

Research Article

Immune Cells Quantitative Abnormalities Associated with Severe COVID-19

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Abstract

COVID-19 spread to countries around the world. It is essential to set up suspected biomarkers for rapid management of the disease. This study aimed to determine quantitative abnormalities of peripheral blood immune cells associated with COVID-19 and establish those associated with the severity of COVID-19 in Togo. In a retrospective analytical study from May to September 2022; 851 symptomatic and 194 asymptomatic COVID-19 patients medical records were recovered. Their complete blood count data were analyzed using Chi-square test, Mann-Whitney U test, univariate and multivariate binary logistic regression analysis. Symptomatic COVID-19 patients had low and high platelets count, high white blood cells count, high neutrophils, low and high lymphocytes count and high eosinophils count compared to asymptomatic ones ($p < 0.0001$). Complete blood count abnormalities as low platelets count [OR = 2.75; 95% CI (1.55-4.88)] or high platelets count [OR = 3.12; 95% CI (1.23-7.91)], high white blood cells [OR = 9.87; 95% CI (4.96-19.61)], high neutrophils count [OR = 9.92; 95% CI (4.80-20.45)] and low lymphocytes count [OR = 3.83; 95% CI (2.20-6.65)] were associated with COVID-19 severity. It is important to consider immune cells profile for the diagnosis and follow-up of COVID-19 patients in Togo.

Keywords: Severe COVID-19; Immune Cells; Quantitative Abnormalities; Togo

Introduction

Coronavirus Infectious Disease 2019 (COVID-19), a disease caused by the SARS-CoV-2 virus (Severe Acute Respiratory Syndrome 2), appeared for the first time in December 2019 in Hubei province in Wuhan (China) in patients who have severe unexplained pneumonia [1]. COVID-19 spread to other Chinese regions and then to other countries around the world, which led to

it being classified as a pandemic [2].

SARS-CoV-2 virus uses Angiotensin-Converting Enzyme 2 (ACE2) as a receptor, to infect host cells [3]. The internalization of ACE2 by SARS-CoV-2 causes disruption of the Renin Angiotensin Aldosterone System (RAAS) resulting in a decrease in the formation of angiotensin 1 and an accumulation of angiotensin 2 with its negative effects (inflammation, fibrosis, coagulation, vasoconstriction). This leads to lesions mainly affecting organs and tissues that strongly express this enzyme, partly explaining the damage to several organs seen during COVID-19 [4]. Most people infected with SARS-CoV-2 have mild to moderate respiratory illness and recover without needing special treatment. However, some patients become seriously ill and need medical attention [5]. The clinical presentation of this disease varies from asymptomatic forms or mild flu-like symptoms to severe pneumonia with acute respiratory distress and possibly death [6]. Old people and those with an underlying medical condition,

such as cardiovascular disease, diabetes, chronic respiratory disease, or cancer, are at higher risk of developing the severe form of the disease [7]. Indeed, the pathophysiology of COVID-19 is complex involving cytokines storm which leads to inappropriate immune reactions together with enzymes, receptors and immune cells [8]. These immune cells are part of the Complete Blood Count (CBC) routinely requested for COVID-19 patients and therefore constitute a therapeutic follow-up examination of hospitalized patients. Some previous studies have shown that the number of lymphocytes were significantly decreased in COVID-19 patients who needed hospitalization [9]. Given that COVID-19 has become an emerging disease, we aimed in this study to determine the quantitative abnormalities of peripheral blood immune cells associated with COVID-19 and establish those associated with the severity of the disease in Togo.

Material and Methods

Study Type, Area and Period

This was a retrospective analytical study carried out from May to September 2022 at the “Centre Hospitalier Régional Lomé Commune” (CHR-LC) which is the national reference center for the care of COVID-19 patients in Togo.

Study Population

Study population was represented by COVID-19 patients, who were positive by quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and hospitalized at CHR-LC from May 2020 to November 2021. COVID-19 patients were grouped in symptomatic and asymptomatic patients based on the WHO classification according to the severity of the disease. Pregnant women, HIV-positive patients and tumor-related immune-compromised people were excluded from this study.

