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FULL PAPER

Clinical Utility of Platelet Count for Screening of Malaria

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SUMMARY

Light microscopy, immunochromatographic rapid diagnostic tests and molecular methods are widely used to diagnose malaria. The aim of this study was to find variables among commonly available urgent blood tests to identify patients with low probability of having malaria in small-scale healthcare facilities in which none of the described methods is feasible within a short time. Diagnosis of malaria was made by examining both stained thick and thin blood films by light microscopy. Two hundred and eleven samples were included. Reduced platelet count and increased values of C-reactive protein (CRP) and total bilirubin were the variables most strongly associated with malaria ($P<0.0001$). The best screening cut-off values obtained by receiver operating characteristic curve analysis for a negative result for malaria were: platelets ≥185,000 cells/μl; CRP ≤2 mg/dl; total bilirubin ≤0.28 mg/dl. The logistic regression model of log-transformed variables showed how platelet count was the only independent variable related to the odds of having a negative blood film result for malaria (odds ratio: 2.621; 95% confidence interval: 1.441–4.768; $P=0.002$). A platelet count of ≥185,000 cells/μl can be considered a screening value to identify patients with high-probability of a negative blood film result for malaria.

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INTRODUCTION

Malaria is a life-threatening parasitic disease of public health importance in many parts of the world (Talapko *et al*., 2019; World malaria report, 2019). In 2018, an estimated 228 million cases of malaria occurred worldwide, 231 million cases in 2017 and 251 million cases in 2010 (World malaria report, 2019). The highest percentage occurred in the African Region (213 million or 93%), followed by South-East Asia with 3.4% of the cases and the Eastern Mediterranean Region with 2.1% (World malaria report, 2019). In 2018, there were an estimated 405,000 deaths from malaria globally, compared with 416,000 estimated deaths in 2017 and 585,000 in 2010 (World malaria report, 2019).

Malaria is caused by protozoan parasites of the *Plasmodium* genus and six species can infect humans: *Plasmodium malariae*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium knowlesi,* and *Plasmodium ovale spp.* including *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri* (Ashley *et al*., 2018; Fuehrer *et al*., 2014; Sutherland *et al*., 2010; Talapko *et al*., 2019). The clinical presentation of uncomplicated malaria is characterized by flulike signs and symptoms such as fever, chills, cough, headache, myalgias, abdominal pain and diarrhea (Ashley *et al*., 2018). Therefore, every patient reporting prior travel to a malaria-endemic country who complains of any of such non-specific symptoms should be considered potentially affected by malaria. Indeed, in *P. falciparum* malaria, the clinical picture may progress very rapidly to severe malaria, especially in pediatric patients, and is associated with high mortality (Bartoloni *et al*., 2012). Light microscopy of stained blood films by Giemsa, rapid diagnostic tests (RDT) and molecular methods are widely used to diagnose malaria (Amir *et al*., 2018; Moody, 2002; Tangpukdee *et al*., 2009).

In the differential diagnosis of febrile patients returning from travel to tropical regions, Dengue and Chikungunya (caused by Dengue virus and Chikungunya virus, respectively) also have to be considered: all three diseases share both endemicity and clinical presentation, which can result in missing the diagnosis of a possible co-infection (Salam *et al*., 2018). Several reports have shown that a high level of suspicion should be maintained for Dengue and Chikungunya even in non-endemic countries like Italy (Burdino *et al*., 2011; Burdino *et al*., 2015). The diagnosis of Dengue and Chikungunya in the viremic phase (lasting a few days after infection) relies on viral genome detection, viral culture and serologic testing, whereas later it relies on serologic testing (Burdino *et al*., 2016; Mardekian *et al*., 2015).

There are health care settings in which none of the described methods for diagnosis of malaria is feasible within a short time. Therefore, since it has been described that malaria causes alterations in several laboratory parameters (Bhardwaj *et al*., 2019; Casalino *et al*., 2002; Paintsil *et al*., 2019; Taylor *et al*., 2010; Ullah *et al*., 2018), the aim of this study was to find possible variables, among the urgent blood tests commonly available in small-scale healthcare facilities, to identify patients with a low probability of having malaria.

