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Impact of the 1997 revised Centers for Disease Control criteria on case rates of legionellosis in Taiwan: review of 38 cases at a teaching hospital, 1998-2002

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In 1997, the United States Centers for Disease Control and Prevention (CDC) published revised case definitions for legionellosis which eliminated the previously used category of "probable case" based on a single indirect fluorescence antibody (IFA) titer. This study evaluated the influence of revision on the case rates of legionellosis in Taiwan. From 1988 to 2002, 4615 patients with pneumonia were tested for legionellosis in our hospital. The testing methods included IFA assay for serum specimens and direct fluorescence antibody (DFA) assay for sputum specimens. Using the revised criteria, Legionnaires' disease (LD) was diagnosed by DFA in 27 cases and by IFA in 11 cases. The most common underlying conditions were cigarette smoking (44.7%), chronic obstructive pulmonary disease (28.9%) and corticosteroid use (26.3%). The clinical features were nonspecific, including fever (73.7%), dyspnea (63.2%), cough (63.2%) and leukocytosis (63.2%). The overall mortality rate was 18.4%, and the directly LD-attributable mortality rate was 10.5%. Nasogastric tube insertion, endotracheal intubation, congestive heart failure before the onset of LD, inappropriate antimicrobial therapy, respiratory failure and absence of fever during the LD course were significantly associated with LD-attributable mortality. Older age (>70 years) was not associated with higher mortality (p=0.053). Using the revised diagnostic criteria in our series, the positive rate of case identification by IFA was 0.26%, while use of the previous case definitions resulted in a positive rate of 7.6% (including probable and definitive cases). Recognition that the original CDC criteria of IFA titer >1:256 or elevation of IFA titer <4-fold in paired sera could not adequately define an LD etiology has led to a dramatic lowering of case rates among studies after the criteria revision in Taiwan and elsewhere. Assays that are faster, more sensitive and less technician dependent are needed to diagnosis this disease.

Key words: Diagnosis, indirect fluorescent antibody technique, legionellosis, Legionnaires' disease

Legionellosis is an infection caused by *Legionella* spp. The clinical spectrum of legionellosis can range from asymptomatic seroconversion, to Pontiac fever and Legionnaires' disease (LD). Pontiac fever is a self-limited flu-like disease, while patients with LD may develop severe pneumonia with multiorgan involvement [1-3]. Patients with impaired cellular immunity and a weakened natural airway protection mechanism are predisposed to acquiring LD [4]. The clinical manifestations of LD are nonspecific and include fever, non-productive cough, myalgias, rigors, dyspnea, diarrhea, headache and delirium [5].

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In Taiwan, data are limited concerning the clinical presentations or epidemiology of legionellosis [6-12]. Lin et al studied 223 patients with pneumonia treated at National Taiwan University Hospital (NTUH) from 1992 to 1993, and identified 21 (9.4%) LD cases (13 probable cases and 8 definitive cases) using the old case definitions [13]. In 1997, the United States Centers for Disease Control and Prevention (CDC) published revised case definitions for legionellosis which eliminated the category of "probable case", defined as single indirect fluorescence antibody (IFA) titer ≥1:256 or elevation of IFA titer <4 fold in paired serum but final titer ≥256 [14]. The National Institute of Preventive Medicine in Taiwan, as the referral laboratory for communicable diseases, has followed the new CDC definitions since January 1998. However, there has been no reported study of LD from Taiwan since the new case

definitions have been adopted. This study reviewed the characteristics of all LD cases diagnosed at our hospital from 1988 to 2002 using the revised definitions, and compared case rates obtained using the 2 criteria sets.

