



Allergenic fungal spores and hyphal fragments in the aerosol of Havana, Cuba

Michel Almaguer · Lilivet Díaz · María Fernández-González · Elayne Valdéz

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Abstract Fungal spores have a recognized allergenicity, but in recent years, fungal fragments have also been reported as inducers of Type I allergy. However, airborne hyphae are scarcely studied in tropical regions. The objective of this research was to analyze the fungal composition of the Havana aerosol during 2017 and 2018, emphasizing the presence of hyphae fragments. Air sampling was performed using a Lanzoni VPPS-2000 sampler. The fungal propagules collected were identified and counted following the recommendations of the Spanish Aerobiology Network in two longitudinal transects of each daily slides. In 2017, the spore count and hyphae count were higher than in 2018 (108,607 spores and 3822 fungal fragments vs. 40,896 spores and 2268 fungal fragments). *Cladosporium* was the most abundant spore (77.2–76.6%), predominating in the month of May. *Leptosphaeria* ascospores were identified at lower levels (16.2–17.9%) but also abundant during May. The behavior of the hyphal fragments does not show great variations throughout the year. The contribution of this component to the aerosol ranged between 3.4

and 5.3% in 2017 and 2018, respectively. The intradiurnal periodicity showed a diurnal pattern for *Cladosporium* and nocturnal for *Leptosphaeria*, while the hyphae pattern does not show great variations. The temperature and relative humidity correlated with the concentration of the detected airborne fungi propagules. The composition of the fungal aerosol in Havana during the 2 years was characterized, including both spores and hyphal fragments, since both structures can be related to respiratory allergy pathologies.

Keywords Airborne fungi · Spores · Non-viable methodology · Havana · Fungal fragments

1 Introduction

Fungi are ubiquitous organisms, that represent approximately 25% of terrestrial biomass, detected in a wide variety of ecosystems. They have important ecological roles as saprotrophs, mutualistic symbionts, parasites or hyperparasites. Based on their lifestyle, fungi typically reproduce by spores, formed through sexual or asexual processes and involved in survival and dispersal processes. Long-range dispersal of airborne spores over thousands of kilometers is known to occur in nature (Levetin et al. 2015). The production, release and dispersion of spores in the atmosphere are determined by the complex interaction between biological and environmental factors such as:

M. Almaguer (✉) · L. Díaz · E. Valdéz
Department of Microbiology and Virology, University of Havana, Havana, Cuba
e-mail: michelalm@fbio.uh.cu;
michel.almaguer@gmail.com

M. Fernández-González
Department of Plant Biology and Soil Sciences,
University of Vigo, Vigo, Spain

geographical location, air pollution, weather conditions, human activity and local source of vegetation (Grinn-Gofroń and Bosiacka 2015; Ščevková et al. 2019). But in addition to the spores, fragments of the mycelium, fractured conidiophores or other mycelial structures can also be dispersed through the air (Green et al. 2006). These fungi propagules generally come from fungal colonies that grow on various substrates. Their presence began to be documented since last century in works conducted in England by Pady and Gregory (1963) and Harvey (1970). In the USA, Pady and Kramer (1960) reported airborne concentrations of up to 1700 hyphae/m³. The interest of this type of studies was intensified after experimental and epidemiological researches demonstrated its relationship with the severity of asthma. Therefore, several authors raised the need to include them in the aerobiological analysis (Green et al. 2005; Aparna et al. 2015). In the Caribbean area, sensitization to hyphal fragments is comparable to basidiospores incidence, even more prevalent than conidial sensitization (Rivera-Mariani and Bolaños-Rosero 2012).

In Cuba, particularly in Havana, continuous monitoring of the atmosphere has allowed to know the predominant fungi (Almaguer et al. 2015). *Cladosporium* Link is the most represented, followed by *Leptosphaeria* Ces. & From Not., and to a lesser extent, *Aspergillus* P. Micheli ex Haller, *Penicillium* Link and *Alternaria* Nees (Almaguer et al. 2015). Their impact on the development of respiratory allergies is well known (González-Díaz et al. 2016). However, the potential of hyphal fragments to cause or aggravate allergic diseases is still the subject of constant research. Therefore, the objective of this work is to provide the first systematic counts of hyphae atmospheric fragments related to data about the concentration of the most abundant spore types in the Havana atmosphere.

