Original Article

Modulation of Telocytes in Women with Preeclampsia: A Prospective Comparative Study

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Abstract

Background: Telocytes (TCs) are networking cells with enigmatic functions. Placenta is a noninnervated organ with the TCs could have function of signal transmission to placental myofibroblasts, being likely a regulator for maternal blood flow. Preeclampsia (PE) is a disease complicating the second half of pregnancy associated with hypoxia probably due to failure of vascular remodeling of spiral arteries resulting in poor placental perfusion. We hypothesized that disturbance in the morphology of TCs may have a role in the pathogenesis of PE. **Materials and Methods:** Women with normal or physiological pregnancy (Group I; 15 women) and with PE (Group II; 15 women) participated in this study. Specimens were obtained from the central cotyledons and the superficial myometrium beneath the implantation sites processed for light microscopy and stained with Hematoxylin and Eosin, toluidine blue, masson trichrome, and CD117. **Results:** The villi of group II has thick-walled blood vessels with increased peri-villous fibrinoid deposition, reduced areas of vasculosyncytial membrane and apparent increase in connective tissue density. Morphometric study and statistical analysis revealed a significant increase in the mean number of syncytial knots and significant decrease in placental (villous and decidual) and myometrial TCs and extravillous trophoblasts (EVTs) beneath the placental implantation site in Group II ($P < 0.011$) in comparison with group I. **Conclusions:** PE is associated with significantly low number of placental TCs interestingly with low number of EVTs. Further studies are needed to support our findings.

Keywords: Placenta, preeclampsia, telocytes

Introduction

Telocytes (TCs) are interstitial cell type first reported by a Noble Prize winner Santiago Ramón y Cajal. He described them as interstitial neurons owing to their location between the nerve ganglia and smooth muscles of the intestine.[1] TCs have telopodes, long and thin cytoplasmic extensions, providing a structural support for homocellular or heterocellular junctions contributing to maintain local homeostasis, tissue repair, remodeling, and intercellular signaling.[2] Their characteristic morphology allows for the formation of intercellular junctions with various cells in the surrounding normal and diseased tissues, suggesting a potential functional relationship with the development of abnormalities.[3] Several organs have TCs: Cavitary organs such as the heart (endo, myo and pericardium), stomach, gall bladder, uterus and fallopian tubes, or noncavitary organs such as lungs, pleura, pancreas, mammary gland, and placenta.[4]

TCs function is poorly understood and remains an avenue for research. TCs act as pacemaker to regulate the peristaltic movement of gastrointestinal tract.[5] Furthermore, they regulate the proliferative and supporting abilities, regulating the regeneration of human skeletal muscles. TCs functions in the female reproductive system include regulation of peristaltic movement of the fallopian tubes and the hormone actions.[6] However, they appear to have other regulatory roles in the reproductive system. Morphological and functional changes of TCs occur to cope with different physiological conditions as pregnancy, puerperium and menopause.[7]

Placenta is a noninnervated organ with the TCs could have function of signal transmission to placental myo-fibroblasts,

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being likely a regulator for maternal blood flow effects on fetal growth and development.[8] TCs and myofibroblasts generate nitric oxide, thereby modulating the tone of perivascular contractile sheets.[9]

A major cause for maternal and fetal morbidity as well as mortality is preeclampsia (PE). It is a hypertensive disorder with pregnancy in the second half of gestation. Complications of PE include cerebral hemorrhage, coagulation disorders, liver and kidney damage, placental abruption, preterm birth, and intra uterine growth retardation.[4] Theories for the pathogenesis of PE belong to poor placentation, resulting in inadequate remodeling of spiral arteries, and development of hypoxia.[10] These theories include the immune theory,[11] oxidative stress theory, and abnormal angiogenesis theory.[12] Given the heretofore regulatory actions of TCs, we hypothesized that disturbance in the function of TCs may have a role in the pathogenesis of the hypoxia and ischemia of PE. This research was performed to prove our hypothesis by examining the TCs in the placentae of normal pregnancies or pregnancies with PE.

