



# Elevated Red Cell Distribution Width Levels in Patients with Pseudoexfoliation Syndrome and Pseudoexfoliation Glaucoma

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## Abstract

**Objectives:** The purpose of this study was to assess the levels of red cell distribution width (RDW) in patients with pseudoexfoliation syndrome (PEX) and to compare the RDW values of patients with PEX, PEX glaucoma (PXG), and healthy controls.

**Methods:** In total, 45 patients with PEX, 14 patients with PXG, and 43 age- and sex-matched healthy control subjects were enrolled in this retrospective study. Complete ophthalmologic examination and complete blood count measurements were performed of all subjects. Complete blood counts were performed within 1 h of blood collection.

**Results:** RDW levels were significantly higher in patients with PEX and PXG than in those with controls ( $p=0.037$  and  $p<0.001$ , respectively). Furthermore, a significant difference was found in RDW values between PXG and PEX groups ( $p=0.028$ ). RDW levels were gradually increased from control group to PXG group ( $p<0.001$  for one-way ANOVA). Multivariate logistic regression analysis revealed that RDW was independently associated with the presence of PEX/PXG (odds ratio 1.698, 95% confidence interval 1.077–2.677,  $p=0.023$ ).

**Conclusion:** The present study for the first time provides evidence that RDW may be an useful marker for predicting the presence of PEX and progression to PXG.

**Keywords:** Inflammation, pseudoexfoliation glaucoma, pseudoexfoliation syndrome, red cell distribution width.

## Introduction

Pseudoexfoliation syndrome (PEX), characterized with progressive accumulation of an abnormal extracellular fibrillar material on anterior structures of the eye and extraocular tissues, including periphery of blood vessels, skin, and visceral organs, is an age-related disorder of extracellular matrix and considered as the most common identifiable cause of secondary open-angle glaucoma (1). It is also linked to a broad range of ocular complications including glaucoma and perioperative problems during cataract surgery (2). Although the exact pathophysiologic mechanism of PEX has

not yet been clarified, it has been presumed to be associated with multifactorial systemic biochemical and pathological processes induced by several growth factors and molecules that play roles in inflammation and oxidative stress (3–8).

Red cell distribution width (RDW) is a laboratory measure of the variability in the size of circulating erythrocytes and is a readily available component of the routine complete blood count parameters. Several studies reported that increased RDW levels are significantly associated with adverse events and poor prognosis in various cardiovascular and cerebrovascular diseases (9–17). Given the association of PEX with various diseases, particularly cardiovascular or

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cerebrovascular diseases, and the role of inflammation and oxidative stress in the pathogenesis of PEX (3, 4, 18–22), we hypothesized that RDW may be related with the presence of PEX or PEX glaucoma (PXG). In a previous study, RDW was significantly associated with the presence of retinopathy (23). Recently, we have shown the increased RDW level in children with seasonal allergic conjunctivitis (24). However, whether RDW level is associated with PEX/PXG has not been explored yet. Accordingly, the aim of this study was to evaluate serum levels of RDW in patients with PEX/PXG in comparison with those of age- and sex-matched healthy subjects.

## Methods

The clinical and laboratory data of 45 patients with PEX, 14 patients with PXG, and 43 age- and sex-matched healthy subjects were retrospectively assessed at our hospital in the study. The diagnosis of PEX was based on the presence of typical exfoliation material on the anterior lens capsule or pupillary margin after the pupillary dilatation by pharmacological agents in one or both eyes, with a normal optic disc and visual field findings in patients with an intraocular pressure (IOP) <21 mmHg. Control subjects had no history of ocular disease (except for refractive error, strabismus, and cataract) and elevated IOP (more than 21 mmHg) and no evidence of exfoliation material on the anterior lens capsule or pupillary margin. They had normal optic disc and visual fields. All PXG patients had a glaucomatous optic neuropathy and visual field damage, PEX material on the anterior lens capsule or at the pupillary border, transillumination defects near the pupil, increased pigmentation or PEX material at the angle or both, and an IOP of more than 21 mmHg without treatment. Patients who experienced any of the following conditions were excluded: Systemic infectious, inflammatory, oncological, hematological, or rheumatologic diseases; renal failure; liver or thyroid dysfunction; chronic obstructive pulmonary disease; anemia (a hemoglobin level <13.0 g/dL in men and <12 g/dL in women, according to the World Health Organization recommendations); chronic or recurrent inflammatory eye disease; ocular trauma; ocular infection; and missing laboratory data. This submission has received the Institutional Review Board/Ethics Committee approval. A described research adhered to the tenets of the Declaration of Helsinki.

