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Original Article

Histological classification of microvascular invasion to predict prognosis in intrahepatic cholangiocarcinoma

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Abstract: Similar to hepatocellular carcinoma, microvascular invasion (MVI) is also one of the most significant prognostic factors of intrahepatic cholangiocarcinoma (ICC). However, there has not been any literature that had mentioned the histologic classification of microvascular invasion in intrahepatic cholangiocarcinoma. We evaluated the significance of MVI classification in this study and analyzed the prognosis based on MVI classification. We herein enrolled 108 patients who were diagnosed with ICC and then underwent surgical exploration from February 2005 to August 2015 at our hospital. We examined them with microvascular invasion (n=43) for four features: the number of invaded microvascular, the maximum number of invading carcinoma cells, the farthest distance from the tumor, and vessel with muscular wall. Thus, Patients were classified into low MVI and High MVI groups according to them. Of the total 108 patients, 65 patients told no detectable MVI, whereas 30 (27.8%) had low MVI, and 13 (12.0%) had high MVI. The median follow-up period lasted 15 months. In the analysis of overall survival, high MVI group showed significantly less positive outcomes than the patients without MVI and the low MVI group, and so did the low MVI group and the patients without MVI. Furthermore, high MVI and low MVI were independent factors for overall survival in ICC patients. We put forward a novel histologic evaluation of ICC which can preferably predict the risk of survival of patients with MVI after curative resection.

Keywords: Intrahepatic cholangiocarcinoma, microvascular invasion, prognosis, histologic classification, clinicopathologic features

Introduction

Intrahepatic cholangiocarcinoma (ICC) which arise from bile duct epithelium within the liver is the second most frequent primary hepatic malignancy after hepatocellular carcinoma (HCC). It accounts for about 10% of primary liver malignancies with an increasing incidence during the past decade [1-3]. In general, the prognosis for patients with ICC is poor, One- and 5-year survival rates in those with unresectable disease are reported to be 23% and 3%, respectively [2, 4]. Surgery continues to be the only modality shown to prolong survival with the advancement in surgical techniques and perioperative management, and post-resection overall survival rates of 70-80% at 1 year [4, 5] and 30-35% at 5 years [5, 6] have been reported. However, patients' prognosis still remained unsatisfactory because of the incidence of intrahepatic or extrahepatic recurrence after hepatic resection.

Vascular invasion is a significant indicator for a poor postresection outcome in HCC and ICC patients. Based on the AJCC/UICCA staging system [7], only the vascular invasion of HCC and ICC influence the classification of specific tumor in all kind of cancer. We classify tumors into T1 or T2 classification depends on the detection of vascular invasion in HCC and ICC patients. Vascular invasion generally includes macroscopic vascular invasion and microvascular invasion. Macroscopic vascular invasion, such as a tumor thrombus in the major portal or hepatic vein, which is known to be a crucial risk factor for survival after liver resection or transplantation in HCC and ICC patients could be detected by various imaging procedures before treatment. On the contrary, microvascular invasion (MVI), which is visible only on

Table 1. Summary of clinicopathologic findings of ICC

Clinical data	
Sex (male/female)	55/53
Age (y)	60.7±10.3
HBsAg (+) (%)	37
Child-Pugh classification (A/B/C)	102/6/0
CA199 (ng/ml)	2121.9±8643.2
Type of resection (anatomical (%))	59.3
Pathologic data	
Tumor size (cm)	5.8±2.8
Differentiation (well/moderate/poor)	3/53/52
MVI (%)	39.8
Gross classification (MF/other type)	83/25
Liver cirrhosis (%)	9.2
Capsule infiltration (%)	21.3
Perineural invasion (%)	86.1

microscopy, is difficult to detect by radiographic images or macroscopy. Recently, MVI in HCC patients has gained extensive attention. Some articles have mentioned the prediction of MVI for HCC patients preoperatively. Furthermore, few articles have confirmed that the classification of MVI could predict prognosis in HCC patients [8-11].

Though, sporadic papers have reported that presence of microvascular invasion is an independent predictor of poor survival [12, 13], however, there is not any literature that has mentioned the histologic characteristics classification of microvascular invasion in intrahepatic cholangiocarcinoma. Therefore, the current study aims to investigate MVI in detail and to propose a new histologic classification of MVI of ICC patients in China.

