

# A DIAGNOSTIC STUDY OF NOCARDIOSIS PATIENTS BEING CONFINED IN SHAREATI TRAINING HOSPITAL IN TEHRAN, USING CULTURAL & SEROLOGICAL METHODS

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## ABSTRACT:

The present investigation was carried out to detect nocardiosis in immunocompromised patients confined in the pulmonary ward of Tehran's Shareati Training Hospital through the use of indirect immunofluorescence assay (IFA) and bacterial culture methods. The comparison of the two methods and the correlation between the antibody titer and the statistical and epidemiological data were also investigated.

One hundred and one patients with advanced symptomatic pulmonary infection were studied in the course of a twenty-month period. Individual patients' sputum, BAL (bronchoalveolar lavage) and blood sera were tested. From each sample three thin smears were prepared for microscopic observations. The samples were cultured in Sabouraud's dextrose, blood and paraffin agar. The detection of antibody against *Nocardia asteroides* was carried out in all study groups, using the IFA method. The medical history of patients was also obtained through questionnaires for further analysis.

Fortyone patients suspected for Nocardiosis with an antibody titer ranging from  $\frac{1}{4}$  to  $\frac{1}{512}$ , detected by IFA method, included 26 (63.4%) men and 15 (14.8%) women. The age of the patients varied from 7-80 years. Those with reasonable antibody titers included 15 (36.5%) housewives and 9 (21.9%) workers. *Nocardia asteroides* was isolated from only one patient suffering from Wagner vasculitis with an antibody titer of 1/512 in serum. Furthermore, *in-vitro* investigation for the differentiation of the isolates was performed and confirmed the notion that the organism which grew on the primary media was, indeed, the *Nocardia asteroides* complex.

Our results revealed that the bronchopulmonary infections, which occur in high-risk patients T-cell deficiencies, long term corticosteroid therapy, immunocompromised hosts, HIV infection, organ transplantation- was an important index for the primary diagnosis of Nocardiosis. As the important finding of the present research, antibody titer of 1/64 could be taken as a cut-off value for diagnosis of patients infected with *Nocardia*, though lower titers should not be totally ignored.

**KEYWORDS:** Pulmonary Nocardiosis, Indirect Immunofluorescence Assay, immunocompromised, *Nocardia asteroides* complex

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## INTRODUCTION

In recent years the increasing incidence and changing spectrum of infectious diseases due to immunodeficiency phenomenon have attracted the scholar's attention to such opportunistic microorganisms as *Nocardia asteroides*<sup>1-5</sup>. *Nocardia asteroides* most commonly infect humans through the respiratory tract<sup>6-9</sup>. Infections caused by *Nocardia asteroides* result from the organism's ability to evade bactericidal mechanisms of the host<sup>10-12</sup>. Systemic immunosuppression, especially cell mediated

immunity dysfunction is an important predisposing factor in Nocardial attack of the lung which mostly occurs in kidney<sup>4-5, 13-15</sup>, heart<sup>16-17</sup>, liver<sup>5</sup> and lung graft recipients<sup>18</sup>, as well as the bone marrow transplant patients<sup>19</sup>. The disease has also been noted in the non-immunocompromised patients<sup>9, 20-21</sup>. Previous studies have demonstrated Nocardiosis in a variety of disorders including systemic lupus erythematosus and pemphigus<sup>12, 22-23</sup>, HIV<sup>8, 21, 23-24</sup>, chronic myelogenous leukemia with lung carcinoma<sup>24-25</sup>, Cushing's syndrome with bronchogenic carcinoma<sup>26</sup>, Evans' syndrome<sup>27</sup>, and repeated pulmonary infection by *Nocardia asteroides* complex in a patient with bronchiectasis<sup>9, 17, 21</sup>.

*Nocardia* is often reported to have caused pulmonary infection in immuno-compromised patients in different regions of Iran<sup>13, 26</sup>. Being reported in soil, this bacterium has been isolated from the soil in different parts of the country, which proves its study necessary. On the other hand, there might have been considerable cases of such infections in the high-risk patients, which have been neglected to date.

The purpose of this study was to detect the *Nocardia* species in the high-risk patients suffering from pulmonary infection due to immunodeficiency disorder, using the indirect immunofluorescence assay (IFA) and bacterial culture methods. A comparative study of the methods employed was carried out and the correlation between the patient's antibody titer and the statistical and epidemiological findings was determined.

## METHODOLOGY

### Subjects

The study population comprised the following group of individuals:

1. The experimental group: Hundred and one patients afflicted with advanced pulmonary infection- high risk - hospitalized at the pulmonary unit of Tehran's Shareati Training Hospital.
2. The control group (including two sub-populations):

- a) 72 individuals, the possibly exposed including the hospital's medical staff, i.e., doctors and nurses along with the maintenance and the janitorial staff.
- b) 106 individuals with no prior or possible exposure, the non-exposed, e.g. blood donors.

