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Bladder Cancer Diagnosis: A Comparative Study of Cystoscopy and Urine Cytology in a Nigerian Cohort

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Author for Correspondence*: imammkabir@yahoo.com/ imkabir.mls@buk.edu.ng<https://dx.doi.org/10.4314/sokjmls.v10i1.34>**Abstract**

Bladder urothelial cancer is the first most common cancer of the urinary system, the 10th most common in men and the 17th in women, with its incidence being 3-4 times higher in men than in women. In addition, Tobacco smoking increases the risk of bladder cancer incidence and recurrence. Urine cytology, cystoscopy and biopsy are the three most common techniques employed in diagnosing bladder cancer. Cystoscopy and biopsy are mostly inconvenient and invasive, in addition to being expensive, especially in resource-limited settings. Therefore, there is a need to evaluate the possibility of adopting urine cytology as a screening method for bladder cancer diagnosis, while understanding the association between smoking and risk of bladder cancer. We studied the morphological features of exfoliated cells in the urine of suspected bladder cancer patients and that of apparently healthy subjects to determine its efficacy as a standard diagnostic procedure. The urine samples were aseptically collected from 40 adult patients suspected of having bladder cancer, and 10 healthy adults from Abubakar Imam Urology Center, Kano, Nigeria. Samples were analyzed by Papanicolaou and Diff Quick staining techniques. Biopsy and cystoscopic samples were collected only from the test subjects. With 26% of the subjects showing an unsatisfactory cytological finding, 36% were negative for high-grade urothelial cancer (NHGUC), 18% were suspicious for high-grade urothelial cancer (SHGUC), and 20% had high-grade urothelial cancer (HGUC). Cystoscopy reveals that 57.5% showed signs of malignancy, while bladder

biopsy revealed that 50% of subjects showed no evidence of primary tumor (T0), 25% had noninvasive carcinoma (Ta), 5% had carcinoma in situ (TIS), and 20% had invasive carcinoma (T2). The majority (36%) of the subjects were found to be negative for bladder cancer. Also, the majority of those diagnosed with HGUC were found to be moderate to severe smokers between the ages of 67-75 years, and none among those aged 45-55 were found to have HGUC. Urine cytology remains a useful tool in evaluating suspected urothelial malignancies and can serve as an adjunct to cystoscopy and biopsy, especially in resource-limited settings. Smoking and old age were found to be among the predisposing factors to the development of bladder urothelial cancer.

Keywords: Bladder urothelial cancer, Urine cytology, Cystoscopy, Biopsy, Smoking.

Introduction

Bladder urothelial cancer is the first most common cancer of the urinary system (Abubakar *et al.*, 2024), the 10th most common in men and the 17th in women, with its incidence being 3-4 times higher in men than in women. The survival of humans depends to a large degree on the crucial functions and processes performed by the kidneys (Sullivan *et al.*, 1998). Urine formed by the nephrons of the kidneys is transported to the urinary bladder through the ureters before it is expelled through the urethra. The bladder is a sac-like organ located in the extra-peritoneal space of the pelvis behind the pubic bones and extends into the abdomen when filled with urine (Sellers *et al.*, 2018; Bolla *et al.*, 2023).

Urine Cytology is a stress-free, readily available noninvasive diagnostic test with historically high specificity for bladder cancer (Yafi *et al.*, 2015). It involves microscopic evaluation of morphologic features of shed urothelial cells (Abdulwahab *et al.*, 2019). It is used in conjunction with cystoscopy and biopsy as a standard for the initial assessment of patients suspected of bladder cancer (Yafi *et al.*, 2015). Urinary cytology is currently an essential procedure for monitoring all patients with urothelial neoplasms. It can decrease the frequency with which patients need to be subjected to cystoscopy (Sullivan *et al.*, 2010).

Examination of urinary specimens is increasingly recognized as an essential component of detection and monitoring for patients with bladder neoplasms. Among the available techniques urinary cytology is the most useful (Murphy 1990). Although urine cytology is used for the early detection and surveillance of urothelial carcinoma, causes and outcomes of cytologic-histologic discrepancies, the accuracy of urinary cytology differs clearly between untreated and treated patients with both high and low-grade tumors (Raab *et al.*, 2007, Comploj *et al.*, 2013).

