Original Article

Analysis of predictive parameters for the development of radiation-induced pneumonitis

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Abstract:

INTRODUCTION: Prevention and effective treatment of radiation-induced pneumonitis (RP) could facilitate greater use of radiation therapy (RT) for lung cancer. The purpose of this study was to determine clinical parameters useful for early prediction of RP.

METHODS: Blood sampling, pulmonary function testing, chest computed tomography, and bronchoalveolar lavage (BAL) were performed in patients with pathologically confirmed lung cancer who had completed ≥60 Gy of RT, at baseline, shortly after RT, and at 1 month posttreatment.

RESULTS: By 3 months post-RT, 11 patients developed RP (RP group) and the remaining 11 patients did not (NRP group). RT significantly increased total cell counts and alveolar macrophages in BAL of the NRP group, whereas lymphocyte count was increased in both groups. Matrix metallopeptidase-9 (MMP-9) increased and vascular endothelial growth factor decreased significantly in the BAL fluid (BALF) of the RP group following RT. Serum surfactant protein D (SP-D) increased significantly in the NRP group. SP-D in BALF from the RP group increased significantly with a subsequent increase in serum SP-D. Pulmonary dilution decreased similarly in both groups of patients.

CONCLUSIONS: Increased SP-D in BALF, rather than that in serum, could be useful biomarkers in predicting RP. The MMP-9 in BALF might play a role in the pathogenesis of RP. Pulmonary dilution test may not be predictive of the development of RP.

Keywords:

Bronchoalveolar lavage, matrix metallopeptidase-9, radiation-induced pneumonitis, surfactant protein D, vascular endothelial growth factor

The incidence of chest radiation therapy (RT) is increasing in association with the increased incidence of lung cancer worldwide. Although RT would ideally be performed without damaging normal tissue, a variety of normal lung tissue damage may occur since lung tissue is highly sensitive to radiation. Lung damage following RT is classified as radiation-induced pneumonitis (RP) when it develops one to 3 months following RT, and as radiation fibrosis when it develops three to 6 months following RT. Both types may overlap and may be hard to distinguish.

The incidence of RP is increased following ≥40 Gy of radiation exposure. With >60 Gy radiation exposure, severe disease may occur. Generally, 10%–20% of radiated patients develop moderate to severe RP and mortality is estimated to be around 50% in severe RP.[1-6] Prevention and effective treatment of RP could facilitate greater use of RT for lung cancer. Clinical markers for risk of development of RP could be helpful.

Recently, a number of cell types and mediators have been proposed to be involved in the pathogenesis of pulmonary fibrosis, including fibrosis following RT. An animal model demonstrated that

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radiation directly induces toxic oxidant production from lung tissue, which causes pulmonary cell death. Pulmonary cells highly sensitive to radiation include Type II alveolar epithelial cells, vascular endothelial cells, and alveolar macrophages (AMs). Following pulmonary damage caused by radiation, inflammatory cells infiltrate lung tissue and interact with pulmonary constitutive cells, leading to the development of interstitial pneumonitis. Fibroblasts increase in the interstitium, as part of the repair process for damaged tissue, and produce extracellular matrix and collagen, which induce fibrosis. These fibrotic cycles are regulated by cytokines and growth factors produced by inflammatory and constitutive cells.[7-9] Among cytokines, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), platelet-derived growth factor, and transforming growth factor-T-β (TGF-T-β) increase in a murine model and in human peripheral blood in response to radiation.[10,11] Matrix metallopeptidase (MMP) and vascular endothelial growth factor (VEGF) have also been suggested to be involved in pulmonary fibrosis.[12-18] Recently, serum sialylated carbohydrate antigen-6 (KL-6) and surfactant protein D (SP-D) have been considered as useful biomarkers to determine the activity of pulmonary fibrosis at clinic level. Serum KL-6 and SP-D levels are associated with greater sensitivity in the diagnosis of RP than serum lactate dehydrogenase.[19,20] In addition, serum KL-6 level significantly correlates with severity and responses to therapy in pulmonary fibrosis.[21] Nonetheless, the role of these parameters in the prediction of RP remains unclear. This study was designed to determine the clinical parameters that are useful for the early prediction of RP.