## METHODS

Blood, sputum and, in some cases, lavage samples were obtained from the experimental group. However, only serum samples (5ml) were drawn from the two control subgroups for the purpose of indirect immunofluorescent assay (IFA). Samples were subsequently sent to the laboratory for the following microbiological examinations i.e. direct slide, culture and the IFA. Patients were, also, requested to complete some questionnaires.

Microbiological identification and differentiation were conducted no more than two hours subsequent to sample taking. Positive patient samples were re-examined at various, less diluted, titers.

## RESULTS

Out of the 101 patients in the present study, 41 had the following range of antibody titers 1/4 to 1/512 (Fig-1), however, in only one of the patients was *Nocardia* organism isolated and detected via both culture and the IFA techniques. The patient was a 28 year old male, confined in the Rheumatology ward, with one and half year history of Wagner vasculitis, immunosuppression drug use, herpes zoster infection and subsequent pulmonary infection. Bacteriologic examination of the patient's sputum, employing solid culture media (blood, Sabouraud's dextrose and paraffin agar), in addition to that of direct slide culture revealed the typical *Nocardia* colonies.

For the determination of the bacteria up to the species level, the substrate hydrolysate and differential tests were carried out. *Nocardia asteroides* was identified, based on the absence of clear zones around the Tyrosine, Xanthine,

Hypoxanthine and Casein substrates contained in the specific culture media.

IFA employing the patient's serum was carried out initially and subsequently three months thereafter. High antibody titer was detected at both trials. It must be mentioned that all individual patient samples contaminated with mycobacterium species were eliminated from the population study in order to prevent the possibility of any cross-reactions. The isolated *Nocardia* strain was employed in the IFA as the antigen source. The results of the IFA in healthy individual blood donors (the non-exposed) and the hospital workers (the possibly exposed) revealed no antibody-antigen reaction (Table-I).

The study population comprises the following groups of patients.

1. 26 (63.4%) males, 15 (36.6%) females
2. 15 (36.5%) housewives, 9(21.9%) workers
3. 07 (17.0%) office workers
4. 05 (12.1%) students
5. 05 (12.1%) free lancers
6. 25 (60.9%) individuals within the mean age group of 17-55
7. 26 (63.4%) patients with a history of immunosuppressive drug use
8. 13 (31.7%) individuals with a history of respiratory illnesses.
9. 06 (14.6%) individuals with familial history of respiratory illnesses.
10. 10 (24.3%) cigarette smokers.

The following data represents the different types of concurrent illnesses noted in the patient population.

Table-I: The percentage and frequency distribution of Study Population on the basis of IFA results

Study Group	Positive	(%)	Negative	(%)	Total
Advanced pulmonary infection	41	40.6	60	59.4	101
Occupational exposed (medical staff)	0	0	72	100	72
Non-exposed (blood donors)	0	0	106	100	106
<b>Total</b>	<b>41</b>	<b>14.7</b>	<b>234</b>	<b>85</b>	<b>279</b>

1. 36 (78.8%) respiratory infection cases
2. 18 (43.9%) blood -related illnesses
3. 16 (39.0%) non-respiratory infections
4. 10 (24.4%) transplant patients.

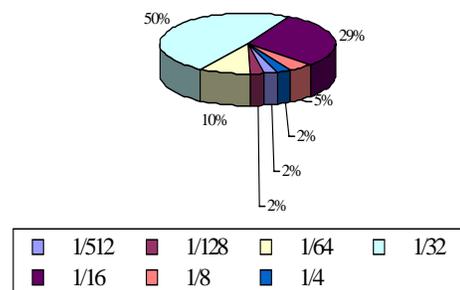
It is worth noting that some of the aforementioned illnesses were concurrently present in some of the patients, and that, to our regret, 9 (22.0%) of the patients passed away in the course of the study following 1 to 3 months of hospitalization. These patients had the following antibody titers 1/16, 1/32 and 1/128.

### DISCUSSION

In light of the presence of 40 percent positive antibody titer cases, it can be said with certainty that the incidence of Nocardiosis is not as rare an occurrence as once thought and that IFA can be proposed as a suitable technique in the diagnosis of such cases. In addition to the possibility of an early diagnosis through IFA, further advancement of the disease and its deadly consequences can be avoided.

