

BMJ Open Factors associated with thrombocytopenia in patients with dengue fever: a retrospective cohort study

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ABSTRACT

Objective Some patients with dengue fever tend to develop thrombocytopenia during the course of infection and are thus vulnerable to haemorrhagic manifestations and other complications. However, the factors associated with the development of thrombocytopenia are unknown. We aimed to identify factors associated with an increased risk of thrombocytopenia and haematological changes in patients with confirmed dengue fever.

Design Retrospective cohort study.

Setting Brazilian multicentre primary care databases.

Participants 387 patients had positive laboratory serological confirmation of dengue infection during 2014. The data were identified from two databases: Notification of Injury Information System (SINAN) and Municipal Laboratory.

Main outcome measure The presence of thrombocytopenia (platelet count $<150 \times 10^9/L$). The associations of factors that predisposed patients to thrombocytopenia and haematological changes were analysed using logistic regression. ORs and 95% CIs were calculated.

Results Among 387 patients, 156 had both dengue and thrombocytopenia. The risk factors associated with thrombocytopenia included male sex (OR: 1.77, 95% CI: 1.16 to 2.71, $p=0.007$), age of 46–64 years (OR: 2.20, 95% CI: 1.15 to 4.21, $p=0.009$) or ≥ 65 years (OR: 3.02, 95% CI: 1.40 to 6.50, $p=0.002$), presence of leucopenia (OR: 6.85, 95% CI: 4.27 to 10.99, $p<0.001$) and high mean corpuscular haemoglobin (MCH) levels (OR: 2.00, 95% CI: 1.29 to 3.12, $p=0.005$).

Conclusion Older age, male sex, presence of leucopenia and high MCH levels were identified as risk factors associated with the development of thrombocytopenia in this population.

BACKGROUND

Dengue virus (DENV) infection occurs via the transmission of a DENV serotype (DENV 1–4) by the *Aedes aegypti* mosquito. All four serotypes may cause either asymptomatic infection or classic symptoms of dengue fever. These symptoms may vary from febrile pain to more severe manifestations such as altered

Strengths and limitations of this study

- This study investigated demographic factors and haematological changes associated with the development of thrombocytopenia.
- The use of serological and haematological tests, plus the date of the beginning of the infection, guaranteed the accuracy of the diagnosis.
- The databases used are subject to errors and under-reporting because they are not for research purposes.

vascular endothelial permeability, plasma leakage, decreased platelet levels, bleeding, dangerously low blood pressure and shock, which may lead to death.^{1 2}

Platelets are an important blood component involved in coagulation. Patients infected with DENV tend to develop thrombocytopenia during the course of infection, which renders them vulnerable to bleeding manifestations and other severe complications.^{3 4} DENV induces bone marrow depression and decreases platelet production and can infect megakaryocytes directly or induce the release of antibodies that attack and thus destroy platelets.^{5–8}

Several studies have identified haematological changes in patients with dengue. The main reported changes include thrombocytopenia in 40%–79% of cases, leucopenia in 30%–69%^{9–12} and changes in lymphocyte populations, including lymphocytosis in 31.9% and lymphocytopenia in 67.2% of cases.¹⁰

The relationship between thrombocytopenia and other haematological changes has not been completely explored. Accordingly, no clear associations have been established, although some authors have suggested a pattern of clinical laboratory characteristics.^{4–7 13–15} In this study, we aimed to verify

the existence of a potential relationship between thrombocytopenia, haematological changes and other factors such as age, sex and ethnicity in patients with dengue.

METHODS

Study design

This was a retrospective study based on two databases affiliated with the public health system in the city of Campinas, São Paulo, Brazil. Campinas has a population of 1 080 113 inhabitants¹⁶ and has experienced consecutive dengue epidemics. For this study, 2014 was selected as the reference year, during which 48 290 cases of dengue were reported.¹⁷

Data source

The study data were obtained from two information sources at the Department of Epidemiological Surveillance within the Municipal Health Department of Campinas. The first database, the Notification of Injury Information System (*Sistema de Informação de Agravos e Notificação*, SINAN),¹⁸ was used to identify reported patients with dengue. The second database, the Municipal Laboratory of Campinas (MLC), was used to locate laboratory test results, confirm dengue infection and access patients' blood counts.