Material and methods

Study population

The current study cases included patients who underwent surgical resection and were diagnosed with ICC according WHO classification at the Affiliated Drum Tower Hospital of Medical School of Nanjing University (Nanjing, China) from February 2005 to August 2015. Exclusion criteria included: 1) macroscopic vascular invasion (n=11), 2) palliative resection (n=2), 3) been reconsidered as combined hepatocellular and cholangiocellular carcinoma by histological findings (n=3), 4) death in hospital (n=4), and 5) losing to follow-up (n=17). Of the total 145 patients, 37 patients were excluded due to the above criteria, and the remaining 108 patients were enrolled in this study. (**Table 1**) summarizes the clinicopathologic characteristics of the 108 ICC patients we enrolled.

Histopathological evaluation and classification

Representative 4-μm serial sections of the tumor were prepared from formalin fixed paraffin embedded tissue blocks for routine hematoxylin-eosin stain. All the histopathological evaluations were retrospectively performed by two experienced pathologists (C.J and S.J). We defined MVI of ICC as clusters of carcinoma cells in the vessel of peritumoral area that only could be detected on microscopy, which is based on Chinese pathologist's criteria of MVI in ICC patients [14]. The slides which contain cancer area or noncancerous liver were examined equally and carefully. When MVI was certified positive, we evaluated 4 features of MVI: the number of invaded vessels, the maximum number of invading carcinoma cells, the farthest distance from the tumor, and the vessel with muscular wall (**Figure 1**) referring to the similar study in HCC [15]. In addition to the evaluation of MVI, the slides with tumor were also examined for differentiation, resection margin, capsule infiltration, gross classification, number of nodules, TNM stage and T classification. Noncancerous liver slices were evaluated for indication of liver cirrhosis, cholangitis and cholangiolithiasis. Gross classification was based on the criteria of the Liver Cancer Study Group of Japan [16]. TNM stage and T classification was based on the 7th edition of the American Joint Committee on Cancer (AJCC) cancer staging system [7].

Clinical data evaluation

All the patients enrolled in the study were retrospectively assessed in age, sex, preoperative Child-Pugh classification, type of resection (anatomical or nonanatomical), maximum tumor size, serum carbohydrate antigen 19-9, glutamate pyruvate transaminase (ALT)/glutamic-oxaloacetic transaminase (AST), and hepatitis B surface antigen (HBsAg). The postoperative data we evaluated included overall survival and disease-free survival. The median