Data Collection

A total of 1168 COVID-19 patient's data were recovered. After database scouring, 50 HIV and/or cancer patients and 73 pregnant women were excluded. From the remaining 1045 patient's data, 851 were classified symptomatic whereas 194 were classified asymptomatic.

Collected data included socio-demographic characteristics such as age, sex, clinical signs, occupation; comorbidities such as diabetes, hypertension, asthma; complete blood count parameters such as haemoglobin, White Blood Cells (WBC), platelets, neutrophils, lymphocytes, eosinophils, monocytes count and clinical symptoms such as fever, asthenia, cough, rhinitis, myalgia, dyspnea, angina.

Statistical Analysis

The study data were entered into Excel spreadsheet and processed with SPSS software (IBM SPSS Statistics 21; Armonk, NY) and GraphPad Prism 8 5.02 software for Windows. The Pearson/Fisher Chi-square test (χ^2) test was used to assess differences in distribution of variables between symptomatic and asymptomatic COVID-19 patients. After D'Agostino omnibus normality test, the Mann-Whitney U-test was used to compare medians of quantitative parameters. A p-value below 0.05 was considered statistically significant.

Univariate and multivariate logistic regression analysis were used to assess the strength of association between asymptomatic and symptomatic COVID-19 (severity of the disease) with different variables. The Odds Ratios (OR) and adjusted Odds Ratios (aOR) were calculated and the risk factor was determined with a 95% Confidence Interval (CI). An OR/aOR < 1 was interpreted as protective factor, an OR/aOR > 1 was interpreted as a risk factor and an OR/aOR = 1 was interpreted as no association.

Ethics Statement

This study received ethical approval from the ethical board (Comité de Bioéthique pour la Recherche en Santé, CBRS) of the Togo Health Ministry under the registration number 029/2022/CBRS. Also, an authorization was received from the “Centre Hospitalier Régional Lomé Commune” (CHR-LC) before collecting COVID-19 patient's data.

Results

Socio-Demographic Characteristics of The Study Population

Male (57.60%) were more represented in the study population than female (42.40%) with a sex ratio M/F=1.36. Most of asymptomatic patients (53.10%) were aged 34 years or less whereas most of symptomatic patients (29.96%) were aged 63 to 92 years. The pupils-student-unemployed group (24.21%) was more affected, followed by artisans-shopkeepers-entrepreneurs (22.30%) and the retired (9.76%). Concerning the comorbidities, hypertension and diabetes were more found in COVID-19 patients with 31.10% and 19.62% respectively, while asthma was found at 3.63%. All symptomatic patients had fever, rhinitis, cough, angina and angina whereas 92.47% had asthenia and 86.84% had myalgia.

Haemoglobin, White Blood Cells and Platelets Count in Symptomatic and Asymptomatic COVID-19 Patients

Haemoglobin levels were significantly lower ($p<0.0001$) in symptomatic patients compared to asymptomatic patients. But white blood cells count was significantly high in symptomatic patients compared to asymptomatic patients with a $p<0.0001$. No significant difference was observed for platelets count (Fig. 1).