Materials and Methods

Case definitions

An LD case was defined as a person with pneumonia, diagnosed by chest radiography, meeting either of the following criteria: (1) detection of *Legionella* in respiratory secretion by direct fluorescence antibody (DFA) test; (2) a 4-fold increase, to ≥1:128, in antibody titer to *Legionella* in serum between the acute phase and the convalescent phase [14]. Patients who had been admitted continuously for at least 10 days before the onset of illness were regarded as having hospital-acquired infection [15]. Death without other obvious causes during the course of disease was considered as directly attributable to LD. A total of 38 cases met the above definition and were included in this study. The medical records of these patients were carefully reviewed to obtain information relating to demographic characteristics, clinical course, complications, treatment and outcome.

Laboratory methods

We retrospectively reviewed all cases requesting *Legionella* tests in the Infectious Disease Laboratory of the Taipei Veterans General Hospital from 1988 to 2002. *Legionella* serum antibody titer was measured by IFA test (Zeus Scientific, Raritan, NJ, USA) and *Legionella* sputum antigen was detected by DFA test (Zeus Scientific) according to the manufacturer's manual.

Statistical analysis

The collected data were statistically analyzed using a commercial computer software package (SPSS, version 11.0; SPSS Inc., Chicago, IL, USA). For categorical data, variables were compared with 2-tailed chi-squared test. A p value of 0.05 or less was considered statistically significant.

Results

From May 1988 to December 2003, 4710 serum specimens and 3217 sputum specimens obtained from 4164 patients with the clinical diagnosis of pneumonia were checked for legionellosis in our laboratory. The serum *Legionella* antibody titer was checked by IFA test. A total of 441 cases had more than 1 specimen, and only 11 of these patients met the definitive diagnostic criteria of paired titer for legionellosis. Sputum samples were subjected to DFA for diagnosing *Legionella* infection and 27 positive DFA results were obtained from 26 patients. One patient had 2 LD episodes in 2 years and these 2 episodes accounted for 2 diagnoses in the analysis. There was no duplicated case between the DFA- and IFA-diagnosed patient groups and the review of clinical characteristics included a total of 38 cases.

The demographic data are summarized in Table 1. Among these 38 cases, 30 (78.9%) were men and 29 (76.3%) of them were greater than 60 years old. The most common underlying conditions were cigarette smoking (44.7%), chronic obstructive pulmonary disease (28.9%), corticosteroid use (26.3%), diabetes mellitus (23.7%), and congestive heart failure (23.7%).

Table 1. Gender, age distribution and underlying disorders of 38 patients with Legionnaires' disease

	No. of cases (%)
Gender	
Male	30 (78.9)
Female	8 (22.1)
Age (years)	
0-10	1 (0.26)
11-20	0
21-30	2 (0.52)
31-40	2 (0.52)
41-50	2 (0.52)
51-60	2 (0.52)
61-70	10 (26.3)
71-80	9 (23.7)
81-90	10 (26.3)
Underlying disorder	
Cigarette smoker	17 (44.7)
Chronic obstructive pulmonary disease	11 (28.9)
Corticosteroid user	10 (26.3)
Diabetes mellitus	9 (23.7)
Congestive heart failure	9 (23.7)
Nasogastric tube	8 (21.1)
Cerebral vascular accident	8 (21.1)
Endotracheal tube	7 (18.4)
Pulmonary tuberculosis	6 (15.8)
Alcohol use	5 (13.2)
Malignancy	3 (0.78)
Hematologic	2 (0.52)
Lung cancer	1 (0.26)
Post-chemotherapy	2 (0.52)
Post heart transplantation	1 (0.26)
Liver cirrhosis	1 (0.26)

Table 2. Clinical presentations of 38 patients with Legionnaires' disease

Symptoms/laboratory data	No. of cases (%)
Dyspnea	24 (63.2)
Cough	24 (63.2)
Chest pain	4 (10.5)
Sputum, non-purulent	13 (34.2)
Sputum, purulent	21 (55.3)
Fever	28 (73.7)
Rigor	11 (28.9)
GI symptoms	14 (36.8)
Myalgia	4 (10.5)
CNS symptoms	14 (36.8)
Shock	10 (26.3)
Elevated hepatic transaminase	18 (47.4)
Leukocytosis	24 (63.2)
Hyponatremia	17 (44.7)
Hypoxemia	20 (52.6)