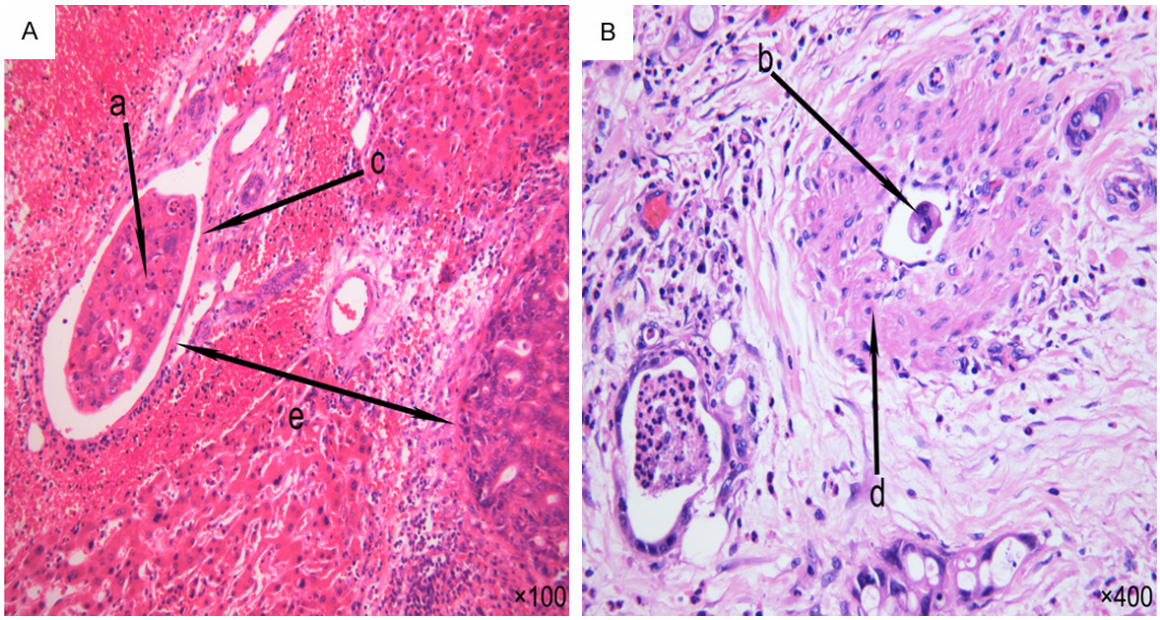

Figure 1. Hematoxylin and eosin staining of MVI in ICC (A, B). Clusters of carcinoma cells are observed in the portal vein and hepatic vein of the surrounding liver tissue (c, d). The numbers of carcinoma cells are 75 (a), and 3 (b). Histologic evaluation of MVI with a focus on 4 points: number of the invaded vessels (c), the maximum number of invading carcinoma cells, the farthest distance from the tumor (e), and the vessel with muscular wall (d).

overall survival and disease-free survival after resection were 15 months (range: 3-109 months).

Patient follow-up

After the operative treatment, all patients were followed up regularly by the serum levels of AFP, liver function and abdominal ultrasonography every month in the first half a year, then every three months in the next one and a half years and every half a year in the later time. Contrast-enhanced computed tomography (CT) was performed every 4 months. Recurrence should be confirmed by at least two imaging modalities, such as CT and enhanced magnetic resonance imaging (MRI). After the detection of a recurrence, further treatment such as repeat hepatectomy, radiotherapy or chemotherapy would be undertaken. Overall survival (OS) was defined as the time interval between the operation and the date of the death. Disease-free survival (DFS) was defined as the period after the operation when a recurrence could be detected. Follow-up data were collected until August 31, 2015.

Statistical analysis

Clinicopathologic findings were evaluated using Tukey-Kramer HSD and Pearson chi-square test, the Kruskal-Wallis test followed by Nemenyi test was used when the data distribution was skewed. Survival curves were calculated using the Kaplan-Meier method and compared using the log-rank test. All variables found to be significant on univariate analysis ($P<0.05$) were entered into a step-down Cox proportional hazard regression analysis. A *P* value less than 0.05 was considered statistically significant. When all combinations of comparative analysis were performed, a *P* value less than 0.017 was considered statistically significant according to Bonferroni correction. Statistical analysis was performed by SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

Results

Histologic features of MVI in ICC and correlation with prognosis

Of the 108 patients enrolled in this study, MVI was found positive in 43 patients (39.8%). We then conducted the investigation of the histologic features of MVI and correlation with prognosis (**Table 2**). However, univariate analysis of overall survival and disease-free survival of all the 108 patients presented that MVI-positive group showed significant poorer overall survival than MVI-negative group (P=0.001). While dis-

Table 2. Univariate analysis of overall survival and disease-free survival associated with histologic features of MVI in ICC

Factors	No.	Overall survival		Disease-free survival	
		Median time (month)[a]	*P*	Median time (month)[a]	*P*
Number of vessels					
1	19	14 (4-58)	0.347	12 (2-58)	0.194
≥2	24	13 (3-27)		5 (1-18)	
≤3	34	14 (4-58)	0.673	8 (1-58)	0.020*
>3	9	11 (3-27)		4 (1-8)	
≤5	38	14 (4-58)	0.213	8 (1-58)	0.042*
>5	5	11 (5-16)		4 (2-5)	
Maximum no. of carcinoma cells					
≤50	14	7 (3-53)	0.397	5 (1-53)	0.951
>50	29	14 (4-58)		6 (1-28)	
≤250	32	13 (3-58)	0.381	6 (1-58)	0.619
>250	11	13 (4-18)		8 (1-18)	
≤500	38	13 (3-58)	0.140	6 (1-58)	0.975
>500	5	7 (4-14)		8 (4-14)	
Farthest distance from the tumor					
≤1 mm	19	14 (3-58)	0.222	6 (1-58)	0.267
>1 mm	24	13 (4-22)		6 (1-18)	
≤3 mm	30	15 (3-58)	0.048*	6 (1-58)	0.594
>3 mm	13	11 (4-15)		5 (2-14)	
≤5 mm	34	14 (3-58)	0.100	6 (1-58)	0.774
>5 mm	9	7 (4-14)		5 (2-14)	
Vessel with muscular wall					
Positive	10	15 (6-53)	0.335	6 (1-53)	0.450
Negative	33	13 (3-58)		5 (1-58)	

