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ORIGINAL ARTICLE

Diagnostic significance of C-reactive protein and hematological parameters in acute toxoplasmosis

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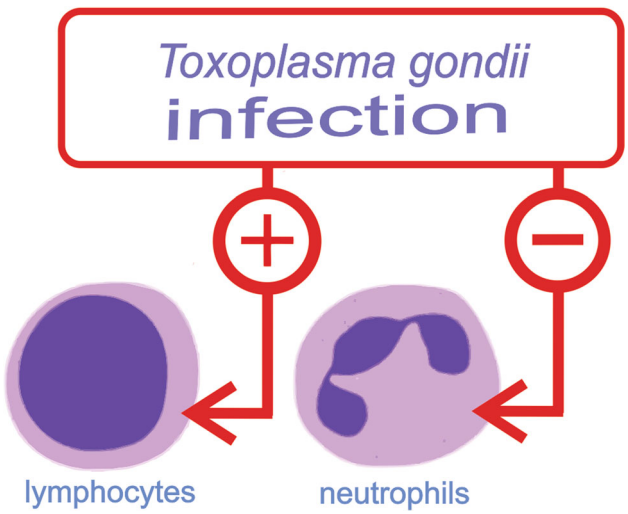
Abstract Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, which can be acquired by oral contact and may cause severe health problems especially for pregnant (congenital toxoplasmosis) and immunocompromised patients. This study aimed to verify the diagnostic significance of hematological parameters and C-reactive protein (CRP) for toxoplasmosis acute detection. A case-control study was carried out between December 2017 and May 2018, in samples of convenience independent of age and sex. The case group was formed by 25 patients with positive anti-*Toxoplasma gondii* IgG/IgM antibody and the control group was formed by 21 patients with non-positive anti-*Toxoplasma gondii* IgG/IgM antibody. The results of the hematological parameters and CRP were analyzed in these patients. The patients with *Toxoplasma gondii* IgM antibody reagent showed higher lymphocytes counting and lower neutrophils counting than the control group. C-reactive protein levels were not different between the groups case and control. ROC curve analysis highlighted that the *cut-off* value of $> 32.00\%$ for lymphocytes and $< 57.50\%$ for neutrophils were able to produce specificity higher than 90% for IgM antibody detection. The Naïve Bayes classifier was considered suitable (AUC $\approx$ 0.700) to separate both groups according to their white cell counting. Changes in lymphocytes and neutrophils may be useful parameters for toxoplasmosis identification and may be used as a tool in the complementary diagnosis of toxoplasmosis.

Graphic abstract



Keywords Toxoplasmosis · Complementary diagnosis · Hematological profile · IgG · IgM

Introduction

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, an intracellular parasite that is the unique specie able to produce the disease in all the hosts (Aguirre et al. 2019). Toxoplasmosis is acquired by oral contact with infecting

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forms eliminated in feline feces, who contaminate the soil and the water (Escotte-Binet et al. 2019). The ingestion of raw or undercook meat contaminated with parasite cysts is an important way of acquiring the disease (Paraboni et al. 2019; Sroka et al. 2020), while the congenital transmission is also extensively reported and represents a public health problem (de Melo et al. 2020). The prevalence of toxoplasmosis in the world varies from 10 to 80%, according to the geographical aspects and risk factors associated with social burdens. Moreover, it has been found a higher prevalence in Latin America and in tropical African countries (Robert-Gangneux and Dardé 2012). In many regions of Brazil toxoplasmosis outbakes have been reported in the last decade (Carmo et al. 2010; Nunes do Rego e Silva et al. 2019; Paraboni et al. 2019).

The *T. gondii* infection usually did not produce symptoms in healthy people; however, cervical lymphadenopathy or ocular disease can occur (Petersen and Liesenfeld 2007). In immunocompromised patients, *T. gondii* infection can produce severe clinical condition when cause pneumonia and disseminated disease, including death (Abbasi et al. 2020). In pregnant, the parasite can lead to spontaneous abortion, premature birth, fetal death or several sequels in the eyes and brain of the fetus, including hydrocephalus, chorioretinitis, intracerebral calcifications, mainly if the infection occurs in the first months of the gestation (Khan and Khan 2018).