Methods

Subjects

This study received ethical approval from the Special Committee of Toho University Ohashi Medical Center (project registration number 23-11) to proceed between 2011 and 2013 and each patient provided written informed consent to participate. Eligible participants were adults with pathologically confirmed lung cancer Stage IIIb or IV who had completed ≥60 Gy of RT. Participants with pulmonary fibrosis before radiotherapy, with systemic severe comorbidities including cardiac and collagen disease, who were current smokers, who had obvious infection, or with medications that frequently cause drug-induced pulmonary diseases were excluded from the study.

Study design

Blood sampling, pulmonary function testing, chest computed tomography (CT), and bronchofiberscopy (BF) were performed for all patients at baseline, shortly after RT and at 1 month after RT. To determine RP, additional chest CT was performed when the development of RP was clinically suspected throughout the study period and up to 3 months posttreatment. Two experienced respiratory physicians and two radiologists evaluated cases for diagnosis of RP. RP was defined as the presence of abnormal shadows in radiated lung fields in the absence of other causes based on clinical examinations. BF (BF-1T30, Olympus Co. Ltd.; Tokyo, Japan) was performed under standard premedication and local anesthesia. Bronchoalveolar lavage (BAL) was performed at a selected site in the tumor-free area of the radiation fields to minimize the effect of cancer cells. The same segment was lavaged for subsequent BAL. Total cell counts, cellular components, and concentrations of TNF-α, IL-1β, MMP-9, VEGF, KL-6, and SP-D in BAL fluid (BALF) were determined. Simultaneously, serum KL-6 and SP-D were determined and pulmonary function testing (FUDAC-77®, Fukuda Denshi Co. Ltd.; Tokyo, Japan) was performed. Total cell count was counted using a Bürker chamber and the differential cell count was evaluated in light microscopy on May-Grunwald-Giemsa-stained slides. TNF-α was measured by chemiluminescence enzyme immunoassay (R & D Systems, Minneapolis, MN, USA), IL-1β was measured by enzyme immunoassay (EIA) (R & D Systems). MMP-9 was measured by EIA (Daiichi fine chemical Co. Ltd.; Toyama, Japan). VEGF was measured by enzyme-linked immunosorbent assay (R & D Systems). SP-D and KL-6 were measured by EIA (Eidea Co. Ltd.; Tokyo, Japan). Detection limits were 0.55 pg/mL, 0.125 pg/mL, 3.13 ng/mL, 15.6 pg/mL, 17.3 pg/mL, and 0 ng/mL, respectively. These results were compared between participants who did not develop RP (NRP) and RP.

Analysis

Results are expressed as means ± standard deviation. The baseline data between the RP group and NRP group were examined by binary logistic regression analysis, with all predictors taken into account. Differences between groups were examined for statistical significance using Wilcoxon signed-rank test and those within groups were examined using Wilcoxon rank-sum test. Sex ratio, pathology of lung cancer, and concomitant chemotherapy ratio between groups were examined by Fisher's exact probability test. All statistical analyses were performed using SPSS Statistics (Japan IBM; Tokyo, Japan). $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Thirty-four participants were entered into the study. Five participants experienced exacerbation of lung cancer during the study period, two participants had

RT interrupted due to pneumonia, and five participants rejected repeat bronchoscopy and were excluded from the study. Twenty-two participants completed the study protocol and were analyzed. All cases had partial response to treatment. Of these 22, 11 developed RP (RP group) and the remaining 11 did not (NRP group) by 3 months after RT. In the RP group, the location of lung cancer was in the right upper lobe in 5, right middle lobe in 1, right hilum in 1, left upper lobe in 3, and left lower lobe in 1. In the NRP group, the location of lung cancer was in the right upper lobe in 1, right middle lobe in 1, right lower lobe in 3, right hilum in 1, left upper lobe in 3, and left lower lobe in 2. In the RP group, RP developed 59.0 ± 19.9 days after the end of RT and there was no mortality; all cases improved spontaneously (n = 7) or after administration of prednisolone (n = 4). Patient characteristics are shown in Table 1. There were no significant differences between RP and NRP groups in terms of mean age, sex, Brinkman index, and pathology of lung cancer and concomitant chemotherapy ratio. The results of binary logistic regression analysis that compared the baseline data between the RP group and NRP group, with all predictors taken into account, were not significant.

