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Original Article

Clinical features and dynamic ordinary laboratory tests differentiating dengue fever from other febrile illnesses in children

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Abstract *Background:* Dengue fever is not easily to be diagnosed before presentation of the classic symptoms. The study aimed to investigate the clinical features and dynamic laboratory tests in pediatric patients to facilitate dengue diagnosis.
Methods: This retrospective study examined the medical records of all pediatric patients who were clinically suspected to have dengue from June to December 2014. Laboratory-positive dengue cases were confirmed by detecting non-structural protein NS1, reverse transcription-polymerase chain reaction of dengue virus, and dengue-specific IgM seroconversion.
Results: Of the 317 pediatric cases clinically suspected of dengue, 205 were laboratory-positive and 112 were laboratory-negative. In laboratory-positive cases, the most common clinical manifestation was skin rash in 156 (76.1%). Leukopenia occurred on days 1—5; thrombocytopenia, on days 2—7; prolonged activated partial thromboplastin time (aPTT), on days 1—4; and elevated transaminase levels, on days 3—11; and low CRP, on days 0—14. The specificity and positive predictive value (PPV) of combining of rash, itching and petechiae increased up to 100%. The PPV of combining of leukopenia, thrombocytopenia, and elevated transaminase levels reached 100% on day 2 as well as days 6—8.
Conclusion: Leukopenia, thrombocytopenia, elevated aPTT, elevated transaminase levels, and low CRP could be used to differentiate dengue fever from other febrile illnesses. During

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dengue epidemics, combinations of the symptoms and laboratory findings are helpful to physicians for accurate diagnosis of dengue fever.

Introduction

Dengue fever is the most prevalent systemic viral infection transmitted by mosquitoes.[1,2] It can be caused by any one of the five different serotypes of the dengue virus.[3] It is endemic to more than 100 countries in the tropical and subtropical regions of the world, especially tropical Asia, Central and South America, and the Caribbean. The incidence of dengue fever has been estimated to be over 390 million infections per year, of which 96 million are symptomatic.[4] The annual number of dengue fever cases reported to the World Health Organization have continued to increase in the past 50 years,[1] with a geographic expansion to new countries. The rapidly expanding global footprint of dengue is a public health challenge with an economic burden.

The clinical manifestations of dengue infection range from asymptomatic to a life-threatening shock or hemorrhage.[5,6] Both viral and host factors are thought to be responsible for the manifestations of dengue in infected patients. The typical clinical manifestations of acute febrile phase of dengue usually last for 2—7 days and are often accompanied by headache, retro-orbital pain, bone pain, myalgia, arthralgia, petechiae, and rash.[5] Other associated symptoms such as abdominal pain, nausea or vomiting, and diarrhea were also noted. Most adults with dengue infection are symptomatic.[7] In contrast, most pediatric patients are asymptomatic or minimally symptomatic.[8,9] Since dengue is not easily to be diagnosed before presentation of its classic features,[10] it is important to consider dengue in persons with related laboratory manifestations and travel history or living in endemic areas. Additionally, dengue fever is a dynamic disease.[1] Being aware of the dynamic features of dengue may facilitate the clinicians to make diagnosis and management.

An epidemic of dengue fever plagued the southern Taiwanese city of Kaohsiung in 2014. Some pediatric patients presented with fever and other unspecific manifestations, which made the diagnosis difficult. The dengue rapid test had not yet been available in 2014. The aim of this study was to investigate the clinical features and dynamic performance of laboratory tests among pediatric patients to help clinicians differentiate dengue fever from other febrile illnesses.

