Platelet-rich plasma (PRP) a biogenic stimulator in treatment of primary atrophic rhinitis

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Abstract

Background: Primary atrophic rhinitis (1ry AR) is a chronic nasal disease characterized by the loss of mucociliary clearance and presence of viscid secretions and dried crusts that causes a characteristic foul odor, usually bilateral. A large range of treatment modalities had been tried, yet there is still no agreement upon a curative treatment with long-lasting success. The purpose of the study was to assess the value of platelet-rich plasma as a biogenic stimulator for healing acceleration in primary atrophic rhinitis.

A total of 78 cases clinically diagnosed to have primary atrophic rhinitis were included. Nasal endoscopy, Sino-Nasal Outcome Test-25 questionnaire, mucociliary clearance assessment by saccharine transit time test, and biopsy specimens were achieved before 1 month and 6 months after the application of platelet-rich plasma in group A (cases) and platelet-poor plasma in group B (controls).

Results: All patients in group A showed endoscopic improvement and reduction in the incidence of the most frequently encountered symptoms before platelet-rich plasma injection including nasal crusts, 36 (92.30%); foetor, 31 (79.48%); nasal obstruction, 30 (76.92%); anosmia, 17 (43.58%); and epistaxis, 7 (17.94%) to nasal crusts, 9 (23.07%); foetor, 13 (33.33%); nasal obstruction, 14 (35.89%); anosmia, 13 (33.33%); and epistaxis, 3 (7.69%), 6 months after, and this was reflected in the reduction of the Sino-Nasal Outcome Test-25 scores which was averaged 40 before platelet-rich plasma to 9, 6 months after. Similarly, the mucociliary clearance time was significantly reduced after platelet-rich plasma injection; saccharine transit time test was initially averaged at 1980 s and got reduced to 920 s, 6 months after platelet-rich plasma injection.

Conclusion: The use of platelet-rich plasma as a biogenic stimulator is a possible innovative less invasive approach that can be effective in repairing tissue dystrophy through further future studies.

Keywords: Primary atrophic rhinitis, Platelet-rich plasma, Mucociliary clearance, Saccharine transit time test

Background

Primary atrophic rhinitis (1ry AR) is a chronic nasal mucosal disease, characterized by extensive fibrosis leading to atrophic changes of the nasal mucosa and the underlying bone of the nasal turbinates resulting in the presence of viscid secretions and dried crusts that causes a characteristic foul odor, usually bilateral [1]. It is mainly seen in Africa, Asia, Eastern Europe especially Egypt,

India, Saudi Arabia, Philippines, and Poland [2, 3]. Where it can affect 0.3–1.0% of the population with special predominance in teens, and young adults show higher prevalence among females (F:M = 5.6:1) [4] and highly attributed to poverty and poor hygienic practices [5]. The exact etiology of primary atrophic rhinitis is still a matter of dispute; out of the various proposed etiologies, the theories of chronic bacterial rhinosinusitis most commonly caused by *Klebsiella ozaenae* [6], and autoimmunity is the most accepted [3, 7]. A diagnosis of primary atrophic rhinitis is essentially clinical; early stage of

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disease may consist of foetor only, with nasal crustations. During late stages, full picture is usually seen and consists of a clinical trio of foetor, thick greenish crustations, and widened nasal cavities. Other symptoms include anosmia, headache, and epistaxis [1]. The histopathological picture consists of patches of squamotransformation of the normal respiratory epithelium which is pathognomonic for atrophic rhinitis seen in more than 80% of cases [8]. Metaplastic changes ranging from stratified squamous epithelium partial to severe in degree with or without keratinization [9]. Lamina propria and submucosa show chronic cellular infiltration, granulation, fibrosis, and glandular atrophy [10]. Two definite types of vascular changes are characteristic in AR: type I is more common (50–80%), where endarteritis obliterans, periarteritis, and periarterial fibrosis of the terminal arterioles are seen. Type II is less common (20–50%) and is associated with vasodilatation of the capillaries [11]. At the electron microscopic level, the cilia are either atrophied or completely lost affecting the mucociliary clearance property of the respiratory epithelium [12]. A large range of treatment modalities had been tried, e.g., local, systemic (medical), or surgical; however, the most adopted treatment is conservative (e.g., nasal irrigation, washes, drops, e.g., glucose-glycerin, mentholparaffin, antibiotics, and vasodilators) [13]. Many surgical modalities with aims like closure of the nasal cavities, e.g., the Young's operation and its modifications, or reducing the size of the nasal cavities, e.g., implants that may be autologous bone (cortical or cancellous chips), cartilage, muscle, fat; homologous lyophilized bone, fat, human placenta extract; or may be synthetics such as teflon, acrylics, silicone or silastic grafts, and many others, have been tried [14–16].