Cellular profiles from bronchoalveolar lavage

Results of cellular profiles from BAL are shown in Figure 1. In the NRP group, total cellular counts and fractions of lymphocytes in BAL were significantly increased at 1 month post-RT compared with pre-RT. Significant differences did not occur in total cellular counts or fractions of AMs in BAL, while lymphocyte fractions increased significantly at 1 month post-RT in the RP group. AM fraction at 1 month post-RT was significantly higher in the NRP group compared with the RP group. Lymphocyte fractions from BAL were comparable between the two groups.

Inflammatory markers in bronchoalveolar lavage fluid

Figure 2 shows the results of testing for inflammatory markers in BALF. Inflammatory markers in the NRP group did not change significantly following RT. In the RP group, MMP-9 in BALF increased significantly at 1 month after RT compared to pretreatment. By contrast, in these patients, VEGF in BALF decreased significantly at 1 month after RT compared to pretreatment. SP-D in BALF of patients in the RP group increased significantly shortly after RT compared with pretreatment. At 1 month after RT, concentrations of MMP-9 and VEGF in BALF of patients in the RP group were significantly higher and lower compared to those in the NRP group, respectively. There were no significant differences in concentrations of IL-1β and TNF-α in BALF within and between groups (data not shown).

Table 1: Patient characteristics*

	NRP	RP
Patients (*n*)	11	11
Sex male/female	9/2	7/4
Age (yrs)	73.2±8.9	72.0±8.6
Brinkman index	926.4±650.9	673.3±428.6
Pathology (A/Sq/S/L/U)	5/4/1/0/1	3/4/3/0/1
Chemotherapy (*n*)	6	5

Data represented as mean±SD. *NRP = Subjects who did not develop radiation-induced pneumonitis, RP = subjects who developed radiation-induced pneumonitis, A = Adenocarcinoma, Sq = Squamous cell carcinoma, S = Small cell carcinoma, L = large cell carcinoma, U = Unclassified carcinoma

