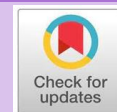


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Correlation between Probable or Non-Probable Leptospirosis with Laboratory Findings: Based on Leptospirosis Case Definition and Faine Criteria

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Abstract

The incidence of leptospirosis is increasing globally, and developing countries are no exception. Leptospirosis cases are called the tip of the iceberg phenomenon, even though misdiagnosis, underdiagnosis, and underreporting still occur in health services. Thus, it leads to delays in leptospirosis treatment and may result in increased mortality rate from severe leptospirosis infection (Weil's disease). This study was to establish an accurate diagnosis by optimizing the Faine criteria. This study used an analytical observational design with a cross-sectional approach to examine faine criteria and laboratory examinations. We collected data from medical records from the Karanganyar General Hospital and the PKU Muhammadiyah Surakarta Hospital. We processed the data using SPSS version 25. The total number of samples was 42. They were divided into women (19%) and men (81%). Based on the definition category of leptospirosis cases, there were 2.4% probable group (score criteria faine part A 20-25) and 97.6% not-probable group (score criteria faine part A <20). Bivariate analysis (Chi-Square test) showed that there was no significant correlation between Faine Part A criteria and serological tests in both groups ($p=0.874$) as well as Hb ($p=0.522$), thrombocytopenia ($p=0.265$), leukocytosis ($p=0.197$), and neutrophilia ($p=0.710$). Loss of sodium and potassium didn't show significant data (hyponatremia $p=0.174$; hypokalemia $p=0.311$; hypocalcemia $p=0.131$) not as in tropical diseases. The approach to diagnosis of leptospirosis cannot be performed using only Part A criteria, Faine, even though the patient was included in the probable definition category, even though the Faine Part A criteria score is 20-25 or ≥ 26 .

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INTRODUCTION

Leptospirosis is an acute infectious disease of humans and animals (zoonosis) caused by the microorganism *Leptospira* spp.¹ *Leptospira* bacteria are most commonly transmitted to humans through contact with infected animals. This occurs directly, either through direct animal-to-human contact or indirectly through contact with the animal's urine, soil, or water.^{2,3}

The World Health Organization (WHO) estimates that approximately 873,000 cases of leptospirosis emerge annually, resulting in over 40,000 deaths. In the United States, the incidence of leptospirosis ranges from 100 to 200 cases per year. In comparison, the incidence of leptospirosis in tropical climates is nearly ten times that of moderate climates.² The Indonesian Ministry of Health reports that 920 cases of leptospirosis have been diagnosed, with 112 resulting in death. These cases have been documented in nine provinces: Banten, Jakarta, West Java, Central Java, Yogyakarta, Maluku, South Sulawesi, North Kalimantan, and the rest of Indonesia. These numbers are relatively low in comparison to the annual morbidity rate of leptospirosis in Indonesia, which has been estimated at 39.2 per 100,000 people.⁴ However, the case fatality rate during 2018 was 17.8% among an estimated 895 human cases.⁵

The diagnosis of leptospirosis is typically based on clinical features and a history of risk exposure.⁶ Conventional laboratory methods for the diagnosis of leptospirosis rely on the examination of patient immune response parameters. The Dark-Ground Microscope (DGM) is the optimal approach for the visualisation of *Leptospira* organisms on culture media. However, this examination has intrinsic limitations as a diagnostic tool, including the potential for false-negative results due

to low concentrations of the organisms in the specimen and false-positive results due to artifacts and the presence of fibrin.¹ Serological analysis employing the microscopic agglutination test (MAT) has been demonstrated to have greater sensitivity in detecting cases of leptospirosis. Due to the inherent complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infections have been developed to facilitate prompt diagnostic confirmation and initiate effective treatment. IgM antibodies, which are produced in the initial phase (after 4-7 days) of the infection, can be identified within the first week of illness, thus providing the opportunity to diagnose the disease and initiate appropriate therapy.^{1,7,8}