2 Materials and methods

2.1 Study zone

The study was carried out in Havana (located on the northwestern coast of the island of Cuba, at an altitude of 24 m above sea level). It is the largest city in Cuba and has a population of more than 2 million inhabitants (approximately 20% of the population in Cuba). The

climate of Cuba is subtropical, characterized by the alternation of two weather seasons throughout the year based on accumulative rainfall: a dry (November–April) and a rainy season (May–October). Havana's climate exhibits mid-continental characteristics: somewhat cold winters and warm, humid summers. In addition, most of the precipitation occurs during the warmer months, and there are rather large variations in yearly temperature and precipitation (Lecha et al. 1994). The meteorological data correspond to the Casablanca station of the Institute of Meteorology, located in the city. In the 2 years of study, the temperature showed few differences, with an average value close to 25 °C (average T^a), the same as the relative humidity (76% RH average). The year 2018 was slightly rainier than 2017 (1221 mm vs. 1131 mm). The predominant wind direction in both years was NE with average values of 47.3 and 49.5 km/h of wind maximum speed. No dense vegetation was present near the location of the volumetric sampler, although there were numerous gardens with a wide variety of trees, shrubs and ornamental plants that are part of the city's layout.

2.2 Sampling and counting

Light microscopy was used to analyze samples collected using a Lanzoni VPPS 2000 Hirst-type volumetric sampler (Lanzoni s.r.l., Bologna, Italy), located on the roof of the Biology Faculty of the University of Havana, 35 m above ground level (23° 08' N; 82° 23' W). Sampling was carried out continuously during 2 years (operating 24 h/day), at 10 L per minute, from January 1, 2017, to December 31, 2018. The spores of *Cladosporium*, *Leptosphaeria*, the rest of spores as “other spores” and the hyphal fragments were counted by direct microscopy, using the methodology proposed by the Spanish Aerobiology Network, based on the observation of two longitudinal sections of microscopic slide at a magnification of 400 × (Galán et al. 2007). The results of the concentrations were expressed as spores or fragments of hyphae per cubic meter of air (spores/m³ or hyphae/m³), while for total counts spores or hyphae were used.

2.3 Statistical analysis

A nonparametric ANOVA with a simple classification by Kruskal–Wallis ranks, complete with

nonparametric SNK, was applied to verify the difference between the monthly concentrations. The Mann–Whitney U test was used to compare spore/hyphae and the main meteorological parameters between years. To determine the influence of weather-related variables on airborne spore or hyphae counts, Spearman's test was used since data distribution was non-normal. The STATISTICA 8.0. software package for Windows was used for this purpose ($p < 0.01$ and $p < 0.05$).

3 Results

An annual spores integral of 108,607 were counted during 2017, of which *Cladosporium* represents 77.2% and *Leptosphaeria* 16.2%. The total fungal hyphal fragments detected were 3822 fragments (Fig. 1). In the year 2018, the amount of total spores (40,896 spores) and hyphae (2268 fragments) was drastically reduced, although the predominance of *Cladosporium* (76.6% of spores) and *Leptosphaeria* (17.9% of spores) was comparable, with a slight increase in *Leptosphaeria*'s incidence. Differences were detected in both total spores and hyphal fragments between the 2 years ($U = 38,010.00^{**}$, $U = 48,579.00^{**}$, respectively). However, from the climatic point of view, both years only differed in the wind speed and minimum relative humidity values ($U = 56,997.00^{**}$, $U = 56,997.00^{**}$, respectively).

The spore types and the hyphal fragments showed their highest values in the month of May, but the daily maximum concentrations were recorded at different times of the year. *Cladosporium* reached 2665 spores/ m^3 on 05/19/2017 and 1690 spores/ m^3 on 04/27/2018, *Leptosphaeria* reached 345 spores/ m^3 on 10/7/2017 and 170 spores/ m^3 on 11/2/2018. Hyphal accounted

for 197 hyphae/ m^3 on 10/25/2017 and 72 hyphae/ m^3 on 01/13/2018 (Fig. 2).

With regard to the intraday distribution, *Cladosporium* spores showed a marked daytime pattern, with a higher concentration between 11 and 12 a.m. in each of the analyzed years (Fig. 3). On the contrary, *Leptosphaeria* was more abundant in the predawn hours and early morning, while the fungal fragments do not reflect a clear pattern.