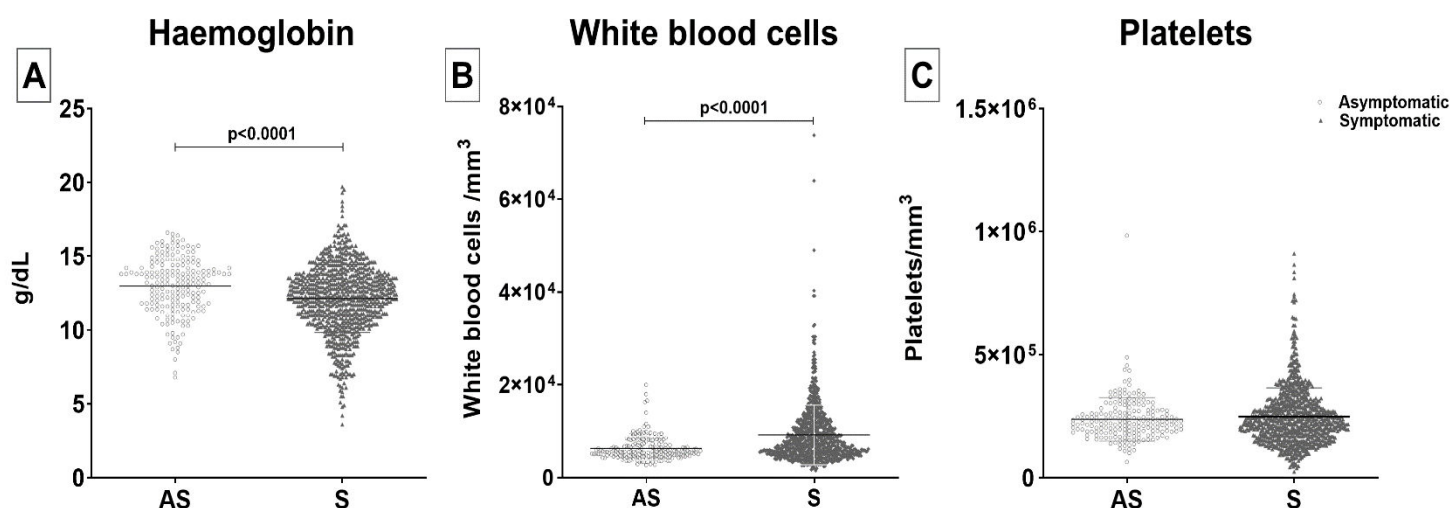


Figure 1: Haemoglobin (A) concentration, white blood cells (B) and platelets (C) count of symptomatic (n=851) and asymptomatic (n=194) COVID-19 patients. The Mann-Whitney U-test was used for comparisons of medians.

Immune Cells Count in Symptomatic and Asymptomatic COVID-19 Patients

COVID-19 symptomatic patients had a significantly high count of neutrophils ($p<0.0001$), lymphocytes ($p=0.0047$) and eosinophils ($p=0.0086$) when compared to asymptomatic patients. No significant difference was found for monocytes count between symptomatic and asymptomatic patients (Fig. 2).

