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# Factors associated with thrombocytopenia in patients with dengue fever: a retrospective study



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# Factors associated with thrombocytopenia in patients with dengue fever: a retrospective 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 hemorrhagic 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 hematological changes in patients with confirmed dengue fever.

**Design:** retrospective study;

**Setting:** Brazilian multicenter primary care databases;

**Participants:** 387 patients with positive laboratory serologic 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:** presence of thrombocytopenia (platelet count<150,000/mm$^3$). The associations of factors that predisposed patients to thrombocytopenia and hematological changes were analyzed using logistic regression. Odds ratios (ORs) and 95% confidence intervals (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: 1.16–2.71, p=0.007), age of 46–64 years (OR: 2.20, 95% CI: 1.15–4.21, p=0.009) or ≥65 years (OR: 3.02, 95% CI: 1.40–6.50, p=0.002), presence of leukopenia (OR: 6.85, 95% CI: 4.27–10.99, p<0.001), and high mean corpuscular hemoglobin (MCH) levels (OR: 2.00, 95% CI: 1.29–3.12, p=0.005).

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

**Keywords:** *Aedes aegypti*; arboviruses; public health; thrombocytopenia

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**Strengths and limitations of this study**

- Our findings contribute to improving the knowledge regarding factors associated with development of thrombocytopenia and hematological changes in individuals with confirmed dengue.
- The databases used are not for research purposes; therefore, this study is subject to some data entry errors and underreporting.
- The source population included a large number of patients with suspected dengue, but few patients underwent serologic examination to confirm dengue infection, which greatly reduced the size of the included sample.
- Accurate diagnosis of dengue fever is assured as we used results of serological and hematological examinations and date of the onset of infection, and there were at least two blood samples available.

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## 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 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 hematological changes in patients with dengue. The main reported changes include thrombocytopenia in 40–79% of cases, leukopenia in 30–69%[9-11], and changes in lymphocyte populations, including lymphocytosis in 31.9% and lymphocytopenia in 67.2% of cases[10].

The relationship between thrombocytopenia and other hematological 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 12-14]. In this study, we aimed to verify the existence of a potential relationship between thrombocytopenia, hematological changes, and other factors such as age, sex, and ethnicity in patients with dengue.

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## METHODS

### Ethical aspects

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 October 8, 2015 (CAAE: 46934815.0.0000.5426) according to Resolution CNS 466/12 of the National Health Council.

### 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 inhabitants[15] 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[16].

### 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)[17], 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 nonstructural protein 1 [NS1], IgM/IgG serological tests or IgM enzyme-linked immunosorbent assay [ELISA]) was performed at the Adolfo Lutz Institute. The results were transferred to the Epidemiological

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Surveillance Department via several reports according to the order of testing. Hemoglobin analyses were performed at the MLC.

**Eligibility criteria**

We included all reported and registered cases of dengue in SINAN for Campinas, Sao 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 <150,000/mm$^3$in two tests (normal range without thrombocytopenia: ≥150,000–400,000/mm$^3$). We excluded all patients with incomplete data (e.g., 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 serologic test results for dengue and available blood counts in the system, to be included in the study.

**Variables**

The predictive variables included hematological 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 days1 to 9 after symptom onset.

The reference values were as follows: leukocytes, 4,500–11,000/μL; erythrocytes, 4.10–5.90×10$^6$; hemoglobin**,** 12.3–17.5g/dL; hematocrit, 36–50%; mean corpuscular hemoglobin (MCH), 27–29pg; mean corpuscular volume (MCV), 77–92fL; mean

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corpuscular hemoglobin concentration (MCHC), 30–35g/dL; and red blood cell distribution width (RDW), 10–15%[18].

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 odds ratios (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 analyzed deterministically[19]. 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 hematological changes such as leukocyte, erythrocyte, and platelet counts, hemoglobin level, hematocrit, MCH, MCV, MCHC, RDW, and blood collection dates. The chi-square test was used to analyze categorical variables[20]. The adjusted ORs were calculated using a logistic regression, which was adjusted by sex, age, and sample

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collection date. We also calculated the 95% confidence intervals (CIs). The medians of blood count variables were compared. The Kruskal–Wallis test was used to compare the hematological 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 were available[20]. Therefore, no data were imputed or attributed to observations. All analyses were performed using Stata, version 14.1 (Stata Corp LLC, College Station, TX, USA).

