Correlation analysis of serum placental growth factor, pregnancy-related plasma protein-A and disease severity in patients with hypertensive disorder in pregnancy

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Abstract. – OBJECTIVE: To analyze the correlation of serum placental growth factor (PLGF), pregnancy-associated plasma protein-A (PAPP-A) and disease severity in patients with hypertensive disorders in pregnancy (HDP).

PATIENTS AND METHODS: Altogether 88 pregnant women with hypertensive disorder who underwent prenatal examination and delivery in our hospital from March 2017 to February 2019 were selected and included as the research group (n=88), and 62 healthy pregnant women who underwent prenatal examination during the same period were included as the normal control group (n=62). Enzyme-linked immunosorbent assay (ELISA) was used to detect the expression levels of PAPP-A and PLGF in the serum of the two groups. The correlation of the expression levels of PAPP-A and PLGF with the severity of HDP was analyzed. The occurrence of adverse pregnancy outcomes in the two groups was compared, and the relationship of the expression levels of PAPP-A and PLGF with adverse pregnancy outcomes was compared.

RESULTS: PAPP-A expression level in serum of pregnant women in the research group was significantly higher than that in the control group, while PLGF expression level was significantly lower than that in the control group (p<0.001). PAPP-A expression level was positively correlated with HDP severity (r=0.753, p<0.001), while PLGF expression level was negatively correlated with HDP severity (r=-0.929, p<0.001). The incidence of adverse pregnancy outcomes in the research group was significantly higher than that in the control group (p<0.05). The serum PAPP-A level of patients in the group with adverse pregnancy outcomes was significantly higher than that in the group without adverse pregnancy outcomes, while PLGF level was significantly lower than that in the group without adverse pregnancy outcomes (p<0.001).

CONCLUSIONS: In conclusion, the expression levels of PAPP-A and PLGF in serum were closely related to the severity of HDP and could be used as indicators for disease monitoring.

Key Words: Hypertensive disorder in pregnancy, Placental growth factor, Pregnancy-associated plasma protein-A, Disease severity, correlation analysis.

Introduction

Hypertension is the most common medical disease during pregnancy, and it complicates pregnancy in about 15% of pregnant women; HDP is the leading cause of maternal death worldwide¹, where 16.7% of pregnant women are affected². In the United States, HDP causes maternal deaths accounting for 10-15% of all pregnant women³. Research has shown that HDP can lead to premature birth, low birth weight, birth asphyxia, stillbirth, early neonatal death and other adverse pregnancy outcomes, and the incidence and mortality rate are relatively high⁴-⁶. Prevention of HDP has always been a hot research topic, and the management of HDP patients is also the key. Strengthening the monitoring of disease severity and optimizing delivery time can reduce organ damage of pregnant women and prevent fetal adverse events⁷. Ambulatory blood pressure monitoring is helpful to distinguish true hypertension from “white coat” hypertension⁸, but this method is difficult to realize for pregnant women. There-
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Therefore, it is of great significance to find a serological marker to monitor the disease severity. PLGF is a dimer glycoprotein and a member of vascular endothelial growth factor (VEGF) family. The circulating level increases significantly during pregnancy and it plays an important role in promoting the development and maturation of placental vascular system. Research has shown that, endothelial dysfunction and vasospasm caused by preeclampsia mainly originate from placenta, where abnormal development of blood vessels leads to hypoperfusion. This relatively low oxygen state leads to the release of anti-angiogenic factors into maternal blood circulation, resulting in changes in endothelial function of maternal system and hypertension. PAPP-A is a kind of metalloprotease. The low level of PAPP-A in serum in early pregnancy can predict HDP and adverse pregnancy outcomes. The above studies showed that PLGF and PAPP-A are closely related to HDP. Moreover, PLGF and PAPP-A can both be used as biomarkers for predicting HDP, and detection of both indicators can improve the prediction effect. However, there is still little research on the relationship between the two indicators and the severity of the disease, and its monitoring effect on HDP is unclear. Therefore, we believe that PLGF and PAPP-A may be closely related to the severity of HDP, and they are expected to be used as monitoring indicators of HDP and provide a basis for clinical treatment. However, there are few clinical studies on the relationship between the two indicators and the severity of the disease, and its monitoring effect on HDP is not clear. For this reason, this study will investigate whether this hypothesis is true.

To sum up, we will study the correlation of the expression levels of PLGF and PAPP-A in serum of HDP patients with the severity of the disease and the relationship between the two and adverse pregnancy outcomes, providing valuable reference for clinical management and treatment of HDP.

