

Which is more important for the intensity of intra-amniotic inflammation between total grade or involved anatomical region in preterm gestations with acute histologic chorioamnionitis?

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Objective

There is no data on which is more important for the intensity of intra-amniotic inflammation (IAI) between total grade or involved anatomical region in acute histologic chorioamnionitis (acute-HCA) of preterm-gestations. The objective of current study is to examine this issue.

Methods

The intensity of IAI was measured by amniotic fluid (AF) white blood cell (WBC) count and matrix metalloproteinase-8 (MMP-8) concentration in 225 singleton preterm-gestations (<36 weeks) who had acute-HCA including chorio-decidual involvement and delivered within 5 days of amniocentesis. Acute-HCA was defined in the presence of acute inflammatory changes in each anatomical region (i.e., chorio-decidual, amnion or chorionic plate). Patients were divided into 6 groups according to total grade (i.e., 1–6) and the presence or absence of chorio-decidual restriction (i.e., chorio-decidual restriction vs. extension beyond chorio-decidual) of acute-HCA.

Results

There was no significant difference in a median AF WBC and MMP-8 between the two groups (group-1, cases with total grade 1 vs. group-2, cases with total grade 2) among cases with chorio-decidual restriction (each for $P>0.05$) and between the four groups (group-3, cases with total grade 2 vs. group-4, cases with total grade 3 vs. group-5, cases with total grade 4 vs. group-6, cases with total grade 5–6) among cases with extension beyond chorio-decidual (each for $P>0.05$). However, group-3 (cases with extension beyond chorio-decidual) had a significantly higher median AF WBC and MMP-8 than group-2 (cases with chorio-decidual restriction) among cases with total grade 2 (each for $P<0.05$).

Conclusion

Involved anatomical region is more important than total grade for the intensity of IAI in acute-HCA of preterm-gestations.

Keywords: Amniotic fluid; Chorioamnionitis; Grade; Inflammation; Intensity; Region

Introduction

Clinically responsive placental examination, which includes the stages about the involved anatomical region and the grades about the severity in acute histologic chorioamnionitis (acute-HCA), is known to enhance clinico-pathologic correlation [1]. Indeed, previous study demonstrated that increasing histologic stages of inflammation in fetal membranes were associated with an increasing rate of funisitis, perinatal mortality and preterm birth [2]. Moreover, sepsis in the newly born

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infant more frequently developed with the higher placental inflammatory score [3].

The grading system in acute placental inflammation has been known to play an important role in the assessment for the intensity of inflammation and infection in the fetus and amniotic cavity as following: 1) there was a strong association between the presence and severity of inflammation in each anatomical region of placenta (i.e., chorion-decidua, amnion, chorionic plate and umbilical cord) and the results of amniotic fluid (AF) culture [4]; 2) both the presence and greater severity of acute-HCA were associated with a higher frequency of positive AF culture and an elevated AF white blood cell (WBC) count [5]; 3) the degree of leukocytic infiltration in the fibrin layer under the chorion or in the chorionic membrane of term placentas, correlated positively with the levels of AF cytokines (i.e., tumor necrosis factor, interleukin-1 [IL-1], and IL-6) [6]; and 4) umbilical venous IL-6 levels in preterm and term neonates correlated with the severity of acute placental inflammation [7]. Moreover, the identification of involved anatomical region in the placenta and umbilical cord by acute inflammatory changes has been reported to be an essential part for the evaluation of the presence or intensity of intra-amniotic, maternal and fetal inflammatory responses as following: 1) fetal and intra-amniotic inflammatory responses were more intense in patients with funisitis than in those without this lesion [8-10]; 2) the involvement of the amnion in acute-HCA was associated with a more intense fetal and intra-amniotic inflammatory response than chorionitis alone [11]; 3) the intra-amniotic inflammatory response was stronger when inflammation was present in both the chorionic plate and chorio-decidua, than when it was restricted to the chorio-decidua only, which was exposed to the cervical canal in placenta previa [12]; 4) an inflammation restricted to chorio-decidua, an initial stage in ascending intrauterine infection pathway, was associated with a more intense and frequent inflammation in AF, but not fetus and mother, than placenta without any inflammation [13]; and 5) an inflammation in the chorion provided a clinically acceptable minimum threshold for the reporting of pathologic changes [2]. However, there is a paucity of information regarding which is more important for the intensity of intra-amniotic inflammation (IAI) between total grade or involved anatomical region in acute-HCA of preterm gestations. The objective of current study is to examine this issue.

