J Clin Periodontol 2017; 44: 150–157 doi: 10.1111/jcpe.12657

Journal of Clinical Periodontology

The role of platelets in inflammatory immune responses in generalized aggressive periodontitis

Yalin Zhan[1], Ruifang Lu[1], Huanxin Meng[1], Xian'e Wang[1], Xiaojun Sun[2] and Jianxia Hou[1]

[1]Department of Periodontology, Peking University School and Hospital of Stomatology & National Engineering Laboratory for Digital and Material Technology of Stomatology & Beijing Key Laboratory of Digital Stomatology, Beijing, China; [2]Department of Stomatology, The First Hospital of Shanxi Medical University, Taiyuan, Shanxi, China

Zhan Y, Lu R, Meng H, Wang X, Sun X, Hou J. The role of platelets in inflammatory immune responses in generalized aggressive periodontitis. J Clin Periodontol 2017; 44: 150–157. doi: 10.1111/jcpe.12657.

Abstract

Aim: To investigate the relationship between inflammatory markers and platelet size in generalized aggressive periodontitis (GAgP).

Material and Methods: Periodontal, inflammatory and platelet indices were compared between 59 GAgP patients and 59 healthy subjects. Gingival biopsies from five patients and five healthy subjects were examined by immunohistochemistry and electron microscopy. Changes in patient periodontal and platelet indices were re-evaluated at 3 months after periodontal therapy.

Results: Platelet size was decreased significantly in GAgP patients compared to healthy subjects ($p \leq 0.003$). Weak negative correlations between platelet size and periodontal parameters were found in GAgP patients ($p \leq 0.025$). Platelet aggregates and adhesion to the endothelium or leucocytes were found in venules and connective tissues of gingival biopsies from GAgP patients. Mean platelet volume (MPV) and platelet large cell ratio increased after periodontal therapy in GAgP patients ($p \leq 0.038$). The increase in MPV was related to the decrease in bleeding index in GAgP patients after periodontal therapy ($p < 0.001$; $r = 0.357$).

Conclusion: Platelet size was reduced in GAgP patients compared to healthy controls, possibly due to the consumption of large platelets at sites of periodontal inflammation. Platelets may be involved in host responses to periodontal infection in GAgP.

Key words: generalized aggressive periodontitis; inflammation; mean platelet volume; platelet large cell ratio

Accepted for publication 18 November 2016

Platelets are small cell fragments that circulate in the bloodstream. Emerging evidence suggests that platelets may be an important component of the immune system (Weyrich & Zimmerman 2004, Li et al. 2013). In addition to their well-known roles in haemostasis and thrombosis, platelets participate in a wide variety of processes involving tissue injury, immune responses and repair that underlie diverse diseases, such as atherosclerosis, autoimmune disorders, inflammatory lung and bowel disorders, host defence responses and sepsis (Zarbock et al. 2006, Vowinkel et al. 2007, Boilard et al. 2010, Linden & Jackson 2010, Smith & Weyrich 2011, Li et al. 2013, Kuo et al. 2015, Thomas & Storey 2015).

Platelet count, platelet large cell ratio (PLCR) and mean platelet volume (MPV) are quantitative measures of the variability in platelet number and size, indicating subclinical platelet function (Vagdatli et al. 2010). Recent studies have suggested MPV as a marker of inflammation,

Conflict of interest and source of funding statement

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

This study was supported by research funds from the National Natural Science Foundations of China (NSFC): 81570980; 81271149; 81300879.

disease activity and the efficacy of anti-inflammatory treatment in several inflammatory disorders including familial mediterranean fever, ankylosing spondylitis, rheumatoid arthritis, ulcerative colitis, active inflammatory bowel disease and synovitis of knee osteoarthritis (Coban & Adanir 2008, Kisacik et al. 2008, Shen et al. 2009, Yüksel et al. 2009, Yazici et al. 2010a,b, Balbaloglu et al. 2014), reflecting the role of platelets as mediators of inflammation and immune responses.

Periodontitis is an inflammatory disease of host responses against bacterial challenges, which leads to the destruction of periodontal tissues and has adverse effects on systemic health (Armitage 1999). Previous work by our group showed that the decrease in MPV was related to the severity of periodontal inflammation in chronic periodontitis (Wang et al. 2015). However, in a previous study of generalized aggressive periodontitis (GAgP), although mean MPV was lower in patients compared to healthy controls, the difference was not statistically significant (Shi et al. 2008). Generalized aggressive periodontitis, a subform of periodontitis, is characterized by rapid and extensive destruction of the periodontium in otherwise healthy young patients (Armitage 1999), who are not suffering from any systemic disease or condition such as diabetes mellitus or coronary heart disease. Because of this apparent difference in chronic and generalized aggressive periodontitis, this study has re-evaluated the possible role of platelets in generalized aggressive periodontitis using a longitudinal, intervention study.

Material and Methods

Ethics statement

The study was approved by the Ethics Committee of Peking University Health Science Center (IRB00001052-08010). Written informed consent was obtained prior to the enrolment of each subject.

Subjects

In total, 59 patients with GAgP were recruited consecutively from the Periodontology Department, Peking University School and Hospital of Stomatology. At baseline, the inclusion criteria for the GAgP group were according to the 1999 International Classification of Periodontal Diseases (Armitage 1999): onset of periodontal disease at <35 years of age; at least 20 functional teeth remaining in the mouth; at least eight teeth with probing depth (PD) >6 mm, at least three of which are not first molars or incisors; and alveolar bone loss.