Key words:
Malaria, Platelet Count, Microbiology, Diagnosis, Biostatistics.

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MATERIALS AND METHODS

Design of the study

We performed a retrospective analysis of results of blood samples drawn from patients admitted to S.S. Antonio e Biagio e Cesare Arrigo General Hospital of Alessandria, Italy, in the period from August 2011 to October 2019, already processed as part of routine clinical care. Inclusion criteria were: only the first request from patients with suspected malaria inclusive of microscopic examination of blood smear for identification of parasites and urgent blood tests such as complete blood count, C-reactive protein (CRP), total bilirubin, sodium, potassium, alanine aminotransferase (ALT) and creatinine, all requested simultaneously by the attending physician. Exclusion criteria: any further sample drawn from the same positive patient as well as any first request not including concurrently all the tests listed above.

Malaria diagnosis

The diagnosis of malaria along with species identification and determination of parasitemia were made by gold standard technique, such as examination by light microscopy of both stained thick and thin blood films (Mathison *et al*., 2017) from blood samples collected in ethylenediaminetetraacetic acid tubes obtained via venipuncture. Thick films were prepared by dropping 50 µl of blood on the glass slide and then spreading it into a circle of around 2 cm in diameter. Thin films were made by dropping 2-5 µl of blood on the glass slide and then spreading it so as to progressively decrease its thickness toward the feathered edge, then fixing in absolute methanol and drying completely prior to staining (Mathison *et al*., 2017). Both thick and thin films were then stained with Giemsa, and thin film was used for both species identification and calculation of percent parasitemia.

Statistical Analysis

Values are expressed as median and interquartile range (IQR) or percentages, as appropriate. A univariate analysis was performed by comparing median values by means of Mann-Whitney U test. A receiver operator characteristic (ROC) curve analysis was performed to look for the best cut-off values by means of Youden index, among the variables having *P* values <0.001 in the univariate analysis. A subsequent binary logistic regression analysis was performed to develop a predictive model of blood film result for malaria. Due to the skewness of the distribution, the natural log transformation was used for the variables included in the regression model. The predictive value of the model was evaluated by a ROC curve. SPSS statistical package, release 17.0 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. The significance level was set at $P \leq 0.05$.

Ethical considerations

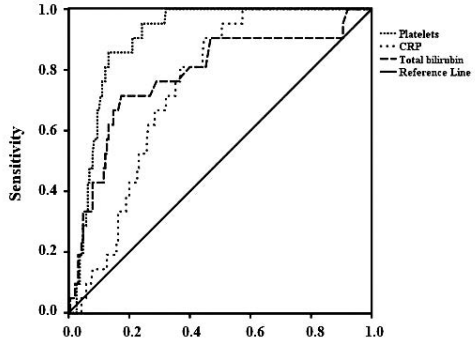
The research complied with all relevant international guidelines and institutional policies. Ethical approval was not needed because this is a retrospective analysis of data from samples collected as part of standard care and those included in the database were de-identified before access. No personal information was stored in the study database. No patient intervention occurred with the obtained results.

Table 1 - *Characteristics of the whole population and comparison of median values of urgent blood tests according to blood film result.*

