



ORIGINAL ARTICLE

# Association between weather seasonality and blood parameters in riverine populations of the Brazilian Amazon<sup>☆,☆☆</sup>



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## KEYWORDS

Iron homeostasis;  
Biomarkers;  
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## Abstract

**Objective:** To analyze the seasonality of blood parameters related to iron homeostasis, inflammation, and allergy in two riverine populations from the Brazilian Amazon.

**Methods:** This was a cross-sectional study of 120 children and adolescents of school age, living in riverine communities of Porto Velho, Rondonia, Brazil, describing the hematocrit, hemoglobin, ferritin, serum iron, total white blood cell count, lymphocytes, eosinophils, C-reactive protein, and immunoglobulin E levels in the dry and rainy seasons. The chi-squared test and the prevalence ratio were used for the comparison of proportions and mean analysis using paired Student's *t*-test.

**Results:** Hemoglobin (13.3 g/dL) and hematocrit (40.9%) showed higher average values in the dry season. Anemia prevalence was approximately 4% and 12% in the dry and rainy seasons, respectively. Serum iron was lower in the dry season, with a mean of 68.7 mcg/dL. The prevalence of iron deficiency was 25.8% in the dry season and 9.2% in the rainy season. Serum ferritin did not show abnormal values in both seasons; however, the mean values were higher in the dry season (48.5 ng/mL). The parameters of eosinophils, lymphocytes, global leukocyte count, C-reactive protein and immunoglobulin E showed no seasonal differences. C-reactive protein and immunoglobulin E showed abnormal values in approximately 7% and 60% of the examinations, respectively.

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**PALAVRAS-CHAVE**

Homeostasia do ferro;  
Biomarcadores;  
Mudanças climáticas

**Conclusion:** Hematological parameters of the red cell series and blood iron homeostasis had seasonal variation, which coincided with the dry season in the region, in which an increase in atmospheric pollutants derived from fires is observed.

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**Relação da sazonalidade climática com parâmetros sanguíneos de ribeirinhos residentes na Amazônia brasileira****Resumo**

**Objetivo:** Analisar a sazonalidade climática de parâmetros sanguíneos relacionados à homeostase do ferro, inflamação e alergia em duas populações ribeirinhas da Amazônia Brasileira.

**Método:** Realizou-se um estudo transversal em 120 crianças e adolescentes em idade escolar, residentes em comunidades ribeirinhas de Porto Velho, Rondônia. Foram analisados hematócrito, hemoglobina, ferritina, ferro sérico, leucometria global, linfócitos, eosinófilos, proteína C-reativa e Imunoglobulina E nas estações seca e chuvosa. Utilizou-se o qui-quadrado e a razão de prevalência para a comparação das proporções, além do teste t de student pareado para a análise de médias.

**Resultados:** Hemoglobina (13,3 g/dL) e hematócrito (40,9%) apresentaram maiores valores médios no período de seca. A prevalência de anemia foi cerca de 4% e 12% na seca e na chuva, respectivamente. O ferro sérico foi menor no período de seca com média de 68,7 mcg/dL. A prevalência de deficiência de ferro foi em média 25,8%, na seca, e 9,2%, na chuva. A concentração sérica de ferritina não apresentou valores alterados em ambos os períodos, no entanto os valores médios apresentaram-se mais elevados na seca (48,5 ng/mL). Os parâmetros dos eosinófilos, linfócitos, leucometria global, proteína C-reativa e Imunoglobulina E não apresentaram diferenças sazonais. A Proteína C-reativa e a Imunoglobulina E apresentaram valores alterados em cerca de 7% e 60% dos exames realizados, respectivamente.

**Conclusão:** Os parâmetros hematológicos da série vermelha e a homeostasia ferro sanguíneo apresentaram variação sazonal, que coincide com o período de seca na região, no qual observa-se aumento dos poluentes atmosféricos derivados das queimadas.

