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Original Article

Clinical prediction of endemic rickettsioses in northern Taiwan — relevance of peripheral blood atypical lymphocytes

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Background and Purpose: Several rickettsioses are endemic in Taiwan. They are under-reported not only because of ignorance but also due to difficulty in recognition caused by their nonspecific manifestations, which overlap with other acute febrile illnesses. We conducted a retrospective study to delineate distinctive clinical features of rickettsiosis, in order to develop a system for differential diagnosis of rickettsiosis.

Methods: Patients admitted to Chang Gung Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan, with suspected rickettsiosis during the period from January 2004 to May 2006 were included. Clinical suspicion was based on the presence of acute fever with eschar formation, relevant contact history, poor response to broad-spectrum empiric antibacterial therapy, unexplained thrombocytopenia, leukopenia, or abnormal liver biochemistry, or unexplained major organ involvement. Serum samples were sent to the Centers for Disease Control, Taiwan, for serologic diagnosis of the 3 rickettsioses endemic to Taiwan — scrub typhus (Tsutsugamushi's disease), murine typhus (endemic typhus) and Q fever. Serologically confirmed and excluded cases were compared for signs and symptoms, risk factors, laboratory findings and response to treatment.

Results: Among 138 suspected cases, 88 were excluded from the study because of incomplete serological tests or insufficient information, 28 were confirmed to have one of the 3 rickettsioses and 22 were negative for all of them. Distinct features among confirmed cases, compared to controls, were eschar formation, relevant contact history, and presence of atypical lymphocytes in peripheral blood. Normal or low leukocyte count, thrombocytopenia and relative bradycardia were not significant in predicting diagnosis. We propose a predictive system for tentative diagnosis of rickettsiosis based on relevant clinical attributes. This system has a positive predictive value of 80% and a negative predictive value of 100%.

Conclusions: The predictive scoring system may allow institution of appropriate treatment for rickettsiosis in a more timely manner. However, a low probability of diagnosis should prompt vigorous search for other etiologies.

Key words: Differential diagnosis; Lymphocytes; Q fever; Rickettsia infections; Scrub typhus; Typhus, endemic flea-borne

Introduction

Rickettsioses are zoonotic bacterial infections transmitted to humans by either arthropods or infected aerosols. Patients have usually incurred vector bites during outdoor activities or from close contact with domestic animals in endemic areas. The incidence of rickettsiosis correlates to vector abundance and atmospheric temperatures [1-3]. The diseases have a geographical and seasonal distribution worldwide, but occur throughout the year in tropical areas. Taiwan is affected by 3 rickettsioses — scrub typhus, murine typhus and Q fever — with several regions recognized as endemic [2,4,5].

Most patients with rickettsiosis present a benign acute febrile illness with headache, myalgia, and skin rashes. Severe complications and fatalities are occasionally seen. In endemic areas, diagnosis of rickettsiosis is usually made by consistent clinical and laboratory features with relevant epidemiological

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clues. Serologic tests tend to lack sensitivity, especially in the early stages of disease. Delayed seroconversion is not uncommon. Serology provides at best a retrospective confirmation only [6]. In contrast, polymerase chain reaction may offer higher sensitivity, but it is expensive and not readily available in most places [7-13].

Patients with mild illness might not seek medical attention. For severe cases, early recognition with prompt treatment can shorten the hospital stay and preclude serious complications or fatality. Tetracyclines are the drugs of choice for rickettsiosis and result in rapid recovery in the majority of patients. The aim of our study was to delineate relevant clinical features of rickettsiosis and to establish a predictive system for clinical diagnosis of rickettsioses, to facilitate their differential diagnosis.

Methods

We performed a retrospective study of suspected cases of rickettsiosis admitted to Chang Gung Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan, between January 2004 and May 2006. Suspicion of rickettsiosis was based on the clinical judgment of attending physicians. Rickettsiosis was considered as possible in patients with acute fever and any of the following — an inoculation lesion (the eschar), a history of relevant environmental exposure, poor response to broad-spectrum antibacterial therapy, unexplained thrombocytopenia, unexplained leukopenia, unexplained abnormal liver biochemistry, and unexplained major organ involvement.

Exposure history was categorized as high risk, low risk, or no risk. Patients from endemic areas, that is Pescadores islands, Orchid islands, Hualien county, and Taitung county, and patients with close animal contact were classified as high risk. Patients living in rural areas and patients with outdoor activities in the wilderness were classified as low risk. Patients with none of the above exposures were classified as no risk.