Materials and methods

1. Study design

Study population consisted of 225 singleton preterm gestations (<36 weeks) who had acute-HCA including chorio-decidua involvement and delivered within 5 days of amniocentesis at the Seoul National University Hospital between January 1993 and December 2007. This criterion of amniocentesis-to-delivery interval was used to preserve a meaningful temporal relationship between the results of AF studies and the placental pathologic findings at birth. The intensity of IAI was measured by AF WBC count and matrix metalloproteinase-8 (MMP-8) concentration. Patients were divided into 6 groups according to total grade (i.e., 1–6) and the presence or absence of chorio-decidua restriction (i.e., chorio-decidua restriction vs. extension beyond chorio-decidua) of acute-HCA as following: 1) group-1, chorio-decidua restriction with total grade 1; 2) group-2, chorio-decidua restriction with total grade 2; 3) group-3, extension beyond chorio-decidua with total grade 2; 4) group-4, extension beyond chorio-decidua with total grade 3; 5) group-5, extension beyond chorio-decidua with total grade 4; 6) group-6, extension beyond chorio-decidua with grade 5-6. At our institution, trans-abdominal amniocentesis for retrieval of AF was routinely offered to all patients who were admitted with the diagnosis of preterm labor and intact membranes (PTL) or preterm premature rupture of membranes (preterm-PROM). AF was analyzed for microbiologic and inflammatory status, and fetal lung maturity. Amniocentesis was performed to assess fetal lung maturity in patients with maternal-fetal indication such as pre-eclampsia. Moreover, we have routinely recommended and performed placental pathologic examination in all preterm gestations. Written informed consent was obtained from all patients. The Institutional Review Board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protections of the Department of Health and Human Services of the United States. Many of patients in this study were included in our previous studies.

2. Clinical characteristics and pregnancy outcomes

The demographic and clinical characteristics of the mothers and their neonates were examined through a review of the

medical records. We investigated maternal age, parity, the cause of preterm delivery, gestational age (GA) at amniocentesis, GA at delivery and birth weight.

3. Placental pathology

Placental tissue samples obtained for pathologic evaluation included chorio-amniotic membrane roll (chorio-decidua and amnion) and chorionic plate. These samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections of prepared tissue blocks were stained with hematoxylin and eosin. Pathologists were masked to the clinical information. Acute-HCA was defined in the presence of acute inflammatory changes on examination of chorio-amniotic membrane roll and chorionic plate of the placenta. The presence of acute inflammatory change was classified as grade 1 or 2 in each anatomical region of placenta (chorio-decidua, amnion, and chorionic plate) according to previously published criteria [14]. Chorio-decidua restriction in acute-HCA was diagnosed in the presence of at least 1 focus of >5 neutrophils in only a chorio-decidua. Extension beyond chorio-decidua in acute-HCA was defined in the presence

of inflammation in amnion or chorionic plate in addition to chorio-decidua: Inflammation of the amnion was diagnosed in the presence of at least 1 focus of >5 neutrophils in the amnion; and inflammation of the chorionic plate was diagnosed in the presence of more than 1 focus of at least 10 neutrophilic collections or diffuse inflammation in subchorionic fibrin, or diffuse and dense inflammation, neutrophilic infiltration into connective tissue of the placental plate, or placental vasculitis, with the use of criteria previously published [14]. Total grade was used to determine the severity of acute-HCA from 1 to 6 according to the criteria previously reported [14].