Platelet-rich plasma (PRP) is a platelet-rich concentrate of plasma proteins developed by dual-speed centrifugation of the whole blood. It has a higher concentration of growth factors and other cytokines [17]. Its numerous advantages include safety being autologous, do not need lengthened or complicated preparation, and the increased delivery of platelets and growth factors in treated areas; it can provide otorhinolaryngologists with a healing promoting packing material of the nose and ear or as a biogenic stimulator with autologous grafting materials. However, there are few published data on the application of platelet-rich plasma: only 11 studies on various subspecialties (otology, rhinology, and laryngology) were revealed in the literature [18].

Since no treatment modality whether alone or combined provided long-lasting cure of 1ry AR as no one addressed the reversal of basic pathologic alteration in the microanatomy [19], we were derived to challenge the value of platelet-rich plasma as a biogenic stimulator for healing acceleration aiming to suggest a new treatment modality that might reverse the atrophic changes of nasal mucosa in primary atrophic rhinitis.

Methods
This study was carried out over the previous 3 years to assess the efficacy of autologous PRP on tissue regeneration in patients with primary atrophic rhinitis. A total of 78 cases clinically diagnosed to have primary atrophic rhinitis were randomly selected into two equal groups (39 patients) each: group A and group B. Patients with history of previous nasal trauma, nasal surgeries, systemic diseases known to cause secondary atrophic rhinitis, platelet-related hematologic disorders, or younger than 16 years old were excluded from the study. The aim of the procedure was clearly explained, and all patients gave their informed written consents.

Nasal endoscopy was done to confirm diagnosis, and clinical signs were recorded in all the cases; subjective scoring using the Sino-Nasal Outcome Test (SNOT-25) questionnaire [20] and mucociliary clearance assessment by saccharine transit time test (STT) according to Rodrigues et al. [21] (Fig. 1) were done before, 1 month and 6 months after the application of treatment.

Platelet-rich plasma (PRP) was prepared by drawing 10 ml of blood sample from the patient. The blood was centrifuged at 3000 rpm for 10 min "soft spin" to separate the blood into red blood cells, platelet-poor plasma (PPP), and a buffy coat. This was again centrifuged at 4000 rpm for 10 min "hard spin" to separate platelet-rich plasma from platelet-poor plasma (Fig. 2). Approximately 1 ml of platelet-rich plasma was obtained from 10 ml blood after degranulating the platelet by adding 0.5 ml of calcium chloride injected using 1 ml insulin syringes into inferior turbinate, middle turbinate, floor, and septal area of both nostrils in group A, and 1 ml of platelet-poor plasma (placebo treatment) was injected in the same manner in group B, and the patients were discharged from the hospital in the same day with oral antibiotics and anti-inflammatory agents for 1 week.