## 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, 7,336 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 231 (59.7%) did and did not have thrombocytopenia, respectively (**Figure 1**).

**Figure 1**

For the 387 included patients, blood was collected from 203 (52.4%) during days 1–3 days after the initial symptom onset, from143 (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: 35.5–43.5; median platelet count, 109,000/mm$^3$, interquartile range

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[IQR]: 89.7–126.2).The following factors were associated with thrombocytopenia: male sex (OR: 1.77, 95% CI: 1.16–2.71, p=0.007) and an age of 46–64 years (OR: 2.20, 95% CI: 1.15–4.21, p=0.009) or ≥65 years (OR: 3.02, 95% CI: 1.40–6.50, p=0.002), as shown in **Table 1**.

**Table 1**

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**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 (1.08–2.45) | 0.021 | 1.77 (1.16–2.71) | 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 (0.88–2.74) | 0.131 | 1.71 (0.95–3.06) | 0.072 |
| **46–64** | 43 (27.5) | 52 (22.5) | 1.94 (1.03–3.66) | 0.040 | 2.20 (1.15–4.21) | 0.018 |
| **≥65** | 16 (10.3) | 13 (5.6) | 2.89 (1.20–6.96) | 0.018 | 3.02 (1.40–6.50) | 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 (0.99–2.66) | 0.053 | 1.63 (0.98–2.70) | 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 (0.53–2.26) | 0.811 | 1.18 (0.54–2.58) | 0.677 |
| **Higher education incomplete** | 17 (23.6) | 22 (20.8) | 1.37 (0.62–3.03) | 0.441 | 1.22 (0.49–3.04) | 0.667 |
| **Higher education complete** | 8 (11.1) | 4 (3.8) | 3.54 (0.97–12.89) | 0.055 | 3.34 (0.86–13.04) | 0.082 |

OR, odds ratio; CI, confidence interval.
*Analysis adjusted by sex, age, and collection date (from 1 to 9 days after symptom onset).

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The presence of leukopenia (OR: 6.85, 95% CI: 4.27–10.99, p<0.001) and a high MCH level (OR: 2.00, 95% CI: 1.29–3.12, p=0.005) were identified as hematological changes associated with thrombocytopenia, as shown in **Table 2**.

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**Table 2: Hematologic 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 (/µL)** | | | | | | |
| Reference range** | 51(32.7) | 172 (74.5) | 1.00 | | 1.00 | |
| Changed range | 105 (67.3) | 59 (25.5) | 6.00 (3.84–9.38) | <0.001 | 6.85 (4.27–10.99) | <0.001 |
| **Erythrocytes (×10⁶)** | | | | | | |
| Reference range** | 141 (90.4) | 205 (88.7) | 1.00 | | 1.00 | |
| Changed range | 15 (9.6) | 26 (11.3) | 0.84 (0.43–1.64) | 0.607 | 0.73 (0.37–1.47) | 0.379 |
| **Hemoglobin (g/dL)** | | | | | | |
| Reference range** | 125 (80.1) | 197 (85.3) | 1.00 | | 1.00 | |
| Changed range | 31 (19.9) | 34 (14.7) | 1.44 (0.84–2.46) | 0.185 | 1.50 (0.87–2.60) | 0.145 |
| **Hematocrit (%)** | | | | | | |
| Reference range** | 136 (87.2) | 197 (85.2) | 1.00 | | 1.00 | |
| Changed range | 20 (12.8) | 34 (14.7) | 0.85 (0.47–1.54) | 0.597 | 0.81 (0.43–1.51) | 0.507 |
| **Mean corpuscular hemoglobin(pg)** | | | | | | |
| Reference range** | 50 (32.0) | 119 (51.5) | 1.00 | | 1.00 | |
| Changed range | 106 (68.0) | 112 (48.5) | 2.25 (1.47–3.44) | <0.001 | 2.00 (1.29–3.12) | 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 (0.51–1.39) | 0.500 | 0.84 (0.50–1.41) | 0.508 |
| **Mean corpuscular hemoglobin concentration (g/dL)** | | | | | | |
| Reference range** | 140 (89.7) | 209 (90.5) | 1.00 | | 1.00 | |
| Changed range | 16 (10.3) | 22 (9.5) | 1.09 (0.55–2.14) | 0.812 | 0.93 (0.46–1.90) | 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 (0.46–2.26) | 0.962 | 1.03 (0.46–2.31) | 0.948 |