## Laboratory Parameters

Blood samples were collected in all patients after fasting at least 8h. Complete blood count parameters were analyzed immediately after collection using a Beckman-Coulter LH 780 Analyzer (Beckman Coulter Inc., Miami, FL, USA).

## Statistical Analysis

All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL). Distribution of data was determined by Kolmogorov–Smirnov test. Continuous variables were expressed as mean±SD and categorical variables as frequency and percentage. Continuous variables were compared with the student's t-test or Mann–Whitney U-test, as appropriate and categorical variables were compared using Pearson's Chi-square test for three groups. One-way ANOVA and Kruskal–Wallis variance analyses were used for multigroup comparison of continuous variables. If the differences were significant, pair-wise comparisons would be based on the Mann–Whitney U-test or student's t-test, with Bonferroni correction to establish which subgroups were different. Receiver operating characteristic (ROC) analysis was also performed to determine the cutoff threshold and quantify the accuracy of RDW to predict PEX and PXG. Sensitivity, specificity, and the area under the ROC (AUROC) curve were used for an overall estimation of the accuracy of the classifier. All of the reported P values were two-tailed, and those <0.05 were considered to be statistically significant.

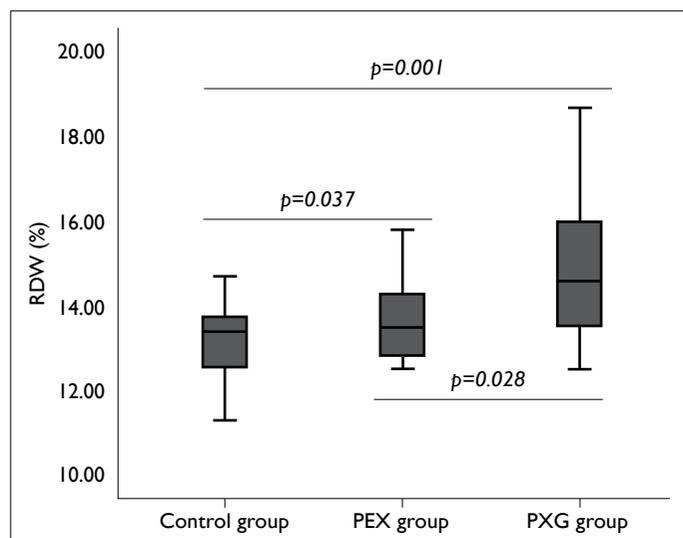
## Results

Baseline demographic and blood sampling characteristics on the participants are shown in Table 1. No statistically significant difference was observed in terms of the age, sex distribution, diabetes mellitus, hypertension, and lipid parameters. Lymphocyte counts were gradually decreased, while neutrophil-to-lymphocyte ratio (NLR) values were gradually increased from control to PXG groups ( $p=0.007$  and  $p<0.001$ , respectively). There was a significant difference in RDW levels between PEX and control groups ( $p=0.037$ ) and PXG and control groups ( $p<0.001$ ). Furthermore, a significant difference was found in RDW values between PXG and PEX groups ( $p=0.028$ ). RDW levels were gradually increased from control group to PXG group ( $p<0.001$ ) (Fig. 1). According to the ROC analysis, the AUROC value of the RDW to distinguish PEX patients and controls was found to be 0.619. The best cutoff value was 12.85, with a sensitivity of 73.3% and a specificity of 41.9% (Fig. 2a). In addition, AUROC value of RDW to distinguish patients with PXG and controls was found to be 0.798. The cutoff value was 13.45, with a sensitivity of 78.6% and a specificity of 55.8% (Fig. 2b). Multivariate logistic regression analysis comparing PEX and PXG patients with controls indicated that elevated RDW level was a significant risk factor for PEX/PXG status (odds ratio =1.698; 95% confidence interval =1.077–2.677,  $p=0.023$ ) (Table 2).

