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Review Article

Recurrent aphthous stomatitis – Etiology, serum autoantibodies, anemia, hematinic deficiencies, and management

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Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases characterized by recurrent and painful ulcerations on the movable or nonkeratinized oral mucosae. Clinically, three types of RAS, namely minor, major, and herpetiform types, can be identified. RAS more commonly affects labial mucosa, buccal mucosa, and tongue. Previous studies indicate that RAS is a multifactorial T cell-mediated immune-dysregulated disease. Factors that modify the immunologic responses in RAS include genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficiencies, systemic diseases, hormonal imbalance, mechanical injuries, and stress. Our previous study found the presence of serum gastric parietal cell antibody, thyroglobulin antibody, and thyroid microsomal antibody in 13.0%, 19.4%, and 19.7% of 355 RAS patients, respectively. We also found anemia, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia in 20.9%, 20.1%, 4.8%, 2.6%, and 7.7% of 273 RAS patients, respectively. Therefore, it is very important to examine the complete blood count, serum autoantibody, hematinic, and homocysteine levels in RAS patients before we start to offer treatments for RAS. Because RAS is an immunologically-mediated disease, topical and systemic corticosteroid therapies are the main treatments of choice for RAS.

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Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common oral mucosal diseases characterized by recurrent and painful ulcerations on the movable or nonkeratinized oral mucosa. The prevalence of RAS in the general population varies from 5% to 66% with a mean of 20%.¹ Kleinman et al.² reported a point prevalence of 1.23% and a lifetime prevalence of 36.5% for RAS in 40,693 USA school children. In Taiwan, the prevalence of RAS is 10.5% in the general population.³

Classification of recurrent aphthous stomatitis

Three types of RAS, namely minor, major, and herpetiform types, are recognized.¹ Minor RAS is the most common type that occurs in 80% of RAS patients. The oral ulcerative lesions of minor RAS measure between 3 mm and 10 mm in diameter. They usually arise from the nonkeratinized oral mucosae with the buccal and labial mucosae being affected frequently. The lesion may be preceded by an erythematous macule with the prodromal symptoms of burning or stinging for a few hours to one or two days. Then, the oral ulceration appears and is subsequently covered by a yellow-white fibrinopurulent pseudomembrane. The oral ulcers heal without scarring in 7–14 days. Although the minor RAS lesion is small, the pain is often out of proportion for the size of the ulceration.¹

The oral ulcerative lesions of major RAS measure from 1 cm to 3 cm in diameter. They usually take 2–6 weeks to heal and may lead to scarring. The labial mucosa, soft palate, and tonsillar fauces are most frequently involved. In severe cases, the repeated scarring processes may result in a limitation of mouth opening.¹

Herpetiform RAS has the greatest number of oral lesions and the most frequent recurrences. Their oral ulcerative lesions vary from 1 mm to 3 mm in diameter and some of them may coalesce into larger irregular ulcerations. The oral ulcerations heal between 7 and 10 days. The herpetiform RAS has a female predilection and a typical onset in adulthood. Herpetiform RAS oral lesions may be confused with those of herpes simplex virus (HSV) type 1 (HSV-1 or HHV-1) infection. However, the herpetiform RAS lesions are commonly found on nonkeratinized oral mucosae and the HSV-1 lesions are often present on keratinized oral mucosae such as gingiva and hard palate.¹

Etiology of recurrent aphthous stomatitis

RAS belongs to the group of chronic, inflammatory, ulcerative diseases of the oral mucosa. Up to date, the etiopathogenesis of this disease remains unclear; however, it is considered to be multifactorial.⁴ The results of previous studies indicate that genetically mediated disturbances of the innate and acquired immunity play an important role in the disease development. Factors that modify the immunologic responses in RAS include genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficiencies, systemic diseases, hormonal imbalance, mechanical injuries, and stress.⁴

For the genetic predisposition, the positive family history of the RAS was reported in 24%–46% of RAS cases.^{5,6} The patients with a positive family history of RAS suffer more frequent recurrences and more severe course of the disease compared to those with a negative RAS family history.^{7–9} Furthermore, in both RAS and Behcet's disease, the risk of the disease development was higher in monozygotic twins than in dizygotic twins.^{9,10} The genetic risk factors that modify the individual susceptibility to RAS include various DNA polymorphisms in patients, especially those related with the alterations in the metabolism of interleukins (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12), interferon (IFN)- γ , and tumor necrosis factor (TNF)- α , can modify the individual susceptibility to RAS.⁴ Our previous studies showed a strong association of HLA-DRw9 with RAS in Chinese patients and a strong association of anti-epithelial cell antibodies with HLA-DR3 or DR7 phenotype in RAS patients.^{11,12}