OR, odds ratio; CI, confidence interval.

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

**Reference values: leukocytes: 4,500–11,000/µL; erythrocytes: 4.10–5.90×10⁶; hemoglobin: 12.3–17.5g/dL; hematocrit: 36–50%; mean corpuscular hemoglobin: 27–29pg; mean corpuscular volume:77–92fL; mean corpuscular hemoglobin concentration: 30–35g/dL; red cell distribution width: 10–15%.

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

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Of the median values of the evaluated hematological 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 leukocyte count than those without thrombocytopenia (3,750/µL, IQR: 2,790–4,725/µL vs. 5,760/µL, IQR: 4,480–7,520). All other median values, particularly the hematocrit (42.5%, IQR 40.6%–44.9%), erythrocyte count ($4.89\times10^6$, IQR: 4.60–$5.19\times10^6$), hemoglobin (14.50 g/dL, IQR: 13.33–15.28 g/L), and MCH (29.60 pg, IQR: 28.55–30.50pg), 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 leukocyte count and MCHC but an elevated MCH level (**Table 3**).

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**Table 3: Quantitative hematological 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** | 4,800 (3,480–6,560) | 3.750 (2.790–4.725) | 5.760 (4.480–7.520) | 0.0001 |
| **Erythrocytes** | 4.76 (4.50–5.10) | 4.89 (4.60–5.19) | 4.68 (4.44–5.03) | 0.0005 |
| **Hemoglobin** | 13.9 (13.0–14.8) | 14.50 (13.33–15.28) | 13.60 (12.80–14.40) | 0.0001 |
| **Hematocrit** | 41.50 (39.30–43.90) | 42.5 (40.6–44.9) | 40.70 (38.40–42.90) | 0.0001 |
| **Mean corpuscular hemoglobin** | 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 hemoglobin concentration** | 33.60 (32.80–34.30) | 33.80 (33.03–34.40) | 33.40 (32.70–34.30) | 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 |

IQR, interquartile range.

*Kruskal–Wallis test.

Normal reference values: leukocytes: 4,500–11,000/μL; erythrocytes: 4.10–5.90×$10^6$; hemoglobin: 12.3–17.5 g/dL; hematocrit: 36–50%; mean corpuscular hemoglobin: 27–29 pg; mean corpuscular volume: 77–92 fL; mean corpuscular hemoglobin concentration: 30–35 g/dL; red cell distribution width: 10–15%.

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## 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. 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, leukopenia, and high MCH levels as factors associated with an increased risk of thrombocytopenia. Moreover, the median hematocrit, erythrocyte count, hemoglobin, and MCH levels were higher in patients with thrombocytopenia than in those with normal platelet counts.

### Comparison with previous studies

The prevalence of dengue in this study, 5.2%, was higher than that reported in other studies (2.4%)[21 22]. However, our determined prevalence was lower than that reported at a hospital in Saudi Arabia (79%) during 2006[11].

The median platelet count in patients with thrombocytopenia in our study was not as low as the reference value for severe cases (109,000/$mm^3$ vs. <40,000/$mm^3$)[23]. Thrombocytopenia (<100,000/$mm^3$) is less common in patients with dengue than in inpatients with other

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arbovirus infections[23-26]. 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[23-26].

Another retrospective study did not identify any sex-specific differences in the prevalence of thrombocytopenia [27]. 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. Several studies have shown clear differences in the prevalence of thrombocytopenia with respect to age. 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[28]. Age-related differences in immune function possibly affect the balance between protective and detrimental host immune responses[4 10 29]. The observed difference in mortality may also be related to the prevalence of chronic diseases, such as diabetes or heart disease[28],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 hemorrhagic manifestations when used during the course of a DENV infection [23-26 28 30 31].