Patients and Methods

Clinical Data Collection

Altogether 88 pregnant women with hypertensive disorder who underwent prenatal examination and delivery in our hospital from March 2017 to February 2019 were selected and included as the research group (n=88), and 62 healthy pregnant women who underwent prenatal examination during the same period were included as the normal control group (n=62). The gestational period was 30-41 weeks. The study was approved by the Medical Ethics Committee of our hospital.

Inclusion and Exclusion Criteria

Inclusion criteria: patient diagnosed with hypertensive disorder in pregnancy in our hospital after the 20th week of pregnancy; patients met the diagnostic criteria according to the relevant guidelines of German Society of Obstetrics and Gynecology (DGGG) in 2013; pregnant women underwent prenatal examination and delivery in our hospital; patients with complete clinical data; patient and their family members were informed and signed an informed consent form.

Exclusion criteria: patients carrying multiple fetuses; patients aged <18 years; patients with history of hypertensive disorder in pregnancy; patients with hypertension, diabetes mellitus, cardiovascular diseases, liver and kidney dysfunction, poor compliance and communication disorders.

Sample Collection and Detection

A total of 5 ml fasting venous blood was collected from pregnant women on the second day of admission and after diagnosis. The samples were placed at room temperature for 30 min, and centrifuged at 3000 g at 4°C for 10 min. The supernatant was obtained and put in a refrigerator at -80°C for examination.

The expression levels of PAPP-A and PLGF in serum were detected by enzyme-linked immunosorbent assay (ELISA) (PAPP-A ELISA kit, Shanghai Jingkang Bioengineering Company, item number: JK-(a)-5280; PLGF ELISA kit, Gene Tex, item number: XY-70R-35338). Specific Gal-3 was precoated on a 96-well microplate. A standard substance and a detection sample were added to the wells, then biotinylated Gal-3 was added, the sample was fully washed to remove unbound biotinylated antibody, horseradish peroxidase (HRP)-labeled avidin was added, TMB substrate was added for color development after washing again. TMB turned blue under catalysis and yellow under the action of acid. The absorbance (OD value) was measured by microplate reader at 450 nm wavelength, and the corresponding concentration was converted from the standard curve.

Outcome Measures

Main outcome measures: PAPP-A and PLGF expression levels in serum of pregnant women in the two groups were observed. The expression levels of PAPP-A and PLGF in serum of Gest-
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Observational Hypertension (GH), Mild Preeclampsia (MP) and Severe Preeclampsia (SP) patients were observed, and the correlation of the expression levels of PAPP-A and PLGF in serum of patients with HDP severity was observed. The disease severity criteria are shown in Table I.

Secondary outcome measures: the adverse pregnancy outcomes of the two groups of pregnant women were observed. The relationship of PAPP-A and PLGF expression levels in patients’ serum with adverse pregnancy outcome was observed.

**Statistical Analysis**

SPSS 20.0 (Cabit Information Technology Co., Ltd., Shanghai, China) was used to carry out statistical analysis on the collected data. Prism 7 (SOFTHEAD, Shenzhen, China) was used to visualize the figures of the data. The counting data was expressed by rate (%) and the Chi-square test was used for comparison, which was expressed by \( \chi^2 \). Measurement data were expressed by (Meas±SD). The comparison of normal distribution data between the two groups used independent sample \( t \)-test. The comparison between the two groups before and after treatment used paired \( t \)-test, which was expressed by \( t \). The correlation between PAPP-A and PLGF expression levels in patients’ serum and HDP severity was analyzed by Spearman correlation analysis. When \( p < 0.05 \), there was a statistical difference between the two groups.

**Results**

**Comparison of General Clinical Data Between Two Groups of Pregnant Women**

By comparing the general clinical data of the two groups of pregnant women, it was found that there was no statistical difference in age, Body mass index (BMI), smoking history, drinking history, residence, educational level, gestational weeks, pregnancy times, production times and abortion times between the two groups of pregnant women \((p>0.05)\), as shown in Table II.

**PAPP-A and PLGF Expression Levels in Serum of Pregnant Women in Two Groups**

By comparing the expression levels of PAPP-A and PLGF in the two groups of pregnant women’s serum, it was found that the expression level of PAPP-A in the research group was \((14.28±3.28)\) mU/L, which was significantly higher than that in the control group \((7.12±2.15)\) mU/L \((p<0.001)\), while the expression level of PLGF in the research group was \((128.42±13.26)\) pg/ml, which was significantly lower than that in the control group \((286.25±12.78)\) pg/ml \((p<0.001)\), as shown in Figure 1.

