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# Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border

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## Abstract

**Background:** Malaria is a major mosquito-borne public health problem in Thailand with varied haematological consequences. The study sought to elucidate the haematological changes in people who suspected malaria infection and their possible predictive values of malaria infection.

**Methods:** Haematological parameters of 4,985 patients, including 703 malaria-infected and 4,282 non-malaria infected, who admitted at Phop Phra Hospital, Tak Province, an area of malaria endemic transmission in Thailand during 2009 were evaluated.

**Results:** The following parameters were significantly lower in malaria-infected patients; red blood cells (RBCs) count, haemoglobin (Hb), platelets count, white blood cells (WBCs) count, neutrophil, monocyte, lymphocyte and eosinophil counts, while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), neutrophil-lymphocyte ratio (NLR), and monocyte-lymphocyte ratio (MLR) were higher in comparison to non-malaria infected patients. Patients with platelet counts < 150,000/uL were 31.8 times (odds ratio) more likely to have a malaria infection. Thrombocytopenia was present in 84.9% of malaria-infected patients and was independent of age, gender and nationality (P value < 0.0001).

**Conclusion:** Patients infected with malaria exhibited important changes in most of haematological parameters with low platelet, WBCs, and lymphocyte counts being the most important predictors of malaria infection. When used in combination with other clinical and microscopy methods, these parameters could improve malaria diagnosis and treatment.

**Keywords:** Complete blood count, Phob Phra, Malaria infection, Thrombocytopenia

## Background

Changes in haematological parameters are likely to be influenced by any disease condition including endemic diseases, such as malaria, that can affect health of mankind with various clinical presentations. Malaria is a major cause of deaths in the tropical area of the world. Two hundred and nineteen million cases were reported worldwide in 2010 [1]. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. These changes involve the major cell types such as RBCs, leucocytes

and thrombocytes [2-5]. Malaria infected patients tended to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-malaria infected patients [2-4,6-8]. One study showed patients with higher WBCs count compared with community controls [9]. The most common complication during malaria infection is thrombocytopenia [6,10-12]. Persons with platelet counts < 150,000/ $\mu$ L were 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/ $\mu$ L [6]. A previous study found that the ratio of monocytes to lymphocytes correlated with risk of clinical malaria during follow-up [5]. In Thailand, about five million people are in the area of high malaria transmission mostly in border between Thailand

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and Burma [13]. Clinical diagnosis is widely used for diagnosis of malaria especially in these areas.

Fever and other signs and symptoms are known to be sensitive measures of malaria infection but they lack specificity and positive predictive values especially in areas where malaria is less prevalent [3,14] and it may be difficult to distinguish the sign and symptom of disease from other viral or bacterial infections [15]. Typically, microscopic slide examination of peripheral blood remains the most widely used test and is the gold standard for detecting malaria infection [16]. However, due to it requires technical expertise and is time-consuming in smear examinations. Haematological changes during malaria infection, such as thrombocytopenia and leucocytosis or leucopenia are well recognized. Diagnostic value of these haematological alterations may be easily obtained and useful in people living in malaria endemic areas.

Phop Phra is a district (Amphoe) in the southwestern part of Tak Province, northern Thailand. It is in part of the Thailand-Myanmar border and is the most common destination for ethnic minorities from Myanmar who migrate into Thailand for agricultural work. This province has the highest number of malaria cases for 10 consecutive years. The present study aim to examine the occurrences of haematological changes and their diagnostic values in people infected with malaria in Phop Phra Hospital. Haematological parameters including WBCs, RBCs, platelets, red cell distribution width (RDW), MCV, MCH, MCHC, and Hb level of people infected with malaria were compared with uninfected people.