**Table 1.** Baseline clinical and laboratory characteristics of patients among three groups

Characteristic	Study groups			p
	Control (n=43)	PEX (n=45)	PXG (n=14)	
Age (years)	65.2±4.9	67.4±7.9	69.3±5.76	0.172
Women, n (%)	26 (60.5)	27 (60.0)	6 (42.9)	0.473
Neutrophil count (×10 <sup>9</sup> /L)	3.32±0.94	3.68±1.09	3.69±0.89	0.195
Lymphocyte count (×10 <sup>9</sup> /L)	2.19±0.61	1.84±0.54	1.78±0.43	0.007
NLR	1.56±0.58	2.08±0.64	2.19±0.78	<0.001
Hypertension, n (%)	20 (46.5)	25 (55.6)	11 (78.6)	0.111
Diabetes mellitus, n (%)	8 (18.6)	13 (28.9)	5 (35.7)	0.347
Total cholesterol (mg/dL)	207±42	220±41	217±44	0.340
HDL cholesterol (mg/dL)	47±13	45±11	41±8	0.199
LDL cholesterol (mg/dL)	131±37	135±34	152±39	0.156
Triglyceride (mg/dL)	140±63	150±71	141±45	0.745
RDW (%)	13.18±0.83	13.89±1.46	14.95±1.88	<0.001

PEX: Pseudoexfoliation syndrome; PXG: Pseudoexfoliation glaucoma; NLR: Neutrophil-to-lymphocyte ratio; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; RDW: Red blood cell distribution width.



**Figure 1.** RDW levels in controls, PEX group, and PXG group. RDW-red cell distribution width, PEX-pseudoexfoliation syndrome, PXG-pseudoexfoliation glaucoma.