**Correlation Between PAPP-A and PLGF Expression Levels in Patients’ Serum and HDP Severity**

According to the diagnostic criteria of HDP severity, there were 36 GH patients, 30 MP patients and 22 SP patients in this study. The PAPP-A and PLGF expression levels in serum of patients in each group were detected respectively. The results showed that the PAPP-A expression level in SP patients \((16.32±3.92)\) mU/L was the highest, followed by MP patients \((11.14±3.61)\) mU/L, and the PAPP-A expression level in serum of GH patients \((8.26±2.13)\) mU/L was the lowest \((p<0.001)\). The PLGF test results showed that the serum PLGF level of GH patients \((181.52±21.34)\) pg/ml was the highest, followed by MP patients \((119.56±16.58)\) pg/ml, and the serum PLGF level of SP patients \((73.74±11.28)\) pg/ml was the lowest \((p<0.001)\), as shown in Figure 2. Then, we analyzed the correlation between PAPP-A, PLGF expression level

<table>
<thead>
<tr>
<th>Severity of disease</th>
<th>Diagnostic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational</td>
<td>Blood pressure ≥140/90 mmHg but without proteinuria after 20th week of pregnancy</td>
</tr>
<tr>
<td>Hypertension (GH)</td>
<td>20th week of pregnancy</td>
</tr>
<tr>
<td>Mild Preeclampsia</td>
<td>Hypertension and proteinuria in pregnancy</td>
</tr>
<tr>
<td>Mild Preeclampsia (MP)</td>
<td>Met any of the following points: blood pressure ≥ 160/110 mmHg; Creatinine ≥ 79.6 mol/L or urine volume &lt; 500 ml/24; Liver involvement (elevated transaminase, persistent epigastric pain); Pulmonary edema, Blood diseases (thrombocytopenia &lt; 100 Gpt/l, hemolysis); Nervous system symptoms (severe headache, visual impairment); Fetal growth retardation.</td>
</tr>
<tr>
<td>Severe Preeclampsia (SP)</td>
<td>Met any of the following points: blood pressure ≥ 160/110 mmHg; Creatinine ≥ 79.6 mol/L or urine volume &lt; 500 ml/24; Liver involvement (elevated transaminase, persistent epigastric pain); Pulmonary edema, Blood diseases (thrombocytopenia &lt; 100 Gpt/l, hemolysis); Nervous system symptoms (severe headache, visual impairment); Fetal growth retardation.</td>
</tr>
</tbody>
</table>
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and HDP severity by Spearman correlation analysis, and found that PAPP-A expression level was positively correlated with HDF severity (r=0.753, p<0.001), while PLGF expression level was negatively correlated with HDP severity (r=-0.929, p<0.001). We found that by scatter plot, the expression level of PAPP-A in serum had a significant increasing trend with the increase of HDP severity, while the expression level of PLGF in serum had an evident decreasing trend with the increase of HDP severity, as shown in Figure 3.

Adverse Pregnancy Outcomes of Two Groups Of Pregnant Women

We counted the occurrence of adverse pregnancy outcomes of all pregnant women.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Research group (n=88)</th>
<th>Control group (n=62)</th>
<th>t/χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.45±4.65</td>
<td>28.36±4.82</td>
<td>1.393</td>
<td>0.166</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.46±3.21</td>
<td>21.45±2.91</td>
<td>1.971</td>
<td>0.051</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48 (54.55)</td>
<td>27 (43.55)</td>
<td>1.760</td>
<td>0.185</td>
</tr>
<tr>
<td>No</td>
<td>40 (45.45)</td>
<td>35 (56.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>51 (45.31)</td>
<td>39 (40.74)</td>
<td>0.371</td>
<td>0.542</td>
</tr>
<tr>
<td>No</td>
<td>37 (54.69)</td>
<td>23 (59.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>38 (57.95)</td>
<td>30 (62.90)</td>
<td>0.175</td>
<td>0.676</td>
</tr>
<tr>
<td>Rural</td>
<td>26 (42.05)</td>
<td>24 (37.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or above</td>
<td>55 (62.50)</td>
<td>43 (69.35)</td>
<td>0.755</td>
<td>0.385</td>
</tr>
<tr>
<td>Below high school</td>
<td>33 (37.50)</td>
<td>19 (30.65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>64 (48.44)</td>
<td>42 (0.00)</td>
<td>0.436</td>
<td>0.509</td>
</tr>
<tr>
<td>&gt;2</td>
<td>24 (51.56)</td>
<td>20 (0.00)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy times</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>75 (85.23)</td>
<td>57 (91.94)</td>
<td>1.550</td>
<td>0.213</td>
</tr>
<tr>
<td>&gt;2</td>
<td>13 (14.77)</td>
<td>5 (8.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production times</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>75 (86.36)</td>
<td>56 (90.32)</td>
<td>0.540</td>
<td>0.463</td>
</tr>
<tr>
<td>&gt;2</td>
<td>12 (13.64)</td>
<td>6 (9.68)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Expression levels of PAPP-A and PLGF in serum of two groups of pregnant women. A, PAPP-A in serum of pregnant women in research group was significantly higher than that in control group. B, PLGF in the serum of pregnant women in the research group was significantly lower than that in the control group. ***indicates p<0.001.
The results showed that there were 27 cases of premature delivery, 8 cases of placental abruption, 20 cases of fetal distress and 7 cases of perinatal death in the research group, 4 cases of premature delivery, 0 cases of placental abruption, 3 cases of fetal distress and 0 cases of perinatal death occurred in the control group. The occurrence rate of adverse pregnancy outcomes in the research group was significantly higher than that in the control group \((p<0.05)\), as shown in Table III.