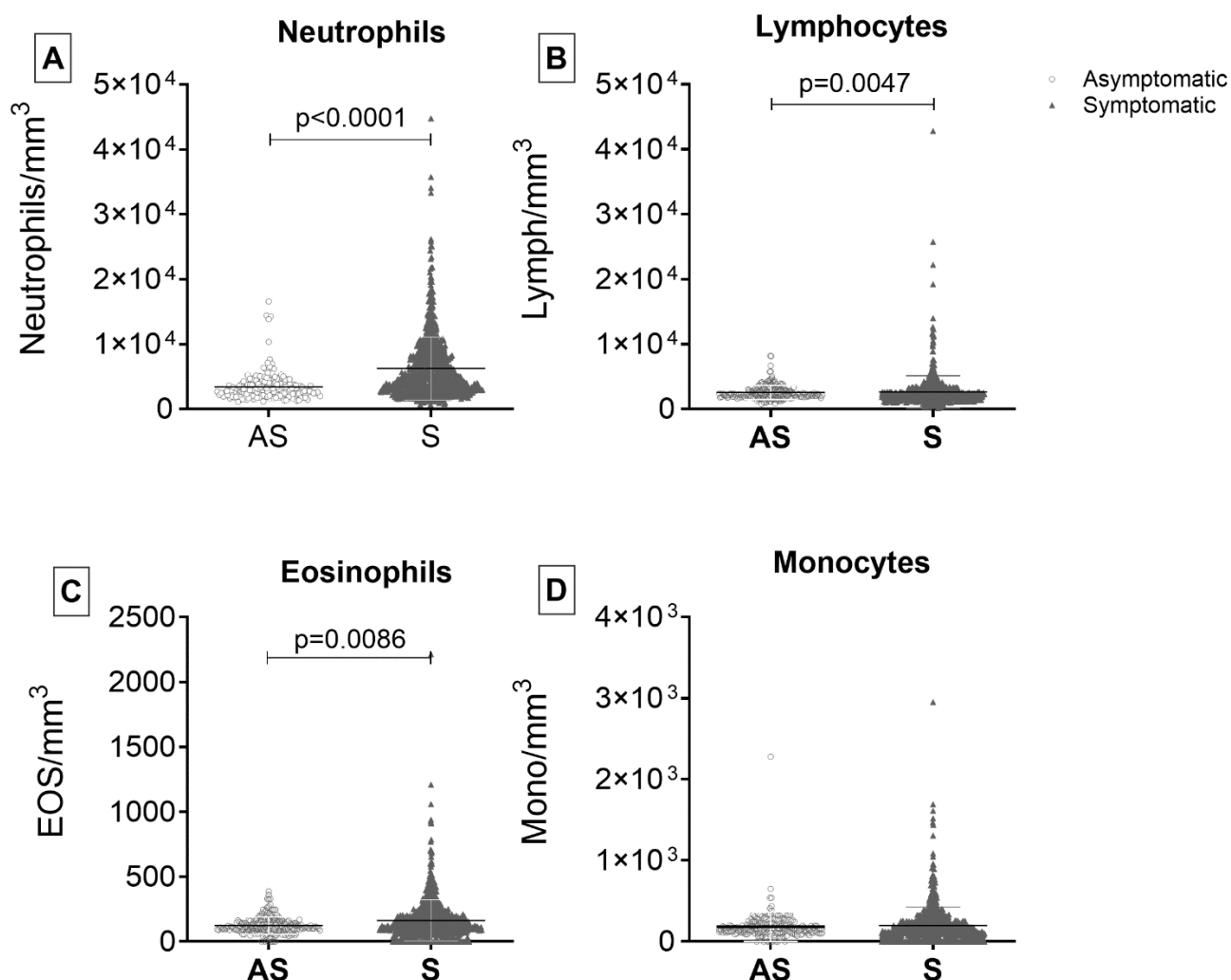


Figure 2: Neutrophils (A), lymphocytes (B), eosinophils (C) and monocytes (D) count in symptomatic (n=851) and asymptomatic (n=194) COVID-19 patients. The Mann-Whitney U-test was used for comparisons of medians.

Association Between Socio-Demographic Characteristics and Severity of COVID-19

Univariate binary logistic regression analysis between socio-demographic characteristics and symptomatic and asymptomatic COVID-19 showed that male gender (OR=1.88; 95% CI [1.35-2.63]); age range [35-47] years (OR=2.54; 95% CI [1.71-3.77]), [48-62] years (OR=5.11; 95% CI [3.26-8.01]) and [63-92] years (OR=21.05; 95% CI [9.98-44.40]) as well as retired patients (OR=24.50; 95% CI [1.51-39.32]) were associated with the severity of COVID-19.

Profession such as managerial and higher intellectual professions (OR=0.162; 95% CI [0.057-0.461]), intermediate professions (OR=0.144; 95% CI [0.051-0.674]), employees (OR=0.215; 95% CI [0.069-0.674]), manual workers (OR=0.157; 95% CI [0.054-0.457]) and Pupils-students-unemployed (OR=0.195; 95% CI [0.062-0.619]) were protective factors against the risk of COVID-19 severity.

Association Between Comorbidities and the Severity of COVID-19

Diabetes [OR = 5.62; 95% CI (3.73-10.78)] and hypertension [OR = 6.34; 95% CI (3.73-10.78)] were associated with the risk of developing the severe form of COVID-19. After adjustment, multivariate binary logistic regression also revealed that diabetes [aOR = 3.61; 95% CI (1.78-7.34)] and hypertension [aOR = 4.76; 95% CI (2.77-8.20)] were indeed high-risk factors for severe outcome of COVID-19.

Association Between Clinical Symptoms and the Severity of the COVID-19

Univariate binary regression analysis showed that fever [OR = 36.07; 95% CI (11.62-115.68)], asthenia [OR = 41.30; 95% CI (10.17-167.63)], rhinitis [OR = 10.78; 95% CI (1.48-78.66)], cough [OR = 19.55; 95% CI (8.57-44.59)] and dyspnoea [OR = 9.75; 95% CI (3.06-31.02)] are symptoms associated with COVID-19.

Multivariate binary logistic regression analysis after adjustment showed that fever [aOR = 21.06; 95% CI (6.57-67.49)], asthenia [aOR = 34.17; 95% CI (8.32-140.32)], rhinitis [aOR = 10.17; 95% CI (1.33-32.61)], cough [aOR = 14.06; 95% CI (6.06-32.61)] and dyspnoea [aOR = 6.93; 95% CI (2.09-23.04)] are symptoms truly associated with COVID-19 disease.