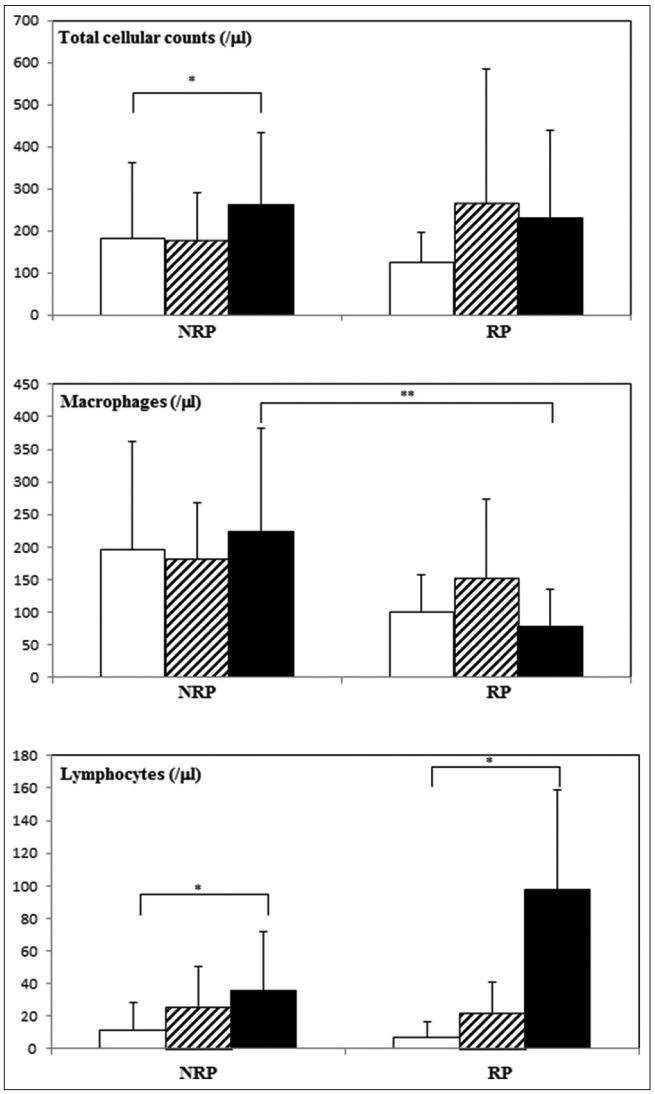

Figure 1: Cellular profiles of bronchoalveolar lavage fluid. In the NRP group, total cellular counts and lymphocyte fractions were significantly increased at 1 month post-RT. In the RP group, lymphocyte fractions increased significantly at 1 month post-RT. Alveolar macrophage fraction at 1 month post-RT was significantly higher in the NRP group compared with the RP group. Bars represent mean ± standard deviation. Open bar: pretreatment; hatched bar: Shortly after RT; closed bar: 1 month after RT. *P < 0.05 and **P < 0.01. NRP: Participants who did not develop radiation-induced pneumonitis; RP: Participants who developed radiation-induced pneumonitis, RT: Radiation therapy

Inflammatory markers in serum

Figure 3 shows the results of testing for serum inflammatory markers. By comparison with pretreatment levels, serum SP-D increased significantly both shortly after RT and 1 month after RT in the NRP group. At 1 month after RT, serum SP-D levels in the RP group increased significantly compared with those at pretreatment and shortly after RT.

Results of pulmonary dilution

Figure 4 shows the results of testing for diffusing capacity of the lungs for carbon monoxide (DL_{CO}). Pulmonary dilution of both groups decreased similarly and significantly at 1 month after RT compared with pretreatment. With the exception of DL_{CO}, other pulmonary functions did not show significant differences within and between groups (data not shown).

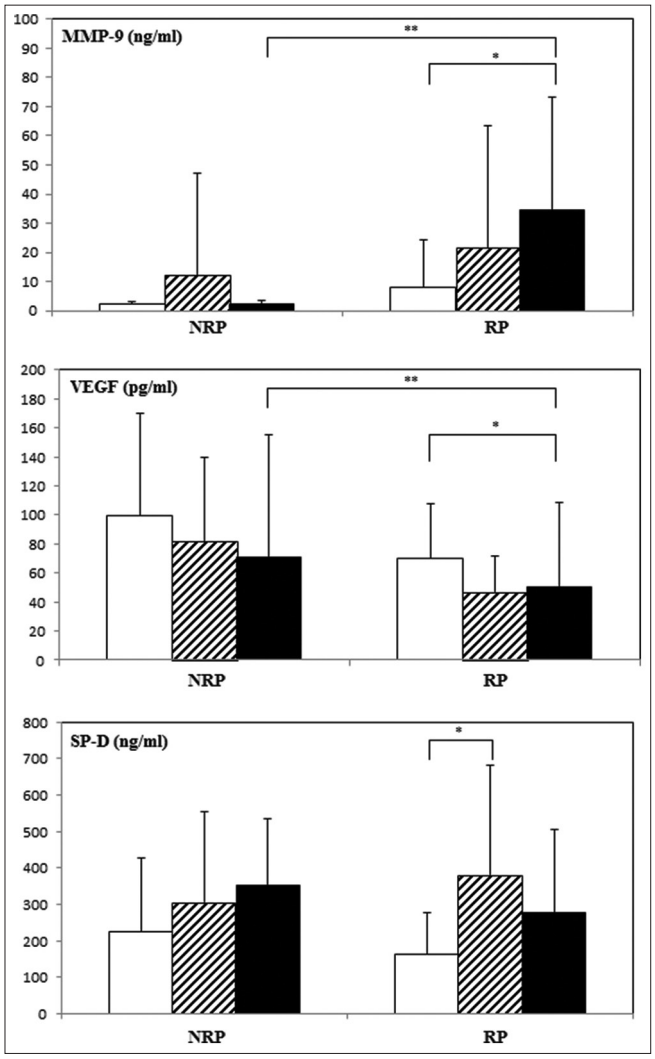

Figure 2: Concentrations of inflammatory markers in bronchoalveolar lavage fluid. In the radiation-induced pneumonitis group, MMP-9 increased significantly and VEGF decreased significantly at 1 month after radiation therapy. SP-D of patients in the radiation-induced pneumonitis group increased significantly shortly after radiation therapy. At 1 month after radiation therapy, MMP-9 and VEGF of patients in the radiation-induced pneumonitis group were significantly higher and lower compared to those in the NRP group, respectively. Bars represent mean ± standard deviation. Open bar: pretreatment; hatched bar: shortly after RT; closed bar: 1 month after radiation therapy. **P* < 0.05 and ***P* < 0.01. MMP-9: matrix metallopeptidase-9; VEGF: vascular endothelial growth factor; SP-D: surfactant protein-D