In total, 59 age-matched healthy subjects were recruited from the staffs and students of the Peking University School and Hospital of Stomatology. Inclusion criteria were teeth with PD ≤3 mm, no site with attachment loss (AL) and no bone loss visible on radiographs.

All subjects were free of systemic disease. Pregnant women, smokers and subjects who had received antibiotics, immunosuppressive drugs or periodontal therapy within the previous 6 months were excluded.

Clinical examination

At baseline, each subject completed a questionnaire. A set of full-mouth periapical radiographs was taken, and a full-mouth clinical periodontal examination was carried out by a calibrated periodontist (XW), including PD and AL, using a William's periodontal probe at six sites (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) of each tooth. To test for bleeding after probing, the probe was carefully and gently introduced into the gingival sulcus. A probing pressure of 0.25 N was applied to assess bleeding after probing. The bleeding index (BI, Mazza et al. 1981) was recorded at 30 seconds after probing on two sides (buccal and lingual) per tooth. Sites with PD >6 mm and AL >5 mm were defined as severe sites (Shi et al. 2008). The percentage of severe sites was calculated.

Blood collection and processing

Fasting venous blood samples were obtained from each subject. A technician who was blinded to case status performed the complete blood cell analysis of blood samples in EDTA-containing tubes in a calibrated Sysmex XS-1,000 automated haematology analyser (Sysmex, Kobe, Japan).

Plasma was separated and stored immediately at −70°C. Plasma levels of interleukin-1β (IL-1β), C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) were measured using enzyme-linked immunosorbent assay kits (IL-1β; R&D Systems, Inc., Minneapolis, MN, USA; CRP; Diagnostic System Laboratories, Inc., Webster, TX, USA; IL-6; R&D Systems, Inc. and TNF-α, Bender Medsystems, Inc., Vienna, Austria), in accordance with the manufacturers' protocols.

Nonsurgical periodontal therapy

After baseline examinations, all patients with GAgP received non-surgical periodontal therapy, including oral hygiene instruction, supragingival scaling and quadrant-based subgingival scaling and root planing under local anaesthesia. Clinical re-evaluation and blood tests were taken at 3 months after non-surgical periodontal therapy. Periodontal parameters were re-evaluated by the same examiner.

Histology and immunohistochemistry of gingival tissues

Gingival tissues were harvested using horizontal incisions made ~2 mm apically to the gingival margin when extracting third molars from five healthy volunteers and when extracting teeth with severe periodontitis from five GAgP patients. Gingival biopsies were fixed in 4% paraformaldehyde and processed in paraffin for routine histology, and sections (5 μm) were stained with haematoxylin and eosin (H&E). The localization of platelets and neutrophils in gingival tissues was detected by immunocytochemistry using a rabbit monoclonal antibody to the platelet-specific marker CD41 (Abcam, Cambridge, UK) and a mouse monoclonal antibody to S100A8/A9 (Abcam), which has been estimated to constitute ~40% of the total cytosolic proteins in neutrophils (Yui et al. 2003), according to the manufacturer's instructions. Briefly, tissue sections were dewaxed with xylene and rehydrated with

descending concentrations of ethanol. Endogenous peroxidase was blocked by treatment with 3% H_2O_2 for 30 min. at room temperature. Microwave antigen retrieval with EDTA antigen retrieval solution (1 mM, pH 9.0) was performed for 5 min. and stopped for 3 min. in triplicate. Sections were incubated with antibodies to CD41 (2 μg/ml; Abcam) and S100A8/A9 (1 μg/ml; Abcam) overnight at 4°C. The primary antibody was replaced with species-specific non-immune IgG for negative controls. The location of platelets and neutrophils was visualized using a Polymer Double Staining Detection Kit (Zhongshan Golden Bridge Biotechnology, Beijing, China). Briefly, after washing with phosphate-buffered saline (PBS, 0.01 M, pH 7.4), sections were incubated with species-specific secondary antibodies labelled with horseradish peroxidase (HRP) and alkaline phosphatase (AP) for 30 min. at room temperature, and DAB for HRP and GBI for AP were used to detect CD41 and S100A8/A9. Sections were finally counterstained with haematoxylin and mounted. Images were captured with a digital microscopic system (Olympus BX51/DP72, Tokyo, Japan). For transmission electron microscopy, gingival tissue samples were fixed in glutaraldehyde (2.5%) and paraformaldehyde (3.3%) in phosphate buffer (pH 7.2). Ultrathin sections were cut, contrast stained with uranyl acetate (50% in acetone) and lead citrate and examined with a JEOL 100-CX transmission electron microscope.

Statistical analysis

Statistical analyses of the data were performed using SPSS 19.0 (SPSS, Chicago, IL, USA). Shapiro–Wilk test and Levene variance homogeneity test were performed to test the normality and variance equality, respectively. Continuous normally distributed data were expressed as mean ± standard deviation (SD), and non-normally distributed data as median (lower to upper quartile). Student's *t*-test and Mann–Whitney *U*-test were used to identify any differences between groups. Gender was analysed using chi-square test. Comparisons of clinical and blood parameters of patients with GAgP between baseline and 3 months after periodontal therapy were performed using paired samples *t*-test or Wilcoxon Signed Ranks test. To account for multiple comparisons, the observed *p*-values were corrected by the Bonferroni correction. Correlation analysis was performed using Pearson or Spearman correlation analysis. A two-tailed *p*-value <0.05 was considered to indicate statistical significance.