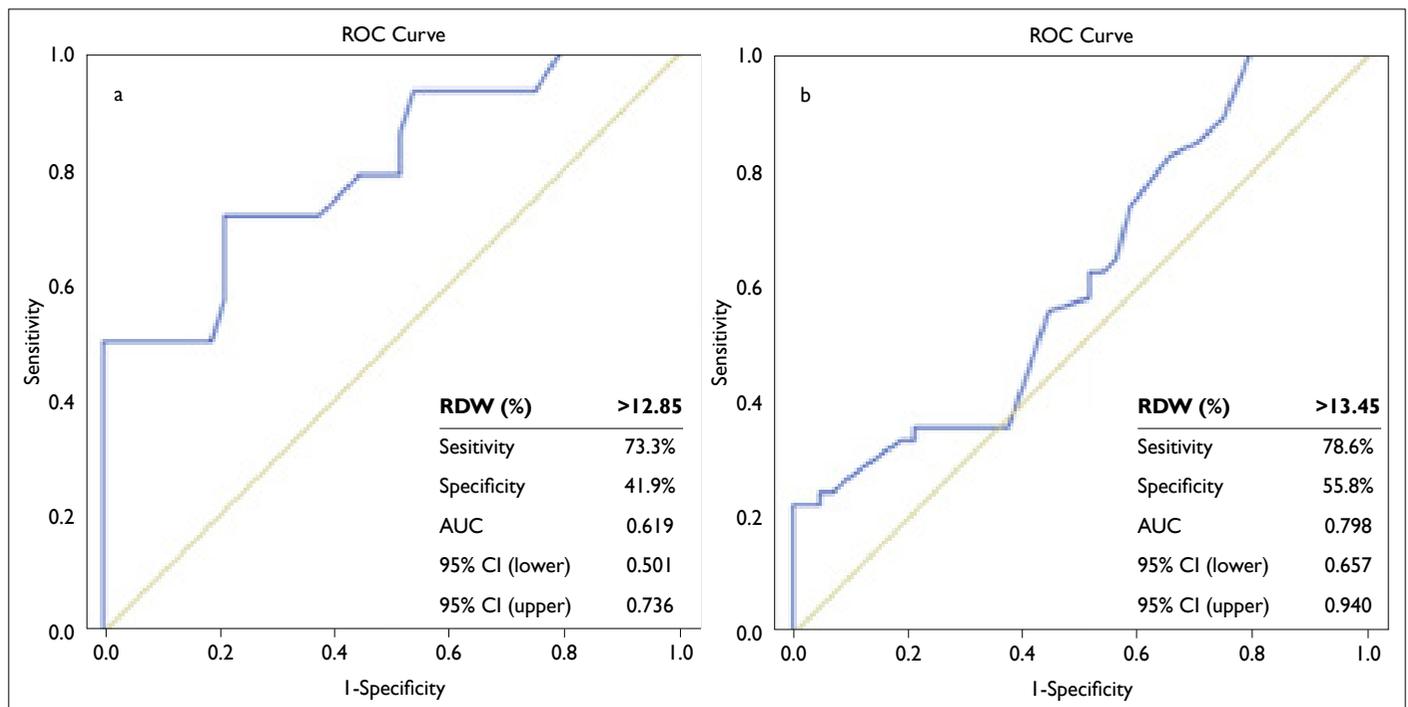
## Discussion

To the best of our knowledge, this is the first published study on the RDW as an inflammation and oxidative stress marker in patients with ocular PEX. The present study was shown increased RDW levels in PEX/PXG groups than the control group. Furthermore, RDW was independently associated with the presence of PEX/PXG.

The pathophysiology of PEX involves both genetic and non-genetic factors. Single-nucleotide polymorphisms in the

coding region of the lysyl-oxidase-like 1 gene, which is responsible for cross-linking of elastin, have been identified as strong genetic risk factors for PEX and PXG (25). Moreover, non-genetic factors including ultraviolet light exposure, dietary factors, infectious agents and trauma, as well as, oxidative stress, hypoxia, and inflammation have been suggested to act as comodulating external factors (4). Stress-induced, temporally restricted subclinical inflammation in anterior segment tissues is detected during the early stages of the fibrotic PEX process (26). According to numerous studies performed previously, inflammatory markers (e.g., alpha-1 antitrypsin, interleukin-6, high-sensitivity C-reactive protein [hs-CRP], and tumor necrosis factor-alpha) and growth factors (e.g., basic fibroblast growth factor, hepatocyte growth factor, connective tissue growth factor, transforming growth factor beta 1, and tumor necrosis factor-β1) were reported to be increased in PEX patients (18–20).

The underlying mechanisms between RDW and PEX/PXG have not yet been clearly demonstrated. Some possible mechanisms may be suggested for this association. One of the possible mechanisms may be related with increased inflammatory activity. It is well known that PEX is related with inflammatory reactions. Previous studies demonstrated that PEX is associated with elevated inflammatory markers such as hs-CRP, NLR, and YKL-40 (20–22). In the present study, we have further found that RDW levels were significantly higher in PEX/PXG group than control group. It was shown that elevated inflammatory cytokines suppress the maturation of erythrocytes, allowing juvenile erythrocytes to enter



**Figure 2.** (a) Receiver operating characteristics (ROC) curve analysis for discrimination between controls and PEX and (b) controls and PXG. PEX-Pseudoexfoliation syndrome, PXG-Pseudoexfoliation glaucoma.

**Table 2.** Independent predictors of PEX/PXG status

Variable	Univariate Analysis		Multivariate Analysis	
	Odds Ratio, 95% CI	p	Odds Ratio, 95% CI	p
Neutrophil count	1.474 (0.963-2.255)	0.074	1.050 (0.328-3.355)	0.933
Lymphocyte count	0.311 (0.142-0.681)	0.004	0.748 (0.105-5.360)	0.773
NLR	4.492 (2.004-10.068)	<0.001	3.329 (0.388-28.544)	0.273
Age	1.059 (0.992-1.130)	0.088	1.055 (0.979-1.138)	0.160
RDW	1.955 (1.279-2.988)	0.002	1.698 (1.077-2.677)	0.023

PEX: Pseudoexfoliation syndrome; PXG: Pseudoexfoliation glaucoma; NLR: Neutrophil-to-lymphocyte ratio; RDW: Red blood cell distribution width; CI: Confidence interval.

into circulation and thereby leading to an increase in heterogeneity of the size, resulting in elevated RDW levels (27).

The other mechanism is based on the relationship between elevated RDW and increased oxidative stress (28). Elevated RDW level may be related with increased oxidative stress in the presence of PEX/PXG. An increase in oxidative stress markers (e.g., 8-isoprostaglandin-F<sub>2α</sub>) and a decrease in antioxidative protective factors (e.g., ascorbic acid) were observed in patients with PEX (6–8). Increasing evidence suggests that the oxidative-antioxidative balance is disturbed in patients with PEX/PXG, both in the anterior segment and throughout the body, and that the resulting oxidative stress constitutes a major mechanism involved in the pathophysiology of this fibrotic process. Significantly reduced levels of antioxidants, such as ascorbic acid, glutathione, trace ele-