The specificity of cytology is greater than 80% (Radkhah *et al.*, 2005), while the sensitivity for high-grade disease and carcinoma-in-situ can be as high as 80 to 90% (Brown 2000).

In this work, we studied the morphological features of exfoliated cells in the urine of suspected bladder cancer patients and that of apparently healthy subjects to determine the efficacy of urine cytology as a standard diagnostic procedure.

Methods

Study Area and Design

The study was conducted at Abubakar Imam Urology Center, 144 Murtala Muhammad Road, Kano, Department of Medical Laboratory Science, Bayero University, Kano and Histopathology Laboratory of Aminu Kano Teaching Hospital, Kano, all in Nigeria. A cross-sectional study design was adopted.

Study Population.

A total of 50 participants were recruited for the study, comprising 40 adult patients suspected of having bladder urothelial cancer, and 10 other adults who visited the study area for other ailments unrelated to the urinary system as controls. All samples were recruited from the outpatient clinic of Abubakar Imam Urology Center, Kano, Nigeria.

Sample collection and processing

An early morning midstream urine was collected into a sterile-labelled container and transported to the laboratory for processing. A dry, clean, grease-free glass slide was labelled and attached to the cytofunnel, and three drops of the cell suspension were dropped gently into the cytofunnel. The assembly is placed into a Cytospin 4 cytocentrifuge (Thermo Fisher Scientific, MA, USA) and spun at 1000 to 1500rpm for 5 minutes to obtain a thick monolayer of cells that were subsequently fixed in 95% alcohol. Two slides were prepared for each sample, one was stained with Papanicolaou and the other with Diff-Quick staining methods.

Sample staining

For the Papanicolaou method, smears were hydrated in descending grades of alcohol, stained in Harris Haematoxylin for 5 minutes, rinsed in tap water, and briefly differentiated in 1% acid alcohol. The smears were blued in Scott's tap water for 5 minutes, dehydrated in 70% alcohol and two changes of 95% alcohol for 2 minutes each, stained with OG-6 for 2 minutes, and rinsed in two changes of 95% alcohol for 2 minutes each. The smears were further stained with EA-50 for 3 minutes, rinsed in 3 changes of 95% alcohol for 10 seconds each, dehydrated in two changes of absolute alcohol for 10 seconds each, cleared in 3 changes of Xylene and mounted in DPX (Papanicolaou, 1942; Kabir *et al.*, 2021).

For the Diff-Quick method, the smears were allowed to air dry, then fixed in methanol for 30 seconds. The fixed smears were dipped in Diff-Quick Solution 1 for 15 seconds and then transferred to Diff-Quick Solution 2 for another 30 seconds. The stained smears were rinsed in distilled water dehydrated, cleared and mounted in DPX.

Ethics approval and consent to participate

Ethical approval (NHREC/17/03/2018) was obtained from the Kano State Ministry of Health research ethics committee. All ethical issues involving the identity and management of the subjects were followed based on the approved guidelines. Written consent was obtained from each subject.

Results

Socio-demographic distribution of the study participant

The study participants were all males within the

age group of 45-95 years. The majority (32.0%) are aged 66-75 years, followed by those aged 45-55 years (28.0%), 56-65 years (20.0%), 76-85 years (16.0%) and the least are those aged 86-95 years accounting for only 4.0%.

Based on the educational status of the participants, the majority (40.0%) had no formal education, 14.0% had primary level education, 20.0% had secondary level of education, and 26.0% had tertiary education (Table 1).

Table 1: Demographic characteristics of the participants

Variables	Category	Frequency	Percentage
Age group	45-55	14	28.0
	56-65	10	20
	67-75	16	32
	76-85	8	16
	86-95	2	4
Educational Level	Primary	7	14
	Secondary	10	20
	Tertiary	13	26
	Non-formal	20	40
Ethnicity	Hausa/Fulani	37	74
	Yoruba	9	18
	Igbo	4	8

Cytologic and Histologic analysis of the participants' samples

Urine cytology was carried out for both test and controls, with 26% showing an unsatisfactory cytological finding, 36% had NHGUC, 18% had SHGUC and 20% had HGUC. Also, 29 (58%) of the subjects showed moderate to severe inflammatory changes. Normal urine smears showing unremarkable epithelial cells were used as controls (Figure 1). Representative slides that show SHGUC and HGUG are shown in Figure 2 and Figure 3 respectively. Cystoscopy and biopsy were carried out for the test subjects only. Cystoscopy reveals that 57.5% showed signs of malignancy, while bladder biopsy revealed that 50% of subjects showed no evidence of primary tumor (T0), 25% had noninvasive carcinoma (Ta), 5% had carcinoma in situ (TIS), and 20% had invasive carcinoma (T2) (Table 2).