Association Between Quantitative Abnormalities of Immune Cells and the Severity of COVID-19

The Table 1 shows results of chi-square test and univariate binary logistic regression between immune cells count and symptomatic/asymptomatic COVID-19. The chi-square test showed a link between symptomatic COVID-19 and low/high platelets and lymphocytes count; high eosinophils, white blood cells and neutrophils count with a p value ≤ 0.0001 .

From univariate binary logistic regression analysis, it was found that complete blood count abnormalities as low platelets count [OR = 2.75; 95% CI (1.55-4.88)] or high platelets count [OR = 3.12; 95% CI (1.23-7.91)], high white blood cells [OR = 9.87; 95% CI (4.96-19.61)], high neutrophils count [OR = 9.92; 95% CI (4.80-20.45)] and low lymphocytes count [OR = 3.82; 95% CI (2.20-6.65)] were associated with COVID-19 severity.

Immune Cells	Infection Status		X ²	Univariate Analysis	
	Asymptomatic n (%)	Symptomatic n (%)	p-value	OR	p-value
Platelets			0.000*		
Normal	175 (21.2)	143 (91.1)		1	
Low	14 (8.9)	58 (92.1)		2.75 (1.55–4.88)	0.001*
High	5 (7.9)	650 (78.8)		3.12 (1.23–7.91)	0.016*
WBC			0.000*		
Normal	166 (24.9)	501 (75.1)		1	
Low	19 (18.8)	82 (96.8)		1.43 (0.84–2.43)	1.85
High	9 (3.2)	268 (96.8)		9.87 (4.96–19.61)	0.000*
Neutrophils			0.000*		
Normal	175 (23.9)	556 (76.1)		1	
Low	11 (20.4)	43 (79.6)		1.23 (0.62–2.44)	0.55
High	8 (3.1)	252 (76.1)		9.92 (4.80–20.45)	0.000*
Eosinophils			0.001*		
Normal	194 (19.5)	803 (80.5)		1	
High	0 (0.0)	48 (100)		NA	
Lymphocytes			0.000*		
Normal	167 (22.4)	579 (77.6)		1	
Low	15 (7.0)	199 (93.0)		3.82 (2.20–6.65)	0.000*
High	12 (14.1)	73 (85.9)		1.76 (0.93–3.31)	0.082
Monocytes			0.658		
Normal	193 (18.6)	844 (81.4)		1	
High	1 (12.5)	7 (87.5)		1.60 (0.19–13.08)	0.66

Table 1: Quantitative abnormalities of immune cells associated with the severity of COVID-19.

Discussion

In this study, men were more represented with frequency of 57.60% and the most exposed people were pupils-students-unemployed (24.21%) followed by Artisans- shopkeepers-entrepreneurs (22.30%). So was in earlier studies where Moueden, et al., found in 2020 a proportion of 67.2% of male and Wang, et al., found 54.3% of males in 2020 [10,11]. This may be due to socioeconomic position, health status and occupation [12]. Indeed, there is a predominance of men in those professions that promotes easy contact with others.

The retired patients were less represented (9.76%) in this study but were the most represented among symptomatic patients (29.96%) with a high risk of severe COVID-19 for over 63 years old patients (OR=24.50; 95% CI [1.51-39.32]). Similarly, Garcia's team found an increased association between mortality due to COVID-19 and age in 2022 [13]. Also, it has been shown that the risk of hospital admission was associated with age with an OR>2 for all age groups above 37.9 (95% CI [26.1-56.0]) [14]. Thus, as age increases, the risk of developing severe COVID-19 become high and could be also explained by the weakening of immunity with age. Concerning the comorbidities, hypertension, diabetes and asthma were found respectively at 31.10%, 19.62% and 3.63% in this study. Huang, et al., found among COVID-19 patients in their study 20% diabetes and 15% hypertension [15]. Also, on a cohort of 5,700 COVID-19 patients in New York City Area, hypertension and diabetes occupied respectively 56.6% and 33.8% of people studied [16]. The rates of patients with hypertension and diabetes are lower in our study than in those studies but hypertension remains the most representative of the comorbidities. Diabetes and hypertension were indeed risk factors associated with the severity of COVID-19. These results suggest that increased severity of COVID-19 observed in patients with diabetes is probably due to a dysregulated immune response which leads to more severe lung pathology. Moreover COVID-19 patients with hypertension would have high risk of mortality [17]. In our study, all symptomatic patients had fever, rhinitis, cough, dyspnea and angina whereas 92.47% had asthenia and 86.84% had myalgia. Moreover, fever, asthenia, rhinitis, cough and dyspnoea were symptoms associated with COVID-19 disease. Indeed, Data from Wuhan population showed that the most frequent clinical features at the onset of illness in COVID-19 patients were fever, fatigue and cough [15]. These data confirm then results found in this study.