Thrombocytopenia was defined as platelet count of less than $150 \times 10^3/mm^3$. Leukopenia was defined as leukocyte count of less than $4 \times 10^3/mm^3$. Abnormal liver function was defined as any abnormality in liver biochemistry. Broad-spectrum antibiotics were defined as antibiotic regimens with coverage for community-acquired Gram-positive and Gram-negative pathogens (for example, first-generation cephalosporins or a combination of penicillin plus an aminoglycoside). Poor response to broad-spectrum antibiotics was defined as symptoms (especially fever) lasting longer than expected for the presumptive diagnosis, under treatment with empiric antibiotics. Major organ involvement was defined as any compromise of central nervous system (depressed conscious level), lung (abnormal pulmonary infiltrates or pleural effusion), cardiovascular system (echocardiographic or serological evidence of myocarditis), liver (hepatomegaly or abnormal liver biochemistry), kidney (acute renal failure), or the reticuloendothelial system (splenomegaly or lymphadenopathy). Relative bradycardia, a pulse-temperature deficit, with the pulse less than that expected for a given temperature, was reported as a relevant clinical feature of rickettsioses [14,15], and was defined using the criteria of Cunha [14]. Serum samples were sent to the Centers for Disease Control (CDC), Taiwan, for confirmatory diagnosis of the 3 rickettsioses endemic in Taiwan. Patients with positive polymerase chain reaction tests or positive indirect immunofluorescence assay were regarded as confirmed diagnoses and were included as cases. Patients negative for all 3 rickettsial tests were included as controls.

Statistical analysis

Cases and controls were compared for signs and symptoms, risk factors, laboratory findings and response to treatment. Categorical variables were compared by use of Fisher's exact test and continuous variables were compared by use of Student's *t* test. A 2-tailed *p* value of <0.05 was considered statistically significant. All statistical calculations were done using the Statistical Package for the Social Sciences (SPSS) for Windows (Version 13; SPSS, Chicago, IL, USA).

Results

Between January 2004 and May 2006, the CDC, Taiwan, received serum samples from 138 patients from Chang Gung Memorial Hospital Linkou Medical Center, Taoyuan, Taiwan, for confirmatory tests for rickettsioses. Eighty eight patients were excluded for a variety of technical reasons, which included insufficient clinical information (n = 6), inadequate serum samples or undetermined test results (n = 33), and incomplete study for the 3 rickettsioses (n = 49). Twenty eight cases were verified via serology or polymerase chain reaction tests, including 17 cases of scrub typhus, 6 cases of Q fever and 5 cases of murine typhus. None of the patients had dual or triple infection. The remaining 22

patients were negative for all 3 rickettsioses and were included as controls. In summary, 28 confirmed cases of endemic rickettsiosis and 22 controls were reviewed. The controls included cases of *Mycobacterium tuberculosis* infection (n = 2), autoimmune disorders (n = 2), pelvic inflammatory disease (n = 1), liver cirrhosis (n = 1), pneumonia (n = 2), and metabolic encephalopathy (n = 2). The other 12 cases had no definitive diagnoses on discharge, and were regarded as having acute viral syndrome.

Age and gender ratio were similar for cases and controls (Table 1). Nine of the 28 cases, compared to none of the 22 controls, were from rural areas. The majority of cases (22/28; 79%) had relevant exposure history, with 14 of 28 cases (50%) reporting high risk exposure. Eight of these 14 had recently traveled to endemic spots, 4 of the 14 had close contact to domestic animals, and 2 of the 14 had hiked into mountainous areas. The remaining 14 cases reported either no relevant exposure or exposure of minimal relevance (such as wild grass around the house or physical proximity to a farm). Six of the 22 controls (27%) reported exposure of minimal relevance. Distinctive memory of insect bites could be traced in only one of the 28 cases. Taken together, the area of residence and contact history differed significantly between cases and controls ($p<0.001$).