Results

In total, 59 GAgP patients and 59 healthy subjects were enrolled in this study, and 47 GAgP patients underwent clinical and haematological re-examinations at 3 months after non-surgical periodontal therapy.

Demographic features and periodontal status of the subjects at baseline

Demographic data and periodontal parameters of patients with GAgP and healthy subjects at baseline are shown in Table 1. There was no significant difference in age, gender or BMI between the groups. High PD, AL, BI and percentage of severe sites in patients with GAgP indicated severe periodontal inflammation.

Comparisons of haematological and inflammatory markers between GAgP patients and healthy subjects at baseline

The percentage and number of neutrophils in GAgP patients were significantly higher than those in healthy subjects ($p \leq 0.002$; Table 2). PLCR and MPV were significantly lower in the GAgP group than in the control group ($p \leq 0.003$;

Table 1. Clinical parameters of the study subjects

	GAgP group (n = 59)	Control group (n = 59)	p-Value
Age (year)	27.90 ± 5.10	26.30 ± 4.50	0.067
Gender (F/M)	34/25	35/24	0.852
BMI	22.37 ± 3.70	21.45 ± 2.17	0.103
Mean PD (mm)	4.95 ± 1.06	1.61 ± 0.20	<0.001*
Mean BI	3.56 (3.33–3.86)	1.14 (1.11–1.21)	<0.001*
Mean AL (mm)	4.97 ± 1.55	0.00 ± 0.00	<0.001*
Severe sites (%)	39.42 ± 19.95	0.00 ± 0.00	<0.001*

BMI, body mass index; PD, pocket depth; BI, bleeding index; AL, attachment loss.
Data are presented as mean ± SD, number of subjects or median (lower-upper quartile). Between-group comparisons were performed using *t*-test, chi-square test or Mann–Whitney *U*-test.
*Significant after Bonferroni correction.

Table 2. Haematological characteristics of the study subjects

	GAgP group (n = 59)	Control group (n = 59)	p-Value
WBC ($\times 10^9$/l)	6.04 ± 1.92	5.52 ± 1.07	0.073
NEUT%	64.60 ± 8.28	57.87 ± 7.40	<0.001*
LYM%	29.80 ± 8.03	35.10 ± 7.01	<0.001*
NEUT ($\times 10^9$/l)	4.01 ± 1.71	3.22 ± 0.85	0.002*
LYM ($\times 10^9$/l)	1.72 ± 0.44	1.91 ± 0.45	0.018
PLT ($\times 10^9$/l)	212.83 ± 48.36	232.83 ± 51.48	0.032
PLCR	0.18 (0.14–0.22)	0.22 (0.17–0.30)	0.001*
MPV (fl)	8.90 (8.40–9.50)	9.40 (8.70–10.50)	0.003*
IL-1β Conc. in plasma (pg/ml)	7.21 (4.46–11.42)	2.79 (1.72–7.25)	<0.001*
IL-6 Conc. in plasma (pg/ml)	1.48 (0.27–3.46)	0.08 (0.04–0.67)	<0.001*
CRP Conc. in plasma (mg/l)	1.92 (0.56–5.02)	0.46 (0.24–1.08)	<0.001*
TNF-α Conc. in plasma (pg/ml)	0.85 (0.30–1.99)	0.57 (0.18–2.14)	0.303

WBC, white blood cell; NEUT%, percentage of neutrophil granulocyte; LYM%, percentage of lymphocyte; NEUT, number of neutrophil granulocyte; LYM, number of lymphocyte; PLT, number of platelet; PLCR, platelet large cell ratio; MPV, mean platelet volume; IL-1β, interleukin-1β; IL-6, interleukin-6; CRP, C-reactive protein; TNF-α, tumour necrosis factor-alpha; Conc., concentration.
Data are presented as mean ± SD or median (lower-upper quartile). Between-group comparisons were performed using *t*-test or Mann–Whitney *U*-test.
*Significant after Bonferroni correction.

Table 2). Patients with GAgP showed accompanying systemic inflammation, with elevated levels of inflammatory markers. Plasma levels of IL-1β, IL-6 and CRP were elevated significantly in GAgP patients compared to healthy subjects ($p < 0.001$; Table 2).

Correlations between platelet indices and periodontal parameters in GAgP patients at baseline

Platelet large cell ratio and MPV showed significant, weak correlations with periodontal parameters (mean PD, BI and AL) in GAgP patients ($p \leq 0.025$; Table 3).

Changes in clinical and haematological parameters after non-surgical periodontal therapy in GAgP patients

Inflammatory conditions were controlled at 3 months post-therapy in GAgP patients, as manifested by significant reductions in PD, BI, percentage of severe sites and percentage of neutrophils ($p \leq 0.001$; Table 4). Moreover, MPV and PLCR in GAgP patients increased significantly at 3 months after periodontal therapy compared with baseline ($p \leq 0.038$; Table 4). After periodontal therapy, PLCR and MPV in patients increased to values not different from those for healthy subjects ($p = 0.537$ and 0.474, respectively; Tables 2 and 4).

Partial correlation between changes in platelet indices and periodontal parameters after periodontal therapy

After controlling for age, gender and BMI as potential confounders, stepwise multiple regression analysis demonstrated a weak partial correlation between the change in BI and the change in MPV in GAgP patients after periodontal therapy ($r = 0.357$, $p < 0.001$; Fig. 1).