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**Introduction**

In 2010, Brazil faced a severe drought throughout the country. In the so-called "deforestation arc," the drought directly interfered in the increase of forest fires. In the municipality of Porto Velho, the highest proportion of forest fires occurred in August and September and, consequently, a high concentration of the mean levels of particulate matter (PM) was observed, recorded by the air monitoring station in Porto Velho, with an approximate peak of 240  $\mu\text{g}/\text{m}^3$  in the month of September. In 2011, PM concentrations did not exceed the monthly mean of 10  $\mu\text{g}/\text{m}^3$  during the entire rainy season.<sup>1</sup>

The scientific literature currently presents several toxicological mechanisms through which PM can cause health damage, mainly damage to the respiratory and cardiovascular systems, triggered by inflammatory, immunological, and genotoxicity responses.<sup>2</sup> The effects involve an inflammatory response with a serum increase in C-reactive

protein and cytokines<sup>3-5</sup> and increased airway reactivity through immunoglobulins,<sup>6</sup> as well as alterations in several hematological parameters such as increased eosinophil adhesion<sup>7</sup> and altered leukocyte count,<sup>8,9</sup> hemoglobin, and hematocrit.<sup>10,11</sup>

The Brazilian Amazon region historically suffers from forest fires during the dry season. In these regions, some ecological studies have shown that biomass burning is one of the main risk factors for increased morbidity and mortality due to respiratory diseases in children and the elderly, especially in the "deforestation arc."<sup>12-14</sup> More recently, mortality rates from cardiovascular disease in the elderly were associated with exposure to PM<sub>2.5</sub>.<sup>15</sup>

Therefore, the present study aimed to analyze the climatic seasonality of blood parameters related to iron homeostasis, inflammation, and allergy in the southern Brazilian Amazon region, characterized by high levels of atmospheric pollutants from forest fires in the dry season, since no studies assessing the behavior of hematological

parameters related to this seasonal feature of the region were retrieved.

## Material and method

### Study design

A cross-sectional descriptive study, with a quantitative approach, was carried out in schoolchildren living in the communities of Cuniã and Belmont, located in the city of Porto Velho, Rondônia, in the southern Brazilian Amazon region, comparing the values of blood parameters in relation to the seasonal exposure to pollutants from forest fires in the region.

### Study population and area

The study population comprised individuals from a census carried out in the communities of Cuniã and Belmont between June and September of 2010. Children and adolescents between the ages of 6 and 16 years, who had been living in the community for at least one year, were considered eligible for the study and were recruited to undergo blood tests in two stages: the first in the dry season (October and November, 2010) and the second in the rainy season (May, 2011). Only individuals who participated in both stages and who did not have an infectious disease at the time of the examinations (cold, flu, and malaria, among others) were included, totaling 120 individuals.

The months of the year were grouped as dry and rainy months, based on the mean monthly rainfall records of the National Institute of Meteorology. The dry season refers to the months of June to November of 2010. The rainy season refers to December of 2010 through May of 2011.

The climatic seasonal variation is a characteristic feature of the region, being limited to two defined seasons: dry and rainy. These communities are located in isolated rural areas; since the 1970s, they suffer the direct influence of pollutants resulting from forest fires in the region during the dry season. Although the Belmont community suffers the influence of urban pollutants, both communities are very similar in their habits and living conditions.

The riverine community of Belmont is located near the fluvial port of Porto Velho and is distributed around the "Belmont road", which is approximately 20 km long. The community, despite its rural characteristics, suffers great urban influence due to its proximity to downtown Porto Velho (approximately 5 km). The extractive reserve (Resex) of Cuniã is located at approximately 190 km from the capital, Porto Velho. As the access to the community occurs basically through the river, this results in the semi-isolation of the population, which is even worse in the dry season, especially terms of medical care.<sup>16</sup>

### Blood tests

The blood and serum samples were collected by technicians from the clinical laboratory of the Hospital 9 de Julho in Porto Velho, and processed in an automated device. For blood analysis, an automated hemogram was performed

using the electrical impedance method, from which hematocrit, hemoglobin, general leukometry, eosinophils, and lymphocytes were selected. Hematocrit and hemoglobin were selected to help evaluate iron homeostasis. The white blood cell parameters – general leukometry, eosinophils, and lymphocytes – were selected to complement the evaluation of the individuals' inflammatory and allergic processes.