Statistical analysis indicates that temperature and relative humidity were the most significant parameters in the airborne concentration of spores and hyphal fragments, while wind and rain speed had a minor influence (Table 1). The highest correlation for *Cladosporium* spores was obtained with the maximum temperature in 2017 (0.338**) and with the minimum temperature in 2018 (-0.306^{**}), in the first case was positive and in the second was negative. For *Leptosphaeria*, the average relative humidity was the parameter most correlated (0.485** and 0.464**, respectively). Finally, for hyphal fragments, the correlation was higher with the minimum relative humidity in 2017 (-0.420^{**}) and with the minimum temperature in 2018 (-0.402^{**}), in both cases negative.

4 Discussion

Cladosporium has been reported as the predominant genus in the atmosphere worldwide (Fernández-Rodríguez et al. 2017; Ramos and Meza 2017; Pusz et al. 2018). However, some studies of tropical areas also noted the great abundance of airborne ascospores (Quintero et al. 2010). In this work, it has been demonstrated that the fungal aerosol of Havana is mainly formed by the *Cladosporium* and *Leptosphaeria* spores, in accordance with previous studies carried out in Cuba (Almaguer et al. 2015) and in other tropical countries (Quintero et al. 2010; Rocha et al. 2013). Both genera have a recognized allergenicity (Levetin et al. 2015; Rivera-Mariani and Bolaños-Rosero 2012), and in a study conducted in Havana, *Cladosporium* was isolated from a large number of nasal samples from patients with asthma and / or rhinitis (Barrios et al. 2019).

The total annual spore integral in 2017 practically tripled those recorded in 2018. This result was obtained with the same methodology of sampling