Platelet localization in inflammatory gingival tissues of patients with GAgP

Aggregates of CD41-positive platelets were observed in inflamed gingivae (Fig. 2c–f). Platelets were adhered to the vessel wall, and neutrophils were attached to the adhered platelets and vessel wall (Fig. 2d). The distribution of the platelet aggregates in connective tissue was consistent with the area of tissue inflammation and the extent of inflammatory cell infiltration (Fig. 2e,f). Some of these platelets were co-localized with, and attached to, leucocytes, including neutrophils (Fig. 2d,f). All gingival specimens from GAgP patients showed similar histological appearance. Few or no platelets or neutrophils were found in control healthy gingival tissue (Fig. 2a,b). Large platelets, manifested as extension and formation of protrusions, were confirmed in inflamed gingivae of GAgP patients by transmission electron microscopy. Extended platelets were aggregated in microvascular vessels (Fig. 3a) and were attached to undamaged endothelium and leucocyte (Fig. 3b).

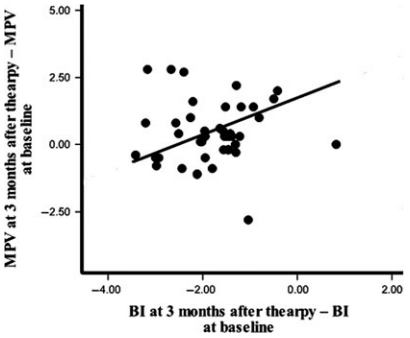

Fig. 1. Partial correlation between the change in mean platelet volume (MPV) and the change in bleeding index (BI) at 3 months after non-surgical periodontal therapy in GAgP patients ($n = 47$, $r = 0.357$, $p < 0.001$).

Table 3. The relationships between platelet indices and periodontal parameters in GAgP group at baseline ($n = 59$)

	PLCR		MPV	
	r	*p*-Value	*r*	*p*-Value
Mean PD	−0.314	0.012	−0.330	0.008
Mean BI	−0.412	0.001	−0.416	0.001
Mean AL	−0.282	0.025	−0.313	0.012

PLCR, platelet large cell ratio; MPV, mean platelet volume; PD, pocket depth; BI, bleeding index; AL, attachment loss.
Correlation analysis was performed using Pearson or Spearman correlation analysis.

Table 4. Changes in periodontal and haematological parameters at 3 months after non-surgical periodontal therapy of GAgP group

	Baseline ($n = 47$)	After therapy ($n = 47$)	*p*-Value
Mean PD (mm)	5.16 ± 0.93	3.15 ± 0.56	<0.001*
Mean BI	3.72 ± 0.37	2.11 ± 1.28	<0.001*
Severe sites (%)	38.27 ± 20.53	3.41 ± 4.23	<0.001*
NEUT %	63.27 ± 8.40	57.77 ± 8.57	0.001*
PLCR	0.19 (0.16–0.26)	0.25 (0.17–0.31)	0.038
MPV (fl)	9.00 (8.50–9.75)	9.70 (8.65–10.80)	0.009*

PD, pocket depth; BI, bleeding index; NEUT%, percentage of neutrophil granulocyte; PLCR, platelet large cell ratio; MPV, mean platelet volume.
Data are presented as mean ± SD or median (lower-upper quartile). Comparisons were performed using the paired-samples *t*-test or Wilcocon Signed Ranks test.
*Significant after Bonferroni correction.

Discussion

In this study, low MPV and PLCR were found in GAgP patients. A possible explanation was the consumption of reactive large platelets at sites of periodontal inflammation. The increase in MPV in response to non-surgical periodontal therapy was related to the control of periodontal inflammation in patients with GAgP.

The role of platelets in various inflammatory disorders has been reported and laboratory markers of platelet function, such as MPV, have been investigated in several inflammatory disorders including familial mediterranean fever, ankylosing spondylitis, rheumatoid arthritis, ulcerative colitis, active inflammatory bowel disease and synovitis of knee osteoarthritis (Coban & Adanir 2008, Kisacik et al. 2008, Shen et al. 2009, Yüksel et al. 2009, Yazici