Materials and Methods

The research protocol was approved by the Scientific Research Ethics Committee of Sohag University, Sohag, Egypt. This study included 30 women admitted in the labor ward for caesarian section delivery at Obstetrics and Gynecology Department, Sohag University Hospitals, Sohag, Egypt from September 2018 to May 2019. Women were counseled for participation and signed a written informed consent. Women with normal or physiological pregnancy were assigned to Group I (G1; 15 women, and those with PE were assigned to Group II (GII; 15 women). Women were included provided they had normal pregnancy or PE, normal body mass index, aged 20–35 years and were at 34–40 weeks of gestation, except for those with severe medical conditions or congenital fetal malformations.

Diagnosis of preeclampsia

Women with PE fulfilled the criteria if they had hypertension (>140/90 mmHg) and proteinuria (> 0.3 g/day or ≥+1 by dipstick urine analysis at ≥20 weeks of gestation). If no proteinuria, hypertension associated with thrombocytopenia, renal or liver impairment, or pulmonary edema was sufficient to diagnose PE "American College of Obstetricians and Gynecologists Practice Bulletin No. 202," (2019).

Specimen preparation

Tissue preparation and histological stains were done according to Bancroft (2008).[13] Specimens measuring 0.5 cm × 0.5 cm from the maternal surface of the central cotyledons were taken including small pieces from the superficial myometrium beneath the implantation sites. Specimens underwent washing with normal saline, followed by immediate fixation in 10% neutral formalin for 24 h. During preparation, specimens underwent dehydration in ascending grades of alcohol, clearance in xylene, impregnation and then embedded into paraffin wax. Sections of 5 um thickness were prepared using microtome (LEICA RM 2125) and were stained with Hematoxylin and Eosin for general histological study and with toluidine blue for demonstration of TCs.[14] The demonstration of collagen fibers took place with Masson staining.

Protocol for immunohistochemical reaction

Detection of TCs took place using C-kit monoclonal antibody (CD117; ScyTec, RA0168-C.-IFU-RUO). We carried out the steps following the manufacturer's instructions and others' example.[14] Samples underwent deparaffinization and rehydration, followed by boiling the sections in 10 mmol/L citrate buffer (pH 6.2) in a microwave oven for two cycles, 3 min each to retrieve the antigen, with replacement of the lost buffer between cycles. The endogenous peroxidase in the sections was blocked by 2% hydrogen peroxide for 5 min, followed by overnight incubation with a 1:150 diluted anti-c-kit antibody at 4°C in refrigerator. Next morning, we subjected the sections to biotinylated as a secondary antibody in a humidified chamber, where enzyme conjugate streptavidin was applied. The slides were stained by substrate-chromogen mixture and then counterstained using Hematoxylin reagent. Next, we rinsed the slides in distilled water and did the dehydration in ascending grades of alcohol, followed by clearance with xylene and mounting with cover slip. Negative control was achieved with the omission of the primary antibody. Brownish cytoplasmic deposits and blue nuclei were the positive TCs for c-kit.

Morphometric analysis

For each case, TCs were counted in five high power fields (×400) of the chorionic villi, endometrium, and myometrium.[15] Syncytial knots were also counted in three fields (×200) for each case in both groups.[16] Furthermore, extravillous trophoblast (EVT) cells were counted in five high power fields (×400) for the implantation site (superficial myometrium). All counted cells/structures were done in nonoverlapping fields.

Statistical analysis

SPSS statistical package (version 16.0) was used for all statistical analyses. Calculations were done by using the statistical package SPSS for Windows, version 10.0 (SPSS, Chicago, Illinois, USA).Data were presented as means ± standard deviation. $P \leq 0.05$ indicated statistical significance. Data analyses of different groups were performed using Student's t-test with a statistical significance of $P < 0.05$.

Results

General morphology

The placenta consists of fetal part and maternal part. The fetal part includes the chorionic villi and the maternal part represented by the decidua. The chorionic villi starting from the fetal chorionic plate to the maternal side include; stem, mature intermediate, terminal and anchoring villi. The latter are connected to the decidua carrying with them the EVTs. The villi are formed of connective tissue core containing the fetal vessels and covered by syncytiotrophoblast cells. The fibrous villous

stroma is dense in the stem villi and to a lesser extend in mature intermediate villi but it was scanty stroma in terminal villi. Dense areas, the syncytial knots appeared as syncytial dense apoptotic nuclei on the terminal villous surface. The maternal part of the placenta consists of connective tissue stroma and decidual cells observed as large polygonal cells with central vesicular one or two nuclei and vacuolated cytoplasm [Figure 1a and d].