Discussion

The major findings of the present study are as follows: (i) RT significantly increased total cell and AM counts in the airways of NRP patients and significantly increased lymphocytes in both groups, (ii) MMP-9 increased significantly and VEGF decreased significantly in the airways of patients with RP, (iii) SP-D increased significantly in the serum, but not BALF, of patients without RP, whereas SP-D in BALF increased significantly with a subsequent significant increase in serum SP-D in patients with RP, (iv) pulmonary dilution decreased similarly in both groups of patients.

A previous report showed RT increases BAL lymphocytes equally between patients who develop RP and those who do not following RT for breast cancer.[22] Lymphocyte fractions in BALF following RT were comparable between the two groups in our study. In contrast to effector T-cells, which promote immune activation

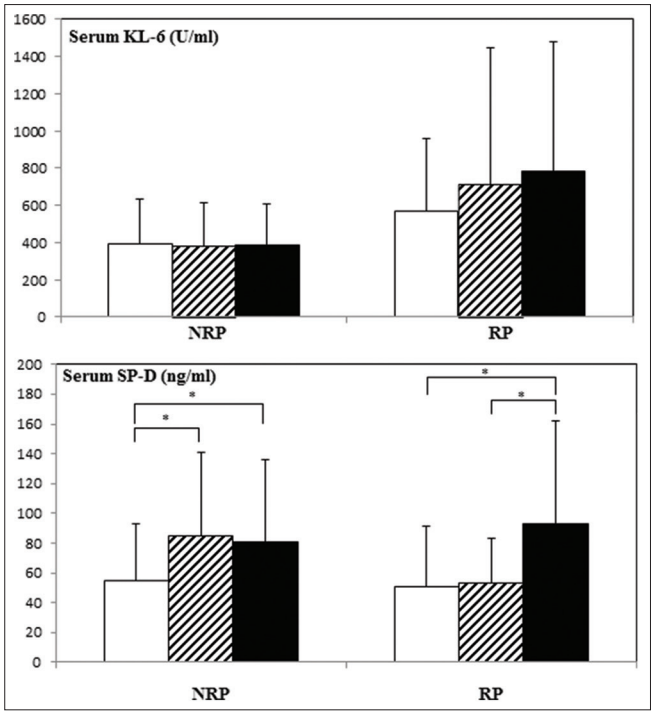

Figure 3: Concentrations of inflammatory markers in serum. surfactant protein-D increased significantly both shortly after radiation therapy and 1 month after radiation therapy in the NRP group. surfactant protein-D in the radiation-induced pneumonitis group increased significantly at 1 month after radiation therapy. Bars represent mean ± standard deviation. Open bar: pretreatment; hatched bar: shortly after radiation therapy; closed bar: 1 month after radiation therapy. **P* < 0.05. KL-6: sialylated carbohydrate antigen-6

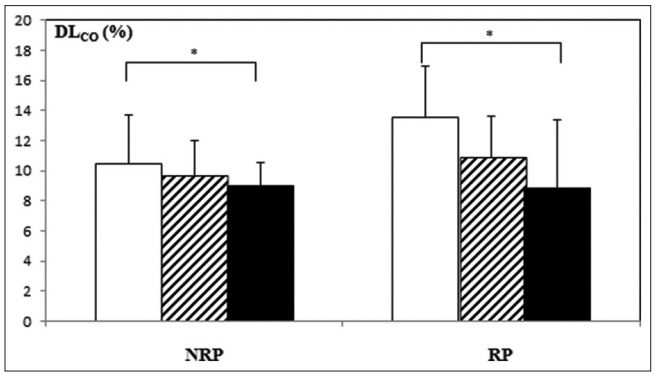

Figure 4: Pulmonary dilution. Both groups decreased similarly and significantly at 1 month after radiation therapy. Bars represent mean ± standard deviation. Open bar: pretreatment; Hatched bar: shortly after radiation therapy; closed bar: 1 month after radiation therapy. *$P < 0.01$. DL_{CO}: Diffusing capacity of the lungs for carbon monoxide

and resultant tissue damage, regulatory T-cells (Treg), counterbalance effector T-cells and prevent tissue damage, a phenomenon that has attracted attention.[23] Treg seems to be involved in the pathogenesis of several pulmonary diseases.[24-26] The depression of Treg significantly correlates with the severity of idiopathic pulmonary fibrosis (IPF).[27] It may be possible that Treg dominantly increased as part of the increase in total lymphocytes and prevented the development of pulmonary damage in the NRP group in the present study.