4. Amniotic fluid

AF was cultured for aerobic and anaerobic bacteria and genital mycoplasmas (*Ureaplasma urelyticum* and *Mycoplasma hominis*) and analyzed for WBC count according to the methods previously described [15,16]. Of 225 cases, AF WBC count results were available in 208 cases. The remaining fluid was centrifuged and stored in polypropylene tubes at -70°C. MMP-8 concentrations in stored AF were measured with a commer-

Table 1. Clinical characteristics and pregnancy outcomes according to total grade and the presence or absence of chorio-decidua restriction among 225 preterm gestations (GA at delivery <36 weeks) with acute histologic chorioamnionitis including chorio-decidua involvement

Involved anatomical region	Chorio-decidua restriction (n=109)				Extension beyond chorio-decidua (amnion or chorionic-plate) (n=116)					
	Grade 1	P ^{a)} -value	Grade 2	P ^{b)} -value	Grade 2	Grade 3	Grade 4	Grade 5-6	P ^{c)} -value	P ^{d)} -value
Group	Group-1 42.2% (95/225)		Group-2 6.2% (14/225)		Group-3 14.7% (33/225)	Group-4 15.6% (35/225)	Group-5 10.7% (24/225)	Group-6 10.7% (24/225)		
Mean maternal age (yr, ±SD)	30.0±4.1	NS	30.7±3.2	NS	29.7±4.9	30.6±3.6	32.1±4.0	31.4±5.0	NS	NS
Nulliparity (%)	55.8	NS	71.4	NS	48.5	40.0	41.7	37.5	NS	NS
Causes of preterm delivery	-	NS	-	NS	-	-	-	-	NS	<0.05
Preterm labor (%)	36.8	-	21.4	-	36.4	51.4	41.7	25.0	-	-
Preterm-PROM (%)	41.1	-	64.3	-	57.6	48.6	58.3	62.5	-	-
Maternal-fetal indication (%)	22.1	-	14.3	-	6.1	0	0	12.5	-	-
Positive AF culture (%) ^{e)}	20.7 (19/92)	NS	23.1 (3/13)	NS	51.6 (16/31)	27.3 (9/33)	33.3 (8/24)	47.8 (11/23)	NS	<0.05
Median GA at amniocentesis (wk), (range)	32.9 (19.7-35.9)	NS	33.1 (23.1-35.7)	NS	32.0 (23.0-35.1)	29.0 (23.0-35.1)	30.2 (21.6-35.1)	26.9 (16.9-34.7)	<0.05	<0.001
Median GA at delivery (wk), (range)	33.1 (19.7-35.9)	NS	33.7 (23.6-35.9)	NS	32.0 (23.4-35.3)	29.1 (23.3-35.7)	30.5 (21.6-35.4)	27.2 (17.4-35.1)	<0.05	<0.001
Mean birth weight (g), ±SD	1,833±712	NS	1,818±594	NS	1,540±533	1,401±554	1,434±557	1,245±750	NS	<0.005

GA, gestational age; preterm-PROM, preterm premature rupture of membranes; SD, standard deviation; NS, not significant; AF, amniotic fluid. ^{a)}P-value: comparison between group-1 and group-2; ^{b)}P-value: comparison between group-2 and group-3; ^{c)}P-value: comparison among group-3, group-4, group-5 and group-6; ^{d)}P-value: comparison among all study groups; ^{e)}Of 225 cases who underwent amniocentesis within 5 days of birth, AF culture results were available in 216 cases.

cially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech Inc., Little Chalfont, Bucks, UK). The sensitivity of the test was <0.3 ng/mL. Both intra- and inter-assay coefficients of variation were $<10\%$. Details about this assay and its performance have been previously described [8]. Of 225 cases, 213 patients were included in the analysis of AF MMP-8, because the test of AF MMP-8 concentration was not performed in 12 cases due to the limited amount of remaining AF.

5. Statistical analysis

Comparisons of continuous variables were performed with Mann-Whitney U test between the two groups and with Kruskal-Wallis test among the three groups and over. The Fisher's exact test or Pearson's chi-square test was used for the comparisons of proportions. Data was analyzed using SPSS Statistics ver. 20.0 (IBM Corp., Somers, NY, USA). Statistical significance was defined as a $P < 0.05$.

Results

1. Clinical characteristics and pregnancy outcomes

Table 1 demonstrated clinical characteristics and pregnancy outcomes according to total grade and the presence or absence of chorio-decdua restriction in preterm gestations with acute-HCA including the involvement of chorio-decdua. There was a significant difference in GA at amniocentesis and GA at delivery among the four groups with extension beyond chorio-decdua (group-3 vs. group-4 vs. group-5 vs. group-6, each for $P < 0.05$) (Table 1). However, no difference was found in any clinical characteristics and pregnancy outcomes between the two groups with chorio-decdua restriction (group-1 vs. group-2, each for $P > 0.05$) (Table 1). Moreover, there was no significant difference in any clinical characteristics and pregnancy outcomes between group-2 with chorio-decdua restriction and group-3 with extension beyond chorio-decdua among cases with total grade 2 (each for $P > 0.05$) (Table 1).