Abbreviations: GI = gastrointestinal; CNS = central nervous system

The clinical features of patients with LD are shown in Table 2. The most common presentations were fever (73.7%), dyspnea (63.2%), cough (63.2%), leukocytosis (63.2%), purulent sputum (55.3%) and hypoxemia (52.6%). The radiologic patterns on chest films varied, with 50% of cases showing bilateral patchy infiltration, 36.8% unilateral patchy infiltration, 7.9% local interstitial infiltration, and 5.3% diffuse interstitial infiltration.

Thirty of 38 patients (78.9%) received appropriate treatment for LD. The most commonly used antimicrobial agents were erythromycin with or without rifampicin and ciprofloxacin. Among the appropriately treated patients, 4 died but only 1 of these deaths was directly attributed to LD. Among the patients who were not appropriately treated (8/38), 3 died and all of these deaths were directly attributed to LD. The overall case mortality rate was 18.4% (7/38) and mortality rate directly attributable to LD was 10.5% (4/38).

As shown in Table 3, pre-existing nasogastric tube insertion (p=0.024), endotracheal intubation (p=0.015), and congestive heart failure (p=0.035) before the onset of LD were significantly associated with mortality directly attributable to LD. During the course of LD, inappropriate treatment (p=0.024), respiratory failure (p=0.032) and absence of fever (p=0.048) were the significant factors which adversely influenced the outcome of LD. Although not statistically significant (p=0.053), age greater than 70 years was associated with a higher LD-related mortality. LD-related mortality rates did not differ between hospital-acquired and community-acquired groups (p=0.065). In contrast, hospital-acquired LD had a statistically higher overall mortality rate (p=0.014) than community-acquired LD. There was no significant difference in the clinical features and underlying conditions of patients with community-acquired and hospital-acquired LD.

Discussion

Legionella is an important pathogen for both community-acquired and nosocomial pneumonia [4]. It is difficult or even impossible to distinguish LD from other types of pneumonia by clinical presentations or chest radiography pattern [16,17]. Only laboratory tests can make a definitive diagnosis of LD. Widely accepted tests for the diagnosis of LD include: isolation of *Legionella* by culture; detection of *Legionella* by DFA testing of lung tissue or respiratory secretions; 4-fold increase in paired serum *Legionella* antibody titer between the acute phase and the convalescent phase with final titer ≥1:128; and detection of antigen in urine [14].

Prior to 1997, the case definitions for legionellosis of the CDC included the "probable case" diagnosis based on elevated IFA titer of ≥1:256. In this series, 306 of 4615 patients with pneumonia in the series met the original criteria for "probable case", including 282 patients who had a single titer ≥256 (selected from 3724 patients who had single IFA test) and 24 patients who had a final titer ≥256 but less than a 4-fold rise in paired serum (identified from 440 patients who had 2 or more IFA tests). If these "probable cases" were added to the 11 "definitive cases", (identified from 440 patients who had 2 or more IFA tests), the total case number diagnosed according to the original criteria for IFA was 317. The positive IFA test rate for LD among these patients was 7.61% (317/4164). A survey conducted by the National Institute of Preventive Medicine in Taiwan studied 487 pneumonia patients treated from 1993 to 1994 and reported a positive rate of IFA test for LD of 8.6% [7]. Another study performed at NTUH reported the positive rate of IFA test for LD was 9.4% in 223 selected pneumonia patients treated from 1993 to 1994 [13]. Both studies used the original definitions and the positive rates were similar to ours.