The following parameters were analyzed from the serum: iron, ferritin, C-reactive protein (CRP) and immunoglobulin E (IgE). The iron homeostasis parameters, serum iron and ferritin, were obtained using the modified Goodwin and immunoturbidimetry methods, respectively. CRP measurement was obtained using the latex agglutination method and was used for inflammation assessment. For the allergy evaluation, multiple IgE for inhalant allergens (HX2) was used, which includes sensitization by house dust (H1), dust mites (D1 and D2), and cockroaches (I6), and the method of analysis used was enzymatic fluorescence – Immunocap (Thermo Fisher Scientific Inc, Uppsala, Sweden).

### Statistical analysis

Databases were created containing all the information collected on the field during the two campaigns that were carried out. The chi-squared test and prevalence ratio were used for the comparison of proportions, in addition to the paired Student's *t*-test for the analysis of means during the dry and rainy seasons. Comparisons between the communities were made using Student's *t*-test for independent samples. The analyses were performed using the statistical software SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, version 20.0, NY, USA).

### Protocols

The study was approved by the Ethics Committee of Escola Nacional de Saúde Pública da Fundação Oswaldo Cruz (CEP/ENSP 89/11 – CAAE 00830031000-1). All participants had the consent of a parent or guardian, who signed the free and informed consent form.

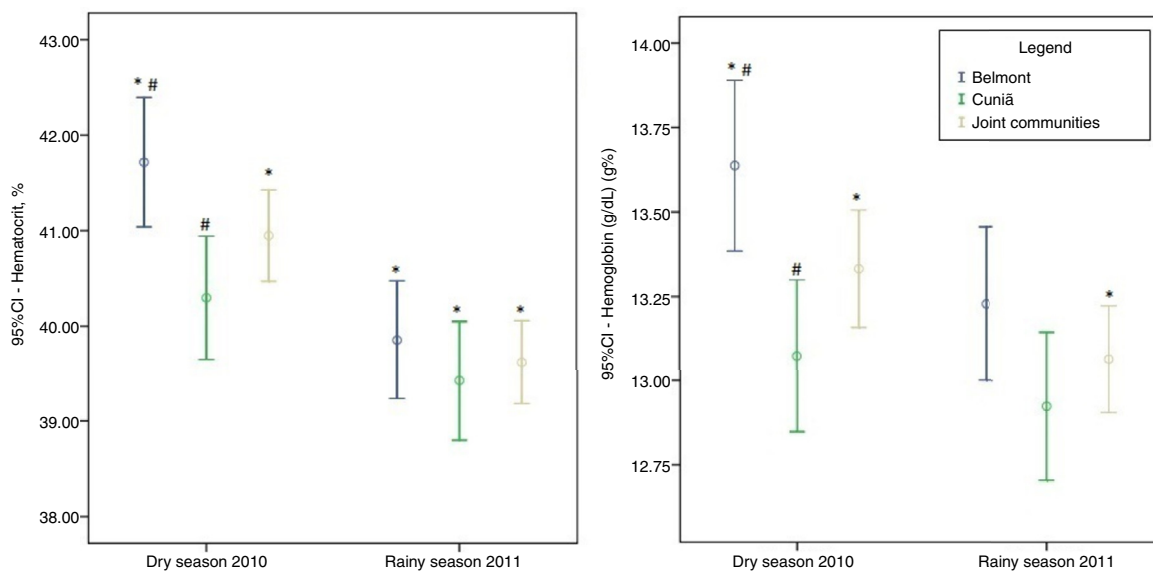
## Results

### General characteristics of the study population

A total of 120 children and adolescents participated in the collections in both periods, totaling 55 tests carried out in Belmont and 65 in Cuniã. Approximately 70% of the study population consisted of children (>12 years of age); the mean age was 10 years in the dry season and 11 years in the rainy season in both communities. Both communities had a higher proportion of female subjects, totaling 52% of the population of Belmont and 60% of the population of Cuniã. The diagnosis of intestinal parasitosis was positive in 38% of the population, 43% for Cuniã and 31% for Belmont.