In accordance with previous reports indicating that MMP-9 increases in the BALF of IPF patients,[14,28] our study also found that MMP-9 increased significantly in the airways of patients with RP. MMP-9 does not constitutively express in normal lung tissue, while its expression is induced in inflamed tissue. MMP-9 activates TGF-β and thus promotes fibrosis.[29] Although an exact role of MMP-9 in the development of pulmonary fibrosis is not fully established, recent studies have indicated that MMP-9 could be a potential biomarker of fibrotic pulmonary diseases.[30] Our study also indicates that MMP-9 might be a representative biomarker of RP.

VEGF is a representative cytokine involved in the pathogenesis of pulmonary fibrosis.[31] VEGF as well as AMs significantly decreased in the BALF of patients in the RP group compared with those in the NRP group. VEGF also induces angiogenesis, a process in which tissue responses to oxygen play a critical role.[32] Tissue hypoxia increases the expression of VEGF through stabilization of hypoxia inducible factors (HIFs) and thus causes angiogenesis. Hypoxic AMs express and produce VEGF through HIFs.[33] VEGF knockout mice develop severe pulmonary fibrosis due to the activation of pulmonary fibroblasts, suggesting the inhibitory role of VEGF produced from hypoxic AMs in regulating pulmonary fibroblasts.[34] Decreased AMs and VEGF in the airway of patients with RP might have failed to inhibit pulmonary fibroblasts in the present study.

SP-D is a potential biomarker of RP since it increases in both serum and BALF.[35,36] In our study, serum SP-D, but not that in BALF, increased significantly shortly after and at 1 month after RT in the NRP group. In the RP group, SP-D in BALF increased significantly shortly after RT and serum SP-D increased significantly at 1 month after RT. Since SP-D is so hydrophilic, it easily moves from tissue to blood leading to correlation between serum and BALF levels.[37] Nonetheless, concentrations of SP-D in BALF and serum did not simultaneously increase in the present study. SP-D plays a critical role in control of infection and immune regulation in lung tissue. It may contribute to tissue homeostasis, preventing fibrosis by regulating MMP-9 activity in lung tissue.[38] In our study, MMP-9 increased significantly in the airway of RP patients. SP-D might persist in the airway to prevent MMP-9 activity and subsequently diffuse into the blood. It may be more appropriate to determine SP-D in BALF than in serum to predict the development of RP.

A few studies had evaluated pulmonary dilution in RP. Severity of RP significantly correlates with pulmonary dilution.[39] Pulmonary dilution testing has been shown to be useful for early detection of the development of RP.[40] It has also been reported that radiological abnormalities precede the decrease in pulmonary dilution.[41] By comparison with baseline, RT similarly and significantly decreased pulmonary dilution in both groups in the present study, indicating that the utility of pulmonary dilution in the early detection of RP may be low.

The study had several limitations. As the data came from a relatively small number of patients with a variety of underlying diseases, the results could have been influenced by confounders, such as existing lung disease and type of concurrent chemotherapy. Further analyses utilizing less invasive methods, such as induced sputum and parameters, such as Treg number in BALF, are required to better establish the predictors for RP. Another limitation was the lack of measurement of urea or total protein concentration levels in BALF. BALF requires a certain technique of collection to obtain sufficient samples and is diluted initially. A diluted BALF sample needs to be corrected by urea or protein concentration for accurate comparison between samples; however, we were not able to do this because of lack of data.

Conclusions

In conclusion, increased SP-D in BALF, rather than that in serum, could be useful biomarkers in predicting RP. MMP-9 might play a role in the pathogenesis of RP.

Pulmonary dilution test may not be predictive of the development of RP.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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