Respiratory infection is ensued upon the entry of the microorganism into the respiratory system<sup>1,28-29</sup>. In healthy individuals the immune system combats the invader; however in the susceptible individual the disease can become aggressive and lead to either chronic and or acute states<sup>7,10,31-32</sup>. The conventional procedures for the detection of this microorganism are culture method<sup>29,33-35</sup>, direct slide culture observation and serological procedures<sup>36-39</sup>. Various advantages and disadvantages are

Fig-1: Distribution of anti *Nocardia* antibody titer in high risk patients suspicious to Nocardiosis



associated with such procedures. What follows is a description of some of these, which could help in our search for a more advantageous and less flawless a technique.

### 1. Culture method

- The ease of operation associated with this technique has made it the most conventional technique utilized in laboratories with limited provisions, i.e., lack of expensive equipment. However, due to the usual 7days time period for colony development, this is not an appropriate technique for the detection of *Nocardia* strains.
- Although thorough the use of selective media e. g. Paraffin agar, the growth of other microorganisms can be prevented <sup>40-41</sup>, this technique lacks the sensitivity for the detection of the *Nocardia asteroides* and thereby allows the growth of other organisms which could interfere with the species detection.

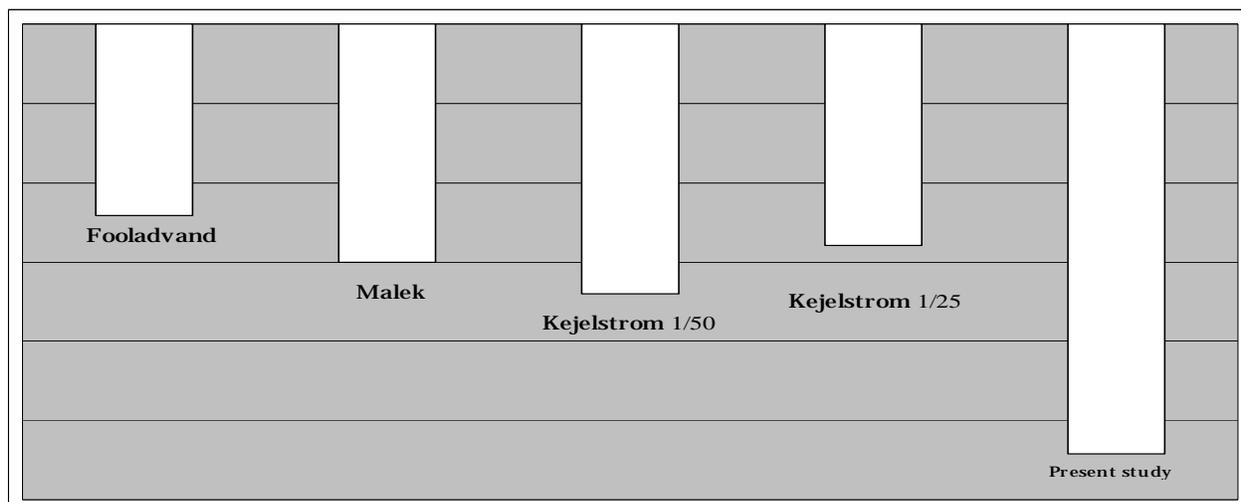
- In the case of antibiotic containing culture media, used for the growth of fungi, *Nocardia* growth is prevented.
- The use of chemicals e.g., mucolytic and antiseptic agents containing such compounds as sodium or potassium N-acetyl cysteine, and mixtures of Benzylchlonium chloride in trisodiumcitrate are toxic to the *Nocardia* species.

### 2. Direct slide sample technique

- As a result of mixing of sputum with mouth secretions more aggressive procedures such as bronchoscopy and bronchobiopsy and etc... are used, although, the use of this technique is rather routine in the detection of *Nocardia* for sputum samples.
- The employment of aggressive techniques and lavage samples however, beneficial have been rather slow and insensitive in the detection of *Nocardia* species. The need for more definite and less time consuming serological techniques, therefore, becomes ever more felt.

Fig-2: The IFA results in patients with Nocardiosis on the base of previous studies

Fooladvand	Maleki	Kjelstrom	Kjelstrom	Present study	Researcher
1/64	1/32	1/50	1/25	1/512	Antibodytiter (MIN)
-1.204	-1.505	-1.699	-1.398	-2.709	Log (Antibody)



AVERAGE of Log (Antibody) = -1.703      Antibody = 0.0198 =  $\frac{1}{505 \# \frac{1}{64}}$

More acceptable results have been obtained through the use of such serological techniques as the DNA-DNA hybridizing, PCR<sup>42-43</sup>, ELISA, Western Blot and the Enzyme Immunoassay IFA, by numerous researchers<sup>23, 36-38, 44-45</sup>.