Biopsy specimens were obtained from the undersurface of middle third of inferior turbinate with the help of 0° rigid nasal endoscope under local anesthesia before, 1 month and 6 months after the application of the treatments in both groups. Sections were prepared by routine paraffin processing, and hematoxylin-eosin staining was done, and the preparations were evaluated after being coded using light microscopy (Olympus BX51) by the same pathologist for detection of histopathological changes in the epithelium and submucosa, including epithelial metaplasia, goblet cell loss, cilia loss, inflammatory infiltration, and vascular changes; histopathological analysis was done using previous studies as reference for scoring over 3: 1 = minimal, 2 = moderate, and 3 = severe [22]. Mean, standard deviation, median, and lowest
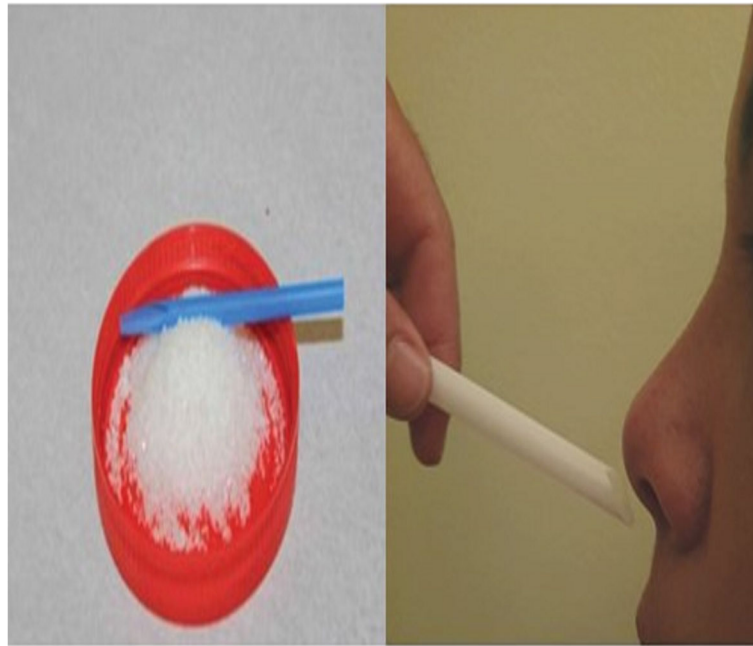
Fig. 1 Saccharine transit time test (STT)

and highest values were used in the descriptive statistics of the data. The distribution of the variables was controlled with Kolmogorov Smirnov test. The Mann-Whitney U test was used for quantitative analysis. The p value ≤ 0.05 was considered the cut-off value for significance. The SPSS 22.0 program was used in analysis.

Results

Through the 78 subjects encountered and completed this study, the maximum incidence was in the third and fourth decades; the mean age was 28 ± 0.45 years (range 17–70 years); 29 cases were males, and 49 were females (M:F ratio was 1:1.68) (Table 1).

The mean duration of symptoms was 6 ± 0.52 years (range 2–54 years). The most common symptoms were nasal crusts, 74 (94.87%); foetor, 71 (91.02%); nasal obstruction, 59 (75.64%); anosmia, 30 (38.46%); and epistaxis, 11 (14.10%). In Group A, the incidence was nasal crusts, 36 (92.30%); foetor, 31 (79.48%); nasal obstruction, 30 (76.92%); anosmia, 17 (43.58%); and epistaxis, 7 (17.94%); all patients felt improvement in symptoms within 1 month of PRP, with nasal crusts, 21 (53.84%); foetor, 25 (64.10%); nasal obstruction, 21 (53.84%); anosmia, 15 (38.46%); and epistaxis, 7 (17.94%). Six months after PRP, there was further improvement in symptoms with nasal crusts, 9 (23.07%); foetor, 13 (33.33%); nasal obstruction, 14 (35.89%); anosmia, 13 (33.33%); and epistaxis, 3 (7.69%), and it was worth noting that those who did not respond well to the PRP treatment were having durations of symptoms longer than 4 years. As regrade group B, there were minimal changes in the results obtained at the start of the study and 6 months after PPP injection where the incidence of the most common symptoms was nasal crusts, 37 (94.87), foetor, 35 (91.02%); nasal obstruction, 29 (75.64%); anosmia, 16 (41.02%); and epistaxis, 5 (14.10%).

None of the patients showed any local or systemic complication in response to the proposed treatment for each group.

Histopathology

Squamous metaplasia was seen in 80.4% of cases. Partial squamous metaplasia, total squamous metaplasia with marked absence of goblet cells, and total squamous metaplasia with keratinization were seen in 42.33%, 33.88%, and 12.66% of cases, respectively, and denuded epithelium in 3.44%. In tunica propria, granulation tissue was noted in 39.22% of cases, chronic inflammatory cell infiltrates mainly lymphocytes in 38.44% of cases and fibrosis in 24.53% of cases. Basement membrane was thickened in 58.30% cases, ill-defined in 32.10%, and normal in 8.10% cases. The changes in the blood vessels were observed as reduced vascularity (56.66%), dilated blood vessels (22.33%), endarteritis (14.33%), and periarteritis (5.44%). Mucosal glands were reduced both in number and size in (58.66%) of cases and were absent in 34.14% of cases (Table 2).