Examination of G II revealed that the core of stem villi has thick-walled blood vessels. The villi were covered with thick trophoblast covering with increased perivillous fibrinoid deposition. The terminal villi showed reduced areas of vasculosyncytial membrane. The mature intermediate and terminal villi showed apparent increase in connective tissue density when compared to GI [Figure 1b, c and e].There was apparent increase in the syncytial knotting of all types of villi compared to the control group. Morphometric study and statistical analysis revealed a significant increase in the mean number of syncytial knots in GII compared to GI [Figure 1f].

Telocyte examination

Villous telocytes

On examination of toluidine Blue and C-kit stained sections of G1, TCs were identified as flattened cells having long, tortuous, and thin cytoplasmic processes observed in the perivascular and subsyncytial regions of stem and mature intermediate villi [Figure 2a and b].

In PE Group (GII), TCs were frequently seen smaller with shorter processes and less numerous than those observed in GI [Figure 2c and d]. Morphometric study and statistical analysis revealed a significant decrease in the mean number of villous C-Kit positive TCs in GII than GI ($P < 0.011$) [Figure 2e].

Endometrial telocytes

The decidua of GI consists of connective tissue stroma containing anchoring villi, superficial band of TCs and aggregations of decidual cells [Figure 3a and b]. In PE group, the decidua showed less numerous TCs compared with those in GI [Figure 3c and d]. Morphometric study showed a significant decrease in the mean number of decidual C-Kit positive TCs in GII compared with GI ($P < 0.001$) [Figure 3e].

Myometrial telocytes

The superficial myometrium of placental bed of GI showed bands of TCs in between the smooth muscles and around the blood vessels and aggregations of giant EVTs around, and within, myometrial blood vessel walls [Figure 4a and b]. In the PE group, both TCs and intramural EVTs were less numerous and usually associated with thicker walled-myometrial blood vessels [Figure 4c and d]. Morphometric study revealed that there was a significant decrease in the number of myometrial C-Kit positive TCs and EVTs in GII compared with the control group ($P < 0.001$) [Figure 4e and f].

Discussion

A compiling fact for us to act is that TCs are involved in multiple systems in human body including the reproductive system. The hypothesis-driven research and physiology