## Methods

Data collection used in this study was approved by The Phob Phra Hospital and The Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects of Walailak University. The data were collected from the Medical Technology Laboratory Unit, Phob Phra Hospital, Tak Province during January to December 2009. Laboratory records of people suspected with malaria infection such as fever, other signs and symptoms as medical doctor recommended were reviewed. Four thousand nine hundred and eighty five patients with full data recorded were included in this analysis. This included 703 malaria and 4,282 non-malaria cases. The data were retrieved from the venous blood sample drawn into EDTA tubes for preparation of the thick and thin smears and automated for determination of Complete Blood Counts (CBCs). Blood counts were performed using BC-5200 Haematology Analyzer (Mindray, Nanshan, Shenzhen, China). The Analyzer provided data on WBCs, RBCs, Hb level, platelet counts, MCV, MCH, MCHC, RDW and five part differentials. The Haematology Analyzer have an internal quality control (IQC) two-times daily (First time in the morning before running

sample and second time in the afternoon) and also external quality control (EQC) three times per year with Department of Medical sciences. Blood slides were prepared and stained with Giemsa. Microscopic abnormality of blood in smear and presence or absence of malaria were determined, the species and the number of asexual parasites. Data analysis was performed using SPSS ver. 11.5 (SPSS Inc., Chicago, IL, USA). Normally distributed of continuous data were determined using The Kolmogorov-Smirnov Test. Comparing between 3 groups of continuous data was using a One-way ANOVA, whereas data not conforming to normal distribution were compared by a Kruskal-Wallis Test. Categorical data were compared using Pearson Chi-Square Test. Association between two continuous data was assessed by Pearson Correlation, whereas data not conforming to normal distribution were compared by Spearman's rank correlation. Diagnostic accuracy of haematological parameters was measured by calculating sensitivity, specificity, predictive values and odds ratios and 95% confidence intervals.

## Results

Four thousand nine hundred and eighty five patients were included in this study. The median age was 26 years (18-40) and 52.4% were males. Seven hundred and three ( $n = 4,985$ ) of the subjects had malaria infection confirmed by microscopy while the remaining were non-infection and were used as controls. In malaria-infected patients, three hundred and fifty-two (50.1%) had *Plasmodium falciparum* infection, whereas three hundred and fifty-one (49.9%) had *Plasmodium vivax* infection. There were significant differences between age, gender, and nationality of patients with group of patients ( $P$  value  $< 0.0001$ ). Malaria positive patients were Burma 50.5%, followed by Thai 49.2% and other nationalities 0.3%, respectively (Table 1).

The median values of haematological parameters of falciparum malaria, vivax malaria and non-malaria infected group were compared using Kruskal-Wallis Test due to non-parametric distribution of data. Median values of RBCs, platelets, WBCs, and all absolute leukocyte components counts were significantly lower in patients with falciparum malaria compared to those with vivax malaria and non-malaria infected groups. Conversely, median of MCV, MCH, MCHC, NL ratio, and ML ratio were significantly higher in falciparum malaria than those with vivax malaria and non-malaria infected groups. A median value of Hb was lower in patients with falciparum malaria compared to those with vivax malaria and non-malaria infected groups. Monocyte and eosinophil counts were higher in patients with vivax malaria compared to those with falciparum malaria and non-malaria infected groups. There was no significant difference in median of RDW between three groups (Table 2).

**Table 1 General characteristics**

	<i>Plasmodium falciparum</i> n = 352	<i>Plasmodium vivax</i> n = 351	Non-malaria n = 4,282	P value
Demographic				
Age, median (IQR)	26 (18-40)	23 (16-36)	16 (7-35)	<0.0001*
Male/female, n (%)	215 (61.1)/137 (38.9)	209 (59.5)/142 (40.5)	2,190 (51.1)/2,092 (48.9)	<0.0001**
Thai/Burma/Others, n (%)	160 (45.5)/192 (54.5)	186 (53)/165 (47)	3,230 (75.4)/1,052 (24.6)	<0.0001**

\*Comparison of 3 groups using The Kruskal Wallis Test, \*\*Comparison of 3 groups using Pearson Chi-Square, IQR = interquartile range.