The mean percent of change in epithelial metaplasia in group A was 1.83 ± 0.75 in the initial biopsy (before PRP), 1.63 ± 0.45 (1 month after PRP), and 1.38 ± 0.32
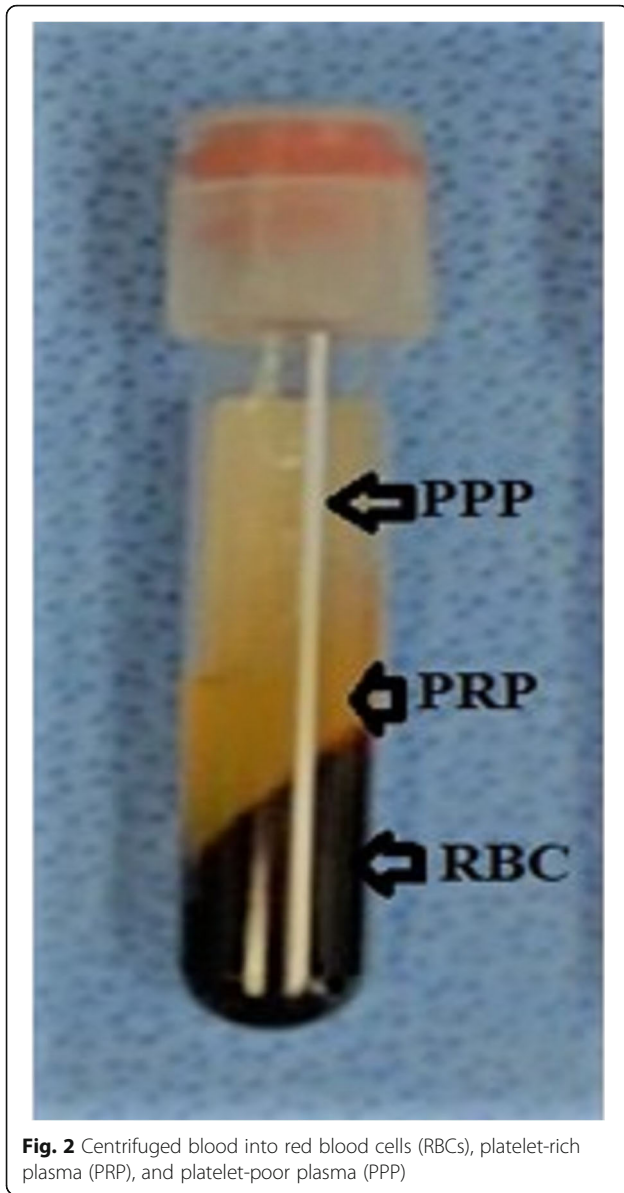
Fig. 2 Centrifuged blood into red blood cells (RBCs), platelet-rich plasma (PRP), and platelet-poor plasma (PPP)

(6 months after PRP). The mean percent of change in epithelial metaplasia in group B was 1.93 ± 0.52 in the initial biopsy (before PPP), 1.91 ± 0.45 (1 month after PPP), and 1.92 ± 0.32 (6 months after PPP) (Fig. 3a).

The mean percent of change in goblet cell loss in group A was 2.19 ± 0.52 in the initial biopsy (before

Table 1 The demographic data for both groups

	Group A	Group B
Mean age ± SD	28.2 ± 4.6	28.4 ± 4.9
M	16	13
F	23	26
Total	39	39

PRP), 1.93 ± 0.45 (1 month after PRP), and 1.83 ± 0.32 (6 months after PRP). The mean percent of change in goblet cell loss in group B was 2.21 ± 0.75 in the initial biopsy (before PPP), 2.25 ± 0.45 (1 month after PPP), and 2.22 ± 0.32 (6 months after PPP) (Fig. 3b).

The mean percent of change in inflammatory infiltration in group A was 2.17 ± 0.75 in the initial biopsy (before PRP), 1.63 ± 0.52 (1 month after PRP), and 1.30 ± 0.55 (6 months after PRP). The mean percent of change in inflammatory infiltration in group B was 2.17 ± 0.45 in the initial biopsy (before PPP), 2.04 ± 0.52 (1 month after PPP), and 2.11 ± 0.32 (6 months after PPP) (Fig. 3c).