Table 2: Cytologic, Histologic and Cystoscopic grading of the respondents

Intervention	Grading	Percentage
Cytology		
	Unsatisfactory	13(26%)
	NHGUC	18(36%)
	SHGUC	9(18%)
	HGUC	10(20%)
	Total	50(100%)
Histology		
	TO	20(50%)
	Ta	10(25%)
	TIS	2(5%)
	T2	8(20%)
	Total	40(100%)
Cystoscopy		
	Shows malignancy	23(57.5%)
	No malignancy	17(42.5%)
	Total	40(100%)

Bladder urothelial cancer risk associated with tobacco smoking

Smoking was graded as mild (1-10 cigarettes/day), moderate (11-30 cigarettes/day), and severe (above 40 cigarettes/day). Non-smokers constitute 20% of the study population, of which none was found to be positive for bladder cancer. Mild smokers constitute 18% of the study population and among the total number of subjects with HGUC, only 10% of them are positive for HGUC. Moderate smokers constitute 34% of the study population and 50% of subjects with HGUC. While severe smokers constitute 28% of the study population and 40% of subjects with HGUC.

Table 3: Cancer grading concerning smoking habits of the respondents

Smoking habits	Cancer grade				
	Unsatisfactory	NHGUC	SHGUC	HGUC	Total
Non-smokers	4	6	-	-	10
Mild	2	3	3	1	9
Moderate	5	5	2	5	17
Severe	2	4	4	4	14
Total	13	18	9	10	50

Relationship between age and bladder urothelial cancer

The majority (36%) of the subjects were found to be negative for bladder urothelial cancer. Also, the majority of those diagnosed with HGUC were found to be within the age of 67-75 years (50%). None among those aged 45-55 were found to have HGUC.

Table 4: Cancer grading with respect to age of the respondents

Age group	Cancer grade				Total
	Unsatisfactory	NHGUC	SHGUC	HGUC	
45-55	6	6	2	-	14
56-65	3	5	-	2	10
67-75	4	5	2	5	16
76-85	-	1	5	2	8
86-95	-	1	-	1	2
Total	13	18	9	10	50