Methods

Patients

We retrospectively reviewed all clinically suspected cases of dengue in pediatric patients aged below 18 years at the Kaohsiung Chang Gung Memorial Hospital from June to December 2014. The clinical features were collected from the medical records. The laboratory tests of blood including white blood cell (WBC) count, hematocrit (Hct) percentage, platelet (PLT) count, prothrombin time (PT), activated partial thromboplastin time (aPTT), transaminase levels, and C-reactive protein (CRP) were analyzed chronologically from the onset of symptoms. Blood samples were serially collected during the course of illnesses. The first febrile day was designated day 0. Cut-off values for laboratory tests were defined as follows: leukopenia, WBC count $<4000/mm^3$; thrombocytopenia, platelet count $<150{,}000/mm^3$; prolonged aPTT, >38 s; elevated serum transaminase levels, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels $>$ 80 U/L (two times the upper limit of normal); and low CRP, $<$ 20 mg/L. Clinically suspected dengue was defined as the presence of documented fever of ≥ 38 °C and two or more symptoms or signs of anorexia, nausea, vomiting, rash, headache, bone pain, myalgia, hemorrhagic manifestations, leukopenia, or thrombocytopenia or for clinically suspected dengue by a physician.[11] Laboratory-positive dengue cases were defined as positive laboratory results confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) of dengue virus, dengue-specific IgM seroconversion, and nonstructural protein NS1. Severe dengue is defined according to 2009 World Health Organization (WHO) classification as evidence of severe plasma leakage with shock and/or fluid accumulation with respiratory distress, severe bleeding, or severe organ impairment.[1] Additional tests included virus isolation from oropharynx, influenza rapid test from oropharynx, respiratory syncytial virus (RSV) Ag test from oropharynx, adenovirus Ag test from nasopharyngeal aspirate, and rotavirus Ag test from stool. Rapid influenza diagnostic tests (Formosa One Sure Flu A/B Rapid test kit, Formosa biomedical, Taiwan) were used to detect influenza virus infection. This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (103-5935B).

Dengue diagnosis

Laboratory-positive dengue cases were defined as: (1) positive test of real-time one-step RT-PCR; (2) positive seroconversion of dengue-specific IgM or ≧four-fold increase in dengue-specific IgG in appropriately timed paired serum samples where cross-reaction to Japanese encephalitis virus (JEV) had been excluded; (3) high-titer (optical density (OD) 405 nm value >2.0) dengue-specific IgM and IgG in a single serum specimen where cross-reaction to JEV had been excluded; and (4) positive test of dengue NS1-specific IgG antibodies. A one-step SYBR Green I real-time RT-PCR (QuantiTect SYBR Green RT-PCR kit; Qiagen, Hilden,

Germany) was performed in the Mx4000™ quantitative PCR system (Stratagene, La Jolla, CA) to detect dengue virus in acute-phase serum samples (Days 1–7) as described previously.[12,13] For all of the serum samples, serological diagnosis of specific antibodies based on envelope and membrane (E/M)-specific capture IgM and IgG enzyme-linked immunosorbent assay (ELISA) was performed as described previously.[12,13] A nonstructural protein NS1 serotype-specific indirect IgG ELISA was used to analyze dengue NS1-specific IgG antibodies (Centers for Disease Control (CDC), Taiwan).

Statistical analysis

We used IBM SPSS version 19 (SPSS Inc., Chicago, IL, USA) for statistics and GraphPad Prism (version 7 for Windows, GraphPad Software, La Jolla California USA) for figures plotting. Statistical significance of differences in categorical variables, such as gender, hospitalization, clinical manifestations, was determined by the chi-square test. Statistical significance of differences in continuous variables, such as age, length of hospital stay, values of laboratory tests at days from onset, was determined by the Mann–Whitney *U*-test. *P* value < 0.05 was considered statistically significant.

Results

A total of 317 pediatric patients were clinically suspected of dengue, and the Taiwan Center of Disease Control (CDC) was notified of the epidemic (Table 1). Of these, 205 were laboratory-positive dengue cases. Dengue virus type 1 was the most common serotype in this epidemic.[14] The other 112 laboratory-negative cases were negative for PCR, NS1, or dengue-specific IgM seroconversion. Several viruses were identified in the laboratory-negative cases including influenza virus, parainfluenza virus, respiratory syncytial virus, adenovirus, enterovirus, and rotavirus. No concurrent viral or bacterial co-infection was identified. The population of male patients was more than that of female patients in both groups (121 males (59.0%) in laboratory-positive group and 61 males (54.5%) in laboratory-negative group). The median ages of laboratory-positive and laboratory-negative patients were 10 and 9 years respectively ($P = 0.023$). Nearly 60% of all patients were hospitalized, and the median length of hospital stay was 4.5 days in laboratory-positive cases and 4 days in laboratory-negative cases.