Three criteria have been established for defining cases of leptospirosis. These are as follows: 1) Suspect cases, 2) Probable cases, and 3) Confirmed cases (Table 1). Furthermore, the World Health Organization (WHO) introduced the Faine criteria for diagnosing leptospirosis cases, which are divided into three parts: 1) Part A (based on clinical history), 2) Part B (based on epidemiologic history), and 3) Part C (laboratory parameters as support for Part B) (Table 2).⁹ However, in Indonesia, there has been an increase in leptospirosis cases, a phenomenon that is referred to as "the tip of the iceberg" despite misdiagnosis, underdiagnosis, and underreporting in health services.¹⁰

Therefore, this study aims to determine the correlation between probable group (score criteria faine part A 20-25) or not-probable group (score criteria faine part A <20) and laboratory findings to facilitate the accurate diagnosis of leptospirosis using the Faine criteria. This will enable earlier diagnosis in primary care facilities, thus preventing the worsening or death of leptospirosis cases.

Table 1. The following three definitions of leptospirosis cases are according to the Book of Internal Medicine, UI Volume I.

Definition	Criteria
1. Suspect Case	• Acute fever with or without headache; • Myalgia; • Weakness (malaise); • Conjunctival hiperemis; • Ciliary suffusion; • Additionally, the individual has a documented history of exposure to a potentially contaminated environment within the last two weeks.
2. Probable Case (Two of the following clinical signs and symptoms were identified)	• Gastrocnemius muscle pain; • jaundice (skin and sclera); • Bleeding manifestation; • Dyspneu; • Oliguria or anuria; • Cardiac arrhythmia; • Cough with or without blood (haemoptysis); • Skin rash. In addition, have laboratory results: • Thrombocytopenia < 100.00 cell/mm • Leucocytosis with neutrophilia >80% • An increase in total bilirubin levels by > 2% or an increase in the levels of other enzymes, including aspartate aminotransferase (AST), amylase, lipase, and creatinine phosphokinase (CPK). • Rapid diagnostic tests (RDTs) are utilized to ascertain the presence of anti-leptospiral IgM antibodies.
3. Confirmed case (Probable cases are accompanied by one of the outcomes below)	• Isolation of <i>Leptospira</i> bacteria from clinical specimens; • The results of the Polymerase Chain Reaction (PCR) are positive; • A shift from a negative to a positive result on the Microscopic Agglutination

Test (MAT) indicates seroconversion.

MATERIALS AND METHODS

METHODS

Study Designs and Patients

This study utilized an analytic observational study design with a cross-sectional approach. Data were collected from two hospitals, namely PKU (*Pembina Kesejahteraan Umat*) Muhammadiyah Surakarta Hospital and Karanganyar Regency General Hospital. A non-probability purposive sampling technique was employed to select participants. Data were gathered from.

June 2022 to June 2023. Patient data were collected for individuals ≥18 years of age, hospitalized patients, and those with laboratory results. Patients with a history that have similar and potentially overlapping symptoms or laboratory results, such as uncontrolled diabetes mellitus, uncontrolled hypertension, heart disease, neuroinfectious diseases, and autoimmune diseases, were excluded from the study.^{11–15} Additionally, patients with positive laboratory results on dengue fever or typhoid fever serology tests were excluded because these infectious diseases have the same laboratory changes as leptospirosis to avoid overlap and to avoid bias in the category of leptospirosis case definitions.^{16–18} Furthermore, the results of the patient’s history, such as duration of fever, history of muscle pain, and history of activity in contaminated environments, and physical examination upon admission to the emergency department such as conjunctival suffusion, icteric sclera, epigastric pain, hepatosplenomegaly, gastrocnemius muscle pain, and jaundice were utilized in classifying the patient’s Faine criteria.

Patients were categorized as probable or not probable definition. To obtain the data group of confirmed leptospirosis patients, it was ensured that probable group (sample with probable definition category but the Faine Criteria (Part A) score between 20-25) or not probable group (Sample with probable definition category but the Faine Criteria (Part A) score < 20) had confirmed positive results on the IgM/Rapid leptospirosis antibody test. Furthermore, we gathered additional laboratory data, including hemoglobin, platelets, leukocytes, neutrophils, sodium, potassium, and calcium, to contrast the outcomes of probable group (sample with probable definition category but the Faine Criteria (Part A) score between 20-25) or not probable group (Sample with probable definition category but the Faine Criteria (Part A) score < 20) with confirmed positive IgM/rapid results with those of patients with confirmed negative IgM/rapid leptospirosis results.