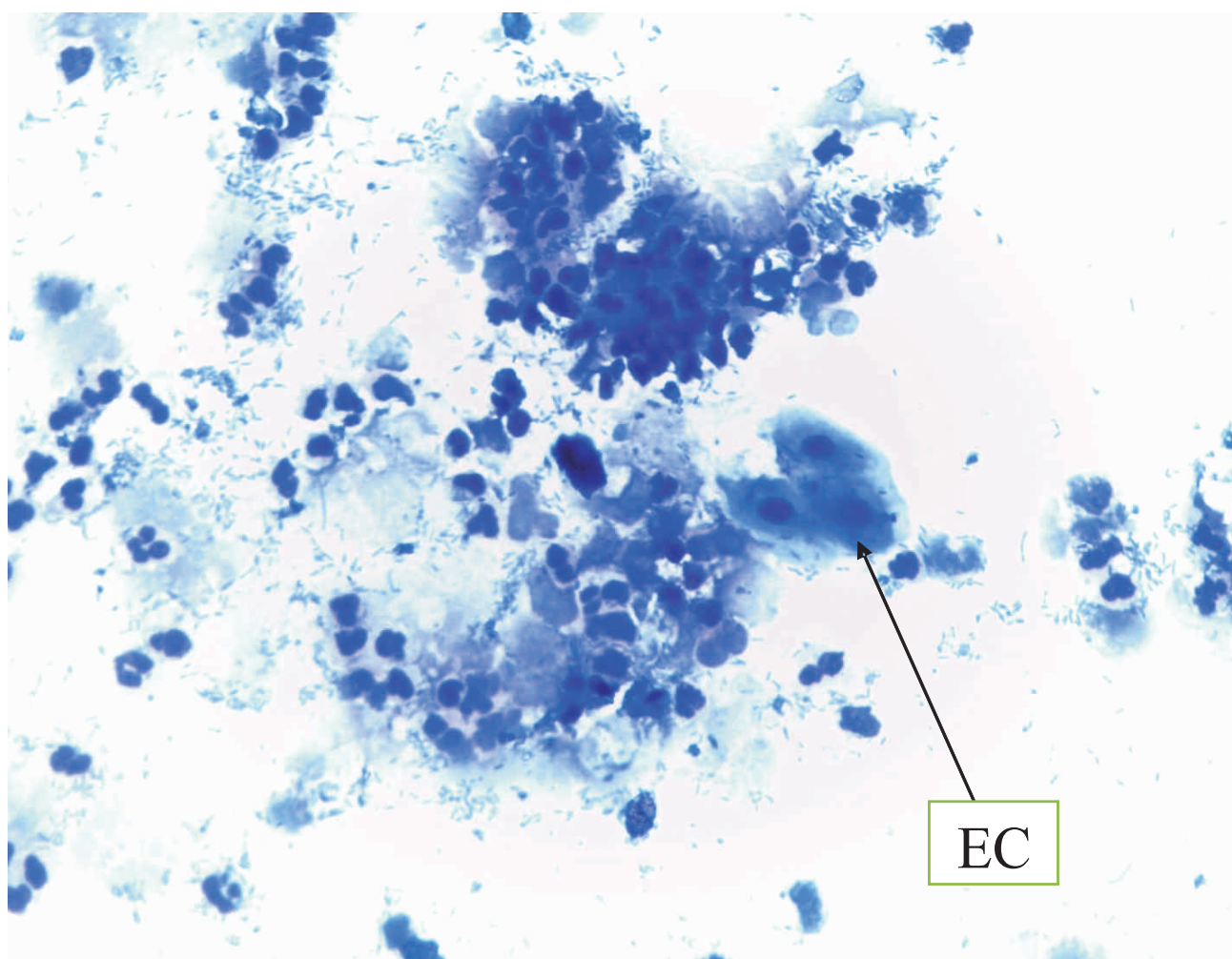


Figure 1: Normal urine smear showing unremarkable epithelial cell (EC) (× 400 magnification, Diff Quick)