The evaluation of immune cells profile of COVID-19 patients in this study revealed hyperleukocytosis ($p = 0.000$) linked to neutrophil polynucleosis ($p = 0.000$), eosinophil polynucleosis ($p = 0.001$) and lymphocytosis ($p = 0.000$) in symptomatic COVID-19 patients compared to asymptomatic patients. Also, univariate binary logistic regression analysis showed that low platelets count [OR = 2.75; 95% CI (1.55-4.88)] or high platelets count [OR = 3.12; 95% CI (1.23-7.91)], high neutrophils count [OR = 9.92; 95% CI (4.80-20.45)] and low lymphocytes count [OR = 3.83; 95% CI (2.20-6.65)] were associated with the severity of COVID-19 disease. In previous studies, hyperleukocytosis was often observed in hospitalized COVID-19 patients. Sayad, et al., found that the mean white blood cell count in all severely affected patients was $9 \times 10^9/L$, the mean white blood cell count in survivors was $7.6 \pm 3.5 \times 10^9/L$ compared to $10.5 \pm 5.6 \times 10^9/L$ ($p = 0.008$) in non-survivors and 41% of non-survivors had a higher white blood cell count than normal ($>11 \times 10^9/L$) compared to 11.4% in survivors [18]. These results support publications that have reported elevated white blood cell counts and decreased lymphocytes counts as an indicator of clinical deterioration in COVID-19 patients [19,20]. Moreover, COVID-19 is an inflammatory disease and induces activation of antiviral immune responses and causes uncontrolled inflammatory responses characterized by the release of pro-inflammatory cytokines in patients with severe COVID-19, resulting in lymphopenia, lymphocytes dysfunction and abnormalities in granulocytes and monocytes [21]. Also, the cytokines storm observed in COVID-19 patients has been reported to underlie the induction of lymphocytes apoptosis. Thus, TNF α , IL-6 and other pro-inflammatory cytokines can induce lymphocytes deficiency [21].

Univariate regression analysis revealed that both low and high platelets count in patients with severe COVID-19, but more thrombocytopenia was observed with a $p=0.0001$. Yang, et al., in their study found that thrombocytopenia was present in 20.7% of cases, mortality due to COVID-19 was related to advanced age which in turn predisposed to thrombocytopenia and the lower the platelets count, the higher the in-hospital mortality rate [22]. Also, Tang, et al., found that 57.1% of non-survivors COVID-19 patients had thrombocytopenia [23]. Indeed, the thrombocytopenia during COVID-19 would be due to SARS-CoV-2 inhibition of hematopoiesis in the bone marrow through some receptors to cause decreased primary platelets formation [24]. Also, platelets can internalize viral infectious agents such as HIV in vitro. The latter are sheltered by the platelets which are eliminated by the tissue monocytes/macrophages in both physiological and inflammatory contexts [25]. This could explain the monocytopenia observed then in this study ($p<0.0001$).

Conclusion

This study aimed to determine the quantitative abnormalities of immune cells associated with COVID-19 severity. We found that low or high platelets count, high white blood cells count, high neutrophils count and low lymphocytes are quantitative abnormalities of immune cells associated with the severity of COVID-19 in Togo. It is therefore important to consider the immune cells profile in the complete blood count examination for the diagnosis and follow-up of COVID-19 patients in Togo.

Conflict of Interests

The authors declare no conflicts of interest.

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