### Red blood cells

Anaemia was defined as Hb level <11g/dl for both males and females based on the WHO cutoff value. The median of Hb in patients with falciparum malaria (11.7 g/dL) was significantly lower than those with vivax malaria (11.9 g/dL) and non-malaria (11.8 g/dL) groups (P value = 0.041). One hundred and thirty-four (38.1%) of patients with falciparum malaria; one hundred and fifteen (32.8%) of patients with vivax malaria; one thousand three hundred and sixty-five (31.9%) of patients with non-malaria groups had anaemia. There was no significant association between status of malaria infection and Hb cut-off (P value = 0.057). The median of RBCs count was significantly lower in patients with falciparum malaria ( $4.33 \times 10^6/\mu\text{L}$ ) than non-falciparum malaria ( $4.45 \times 10^6/\mu\text{L}$ ) and non-malaria ( $4.63 \times 10^6/\mu\text{L}$ ) groups (P value <0.0001). Patients with RBCs count less than  $4 \times 10^6/\mu\text{L}$  was frequently seen in patients with falciparum malaria (32.4%) compared to those with vivax malaria (22.2%) and non-malaria (13.6%) groups (P value <0.0001). The median of MCV, MCH, and MCHC in both patient with falciparum malaria and vivax malaria were significantly

higher than those with non-malaria group (P value < 0.001). There was no significant association between status of malaria infection and MCV > 100fL, MCH > 32pg/cell, and MCHC >37g/dL cutoff value (P value > 0.05).

### White blood cells

Leucopenia was defined as total WBCs count < 4,000/ $\mu\text{L}$ . There was significant difference in total WBCs count between three groups of patients (P value <0.0001). Leukocyte components were also significantly affected. Neutrophil, lymphocyte, monocytes, eosinophil and basophil counts were all significantly decreased in patients with falciparum malaria and vivax malaria as compared to those with non-malaria group (P value < 0.0001). Neutrophil count > 2,800/ $\mu\text{L}$  was frequently seen in the malaria-infected group (27.9%) than the non-malaria infected group (19.5%) (P value <0.0001). Lymphocytes < 800 / $\mu\text{L}$  (lymphocytopenia) was frequently seen in the malaria-infected group (20.1%) than the non-infected group (4.6%) (P value < 0.0001). There were significant differences in NL ratio and ML ratio between

**Table 2 Haematological values in study population**

Variable	<i>Plasmodium falciparum</i> Median (IQR)	<i>Plasmodium vivax</i> Median (IQR)	Non-malaria Median (IQR)	P value*
RBC ( $\times 10^6/\mu\text{L}$ )	4.33 (3.78–4.8)	4.45 (4.04–4.88)	4.63 (4.25–5.02)	<0.0001
Haemoglobin (g/dL)	11.7 (9.8–13.1)	11.9 (10.4–13)	11.8 (10.5–13.1)	0.041
MCV (fL)	83.45 (78–87.7)	82.2 (75–86.8)	81.7 (74.6–87.3)	0.001
MCH (pg/cell)	27 (25–28.9)	26.9 (24–28.7)	26.3 (23.5–28.6)	<0.0001
MCHC (g/dL)	32.6 (31.6–33.4)	32.5 (31.7–33.3)	26.3 (23.5–28.6)	<0.0001
RDW (%)	12.8 (12.1–13.9)	12.8 (12.1–13.9)	12.7 (12–14)	0.967
Platelet ( $\times 10^3/\mu\text{L}$ )	72.5 (44–112)	89 (63–133)	242 (181–317)	<0.0001
WBC ( $\times 10^3/\mu\text{L}$ )	5.76 (4.52–7.99)	5.59 (4.48–7.18)	8.71 (6.17–12.4)	<0.0001
Neutrophil ( $\times 10^3/\mu\text{L}$ )	3.82 (2.77–5.18)	3.44 (2.63–4.51)	5.08 (3.23–8.04)	<0.0001
Lymphocyte ( $\times 10^3/\mu\text{L}$ )	1.28 (0.83–1.98)	1.31 (0.9–1.89)	2.29 (1.55–3.41)	<0.0001
Monocyte ( $\times 10^3/\mu\text{L}$ )	0.4 (0.25–0.61)	0.4 (0.26–0.6)	0.57 (0.38–0.84)	<0.0001
Eosinophil ( $\times 10^3/\mu\text{L}$ )	0.17 (0.73–0.31)	0.2 (0.08–0.37)	0.24 (0.12–0.47)	<0.0001
Basophil ( $\times 10^3/\mu\text{L}$ )	0.05 (0.04–0.06)	0.06 (0.04–0.07)	0.08 (0.06–0.11)	<0.0001
NL ratio	2.8 (2.81–4.88)	2.67 (1.69–4.22)	2.17 (1.2–3.95)	<0.0001
ML ratio	0.3 (0.3–0.43)	0.29 (0.19–0.47)	0.24 (0.17–0.38)	<0.0001