The mean percent of change in vascular ectasia in group A was 2.17 ± 0.75 in the initial biopsy (before PRP), 2.11 ± 0.45 (1 month after PRP), and 1.93 ± 0.45 (6 months after PRP). The mean percent of change in vascular ectasia in group B was 1.83 ± 0.75 in the initial biopsy (before PPP), 1.93 ± 0.45 (1 month after PPP), and 2.17 ± 0.32 (6 months after PPP) (Fig. 3d).

No statistically significant difference was found when the incidence in the three biopsies was compared to each other in the same group or when compared to the results obtained in group B ($p > 0.05$).

Sino-nasal outcome test (SNOT-25)
In group A, SNOT-25 scores were averaged 40 initially (before PRP), with improvement to 11 (1 month after PRP) and to 9 (6 months after PRP). Where in group B, SNOT-25 scores were averaged 38 initially (before PPP), 37 (1 month after PPP), and 39 (6 months after PPP). A statistically significant difference was found when the SNOT-25 scores of the same case were compared to each or when compared to the results obtained in group B ($p < 0.05$).

Saccharine transit time test (STT)
In group A, mucociliary clearance time was significantly reduced after PRP injection. STT was initially averaged at 1980 s with improvement to 1060 s after 1 month and 920 s after 6 months of PRP injection. Where in group B, there was no change in the initial average STT (1970 s) after 1 and 6 months of PPP injection. A statistically significant difference was found when the STT of the same case was compared to each or when compared to the results obtained in group B ($p < 0.05$).

Discussion
Atrophic rhinitis was described initially as early as 1876 by Fraenkel [23], although various medical and surgical treatments have been tried yet there is still no agreement upon a curative treatment. Since the clinical picture can be explained by the metaplasia of the normal ciliated columnar epithelium to nonciliated squamous epithelium and loss of mucociliary clearance, then the aim of

Table 2 Histopathological features in group A, before, 1 month and 6 months after PRP treatment

|	Histopathological features	(%) before PRP	(%) 1 month after PRP	(%) 6 months after PRP
A. Status of the epithelium	1. Partial squamous metaplasia	42.33	41.33	39.33
	2. Total squamous metaplasia	33.88	32.66	32.66
	3. Denuded epithelium	3.44	1.88	0.64
	4. Total squamous metaplasia with keratinization	12.66	10.66	10.66
B. Tunica propria	1. Granulation tissue	39.22	38.66	34.66
	2. Chronic inflammatory cellular infiltrate	38.44	34.22	34.88
	3. Fibrosis	24.53	21.22	21.22
	4. Granulation tissue with fibrosis	24.53	23.22	23.22
	5. Chronic inflammatory cellular infiltrate with fibrosis	38.44	35.22	31.88
	6. Basement membrane			
	Thickened	58.30	56.22	56.22
	Ill-defined	32.10	29.88	29.88
	Normal	8.10	15.88	17.22
	1. Absence of glands	23.14	19.27	17.88
C. Mucous glands	2. Reduction in number and size of glands	58.66	49.22	48.62
	3. Normal glands	11.66	16.22	16.22
D. Blood vessels	1. Reduced vascularity	56.66	49.22	40.88
	2. Endarteritis	14.33	13.22	12.28
	3. Periarteritis	5.44	4.66	4.66
	4. Dilated blood vessels	22.33	20.22	18.88
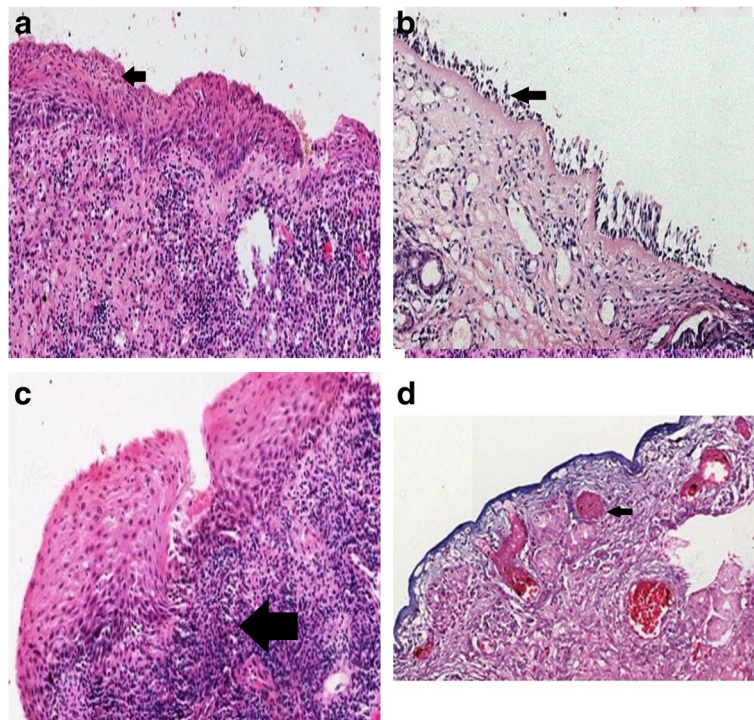
Fig. 3 a Squamous cell metaplasia (arrow); H&E, original, magnification × 200. **b** Erosion of the respiratory epithelium with cilia and goblet cell loss (arrow); H&E, original, magnification × 200. **c** Granulation tissue with abundant inflammatory cells (arrow); H&E, original, magnification × 200. **d** Stromal fibrosis, thick-walled vascular structures with abundant hemorrhage, and end arteritis obliterans (arrow); H&E, original, magnification × 200