2. AF MMP-8 concentrations and WBC counts

Figs. 1, 2 show AF MMP-8 concentrations (Fig. 1) and AF WBC

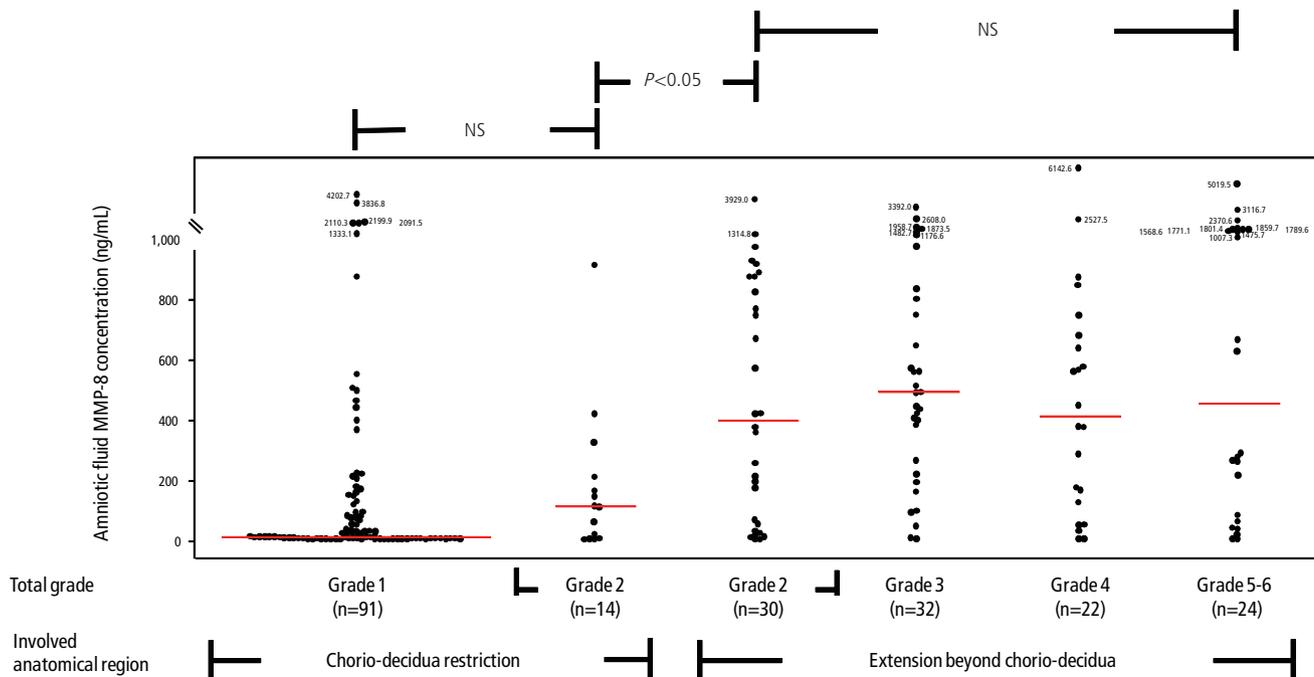


Fig. 1. Amniotic fluid matrix metalloproteinase-8 (MMP-8) concentrations according to total grade and the presence or absence of chorio-decdua restriction among preterm gestations with acute histologic chorioamnionitis including the involvement of chorio-decdua (median [range], ng/mL; group-1: 10.1 [0.3-4,202.7] vs. group-2: 107.85 [0.3-909.4] vs. group-3: 393.1 [0.3-929.0] vs. group-4: 486.15 [0.4-3,392.0] vs. group-5: 408.6 [0.6-6,142.6] vs. group-6: 454.0 [0.5-5,019.5]) (each and all P -value is shown in graph). NS, not significant.