**Table III.** Incidence of adverse pregnancy outcomes of two groups of pregnant women.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Research group ((n=88))</th>
<th>Control group ((n=62))</th>
<th>(\chi^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature birth</td>
<td>27 (30.68)</td>
<td>4 (6.45)</td>
<td>13.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>8 (9.09)</td>
<td>0 (0.00)</td>
<td>5.954</td>
<td>0.015</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>20 (22.73)</td>
<td>3 (4.84)</td>
<td>8.966</td>
<td>0.003</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>7 (7.95)</td>
<td>0 (0.00)</td>
<td>5.173</td>
<td>0.023</td>
</tr>
<tr>
<td>Total</td>
<td>62 (70.45)</td>
<td>7 (11.29)</td>
<td>51.26</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Figure 2.** Expression levels of PAPP-A and PLGF in serum under various HDP severity levels. A, PAPP-A detection results showed that the expression level of PAPP-A was highest in SP patients, followed by MP patients, and PAPP-A was lowest in serum of GH patients. B, PLGF test results showed that the expression level of PLGF of GH patients was the highest, followed by MP patients, and serum PLGF of SP patients was the lowest. ***indicates ***\(p<0.001\).

**Figure 3.** Scatter plot of correlation of PAPP-A and PLGF expression level with HDP severity in serum. A, The expression level of serum PAPP-A had a significant increasing trend with the increase of HDP severity. B, The expression level of serum PLGF had a significant decreasing trend with the increase of HDP severity.
**Relationship Between PAPP-A and PLGF Expression Levels in Patients’ Serum and Adverse Pregnancy Outcomes**

According to adverse pregnancy outcomes, patients were divided into group with adverse pregnancy outcomes and group without adverse pregnancy outcomes. By detecting PAPP-A and PLGF in serum of the two groups of patients, it was found that PAPP-A of patients with adverse pregnancy outcomes (15.02±4.47) mU/L was significantly higher than that of patients without adverse pregnancy outcomes (9.21± 1.96) mU/L \((p<0.001)\), while PLGF of patients with adverse pregnancy outcomes (81.35±14.83) pg/ml was significantly lower than that of patients without adverse pregnancy outcomes (172.74± 23.68) pg/ml \((p<0.001);\) Figure 4.

**Discussion**

HDP is the most common complication during pregnancy. It causes a global disease burden, which is reported in 5%-10% of pregnancies. Due to the increasing incidence of heart disease and metabolic diseases in pregnant women, it is believed that the incidence of HDP is also increasing\(^1\). Immunodeficiency, placental hypoplasia and trophoblastic infiltration, placental ischemia, oxidative stress and thrombosis are all considered as key factors for the disease development\(^2\). Preeclampsia is a potential serious complication of pregnancy, and its mortality rate accounts for 9%-26% of the world’s pregnant women. Early detection, prevention and management have become current research hotspots\(^3\). Therefore, the prevention of HDP and the study of disease severity are of great significance for clinical treatment.