	Whole sample (n=211)		*Positive for malaria (n=21)*		*Negative for malaria (n=190)*		
Variable	*Median*	*IQR*	*Median*	*IQR*	*Median*	*IQR*	*P*
Age (years)	38	24-52	42	12.5-49	37.5	25-53	0.461
Red blood cell count (cells/mm³)	4,500,000	3,930,000-4,920,000	4,290,000	3,715,000-4,555,000	4,555,000	3,945,000-4,930,000	0.163
Hemoglobin (g/dl)	13	11.1-14.2	12.2	10.3-13.2	13.2	11.2-14.3	0.051
Hematocrit (%)	39.2	33.6-42.4	35.8	31.6-39.2	39.5	33.7-42.5	0.080
Platelets (cells/µl)	203,000	138,000-273,000	89,000	53,000-115,000	213,000	164,750-282,250	<0.0001
White blood cell count (cells/mm³)	6380	4490-10,110	4710	4220-6470	6665	4675-10,262	0.053
Neutrophils (cells/mm³)	3880	2570-6940	2740	1900-4055	4010	2680-7207	0.006
Eosinophils (cells/mm³)	60	20-130	20	15-55	70	30-152	0.001
Basophils (cells/mm³)	30	20-50	30	20-50	30	20-50	0.639
Lymphocytes (cells/mm³)	1290	820-1960	1220	770-1970	1295	817-1945	0.760
Monocytes (cells/mm³)	430	270-590	400	270-575	430	275-590	0.783
Neutrophils/lymphocytes ratio	2.9	1.7-6.4	1.7	0.9-6.1	3.2	1.8-6.4	0.034
C-reactive protein (mg/dl)	3.3	0.7-9.8	9.7	5.3-13.9	2.9	0.6-8.3	<0.0001
Total bilirubin (mg/dl)	0.5	0.4-0.7	1.1	0.6-1.8	0.5	0.4-0.6	<0.0001
Na (mEq/l)	139	136-140	136	134.5-140	139	136-140	0.106
K (mEq/l)	4.2	3.8-4.4	4	3.8-4.2	4.2	3.8-4.4	0.134
ALT (IU/l)	23	16-39	37	19.5-57.7	23	15-37	0.032
Creatinin (mg/dl)	0.8	0.6-0.9	0.8	0.6-0.9	0.7	0.6-0.9	0.976

RESULTS

During the period considered, a total of 211 samples from as many patients were included in the study; 60.2% (127/211) were males. The requesting wards were: Emergency Medicine: 52.6% (111/211); Infectious Diseases: 17.1% (36/211); Intensive Care Unit: 10.9% (23/211); Pediatrics: 7.6% (16/211); Internal Medicine: 6.6% (14/211); Hematology: 5.2% (11/211). Twenty-one out of 211 patients (9.9%) were positive and the species identified were: *Plasmodium falciparum*: 85.7% (18/21); *Plasmodium ovale*: 9.5% (2/21); *Plasmodium vivax*: 4.8% (1/21) with a median parasitemia of 0.6% (IQR: 0.007%–1.5%). The characteristics of the whole population and the comparison of median values of urgent blood tests according to blood film result are described in *Table 1*. Reduced values of platelets and increased values of CRP and total bilirubin were the variables most strongly associated with blood film result; *Figure 1* shows the areas under the curve (AUCs). The best diagnostic cut-off found for the variables considered were: platelets: 124,000 cells/µl (85.7% sensitivity; 86.8% specificity); CRP: 3.6 mg/dl (90.5% sensitivity; 55.3% specificity); total bilirubin: 0.78 mg/dl (71.4% sensitivity; 82.6% specificity). Considering the same variables as a screening tool (100% sensitivity), the values found associated with a negative blood film result for malaria were: platelets ≥185,000 cells/µl; CRP ≤2 mg/dl; total bilirubin ≤0.28 mg/dl. On the other hand, since none of the three selected variables showed enough specificity to be used as a diagnostic test, the binary logistic regression model was built using negative blood film result for malaria as outcome (*Table 2*). The explained variance of the model calculated by Nagelkerke R-Square was 33.2% and the *P* value for the Hosmer-Lemeshow goodness-of-fit test was 0.979, indicating good discrimination and calibration. As conceivable, the ROC curve plotted by using the predicted probabilities of the model gave substantially the same area under the curve (AUC) of platelets alone: AUC: 0.890; 95% confidence interval: 0.829–0.951; *P*<0.0001.



Variable	AUC	95% CI	*P*
Platelets (cells/µl)	0.902	0.856-0.947	<0.0001
C-reactive protein (mg/dl)	0.741	0.663-0.819	<0.0001
Total bilirubin (mg/dl)	0.781	0.665-0.897	<0.0001

AUC: area under the curve; CI: confidence interval.