The overall decrease in the leukocyte count observed in patients with dengue is mainly due to a decrease in the population of granulocytes (e.g., neutrophils)[32]. The ability of DENV to suppress white blood cell production in the bone marrow may explain mechanistically the appearance of leukopenia in patients with dengue[33].

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Patients with thrombocytopenia were approximately seven times more likely to exhibit a shift to leukopenia than those without thrombocytopenia. Most reports that describe frequent hematological changes during dengue infection noted that leukopenia is commonly observed[34].

One systematic review revealed that several clinical and laboratory measures could potentially distinguish people with dengue from those with other febrile viral diseases [35]. An increased hemoconcentration and hematocrit are commonly observed in patients with dengue infection[26]. Plasma extravasation leads to a high hematocrit value, which is the initial abnormality associated with dengue infection. A hematocrit value >20% over the baseline value is an important diagnostic criterion for dengue[23 36]. Hemoconcentration tends to occur in patients with hemorrhagic dengue. This tendency is defined solely based on the patient's initial hematocrit value.

In this study, patients with dengue and thrombocytopenia had a statistically higher median hematocrit 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 two-fold 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 hematological parameters that could be used to differentiate dengue and malaria in endemic areas of Thailand[29]. In our study, we found that most hematological alterations exhibited differences in sensitivity and specificity with respect to dengue and malaria. However,

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a 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 differential diagnosis of dengue fever and other febrile viral infections[29].

**Strengths and limitations of the study**

Although other studies[3 4] have investigated the presence of thrombocytopenia as the main hematological alteration in patients with dengue, ours is the first study to examine additional factors such as sex, age, ethnicity, education level, and hematological 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[37]. 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 hematological test results, the

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onset of infection, and the availability of more than one blood samples. 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 decentralized 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, hospitalization, and death.

## 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 etiology, 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 leukocyte counts and an elevated hematocrit were the most frequently observed hematological alterations during dengue, MCH may be a novel parameter worthy of monitoring and further exploration.

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## LIST OF ABBREVIATIONS

CI: confidence intervals

DENV: Dengue virus

DENV 1–4: virus serotypes

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

MLC: Municipal Laboratory of Campinas

OR: odds ratios

RDW: red blood cell distribution width

SINAN: Notification of Injury Information System

**Figure 1title:** Flow diagram of the stages of sample composition.

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## DECLARATIONS

**Authors' Contributions:** 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.

**Conflict of Interest Statement:** The authors declare that they have no conflicts of interests.

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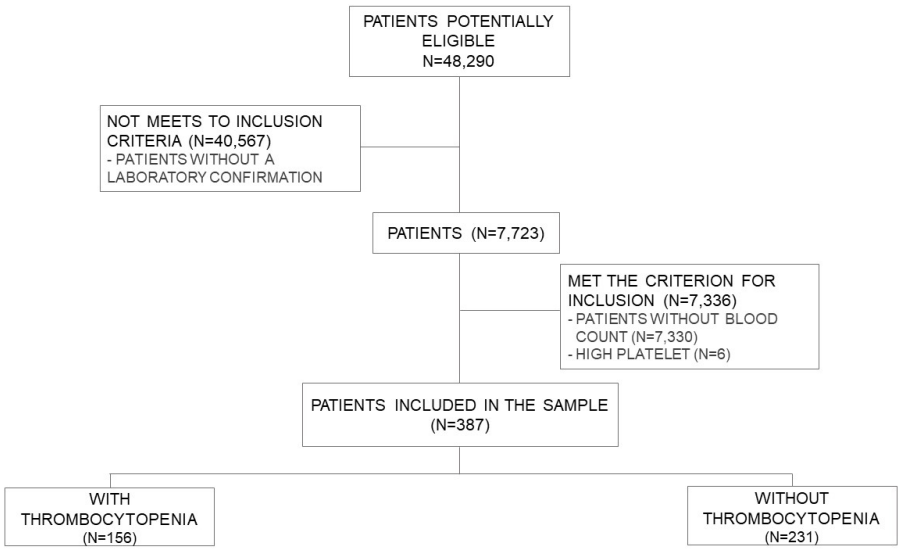


Figure 1 title: Flow diagram of the stages of sample composition.