Serological diagnosis plays a relevant role in the identification of *T. gondii* infections (Ybañez et al. 2020). The detection of IgM antibody anti-toxoplasmosis is the main analysis performed in the clinical routine for diagnostic of active recent infection, while the presence of IgG antibody anti-toxoplasmosis is related to a previously exposure and it is found in chronic cases (Pomares et al. 2017). Concomitantly with antibodies against *T. gondii*, other laboratory analyses may contribute to the accuracy of diagnostics. The presence of low avidity of IgG antibodies can be applied for the diagnosis of a recent toxoplasmosis infection (Rahbari et al. 2012). Also, molecular methods such as polymerase chain reaction may be useful for the detection of the infection by amplification of conserved sequences of parasite genes (Marín et al. 2018). Despite the availability of these laboratory analyses for toxoplasmosis diagnostic, the confirmation of ocular toxoplasmosis is usually clinical and when the clinical examination is not sufficient to confirm its presence, the laboratory diagnostic involves the analyze of ocular fluids (Greigert et al. 2019a; Rahimi-Esboei et al. 2018; Rahimi Esboei et al. 2019) using polymerase chain reaction and immunoblot techniques (Greigert et al. 2019b). Thus, there is an interest in finding new markers and predictors of disease useful for ocular toxoplasmosis diagnostics and monitoring, and some routine laboratory tests may be better explored for this purpose.

Simple techniques commonly available in most of the clinical laboratories may have critical importance in suggest the presence of infection by *T. gondii,* mainly when other methodologies were not available. In this context, the analysis of blood cells differential counting and C-reactive protein (CRP) are inserted. CRP is an essential component of the non-specific immune response, which is increased during infections and inflammation (Sproston and Ashworth 2018). Currently, new applications have been proposed for CRP measurements. Some recent investigations involving the use of CRP highlights the role of lymphocyte/CRP ratio for risk stratification in patients with intrahepatic cholangiocarcinoma (Lu et al. 2020); the use of CRP values for differentiation between severe malaria from uncomplicated malaria (Bhardwaj et al. 2019); and the use of CRP/albumin ratio in coronary artery disease detection (Tanriverdi et al. 2020). In the literature, there is a lack of investigations that explores the use of CRP and differential leucocytes counting for toxoplasmosis diagnostic. Considering these aspects, this work aimed to evaluate the significance of hematological parameters and CRP levels in the diagnostic of acute toxoplasmosis.

Materials and methods

A case-control study was performed in convenience samples obtained from patients attended at the clinical analysis laboratory of the Santa Terezinha Hospital Foundation of Erechim/RS (FHSTE), between December 2017 and May 2018. The samples were separated within two groups: the samples which showed serological tests positives to anti-*T. gondii* IgG/IgM antibody were enrolled in the case group. The control group was formed by samples with serological tests negatives (negative anti-*T. gondii* IgG/IgM antibody). All the measurements were performed by chemiluminescence methodology (Beckman Coulter®).

Hematological parameters were obtained in a XN 1000 Sysmex and the reference values were defined according to the age and sex of the patients. CRP levels were obtained from immunoturbidimetry technique in a Bioplus 2000 equipment (Biotecnica) with CRP turbilate diagnostic kit (Biotécnica®) and the analyses were performed according to the manufacturer's protocol. The reference value was considered normal when below 6 mg/L. The study was approved by the URI-Erechim Ethical Committee under the number 38135014.8.0000.5351.

The data was analyzed using the *t* test or Mann-Whitney test considering as significant the difference when $p < 0.05$. The normality of the data was assessed by Shapiro–Wilk test. Receiver Operating Characteristic Curves

(ROCs) were produced aiming to evaluate the efficacy of CRP or hematological parameters into differentiate the IgM reactive of non-reactive and Youden's *J* statistic was used to define the optimized cut-off values. All analyses were performed in GraphPad Prism 6.0 software.

Furthermore, machine learning (ML) techniques were employed for the statistical classification of the data into two groups (IgM reactive and non-reactive) by using Orange (Demšar et al. 2013). Firstly, the absolute number of the hematological parameters were obtained and then normalized through mean centering and standard deviation scaling in order to avoid large differences among the individual values. The normalized data was checked for pairwise intercorrelation of the variables by using the Pearson's correlation coefficient (r), which was considered suitable if it was lower than 0.80. Finally, the data was evaluated through the following supervised methods using default parameters: neural network, random forest, support vector machine (SVM), k-nearest neighbor (k-NN), naive Bayes and logistic regression (logreg). An internal validation method, leave-one-out (LOO) was employed to evaluate the robustness of the models. The data was evaluated according to the following metrics: Area Under the ROC-Curve (AUC), Recall (sensibility) and Precision.