The Table 1 was used to define which patients are probable cases based on the criteria of each case status. All samples used were probable cases according to the definition of the table, with at least 2 signs and symptoms, and or with IgM/Rapid test results.

Meanwhile, the World Health Organization has established criteria for the diagnosis of leptospirosis, known as the Faine criteria. These criteria are divided into three parts.

In accordance with the Faine criteria Table 2, the score of each sample will be obtained with the score category of Part A, or the score of Part A and Part B was 26 or more. Furthermore, the total score of Parts A, B, and C was 25 or more, and the score of 20-25 was Possible Leptospirosis, so a presumptive diagnosis of leptospirosis can be made.

Table 2. The World Health Organisation's Faine criteria are divided into three parts.

Faine Criteria	Score
1. Part A: Based on Clinical data	
• Headache	2
• Fever	2
• If fever $\geq 39^{\circ}\text{C}$	2
• Conjunctival suffusion	4
• Meningism	4
• Myalgia (especially gastrocnemius muscles)	4
• Conjunctival suffusion + meningism + Myalgia	10
• Jaundice	1
• Albuminuria/Nitrogen retention	2
• Haemoptysis/dyspnea	2
2. Part B: Based on Epidemiological history	
• Rainfall	5
• Contact with contaminated environment	4
• Animal Contact	1
3. Part C: Bacteriological and Laboratory Findings	
• Isolation of leptospira in culture – Diagnosis certain	
- PCR ^a	25
• Positive serology	
- ELISA ^b IgM positive	15
- SAT ^c positive	15
- Other rapid test	15
- MAT ^d – single positive in high titer	15
- MAT – rising titer/seroconversion	25

Presumptive diagnosis of leptospirosis is made of:

- Part A or Part A and Part B score: 26 or more
- Total score of Parts A, B, and C: 25 or more
- Possible Leptospirosis if the score is between 20-25

Statistical Analysis

The characteristics of the study and the laboratory results were described using the format “number (n) and percentage (%)”. The data were analyzed univariately to determine associations between the variables. The results were then analyzed bivariately with the chi-square test to assess the correlation between probable/not probable leptospirosis patients with positive

IgM/Rapid test results and laboratory variables, namely hemoglobin, platelets, leukocytes, neutrophils, sodium, potassium, and calcium, using SPSS version 25.

RESULTS AND DISCUSSION

Table 3. Characteristics of the study

Characteristic	Number (n)	Percentage (%)
Gender		
Female	8	19%
Male	34	81%
Serologic Test		
IgM (+)	41	97.6%
Rapid test (+)	1	2.4%
Faine score (Part A)		
Probable (Score 20-25) ^a	1	2.%
Not Probable (Score <20) ^b	41	97.%
Hemoglobin		
Anemic	12	28.%
not anemic	30	71.4%
Thrombocytes		
Thrombocytopenia	23	54.8%
Not thrombocytopenia	19	45.2%
Leukocytes		
Leukocytosis	16	38.1%
Not leukocytosis	26	61.9%
Neutrophil		
Neutrophilia	37	88.1%
Not neutrophilia	5	11.9%
Sodium (Na⁺)		
Hyponatremia	15	35.7%
Not hyponatremia	27	64.3%
Potassium (K⁺)		
Hypokalemia	21	50%
Not hypokalemia	21	50%
Calcium (Ca²⁺)		
Hypocalcemia	13	31%
Not hypocalcemia	29	69%

^aProbable Sample with probable definition category but the Faine Criteria (Part A) score between 20-25;
^bNot Probable= Sample with probable definition category but the Faine Criteria (Part A) score < 20.

We obtained a total of 42 samples that met the restriction criteria for the period June 2022 to June 2023 at PKU Muhammadiyah Surakarta Hospital and Karanganyar Regency General Hospital. The 42 samples consisted of women (n=8; 19%) and men (n=34; 81%) (Table 3). Based on the Faine criteria (part A), we

found that only one sample whose probable definition category was probable leptospirosis (n=1; 2.4%) and 41 samples whose probable definition category was not probable leptospirosis (n=41; 97.6%). However, all samples we obtained had positive serology test results, both IgM (n=41; 97.6%) and Rapid test (n=1; 2.4%).