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| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-7 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6-7 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | |
| Bias | 9 | Describe any efforts to address potential sources of bias | |
| Study size | 10 | Explain how the study size was arrived at | 7 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6-7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 7-8 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 7-8 |
| | | (*c*) Explain how missing data were addressed | 7-8 |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | 7 |
| | | (*e*) Describe any sensitivity analyses | 7-8 |
| **Results** | | | |

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| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | 8 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 9-10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| Outcome data | 15* | Report numbers of outcome events or summary measures | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 10-14 |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10-14 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 15 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 17-18 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 15-18 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 17-18 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 20 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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

## Authors

Bruna Mateus de Castilho, Marcus Tolentino Silva, André Ricardo Ribas Freitas, Izabela Fulone, Luciane Cruz Lopes

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



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# 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 hemorrhagic 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 hematological changes in patients with confirmed dengue fever.

**Design:** retrospective **cohort** study;

**Setting:** Brazilian multicenter primary care databases;

**Participants:** 387 patients with positive laboratory serologic 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:** presence of thrombocytopenia (platelet count < 150,000/mm$^3$). The associations of factors that predisposed patients to thrombocytopenia and hematological changes were analyzed using logistic regression. Odds ratios (ORs) and 95% confidence intervals (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: 1.16–2.71, p=0.007), age of 46–64 years (OR: 2.20, 95% CI: 1.15–4.21, p=0.009) or ≥ 65 years (OR: 3.02, 95% CI: 1.40–6.50, p=0.002), presence of leukopenia (OR: 6.85, 95% CI: 4.27–10.99, p < 0.001), and high mean corpuscular hemoglobin (MCH) levels (OR: 2.00, 95% CI: 1.29–3.12, p=0.005).

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

**Keywords:** *Aedes aegypti*; arboviruses; public health; thrombocytopenia

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**Strengths and limitations of this study**

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

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## 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 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 hematological changes in patients with dengue. The main reported changes include thrombocytopenia in 40–79% of cases, leukopenia 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 hematological 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, hematological changes, and other factors such as age, sex, and ethnicity in patients with dengue.

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## METHODS

### Ethical aspects

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 October 8, 2015 (CAAE: 46934815.0.0000.5426) according to Resolution CNS 466/12 of the National Health Council.

### 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 inhabitants[16] 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 nonstructural protein 1 [NS1], IgM/IgG serological tests or IgM enzyme-linked immunosorbent assay [ELISA]) was performed at the Adolfo Lutz Institute. The results were transferred to the Epidemiological

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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, Sao 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 <150,000/mm$^3$in two tests (normal range without thrombocytopenia: ≥150,000–400,000/mm$^3$). We excluded all patients with incomplete data (e.g., 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 serologic test results for dengue and available blood counts in the system, to be included in the study.

**Variables**

The predictive variables included hematological 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 days1 to 9 after symptom onset.

The reference values were as follows: leukocytes, 4,500–11,000/μL; erythrocytes, 4.10–5.90×10$^6$; hemoglobin**,** 12.3–17.5g/dL; hematocrit, 36–50%; mean corpuscular hemoglobin (MCH), 27–29pg; mean corpuscular volume (MCV), 77–92fL; mean

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corpuscular hemoglobin concentration (MCHC), 30–35g/dL; 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 odds ratios (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 analyzed 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 hematological changes such as leukocyte, erythrocyte, and platelet counts, hemoglobin level, hematocrit, MCH, MCV, MCHC, RDW, and blood collection dates. The chi-square test was used to analyze categorical variables[21]. The adjusted ORs were calculated using a logistic regression, which was adjusted by sex, age, and sample

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collection date. We also calculated the 95% confidence intervals (CIs). The medians of blood count variables were compared. The Kruskal–Wallis test was used to compare the hematological 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 were available.[21] Therefore, no data were imputed or attributed to observations. All analyses were performed using Stata, version 14.1 (Stata Corp LLC, College Station, TX, USA).