Dengue confirmation testing (DENV non-structural protein 1 (NS1), immunoglobulin (Ig)M/IgG serological tests or IgM ELISA) was performed at the Adolfo Lutz Institute. The results were transferred to the Epidemiological Surveillance Department via several reports according to the order of testing blood count analyses performed at the MLC.

Eligibility criteria

We included all reported and registered cases of dengue in SINAN for Campinas, São Paulo State during January–December 2014. Cases with positive laboratory confirmation of dengue according to the NS1, IgM/IgG serology or IgM ELISA results were included. We defined thrombocytopenia as a platelet count $<150 \times 10^9/L$ in two tests (normal range without thrombocytopenia: $\geq 150\text{--}400 \times 10^9/L$). We excluded all patients with incomplete data (eg, no laboratory confirmation and/or blood count data). We also excluded patients with thrombocytosis, which may have been a confounder in this study.

All SINAN records and MLC blood counts were considered. In other words, a patient needed to be registered in SINAN, with available positive serological test results for dengue and available blood counts in the system, to be included in the study.

Variables

The predictive variables included haematological changes detected via blood counts performed at the MLC. For all included patients, at least two platelet counts obtained during the course of illness were available. Blood samples were collected from day 1 to 9 after symptom onset.

The reference values were as follows: leucocytes, $4.5\text{--}11.0 \times 10^9/L$; erythrocytes, $4.10\text{--}5.90 \times 10^{12}/L$; haemoglobin, 123–175 g/L; haematocrit, 36%–50%; mean corpuscular haemoglobin (MCH), 27–29 pg; mean corpuscular volume (MCV), 77–92 fL; mean corpuscular haemoglobin concentration (MCHC), 300–350 g/L; and red blood cell distribution width (RDW), 10%–15%.¹⁹

We verified whether the changes in each erythrogram variable yielded values below or above the reference values for adults. Sex, age, ethnicity and education level were considered potential confounding variables and were treated as such in the statistical analysis.

Sample size

To determine the sufficiency of the sample for the analysis, we assumed that thrombocytopenia would be present in 50% of the population and that the predictive variables would have ORs of 1.8. At a power of 80% and significance level of 5%, we estimated that the sample should comprise at least 378 subjects.

Patient and public involvement

There was no patient or public involvement in this study.

Statistical analysis

The reports and laboratory confirmations of dengue were analysed deterministically.²⁰ All variables were described and stratified according to the presence or absence of thrombocytopenia (dependent variable). Tests were used to detect differences among the following independent variables: sex, age, ethnicity, education level and haematological changes such as leucocyte, erythrocyte, and platelet counts, haemoglobin level, haematocrit, MCH, MCV, MCHC, RDW and blood collection dates. The χ^2 test was used to analyse categorical variables.²¹ The adjusted ORs were calculated using a logistic regression, which was adjusted by sex, age and sample collection date. We also calculated the 95% CIs. The medians of blood count variables were compared. The Kruskal-Wallis test was used to compare the haematological values of patients with and without thrombocytopenia, assuming a non-normal data distribution.

As some of the consulted records had incomplete data, the analyses were restricted to individuals for whom complete information was available.²¹ Therefore, no data were imputed or attributed to observations. All analyses were performed using Stata V. 14.1 (Stata Corp LLC).

RESULTS

Sample composition

All patients with serologically confirmed dengue fever who were included in the SINAN registry were considered eligible for this study. Of these patients, 7336 were excluded because of a lack of available laboratory test data. Finally, 387 patients with confirmed dengue fever were included in the analysis, of whom 156 (40.3%) and