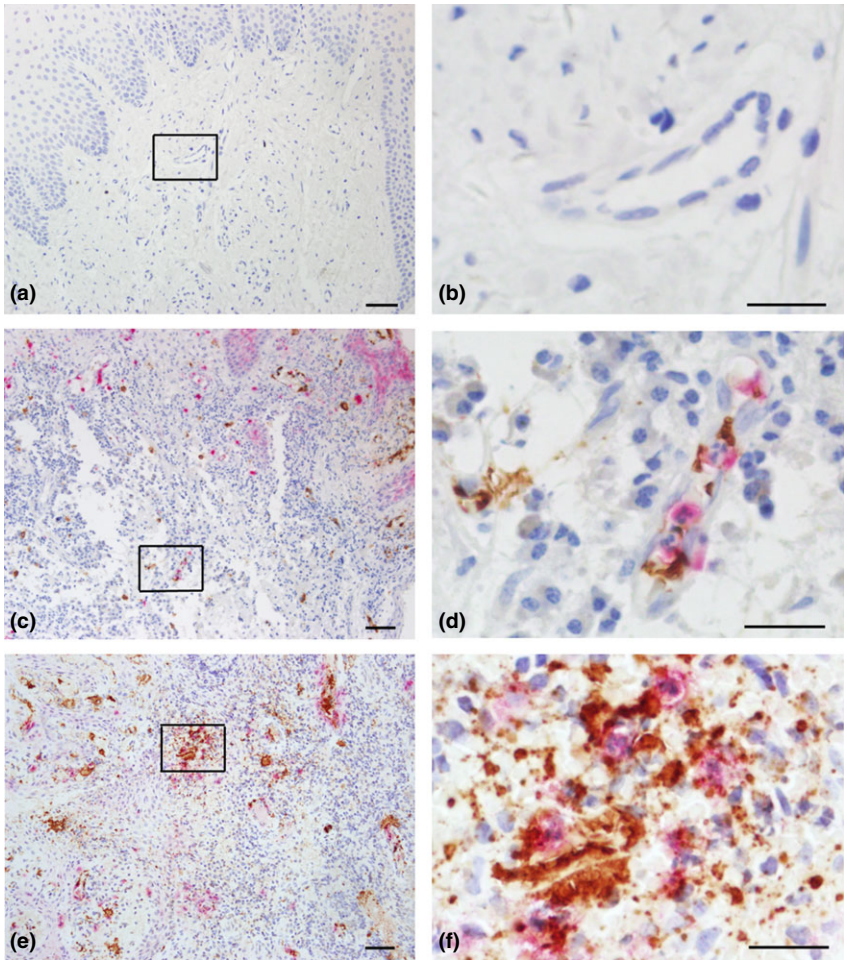

Fig. 2. Histological and immunohistochemical appearance of gingival tissue samples from GAgP patients and healthy subjects. (a, b) Histological images of almost no platelets (CD41, brown) or neutrophils (S100A8/A9, red) in healthy gingival tissue. (c, d) Representative histological images of platelet (CD41, brown) aggregates in inflamed gingival tissue and platelet adhesion to the vessel wall and neutrophils (S100A8/A9, red) in the vessels. (e, f) Platelet (CD41, brown) aggregates in connective tissue were co-localized with inflammatory cell infiltration, with some of the platelets adhering to neutrophils (S100A8/A9, red). The boxes in a, c and e highlight the areas imaged at higher magnifications in b, d and f, respectively. Scale bars, 50 μm (a, c and e) and 20 μm (b, d and f).

et al. 2010a,b, Balbaloglu et al. 2014). The results of this study showed that periodontal inflammation was associated with low MPV and PLCR in GAgP patients and were consistent with data from other inflammatory diseases (Kapsoritakis et al. 2001, Kisacik et al. 2008, Shen et al. 2009, Yüksel et al. 2009) and our previous finding in severe chronic periodontitis (Wang et al. 2015).

In a previous cross-sectional study of patients with aggressive periodontitis, MPV was also decreased in patients compared with healthy subjects (Shi et al. 2008) but the difference did not reach statistical significance. It is difficult to explain why MPV was not significantly reduced in the previously published study of 150 patients, especially as there were no apparent differences in the overall clinical periodontal measures of disease between the patient cohorts studied. However, in this study, decreases in PLCR and MPV correlated with the severity of periodontal inflammation in GAgP patients. Furthermore, a reverse shift of MPV and PLCR to values similar to those of healthy subjects was found after clinically verified, successful periodontal therapy, suggesting that platelet size may be an indicator of inflammation and the efficacy of treatment in GAgP patients. The post-therapy increase in MPV was related to the decrease in BI, further indicating a connection between platelet size and the intensity and activity of periodontal inflammation in GAgP.

Consistent with data derived from studies of other inflammatory disorders, MPV could be recognized as a marker of inflammation, disease activity and an indication of the efficacy of non-surgical treatment in GAgP. However, the size of platelets in the circulating blood is dependent on the intensity of inflammation, with contrasting MPV features in high- and low-grade inflammatory disorders (Kapsoritakis et al. 2001, Coban & Adanir 2008, Kisacik et al. 2008, Shen et al. 2009, Yüksel et al. 2009, Yazici et al. 2010a,b). In addition to the clinical evidence of periodontal inflammation (PD, BI and AL), patients with GAgP had significantly higher number and percentage of neutrophils and plasma levels of IL-1β, IL-6 and CRP compared to healthy subjects, indicating high inflammatory status in GAgP patients. The finding that inflammation accompanied the decrease in MPV and PLCR in GAgP is similar to data obtained from studies of other high-grade inflammatory diseases (Kapsoritakis et al. 2001, Kisacik et al. 2008, Shen et al. 2009, Yüksel et al. 2009). The cause of the reduced platelet size in the circulation of GAgP patients remains unknown, but it may be a consequence of inflammation, similar to what has been suggested in other inflammatory diseases, such as rheumatoid arthritis (Kisacik et al. 2008), ulcerative colitis (Yüksel et al. 2009) and ankylosing spondyloarthritis (Kisacik et al. 2008), in which low MPV may be related to the increased consumption of large activated platelets at sites of inflammation. Consumption of platelets in inflamed gingivae was inferred from histopathological observations revealing platelet aggregates and platelet adhesion to the endothelium or leucocytes in gingival biopsies of patients with GAgP. Platelets localized in inflamed gingivae were large platelets, as evidenced by their morphology on electron microscopy.