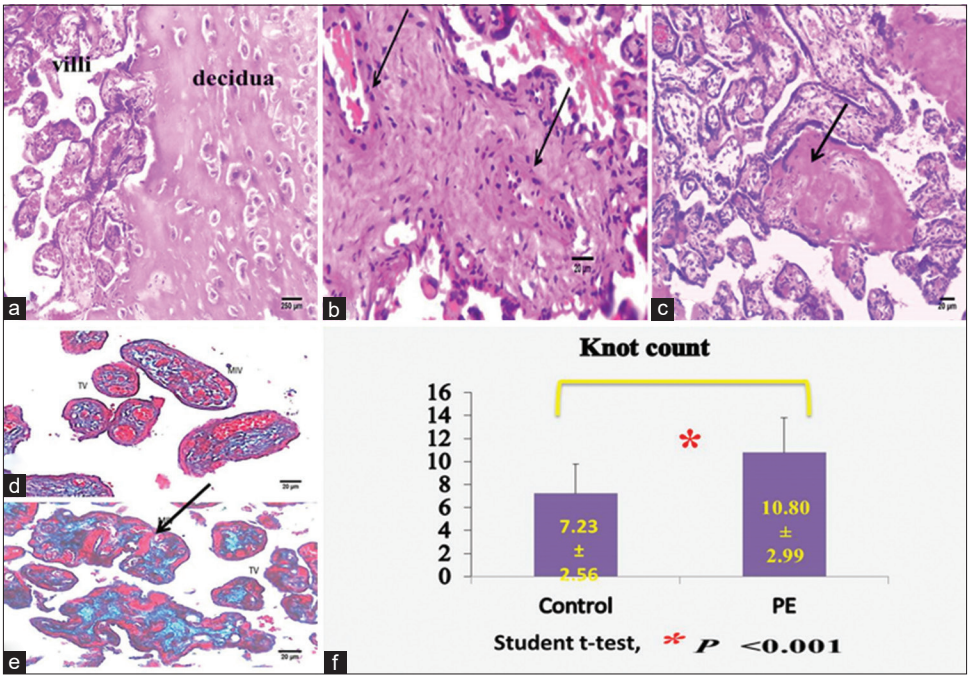

Figure 1: Photomicrographs of placentae of G1 (a and d) and G11 (b, c and e) and graphical presentation of knot count (f). A shows normal placenta consists of chorionic villi and decidua. (b) Shows thick walled blood vessels of stem villus (arrow). (c) Shows increased fibrinoid deposition within the villi (arrow) (×H and E,). (d and e) Show the collagen fibers within the mature intermediate villi and terminal villi in G1 and G11 respectively being denser in G11. Note, the massive fibrinoid deposition in G11 (arrow). (Masson trichrome stain). (f) Shows graphical presentation of the student's *t*-test result for data analysis of the knot count of both groups (G1 and G11) being significantly higher in G11 compared to G1. $P \leq 0.05$ indicated statistical significance

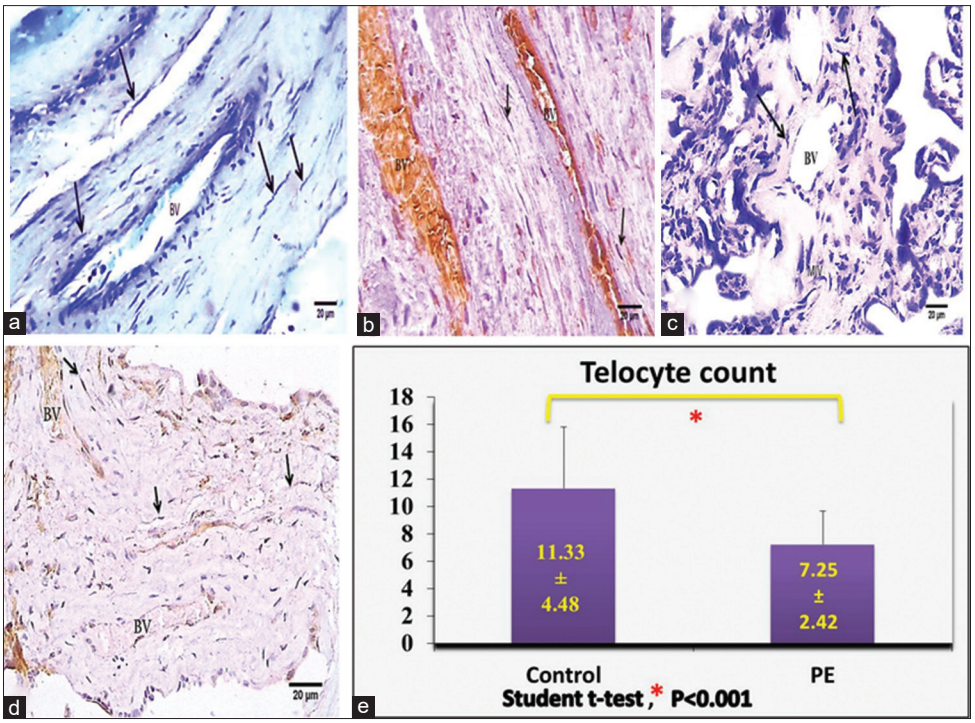

Figure 2: Photomicrographs of placentae of G1 (a and b) and G11 (c and d) and graphical presentation of villous telocytes count (e). (a-d) Show stem villi with parallel telocytes (arrow) around the blood vessels being less numerous and with shorter processes in G11. (a and c Toluidine Blue, b and d c-Kit immuno-stain). (e and f) Shows graphical presentation of the student's *t*-test result for data analysis of the telocytes count of both groups (G1 and G11) being significantly lower in G11 compared to G1. $P \leq 0.05$ indicated statistical significance