Table 1 Demographic and clinical manifestations of suspected dengue patients.

	Lab-positive n = 205	Lab-negative n = 112	*P*-value
Gender (male:female)	121:84	61:51	0.433
Age[a] (years)	10 (1–18)	9 (1–18)	0.023
Hospitalization (%)	55.6%	58.9%	0.569
Length of hospital stay (days)[a]	4.5 (1–10)	4 (2–28)	0.389
Clinical manifestations			
Headache	66 (32.2%)	37 (33.0%)	0.879
Retro-orbital pain	13 (6.3%)	15 (13.4%)	0.034
Bone pain	11 (5.4%)	6 (5.4%)	0.997
Myalgia	66 (32.2%)	46 (41.1%)	0.114
Abdominal pain	50 (24.4%)	20 (17.9%)	0.180
Nausea or vomiting	67 (32.7%)	33 (29.5%)	0.556
Diarrhea	27 (13.2%)	22 (19.6%)	0.128
Cough	54 (26.3%)	60 (53.6%)	<0.001
Sore throat	19 (9.3%)	21 (18.8%)	0.015
Rhinorrhea	43 (21.0%)	42 (37.5%)	0.002
Rash	156 (76.1%)	50 (44.6%)	<0.001
Itching	59 (28.8%)	18 (16.1%)	0.012
Petechiae	30 (14.6%)	7 (6.3%)	0.026
Duration of fever[a]	5 (2–7)	4 (1–15)	0.007

[a] Median (range).
Lab-positive, laboratory-positive cases; Lab-negative, laboratory-negative cases.

The most common clinical manifestation of laboratory-positive dengue cases was skin rash in 156 patients (76.1%) (Table 1). Both headache and myalgia were present in 66 patients (32.2%), and nausea or vomiting occurred in 67 patients (32.7%). Totally, 76 patients (37.0%) had respiratory tract symptoms such as cough, sore throat, or rhinorrhea. There were statistically significant differences in the occurrences of retro-orbital pain, rash, itching, petechiae, cough, sore throat, and rhinorrhea between laboratory-positive and laboratory-negative cases. The median duration of fever was 5 days in laboratory-positive dengue cases and 4 days in laboratory-negative cases ($P = 0.007$). Most cases were uncomplicated, and only 3 patients in the laboratory-positive group developed severe dengue, of which 2 patients developed dengue shock syndrome and 1 patient had severe organ involvement (AST level > 1000 U/L). None of the patients expired, and there were no sequelae after disease resolution. In addition, no concomitant bacteremia was found in any of the patients.

In laboratory-positive dengue patients, leukopenia occurred on days 1–5 (Fig. 1). The nadir of mean WBC count was $2500 \pm 1000/mm^3$ on day 3. The dynamic change of hematocrit percentage was trivial: The mean hematocrit percentage reached its peak at $40.2 \pm 3.1\%$ on day 5. Thrombocytopenia occurred on days 2–7 (Fig. 2). The nadir of mean platelet count was $89{,}300 \pm 52{,}300/mm^3$ on day 5. Thrombocytopenia was observed up to 93% on day 5.

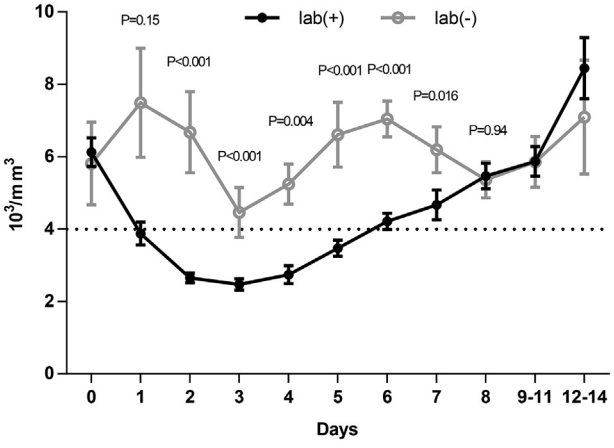

Figure 1. Dynamic mean white blood cell count.