Preeclampsia (PE) is a severe HDP. Its occurrence and development are closely related to soluble vascular endothelial growth factor receptor 1 (sFLT-1), PLGF and soluble endothelial glycoprotein (sEng)\(^4\). Studies have shown that low levels of PAPP-A may be related to placental abnormalities and lead to the development of PE\(^4\). To further explore the relationship between the two markers and HDP, we first found that the expression level of PAPP-A and PLGF in the serum of pregnant women in the research group was significantly higher than that in the control group, while the expression level of PLGF in the research group was significantly lower than that in the control group. Results showed that the levels of PAPP-A and PLGF in serum were differentially expressed between normal pregnant women and HDP pregnant women, which can be used as indicators for distinguishing normal pregnant women from HDP pregnant women. Saffer et al\(^2\) showed that the concentration of PLGF was relatively low in the first three months of pregnancy of normal pregnant women, increasing from the 11th week to the 12th week and reaching a peak at the 30th week. Ratsep et al\(^2\) observed that knocking out PLGF of mice showed placental vascular abnormalities. This study also indicated that the low level of PLGF may indicate pathological changes in placenta, which was related to the occurrence of HDP. Paredes et al\(^2\) showed that the fetal circulation near severe preeclampsia during pregnancy and deliv-

![Figure 4. Relationship between PAPP-A, PLGF expression level in patients’ serum and adverse pregnancy outcome. A, PAPP-A level in serum of patients with adverse pregnancy outcomes group was significantly higher than that of patients without adverse pregnancy outcomes group. B, PLGF level in serum of patients with adverse pregnancy outcomes was significantly lower than that of patients without adverse pregnancy outcomes. ***indicates ***<0.001.](image)
ery showed lower PLGF and higher PAPP-A level, which was similar to our research results.

PLGF and PAPP-A have high predictive value for HDP in early pregnancy. However, the relationship between PLGF and PAPP-A levels in patients with different disease severity from the middle stage to late stage of pregnancy is unclear. Therefore, we have tested PLGF and PAPP-A levels in patients with different disease severity. The results showed that PAPP-A expression level in SP patients was the highest, followed by MP patients. PAPP-A expression level in serum of GH patients was the lowest, while PLGF detection results were the opposite. The results revealed that PLGF and PAPP-A levels are closely related to HDP severity. For this reason, we analyzed the correlation between PAPP-A, PLGF expression level and HDP severity through Spearman correlation analysis. It was found that PAPP-A expression level was positively correlated with HDP severity, while PLGF expression level was negatively correlated with HDP severity. The results showed that serum PAPP-A expression level had a significant upward trend with the increase of HDP severity. However, the expression level of serum PLGF had a significantly decreasing trend with the increase of HDP severity, which is conducive to clinical monitoring of HDP patients and better prevention and management of HDP. PLGF has the function of promoting angiogenesis and enhances the activity of VEGF by competitively binding VEGFR-1 receptor. The imbalance among sFLT1, VEGF and PLGF may affect the pathophysiology of placental diseases, thus leading to the development of pregnancy-related hypertension. The lack of PLGF in HDP patients may be due to the reduction of PLGF expression caused by binding to sFLT1. We speculated that the imbalance of sFLT1 regulation with VEGF and PLGF led to the reduction of angiogenesis, vascular permeability and HDP, and the low HDP level was closely related to the seriousness of the disease. In patients with mild and severe HDP, Xu et al. revealed that the level of serum PAPP-A showed an upward trend, the level of PAPP-A at 34-40 weeks of pregnancy showed an upward trend, the level in the research group was higher than in the healthy control group. This was similar to our research results, which showed that PAPP-A participated in the progress of HDP and was closely related to the severity of the disease and could be used as an HDP monitoring index to monitor the development of the disease.

HDP is related to adverse pregnancy outcomes and neonatal adverse outcomes. We have counted the adverse pregnancy outcomes of all pregnant women. Results showed that the incidence of adverse pregnancy outcomes in the research group was significantly higher than that in the control group, and HDP greatly increased the incidence of adverse pregnancy outcomes. Finally, we detected the expression levels of PAPP-A and PLGF in the serum of patients with adverse pregnancy outcomes and patients without adverse pregnancy outcomes. It was found that the PAPP-A level in the serum of patients with adverse pregnancy outcomes was significantly higher than that of patients without adverse pregnancy outcomes, while the PLGF level in the serum of patients with adverse pregnancy outcomes was significantly lower than that of patients without adverse pregnancy outcomes. Results showed that PAPP-A and PLGF had a certain relationship with adverse pregnancy outcomes. The high level of PAPP-A and low level of PLGF increased the probability of adverse pregnancy outcomes in HDP patients, which may be related to the conclusion that PAPP-A, PLGF and HDP severity have a certain correlation.

There are some limitations in this study. First, we only collected pregnant women's serum for testing, but did not collect tissues for testing. Whether there is any difference in expression between the two is not clear. Second, the correlation between the two indicators has not been explored, and the connection between the two indicators is not clear. Therefore, we will supplement these contents in future studies to further verify this study.

Conclusions

To sum up, we observed that the expression levels of PAPP-A and PLGF in serum are closely related to the severity of HDP and can be used as disease monitoring indicators.

Conflict of Interests

The authors declare that they have no conflict of interest.

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