In addition, our previous study also demonstrated that the phenotype frequencies of HLA-DR5, -DRw8 and -DQw1 as well as the haplotype frequencies of HLA-DR5/DQw1 and HLA-DRw8/DQw1 in patients with the mucocutaneous type of Behcet's disease are significantly higher than those in RAS patients. Moreover, the relative risks of HLA-DR5/DQw1 and HLA-DRw8/DQw1 haplotypes are greater than the relative risks of HLA-DR5, HLA-DRw8, and HLA-DQw1 antigens. These results suggest that some specific HLA-DR/DQ haplotypes may be more important than the individual HLA-DR and HLA-DQ phenotypes in the disease shift from RAS to the mucocutaneous type of BD.¹³ In RAS patients, higher incidences of HLA-A33, HLA-B35 and HLA-B81,¹⁴ HLA-B12,¹⁵ HLA-B51,¹⁶ HLA-DR7 and HLA-DR5^{17,18} are observed when compared to healthy control subjects.

Bacterial (*Streptococcus oralis*, *Helicobacter pylori*) and viral (HSV, varicella-zoster virus, cytomegalovirus, and adenoviruses) antigens have been reported to be potential factors that may modify the immunologic response and subsequently induce recurrent aphthae in predisposed subjects. However, the results of these studies are ambiguous and conflicting.⁴ In addition, Greenspan et al.¹⁹ concluded that neither cell-mediated hypersensitivity to streptococcal or viral antigens nor cross-reactivity between oral mucosal and streptococcal antigens is likely to play a role in the pathogenesis of RAS.

Deficiencies of hematinics (iron, vitamin B12, and folic acid) and zinc have been demonstrated in some RAS patients, but it has not been well explained why the hematinic deficiencies may influence on the course of immune response in RAS.⁴ We tried to explain why anemia and hematinic deficiencies might cause the occurrence of RAS.²⁰ Deficiencies of iron, vitamin B12, or folic acid may lead to anemia in RAS patients. Because RAS patients with anemia have reduced capacity of the blood to carry oxygen to oral mucosa, finally resulting in atrophy of oral mucosa.²⁰ Moreover, iron is essential to the normal functioning of oral epithelial cells,²¹ and both vitamin B12 and folic acid play important roles in DNA synthesis and cell division.^{22,23} Oral epithelial cells have a high turnover rate. Therefore, deficiencies of iron, vitamin B12, and folic acid may result in oral epithelial atrophy. Atrophic

oral epithelium in hematinic-deficient patients may explain why some patients with deficiencies of hematinics are prone to have RAS.²⁰ Furthermore, high blood homocysteine level (due to deficiencies of mainly vitamins B6 and B12 and folic acid) in some RAS patients may result in an elevated frequency of thrombosis in the feeding arterioles that supply the oral epithelial cells.^{24–28} This in turn leads to a breakdown of oral epithelium and finally produces an oral ulceration. Taken these together, anemia, hematinic deficiencies, and a high blood homocysteine level can decrease oral epithelial barrier and thus increase the frequency of RAS occurrence.²⁰ In addition, Volkov et al.²⁹ found that oral vitamin B12 supplementation can improve the symptoms and signs of RAS regardless of the initial serum levels of vitamin B12. This finding also confirms the role of vitamin B12 deficiency in the development of RAS.

Some previous studies mentioned that exposure to some specific ingredients (e.g., chocolate, gluten, cow milk, preservatives, nuts, and food coloring agents) may induce RAS.⁴ However, a previous double-blind study did not confirm the role of specific food ingredient allergy in the development of RAS.³⁰

Some patients with systemic diseases, especially the Behcet's disease, inflammatory bowel diseases (Crohn's disease, ulcerative colitis), celiac disease, and immunodeficiency virus (HIV) disease, are prone to have RAS.^{1,4} Moreover, RAS is one of the criteria for the diagnosis of Behcet's disease.¹ Patients with inflammatory bowel diseases or celiac disease are more likely to have the complications of nutrient deficiencies due to the malabsorption of nutrients. Moreover, these patients tend to have autoimmune reactions that result in oral aphthous ulcerations.⁴ HIV-infected patients are immunocompromised and also have more chance to develop RAS due to the decrease of CD4 lymphocytes and the increase of CD8 lymphocytes.³

Some reports mentioned the association of hormone imbalance with the occurrence of RAS. Women in the luteal phase of the menstrual cycle and in the menopause are prone to have RAS, while those on contraceptives or during pregnancy often have remission of RAS, suggesting the role of hormonal imbalance in the development of RAS.⁴