Laboratory results indicated the presence of anemia were 12 of 42 samples or 28.6% (n=12; 28.6%), thrombocytopenia were 23 of 42 samples or 54% (n=23; 54%), leukocytosis were 16 of 42 samples or 38.1% (n=16; 38.1%), and neutrophilia were 37 of 42 samples or 88.1% (n=37; 88.1%). The remaining abnormalities observed were as follows: Hyponatremia was 15 of 42 samples or 35.7% (n=15; 35.7%), hypokalemia was 21 of 42 samples or 50% (n=21; 50%), and hypocalcemia was 13 of 42 samples or 31% (n=13; 31%).

The results of bivariate analysis of the relationship between the results of serology examination and probable definition category in Table 4 indicated that the majority of leptospirosis samples with positive IgM test results were included in cases that were not probable leptospirosis by Faine Criteria (part A). Specifically, 40 samples (95.2%) exhibited this pattern. One sample (2.4%) with probable definition category exhibited a positive IgM result, while one additional patient (2.4%) had a positive leptospirosis rapid test result in the not probable definition category. The results of the serological examination analysis yielded a probability (*p*) value of 0.874 (*p* > 0.05), indicating that there is no significant relationship between the results of the serological examination and probable definition category (Table 4).

Table 4. Bivariate Analysis of Leptospirosis Serology Test Results for Probable Leptospirosis Cases.

Leptospirosis Cases:					
Variable	Faine Criteria (Part A)				P value*
	Not Probable		Probable ^a		
	n	%	N	%	
Serology test					
IgM (+)	40	95.2	1	2.4	0.874
Rapid test (+)	1	2.4	0	0	

^aProbable= Sample with probable definition category but the Faine Criteria (Part A) score between 20-25;

^bNot Probable= Sample with probable definition category but the Faine Criteria (Part A) score < 20;

*P-value: Bivariate analysis by chi-square test.

Furthermore, the results of bivariate analysis in Table 5 showed that more samples in the not probable definition category had thrombocytopenia (n=23; 54.8%; $p=0.265$) and neutrophilia (n=36; 85.7%; $p=0.710$). Meanwhile, the probable definition category had leukocytosis (n=1; 2.4%; $p=0.197$) and neutrophilia (n=1; 2.4%; $p=0.710$). This analysis showed that leptospirosis samples with no probable definition category can have thrombocytopenia and neutrophilia (Table 5).

Table 5. Bivariate Analysis of Routine Blood Testing Results for Probable Leptospirosis Cases.

Cases.					
Variable	Faine Criteria (Part A)				p value*
	Not Probable ^b		Probable ^a		
	n	%	N	%	
Hemoglobin					
Anemic	12	28.6	0	0	0.522
Not anemic	29	69	1	2.4	
Thrombocytes					
Thrombocyto penia	23	54.8	0	0	0.265
Not Thrombocyto penia	18	42.8	1	2.4	
Leukocytes					
Leukocytosis	15	35.7	1	2.4	0.197
Not leukocytosis	26	61.9	0	0	

Leptospira spp. Bacteria, which are the primary vectors for the bacteria.¹⁹ In Indonesia, the increase in leptospirosis cases is referred to as the "tip of the iceberg phenomenon," despite the continued occurrence of misdiagnosis, underdiagnosis, and under-reporting in health services.¹⁰

A review of score part A faine criteria based on clinical data revealed that the characteristics of samples with leptospirosis showed a higher prevalence of not-probable definition category than probable definition category (Table.3). This discrepancy can be attributed to the incubation period of leptospirosis, which can range from 5 to 14 days or occur between days 2 and 21 after exposure to water or soil. In the leptospirosis phase, symptoms may manifest as a non-specific acute febrile illness or with symptoms such as fever, chills, myalgia, headache, ocular discomfort, nausea, and vomiting.^{6,22} Additionally, red eyes and watery eyes may occur. Without serological confirmation, it is challenging to diagnose leptospirosis in this phase in primary healthcare.^{6,7}