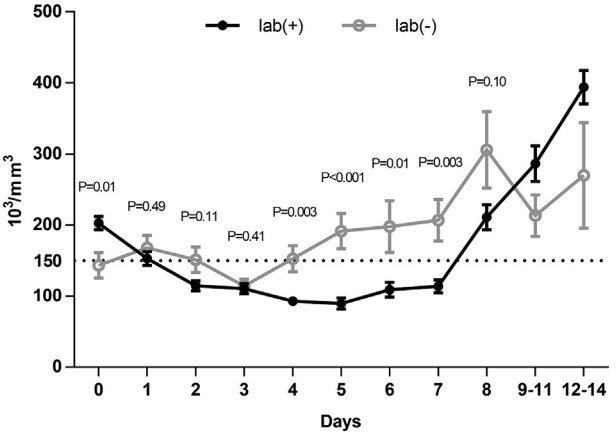

Figure 2. Dynamic mean platelet count.

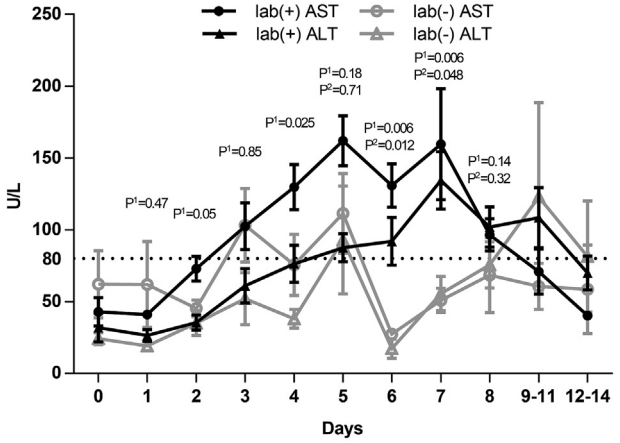

Figure 4. Dynamic mean transaminase levels.

Prolonged aPTT was noted on days 1—4 (Fig. 3). The mean aPTT peaked to 39.8 ± 5.8 s on day 2. Elevated serum transaminase levels (AST or ALT > 80 U/L) presented on days 3—11. The AST levels increased gradually initially, and the mean AST levels peaked to 162.1 ± 100.3 U/L on day 5 (Fig. 4). The ALT levels increased following the AST level, and the mean ALT levels peaked to 134.5 ± 98.4 U/L on day 7. On days 1—5, the mean AST levels were 1.5 times the mean ALT levels. Furthermore, the mean CRP were mildly elevated to 19.0 ± 26.0 mg/L on day 1 and maintained at a low level (<7 mg/L) during the disease course. No significant difference in CRP was noted between severe dengue and non-severe dengue cases ($P > 0.1$).

The difference of dynamic laboratory tests among suspected dengue patients was analyzed. The mean WBC, platelet counts were significantly lower in laboratory-positive cases than laboratory-negative cases on days 2—7 ($P < 0.02$), and days 4—7 ($P \leqq 0.01$), respectively. Patients with laboratory-positive had significantly higher mean aPTT than those with laboratory-negative on day 2 ($P = 0.039$). The mean AST, ALT levels were significantly higher in laboratory-positive cases than laboratory-negative cases on days 2, 4, 6—7 ($P \leqq 0.05$), and days 6—7 ($P < 0.05$), respectively. Laboratory-positive cases had significantly lower CRP than laboratory-negative cases on days 2—6 ($P < 0.03$).

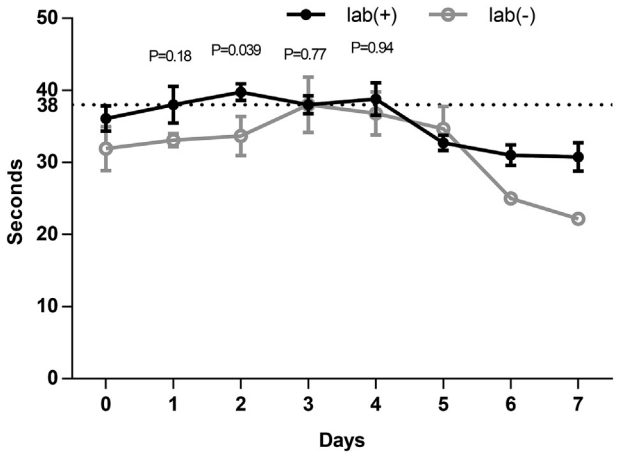

Figure 3. Dynamic mean aPTT.