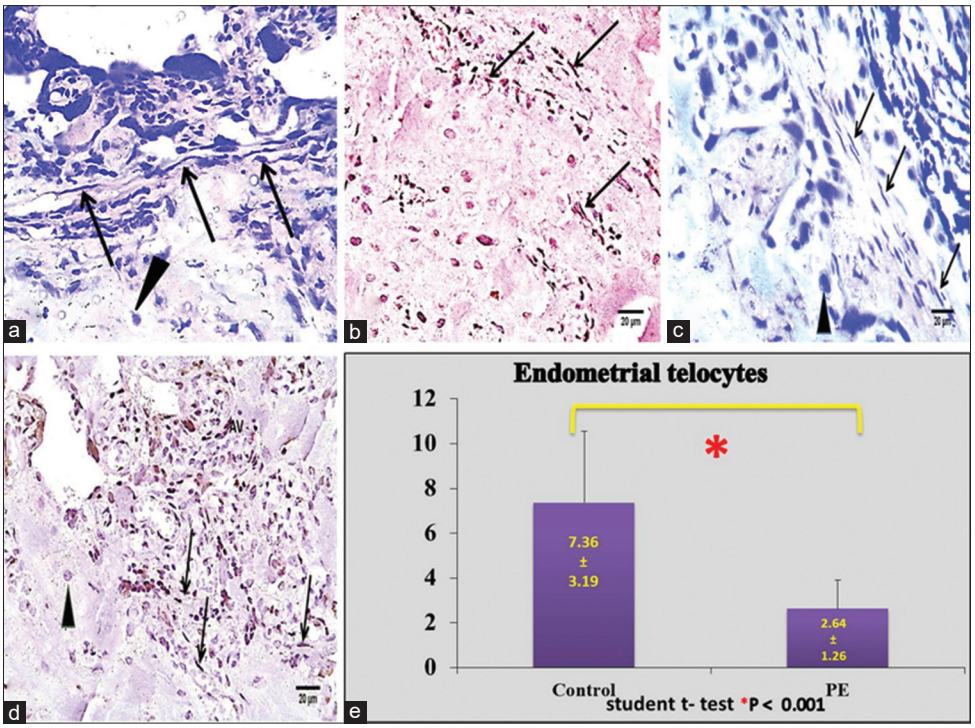

Figure 3: Photomicrographs of placentae of G1 (a and b) and G11 (c and d) and graphical presentation of endometrial telocyte count (e). (a-d) Show anchoring villi (within the decidua with a superficial band of telocytes (arrow) being less numerous and with shorter processes in G11. Note the decidual cells with large central nucleus and abundant cytoplasm within the connective tissue stroma (arrow head) (a and c Toluidine Blue, b and d c-Kit immuno-stain). (e and f) Shows graphical presentation of the Student's *t*-test result for data analysis of the endometrial telocytes count of both groups (G1 and G11) being significantly lower in G11 compared to G1. $P \leq 0.05$ indicated statistical significance