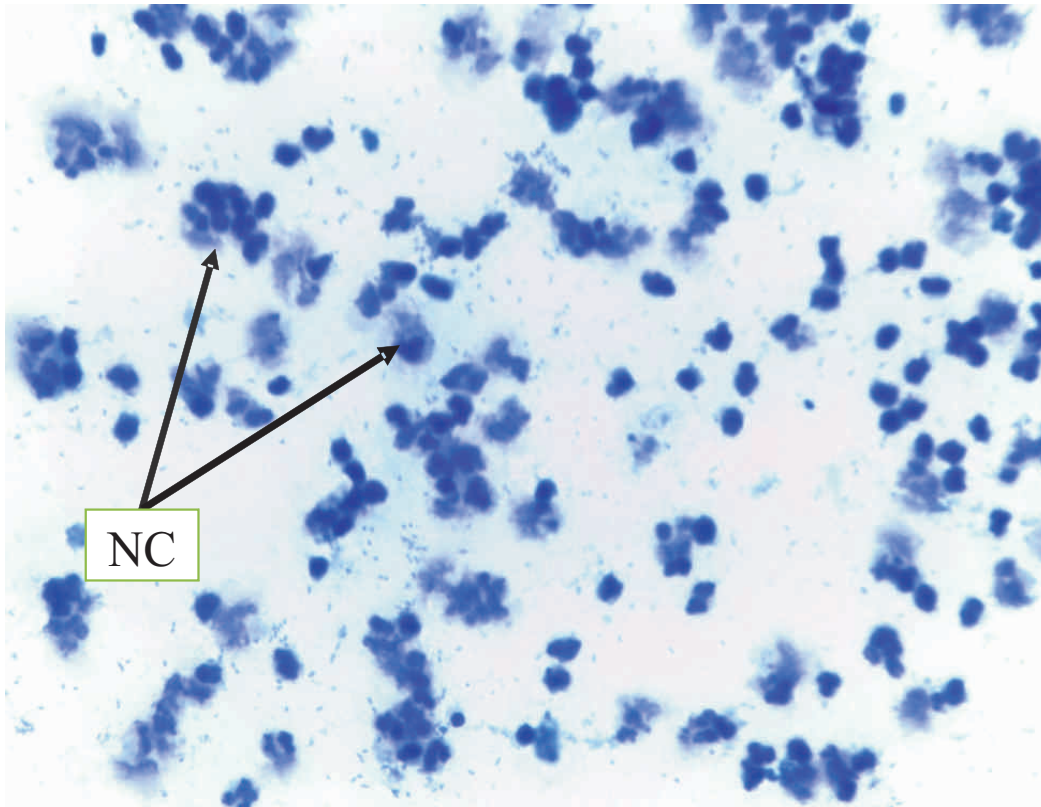


Fig 2: Urine smear showing cytoplasmic changes and unremarkable epithelial cells with increased nucleo-cytoplasmic ratio ($\times 400$ magnification, Diff Quick)

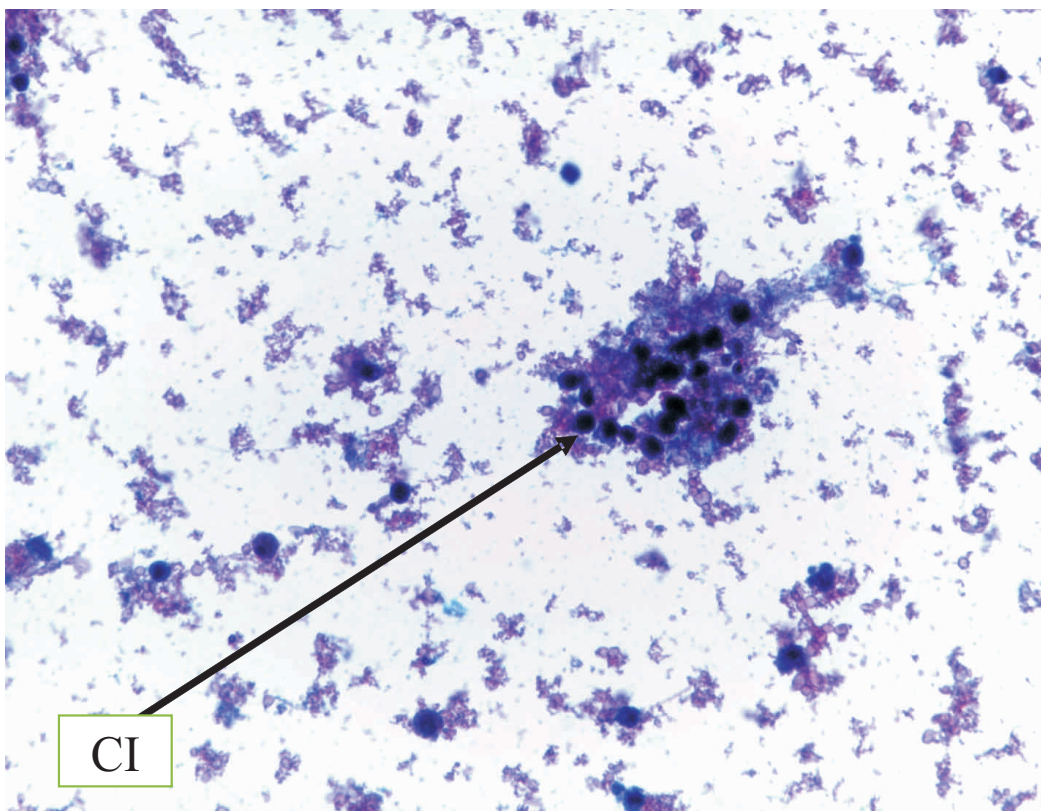


Figure 3: Smear showing cytoplasmic changes of remarkable epithelial cells infiltrated with enlarged nucleus indicating HGUC ($\times 100$ magnification, Papanicolaou stain)

Discussion

For proper diagnosis and management of urinary tract pathology, especially bladder cancer, a triple approach consisting of urine cytology, cystoscopy and biopsy is highly needed, with urine cytology being the first and quickest to do. Even though many studies have reported against the use of urine cytology due to its low sensitivity and always the need for a follow-up diagnosis with cystoscopy and biopsy, urine cytology is still needed especially in low-resource settings and is indispensable as a screening test due to its non-invasive nature as compared with biopsy and cystoscopy.