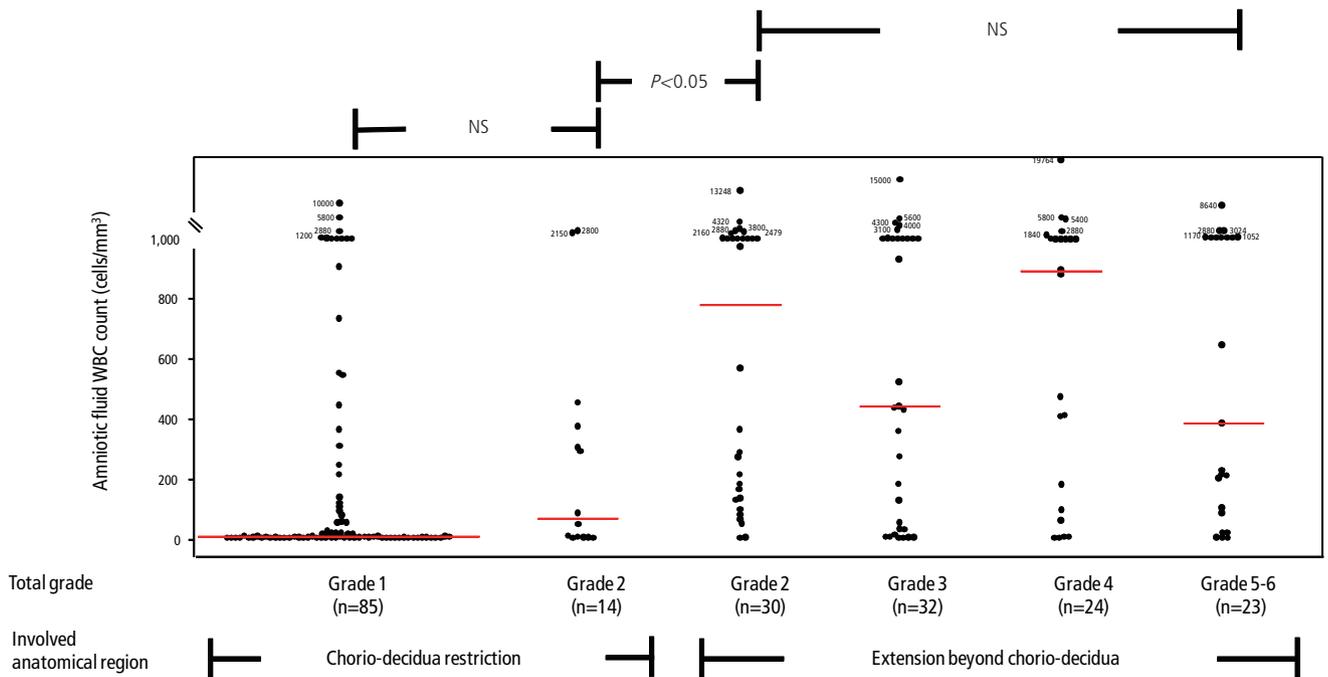


Fig. 2. Amniotic fluid white blood cell (WBC) counts according to total grade and the presence or absence of chorio-decdua restriction among preterm gestations with acute histologic chorioamnionitis including the involvement of chorio-decdua (median [range], cells/mm³; group-1: 3 [0-10000] vs. group-2: 64 [0-2,800] vs. group-3: 773 [0-13,248] vs. group-4: 435 [0-15,000] vs. group-5: 884 [1-19,764] vs. group-6: 380 [0-8,640]) (each and all *P*-value is shown in graph). NS, not significant.

counts (Fig. 2) according to total grade and the presence or absence of chorio-decdua restriction in preterm gestations with acute-HCA including the involvement of chorio-decdua. There was no significant difference in a median AF MMP-8 concentration (Fig. 1) and WBC count (Fig. 2) between the two groups (group-1, total grade 1 vs. group-2, total grade 2) in cases with chorio-decdua restriction (each for *P*>0.05) and among the four groups (group-3, total grade 2 vs. group-4, total grade 3 vs. group-5, total grade 4 vs. group-6, total grade 5–6) in cases with extension beyond chorio-decdua (each for *P*>0.05). However, group-3 with extension beyond chorio-decdua had a significantly higher median AF MMP-8 concentration (Fig. 1) and WBC count (Fig. 2) than group-2 with chorio-decdua restriction among cases with total grade 2 (each for *P*<0.05).