### Blood parameters

A statistically significant reduction in hematocrit was observed during the rainy season for the Belmont community



**Figure 1** Distribution of values and confidence intervals (CI) for hematocrit and hemoglobin, according to community and period. \*Statistically significant result for comparison of means between the periods (dry and rainy seasons) through paired Student's *t*-test and *p*-value <0.05. #Statistically significant result for comparison of means between the communities (in each period) through Student's *t*-test for independent samples and *p*-value <0.05.

and for both communities considered together. The global means, approximately 40.9% in the dry season and 39.6% in the rainy season, were considered to be within the normal range. As for the dry season, there was a difference in hematocrit values between the communities. Regarding the global hemoglobin parameters, for the grouped communities and for the Belmont community, a statistically significant decrease was observed in the rainy season. The highest concentrations of hemoglobin in blood were observed in the Belmont community in both periods; however, a statistically significant difference was observed in concentrations between communities only in the dry season (Fig. 1).

The global hemoglobin means in the dry and rainy seasons were within the normal range and the prevalence of anemia was higher in Cuniã. In the dry season, the prevalence of anemia was around 4%; in the rainy season, it was 12% for all communities. It was observed that the probability of anemia occurrence was 35% lower in the dry season (95% CI: 0.1–0.9).

Considering both communities together, serum ferritin concentrations showed a statistically significant decrease in the rainy season. The mean concentration in the dry season was 48.5 ng/mL, while in the rainy season it was 41.2 ng/mL. Not even the minimum values in both periods can be considered outside of the normal parameters; nevertheless, the serum levels observed in the community of Belmont were higher than in the community of Cuniã and in the group of the communities, in both periods. A statistically significant difference was observed in serum ferritin levels between the communities in both the dry and rainy seasons. Serum iron, with global means of 68.7 mcg/dL in the dry season and 77.5 mcg/dL in the rainy season, showed a statistically significant increase in the rainy season when the communities were considered as a group and in the community of Belmont (Fig. 2).

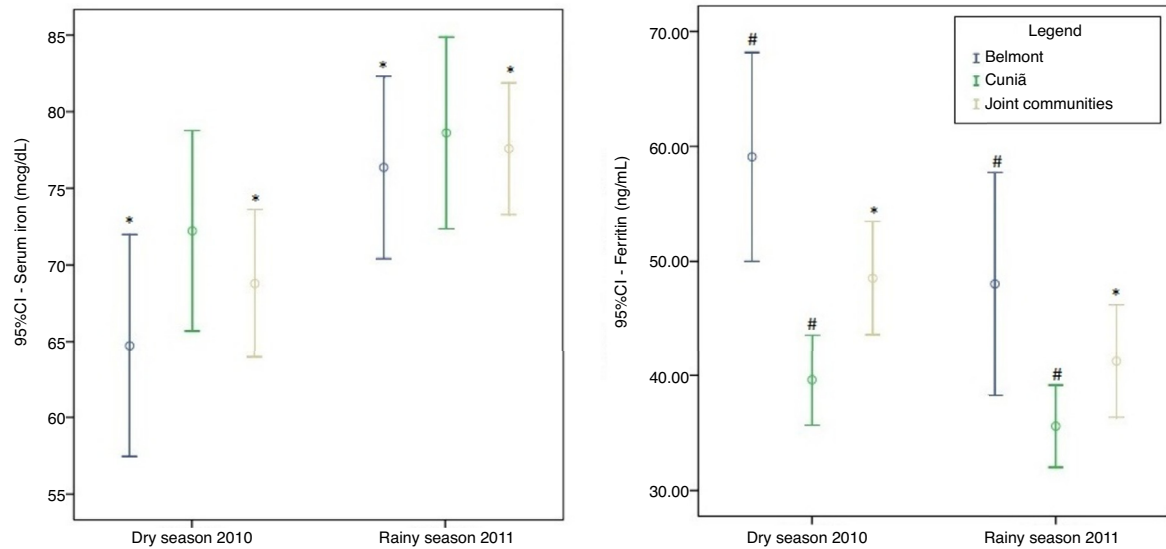
The mean prevalence of altered values, which constitute iron deficiency, was 25.8% in the dry season and 9.2% in the rainy season. Both communities had a higher prevalence of altered values in the dry season; however, the Belmont community had a higher prevalence of iron deficiency (approximately 33% in the dry season and 11% in the rainy season).