[a]Median (range). *$P<0.05$.

ease-free survival showed no significant difference between MVI-positive group and MVI-negative group (P=0.382). Univariate analysis of overall survival and disease-free survival revealed that only the farthest distance from the tumor ≤3 mm or >3 mm showed significant difference in Overall survival (P=0.048). The high-number group of invaded vessels (>3 or >5) showed poorer disease-free survival than the low-number group (≤3 or ≤5) (P=0.020 and P=0.042, respectively). We chose the farthest distance from the tumor ≤3 mm or >3 mm to be the only cut-off point to differentiate MVI degree in ICC patients and then classified all the 108 patients into 3 groups: 1) high-MVI group, in which the farthest distance from the tumor of MVI >3 mm, 2) low-MVI group, in which MVI was observed but the farthest distance from the tumor of MVI ≤3 mm, and 3) non-MVI group, in which MVI was negative in the resected specimens.

The clinicopathologic findings of the high-MVI group were compared with those of the non-MVI group and low-MVI group (**Table 3**). Only the number of nodules in the non-MVI group was significant lower than in high-MVI group and low-MVI group (P=0.009, P=0.003, respectively). There were no other clinicopathologic differences among the three groups.

In the analysis of overall survival, the high-MVI group showed significantly poorer outcomes than non-MVI group (P<0.001) and the low-MVI group (P=0.035). And the low-MVI group showed significantly poorer outcomes than the non-MVI group (P=0.013). There was no significant difference among the three groups in disease-free survival (**Figure 2**).

Table 3. Comparative analysis of clinical findings among three groups

	Non-MVI	Low-MVI	High-MVI	*P*	*P*		
	n=65	n=30	n=13		Non-MVI vs. Low-MVI	Low-MVI vs. High-MVI	Non-MVI vs. High-MVI
Sex (male/female)	36/29	12/18	7/6	0.376[b]	-	-	-
Age	60.6±10.0	61.6±11.5	59.3±10.2	-	0.905[a]	0.793[a]	0.915[a]
HBsAg (+/-)	24/41	8/22	8/5	0.095[b]	-	-	-
Child-Pugh classification (A/B/C)	62/3/0	28/2/0	12/1/0	0.867[b]	-	-	-
CA199 (ng/ml)	2534.5±1050.0	1682.0±4967.5	688.1±1661.5	-	0.926[a]	0.932[a]	0.790[a]
Type of resection (anatomical/nonanatomical)	33/32	23/7	8/5	0.328[c]	-	-	-
Tumor size (cm)	5.4±2.7	6.4±2.7	6.7±3.2	-	0.246[a]	0.932[a]	0.268[a]
No. of nodules (single/multiple)	57/8	18/12	7/6	0.002[c]	0.003[c,**]	0.666[c]	0.009[c,**]
Differentiation (well/moderate/poor)	3/36/26	0/13/17	0/4/9	0.160[b]	-	-	-
Gross classification (MF/other type)	50/15	21/9	11/2	0.776[b]	-	-	-
Capsule infiltration (+/-)	11/54	11/19	1/12	0.116[c]	-	-	-
Perineural invasion (+/-)	56/9	26/4	11/2	0.984[b]	-	-	-

[a]Tukey-Kramer HSD. [b]Pearson chi-square test. [c]Kruskal-Wallis test followed by Nemenyi test. **$P<0.01$.