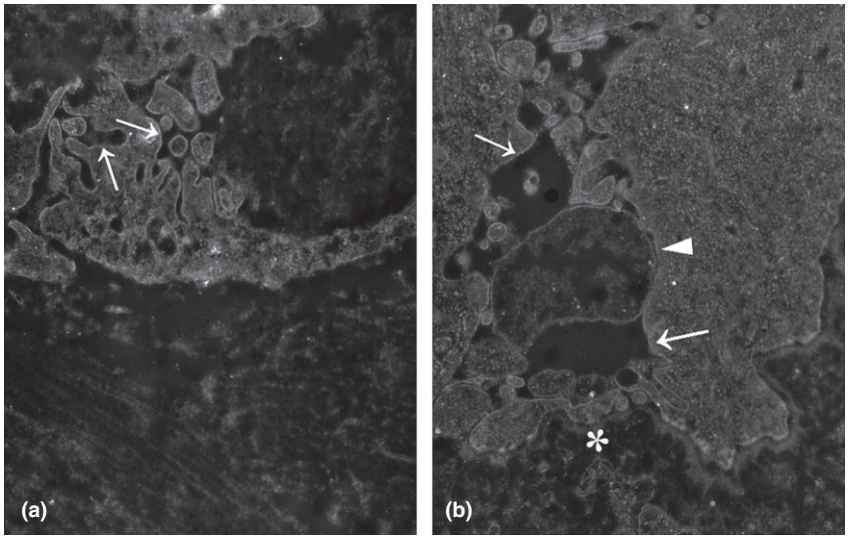

Fig. 3. Ultrastructure of platelets in gingival tissues of GAgP patients. (a) Protrusions extend from platelets (arrows), many of which contacted the endothelium. (b) Extended platelets (arrows) attached directly to the endothelium (asterisk) and to a leucocyte (solid arrowhead). Original magnification: ×1100 (a) and ×3000 (b).

Large platelets manifested as extension and formation of protrusions on electron microscopy and were directly attached to undamaged endothelium and leucocytes in venules. The decrease in MPV and PLCR in the circulation of GAgP patients may be due to the movement of large platelets to inflamed gingivae, where they were consumed.

It is increasingly recognized that platelets, as specialized inflammatory cells, participate in different aspects of the inflammatory immune response, including interactions with endothelium and leucocytes and the release of inflammatory mediators (Zarbock et al. 2006, Vowinkel et al. 2007, Linden & Jackson 2010, Smith & Weyrich 2011, Li et al. 2013, Thomas & Storey 2015). Membrane-expressed CD62P or CD40L on platelets engages its receptor on endothelial cells, resulting in endothelial cell expression of adhesion molecules and the release of inflammatory cytokines for leucocyte chemotaxis and adhesion (Gawaz et al. 2000, Hausding et al. 2013, Ed Rainger et al. 2015, Ghasemzadeh & Hosseini 2015, Gerdes et al. 2016), which is a key step in the activation of endothelial cells and the recruitment of leucocytes to inflammatory sites (Ridley et al. 2003, Phillipson & Kubes 2011, Pitchford et al. 2016). In this study, platelets adhering to the endothelium suggested the interaction between platelets and endothelial cells, which may be an initiating step in periodontal inflammation, resulting in the recruitment of leucocytes to periodontal tissue. Leucocytes have been recognized as major participants in periodontal inflammatory processes. The interaction between platelets and leucocytes is a means for the delivery of molecular signals and a critical step in the activation and recruitment of leucocytes to inflammatory sites for immunopathological responses (Pitchford et al. 2004, 2005, Zarbock et al. 2006, Clark et al. 2007, Vowinkel et al. 2007, Ghasemzadeh & Hosseini 2013, Sreeramkumar et al. 2014, Ed Rainger et al. 2015). In this study, platelets adhering to the surfaces of leucocytes (including neutrophils) were observed in venules, and some of platelets in the extravascular tissues were also attached to leucocytes. The interactions of platelets and leucocytes may be another step in the recruitment of leucocytes to periodontal tissues in GAgP. In addition, platelet aggregates and platelet–leucocyte aggregates contribute to the synthesis and secretion of proinflammatory cytokines that have a known role in inflammation and tissue injury (Thachil 2015, DiVito et al. 2016). Antiplatelet drugs have showed the ability to attenuate inflammation and prevent bone loss in experimental periodontitis (Coimbra et al. 2011, 2014). Observations in this study suggest that platelets may participate in inflammatory immune responses in GAgP. However, the role of platelets in host responses to periodontal infection remains largely unclear and provides an area for future studies.

In conclusion, decreased MPV and PLCR are associated with periodontal inflammation in GAgP patients. Furthermore, MPV and PLCR increased to values similar to those of healthy control subjects after successful periodontal therapy. The detection of platelet–endothelium and platelet–leucocyte interactions in inflamed gingivae suggests a role of platelets in inflammatory immune responses in GAgP patients. A better understanding of the role of platelets in host responses to periodontal infection may lead to novel approaches for the treatment of periodontitis.

Acknowledgements

We thank all enrolled subjects for their participation, support and willingness to discuss their health problems, which are essential for the quality of data.

References

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Balbaloglu, O., Korkmaz, M., Yolcu, S., Karaaslan, F. & Beceren, N. G. (2014) Evaluation of mean platelet volume (MPV) levels in patients with synovitis associated with knee osteoarthritis. *Platelets* **25**, 81–85.

Boilard, E., Nigrovic, P. A., Larabee, K., Watts, G. F., Coblyn, J. S., Weinblatt, M. E., Massarotti, E. M., Remold-O'Donnell, E., Farndale, R. W., Ware, J. & Lee, D. M. (2010) Platelets amplify inflammation in arthritis *via* collagen-dependent microparticle production. *Science* **327**, 580–583.