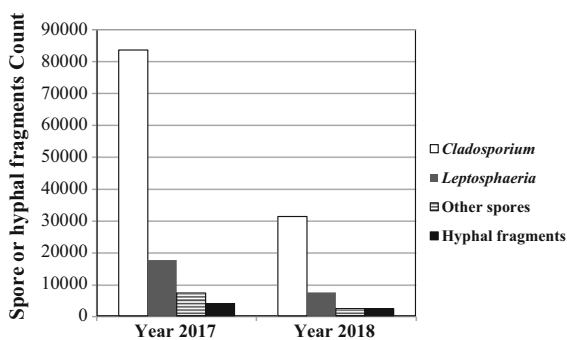


Fig. 1 Spores and hyphal fragments counted in Havana

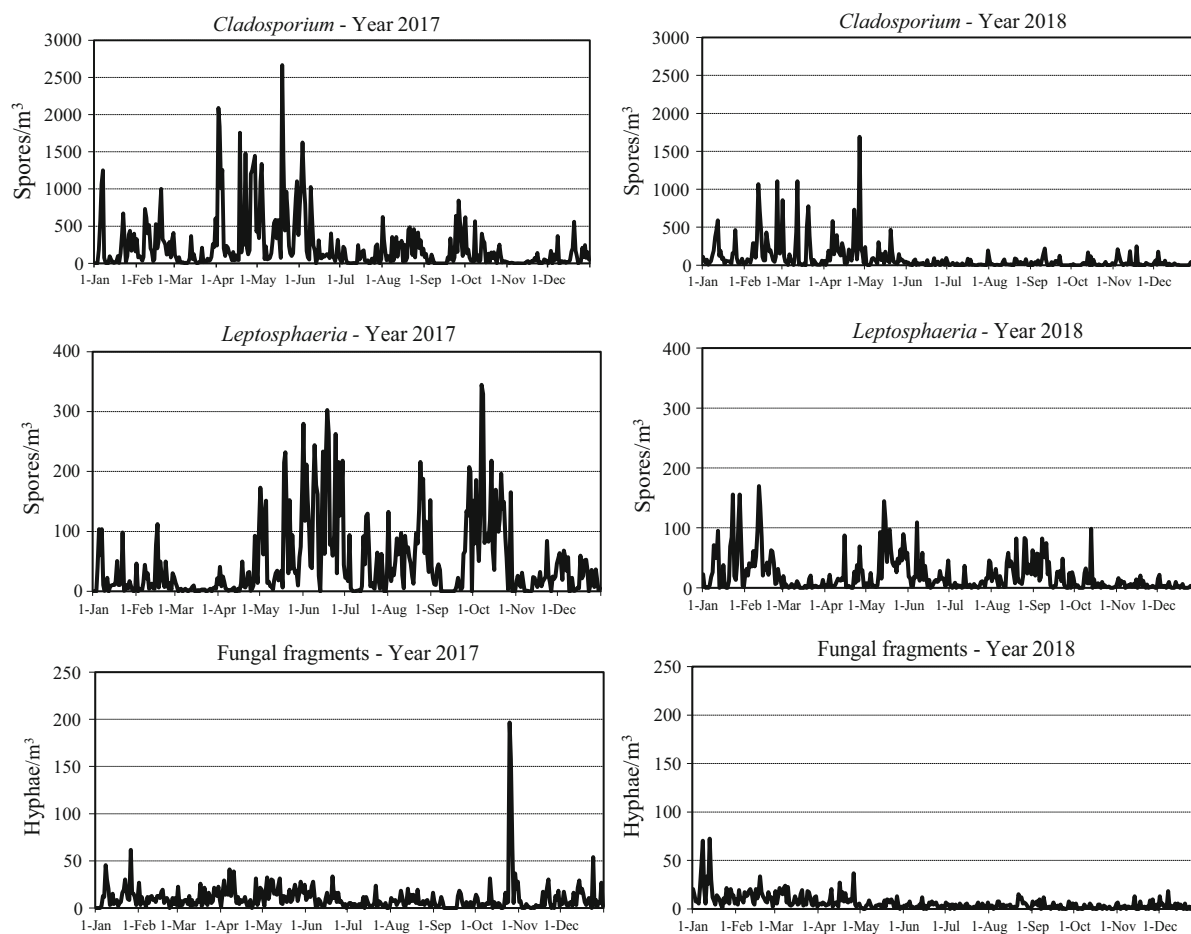


Fig. 2 Daily variation of the concentration of *Cladosporium*, *Leptosphaeria* spores and hyphal fragments counted in Havana

and counting (slide analysis, microscope). Several authors have pointed out that the main causes of these variations between years are due to climatic factors and anthropic action, mainly for the influence of agricultural activities (Green et al. 2006; Elbert et al. 2007; Njokuocha et al. 2019). The findings revealed that taking into account that the average temperature and humidity remained fairly homogeneous throughout the two study years and that the difference in rainfall rate scarcely varied, the indicated differences could be related to the influence of the wind, as statistical correlations suggest. In both years, the predominant direction was NE; however, the maximum wind speed was higher in 2018 than in 2017 with an average of 49.5 km/h (25–111 km/h) and 173 days above the average values compared to 47.3 km/h (15–124 km/h) and 150 days. The fungal content in the air may be directly influenced by the variability of

wind direction and speed over time (Tignat-Perrier et al. 2019).

The spores distribution throughout the year showed a gradual increase from the first months, with maximum values in May at the beginning of the rainy season. This dynamic, in which the concentration of spores increases as the rainy months progress, has also been reported in other countries (Aparna et al. 2015). The increase in *Cladosporium* spores during this period can be related to the progress of summer, since the maximum temperature has been statistically significant. In other geographical areas its predominance has also been cited toward the end of the rainy seasons (Pyrri and Kapsanaki-Gotsi 2015), although sporulation of *Cladosporium* can occur in both dry and wet conditions (Knutsen et al. 2012).

The results obtained for *Leptosphaeria* coincide with the previously reported (Almaguer et al. 2015).