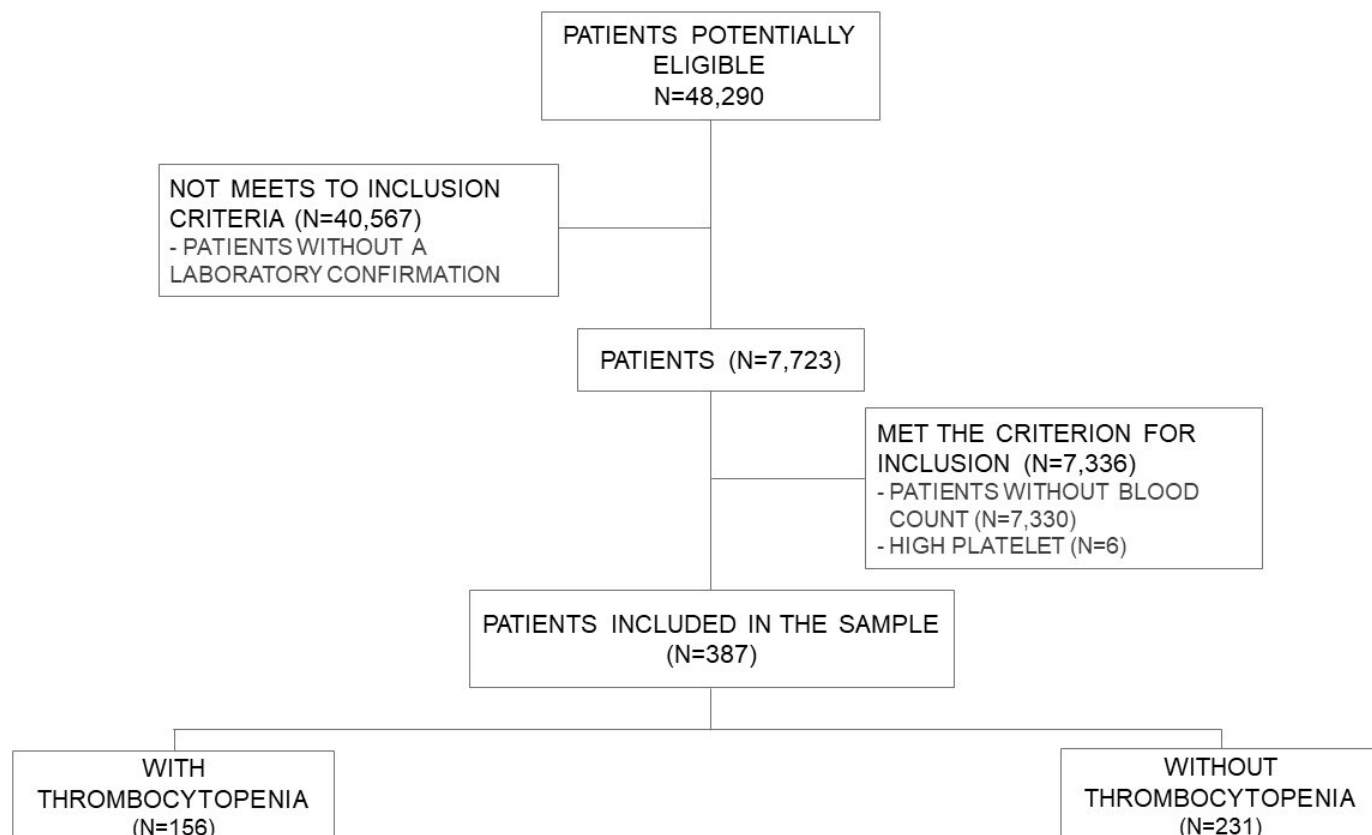


Figure 1 Flow diagram of the stages of sample composition.

231 (59.7%) did and did not have thrombocytopenia, respectively (figure 1).

For the 387 included patients, blood was collected from 203 (52.4%) during days 1–3 days after the initial symptom onset, from 143 (37.0%) patients on days 4–8, and from 41 (10.6%) patients up to 9 days.

The prevalence of thrombocytopenia among patients with confirmed dengue was 40.3% (95% CI: 35.5 to 43.5; median platelet count, $109 \times 10^9/L$, IQR: 89.7–126.2). The following factors were associated with thrombocytopenia: male sex (OR: 1.77, 95% CI: 1.16 to 2.71, $p=0.007$) and an age of 46–64 years (OR: 2.20, 95% CI: 1.15 to 4.21, $p=0.009$) or ≥ 65 years (OR: 3.02, 95% CI: 1.40 to 6.50, $p=0.002$), as shown in table 1.