The predictive value of symptom or sign was not robust (Table 2); however, 93.8% of laboratory-negative cases did not have petechiae (specificity of petechiae: 93.8%). The specificity of combination of petechiae and headache rose to 98.2%. Negative predictive values (NPV) were low in all symptoms and signs. Rash, itching, and petechiae had relatively high positive predictive value (PPV) > 75%. When the symptoms and signs were applied together, PPV of combination of rash and headache arose to 83.1% while those of petechiae and headache increased to 83.3%. Moreover, the specificity and PPV of combination of rash, itching and petechiae increased up to 100%.

During the course of the study, the predictive value of laboratory tests changed dynamically (Fig. 5, and Table 3). Only 19.4% of laboratory-positive cases had leukopenia on day 0 (sensitivity of leukopenia: 19.4%), which increased to >90% on days 2 and 3, and 86.4% on day 4. The specificity of leukopenia reached above 80% after day 6. The PPV of leukopenia was low on days 0—1 and increased up to 100% on day 6. Positive likelihood ratio (LR+) for leukopenia was >2.90 on days 2, 4, 6—7. Negative likelihood ratio (LR—) for leukopenia ranged from 0.14 to 0.24 on days 2—4. The sensitivity of thrombocytopenia was >81% on days 2—7. The PPV of thrombocytopenia increased to >85% after day 4 and reached its peak of 92.9% on day 6. LR+ for thrombocytopenia ranged from 2.00 to 2.55 while LR— for thrombocytopenia ranged from 0.12 to 0.24 on days 4—7. The specificity of prolonged aPTT was >50% on days 0—7. Prolonged aPTT had a PPV of >80% on days 1—4. The sensitivity of elevated transaminases was above 68% on days 5—7. The PPV of elevated transaminases was above 86% on days 4—8. LR— for elevated transaminases ranged from 0.32 to 0.67 on days 4—8. The sensitivity of low CRP (<20 mg/L) was beyond 89% on days 2—9. The PPV of low CRP was above 82% on days 1—3. LR— for low CRP ranged from 0.00 to 0.28 on days 2—8.

The predictive value of combined laboratory tests was also calculated. If we combined leukopenia and thrombocytopenia, a PPV >80% was noted on days 2—8. If leukopenia, thrombocytopenia, and elevated transaminase levels were combined, the PPV reached 100% on day 2 and days 6—8.

Table 2 The predictive value of clinical manifestations.

	Lab-positive n = 205	Lab-negative n = 112	Se (%)	Sp(%)	PPV(%)	NPV(%)
Rash	156	50	76.1	55.4	75.7	55.9
Itching	59	18	28.8	83.9	76.6	39.2
Petechiae	30	7	14.6	93.8	81.1	37.5
Headache	66	37	32.2	67.0	64.1	35.0
Cough	54	60	26.3	46.4	47.4	25.6
Sore throat	19	21	9.3	81.3	47.5	32.9
Rhinorrhea	43	42	21.0	62.5	50.6	30.2
Rash + Headache	54	11	26.3	90.2	83.1	40.1
Petechiae + Headache	10	2	4.9	98.2	83.3	36.1
Rash + Itching + Petechiae	13	0	6.3	100	100	36.8

Lab-positive, laboratory-positive cases; Lab-negative, laboratory-negative cases; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value.