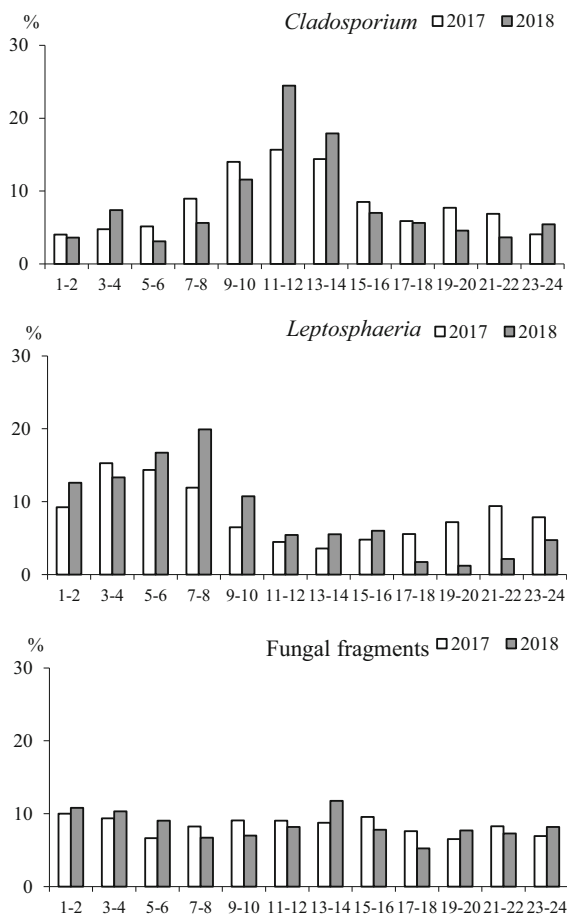


Fig. 3 Intraday variation of *Cladosporium*, *Leptosphaeria* spores and fungal fragment counted in Havana

Relative humidity directly influences their atmospheric dynamics, since it mediates in the mechanisms of ascospores release. In the Caribbean area, Quintero et al. (2010) noted its prevalence during rainy and hot months, whereas in other latitudes a similar behavior has been observed, related to a greater release in periods of abundant rainfall (Dawidziuk et al. 2012).

The maximum daily *Cladosporium* values were recorded around the change of season with levels higher than 1600 spores/m³, while those of *Leptosphaeria* did not reach 350 spores/m³. The predominance of *Cladosporium* versus *Leptosphaeria* has also been pointed out by other authors (Antón et al. 2019), with daily peaks of up to 5746 spores/m³ in the first case and lower in the second (44 spores/m³).

In relation to the intraday behavior, both *Cladosporium* and *Leptosphaeria* nocturnal patterns have been observed in previous studies (Almaguer et al. 2014). The prevalence of *Cladosporium* during the day may be related to high insolation values, since in many anamorphic fungi of septate hyphae, asexual spores are formed in loose chains (Portnoy and Jara 2015). When they dry, the torsion of hyphae favors release of conidia (Elbert et al. 2007). On the contrary, the discharge is active for *Leptosphaeria* since the ascus requires moisture for the release of ascospores. These conditions may happen more commonly at night (Quintero et al. 2010).

Regarding hyphal fragments, although some studies have highlighted their importance as part of the

Table 1 Correlations between *Cladosporium*, *Leptosphaeria* and fungal fragments concentrations, and the main meteorological parameters during the two study years (2017 and 2018)

	Rain	Max T^a	Min T^a	Avg T^a	Max RH	Min RH	Avg RH	Wind speed
2017 ($N = 365$)								
<i>Cladosporium</i>	− 0.019	0.338**	0.0238	0.120*	0.199**	− 0.228**	0.083	0.105*
<i>Leptosphaeria</i>	0.302**	0.415**	0.342**	0.349**	0.422**	0.176**	0.485**	− 0.055
Others spores	0.0074	0.321**	0.249**	0.284**	0.173**	0.013	0.171**	− 0.077
Fungal fragments	− 0.176**	− 0.0124	− 0.242**	− 0.160**	− 0.275**	− 0.420**	− 0.400**	0.215**
2018 ($N = 365$)								
<i>Cladosporium</i>	0.130*	− 0.113*	− 0.306**	− 0.263**	0.163**	0.0061	0.085	0.097
<i>Leptosphaeria</i>	0.373**	0.175**	0.0685	0.055	0.420**	0.276**	0.464**	0.086
Others spores	0.195**	0.252**	0.0914	0.135*	0.198**	0.0737	0.225**	− 0.057
Fungal fragments	− 0.075	− 0.214**	− 0.402**	− 0.343**	− 0.201**	− 0.269**	− 0.286**	0.134*

T^a temperature, RH relative humidity, Min minimum, Avg average, Max maximum

Significant differences are indicated in bold according to Spearman's rank correlation (* $p < 0.05$; ** $p < 0.01$)

aerosol in different regions of the world (Sinha and Kramer 1971; Samir et al. 2014; Tendal and Madsen 2011; Saatcioglu et al. 2016), there is no reference in the aerobiological scientific reports in Cuba. However, Green et al. (2006) argued that such fragments may have a higher content of allergenic proteins than conidia and Afanou et al. (2018) indicated that airborne hyphae contain immunostimulatory macromolecules, which include antigens, allergens, mycotoxins and β -1,3-glucans.