## 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, 7,336 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 231 (59.7%) did and did not have thrombocytopenia, respectively (**Figure 1**).

**Figure 1**

For the 387 included patients, blood was collected from 203 (52.4%) during days 1–3 days after the initial symptom onset, from143 (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: 35.5–43.5; median platelet count, 109,000/mm$^3$, interquartile range

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[IQR]: 89.7–126.2).The following factors were associated with thrombocytopenia: male sex (OR: 1.77, 95% CI: 1.16–2.71, p=0.007) and an age of 46–64 years (OR: 2.20, 95% CI: 1.15–4.21, p=0.009) or ≥ 65 years (OR: 3.02, 95% CI: 1.40–6.50, p=0.002), as shown in **Table 1**.

**Table 1**

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**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 (1.08–2.45) | 0.021 | 1.77 (1.16–2.71) | 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 (0.88–2.74) | 0.131 | 1.71 (0.95–3.06) | 0.072 |
| **46–64** | 43 (27.5) | 52 (22.5) | 1.94 (1.03–3.66) | 0.040 | 2.20 (1.15–4.21) | 0.018 |
| **≥65** | 16 (10.3) | 13 (5.6) | 2.89 (1.20–6.96) | 0.018 | 3.02 (1.40–6.50) | 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 (0.99–2.66) | 0.053 | 1.63 (0.98–2.70) | 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 (0.53–2.26) | 0.811 | 1.18 (0.54–2.58) | 0.677 |
| **Higher education incomplete** | 17 (23.6) | 22 (20.8) | 1.37 (0.62–3.03) | 0.441 | 1.22 (0.49–3.04) | 0.667 |
| **Higher education complete** | 8 (11.1) | 4 (3.8) | 3.54 (0.97–12.89) | 0.055 | 3.34 (0.86–13.04) | 0.082 |

OR, odds ratio; CI, confidence interval.
*Analysis adjusted by sex, age, and collection date (from 1 to 9 days after symptom onset).

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The presence of leukopenia (OR: 6.85, 95% CI: 4.27–10.99, p<0.001) and a high MCH level (OR: 2.00, 95% CI: 1.29–3.12, p=0.005) were identified as hematological changes associated with thrombocytopenia, as shown in **Table 2**.

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**Table 2: Hematologic 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 (/µL)** | | | | | | |
| Reference range** | 51(32.7) | 172 (74.5) | 1.00 | | 1.00 | |
| Changed range | 105 (67.3) | 59 (25.5) | 6.00 (3.84–9.38) | <0.001 | 6.85 (4.27–10.99) | <0.001 |
| **Erythrocytes (×10⁶)** | | | | | | |
| Reference range** | 141 (90.4) | 205 (88.7) | 1.00 | | 1.00 | |
| Changed range | 15 (9.6) | 26 (11.3) | 0.84 (0.43–1.64) | 0.607 | 0.73 (0.37–1.47) | 0.379 |
| **Hemoglobin (g/dL)** | | | | | | |
| Reference range** | 125 (80.1) | 197 (85.3) | 1.00 | | 1.00 | |
| Changed range | 31 (19.9) | 34 (14.7) | 1.44 (0.84–2.46) | 0.185 | 1.50 (0.87–2.60) | 0.145 |
| **Hematocrit (%)** | | | | | | |
| Reference range** | 136 (87.2) | 197 (85.2) | 1.00 | | 1.00 | |
| Changed range | 20 (12.8) | 34 (14.7) | 0.85 (0.47–1.54) | 0.597 | 0.81 (0.43–1.51) | 0.507 |
| **Mean corpuscular hemoglobin(pg)** | | | | | | |
| Reference range** | 50 (32.0) | 119 (51.5) | 1.00 | | 1.00 | |
| Changed range | 106 (68.0) | 112 (48.5) | 2.25 (1.47–3.44) | <0.001 | 2.00 (1.29–3.12) | 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 (0.51–1.39) | 0.500 | 0.84 (0.50–1.41) | 0.508 |
| **Mean corpuscular hemoglobin concentration (g/dL)** | | | | | | |
| Reference range** | 140 (89.7) | 209 (90.5) | 1.00 | | 1.00 | |
| Changed range | 16 (10.3) | 22 (9.5) | 1.09 (0.55–2.14) | 0.812 | 0.93 (0.46–1.90) | 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 (0.46–2.26) | 0.962 | 1.03 (0.46–2.31) | 0.948 |

OR, odds ratio; CI, confidence interval.