Symptoms and laboratory findings of the cases and controls are summarized in Tables 1 and 2, respectively. The duration of fever before admission and many other signs and symptoms were similar for cases and controls. However, eschar was found in 43% (12/28) of the cases, but in none of the controls. The prevalence of eschar was particularly high among patients with scrub typhus (71%). Shock was noted in 2 confirmed cases. Relative bradycardia was common in both groups (68% vs 86%, $p=0.304$). White blood cell count (mean, 8100/mm^3 vs 8663/mm^3) and frequency of thrombocytopenia (57% vs 50%, $p=0.615$) were similar in cases and controls. The proportion of patients with abnormal liver biochemistry was significantly higher in the confirmed cases than among controls (89% vs 64%, $p=0.042$), and the confirmed cases had a higher degree of liver enzyme derangement than controls ($p=0.019$). There were no differences between the groups in C-reactive protein level, serum creatinine, and chest X-ray abnormalities, including the occurrence of pleural effusion.

Atypical lymphocytes were machine counted in our study. Twenty one confirmed cases (75%) had atypical lymphocytes in the peripheral blood either on admission or on follow-up within 5 days of admission. Atypical lymphocytes ranged from 1% to 31% in

Table 1. Demographic features, symptoms and signs of rickettsiosis cases and controls

Variable	Cases (n = 28) No. (%)	Controls (n = 22) No. (%)	*p*
Age (years; mean) [range]	50.5 (26-80)	45.4 (7-76)	0.374
Gender			
Male	18 (64)	10 (45)	0.187
Relevant exposure history	22 (79)	6 (27)	<0.001
Duration of fever (days) [mean]	2-30 (7.2)	0-30 (6.4)	0.215
Symptoms			
Chills	19 (68)	12 (55)	0.336
Malaise	12 (43)	6 (27)	0.374
Headache	15 (54)	12 (55)	1.000
Myalgia	10 (36)	9 (41)	0.774
Arthralgia	3 (11)	1 (5)	0.425
Cough	15 (54)	11 (50)	1.000
Dyspnea	7 (25)	5 (23)	1.000
Nausea/vomiting	8 (29)	6 (27)	1.000
Abdominal discomfort	13 (46)	8 (36)	0.569
Relative bradycardia[a]	19 (68)	18 (86)	0.304
Shock	2 (7)	0 (0)	0.201
Hepatosplenomegaly	5 (18)	2 (9)	0.444
Lymphadenopathy	6 (21)	4 (18)	0.481
Skin rash	10 (36)	6 (27)	0.549
Eschar	12 (43)	0 (0)	<0.001

[a]Relative bradycardia was defined as pulse-temperature deficit [14].

Table 2. Laboratory data in rickettsiosis cases and controls

Variable	Cases (n = 28) No. (%)	Controls (n = 22) No. (%)	*p*
WBC count (/mm^3; mean) [range]	8100 (1900-17,800)	8663 (700-26,200)	0.506
Count <10 × 10^3/mm^3	20 (71)	15 (68)	0.804
Platelet count (× 10^3/mm^3; mean) [range]	146.1 (32-328)	194 (57-590)	0.171
Thrombocytopenia	16 (57)	11 (50)	0.615
Serum creatinine (mg/dL; mean) [range]	1.05 (0.6-2.0)	1.61 (0.5-7.4)	0.883
ALT (U/L; mean) [range]	152 (15-558)	104 (11-459)	0.019
Abnormal liver biochemistry	25 (89)	14 (64)	0.042
CRP (mg/L; mean) [range]	93.58 (2.28-279.5)	98.83 (0.55-354.6)	0.800
LDH (U/L; mean) [range]	223 (116-385)	182 (104-282)	0.439
Atypical lymphocytes in peripheral blood	21 (75)	7 (32)	<0.001
Abnormal chest film[a]	11 (39)	8 (36)	0.959
Pleural effusion	6 (21)	5 (23)	1.000

Abbreviations: WBC = white blood cell; ALT = alanine aminotransferase; CRP = C-reactive protein; LDH = lactate dehydrogenase
[a]Abnormal chest film was defined as any new pulmonary infiltrates or pleural effusions compared with plain chest X-ray.

these patients. There was no difference in the clinical presentation of cases with atypical lymphocytes. For the cases without atypical lymphocytes (n = 7, 25%), white blood cell counts were not followed beyond admission because of their mild illness. In contrast, only 7 of the 20 control patients who had hemogram data had atypical lymphocytes (32%, $p<0.001$).

Seasonal variation of incidence was not obvious, which may be due to the small number of cases in our series. However, we had more control patients in winter; 40.9% of the controls were from the winter season, compared to 21.4% of the confirmed cases ($p=0.136$) [Fig. 1].