The importance of analyzing the fungal propagules that are dispersed through the air, both spores and fungal fragments, is justified by the sensitization that both can produce in the atopic population. This may be related to their dimensions and how they can reach different parts of the respiratory system (Venero et al. 2009). In this work, the hyphal fragment size ranged between 10 and 82 μm . Vincent (2005) noted fungal aeroallergens with dimensions greater than 5 μm will be deposited mostly in the nasopharyngeal and extrathoracic areas, where they can trigger nasal and ocular disorders such as allergic rhinitis and allergic asthma.

The behavior of the hyphal fragments in this study does not show great variations throughout the year. Since their origin can be very diverse, molecular investigations to complement the aerobiological studies should be required to identify their source (Green et al. 2006; Samir et al. 2014). Although hyphal fragment formation is random, their increase was registered in the month of May, with a similar trend that the spore counts. This result contrasts with other investigations which detected maximum levels during July, August and September (Pady and Kramer 1960; Kadam et al. 2009; Pyrri and Kapsanaki-Gotsi 2015). Some authors indicate their abundance during the dry days and with higher temperature, probably because these conditions allow them to detach from the substrate more easily (Kadam et al. 2009; Tendal and Madsen 2011).

It has been estimated that mycelium fragments can contribute to the airborne mycobiota in a variable range from the 6 to 80% of the total fungal particles of the outside air (Kadam et al. 2009; Tendal and Madsen 2011; Pyrri and Kapsanaki-Gotsi 2015). In this work, the contribution of this component to the aerosol has ranged between 3.4% in 2017 and 5.3% in 2018.

The hyphal fragments peak was detected on October 25, 2017. According to weather records, the

last days of this month were characterized by the arrival of several cold fronts. The first one affected the western region at dawn on the 25th, with a maximum wind gust of 57 km/h and maximum winds of 35 km/h (Arias-Mulet et al. 2019). Cold fronts are characterized by a discontinuity in meteorological characteristics such as air temperature and humidity and are rain-producing systems (Lecha et al. 1994). These characteristics could influence the presence of hyphal fragments, taking into account the correlations with the meteorological variables.

Although temperature and relative humidity were the most significant parameters in the airborne concentration of spores, the positive correlation between *Cladosporium* and the maximum temperature in 2017 and negative with the minimum temperature in 2018 indicates the diverse influence of weather in tropical climate. The positive relationship between *Leptosphaeria* and meteorological conditions (temperature, relative humidity and rain) is possibly related to the ascospores release.

The hyphal fragments also do not show a clear intraday pattern, as reported in studies conducted in other geographical areas, in which the highest concentrations were recorded at different hours of the day (Pady and Kramer 1960; Harvey 1970). Rúa-Giraldo (2013) detected its predominance from dawn until noon in summer, while in autumn the highest levels were late in the afternoon.

The present research provides a new insight into the composition of the fungal aerosol of Havana, a neotropical city, since hyphal fragments are studied in addition to the spores. Our results suggest that the mixed composition of various types of all potentially allergic fungal structures should be considered, in order to elucidate the role of fungal aerosols may play in fungal diseases.

5 Conclusions

Fungal fragments were detected for first time in a continuously way in Havana, Cuba, by using a non-viable volumetric sampling method. *Cladosporium* and *Leptosphaeria* spores were abundant during May, while hyphal fragments are constantly represented in the atmosphere throughout the year. The intradiurnal periodicity showed a diurnal pattern for *Cladosporium* and nocturnal for *Leptosphaeria*, while the hyphae

content did not show great variations. Analysis of the influence of major weather-related variables on airborne spore and fungal fragments concentrations showed that temperature and relative humidity were the most significant variables. The results obtained in this study may help in the design of strategies aimed to prevent the allergic respiratory diseases, since all detected fungal bioparticles have an allergenic character.

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Compliance with ethical standards

Conflict of interest There are not conflicts of interest/competing interests.

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