\*Comparison of 3 groups using The Kruskal Wallis Test.

three groups of patients ( $P$  value < 0.0001). High NL ratio (>2.8) was frequently seen in the malaria-infected group than the non-infected group ( $P$  value < 0.0001). High ML ratio (>0.25) was frequently seen in the malaria-infected group than the non-infected group ( $P$  value < 0.0001).

#### Platelets

Thrombocytopenia was defined as platelets count < 150,000/ $\mu$ L. There were 597 patients with Thrombocytopenia (84.9%) in the malaria-infected group. The median of platelet count in patients with falciparum malaria ( $72.5 \times 10^3/\mu$ L) and vivax malaria ( $89 \times 10^3/\mu$ L) were significantly lower than those with non-malaria ( $242 \times 10^3/\mu$ L) group ( $P$  value < 0.0001). Thrombocytopenia was also associated with anaemia in the malaria group ( $r = -0.107$ ,  $P$  value = 0.004) and age ( $r = -0.235$ ,  $P$  value < 0.0001).

#### Diagnostic values of haematological parameters

Most of haematological parameters had good specificity but lack of sensitivity to detect infection of malaria parasite. However, low platelet count with platelet count < 150,000/uL had high sensitivity (85%) and specificity (85%) for diagnosis of malaria infection. This parameter had better odds ratios. Patients with platelets count < 150,000/uL were up to 31.8 (OR) times more likely to have malaria infection than those with normal platelets count ( $P$  value < 0.0001, OR = 31.8, CI = 25.1-39.7) (Table 3). It could be predict that the association between thrombocytopenia and malaria infection would not be significant following adjustment for age, gender, and nationality of patients. Age, gender, and nationality, correlated with risk of malaria infection (Table 4). However, adjusting for these demographic variables did not

confound the association between thrombocytopenia and malaria infection.

#### Discussion

This study confirms that haematological abnormalities in malaria infection are common. Leucopenia was frequently seen in the malaria-infected patients which was confirmed by other studies that have demonstrated leucopenia [6,15] and contrast with another study that had demonstrate leucocytosis [3]. Neutrophil and lymphocyte counts were the most important leukocytic changes associated with malaria infection. The decrease in lymphocyte counts associated with malaria observed in this study may due to reflect redistribution of lymphocytes with sequestration in the spleen [6,17]. The decrease in neutrophil count associated with malaria infection was in contrast with previous studies which suggested that it might be activated neutrophil production or release from the marrow or suppressed peripheral removal [3].

The observation of decreased monocytes counts in this study was also in contrast with previous studies [3,18]. Mononuclear cells, which are activated by Plasmodium during malarial infection, produce inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1 (IL1) and interleukin-6 (IL6). These cytokines stimulate the hepatic synthesis of acute phase inflammatory proteins, including CRP, which increase during malaria infection [17].

Anaemia is one of the most common complications in malaria infection especially in younger children and pregnant women in high transmission areas [19]. The pathogenesis of anaemia during malaria infection is not

**Table 3 Sensitivity, specificity, predictive value and odds ratio of the haematological parameters in diagnosis of Malaria**