Results

Twenty-five patients in the acute phase of toxoplasmosis were attended at the clinical laboratory of FHSTE between December 2017 and May 2018. The age of patients ranged from 13 to 49 years old (27 ± 9) and there were 6 men and 19 women. Twenty-one patients with 25 ± 6 years old formed the control group. The CRP concentration average in the case group was 8.84 ± 15.26 mg/L, and in the control group 3.66 ± 4.87 mg/L, thus was not found a difference between the two groups ($p = 0.7299$).

The hematological analysis showed a different profile in case and control groups. The total white cells counting was not different ($p = 0.2270$), otherwise the distribution of lymphocytes and neutrophils was intensively modified. The presence of acute infection by *T. gondii* promoted a significant increase in the lymphocytes percentage (36.20 ± 9.98%), when compared to the control group (25.57 ± 6.18%). On the other hand, the case group showed a lower neutrophil counting (51.84 ± 12.35%) than the control group (64.38 ± 6.65%). Considering other hematological parameters, the difference between the two groups was not found. Thus, the relative counting of monocytes, basophils and eosinophils were similar in the two groups ($p > 0.05$). These findings are represented in Fig. 1 and suggest strongly that the predominance of lymphocytes is associated with the presence of anti-*T. gondii* IgG/IgM antibodies.

Another data collected from the hematological profile also did not allowed to differentiate the patients of two groups. A significant difference was not found between the groups for thrombocytes counting ($p = 0.3087$); erythrocytes counting ($p = 0.0948$), hemoglobin measurement ($p = 0.3482$) and hematocrit measurement ($p = 0.3928$). In the same way, the analyses of the hematimetric indices were not different among patients with IgG/IgM reagents.

The analysis of ROC curves better explored the differences found in differential white blood cell count. The counting of lymphocytes and neutrophils were able to differentiate both groups (reactive IgG/IgM and non-reactive IgG/IgM) with good accuracy. The ROC curve for lymphocytes showed an area under the curve (AUC) value of 0.8105, whereas for neutrophils the AUC value was 0.8114. The counting of the other white cells showed AUC values slightly higher than 0.50; thus, these parameters demonstrated a smaller efficacy for groups' differentiation. Figure 2a–e summarizes the ROC analyses considering white blood cell count. For CRP analyses, the ROC analysis yielded an AUC value of 0.5305 (Fig. 2f).

A deeper analysis of ROC curves through *Youden's J* statistic showed that for lymphocytes the optimized *cut-off* of $> 32.00\%$ yielded a sensibility of 64.06% and a specificity of 90.48%. According to the same analysis for neutrophils, the *cut-off* value $< 57.50\%$ produced a sensibility of 64.00%, and a specificity of 90.48%. Thus, the use of the information obtained from white cell counting may be a parameter able to improve the specificity for *T. gondii* detection with good specificity values. These values of specificity and sensibility are highlighted by the black arrow in Fig. 2b, c.

The correlation among relative and absolute variables derived from the hematological data was assessed through Pearson's coefficient (Table 1). A critical point was related to the influence of lymphocytes and neutrophils relative values, which showed a r value of -0.971, indicating a strong negative relation between the values. The use of absolute values changed the association between the data. The correlation between lymphocytes and neutrophils absolute numbers changed the r value to 0.106. Thus, the choice of using absolute values of the hematological parameters avoided the intercorrelation between the variables, which might overfit the classification.