The reported sensitivity of IFA was about 80% in culture-positive LD cases [18]. Using the original definitions including definitive or probable cases, the specificity exceeded 99% [19]. In culture-positive LD cases, using the original definitions, the sensitivity rate of IFA was 80% and the specificity rate exceeded 99%

Table 3. Prognostic factors of 38 patients with Legionnaires' disease (LD)

	Total	LD mortality (n = 4)		All mortality (n = 7)	
		No.	*p*	No.	*p*
Gender					
Male	30	4	0.371	6	0.538
Female	8	0		1	
Age >70 years	19	4	0.053	6	0.045[a]
Dyspnea	24	4	0.114	7	0.027[a]
Cough	24	2	0.472	4	0.517
Chest pain	4	1	0.372	1	0.574
Fever	28	1	0.048[a]	4	0.257
Rigor	11	0	0.238	0	0.07
GI symptoms	14	3	0.132	4	0.21
Myalgia	4	0	0.628	0	0.426
CNS symptoms	14	2	0.472	4	0.21
Shock	10	1	0.721	1	0.392
Elevated hepatic	18	3	0.263	3	0.563
Transaminase	20	1		4	
Leukocytosis	24	2	0.472	5	0.483
Hyponatremia	17	0	0.081	2	0.302
Hypoxia	20	3	0.344	5	0.249
Acquisition					
Community	27	1	0.065	2	0.014[a]
Hospital	11	3		5	
Alcohol use	5	1	0.446	1	0.661
Diabetes mellitus	9	0	0.554	1	0.462
CHF	9	3	0.035[a]	4	0.041[a]
Cirrhosis of liver	1	0	0.895	0	0.816
Cigarette smoker	17	1	0.387	2	0.302
COPD	11	1	0.674	1	0.328
CVA	8	2	0.189	3	0.146
NG tube insertion	8	3	0.024[a]	4	0.025[a]
ET tube insertion	7	3	0.015[a]	4	0.013[a]
Malignancy	3	0	0.709	0	0.533
Chemotherapy	2	0	0.798	0	0.661
Corticosteroid	10	1	0.721	1	0.392
LD-specific regimen	30	1	0.024[a]	4	0.146
Respiratory failure	17	4	0.032[a]	5	0.125

Abbreviations: GI = gastrointestinal; CNS = central nervous system; CHF = congestive heart failure; COPD = chronic obstructive pulmonary disease; CVA = cerebral vascular accident; NG = nasogastric; ET = endotracheal

[a] $p<0.05$.

[19,20]. However, based on a single IFA titer ≥1:256, it was impossible to distinguish LD from pneumonia caused by other pathogens or discriminate recently developed from ongoing LD [21,22]. In 1997, the CDC proposed new definitions and the category of "probable case" was abandoned [14]. In this series, the positive rate of IFA was markedly lower when using the revised definitions (0.26%) versus the original criteria (7.6%). While the use of the revised criteria might lead to a false impression that LD is an uncommon disease, it is also less likely to misdiagnose LD when pneumonia is due to other etiologies.

Advanced age, smoking, male gender, chronic lung disease, hematologic malignancies, end-stage renal disease, lung cancer, immunosuppression, and diabetes are known risk factors of LD [4]. Similar risk factors, including smoking, chronic obstructive pulmonary disease and corticosteroid use, were also found in this series. One of the patients had received cardiac transplantation as was previously reported [12]. The most common clinical presentations of LD observed in the present study were nonspecific. Expected features of extrapulmonary symptoms and signs were not uniformly present. Our results on clinical presentations

and underlying conditions were similar to a previous study from Taiwan [13].

Although erythromycin was considered the drug of choice for LD in the past, in vitro data suggested azithromycin and many fluoroquinolones may have better activity [20]. In this series, among the 30 LD patients who received antibiotics, 19 received erythromycin and 4 received ciprofloxacin. Guidelines of empiric therapy for patients hospitalized with community-acquired pneumonia should include antimicrobial agents which are effective for LD [21-24].