the curative treatment with long-lasting success should be the reversal of this basic histopathologic change.

Only one report describing the use of platelet-rich plasma in treatment of atrophic rhinitis was identified. Friji et al. reported 5 patients had an autologous lipoaspirate applied to the inferior, middle turbinate, floor, and septum bilaterally with concomitant injection of platelet-rich plasma into the same areas as a biogenic stimulator to promote adipocytes survival and graft absorption reduction. Six months after the intervention, all 5 patients showed disappearance of nasal crustations and improvement of other symptom; endoscopic examination showed no signs of atrophy with the reappearance of glistening nasal mucosa. Sino-Nasal Outcome Test-20 scores had improved from an average of 36 to an average of 8. In addition, the nasal mucociliary clearance time was significantly shorter after 960 s than before 1995 s intervention [19]. They attributed this to the stimulatory effect of mesenchymal stem cells within the fat graft, yet they could not proof or deny other non-mesenchymal mechanism for their results. The main drawbacks of Friji et al. study are the small number of cases and that the results are not substantiated by histopathological examination [19].

This article aimed at studding the benefit of the platelet-rich plasma as a biogenic stimulator that promotes regeneration to reverse the mucosal dystrophy which is pathognomonic for primary atrophic rhinitis. Although there were few commonalities in the techniques and evaluation methods adopted by Friji et al. study and this study, yet no fat graft was used (to exclude mesenchymal stem cell role), and nasal biopsy histopathologic examination was achieved [19].

All patients showed improvement in the endoscopic picture and felt reduction in the incidence of the most frequently encountered symptoms before PRP, and this was reflected in the SNOT-25 scores which was averaged 40 initially (before PRP) to 9 (6 months after PRP). Similarly, the mucociliary clearance time (evidence of ciliary regeneration) was significantly reduced after PRP injection. STT was initially averaged at 1980 s with improvement to 920 s after 6 months of PRP injection demonstrating mucosal regeneration, coinciding with Friji et al.'s report [19].

The incidence of the histopathological changes in the epithelium and submucosa, including epithelial metaplasia, absence of goblet cell, absence of cilia, inflammatory infiltration, and vascular changes, showed improvement as regards the 1 month and 6 months after PRP biopsies compared to the initial biopsies.

These initial results could not prove the histopathologic alternation towards the mucosal regeneration after the PRP treatment as no statistically significant difference was found when the three biopsies in group A were compared to each other or when compared to the results obtained in group B. Yet the improvement in patient clinical picture by nasal endoscopy, symptoms by SNOT score, and mucociliary clearance by STT can demonstrate it with great similarity to the results obtained through more invasive approaches as the adipocyte-derived stem cells dermofat grafts.

Conclusion

Current managements of atrophic rhinitis are ineffective for long-term cure; in light of this study, the use of PRP as a biogenic stimulator is a possible innovative less invasive approach that can be effective in repairing tissue dystrophy through further future studies.

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