The machine learning techniques employed to classify the outcome into IgM reactive and non-reactive were first tested on the training data (Table 2). According to the table, the learners neural network and random forest had almost a perfect score (AUC $\approx$ 1.000) in classifying the data, which may be overestimated, whereas the other learners pointed scores between 0.849 and 0.798. In order

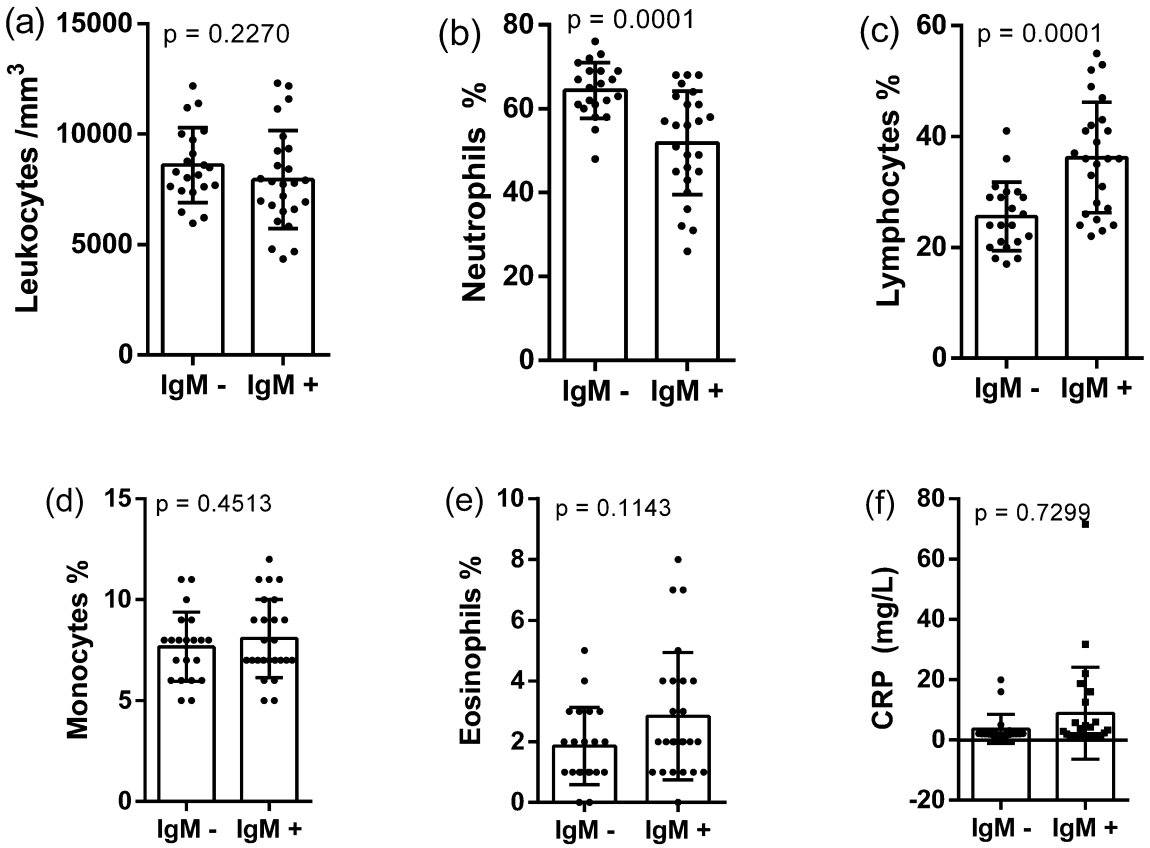

Fig. 1 Differences of white cells counting (**a**–**e**) and CRP (**f**) measurement in patients with IgM− and IgM+ . The values are represented as mean ± standard deviation

to evaluate the robustness of the classification, an internal validation method (LOO) was used (Table 3). The validation pointed out that Naïve Bayes was the best scored technique maintaining metrics values closed to 0.700 (Fig. 3) and therefore it may be suitable a classifier, considering the observed confusion matrix (Fig. 4).

Discussion

Infection by *T. gondii* in immunocompetent hosts produces a complex innate and adaptive immune response, which enables long term parasite persistence (Sasai et al. 2018). The lymphocytes role in *T. gondii* infection is in restriction of parasite replication during the chronic phase. Amongst the adaptive immune subsets, CD8 T lymphocytes are the primary effector cells while CD4 T cells play an essential helper function to maintain long-term immunity (Khan et al. 2019). The action of lymphocytes in *T. gondii* infection starts with its activation by macrophages and dendritic cells, followed by induction of Th1 cells and antigen-specific killer CD8 T cells. These lymphocytes contributes for interferon-γ production, yielding cell-autonomous immunity (Sasai et al. 2018).