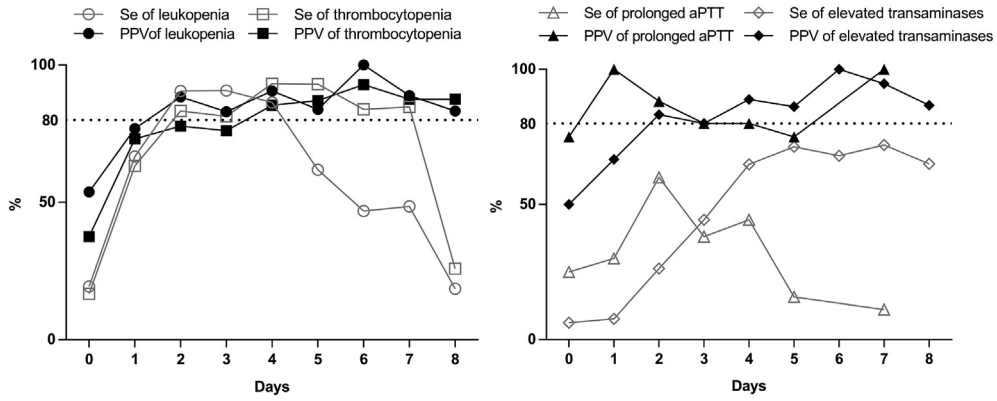

Figure 5. Sensitivity and PPV of leukopenia, thrombocytopenia, prolonged aPTT, and elevated transaminase levels.

Table 3 The dynamic results in each laboratory tests among laboratory-positive and laboratory-negative cases.

		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Leukopenia	Lab(+)	7/36	20/30	38/42	39/43	38/44	26/42	15/32	16/33	5/27
	Lab(−)	6/17	6/13	5/16	8/13	4/15	5/14	0/6	2/12	1/6
Thrombocytopenia	Lab(+)	6/36	19/30	35/42	35/43	41/44	40/43	26/31	28/33	7/27
	Lab(−)	10/17	7/13	10/16	11/13	7/15	6/14	2/6	4/12	1/6
Prolonged aPTT	Lab(+)	3/12	3/10	15/25	8/21	8/18	3/19	0/5	1/9	0/6
	Lab(−)	1/4	0/5	2/8	2/4	2/6	1/4	0/1	0/1	0/0
Elevated transaminases	Lab(+)	2/32	2/26	10/38	16/36	24/37	25/35	17/25	18/25	13/20
	Lab(−)	2/15	1/10	2/14	4/8	3/9	4/7	0/3	1/6	2/5

Lab(+), laboratory-positive cases; Lab(−), laboratory-negative cases.
Denominators represent the number of blood tests.
Numerators represent the positive results in each laboratory finding.

Discussion

There was a dengue outbreak in southern Taiwan city, Kaohsiung, in 2014. More than 14,000 laboratory-confirmed dengue cases were detected in Kaohsiung from June to December 2014.[15] We analyzed the dynamic change of laboratory tests chronologically from the onset of fever. Compared with previous studies of dengue based on single laboratory tests on a random day,[16,17] our study presented with a panorama of dynamic clinical course. Awareness of the dynamic ordinary laboratory tests may assist clinicians to determine the possibility of dengue fever.

Some infectious febrile illnesses resemble dengue fever.[11] A prospective study found that children with dengue were more likely to present with anorexia, nausea, and vomiting and to have a positive tourniquet test than children with other febrile illnesses.[18] No difference in nausea and vomiting between dengue cases and other febrile illnesses was noted in the current study. However, the current study observed that cough, rhinorrhea, and sore

throat were found to a lesser extent in laboratory-confirmed dengue cases than in other febrile illnesses cases. Skin rash, itching, and petechiae were more likely to be present in laboratory-confirmed dengue cases. Further less other febrile illnesses presented with concurrent rash and headache as well as petechiae and headache. Moreover, no case had concomitant rash, itching and petechiae in other febrile illnesses, that is to say, if a patient had a constellation of rash, itching and petechiae, the positive rate of dengue is up to 100% according to the current study.

The WBC, absolute neutrophil, absolute monocyte counts, and platelet counts were lower in children with dengue than in those with other febrile illnesses.[18,19] However, neutropenia was not associated with poor outcomes.[20] In our study, dengue-infected cases had more profound leukopenia, and WBC count reached its nadir 2500/mm^3 on day 3. There was no evidence of concomitant bacterial infection noted during the leukopenia period. No association was noted between WBC counts and the disease severity of dengue fever. In the literature, thrombocytopenia was present in 30—100% of dengue cases.[21—24] In the current study, thrombocytopenia occurred commonly in laboratory-confirmed dengue cases on days 2—7. Dengue-infected cases had more severe thrombocytopenia on days 4—7. And the platelet count reached its nadir on day 5, which was later than WBC count. This phenomenon may facilitate clinicians to consider the possibility of dengue infection.