ments, antioxidative enzymes, and total antioxidative capacity in aqueous humor and serum, suggest a faulty antioxidative defense system in PEX patients. In other words, the oxidative-antioxidative balance is disturbed in patients with PEX as supported by reduced levels of antioxidants such as ascorbic acid, glutathione, trace elements, and antioxidative enzymes in aqueous humor and serum and increased levels of oxidants such as hydrogen peroxide or nitric oxide, as well as, oxidative stress markers (5). Oxidative stress, therefore, appears to represent a modifiable risk factor in the management of patients with PEX/PXG. It is well known that oxidative stress directly damages erythrocytes and leads to shortened erythrocyte survival, resulting in elevated RDW levels (29). The variability in circulation red cell sizes may increase with these mechanisms.

Taken together, this study showed that RDW levels were higher in both patient groups with PEX and PXG than control group. A higher level of RDW (13.45 as a cutoff value) was significantly predictive to distinguish PXG patients than controls. Considering PEX as an early stage of PXG, determining RDW as a significant marker to distinguish PEX and control patients was another important finding of the present study.

### Study Limitations

This study has several limitations. The main limitation of our study was the lack of data on pro-inflammatory cytokines, inflammation (e.g., hs-CRP), and oxidative stress (oxidized phospholipid, free oxygen radicals) markers, which possibly plays a key role in the inflammation process in PEX. Another limitation is the relatively small sample size.

### Conclusions

This study demonstrated that the RDW, the parameter reflecting inflammation, and oxidative stress were significantly higher in patients with PEX/PXG. This study also may shed light on future studies investigating the pathogenesis of RDW in the development of PXG in patients with ocular PEX. Further studies investigating mechanisms playing a role in chronic low-grade inflammation and oxidative stress in patients with PEX are needed.

### Disclosures

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship Contributions:** Involved in design and conduct of the study (BEK, EUK); preparation and review of the study (BEK); data collection (BEK, EUK); and statistical analysis (BEK).

### References

- Schlötzer-Schrehardt UM, Koca MR, Naumann GO, Volkholz H. Pseudoexfoliation syndrome. Ocular manifestation of a systemic disorder? *Arch Ophthalmol* 1992;110:1752–6.
- Schlötzer-Schrehardt U, Naumann GO. Ocular and systemic pseudoexfoliation syndrome. *Am J Ophthalmol* 2006;141:921–37.
- Schumacher S, Schlötzer-Schrehardt U, Martus P, Lang W, Naumann GO. Pseudoexfoliation syndrome and aneurysms of the abdominal aorta. *Lancet* 2001;357:359–60.
- Kurtul BE, Kurtul A, Altıaylık Ozer P, Kabatas EU, Ertugrul GT. Serum Lipid Levels in Pseudoexfoliation Syndrome. *Semin Ophthalmol* 2017;32:281–4.
- Kılıçarslan S, Tekcan A, Demir HD, Yiğit S. Investigation of Association with Clusterin Rs11136000 Gene Polymorphism of Pseudoexfoliation Syndrome [Article in Turkish]. *Bozok Med J* 2017;7:1–6.
- Schlötzer-Schrehardt U. Oxidative stress and pseudoexfoliation glaucoma [Article in German]. *Klin Monbl Augenheilkd* 2010;227:108–13.
- Koliakos GG, Konstas AG, Schlötzer-Schrehardt U, Hollo G, Katsimbris IE, Georgiadis N, et al. 8-Isoprostaglandin F2a and ascorbic acid concentration in the aqueous humour of patients with exfoliation syndrome. *Br J Ophthalmol* 2003;87:353–6.
- Bleich S, Roedel J, Von Ahsen N, Schlötzer-Schrehardt U, Reulbach U, Beck G, et al. Elevated homocysteine levels in aqueous humor of patients with pseudoexfoliation glaucoma. *Am J Ophthalmol* 2004;138:162–4.
- Pascual-Figal DA, Bonaque JC, Redondo B, Caro C, Manzano-Fernandez S, Sánchez-Mas J, et al. Red blood cell distribution width predicts long-term outcome regardless of anaemia status in acute heart failure patients. *Eur J Heart Fail* 2009;11:840–6.
- Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M; for the Cholesterol and Recurrent Events (CARE) Trial Investigators. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. *Circulation* 2008;117:163–8.
- Dabbah S, Hammerman H, Markiewicz W, Aronson D. Relation between red cell distribution width and clinical outcomes after acute myocardial infarction. *Am J Cardiol* 2010;105:312–7.
- Kurtul A, Murat SN, Yarlioglu M, Duran M, Karadeniz M, Ergun G, et al. The association of red cell distribution width with in-stent restenosis in patients with stable coronary artery disease. *Platelets* 2015;26:48–52.
- Kurtul A, Yarlioglu M, Murat SN, Demircelik MB, Acikgoz SK, Ergun G, et al. Red cell distribution width predicts contrast-induced nephropathy in patients undergoing percutaneous coronary intervention for acute coronary syndrome. *Angiology* 2015;66:433–40.
- Akyel A, Celik IE, Oksüz F, Cay S, Karadeniz M, Kurtul A, et al. Red blood cell distribution width in saphenous vein graft disease. *Can J Cardiol* 2013;29:448–51.
- Yarlioglu M, Kurtul A. Association of red cell distribution width with noninfarct-related artery-chronic total occlusion in acute myocardial infarction patients. *Biomark Med* 2017;11:255–63.
- Kim J, Kim YD, Song TJ, Park JH, Lee HS, Nam CM, et al. Red blood cell distribution width is associated with poor clinical outcome in acute cerebral infarction. *Thromb Haemost* 2012;108:349–56.
- Söderholm M, Borné Y, Hedblad B, Persson M, Engström G. Red cell distribution width in relation to incidence of stroke and carotid atherosclerosis: a population-based cohort study. *PLoS One* 2015;10:e0124957.
- Cumurcu T, Ozyurt H, Demir HD, Yardim H. Serum alpha-1-antitrypsin levels in patients with pseudoexfoliative syndrome. *Curr Eye Res* 2008;33:159–62.
- Yildirim Z, Yildirim F, Uçgun NI, Sepici-Dinçel A. The role of the cytokines in the pathogenesis of pseudoexfoliation syndrome. *Int J Ophthalmol* 2013;6:50–3.