The diagnosis of leptospirosis is based on identifying the bacterium or its metabolic products in bodily fluids or tissues via serological testing. Serological testing is divided into genus-specific and serogroup-specific.²³ For a leptospirosis antibody test to be valid, antibodies must be present in the body between the third and tenth day following the onset of symptoms. This may result in a negative serology test result in samples collected during the first week of illness. These results should not be interpreted as evidence of the absence of infection and should be retested 7-14 days after the initial examination.^{8,19}

Bivariate analysis in Table 4 to determine if there was a significant association between the Faine criteria score A and serology tests. The analysis revealed that there was no statistically significant association between the not-probable and probable definition categories that had positive serology test results ($p=0.874$). Antibodies are usually detected between the 6th and 10th day of illness, with a peak within 3-4 weeks.⁶ Consequently, it is recommended that the serum test be repeated on two separate occasions, with a minimum interval of one to two weeks between each test. This approach was based on the understanding that seroconversion, or the development of antibodies in response to an infection, occurs during the disease.¹⁰ A negative serologic test in the early phase of the disease does not mean that the patient is not infected with leptospirosis cause the early phase of the disease may enable seroconversion to be avoided due to its status as an incubation period, it is important to conduct a re-serum examination to ascertain the increase in titer between two samples (seroconversion) and confirm the diagnosis of leptospirosis. The administration of serum should be performed on two occasions, with a minimum interval of one to two weeks between each administration. This is typically based on the date of onset and the estimated time of seroconversion.¹⁰

The gold standard test for leptospirosis is the microscopic agglutination test (MAT). The MAT test is preferred over other screening options to avoid misdiagnosis of other tropical infections, including some difficult differential diagnoses due to overlapping clinical presentations such as dengue fever and dengue hemorrhagic fever, influenza, malaria, enteric fever, toxoplasmosis, hepatitis, and others.^{9,19} Although the MAT

test is the gold standard for leptospirosis, and can differentiate from other differential diagnoses. A study in Thailand showed that the MAT test was not perfect, but quantitative polymerase chain reaction (qPCR) can improve the sensitivity of leptospirosis diagnosis.²⁴ Furthermore, the MAT test is not available in primary care settings, so Faine criteria part A screening is an option in diagnosis.

The World Health Organization (WHO) has established criteria for leptospirosis screening, the Faine Criteria, consisting of Part A (clinical data; score A), Part B (epidemiologic data; score B), and Part C (serology or laboratory data; score C).⁹ In Table 5, 41 samples were categorized as not-probable definition category based on clinical data, and one sample was classified as probable definition category. However, both groups had positive IgM or rapid test serologic results for leptospirosis. The results of the bivariate analysis in Table 4 regarding the results of the serological examination of probable definition category showed that there was no significant results ($p=0.874$), so this can explain that the leptospirosis diagnostic approach with Faine criteria based on Part A (score 20-25) does not have a significant correlation and still requires evaluation with Part B, especially Part C (serology/laboratory data). However, not all primary health care services have facilities for laboratory examination of both leptospirosis IgM serology and MAT tests, so the Faine criteria part A alone is not recommended when used for the Leptospirosis diagnostic approach in primary health care.

Nonspecific laboratory examinations in leptospirosis can be performed, such as blood analysis, urine analysis, and cerebrospinal fluid.^{25,26} Blood analysis showed leukocytosis shifted to the left and