Despite the neutrophils counting reduction verified in our results, this group of cells shows significant activity in the immune response against *T. gondii*. Mice that were neutrophils depleted by a monoclonal antibody showed weaker type 1 immune response against *T. gondii* measured by decreased levels of interferon-γ, interleukin 12 and tumor necrosis factor α. As a result, the parasite lesions in tissues of mice with neutrophil depleted were significantly elevated (Bliss et al. 2001).

There is a lack of robust studies that examine the effect of infections by *T. gondii* on hematological parameters. In rats infected with *T. gondii*, a study reported an increase in lymphocyte counting after 10 days of infection. The lymphocyte counting in the control group was 2768 ± 995 cells/μL, while this parameter in the infected group was 4572 ± 748 cells/μL (Tonin et al. 2013). In an investigation performed in 37 patients with the acute phase of toxoplasmosis, four presented low hemoglobin levels (10.8%), six leukopenia (16.2%), one thrombocytopenia (2.7%) and fourteen lymphocytosis (37.8%), which was the most frequent hematological alteration found (Neves et al. 2009). According to these findings, we reported here the increase in lymphocytes counting as a noteworthy

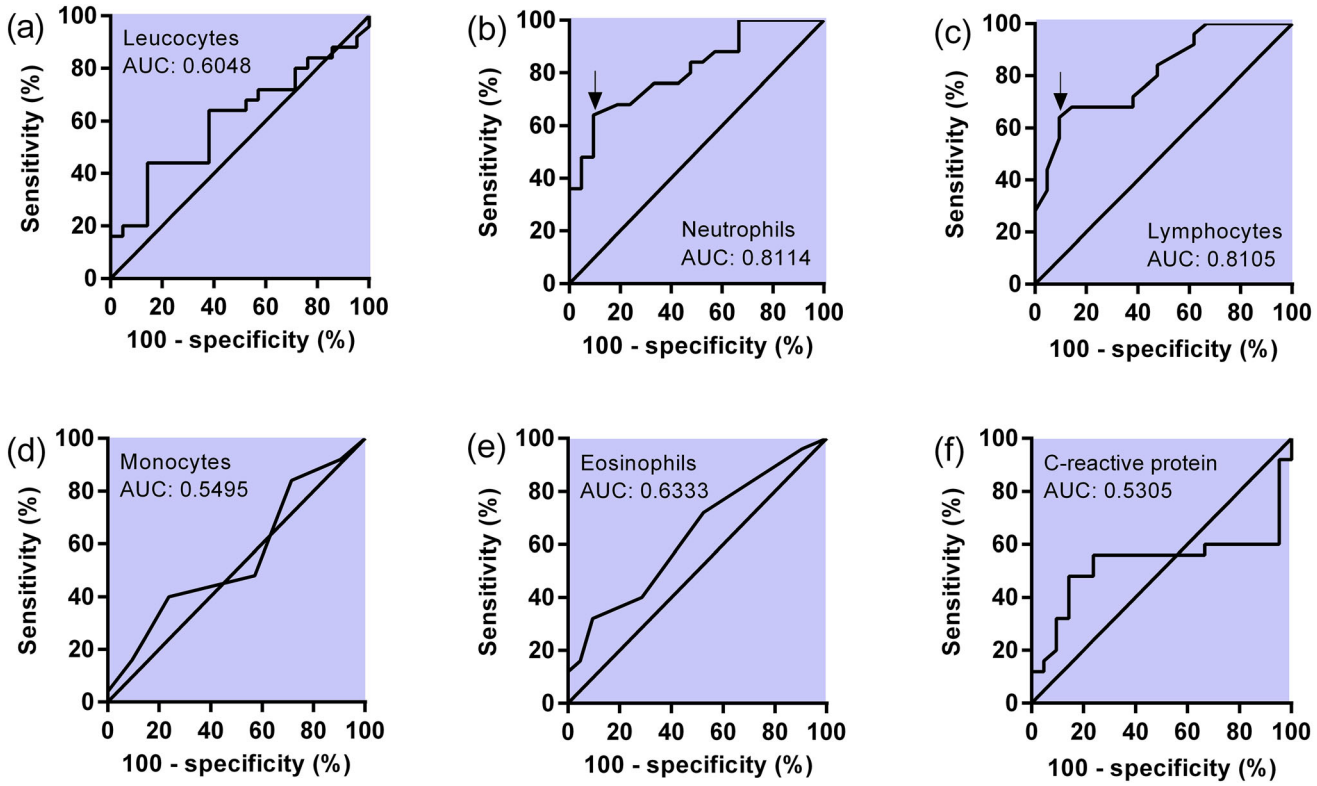

Fig. 2 ROC analyses for white cells counting (**a**–**e**) and CRP (**f**). The arrow in figure (**b**) and (**c**) shows the *cut-off* value optimized by the *Youden's J* statistic

Table 1 Variable intercorrelation estimated through Pearson's coefficient

	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Relative values of hematological differential					
Lymphocytes	1				
Neutrophils	− 0.971	1			
Eosinophils	0.435	− 0.566	1		