The presence of leucopenia (OR: 6.85, 95% CI: 4.27 to 10.99, $p<0.001$) and a high MCH level (OR: 2.00, 95% CI: 1.29 to 3.12, $p=0.005$) were identified as haematological changes associated with thrombocytopenia, as shown in table 2.

Of the median values of the evaluated haematological parameters, we verified that only the values for RDW and MCV did not differ significantly between patients with and without thrombocytopenia ($p<0.05$). Patients with dengue and thrombocytopenia had a lower median leucocyte count than those without thrombocytopenia ($3.75 \times 10^9/L$, IQR: $2.79\text{--}4.73 \times 10^9/L$ vs $5.76 \times 10^9/L$, IQR: $4.48\text{--}7.52 \times 10^9/L$). All other median values, particularly the haematocrit (42.5%, IQR 40.6–44.9%), erythrocyte count ($4.89 \times 10^{12}/L$, IQR: $4.60\text{--}5.19 \times 10^{12}/L$),

haemoglobin (145 g/L, IQR: 133.3–152.8 g/L) and MCH (29.60 pg, IQR: 28.55–30.50 pg), were significantly higher in patients with thrombocytopenia than in those without thrombocytopenia ($p<0.05$). Compared with patients without thrombocytopenia, those with thrombocytopenia exhibited significant decreases in the leucocyte count and MCHC but an elevated MCH level (table 3).

DISCUSSION

Main findings

In our sample of patients with dengue, the prevalence of thrombocytopenia was 40.3%. The risk of thrombocytopenia was proportional to increasing age and related with male sex. Specifically, older people were three times more likely than younger people and almost twice as likely as adults to develop thrombocytopenia. We also identified male sex, leucopenia and high MCH levels as factors associated with an increased risk of thrombocytopenia. Moreover, the median haematocrit, erythrocyte count, haemoglobin and MCH levels were higher in patients with thrombocytopenia than in those with normal platelet counts.

Comparison with previous studies

In this study, the most of patients with dengue were women, differing of other studies, which showed greater prevalence in male individuals.^{12 22–25} The age between 18 and 45 years is also common in other researches.^{12 22 23 25 26}

Table 1 Sociodemographic characteristics of patients with confirmed dengue fever associated with thrombocytopenia

	Patients with dengue and thrombocytopenia n=156 (100%)	Patients with dengue and without thrombocytopenia n=231 (100%)	Unadjusted OR (95% CI)	P value	Adjusted OR* (95% CI)	P value
Sex						
Female	77 (44.9)	141 (61.3)	1.00		1.00	
Male	79 (50.6)	89 (38.7)	1.63 (1.08 to 2.45)	0.021	1.77 (1.16 to 2.71)	0.008
Age (years)						
0–17	23 (14.7)	54 (23.4)	1.00		1.00	
18–45	74 (47.4)	112 (48.5)	1.55 (0.88 to 2.74)	0.131	1.71 (0.95 to 3.06)	0.072
46–64	43 (27.5)	52 (22.5)	1.94 (1.03 to 3.66)	0.040	2.20 (1.15 to 4.21)	0.018
≥65	16 (10.3)	13 (5.6)	2.89 (1.20 to 6.96)	0.018	3.02 (1.40 to 6.50)	0.005
Ethnicity						
White	75 (62.0)	130 (72.6)	1.00		1.00	
Black/brown/indigenous	46 (38.0)	49 (27.4)	1.63 (0.99 to 2.66)	0.053	1.63 (0.98 to 2.70)	0.058
Education level						
Elementary school incomplete	26 (36.1)	46 (43.4)	1.00		1.00	
High school incomplete	21 (29.2)	34 (32.1)	1.09 (0.53 to 2.26)	0.811	1.18 (0.54 to 2.58)	0.677
Higher education incomplete	17 (23.6)	22 (20.8)	1.37 (0.62 to 3.03)	0.441	1.22 (0.49 to 3.04)	0.667
Higher education complete	8 (11.1)	4 (3.8)	3.54 (0.97 to 2.89)	0.055	3.34 (0.86 to 13.04)	0.082

*Analysis adjusted by sex, age and collection date (from 1 to 9 days after symptom onset).