Forty four patients received antirickettsial treatment with either tetracycline or a fluoroquinolone. Data for timing of therapy, response to therapy, and duration of hospital stay are shown in Table 3. The duration between admission and initiation of antirickettsial therapy was longer in the control cases, although the difference did not reach statistical significance. Thirty two percent (9/28) of the confirmed cases and 9% (2/22) of the control patients became afebrile without antirickettsial therapy ($p=0.083$). Hospital stay was significantly longer in the controls. Among patients who were still febrile when therapy was started, confirmed cases had quicker defervescence compared to controls (2.4 days vs 6.9 days, $p=0.057$). The duration of fever could be documented in only 17 of 19 cases who received treatment. Eighty eight percent of confirmed cases (15/17) became afebrile within 3 days after antirickettsial therapy was started. In contrast, only 45% of controls responded to antirickettsial therapy within 3 days ($p=0.014$). Finally, there was no mortality in either group of patients.

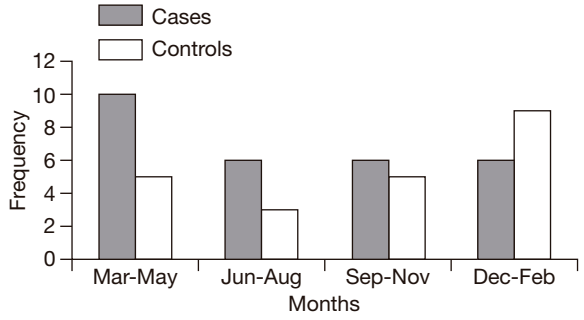

Fig. 1. Seasonal distribution of rickettsiosis cases and controls.

According to these results, we proposed a predictive scoring system for rickettsiosis (Table 4). For laboratory data collected on admission, liver biochemistry should include aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total bilirubin. Repeat of white blood cell differential count within 5 days is recommended if atypical lymphocytes are not seen on admission. A cumulative score of 6 or more has a positive predictive value of 80%, while a cumulative score of 5 or less has a negative predictive value of 100%. Eschar is not used as a factor in this scoring system, as it is virtually pathognomonic. This scoring system should be used with caution. Irrespective of the score, any symptoms or signs indicative of alternative diagnosis should prompt diagnostic and therapeutic efforts for other possible disease entities.

Discussion

Fever, relevant exposure history, eschar, skin rash, normal white blood cell count, thrombocytopenia,

Table 3. Treatment responses in rickettsiosis cases and controls

Variable	Cases (n = 28) No. (%)	Controls (n = 22) No. (%)	*p*
Time between admission and start of antirickettsial treatment (days) [range]	3.75 (1-10)	6.65 (1-27)	0.288
Spontaneous defervescence	9 (32)	2 (9)	0.083
Defervescence after treatment[a] (days) [range]	2.40 (1-8)	6.90 (1-46)	0.057
Defervescence within 72 h[a]	15/17 (88)	9/20 (45)	0.014
Hospital stay (days) [range]	10.10 (3-31)	19.60 (5-47)	0.013

[a]Treatment was initiated for 19 of 28 cases and 20 of 22 controls for whom fever did not subside spontaneously. The duration of fever could be documented in only 17 of 19 cases who received treatment.

abnormal liver biochemistry and lymphadenopathy have been reported as typical features of rickettsiosis [16-21]. Rickettsiosis is often considered as a mild illness. The occurrence of shock in 2 of the 28 confirmed cases reported here emphasizes the potential mortality of the disease. Rickettsioses are still not readily diagnosed in clinical practice because all of the symptoms and laboratory findings are nonspecific. Our study further confirmed this impression. The age and gender ratio in the 2 groups were similar to those reported in previous surveillance studies [4]. Confirmed cases and controls had similar duration of fever before admission, range of white blood cell and platelet counts, and prevalence of skin rash, constitutional symptoms, and major organ involvement. The proportion of cases with abnormal liver biochemistry was also similar to previous reports [22,23]. The perception that thrombocytopenia, a normal white blood cell count, and relative bradycardia are suggestive of rickettsiosis cannot be confirmed in our study. The reason for this discrepancy is probably that we were comparing verified and control cases, all of which had been likely to have rickettsiosis based on clinical assessment. Some of the control patients might have had rickettsial infection, and been excluded due to low sensitivity of the diagnostic tests.