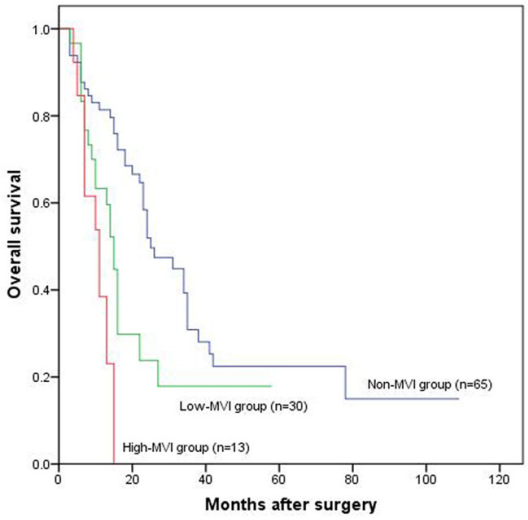

Figure 2. The overall survival curves for patients without MVI and for patients in the high- and low-MVI groups. The high-MVI group showed significantly poorer overall survival than the patients without MVI and the low-MVI group (P<0.001 and P=0.035). The low-MVI group showed significantly poorer overall survival than the patients without MVI (P=0.013).

Univariate and multivariate analysis of survival in ICC patients

On the aspect of overall survival, univariate analysis showed that preoperative CA19-9, number of nodule (single/multiple), TNM stage (I-II or III-IV), T classification (T1-T2 or T3-T4), resection margin (+/-), Liver cirrhosis (+/-), low-MVI, and high-MVI were prognostic factors (**Table 4**). Multivariate analysis including these factors revealed that TNM stage, resection margin, low-MVI, and high-MVI were independent factors for overall survival (P=0.001, P=0.009, P=0.036, P<0.001, respectively) (**Table 5**). For disease-free survival, univariate analysis showed that TNM stage (I-II or III-IV), T classification, and Liver cirrhosis (+/-) were prognostic factors (**Table 4**). Moreover, Multivariate analysis including these factors revealed that TNM stage and Liver cirrhosis were independent factors for disease-free survival (P=0.001, P=0.030, respectively) (**Table 5**).

Discussion

In the present study, we examined MVI of ICC in 4 features: the number of invaded vessels, the maximum number of invading carcinoma cells, the farthest distance from the tumor, and the vessel with muscular wall, which had been inspected in the research of MVI in HCC [8-11]. Though MVI of ICC was a significant prognostic factor for overall survival, only the farthest distance from the tumor of MVI was closely related to the prognosis of ICC patients. Then we demonstrated that the grade based on histologic features of MVI is able to stratify ICC patients into 3 distinct groups with significantly different overall survival.

Several articles had conducted the research of MVI histologically in HCC patients. Roayaie S et al [8] reported that the risk score which depended on the appearance of vessel with

Table 4. Univariate analyses of overall survival and disease-free survival for ICC

	Overall survival	*P*	Disease-free survival	*P*
	Hazard ratio (95% CI)		Hazard ratio (95% CI)	
CA199				
(>40 ng/mL)	1.811 (1.123-2.922)	0.015*	1.634 (0.973-2.746)	0.064
No. of nodules				
(multiple)	1.725 (1.035-2.876)	0.036*	1.521 (0.855-2.704)	0.153
TNM stage				
(I-II or III-IV)	2.785 (1.717-4.519)	<0.001**	2.409 (1.429-4.062)	0.001**
T classification				
(T1-T2 or T3-T4)	2.458 (1.437-4.203)	0.001**	1.943 (1.087-3.472)	0.025*
Resection margin				
(+)	1.692 (1.191-2.405)	0.003**	1.409 (0.966-2.054)	0.075
MVI				
Low-MVI	1.945 (1.139-3.322)	0.015*	1.145 (0.628-2.086)	0.658
High-MVI	4.723 (2.247-9.927)	<0.001**	1.671 (0.732-3.814)	0.223
Liver cirrhosis				
(+)	1.249 (1.027-1.519)	0.026*	1.252 (1.021-1.534)	0.031*

*P<0.05. **P<0.01.