Discussion

Principal findings of the study are as following. Firstly, the difference in total grade had little effect on the intensity of

IAI in the same context of chorio-decdua restriction and in the same context of extension beyond chorio-decdua among patients with acute-HCA. Secondly, extension beyond chorio-decdua was associated with a significantly higher intensity of IAI than chorio-decdua restriction in the same context of total grade 2 among cases with acute-HCA.

Our results show that there was no significant difference in the intensity of IAI between the two groups of patients with chorio-decdua restriction (Figs. 1, 2). IAI has been defined in the case of an elevated AF WBC count ≥ 19 cells/mm³ [17] and in the case of an elevated AF MMP-8 concentration ≥ 23 ng/mL [18] according to the criteria previously published. Considering a median AF MMP-8 concentration and AF WBC count in group-1 or group-2 (AF MMP-8 [ng/mL], 10.1 vs. 107.85; AF WBC count [cells/mm³], 3 vs. 64), no or only a mild inflammation in AF was present in these two groups and ultimately did not cause the difference between the two groups with chorio-decdua restriction (Figs. 1, 2). Indeed, previous studies demonstrated that acute-HCA without amnionitis [11] or chorionic plate inflammation [12] was not associated with an intense IAI.

Our data demonstrated that no significant difference was found in the intensity of IAI according to total grade among cases with extension beyond chorio-decidua in acute-HCA (Figs. 1, 2). An inflammation in amnion or chorionic plate is thought to be the most advanced stage in acute-HCA for the following reason: 1) amnion or chorionic plate, anatomical region beyond chorio-decidua, is located in direct proximity of the amniotic cavity in extra-placental membranes or placental disc; 2) chorionic plate includes large fetal blood vessels that connect to the umbilical cord vessels as a fetal compartment. Therefore, severe intensity of IAI was likely to develop without variation among the four groups according to total grade, because ascending intra-uterine infection had already entered the advanced stages in patients with extension beyond chorio-decidua.

Patients with total grade 2 had a difference in the involved anatomical region as following: i.e., group-2, chorio-decidua restriction vs. group-3, extension beyond chorio-decidua. Therefore, in view of the previous studies about the significance of involved anatomical region in acute-HCA [11-13], one may expect that extension beyond chorio-decidua was associated with a significantly more intense IAI than chorio-decidua restriction although the two groups had the same total grade. Indeed, patients with extension beyond chorio-decidua had a significantly higher median AF MMP-8 concentration and WBC count than those with chorio-decidua restriction among cases with total grade 2 in acute-HCA (Figs. 1, 2). Moreover, it should be noted that extension beyond chorio-decidua was associated with a higher rate of positive AF culture than chorio-decidua restriction among cases with total grade 2 (52% vs. 23%) without reaching a statistical significance (Table 1). Our findings demonstrated that patients with the same total grade 2 could be divided into two heterogeneous groups (chorio-decidua restriction vs. extension beyond chorio-decidua), which had a different intensity of IAI and a different frequency of intra-amniotic infection.

Major strengths of the study are: 1) a large cohort of singleton preterm gestations (n=225) with acute-HCA; 2) it compared a positive culture, MMP-8 concentration and WBC count in AF and therefore, this study examined all potential markers of intra-amniotic infection and inflammation; 3) patients were meticulously divided into as many as 6 groups according to total grade and the presence or absence of chorio-decidua restriction. One potential weakness of the study is that study design was retrospective.

To our knowledge, this study is the first study regarding which is more important for the intensity of IAI between total grade or involved anatomical region in acute-HCA of preterm gestations. Although the standardized classification about grading and staging of acute-HCA has not been made yet, our data may suggest that the classification of acute-HCA should attach more importance to the staging about the involved anatomical region of placenta than to the grading about the severity.

There is a paucity of information about antenatal non-invasive prediction methods for an inflammation in the anatomical region beyond chorio-decidua. Moreover, it should be determined whether the time required for the extension beyond chorio-decidua in acute-HCA is different between patients with PTL and those with preterm-PROM.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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