Monocytes	0.38	− 0.522	0.314	1	
Basophils	0.257	− 0.345	0.29	0.366	1
Absolute values of hematological differential					
Lymphocytes	1				
Neutrophils	− 0.106	1			
Eosinophils	0.439	− 0.023	1		
Monocytes	0.354	0.397	0.246	1	
Basophils	0.255	− 0.169	0.327	0.208	1

implication of toxoplasmosis in individuals with serological tests positives to *T. gondii*.

Pyrimethamine, a drug used for toxoplasmosis treatment, may produce a reduction in the neutrophils counting (Dardé et al. 2018; Dunay et al. 2018). This collateral effect could be the cause of changes in the white cells counting, however none of the study participants were under treatment with pyrimethamine in the moment of blood samples obtaining. Further investigations with more cases are needed to ensure the diagnostic values of changes in white cells counting.

The data analysis driven by ML is a quite new approach in medical care and complementary diagnosis (Heinrichs and Eickhoff 2020; Sidey-Gibbons and Sidey-Gibbons

Table 2 Classification metrics of the machine learning techniques into training set for toxoplasmosis

Method	AUC	CA	F1	Precision	Recall
Neural network	1.000	1.000	1.000	1.000	1.000
Random forest	0.996	0.935	0.935	0.935	0.935
SVM	0.798	0.761	0.761	0.768	0.761
kNN	0.798	0.761	0.758	0.763	0.761
Naive Bayes	0.849	0.739	0.758	0.763	0.761
Logreg	0.811	0.696	0.696	0.696	0.696

Table 3 Classification metrics using the leave-one-out validation of the machine learning techniques into training set for toxoplasmosis

Method	AUC	CA	F1	Precision	Recall
Neural network	0.623	0.609	0.606	0.606	0.609
Random forest	0.650	0.630	0.627	0.628	0.630
SVM	0.659	0.630	0.630	0.629	0.630
kNN	0.590	0.587	0.588	0.593	0.587
Naive Bayes	0.724	0.652	0.652	0.652	0.652
Logreg	0.680	0.652	0.652	0.652	0.652