The values for leukometry, eosinophils, and lymphocytes did not show a seasonal difference for the communities considered as a group or separately. In the dry season, a global mean of 7405.8 mm<sup>3</sup> was observed, whereas in the rainy season, the mean was 7172.5 mm<sup>3</sup>. Eosinophilia occurred in approximately 70% of the tests in both periods for both communities, as well as for the communities as a group. The global means were around 9.9% in the dry and 10.2% in the rainy season. A statistically significant difference was observed between the communities during the dry season. The global means for the distribution of lymphocyte values are around 36.1% in the dry season and 35.1% in the rainy season (Fig. 3).

The proportion of altered CRP tests was about 7% in total, and Belmont showed a higher proportion in the dry season, while Cuniã had a higher proportion in the rainy season. IgE was altered (reagent) in approximately 60% of the tests in both seasons and communities, with a greater probability of alteration in the rainy period in both communities (Table 1).

## Discussion

Serum ferritin levels were higher in the dry season, while serum iron was reduced. Studies have shown changes in iron homeostasis after exposure to PM, cigarette smoke, silica, and ozone, inducing oxidative stress and inflammatory processes in the lower respiratory tract.<sup>2,17,18</sup> Tuluze et al.<sup>18</sup>

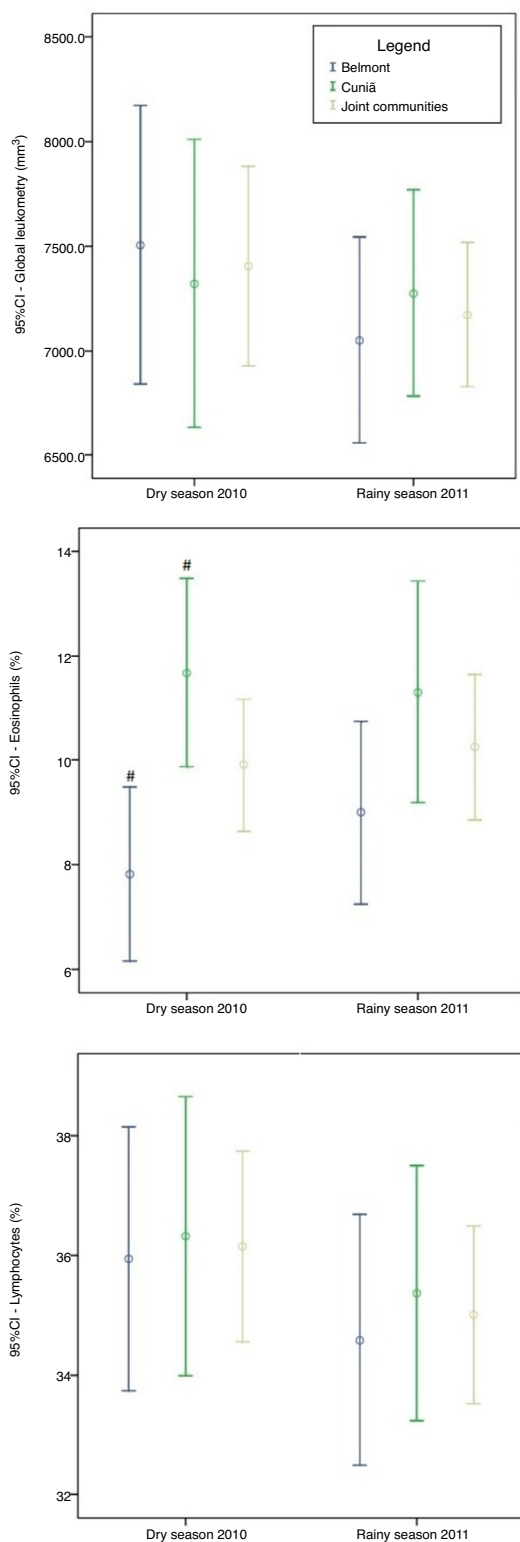


**Figure 2** Distribution of values and confidence intervals (CI) for serum iron and ferritin, according to community and period. \*Statistically significant result for comparison of means between the periods (dry and rainy seasons) through paired Student's *t*-test and *p*-value <0.05. #Statistically significant result for comparison of means between the communities (in each period) through Student's *t*-test for independent samples and *p*-value <0.05.