Clark, S. R., Ma, A. C., Tavener, S. A., McDonald, B., Goodarzi, Z., Kelly, M. M., Patel, K. D., Chakrabarti, S., McAvoy, E., Sinclair, G. D., Keys, E. M., Allen-Vercoe, E., Devinney, R., Doig, C. J., Green, F. H. & Kubes, P. (2007) Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature Medicine* **13**, 463–469.

Coban, E. & Adanir, H. (2008) Platelet activation in patients with familial mediterranean fever. *Platelets* **19**, 405–408.

Coimbra, L. S., Rossa, C. Jr, Guimarães, M. R., Gerlach, R. F., Muscará, M. N., Spolidorio, D. M., Herrera, B. S. & Spolidorio, L. C. (2011) Influence of antiplatelet drugs in the pathogenesis of experimental periodontitis and periodontal repair in rats. *Journal of Periodontology* **82**, 767–777.

Coimbra, L. S., Steffens, J. P., Muscará, M. N., Rossa, C. Jr & Spolidorio, L. C. (2014) Antiplatelet drugs reduce the immunoinflammatory response in a rat model of periodontal disease. *Journal of Periodontal Research* **49**, 729–735.

DiVito, C., Hadi, L. A., Navone, S. E., Marfia, G., Campanella, R., Mancuso, M. E. & Riboni, L. (2016) Platelet-derived sphingosine-1-phosphate and inflammation: from basic mechanisms to clinical implications. *Platelets* **7**, 1–9.

Ed Rainger, G., Chimen, M., Harrison, M. J., Yates, C. M., Harrison, P., Watson, S. P., Lordkipanidzé, M. & Nash, G. B. (2015) The role of platelets in the recruitment of leukocytes during vascular disease. *Platelets* **26**, 507–520.

Gawaz, M., Brand, K., Dickfeld, T., Pogatsa-Murray, G., Page, S., Bogner, C., Koch, W., Schömig, A. & Neumann, F. (2000) Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* **148**, 75–85.

Gerdes, N., Seijkens, T., Lievens, D., Kuijpers, M. J., Winkels, H., Projahn, D., Hartwig, H., Beckers, L., Megens, R. T., Boon, L., Noelle, R. J., Soehnlein, O., Heemskerk, J. W., Weber, C. & Lutgens, E. (2016) Platelet CD40 exacerbates atherosclerosis by transcellular activation of endothelial cells and leukocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology* **36**, 482–490.

Ghasemzadeh, M. & Hosseini, E. (2013) Platelet-leukocyte crosstalk: linking proinflammatory responses to procoagulant state. *Thrombosis Research* **131**, 191–197.

Ghasemzadeh, M. & Hosseini, E. (2015) Intravascular leukocyte migration through platelet thrombi: directing leukocytes to sites of vascular injury. *Thrombosis and Haemostasis* **113**, 1224–1235.

Hausding, M., Jurk, K., Daub, S., Kröller-Schön, S., Stein, J., Schwenk, M., Oelze, M., Mikhed, Y., Kerahrodi, J. G., Kossmann, S., Jansen, T., Schulz, E., Wenzel, P., Reske-Kunz, A. B., Becker, C., Münzel, T., Grabbe, S. & Daiber, A. (2013) CD40L contributes to angiotensin II-induced pro-thrombotic state, vascular inflammation, oxidative stress and endothelial dysfunction. *Basic Research in Cardiology* **108**, 386.

Kapsoritakis, A. N., Koukourakis, M. I., Sfiridaki, A., Potamianos, S. P., Kosmadaki, M. G., Koutroubakis, I. E. & Kouroumalis, E. A. (2001) Mean platelet volume: a useful marker of inflammatory bowel disease activity. *American Journal of Gastroenterology* **96**, 776–781.

Kisacik, B., Tufan, A., Kalyoncu, U., Karadag, O., Akdogan, A., Ozturk, M. A., Kiraz, S., Ertenli, I. & Calguneri, M. (2008) Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. *Joint, Bone, Spine* **75**, 291–294.

Kuo, H. H., Fan, R., Dvorina, N., Chiesa-Vottero, A. & Baldwin, W. M. 3rd (2015) Platelets in early antibody-mediated rejection of renal transplants. *Journal of the American Society of Nephrology* **26**, 855–863.

Li, Z., Yang, F., Dunn, S., Gross, A. K. & Smyth, S. S. (2013) Platelets as immune mediators: their role in host defense responses and sepsis. *Thrombosis Research* **127**, 184–188.

Linden, M. D. & Jackson, D. E. (2010) Platelets: pleiotropic roles in atherogenesis and atherothrombosis. *International Journal of Biochemistry & Cell Biology* **42**, 1762–1766.

Mazza, J. E., Newman, M. G. & Sims, T. N. (1981) Clinical and antimicrobial effect of stannous fluoride on periodontitis. *Journal of Clinical Periodontology* **8**, 203–212.

Phillipson, M. & Kubes, P. (2011) The neutrophil in vascular inflammation. *Nature Medicine* **17**, 1381–1390.

Pitchford, S. C., Momi, S., Giannini, S., Casali, L., Spina, D., Page, C. P. & Gresele, P. (2005) Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* **105**, 2074–2081.