WHO recommended that coagulation profile should be monitored in patients with warning signs.[11] Coagulation profile can be used to facilitate the diagnosis of dengue fever as well as to monitor organ function.[11,25] Lin SW et al. found that aPTT prolongation may be derived from the binding of nonstructural protein NS1 to prothrombin and inhibiting its activation.[26] Nonstructural protein NS1 antigen is a test of acute dengue infection and can be detected in serum on days 1—7 of illness.[9,11] The current study observed that prolonged aPTT developed on days 1—4 in laboratory-confirmed dengue cases. The period of prolonged aPTT may be associated with the period of NS1 antigenemia. Previous studies found that elevated transaminases were mild (2—5 times upper limit of normal values) in most cases.[27—29] Kuo CH et al. found that the level of transaminases increased to maximum levels nine days after the onset of symptoms, then decreased to normal levels within two weeks.[27] We observed that elevated serum transaminases became prominent near day 6 in laboratory-confirmed dengue cases. The mean AST level was about 1.5 times the mean ALT level within the first week. The peak AST levels developed earlier than the peak ALT levels. The CRP usually maintained in low levels in dengue cases.[23,30] Higher CRP for severe dengue cases have been described in adults.[31] In the current study, the CRP were within normal range in most of laboratory-confirmed dengue cases, however, there was no significant difference in CRP between severe dengue and non-severe dengue cases in children.

Although the predictive value of laboratory tests for dengue fever has been investigated,[16,25] the dynamic predictive value of laboratory tests has not been studied yet. We found that leukopenia, thrombocytopenia, prolonged aPTT, elevated transaminases, and low CRP all had high positive predictive value (>80%) for diagnosis of dengue, especially leukopenia on days 2—8, thrombocytopenia on days 4—8, prolonged aPTT on days 1—4, and elevated transaminases on days 2—8, and low CRP on days 1—3, respectively. Combination of leukopenia, thrombocytopenia, and elevated transaminase levels had a PPV 100% on day 2 and days 6—8. LR+ >2 slightly increased the probability of laboratory-positive dengue, which was noted for leukopenia on days 2, 4, 6—7, and thrombocytopenia on days 4—7. LR− <0.3 moderately decreased the probability of laboratory-positive dengue, which was noted for leukopenia on days 2—4, thrombocytopenia on days 4—7, and low CRP on days 2—8, that is to say, absence of leukopenia, thrombocytopenia, low CRP on days 2—4, 4—7, 2—8, respectively, moderately decreased the likelihood of dengue diagnosis in children.

This study had limitations. First, this investigation was conducted during an epidemic. The high prevalence of dengue infection contributed to high positive predictive values. The high positive predictive values will not be replicated in a low prevalence population. Second, only 3 patients (1.5%) of the laboratory-confirmed dengue cases presented with complicated severe dengue, and none of the patients died. The overall disease severity in this outbreak was relatively lower than that in other studies.[32,33] This could be attributed to most of our patients being infected by the dengue virus serotype 1.[14] Patients with dengue virus serotype 2 infections experienced more severe disease than those infected with other serotypes.[34] Another reason is that medical availability is very good and convenient in Taiwan. Furthermore, owing to the high admission rate, close monitoring allows prevention of further morbidity and mortality.

In conclusion, skin rash, itching, and petechiae were more common in laboratory-positive dengue cases than in other febrile illnesses cases. Significantly lower WBC counts on days 2—7, lower PLT counts on days 4—7, lower CRP on days 2—6, higher aPTT on day 2, and higher elevated transaminase level on days 2, 4, 6—7 in dengue cases were found. High diagnostic performance of each of these ordinary laboratory tests was also observed near the first week of illnesses. During dengue epidemics, combinations of the symptoms and dynamic ordinary laboratory tests are helpful to physicians for accurate diagnosis of dengue fever.

Conflicts of interest

None to declare.

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