**Table 1** Proportion of altered tests for C-reactive protein and multiple IgE for inhalants, according to the community and period.

	Dry season		Rainy season		$\chi^2$ ( <i>p</i> -value)	Prevalence ratio (95% CI)
	<i>n</i>	%	<i>n</i>	%		
<b>C-reactive protein</b>						
<i>Belmont</i>						
Altered	6	10.91	3	5.45	1.08 (0.489)	2.00 (0.5–7.6)
Normal	49	89.09	52	94.55		
Total	55	100.00	55	100.00		
<i>Cuniã</i>						
Altered	2	3.08	3	4.62	0.20 (1.000)	0.7 (0.12–3.9)
Normal	63	96.92	62	95.38		
Total	65	100.00	65	100.00		
<i>Global</i>						
Altered	8	6.67	6	5.00	0.30 (0.784)	1.33 (0.5–3.7)
Normal	112	93.33	114	95.00		
Total	120	100.00	120	100.00		
<b>Multiple IgE for inhalant allergens</b>						
<i>Belmont</i>						
Reagent	36	65.45	37	67.27	0.04 (1.000)	0.97 (0.7–1.3)
Non-reagent	19	34.55	18	32.73		
Total	55	100.00	55	100.00		
<i>Cuniã</i>						
Reagent	38	58.46	39	60.00	0.03 (1.000)	0.97 (0.7–1.3)
Non-reagent	27	41.54	26	40.00		
Total	65	100.00	65	100.00		
<i>Global</i>						
Reagent	74	61.67	76	63.33	0.07 (0.894)	0.97 (0.8–1.2)
Non-reagent	46	38.33	44	36.67		
Total	120	100.00	120	100.00		

CI, Confidence Interval; IgE, immunoglobulin E.



**Figure 3** Distribution of the values of global leukometry, eosinophils, and lymphocytes according to community and period. #Statistically significant result for comparison of means between the communities (in each period) through Student's t-test for independent samples and  $p$ -value  $<0.05$ .

observed an increase in ferritin levels in healthy workers responsible for heating apartments using coal in Turkey.

Ghio et al.<sup>17</sup> showed higher serum ferritin levels in pulmonary lavage of individuals with lung inflammation when compared to individuals without inflammation. The inversely proportional association observed may be related to the mobilization of iron stores by the increased intracellular use of iron. Ferritin, although considered a gold standard for the quantification of iron stores, may increase due to inflammatory or infectious processes, which does not mean that there is an increase in iron stores.<sup>19</sup> In turn, iron, which is highly used in metabolic reactions and in the formation of blood elements, can be acutely reduced in the presence of infections or inflammation.<sup>20</sup> The blood parameters of red blood cells, hemoglobin, and hematocrit, showed a seasonal variation with an increase in the dry season, when the concentration of PM is usually higher. This result corroborates the studies by Sorensen et al.,<sup>11</sup> in Germany, who found an increase in the same parameters in university students, related to high concentrations of PM<sub>2.5</sub>, and Neufeld et al.,<sup>10</sup> in India, who also observed an increase in the proportion of blood hemoglobin and hematocrit in women using indoor wood stoves. This increase has been interpreted as a functional adaptation of the body in response to tissue hypoxia<sup>20</sup> caused by the multi-element nature of PM, which could mask, for instance, the diagnosis of anemia. This increase in hemoglobin and hematocrit is also associated with a possible hemoconcentration,<sup>21</sup> suggesting an increase in blood viscosity at this time.

Another important finding is the fact that hematocrit, hemoglobin, and ferritin values were higher in the Belmont community, together with a higher prevalence of iron deficiency, suggesting a greater use of iron stores by individuals in this community. This finding may be related to the influence of urban pollutants in the community, as well as exposure to forest fires, since Belmont is closer to the city of Porto Velho and the city's river port, which may suggest greater exposure to other pollutants, mainly products from burning fossil fuels, in contrast to the community of Cuniã, which is located in the middle of the forest and has no cars.