thrombocytopenia. However, this examination cannot be used in the diagnosis of leptospirosis, but rather for differential diagnosis screening and initial observation, and thrombocytopenia is an important laboratory parameter in the context of leptospirosis. It can be used as an early recognition *a priori* to prevent complications and mortality.²⁷ Furthermore, studies have indicated that platelet counts of less than 46,000 cells/mm³ are correlated with the occurrence of sepsis, which was a severe form of leptospirosis.²⁸ As in the results of bivariate analysis, Table 5. The results of hemoglobin, platelets, leukocytes, and neutrophils in both groups showed non-significant results (hemoglobin $p=0.522$; thrombocytes $p=0.265$; leukocytes $p=0.197$; neutrophils $p=0.710$). Based on the examination results of the two groups, it can be concluded that leptospirosis patients do not always meet the probable febrile criteria and can be a differential diagnosis when thrombocytopenia and neutrophilia are found. If the health facility already has serologic testing as a specific test, the diagnosis of leptospirosis can be confirmed. However, non-specific tests are also important in patients suspected of having leptospirosis, with either a part A+B score (Faine criteria) of probable or non-probable definition category. It is because many patients with leptospirosis present with leukocytosis and thrombocytopenia, which usually do not result in spontaneous bleeding, but may result in gastrointestinal bleeding (melena or hematemesis) or pulmonary bleeding that is difficult to detect.²⁹ In addition, nonspecific testing can be useful when patients with severe leptospirosis (Weil's Disease) have developed multiorgan liver, kidney, lung, and brain.^{29,30}

Hemodynamic changes with decreased systemic vascular resistance, increased cardiac output, and increased

renal vascular resistance occur in tropical diseases. Hyponatremia can occur in tropical diseases due to increased levels of antidiuretic hormone (vasopressin), which causes sodium entry into cells, sodium loss, and osmoreceptor resetting.³¹ Electrolyte disturbances can occur with renal involvement ranging from mild nonoliguric renal dysfunction to complete renal failure. It is associated with decreased expression of sodium-hydrogen exchanger-3, which leads to decreased reabsorption of sodium and fluid in the proximal tubule.²⁹ The evaluation of glycolipoprotein interaction with Na/K-ATPase demonstrated that the indication of natural resistance in leptospirosis is not due to the lack of sensitivity of Na/K-ATPase of renal cells, but rather due to the bioavailability of endotoxins in bacterially infected tissues.³² Consequently, serum sodium and potassium levels are lower among patients with severe leptospirosis than among those with moderate leptospirosis or acute tubular necrosis.³³ Table 6 showed that electrolyte imbalance was not significant for sodium, potassium, or calcium (Na^+ $p=0.174$; K^+ $p=0.311$; Ca^{2+} $p=0.131$). Observations in patients with leptospirosis regarding the loss of sodium and potassium in large amounts occur when the patients are suffering from diarrhea.³¹ In Table 6, not all samples were in diarrhea symptoms or diarrhea that had improved with symptomatic treatment that had been given previously due to the delay in diagnosis, thus electrolyte imbalance cannot be used as the principal examination in the diagnostic approach to leptospirosis. However, it is a useful tool for evaluating the impact of the disease and monitoring patient response to treatment.

STRENGTH AND LIMITATION

The findings of this study have the

potential to enhance the attention and diagnosis of leptospirosis cases, which are becoming increasingly prevalent in Indonesia. The results of this study can serve as a reference for primary care physicians to consider the appropriate criteria and improve the quality of support examinations in primary care. A limitation of this study was the restricted sampling frame, which included only two hospitals. Consequently, the number of samples obtained was relatively small. Additionally, the classification of the fainéant criteria for non-probable and probable leptospirosis groups was based solely on medical records, rather than direct interviews. It is recommended that future researchers conduct direct interviews to enhance the accuracy of group classification.

CONCLUSIONS

The approach to diagnosis of leptospirosis cannot be performed using only Faine Part A criteria, even though the patient is included in the probable definition category, even though the Faine Part A criteria score is 20-25 or ≥ 26 . Because the results of this study showed that the Faine criteria part A score < 20 showed a positive serological test result, it is not sufficient to diagnose leptospirosis only with Faine criteria part A, but at least use part B or part B and part C in the diagnosis of Leptospirosis. Moreover, there is considerable potential for misdiagnosis due to the possibility of overlap with other tropical diseases, resulting in delays or disruption to patient care. In such cases, primary health services can promptly provide appropriate treatment or first-line treatment, if the patient's Faine criteria part A score is within the range of 21–25. This approach is preferable to the alternative of

not treating the patient despite the lack of a confirmed leptospirosis diagnosis.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed to the study by collecting data, analyzing results, and discussing the study.

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