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

**Reference values: leukocytes: 4,500–11,000/µL; erythrocytes: 4.10–5.90×10⁶; hemoglobin: 12.3–17.5g/dL; hematocrit: 36–50%; mean corpuscular hemoglobin: 27–29pg; mean corpuscular volume:77–92fL; mean corpuscular hemoglobin concentration: 30–35g/dL; red cell distribution width: 10–15%.

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

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Of the median values of the evaluated hematological 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 leukocyte count than those without thrombocytopenia (3,750/µL, IQR: 2,790–4,725/µL vs. 5,760/µL, IQR: 4,480–7,520). All other median values, particularly the hematocrit (42.5%, IQR 40.6%–44.9%), erythrocyte count ($4.89\times10^6$, IQR: 4.60–$5.19\times10^6$), hemoglobin (14.50 g/dL, IQR: 13.33–15.28 g/L), and MCH (29.60 pg, IQR: 28.55–30.50pg), 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 leukocyte count and MCHC but an elevated MCH level (**Table 3**).

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**Table 3: Quantitative hematological 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** | 4,800 (3,480–6,560) | 3,750 (2,790–4,725) | 5,760 (4,480–7,520) | 0.0001 |
| **Erythrocytes** | 4.76 (4.50–5.10) | 4.89 (4.60–5.19) | 4.68 (4.44–5.03) | 0.0005 |
| **Hemoglobin** | 13.9 (13.0–14.8) | 14.50 (13.33–15.28) | 13.60 (12.80–14.40) | 0.0001 |
| **Hematocrit** | 41.50 (39.30–43.90) | 42.5 (40.6–44.9) | 40.70 (38.40–42.80) | 0.0001 |
| **Mean corpuscular hemoglobin** | 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 hemoglobin concentration** | 33.60 (32.80–34.30) | 33.80 (33.03–34.40) | 33.40 (32.70–34.30) | 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 |

IQR, interquartile range.

*Kruskal–Wallis test.

Normal reference values: leukocytes: 4,500–11,000/μL; erythrocytes: 4.10–5.90×$10^6$; hemoglobin: 12.3–17.5 g/dL; hematocrit: 36–50%; mean corpuscular hemoglobin: 27–29 pg; mean corpuscular volume: 77–92 fL; mean corpuscular hemoglobin concentration: 30–35 g/dL; red cell distribution width: 10–15%.

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## 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, leukopenia, and high MCH levels as factors associated with an increased risk of thrombocytopenia. Moreover, the median hematocrit, erythrocyte count, hemoglobin, 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. [12 22-25] The age between 18-45 years is also common in other researches. [12 22 23 25 26]

The World Health Organization 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 dengue virus affects bone marrow cells, inhibiting their function to reduce the proliferative capacity of hematopoietic 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

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value for severe cases (109,000/mm$^3$ vs. <40,000/mm$^3$).[31] Other study involving only inpatients with dengue fever also exhibited similar platelet rates.[22] Thrombocytopenia (<100,000/mm$^3$) 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 other studies, thrombocytopenia and platelet dysfunction are also related to the clinical outcome such as skin rush and hemophagocytosis.[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 hemorrhagic manifestations when used during the course of a DENV infection. [31 38]

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

Patients with thrombocytopenia were approximately seven times more likely to exhibit a shift to leukopenia than those without thrombocytopenia. Most reports that describe frequent hematological changes during dengue infection noted that leukopenia 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 hemoconcentration and hematocrit are commonly observed in patients with dengue infection.[34] Plasma extravasation leads to a high hematocrit value, which is the initial abnormality associated with dengue infection. A hematocrit value >20% over the baseline value is an important diagnostic criterion for dengue.[31 43] Hemoconcentration tends to occur in patients with hemorrhagic dengue. This tendency is defined solely based on the patient's initial hematocrit value.