Figure 1 - *Receiver operator characteristic curve analysis for platelets, C-reactive protein (CRP) and total bilirubin in predicting blood film result for malaria.*

Table 2 - *Logistic regression analysis of log-transformed values of: platelets (Log-PLT), C-reactive protein (Log-CRP) and total bilirubin (Log-Bil) for negative blood film result for malaria.*

Variable	*OR*	*95% CI*	*P*
Log-PLT	2.621	1.441-4.768	0.002
Log-CRP	0.562	0.337-1.038	0.067
Log-Bil	0.493	0.220-1.107	0.087

OR: odds ratio; CI: confidence interval.

DISCUSSION

The aim of this study was to evaluate the complete blood count and some of the basic metabolic panel tests as possible screening variables to identify patients with a low probability of having malaria in small-scale healthcare facilities without the possibility of using microscopy, RDT or molecular methods within a short time.

The finding of a low platelet count associated with malaria has already been described by Paintsil (Paintsil *et al*., 2019) on a total of 2076 patients and also by Gupta (Gupta *et al*., 2019) on 159 patients in which the severity of malaria was assessed by the level of thrombocytopenia. Similarly, in a systematic review that evaluated seven studies, Taylor (Taylor *et al*., 2010) found that platelets below $150*10^3$/µl accurately diagnosed malaria and a normal platelet count could be used to exclude it. Similarly, Casalino (Casalino *et al*., 2002), on a total of 783 patients admitted to the ED, found thrombocytopenia to be significantly associated with a diagnosis of malaria. There are reports describing patients affected by malaria with platelet count within the normal range (Ladhani *et al*., 2002; Shaikh *et al*., 2011). Even if in this study we found a platelet count threshold of ≥185,000 cells/µl, thus above the lower limit of normal platelet count (150,000 cells/µl), this threshold has to be considered only an adjunctive tool to estimate the probability of having a blood film negative for malaria, and should not replace examination of both stained thick and thin blood films by light microscopy.

With regard to low values of neutrophils and eosinophils found significantly associated with malaria, the finding of these leukocyte components as significantly decreased in patients with *P. falciparum* and *P. vivax* malaria as compared to those with non-malaria group has been described by Kotepui (Kotepui *et al*., 2014) in a study of 4985 patients.

The significantly higher values of CRP found in patients with malaria match the results of Bhardwaj (Bhardwaj *et al*., 2019), who, in a study of 74 *P. falciparum* patients and 22 healthy controls, found CRP significantly higher in subjects with malaria and even higher in severe malaria vs. uncomplicated malaria. Likewise, Paul (Paul *et al*., 2012) found CRP as predictive of both hospital stay and mortality in 71 patients with malaria.

Finally, the significantly higher levels of total bilirubin found to be associated with a diagnosis of malaria has already been described in the literature; as for low platelet count, both Taylor (Taylor *et al*., 2010) and Casalino (Casalino *et al*., 2002) also found hyperbilirubinemia as predictive of malaria.

The logistic regression model and the ROC curve plotted by using the predicted probabilities showed that platelet

count was the only independent variable related to the odds of having a negative blood film result for malaria.

An association with reduced platelet count and increased bilirubin levels has likewise been reported with regard to Dengue and Chikungunya (Lee *et al*., 2008; Lee *et al*., 2012). Unfortunately, only 13 of our patients were evaluated for Dengue (two were positive, no co-infection with malaria was found) and only three for Chikungunya (all negative); therefore, the sample size was too small for any statistical analysis.

The lack of independent association between low platelet count and positive result for malaria and between total bilirubin or CRP and any result for malaria is most probably due to population heterogeneity: in the present study, 11 out of 211 samples were drawn from patients hospitalized in Hematology, with various degrees of pancytopenia and increased levels of both CRP and total bilirubin for other reasons. Likewise, the 14 patients hospitalized in Internal Medicine had multiple comorbidities. Therefore, this is one of the limitations of this study, along with the sample size, due to the low-prevalence setting.

CONCLUSION

This study showed that a platelet count of ≥185,000 cells/µl can be considered a screening value to identify patients with high-probability of a negative blood film result for malaria.

Conflict of interest statement

All authors have no conflicts of interest to declare.

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