In 2 large-scale surveillance studies conducted by the CDC, determination of the cause of death was also based on the judgment of clinicians [4,25]. In this study, using the revised CDC criteria, the overall mortality rate was 18.4% and mortality rate directly attributable to LD was 10.5%. The results were similar to a previous study from Taiwan which included 13 probable and 8 definitive cases and found overall mortality rates of 14.3% and 9.5%, respectively [13]. Surveillance of LD in the United States from 1980-89 found an overall mortality rate of 24% in confirmed LD [4]. The follow-up survey from 1980-98 revealed a trend of declining crude mortality, from 34% to 12% in confirmed cases [25].

Analysis of the predicting factors of overall and the directly LD-attributable mortality revealed that congestive heart failure, nasogastric tube and endotracheal tube insertion before the onset of LD were significantly associated with both LD-attributable and overall mortality rates. There was statistically significant association of appropriate antibiotic therapy with lower directly LD-attributable but not lower overall mortality. Delay in starting erythromycin therapy has been associated with increased mortality in patients with LD [26]. We assume that LD-specific therapy might reduce the risk of death due to LD, but not due to other causes. Complication with respiratory failure was associated with LD as the cause of death. Although old age (>70 years) has been reported as a poor prognostic factor, it was not significantly associated with LD as a cause of death in this study [13].

Use of the revised 1997 criteria for diagnosis of LD resulted in a decrease in the positive rate due to IFA alone from 7.61% to 0.26%. When using both DFA and IFA assay, the positive rate still dropped from 8.26% to 0.91% with the revised criteria in this study. However, the underlying conditions, clinical presentations, complications and outcome of cases diagnosed with the revised criteria were similar to a study based on the original definitions [13].

Currently available laboratory diagnostic tests for LD are of limited value. The reported sensitivity of sputum culture may be less than 10% by routine culture procedure [7,27]. A previous study found that using buffered charcoal-yeast extract agar, pretreating the specimen with heat or acidification, and not routinely rejecting non-purulent sputum specimens improved recovery of *Legionella* by culture [28]. Although special selective media and effort may increase the positive rate, heavily contaminated specimen or sputum collected after antibiotic usage could further lower the yield [7]. However, culture remains the most specific diagnostic method and is the gold standard for diagnosing legionellosis and provides the material for species identification and antimicrobial sensitivity testing [28]. Improvement of current culture methods is needed. In culture-positive LD cases, the sensitivity of DFA testing of respiratory secretions has ranged from 25-75%, while the specificity was >95% [29]. Dry cough is common in LD cases, resulting in inability to provide a specimen for culture and DFA assay [27]. There were also limitations in collection of paired serum. The 4-fold rise in serum titers may occur over a period of more than 4 weeks and may not occur at all [29]. Patients may die or be lost to follow-up thus leading to lack of data on convalescence titers. Also, clinicians may forget to draw paired sera. Detecting urine antigen by radioimmunoassay or enzyme-linked immunosorbent assay is convenient, fast, and rarely influenced by specimen collection or immune response of the host [30]. The sensitivity was 80-99% but the test was only specific to *L. pneumophila* serogroup 1 [30-32]. Urinary antigen testing detected a recurrent outbreak of nosocomial LD and had a role in controlling these infections in hospital [33]. In the United States, the proportion of LD diagnosed by culture, DFA, or IFA decreased significantly, while the proportion diagnosed by urine antigen testing increased from 0% to 69% from 1980-98 [25]. The Center for Disease Control in Taiwan began requesting urine specimens in addition to sputum and paired sera for reported LD cases in June 2004. Urine antigen detection was adopted as the official routine diagnostic procedure for LD. The relative value of *Legionella* DNA with DNA probe or polymerase chain reaction remains to be established [16,28,34].

For the infectious disease specialist, curing the patient is the first priority. Treatment guidelines for community-acquired and hospital-acquired pneumonia both covered LD in the initial regimen. Current diagnostic methods are limited and should not affect

initial clinical management because they are time-consuming, insensitive or biased to *L. pneumophilia* serogroup 1. Development of additional tests to supplement DFA and IFA assay for legionellosis, and improvements in culture procedure are needed.

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