The WHO has considered thrombocytopenia as one of the indicators for the clinical severity of the disease.²⁷ Although the mechanisms involved in thrombocytopenia during dengue infection are not fully elucidated, it has been suggested that the DENV affects bone marrow cells, inhibiting their function to reduce the proliferative capacity of haematopoietic cells.^{28–29} In addition to the platelet count, the functional disruption of these cells is associated with the immunopathogenesis of dengue and to the fact that the infection induces the consumption of platelets due to disseminated intravascular coagulation, destruction of platelets due to increased apoptosis, lysis by the complement system and involvement of antiplatelet antibodies.^{29–30} The median platelet count in patients with thrombocytopenia in our study was not as low as the reference value for severe cases ($109 \times 10^9/L$ vs $<40 \times 10^9/L$).³¹ Other study involving only inpatients with dengue fever also exhibited similar platelet rates.²² Thrombocytopenia ($<100 \times 10^9/L$) is less common in patients with dengue than in inpatients with other arbovirus infections.^{31–34} This discrepancy is attributed to the pattern of platelet counts over time in patients with dengue fever; the count is typically lowest between 3 and 6 days after the onset of illness, just before the fever begins to subside.^{31–34} According to other studies, thrombocytopenia and platelet dysfunction are also related to the clinical outcome such as skin rash and haemophagocytosis.^{25–35}

Another retrospective study did not identify any sex-specific differences in the prevalence of thrombocytopenia.³⁶ In our sample, although the prevalence of dengue fever was higher among women, men were almost twice

as likely as women to develop thrombocytopenia during the course of infection. Other research that included only inpatients also showed this trend of greater prevalence in men.¹²

Several studies have shown clear differences in the prevalence of thrombocytopenia with respect to age.^{12–37} In our sample, dengue-infected patients aged ≥ 65 years were three times more likely to develop thrombocytopenia than those in other age groups. According to the Ministry of Health, older people are 12 times more likely to die of dengue-related causes than those in other age groups.³⁷ Age-related differences in immune function possibly affect the balance between protective and detrimental host immune responses.^{4–10} The observed difference in mortality may also be related to the prevalence of chronic diseases, such as diabetes or heart disease,³⁷ as well as the chronic use of some drugs among older people. Medications, such as acetylsalicylic acid, that are used to treat heart diseases tend to decrease platelet concentrations and may contribute to thrombocytopenia and haemorrhagic manifestations when used during the course of a DENV infection.^{31–38}

The overall decrease in the leucocyte count observed in patients with dengue is mainly due to a decrease in the population of granulocytes (eg, neutrophils).³⁹ The ability of DENV to suppress white blood cell production in the bone marrow may explain mechanistically the appearance of leucopenia in patients with dengue.⁴⁰

Patients with thrombocytopenia were approximately seven times more likely to exhibit a shift to leucopenia than those without thrombocytopenia. Most reports that

Table 2 Haematological changes in patients with confirmed dengue fever associated with thrombocytopenia