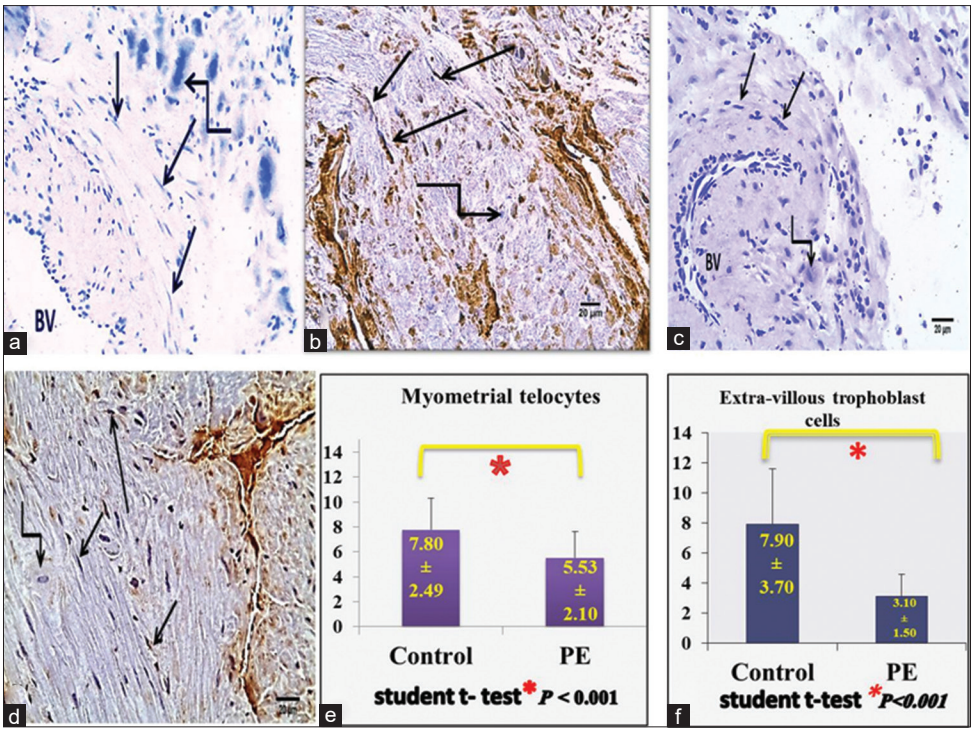

Figure 4: Photomicrographs of placentae of G1 (a and b) and G11 (c and d) and graphical presentation of myometrial telocytes and extravillous trophoblast cell counts (e and f). (a-d) show telocytes (arrow) and aggregation of multinucleated giant extravillous trophoblast cell (angled arrow) around the blood vessels, both cells being less numerous in G11 compared to G1. (a and c Toluidine Blue, b and d c-Kit immuno-stain). (e and f) Show graphical presentation of the student's *t*-test result for data analysis of the myometrial telocytes and extra villous trophoblast cell count of both groups (G1 and G11) being significantly lower in G11 compared to G1. $P \leq 0.05$ indicated statistical significance

suggest a role for TCs in PE; therefore, we conducted this research.

In normal gestation, we found that the placenta consists of the fetal part including the chorionic stem, mature intermediate, terminal, and anchoring villi and the maternal part represented by the decidua. The syncytial knots were demonstrated as plate-like thickenings that bulge gently from the villous surface. Their nuclei are highly condensed. They represent focal areas of syncytiotrophoblast degeneration that occurs in late gestation.[17]

In PE, a prominent feature was the marked decrease of the vasculosyncitial membrane, being the leading site of oxygen transfer. It was associated with increased stromal fibrosis of the chorionic villous corium. These pathological features can cause the fetal hypoxia associated with PE, at least partly.[18] In addition, the placentae with PE have a feature of trophoblastic proliferation observed as an increase in the thickness of trophoblastic covering of the villi. This may be due to ischemia and oxidative stress as postulated by Myren *et al*.[19] The higher number of syncytial knots and the massive fibrinoid deposition within the chorionic villi in PE group could be signs of degeneration due to hypoxia associated with PE. Tomas *et al*. found that the total number of syncytial knots were significantly higher with less villus trophoblast Fas L expression and greater Bcl-2 expression in PE than in control group.[20]

The demonstrated TCs by Toluidine blue stain as well as by immunohistochemical staining with C-Kit antibody located in the perivascular region of the stem, mature intermediate and terminal villi were in accordance with a previous study.[21] In the endometrial implantation site, they were demonstrated as a parallel sheet of cells underlying the anchoring villi, while in the myometrial implantation site they were located around and within the wall of blood vessels. These were thin elongated cells with thin and long cytoplasmic extensions mainly from the two longitudinal poles of the cell. In the present study, TCs' count of the villi and endometrial and myometrial implantation site were significantly lower in PE compared with the control group. The same results were described by the Russian group using different immune-stains and by electron microscopy.[22] The decreased number of villous TCs in PE may contribute to developing the associated fibrosis.[21] TCs were found to be reduced in fibrotic areas in many organs like the heart,[23] interstitial wall fibrosis following salpingitis[1] and the skin of systemic sclerosis.[24]

Our study revealed that the number of EVTs in the perivascular region of superficial myometrium was significantly lower in PE than in the control group. This suggests that the paucity of TCs demonstrated in the endometrium and myometrium may cause the reduced EVTs' migration within the implantation site. Decreased extravillous trophoblast migration and invasion lead to failure of vascular remodeling of spiral arteries resulting in

poor placental perfusion and hypoxia. Whether the decreased TCs are of genetic or developmental etiology warrants further studies.

Conclusions

PE is associated with significantly low number and morphologically altered with consequent impaired function of the TCs. Further studies using a larger sample size should replicate our results. Although electron microscopy is thus far the gold standard for elucidating the TCs,[21] it is recommended that future studies using more than one immuno-histochemical staining are performed to provide more confidence about the exact role and site of this emerging cells.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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