The IFA seems to be an easy, inexpensive and highly sensitive technique for the detection of *Nocardia* species. Through the use of the whole of the *Nocardia* microorganism as the antigen the IFA sensitivity is raised in comparison to other serological testing. The justification for the employment of IFA as “the” technique for the rapid and accurate detection of *Nocardia* is based on the comparison of data with those of other aforementioned techniques. For an appropriate selection of patient population the following steps are recommended:

1. Close scrutiny and follow up of suspected patients, no definite clinical symptoms are associated with pulmonary Nocardiosis for a number of years. This procedure is, however, not a feasible recommendation in the context of Iranian medical community, due

to lack of coherent patient information and/ or file system.

2. Bacterial injection to healthy subjects and studying the course of infection, along with the individual’s immunologic response. Kjelstrom carried out such study in 1993 employing laboratory rats. It is rather obvious that such studies can not be carried out utilizing human subjects, and the extrapolation of findings, using animal subjects, onto humans poses certain difficulties of its own. The “Probability approach” is the route to take. Based on this approach we would not know for sure that the patient is infected with the *Nocardia* species, just that there is a chance or probability of such infection.

**A frequently asked question!** What value can be set as a criterion for antibody titer?

Figure 2, illustrates the antibody titer, which lead to positive results through the employment of the IFA. A good criterion could be the mean value of such titer levels reached through previous and current studies. The mean loga-

Fig-3: statistical finding on experimental group study (high risk) based on antibody titer assay

Antibody (fraction)	1/512	1/64	1/8	1/4	1/16	1/32	1/128
Antibody (decimal)	0.0020	0.0156	0.1250	0.2500	0.0625	0.0313	0.0078
Number of cases	1	4	2	1	12	20	1

	Value (decimal)	1/Value (decimal)	Approx.Value
Average of antibody	0.0475	21.1	1/20
SD of Antibody	0.0414	24.1	
Mean + SD	0.0889	11.2	1/11
Mean - SD	0.0060	165.4	1/165
Mean + 2SD	0.1304	7.7	1/8
Mean - 2SD	-0.0354	-28.2	

	Value (Log)	Approx. antibody
Average of Log (antibody)	-1.439070223	1/27
SD of Log(Antibody)	0.339991992	
(Mean + SD) of Log	-1.099078231	1/13
(Mean - SD) of Log	-1.779062215	1/60
(Mean + 2SD) of Log	-0.759086239	1/6
(Mean - 2SD) of Log	-2.119054208	1/131

rhythm of antibody titers, however, gives us yet a better correlation with the incidence of the disease. As seen in figure 3, mean logarithm value is 1/703 with an equivalent antibody value of 0.0198, and approximate number 1/64.

## CONCLUSION

Out of the 101 patients participating in the study 41 seemed to have the antibody titer sufficient to render them increasingly susceptible to pulmonary Nocardiosis. Men outnumbered women 2:1 and individuals from all ages seemed to be at risk. Construction workers and housewives seemed to comprise a larger proportion of the patient population, due to the greater possibility of exposure to dust. The presence of concurrent illnesses in addition to the use of immunosuppressive agents among the patient population was also another notable finding. Due to the rarity or the infrequency in the incidence of this disease and the existence of an only single positive culture in the study population, it was not feasible to compare the findings of the indirect fluorescent antibody assay with that of the culture method. Hence, no statistical information was obtained and, thereby, presented. Future studies with greater patient population are required in order to observe a statistically significant difference between the two techniques. As for the time being, we shall only suffice with descriptive analyses of our observations. In the course of the study 9 patients (antibody titers: 1/16, 1/32, 1/128) passed away, possibly because of brain abscess resulting from Nocardiosis.

Serologic IFA along with direct slide culture observation and solid media culture method can be employed as aids in the early definitive diagnosis of pulmonary Nocardiosis which, if undetected, could lead to brain abscess and increased mortality. This can be of particular value, in view of the nature of the infection, where the microorganism has the tendency to quickly spread from pulmonary lesions by the way of blood stream and to establish metastatic abscesses in the subcutaneous tissues and in

the central nervous system. Due to the fact that the indirect IFA technique is a rather inexpensive, quick, and easily operated one, as well as, being capable of providing a definite diagnosis of Nocardiosis in many cases; it can be proposed as a valuable tool in the early assessment and clinical diagnosis of the disease in the afflicted individuals. In addition, the following recommendations are proposed by the researchers:

- 1) Conducting other serological studies e.g. ELISA, in order to compare and assess the possible superior sensitivity of the IFA.
- 2) Standardization of IFA as a possible replacement for the conventional culture method, in the hopes of avoiding some of the already mentioned difficulties experienced through this technique.
- 3) The employment of the IFA technique on patients' serum, with established diagnosis of Nocardiosis.
- 4) The determination of the disease initiating antibody titer.

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