Pitchford, S. C., Riffo-Vasquez, Y., Sousa, A., Momi, S., Gresele, P., Spina, D., Page, C. P. (2004) Platelets are necessary for airway wall remodeling in a murine model of chronic allergic inflammation. *Blood* **103**, 639–647.

Pitchford, S., Pan, D. & Welch, H. C. (2017) Platelets in neutrophil recruitment to sites of inflammation. *Current Opinion in Hematology* **24**, 23–31.

Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T. & Horwitz, A. R. (2003) Cell migration: integrating signals from front to back. *Science* **302**, 1704–1709.

Shen, J., Ran, Z. H., Zhang, Y., Cai, Q., Yin, H. M., Zhou, X. T. & Xiao, S. D. (2009) Biomarkers of altered coagulation and fibrinolysis as measures of disease activity in active inflammatory bowel disease: a gender-stratified, cohort analysis. *Thrombosis Research* **123**, 604–611.

Shi, D., Meng, H. X., Xu, L., Zhang, L., Chen, Z. B., Feng, X. H., Lu, R. F., Sun, X. J. & Ren, X. Y. (2008) Systemic inflammation markers in patients with aggressive periodontitis: a pilot study. *Journal of Periodontology* **79**, 2340–2346.

Smith, T. L. & Weyrich, A. S. (2011) Platelets as central mediators of systemic inflammatory responses. *Thrombosis Research* **127**, 391–394.

Sreeramkumar, V., Adrover, J. M., Ballesteros, I., Cuartero, M. I., Rossaint, J., Bilbao, I., Nácher, M., Pitaval, C., Radovanovic, I., Fukui, Y., McEver, R. P., Filippi, M. D., Lizasoain, I., Ruiz-Cabello, J., Zarbock, A., Moro, M. A. & Hidalgo, A. (2014) Neutrophils scan for activated platelets to initiate inflammation. *Science* **346**, 1234–1238.

Thachil, J. (2015) Platelets in inflammatory disorders: a pathophysiological and clinical perspective. *Seminars in Thrombosis and Hemostasis* **41**, 572–581.

Thomas, M. R. & Storey, R. F. (2015) The role of platelets in inflammation. *Thrombosis and Haemostasis* **114**, 449–458.

Vagdatli, E., Gounari, E., Lazaridou, E., Katsibourlia, E., Tsikopoulou, F. & Labrianou, I. (2010) Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia* **14**, 28–32.

Vowinkel, T., Wood, K. C., Stokes, K. Y., Russell, J., Tailor, A., Anthoni, C., Senninger, N., Krieglstein, C. F. & Granger, D. N. (2007) Mechanisms of platelet and leukocyte recruitment in experimental colitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology* **293**, G1054–G1060.

Wang, X. E., Meng, H. X., Xu, L., Chen, Z., Shi, D. & Lv, D. (2015) Mean platelet volume as an inflammatory marker in patients with severe periodontitis. *Platelets* **26**, 67–71.

Weyrich, A. S. & Zimmerman, G. A. (2004) Platelets: signaling cells in the immune continuum. *Trends in immunology* **25**, 489–495.

Yazici, S., Yazici, M., Erer, B., Calik, Y., Bulur, S., Ozhan, H. & Ataoglu, S. (2010b) The platelet functions in patients with ankylosing spondylitis: anti-TNF-alpha therapy decreases the mean platelet volume and platelet mass. *Platelets* **21**, 126–131.

Yazici, S., Yazici, M., Erer, B., Calik, Y., Ozhan, H. & Ataoglu, S. (2010a) The platelet indices in patients with rheumatoid arthritis: mean platelet volume reflects disease activity. *Platelets* **21**, 122–125.

Yui, S., Nakatani, Y. & Mikami, M. (2003) Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. *Biological and Pharmaceutical Bulletin* **26**, 753–760.

Yüksel, O., Helvaci, K., Başar, O., Köklü, S., Caner, S., Helvaci, N., Abayli, E. & Altiparmak, E. (2009) An overlooked indicator of disease activity in ulcerative colitis: mean platelet volume. *Platelets* **20**, 277–281.

Zarbock, A., Singbartl, K. & Ley, K. (2006) Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *The Journal of Clinical Investigation* **116**, 3211–3219.

Address:
Huanxin Meng
Department of Periodontology
Peking University School and Hospital of Stomatology
22 Zhongguancun South Avenue
Haidian District
Beijing 100081
China
E-mail: kqhxmeng@bjmu.edu.cn

Clinical Relevance

Scientific rationale for the study: Evidence suggests the role of platelets as initiators and mediators of inflammatory responses in various inflammatory diseases. However, the role of platelets in generalized aggressive periodontitis (GAgP) remains unclear. Understanding the role of platelets in host responses to periodontal infection may lead to novel therapeutic approaches for periodontitis.

Principal findings: Relatively low mean platelet volume (MPV) and platelet large cell ratio were found in GAgP patients. A reverse shift

of MPV was found after the control of inflammation by periodontal therapy. The recruitment of platelets and the adhesion of platelets to endothelium and leucocytes were observed in inflamed gingivae. A possible explanation for low MPV in GAgP patients was the consumption of large platelets at sites of periodontal inflammation.

Practical implications: MPV may be an indicator of periodontal inflammation, disease activity and the efficacy of treatment in patients with GAgP. Platelets may participate in host responses to periodontal infection. These findings suggest the potential of antiplatelet drugs in the treatment of GAgP.