The presence of atypical lymphocytes in peripheral blood has been reported in some case reports [24,25], and was also observed in this series. Atypical lymphocytosis is a feature of many viral infections and is often present with lymphoproliferative disorders, such as lymphoma. Activation of cytotoxic T lymphocytes and bacterial invasion into mononuclear cells are possible mechanisms for this abnormality in scrub typhus [26-29]. In addition, we have noted other similarities in clinical and laboratory profiles between rickettsial infection and diseases, such as lymphoproliferative disorders or certain viral infections (data not shown).

Seasonal variation of incidence in subtropical countries has been reported in the literature [2,4,5], but is not clear-cut in our study. However, these and our other findings must be considered in the context of

Table 4. Preliminary scoring system for rickettsiosis prediction

Variable	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)	Score
Possible relevant history					
High risk	100	58	43	100	4
Low risk	60	78	75	64	2
No risk	22	25	14	36	0
Abnormal liver biochemistry	63	78	93	32	4[b]
Atypical lymphocytes in peripheral blood by fifth day	78	70	75	73	4[c]
Time of defervescence					
Spontaneous	82	54	35	91	2
Within 72 h of treatment	63	85	88	55	2
Symptoms and signs indicating alternative diagnosis[a]	0	38	0	77	–6

Abbreviations: PPV = positive predictive value; NPV = negative predictive value

[a]For example, blood positive for parasite or bacterial culture, new-onset ascites, positive antinuclear antibodies, skin lesions suggesting alternative diagnosis or for which skin biopsy was indicated, and symptoms of chronic illness, such as profound body weight loss, etc.

[b]Data collected on admission, with cases of liver cirrhosis excluded.

[c]White blood cell differential count was repeated within 5 days if atypical lymphocytes were negative on admission.

the study having a small number of subjects, that were from northern Taiwan, which is not an endemic area for rickettsiosis and has a largely urban population.

The high negative predictive value of our scoring system may help clinicians exclude the diagnosis of rickettsiosis and prompt diagnostic efforts for other disease entities, such as malignant lymphoma. The following features suggest alternative diagnosis — blood positive for parasite or bacterial culture, new-onset ascites, positive antinuclear antibodies, particular skin lesions which necessitate skin biopsy or raise the suspicion of other diagnosis (such as malar rash or discoid lesions), hepatosplenomegaly with symptoms of chronicity (for example, body weight loss), or fever refractory to empiric antirickettsial therapy. Efforts for alternative diagnosis should not be delayed for patients with any of these features. The actual positive or negative predictive values of our prediction system will be validated with a prospective study in the future.

There are several limitations of our study. Firstly, each of the 3 rickettsioses has distinct clinical features. Eschar is a prominent attribute, especially for scrub typhus [30]. Rash is more common in murine typhus [31], and Q fever is associated with a higher incidence of chronicity and endocarditis and with slower defervescence [32-34]. Analysis of all 3 infections combined results in a loss of predictive specificity of data. Secondly, there are drawbacks to serologic diagnosis of rickettsioses [7-13]. For example, there have been reports of local variants in Taiwan, which may not be detected with standard antigens used in the immunofluorescence assay at the CDC, Taiwan [35,36]. Thirdly, most of our suspected cases were not tested for all 3 rickettsioses evaluated in the study, largely because of personal preferences of the in-charge physicians, which may have reduced the frequency of identification of confirmed cases.

More than half of our controls had a final diagnosis of suspected viral infection on discharge. Whether they indeed had acute viral infection was not investigated further. In some previous series, patients were studied as to whether they were still febrile after a period of observation (for example, 5 days), in order to exclude self-limiting viral infections [37,38]. We did not use this exclusion criterion, because many of our positive cases would have been excluded, thus reducing the sample size even further.

In the absence of a more powerful diagnostic tool, a simple, straightforward prediction system is desirable for clinical practice. Because our patients in the control group did not include diseases such as leptospirosis, typhoid fever, and malaria, it may not be used for differential diagnosis of these serious disease entities with similar presentation. We would recommend this scoring system for cases of clinically suspected rickettsiosis only. The scoring system is probably of less value in endemic areas, where epidemiological features are much more relevant for clinical diagnosis than any other clinical or laboratory clues. Prospective, multicenter studies in different areas are required for validation of the scoring system.

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