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学位の種類	博士 (医学)
学位記番号	甲第610号
学位授与年月日	令和2年3月16日
学位授与の要件	自治医科大学学位規定第4条第2項該当
学位論文名	**抗生物質既治療進行性肺炎患者において、現在起炎病原体となっている病原体と定着病原体とを判別する分子技術とその応用**
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論文内容の要旨

1　研究目的

Empiric antibiotics are administered for pneumonia when the causative pathogens are unidentified. However, therapy based on causative pathogen can be challenging with negative result and culture time lag. This circumstance necessitates a salvage method for pathogen identification, especially when antibiotic therapy has failed. Detecting bacterial gene and indexing to human gene can be made by quantitative PCR (HIRA-TAN method). Here, we aimed to preliminarily investigate the HIRA-TAN method in pneumonia with a progressive course despite prior empiric antibiotic therapy.

2　研究方法

This prospective study was conducted for patients who were referred to Dr. Zainoel Abidin Hospital, Aceh, Indonesia, from December 2016 to January 2017, owing to pneumonia with a progressive course. Sputum or exudative pleural effusion was subjected to culture and quantitative PCR targeting both human and bacterial gene HIRA-TAN assay. The HIRA-TAN identified the candidate causative pathogens based on the difference in the cycle threshold (Ct) between the targeted pathogen and the single-copy human gene. The specimen was divided into two portions: one was used for conventional culture and the other was used for the HIRA-TAN. The specimen for the HIRA-TAN was diluted with an equal volume of phosphate-buffered saline and homogenized by vortexing. The first step of the HIRA-TAN is real time PCR. The PCR is a multiplex TaqMan assay performed in 5 separate reactions. The targeted pathogens were *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. coli*, *S. pneumoniae*, *S. aureus*, *H. influenzae*, *M. catarrhalis*, *Proteus spp.*, and *M. tuberculosis*.

The second step of the HIRA-TAN is the evaluation of the quality of the samples by the cycle threshold for the human TNF gene: Ct_{Human}. The TNF gene comprises a single copy in the human genome, and thus its copy number in the reaction directly

reflects the number of human cells. Sputum samples with a Ct_{Human} <27 contain many human cells and have been classified in M2-P3, according to the Miller and Jones' classification, and are expected to provide highly reliable results.

3 研究成果

A total of 27 patients were enrolled in this study. Sputum was collected from 25 patients, and pleural effusion from the remaining 2 patients. All patients were referred from the primary (19 patients) and the secondary (8 patients) health care centers. Accordingly, the predominant type of disease was hospital-acquired pneumonia (HAP: 25 patients, 93%) with the two others were pleuropneumonia and empyema. The patients had median age of 62 years (range: 21-90 years) and were predominantly male (22 patients, 88%). All patients had comorbid disease, including lung cancer and other neoplastic diseases (11 patients, 41%), chronic respiratory diseases such as COPD (9 patients, 33%), cardiovascular disease (3 patients, 11%), cerebrovascular disease (1 patient, 4%), thyroid disorder (2 patients, 8%), and mandibular fracture (1 patient, 4%). As expected from the high rates of HAP and comorbid disease, many patients (82%) had pneumonia with multilobar infiltrates.

The cut-off values that discriminate the pathogens causing pneumonia were determined for *P. aeruginosa, K. pneumoniae, M. catarrhalis*, and *S. pneumoniae* in a previous HIRA-TAN study that investigated untreated pneumonia. We adopted the same cut-off values in this study because it was considered to be a good starting point to preliminarily investigate the utility of the HIRA-TAN in progressive pneumonia without any preceding studies targeting the similar patient group. In many samples, the HIRA-TAN provided 2 pathogens suggestive of being the causative agent, which is also clear from the HIRA-TAN results plotted for each sample. The bacterial culture detected the definite pathogens in nearly half of the samples. Some fastidious are difficult to culture since it is challenging to accurately simulate their natural milieu in the culture medium. Moreover, previous antibiotic history may also prohibit the growth of colonies. The results of the culture-positive samples and those obtained by the HIRA-TAN were in close agreement (Table 3) and the HIRA-TAN provided the likely causative pathogens in the samples with non-diagnostic culture. No fluorescent signal was observed from other probes, so we did not consider another detection. No fluorescent signal was observed from other probes, so we did not consider another detection.

4 考察

In the current study, we aimed to preliminarily evaluate the clinical utility of the HIRA-TAN for pneumonia with a progressive course after a failure of antibiotic treatment. In many cases, the HIRA-TAN identified pathogen(s) with numbers overwhelming those of inflammatory cells, thus judging them as likely causative agents of the pneumonia. This

disparity may be explained by the low performance of culture. We suspect that, in some cases, a trace amount of previous antibiotics that remain may have prohibited the growth of colonies.

Detection of a bacterial genome by PCR does not necessarily indicate that the bacterium is viable in the sample, because PCR detects DNA in both living and dead cells. However, a report of the HIRA-TAN for community-acquired pneumonia (CAP) demonstrated that the copy number of bacterial DNA in a sample quickly decreased after effective antibiotic treatment. Therefore, practically, when a pathogen(s) is detected as the causative pathogen by the HIRA-TAN, it should be considered viable and set as the treatment focus.

