°C for 5 minutes. cDNA was stored at 4°C or -20°C for further use. PCR examination was performed using GoTaq (R) Green Master Mix (Promega, USA). In the first stage, PCR was performed on a total volume of 20 µl containing 10 µl GoTag green master mix, 1 µl primary forward, 1 µl primary reverse, 5 µl nuclease-free water, and 3 µl cDNA template. The primary concentration was 10 pmol/µl. The conditions of PCR amplification were as follows: pre-denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension was at 72°C for 7 minutes, with visualization continuing with 2% gel electrophoresis. In the second stage, 3 µl of DNA copy at the first stage of PCR was used as a DNA template. The reactions and conditions in the second PCR stage were the same as for the first stage except for the primer and template. PCR was also performed for housekeeping of the GAPDH gene for all samples with the same reaction and conditions using PCR for MAGE. To verify the PCR results for MAGE A1 and A3, separate PCR was performed for each subtype of MAGE A1 and A3. The next process was directly sequenced to confirm positive PCR results using a Genetic Analyzer (Applied Biosystems, USA). The results of the sequencing were compared with data from GenBank. Data analysis was performed on a computer using the SPSS software.

Results and Discussion

This study aimed to determine the expression of tumor antigen MAGE A1 and A3 in lung cancer samples. Subjects in this study were patients with suspected lung cancer who underwent a diagnosis process in the RSUD pulmonary room of Dr. Soetomo Surabaya. Samples were taken using the CB procedure and fiber optic bronchoscopy procedure (BAL and FB) from August 2017 to July 2019. The total number of study subjects was 65 people, each sample was examined for histopathology and cytopathology at the Department/SMF Pathology of Anatomy, Faculty of Medicine, Airlangga University/RSUD dr. Soetomo Surabaya. Samples underwent RT-PCR process and gene sequencing at ITD Airlangga University, Surabaya.

From 65 samples and 101 samples, histo PA positive results were obtained in 37 samples (47.7%), MAGE A1 expression was positive in 22 samples (21.8%), MAGE A3 expression was positive in 16 samples (15.8%), and MAGE A1 + MAGE A3 expression was positive in 38 samples (37.6%). In the CB group, PA histo-positive values were obtained in 23 samples (74.2%), MAGE A1 expression was positive in 7 samples (22.58%), MAGE A3 expression was positive in 3 samples (9.67%), and MAGE A1 + MAGE A3 expression was positive in 10 samples (32.25%) (Table 1). In the BAL group, positive histo PA values were obtained in 5 samples (13.5%), MAGE A1 expression was positive in 5 samples (13.51%), MAGE A3 expression was positive in 5 samples (13.51%), and MAGE A1 + MAGE A3 expression was positive in 10 samples (27.03%) (Table 2). In the FB group, positive histo PA values were obtained in 9 samples (27.2%), MAGE A1 expression was positive in 10 samples (30.30%), MAGE A3 expression was positive in 8 samples (24.24%), and MAGE A1 + MAGE A3 expression was positive in 18 samples (54.55%) (Table 3). Overall, the results of the examination in the CB group showed positive histopathological results in 10 (32.25%) samples, MAGE A1 (+) in 7 (22.58%) samples, and MAGE A3 (+) in 3 (9.67%) samples. The results of the examination in the BAL group showed positive histopathological results in 10 samples (27.02%), MAGE A1 (+) in 5 (13.51%) samples, MAGE A3 (+) in 5 (13.51%) samples. While the results of the examination in the FB group showed positive histopathological results in 18 (54.54%) samples, MAGE A1 (+) in 10 (30.30%) samples, and MAGE A3 (+) in 8 samples (24.24%). The results of MAGE A1 and MAGE A3 examinations showed 22 (21.8%) MAGE A1 (+) patients and 16 (15.8%) MAGE A3 (+) patients.