Variable	Sensitivity* (95% CI)	Specificity* (95% CI)	PPV* (95% CI)	NPV* (95% CI)	OR (95% CI)	PLR	NLR
RBC < $4 \times 10^6/\mu$ L	27 (24–31)	86 (85–87)	25 (22–28)	88 (87–89)	2.4 (2–2.9)	2	0.8
Hb < 11 g/dL	35 (32–39)	68 (67–70)	15 (14–17)	87 (85–88)	1.2 (1–1.4)	1.1	0.9
MCV > 100 fL	1 (0.5–2)	98 (97–99)	10 (4–19)	86 (85–87)	0.7 (0.3–1.4)	0.7	1
MCH > 32 pg/cell	3 (2–5)	97 (96–97)	13 (8–19)	86 (85–87)	0.9 (0.6–1.4)	0.9	1
MCHC > 37 g/dL	2 (1–4)	98 (98–99)	18 (11–28)	86 (85–87)	1.4 (0.8–2.4)	1.4	1
Platelets < 150,000/ $\mu$ L	85 (82–87)	85 (84–86)	48 (45–51)	97 (96–98)	31.8 (25.1–39.7)	5.6	0.2
WBCs < 4,000/ $\mu$ L	17 (14–20)	94 (93–94)	30 (26–35)	88 (87–89)	3 (2.4–3.7)	2.7	0.9
Neutrophils < 2,800/ $\mu$ L	35 (32–39)	69 (68–70)	16 (14–17)	87 (86–89)	1.2 (1.0–1.4)	1.1	0.9
Lymphocytes < 800/ $\mu$ L	25 (23–28)	78 (77–79)	17 (15–19)	86 (85–87)	1.4 (1.1–1.6)	1.2	1
Monocytes < 80/ $\mu$ L	3 (2–5)	98 (97–99)	19 (12–26)	86 (85–87)	1.4 (0.9–2.2)	1.4	1
Eosinophils < 40/ $\mu$ L	27 (24–30)	71 (69–72)	13 (11–15)	86 (84–87)	0.9 (0.8–1)	0.9	1
Basophils < 16/ $\mu$ L	89 (86–91)	12 (11–13)	14 (13–15)	86 (83–89)	1.0 (0.8–1.3)	1	1
NL ratio > 2.8	49 (45–52)	61 (60–63)	17 (15–19)	88 (87–89)	1.5 (1.3–1.8)	1	0.8
ML ratio > 0.25	58 (54–62)	54 (53–56)	17 (16–19)	89 (88–90)	1.7 (1.4–1.9)	1.3	0.8

PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio, \*Percent, OR, odds ratio.

**Table 4 The association between risk of malaria and platelet count is independent of age, gender and nationality**

Analysis type	Variable	Malaria positive/negative	
		B*	P value
Univariate	Platelets <150,000	0.024	<0.0001
	Age	-0.01	<0.0001
	Gender	0.373	<0.0001
	Nationality	-1.153	<0.0001
Multivariate	Platelets <150,000	0.024	<0.0001
	Age	0.014	<0.0001
	Gender	-0.115	0.287
	Nationality	0.935	<0.0001
	Constant	-2.926	<0.0001

\*unstandardized variables.

clearly understood. However, It is thought to result from the parasite's primary target is the red blood cell resulting in RBCs destruction, accelerated removal of both parasitized and non-parasitized [20], bone marrow dysfunction [21] and the level of parasitemia [22]. This study, reported a significant reduction of Hb level and RBC count whereas MCV, MCH, and MCHC level in patients infected with malaria were higher as compared to non-malaria infected group. There are no evidence relate to higher level of MCV, MCH, and MCHC and pathogenesis of malaria infection but it has been suggested that people with  $\alpha^+$ -thalassaemia protected malaria infection by a direct interaction between the parasite (*Plasmodium falciparum*) and the altered thalassaemic erythrocyte, resulting in reduced parasite load [23]. Most studies showed that  $\alpha^+$ -thalassaemia homozygotes have better protection against severe malarial anaemia compared to heterozygotes [24,25]. Moreover, patients with microcytosis and higher erythrocyte count associated with  $\alpha^+$ -thalassaemia homozygosity had an advantage against severe malarial anaemia during acute infection with *Plasmodium falciparum* [26].