K. pneumoniae, P. aeruginosa, or both were detected in most patients. On the other hand, *S. pneumoniae*, which is the most common pneumonia pathogen worldwide, was not detected. This suggests that the antibiotics used in the area investigated may be effective for *S. pneumoniae*, pending confirmation. Our data suggests that *K. pneumoniae* and *P. aeruginosa* survived the treatment and became the causative pathogen(s) for the subsequent pneumonia. This finding is slightly different from that of a previous report, in which most of the causative pathogens in HAP and ventilator-associated pneumonia (VAP) were *S. aureus, K. pneumoniae,* or *P. aeruginosa*. This may reflect the difference of hospital ward or geographical variance. However, such information is important for installing region-specific therapeutic strategies.

5 結論

We consider that the HIRA-TAN is useful as an alternative diagnostic test and can provide important information while waiting for confirmation. Early initiation of a pathogen-directed, second-line therapy will become possible by employing the HIRA-TAN as salvage microbiology. However, the study was limited by a small sample size within only one ward, pending confirmation by a larger clinical trial. We conclude that the HIRA-TAN provided valuable information for determining the second-line treatment for pneumonia that fails the initial round of antibiotic therapy.

論文審査の結果の要旨

細菌性肺炎はその起因菌の特定は難しく、普及している迅速診断キットも肺炎球菌などの一部の病原体を対象としたのもしかないため、肺炎患者は起因菌が不明なまま治療をうける場合が多い。日常診療における起因菌の診断法は、肺炎患者の喀痰を培地に塗布し，培養後に増殖した病原体を同定する方法が(喀痰培養検査)主に用いられている。上記の方法は、診断まで時間を要することと、培養できない菌もいることで，また手技も煩雑であり、検査実施者による結果の誤差が生じるなどの問題点がある。現在では，高価・強力・広域スペクトルの抗菌薬が普及しているため，治療に難渋する肺炎は昔から比べると減少はしているものの，耐性菌の出現や医療費の高騰をまねき、またそれら抗菌薬で治癒ができない

場合もよくある。そのため細菌性肺炎の死亡率が現在も高いままである。

病原体の検出においてPCRなどの分子生物学的手法は有用な検査法であるが，呼吸器感染症領域では普及していない。それは，検出感度が高い反面、肺炎球菌や緑膿菌などのような定着型病原菌（無症候で気道に存在しうる病原菌，すなわち常在菌）が検出されることが多く、起因菌との区別ができない問題点がある。実際に、臨床で応用されているPCR法は，マイコプラズマや結核菌のような非定着性病原菌（健常者からは検出されず，検出されれば起因菌と確定できる病原菌）に対してのみが実用されている。しかし、臨床で問題となる多くの重症型市中肺炎や院内肺炎の起因菌は，基本的に定着型病原菌によるものがほとんどである。このような背景を踏まえ、申請者KURNIAWAN氏は、本研究で抗生物質既治療進行性肺炎患者を対象に指導教員らが開発した起因菌と定着菌を区別する迅速診断法（HIRA-TAN法）の実用性を検討した。本迅速診断法は、ヒト細胞と病原体との細胞数比をReal-time PCR結果で表現し，起因菌と定着菌を鑑別することができるとされている。

本研究は、研究目的が明確である。また、研究結果では、HIRA-TAN法を用いれば、既に抗菌治療を行なっている肺炎患者の検体から起因菌を同定することに一定の効果があることを示した。そして、本方法を用いれば、培養が困難な細菌（または経験的抗菌治療を開始後）を速やかに同定することができ、エビデンスに基づく抗菌治療を早期に開始することにつながり、これが本研究の一番のインパクトである。

本研究成果は既にJournal of respiratory investigation誌に英文原著論文として発表しており、学問的に意義がある判断した。一方、研究で使用されているサンプル数が少ないことや、細菌分離培養の成績が十分ではいなど改善するべき点もあった。

また、学位論文に関する審査委員の指摘に対して丁寧に対応しており、2度の修正を重ね、最終的にかなり改善された論文が提出されている。

以上の理由と審査修正の過程を経て、全審査委員が一致して、学位論文として相応しいものと判断した。

最終試験の結果の要旨

最終試験では、申請者 KURNIAWAN 氏に対して、本研究の着想に至った背景と仮説、その仮説に答えるために研究に用いた材料・方法の妥当性と限界、得られた結果と解釈、及び考察について発表、口頭試問を実施した。

KURNIAWAN 氏は、それらに関する質問に対して適切に答え、研究全般及び関連情報について十分に理解していることが確認された。また、論文修正意見に対する適切な対応や研究内容の発表と質疑応答を通して一貫して自信をもった態度で臨んでいたことは、申請者自身が主体的に本研究を遂行したことを示すものである。

以上から、諮問の結果は合格と判定した。