Global leukometry, as well as lymphocytes, eosinophils, multiple IgE, and CRP, did not show seasonal variation. The association between hematological parameters of white blood cells and air pollution still remains contradictory in the scientific literature. While Steinvil et al.<sup>22</sup> found no association between pollution and alterations in these parameters in young adults in Israel, a study carried out with children and adolescents in Iran by Poursafa et al.<sup>9</sup> found an association between atmospheric pollution and an increase in global leukometry, for instance. Moreover, the hematological parameters of the white blood cells are very non-specific with respect to diagnoses. In general, these parameters concern the individual's overall nutritional status and the presence of some inflammatory or infectious process, such as allergies, viruses, and even intestinal parasitosis.<sup>23,24</sup> Therefore, the main reason for this finding may be associated to the fact that all these parameters undergo significant changes in the presence of intestinal parasitosis, since the studied population has a high and constant prevalence of intestinal parasites.

IgE, the gold standard marker for allergy, showed high levels in both periods and in the two analyzed communities.

Amin,<sup>6</sup> in a review on the role of mast cells in allergic inflammation, indicated that continuous exposure to pollutants may promote the constant synthesis of IgE. However, Zavadniak and Rosário<sup>25</sup> discussed a possible blocking in the production of allergen-specific IgE in the presence of parasites due to the production of T-cells. Medeiros et al.<sup>26</sup> observed lower reactivity in skin tests, considered as the most specific allergy tests, in asthmatic children with parasitosis. Moreover, Genov et al.<sup>27</sup> reported that exposure to helminths would cause cross-reactivity by stimulating IgE-mediated immune responses due to the high similarity between tropomyosin (a protein involved in vertebrate and invertebrate muscle contractions, known to be highly allergenic) of helminths and several allergens.

The results found for CRP in the present study differ from other that showed an association between the increase in CRP and the acute effect of air pollution. These effects were observed mainly in adults and elderly patients with cardiovascular diseases,<sup>4,5</sup> in whom a high level of CRP is expected, as well as greater susceptibility to air pollutants due to body fragility caused by the disease. However, Shima et al.<sup>3</sup> observed a significant increase in CRP in healthy schoolchildren. Conversely, Steinvil et al.,<sup>22</sup> Rudez et al.,<sup>28</sup> and Hildebrandt et al.<sup>29</sup> also found no significant alterations in serum CRP concentrations in healthy individuals, even in high atmospheric pollution environments, corroborating the findings of the present study. This fact would be explained by a possible different systemic relationship in the regulation of inflammation in healthy and sick individuals, in addition to the fact that CRP does not participate in all aspects and stages of inflammation.<sup>5,30</sup> Another explanation for the absence of CRP alteration, even in the presence of reagent IgE and eosinophilia, may be related to its short half-life of just a few hours, while the half-life of the other parameters lasts for days.

Among the limitations of this study are its cross-sectional design and the ecological nature of the associations, while the number of tests performed may have been insufficient to demonstrate the seasonal alterations. Another important point is related to the high prevalence of intestinal parasitosis, the chronic nature of other exposures, and the nonspecific character of some biomarkers that may have masked or influenced some of the blood parameters, especially those related to allergy and inflammation. Moreover, the isolation of some communities at certain times of the year (Cuniã in the dry season and Belmont in the rainy season), as well as the low socioeconomic status of both communities can make it difficult to have access to food, physicians, and medication, negatively influencing the population health status.

The results of this study provide health assessments at two very characteristic moments of the Brazilian Amazon, allowing an overview of the possible subclinical effects occurring during periods of more and fewer forest fires, describing their particularities in two riverine communities of Porto Velho. However, it is necessary to carry out more detailed analyses of the variables investigated in the study and a better understanding of the processes that interfere with the dynamics of the health and environment situation in the riverine communities of the Brazilian Amazon.

It can be concluded that the hematological red blood cell parameters and blood iron homeostasis of schoolchildren living in the Brazilian Amazon showed seasonal variations, which coincides with the dry season in the region, when there is an increase in atmospheric pollutants originating from fires. CRP, IgE, global leukometry, lymphocytes, and eosinophils were not sufficiently sensitive for the screening of subclinical effects related to forest fires.

## Conflicts of interest

The authors declare no conflicts of interest.

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