Table 5. Multivariate analysis of clinicopathologic variables associated with recurrence and survival

	HR (95% CI)	*P*
Overall survival		
High-MVI	5.364 (2.487-11.568)	<0.001**
Low-MVI	1.808 (1.041-3.141)	0.036*
TNM stage	2.317 (1.398-3.841)	0.001**
Resection margin	1.592 (1.123-2.255)	0.009**
Recurrence survival		
TNM stage	2.420 (1.432-4.088)	0.001**
Liver cirrhosis	1.270 (1.024-1.576)	0.030*

*P<0.05. **P<0.01.

muscular wall and the farthest distance of MVI from the tumor ≥1 cm was an independent prognostic factor for survival and recurrence. Fujita N [9] et al defined High-MPI as HCC patients with MPI with multiple invaded portal venous vessels (≥2) and more than 50 invading carcinoma cells, and High-MPI was an independent prognostic factor for disease-free survival. However, in the present study, the farthest distance of MVI from the tumor was the only prognostic factor of ICCs with MVI in overall survival. Different pathways of invasion and metastasis between HCC and ICC might account for the contrast above. Beside vascular invasion and hematogenous metastasis, ICC is also prone to metastasize through lymphatic system. We then classified ICCs with MVI into high- , low-, and non-MVI groups according to the farthest distance of MVI from the tumor >3 mm or ≤3 mm. The high-MVI group showed poorer overall survival than the low-MVI group and non-MVI group, and low-MVI group revealed poorer overall survival than the non-MVI group. Moreover, high MVI and low MVI were independent prognostic factors for overall survival. Otherwise, the three groups showed no significant difference in univariate and multivariate analysis of disease-free survival. These results indicate that histologic evaluation of MVI can play a fatal role on predicting the overall survival in ICC. However, unlike HCC, complex pathogenesis and various routes of metastasis make multiple histologic features of MVI unconnected to the prognosis of ICC patients. In addition to MVI, TNM stage, resection margin were independent factors for overall survival and TNM stage and Liver cirrhosis were independent factors for disease-free survival.

The metastasis of lymph node is a significant prognostic factor in ICC which has been identified in many literatures [17-20]. However, lymph node dissection is not routinely performed at the time of ICC resection so far, especially in Western countries [19]. Clancy J. Clark et al reported that data on lymph node status were available for only 49% of patients submitted to surgery for ICC, and that a histological evalua-

tion of lymph nodes was performed in only 14% [17]. The patients enrolled in the present study, only 18 (16.7%) of them had underwent lymph node dissection. We could not recognize the actual condition of the lymphatic metastasis of these ICC patients on account of the low dissection rate of lymph node, so that the lymph node condition was not described in this study. Lack of the inspection of lymph node might influence the veracity and persuasion of this study, moreover, lymphatic metastasis could have a crucial relation to the origin and progression of microvascular invasion. Therefore, further research should select specific samples that majority of the ICC patients have went through lymph node dissection and pay close attention to the connection between lymphatic metastasis and microvascular invasion.

During the last decade, there has been a great quantity of studies aimed to find out the relationship between the circulating tumor cells (CTCs) and specific cancer, especially in breast cancer and lung cancer [21]. Recently, Margherita [22] et al conducted a review which emphasized the most relevant findings of CTCs in the context of stem-like biology associated to liver carcinogenesis. Among most of these literatures, high CTCs were strongly associated with early recurrence and poor overall survival. Furthermore, Sun [23] et al and Kelley [24] et al even proved that the presence of high CTCs correlated with microvascular invasion in HCC patients. Microvascular invasion and CTCs might be the different phases of the primary liver cancer to metastasize. Investigation of primary liver cancer-CTCs is still at their very beginning in comparison with other tumor systems, particularly breast cancer. Our center had carried out the detection of the CTCs in the patients with primary liver cancer. We believe that further research of CTCs would also be helpful to figure out the mechanism of microvascular invasion.

The current study had limitations that should be considered. ICC is the certain cancer with low incidence. Furthermore, quite a number of patients lose the lost the opportunity of surgical therapy because of the highly aggressive biological behavior of ICC. So that, only 108 patients was included in this study, which might affect the general representativeness of the present study. As a result, expanding the sample size is essential for the further study.

In conclusion, we believe that the MVI is a significant prognostic factor for ICC patients, which is similar to the MVI of HCC patients. Furthermore, pathologic evaluation of the degree of MVI in ICC patients is useful for estimating tumor biological behavior and guiding the subsequent treatment and follow-up strategy. We firstly proposed the classification of MVI of ICC patients, and with the improving of the degree of MVI, the overall survival decreased as expected. This stratification will certainly be an important histologic factor and help to predict aggressive behavior in ICC, so that strict observation and adjuvant treatment could be determinate on high-MVI group patients.

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Disclosure of conflict of interest

None.

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