	Patients with dengue and thrombocytopenia (n=156)	Patients with dengue and without thrombocytopenia (n=231)	Unadjusted OR (95%CI)	P value	Adjusted OR* (95%CI)	P value
Leukocytes (×10⁹/L)						
Reference range†	51 (32.7)	172 (74.5)	1.00		1.00	
Changed range	105 (67.3)	59 (25.5)	6.00 (3.84 to 9.38)	<0.001	6.85 (4.27 to 10.99)	<0.001
Erythrocytes (×10¹²/L)						
Reference range†	141 (90.4)	205 (88.7)	1.00		1.00	
Changed range	15 (9.6)	26 (11.3)	0.84 (0.43 to 1.64)	0.607	0.73 (0.37 to 1.47)	0.379
Haemoglobin (g/L)						
Reference range†	125 (80.1)	197 (85.3)	1.00		1.00	
Changed range	31 (19.9)	34 (14.7)	1.44 (0.84 to 2.46)	0.185	1.50 (0.87 to 2.60)	0.145
Haematocrit (%)						
Reference range†	136 (87.2)	197 (85.2)	1.00		1.00	
Changed range	20 (12.8)	34 (14.7)	0.85 (0.47 to 1.54)	0.597	0.81 (0.43 to 1.51)	0.507
Mean corpuscular haemoglobin (pg)						
Reference range†	50 (32.0)	119 (51.5)	1.00		1.00	
Changed range	106 (68.0)	112 (48.5)	2.25 (1.47 to 3.44)	<0.001	2.00 (1.29 to 3.12)	0.002
Mean corpuscular volume (fL)						
Reference range†	126 (80.8)	180 (78.0)	1.00		1.00	
Changed range	30 (19.2)	51 (22.0)	0.84 (0.51 to 1.39)	0.500	0.84 (0.50 to 1.41)	0.508
Mean corpuscular haemoglobin concentration (g/L)						
Reference range†	140 (89.7)	209 (90.5)	1.00		1.00	
Changed range	16 (10.3)	22 (9.5)	1.09 (0.55 to 2.14)	0.812	0.93 (0.46 to 1.90)	0.849
Red cell distribution width (%)						
Reference range†	145 (93.0)	215 (93.1)	1.00		1.00	
Changed range	11 (7.0)	16 (6.9)	1.02 (0.46 to 2.26)	0.962	1.03 (0.46 to 2.31)	0.948

Changed range: values lower or higher than the reference range.

*OR adjusted by sex, age and collection date (from 1 up to 9 days after symptom onset).

†Reference values: leucocytes: 4.5–11.0×10⁹/L; erythrocytes: 4.10–5.90 ×10¹²/L; haemoglobin: 123–175 g/L; haematocrit: 36%–50%; mean corpuscular haemoglobin: 27–29 pg; mean corpuscular volume: 77–92 fL; mean corpuscular haemoglobin concentration: 300–350 g/L; red cell distribution width: 10%–15%.

describe frequent haematological changes during dengue infection noted that leucopenia is commonly observed.⁴¹

One systematic review revealed that several clinical and laboratory measures could potentially distinguish people with dengue from those with other febrile viral diseases.⁴² An increased haemoconcentration and haematocrit are commonly observed in patients with dengue infection.³⁴ Plasma extravasation leads to a high haematocrit value, which is the initial abnormality associated with dengue infection. A haematocrit value >20% over the baseline value is an important diagnostic criterion for dengue.^{31 43} Haemoconcentration tends to occur in patients with haemorrhagic dengue. This tendency is defined solely based on the patient's initial haematocrit value.

In this study, patients with dengue and thrombocytopenia had a statistically higher median haematocrit value than patients without thrombocytopenia, although this change was not associated with a higher risk of developing

thrombocytopenia in our sample. The median MCH value was higher in patients with thrombocytopenia than in those without thrombocytopenia, and a high MCH was associated with a nearly twofold increase in the probability of developing thrombocytopenia among patients with dengue. Currently, MCH is not used to differentiate dengue infection, and few studies have explored an association of a high MCH with thrombocytopenia in patients with dengue.

One study explored haematological parameters that could be used to differentiate dengue and malaria in endemic areas of Thailand.⁴⁴ In our study, we found that most haematological alterations exhibited differences in sensitivity and specificity with respect to dengue and malaria. However, the MCH level greater than the reference values was identified as the most sensitive parameter (78%) for differentiating patients with dengue from those with malaria. Our findings suggest that this parameter maybe useful in the initial

Table 3 Quantitative haematological changes in patients with confirmed dengue fever associated with thrombocytopenia