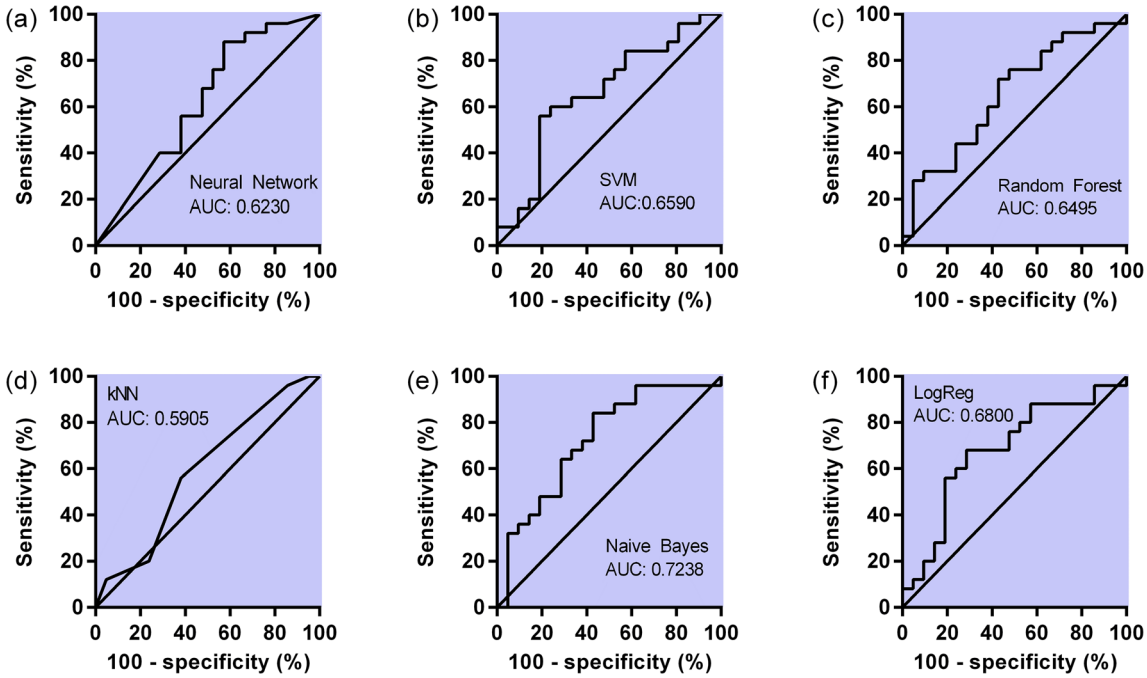

Fig. 3 ROC analyses for different classifiers (**a**–**f**) using all the white cell counting

2019; Watson et al. 2019); however, in the last years, several studies have reported the use of ML with image data or clinical specimens (blood, stool, urine) to help in diagnose cancer (Kourou et al. 2015; Podnar et al. 2019; Salod and Singh 2019; Wu et al. 2019), diabetes and cardiovascular disease (Dinh et al. 2019; Kavakiotis et al. 2017; Shameer et al. 2018) among other conditions (Ayling et al. 2019; Gunčar et al. 2018; Poostchi et al. 2018; Ullah et al. 2019). Considering the infective diseases, ML techniques were used to identify dengue (Hair et al. 2019), bacterial infections (Rawson et al. 2019) and, more recently, COVID-19 (Banerjee et al. 2020; Brinati et al.

SVM

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	12	9	21
	IgM positive	8	17	25
	Σ	20	26	46

Log reg

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	13	8	21
	IgM positive	8	17	25
	Σ	21	25	46

Naive Bayes

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	13	8	21
	IgM positive	8	17	25
	Σ	21	25	46

kNN

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	13	8	21
	IgM positive	11	14	25
	Σ	24	22	46

Neural Network

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	12	9	21
	IgM positive	11	14	25
	Σ	23	23	46

Random Forest

		Predicted		
		IgM negative	IgM positive	Σ
Actual	IgM negative	11	10	21
	IgM positive	7	18	25
	Σ	18	28	46

Fig. 4 Confusion matrix of the LOO validation procedures on different classifiers used to predict the toxoplasmosis IgM antibody

2020). These studies employed mostly Random Forest, Logistic Regression, Naïve Bayes and SVM as learners and included basic blood sample data in their analysis (hematologic data, CRP, alanine and aspartate aminotransferases, among others). Regarding the analysis by ML techniques employing hematological parameters for the clinical investigation of toxoplasmosis, this is the first study to be reported to the best of our knowledge. The Naïve Bayes classifier showed performance metrics values of 0.724 in the AUC and 0.652 for the other metrics at the leave-one-out validation test. In order to evaluate the method's capacity to classify the data correctly, more patients should be included in the dataset.

Conclusions

The lymphocytes counting was higher in patients with acute toxoplasmosis, and neutrophils counting was lower when compared to the control group. These findings were supported by the analysis of ROC curves, in which AUC values were near of 0.80 for these parameters. Furthermore, the machine learning technique Naïve Bayes was considered a good classifier when analyzing all the white cell population (AUC near to 0.700). Changes in the white cells population were proposed as an additional parameter for clinical investigation in toxoplasmosis diagnostic. The data reported here support that white cells profile may be a useful parameter for be considered in toxoplasmosis diagnostic.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animals participants All applicable international and institutional guidelines for the use of patient's data were followed. The study protocol was approved by the ethical committee of the institution (committee of URI-Erechim under the number 2.397.014 and CAAE: 38135014.8.0000.5351). This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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