Platelet abnormalities in malaria are both qualitative and quantitative change. In this study, platelet counts were significantly reduced in malaria-infected people. Thrombocytopenia occurred in 84.9% of malaria-infected patients. These observations may imply that thrombocytopenia may be a marker of *Plasmodium* infection. The association of platelet count and malaria infection has previously been described [3,7,18]. Patients with thrombocytopenia were also likely to have an anaemia ( $r = -0.107$ ,  $P$  value = 0.004) and correlate with age ( $r = -0.235$ ,  $P$  value < 0.0001) as previously reported from a study in Kenya and Nigeria [3,8]. There were various hypotheses about thrombocytopenia occurring in malaria infection. Thrombocytopenia seems to occur

through peripheral destruction [9], excessive removal of platelets by spleen pooling [27,28] as well as platelet consumption by the process of disseminated intravascular coagulopathy (DIC) [29]. Adequate or increased number of megakaryocytes in the bone marrow affects decreased thrombopoiesis an unlikely cause of thrombocytopenia in malaria [27]. Immune-mediated destruction of circulating platelets has been postulated as a cause of thrombocytopenia seen in malaria infection. Platelets have also been shown to mediate clumping of *P. falciparum* infected erythrocytes [30]. This could lead to pseudo thrombocytopenia. Malaria-infected patients have elevated levels of specific immunoglobulinG (IgG) in the blood which binds to platelet-bound malaria antigens possibly leading to accelerated destruction of platelets [10]. The previous study revealed that platelet aggregation, which is the platelet clumps are falsely counted as single platelet by the analyzers thus causing pseudo-thrombocytopenia [3]. Additionally, during malaria infection, endothelial activation was activated and may contribute to loss of barrier function of the endothelium and organ dysfunction. This process may use platelets and their released proteins as an important regulator of endothelial permeability resulting in thrombocytopenia [31].

In this study, the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for all haematoparameter were determined. Among of those parameter, thrombocytopenia (platelet counts < 150,000/ $\mu$ l) had the best sensitivity, specificity and negative predictive value (85% sensitivity, 85% specificity, and 97% negative predictive value) which were higher than the previous studies [11,12] while the positive predictive value was low (48%). The two most reliable haematological parameters for predicting malaria in people from endemic areas were thrombocytopenia and leucopenia. Patients with thrombocytopenia were 31.8 times more likely to have malaria infection than those with normal platelets count. Thrombocytopenia and leucopenia had sensitivity of 85% and 17%, specificity 85% and 94%, respectively to predict malaria infection. Patients with leucopenia (WBCs <4,000/ $\mu$ L) were 2.7 times more likely to have malaria infection than those with normal leucocytes count. High neutrophil counts, leucopenia, and lymphopenia had good specificity but lacked sensitivity to screening of malaria infection. The monocytes to lymphocytes ratio (ML ratio) in this study was high supported by the previous study that the ML ratio, which was measured in peripheral blood was directly correlates with risk of clinical malaria during follow-up [5]. This may due to the major role of the monocytes in the first time of innate immune response by releasing IFN $\gamma$ , to response to malaria infection [32]. When considered the neutrophil to lymphocytes ratio

(NL ratio) in this study, it was also high which supported that the NL ratio was found to correlate with malaria parasitaemia [4].

Limitations of this study included lack of previous medical histories such as other diseases that may have analysis bias such as Hb diseases, anaemia, bacteria or virus infection, which could potentially affect the interpretation of the results.

## Conclusions

In conclusion, the association of haematological parameters and a diagnosis of malaria infection among people living in malaria endemic area was retrieved. The most commonly changed parameters were platelet count, Hb, RBC, MCV, MCH, MCHC, WBC, neutrophil, and lymphocyte counts. Presence of thrombocytopenia in people from endemic areas may be useful as supportive diagnostic criteria for malaria in case with low level of parasite number. Therefore, when used with other clinical and microscopy parameters, it can significantly improve malaria diagnosis and timely further treat for malaria infection. The large sample size used in this study gives this analysis power to detect differences between malaria infected and non-malaria infected people.

## Competing interests

The authors declare that they have no conflict of interest.

## Authors' contributions

MK participated in study design, data analysis, statistical analysis, and manuscript writing and. BP and NP participated in data collection including CBC and malaria microscopy. SD and CC participated in data analysis and manuscript writing. All authors read and approved the final manuscript.

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