The indications for requesting urine cytology are wide and nonspecific for geographical location. For example, in some parts of Africa like Nigeria, urine cytology is mostly requested for the detection of bladder tumors associated with extensive chronic inflammation, carcinoma hidden in the bladder diverticulum, and carcinoma in situ (Abdulwahab *et al.*, 2019, Nwafor *et al.*, 2020). In other parts of the world such as Saudi Arabia and the United States of America, the main indication for urine cytology is bladder-associated haematuria and other genitourinary symptoms (Nwafor *et al.*, 2020).

Globally, men are three to four times more likely to develop bladder urothelial cancer than women as a result of exposure to carcinogens (eg. tobacco and occupational chemicals) as well as anatomical, hormonal, societal and environmental factors (Shariat *et al.*, 2010), this is the reason why the present study focused on recruiting only male subjects. Many studies have also reported a very high proportion of males among their subjects. A study by Abdulwahab *et al.* (2019) reported that the male subjects constitute of 86.2%. In a similar study conducted by Chawla *et al.* (2018), 85% were also males. Also, 84.4% of males were also reported by Sahabi *et al.* (2018).

In this study, the most common cytological diagnosis (36%) among the subjects was those found to be negative for high-grade urothelial carcinoma (NHGUC). This is followed by those positive for high-grade urothelial carcinoma (HGUC) accounting for 20% of the subjects. This

is similar to the 38.0% reported for NHGUC in two different studies by Curling *et al.* (1986) and Planz *et al.* (2005) and lower than the 53.9% reported in another study by Chawla *et al.* (2018).

Moderate to severe inflammatory changes of 58% were diagnosed in the present study. This may have contributed to the lower number of positive malignant smears because inflammation changes mostly result in false-positive reports of urine cytology despite being primarily caused by bacterial or parasitic infections (Siddappa *et al.*, 2012).

An analytic cohort study of 281,394 men and 186,134 women from October 25th, 1995, to December 31st, 2006, revealed that incidence of bladder cancer occurred in 3,896 men and 627 women, with former smokers (hazard ratio: 2.22) and current smokers (hazard ratio: 4.06) having high risk of bladder cancer than those that had never smoked (Freedman *et al.*, 2011). The Be-Well study reported that years of cumulating and chronic cigarette smoking are associated with a higher risk of cancer incidence and recurrence (Kwan *et al.*, 2022).

The present study also found that bladder cancer is common amongst moderate and severe smokers.

The present study found that the majority (36%) of the subjects were found to be negative for bladder cancer. Also, the majority of those diagnosed with HGUC were found to be within the age of 67-75 years (50%), and one among those aged 45-55 were found to have HGUC. The data from this study may not provide clear information concerning the association between age and bladder cancer since the subjects aged 76-95 only constitute 20% of the study population. The American Cancer Society reported that the average age at which people are diagnosed with bladder cancer is 73 (American Cancer Society, 2024).

Conclusion

Urine cytology, even with its variable sensitivity remains a useful tool in evaluating suspected urothelial malignancies and can serve as an adjunct to cystoscopy and biopsy, especially in resource-limited settings. Urine cytology may be recommended as a screening test that may narrow

down the need for cystoscopy or biopsy, hence reducing workload in centres with limited staffing and reducing the burden on patients who might not necessarily require cystoscopy or biopsy.

List of abbreviations

NHGUC: Negative for high-grade urothelial cancer

SHGUC: Suspicious for high-grade urothelial cancer

HGUC: High-grade urothelial cancer

TO: No evidence of primary tumor

Ta: Noninvasive carcinoma

TIS: Carcinoma in situ

T2: Invasive carcinoma

Conflict of interest: The authors declare that they have no competing interests.

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