Table 1. Statistic result of MAGE A1/A3 and histopathologic CB

Statistic	Value	95% CI
Sensitivity	30.43%	13.21% to 52.92%
Specificity	75.00%	34.91% to 96.81%
Positive Likelihood Ratio	1.22	0.32 to 4.70
Negative Likelihood Ratio	0.93	0.57 to 1.50
Disease prevalence	74.19%	55.39% to 88.14%
Positive Predictive Value	77.78%	47.57% to 93.10%
Negative Predictive Value	27.27%	18.79% to 37.80%

Accuracy	41.94%	24.55% to 60.92%
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Table 2. Statistic result of MAGE A1/A3 and histopathologic BAL

Statistic	Value	95% CI
Sensitivity	80.00%	28.36% to 99.49%
Specificity	93.75%	79.19% to 99.23%
Positive Likelihood Ratio	12.80	3.12 to 52.52
Negative Likelihood Ratio	0.21	0.04 to 1.23
Disease prevalence	13.51%	4.54% to 28.77%
Positive Predictive Value	66.67%	32.77% to 89.14%
Negative Predictive Value	96.77%	83.83% to 99.43%
Accuracy	91.89%	78.09% to 98.30%

Table 3. Statistic result of MAGE A1/A3 and histopathologic FB

Statistic	Value	95% CI
Sensitivity	44.44%	13.70% to 78.80%
Specificity	58.33%	36.64% to 77.89%
Positive Likelihood Ratio	1.07	0.45 to 2.55
Negative Likelihood Ratio	0.95	0.48 to 1.87
Disease prevalence	27.27%	13.30% to 45.52%
Positive Predictive Value	28.57%	14.35% to 48.85%
Negative Predictive Value	73.68%	58.77% to 84.62%
Accuracy	54.55%	36.35% to 71.89%

Based on the comparison between MAGE A1/A3 and the results of the PA CB, the sensitivity value was 30.43% and the specificity value was 75.00%. Kim & Kim (2009) reported that MAGE RT-nested PCR showed drastically increased sensitivity (67.9%) compared to cytology (21.4%). Although the study by Kim & Kim showed good sensitivity and specificity of MAGE RT-nested PCR for detection of peripheral lung cancer, the number of patients enrolled was very small, consisting of 28 cancer patients and 14 control cases [15]. Research by Shin et al., (2012) found that MAGE A1-6 RT-nested PCR showed a higher sensitivity (64%) than conventional cytology (14.7%) [16]. When combining the MAGE A1-6 RT-nested PCR method and conventional cytology, a higher sensitivity was obtained, namely 72%. Based on tumor size, the detection rate for tumors smaller than 3 cm was 74%, and for tumors larger than 3 cm. Apart from the relatively small amount of bronchial fluid flushing, the MAGE A1-6 RT-nested PCR method has a high sensitivity to diagnostics of lung cancer, which bronchoscopy cannot detect. A possible explanation for this result is that MAGE A1-6 common primary can detect small numbers of cancer cells in bronchial rinses. Thus, with the increase in the peripheral lung tumor MAGE, A1-6 RT-nested PCR of bronchial flushing fluid can have important clinical significance [10].

High specificity is required in the screening test to avoid false positivity. The diagnostic value of a single marker is relatively low. Expression of individual members of the MAGE family is frequently found in lung cancer, but the level of expression is variable and low. To increase the detection rate of MAGE, several researchers have developed multi-marker RT-PCR. Different choices of many biomarkers will produce different results. Another study by Zhang *et al.* (2019) showed that combining 5 selected biomarkers of SOX2, MAGE, P53, GBU4-5, and PGP9.5 from the SSC group resulted in a sensitivity of 58.8% and a specificity of 66.9% in the validation set [2].

Conclusion

In conclusion, the examination of the tumor antigen MAGE A1 and A3 can be used as a new candidate for lung cancer diagnosis in the future.

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Conflict of interest: None

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Ethics statement: All procedures were in accordance with the ethical standards on human experimentation in the Helsinki Declaration of 1964 and its later derivat. Informed consent was obtained from all patients included in the study.

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