20. Sorkhabi R, Ghorbanihaghjo A, Ahoor M, Nahaei M, Rashtchizadeh N. High-sensitivity C-reactive Protein and Tumor Necrosis Factor Alpha in Pseudoexfoliation Syndrome. *Oman Med J* 2013;28:16–9.
21. Ozgonul C, Sertoglu E, Mumcuoglu T, et al. Prediction of Pseudoexfoliation Syndrome and Pseudoexfoliation Glaucoma by Using Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio. *Ocul Immunol Inflamm* 2016;24:665–70.
22. Türkyilmaz K, Öner V, Kirbas A, Sevim MS, Sekeryapan B, Özgür G, et al. Serum YKL-40 levels as a novel marker of inflammation and endothelial dysfunction in patients with pseudoexfoliation syndrome. *Eye (Lond)* 2013;27:854–9.
23. Carraro MC, Rossetti L, Gerli GC. Prevalence of retinopathy in patients with anemia or thrombocytopenia. *Eur J Haematol* 2001;67:238–44.
24. Kurtul BE, Kabatas EU, Boybeyi SD, Caglar AA, Ozer PA. Increased red cell distribution width levels in children with seasonal allergic conjunctivitis. *Int Ophthalmol* 2018;38:1079–84.
25. Thorleifsson G, Magnusson KP, Sulem P, Walters GB, Gudbjartsson DF, Stefansson H, et al. Common sequence variants in the LOXLI gene confer susceptibility to exfoliation glaucoma. *Science* 2007;317:1397–400.
26. Zenkel M, Lewczuk P, Jünemann A, Kruse FE, Naumann GO, Schlotzer-Schrehardt U. Proinflammatory cytokines are involved in the initiation of the abnormal matrix process in pseudoexfoliation syndrome/glaucoma. *Am J Pathol* 2010;176:2868–79.
27. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, et al; CHARM Investigators. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol* 2007;50:40–7.
28. Zhao Z, Liu T, Li J, Yang W, Liu E, Li G. Elevated red cell distribution width level is associated with oxidative stress and inflammation in a canine model of rapid atrial pacing. *Int J Cardiol* 2014;174:174–6.
29. Friedman JS, Lopez MF, Fleming MD, Rivera A, Martin FM, Welsh ML, et al. SOD2-deficiency anemia: protein oxidation and altered protein expression reveal targets of damage, stress response, and antioxidant responsiveness. *Blood* 2004;104:2565–73.