	Total population, median (IQR)	Patients with dengue and thrombocytopenia (n=156), median (IQR)	Patients with dengue and without thrombocytopenia (n=231), median (IQR)	P value*
Leukocytes	4.80 (3.48–6.56)	3.75 (2.79–4.73)	5.76 (4.48–7.52)	0.0001
Erythrocytes	4.76 (4.50–5.10)	4.89 (4.60–5.19)	4.68 (4.44–5.03)	0.0005
Haemoglobin	139 (130–148)	145 (133–153)	136 (128–144)	0.0001
Haematocrit	41.50 (39.30–43.90)	42.50 (40.60–44.90)	40.70 (38.40–42.90)	0.0001
Mean corpuscular haemoglobin	29.30 (27.90–30.40)	29.60 (28.55–30.50)	28.80 (27.50–30.40)	0.0039
Mean corpuscular volume	87.00 (83.30–90.30)	87.55 (84.63–90.40)	86.20 (82.20–90.20)	0.0781
Mean corpuscular haemoglobin concentration	336 (328–343)	338 (330–344)	334 (327–343)	0.0130
Red cell distribution width	13.50 (13.00–14.10)	13.50 (13.00–14.00)	13.60 (13.00–14.20)	0.4354

Normal reference values: leucocytes: $4.5\text{--}11.0 \times 10^9/\text{L}$; erythrocytes: $4.10\text{--}5.90 \times 10^{12}/\text{L}$; haemoglobin: 123–175 g/L; haematocrit: 36%–50%; mean corpuscular haemoglobin: 27–29 pg; mean corpuscular volume: 77–92 fL; mean corpuscular haemoglobin concentration: 300–350 g/L; red cell distribution width: 10%–15%.

*Kruskal-Wallis test.

differential diagnosis of dengue fever and other febrile viral infections.⁴⁴

Strengths and limitations of the study

Although other studies^{3 4} have investigated the presence of thrombocytopenia as the main haematological alteration in patients with dengue, ours is the first study to examine additional factors such as sex, age, ethnicity, education level and haematological changes in association with the development of thrombocytopenia. Our study was limited mainly by the sample size.

The initial cases during the course of an epidemic must be confirmed via laboratory testing, whereas subsequent cases can be confirmed using clinical–epidemiological criteria.³⁸ Given the potential circulation of other arboviruses (Zika and Chikungunya) in the country, we only included individuals who were seropositive for DENV; this restriction greatly reduced the selected sample because only a few people were subjected to this confirmatory evaluation.

In many observational studies, the serological confirmation of dengue fever and other febrile viruses was performed incorrectly or was poorly described by the authors, and these inconsistencies cast doubt on the distinction between the presence or absence of thrombocytopenia. In our study, patients with and without thrombocytopenia were selected according to the availability of serological and haematological test results, the onset of infection, and the availability of more than one blood sample. These criteria ensured that both subpopulations in this study received the correct differential diagnosis.

Retrospective observational studies are inherently subject to bias due to the incorrect reporting or omission of information. Information is entered into SINAN in a decentralised manner, and many entries into the same system are made at the municipal level. Accordingly, this

database may include incorrect, incomplete or missing data, which would influence the quality of secondary data such as the virus type, disease severity, dengue infection during pregnancy, hospitalisation and death. Also, we did not access medical records and could not assess the variation in the clinical and laboratory features that took place during the course of illness.

CONCLUSIONS

The initial diagnosis of dengue is based solely on the clinical history, and the broad spectrum of disease-related symptoms can easily lead to a misdiagnosis of other infectious diseases of viral aetiology, such as influenza, Zika or other arbovirus infection. As dengue can worsen rapidly, we propose that in daily clinical practice, male patients and older patients should be examined meticulously and monitored frequently and that both follow-up and management protocols should be improved to avoid dengue-related mortality. Moreover, although changes in platelet and leucocyte counts and an elevated haematocrit were the most frequently observed haematological alterations during dengue, MCH may be a novel parameter worthy of monitoring and further exploration.

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Contributors LCL and BMC developed the original study concept and protocol. BMC, MTS and ARRF collected data and performed the data analysis. BMC, LCL, ARRF and IF drafted the manuscript. LCL, BMC, MTS, IF and ARRF reviewed the manuscript and performed editing at all steps.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences of the São Paulo State University, 'Júlio de Mesquita Filho', on 8 October 2015 (CAAE: 46934815.0.0000.5426) according to Resolution CNS 466/12 of the National Health Council.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. No database available. All data generated or analysed during this study are included in this article.

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