In this study, patients with dengue and thrombocytopenia had a statistically higher median hematocrit 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 two-fold 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.

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One study explored hematological parameters that could be used to differentiate dengue and malaria in endemic areas of Thailand.[44] In our study, we found that most hematological alterations exhibited differences in sensitivity and specificity with respect to dengue and malaria. However, 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 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 hematological alteration in patients with dengue, ours is the first study to examine additional factors such as sex, age, ethnicity, education level, and hematological 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 hematological test results, the

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onset of infection, and the availability of more than one blood samples. 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 decentralized 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, hospitalization, 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 etiology, 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 leukocyte counts and an elevated hematocrit were the most frequently observed hematological alterations during dengue, MCH may be a novel parameter worthy of monitoring and further exploration.

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## LIST OF ABBREVIATIONS

CI: confidence intervals

DENV: Dengue virus

DENV 1–4: virus serotypes

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

MLC: Municipal Laboratory of Campinas

OR: odds ratios

RDW: red blood cell distribution width

SINAN: Notification of Injury Information System

**Figure 1 title:** Flow diagram of the stages of sample composition.

## DECLARATIONS

**Authors' Contributions:** 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.

**Conflict of Interest Statement:** The authors declare that they have no conflicts of interests.

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**Data sharing statement:** no database available. All data generated or analyzed during this study are included in this published article.

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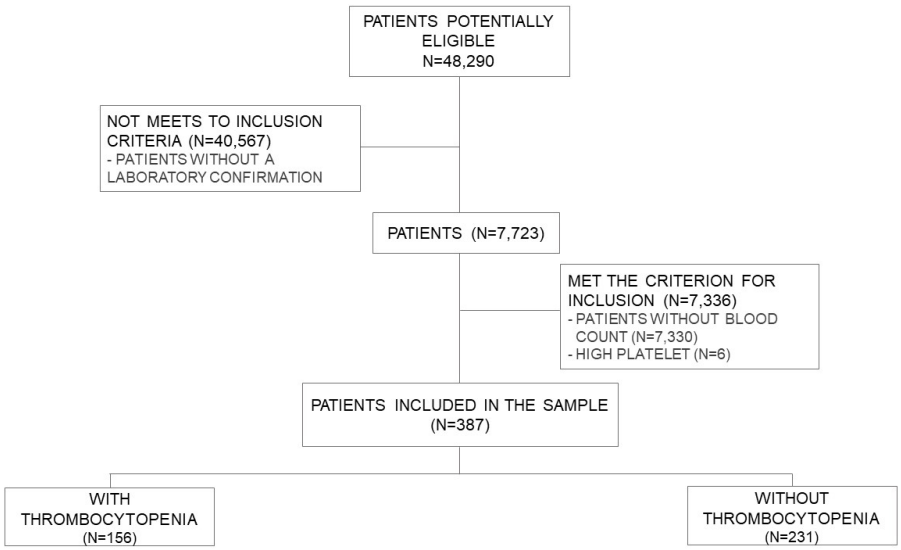


Figure 1 title: Flow diagram of the stages of sample composition.

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**STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***

| Section/Topic | Item # | Recommendation | Reported on page # |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-7 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants | 6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6-7 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5-6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | |
| Study size | 10 | Explain how the study size was arrived at | 7 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6-7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 7-8 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 7-8 |
| | | (*c*) Explain how missing data were addressed | 7-8 |
| | | (*d*) If applicable, describe analytical methods taking account of sampling strategy | 7 |
| | | (*e*) Describe any sensitivity analyses | 7-8 |
| **Results** | | | |

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| | | | |
|---|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | 8 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 8-10 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| Outcome data | 15* | Report numbers of outcome events or summary measures | |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 10-14 |
| | | (*b*) Report category boundaries when continuous variables were categorized | |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 10-14 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 15 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 18-19 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 15-18 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 17-18 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 21 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.



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