Mechanical injuries and stress may also involve in the occurrence of RAS. In some RAS-predisposed patients, oral ulcers may appear on the oral mucosa shortly after mechanical injuries. However, the mechanism of RAS development due to mechanical injuries remains unclear.⁴ Subjects with smoking habit or smokeless tobacco use have the decreased risk of RAS development.³¹ This could be explained by a higher level of oral epithelial keratinization in response to smoking or smokeless tobacco use. These keratinized oral mucosae are resistant to mechanical injuries.³¹ Furthermore, nicotine and its metabolites can also reduce the level of pro-inflammatory cytokines (TNF- α , IL-1 and IL-6) and augment the level of anti-inflammatory IL-10, and thus decrease the chance to develop RAS.³¹ Stress is also considered to be related to the exacerbation of RAS. Stress may induce immune dysfunction that subsequently triggers the onset of RAS episode rather than influences the duration of RAS.⁴

Serum autoantibodies in recurrent aphthous stomatitis patients

Our previous study assessed the serum gastric parietal cell antibody (GPCA), thyroglobulin antibody (TGA), and thyroid microsomal antibody (TMA, also known as thyroid peroxidase antibody or TPO) in 355 RAS patients of different subtypes and in 355 age- and sex-matched healthy control subjects.³² We discovered the presence of serum GPCA, TGA, and TMA in 13.0%, 19.4%, and 19.7% of 355 RAS patients, in 16.7%, 23.3%, and 21.7% of 60 major RAS patients, in 12.2%, 18.6%, and 19.3% of 295 minor RAS patients, in 18.1%, 20.0%, and 21.9% of 160 atrophic glossitis (AG)-positive RAS patients, and in 8.7%, 19.0%, and 17.9% of 195 AG-negative RAS patients, respectively. RAS, major RAS, minor RAS, AG-positive RAS, and AG-negative RAS patients all have a significantly higher frequency of serum GPCA, TGA, or TMA positivity than healthy control subjects (all P -values < 0.001).³² Of 65 TGA/TMA-positive RAS patients whose serum thyroid-stimulating hormone (TSH) levels are measured, 76.9%, 12.3%, and 10.8% of these TGA/TMA-positive RAS patients have normal, lower, and higher serum TSH levels, respectively.³² The aforementioned findings suggest that major RAS patients have slightly higher frequencies of serum GPCA, TGA, and TMA positivities than minor RAS patients. AG-positive RAS patients have slightly higher frequencies of serum TGA and TMA positivities than AG-negative RAS patients. However, the positive rate of GPCA is significantly higher in AG-positive RAS patients (18.1%) than in AG-negative RAS patients (8.7%, $P = 0.014$).³² Our previous study showed the serum GPCA positivity in 47 (26.7%) of 176 AG patients.³³ The GPCA-positive rate is greater in AG patients than in AG-positive RAS patients ($P = 0.081$, marginal significance). Moreover, 76.9%, 12.3%, and 10.8% of these TGA/TMA-positive RAS patients have euthyroid, hyperthyroidism, and hypothyroidism, respectively.³² In addition, 3.9%, 11.8% or 16.6% of 355 RAS patients had the presence of three, two, or one organ-specific autoantibody (including GPCA, TGA and TMA) in their sera, respectively.³²

The presence of serum GPCA, TGA, and TMA positivities do have special impact on the patients' health. Without proper early diagnosis and treatment, GPCA-positive patients are more likely to have pernicious anemia (PA) and to develop autoimmune atrophic gastritis which may subsequently progress to gastric carcinoma,^{34,35} and TGA/TMA-positive patients may develop autoimmune thyroid disease and finally result in thyroid dysfunction.^{36,37} Those TGA/TMA-positive RAS patients with either hyperthyroidism or hypothyroidism should be referred to endocrinologists for further treatment. Moreover, those GPCA-positive patients should be referred to department of gastroenterology for endoscopic examination of stomach to check for the presence of autoimmune atrophic gastritis that can be further treated by medical doctors in that department. In addition, it needs a long-term follow-up study to assess whether GPCA-positive RAS patients with or without treatment may develop gastric carcinoma.

Different types of antibody or autoantibody other than GPCA, TGA and TMA have been reported in RAS

patients.^{38–42} Anti-endomysial (or anti-transglutaminase) IgA and IgG antibodies are found in two RAS patients with concomitant celiac disease.³⁸ Anti-reticulin IgG, anti-reticulin IgA, and anti-endomysial IgA antibodies are demonstrated in 3 (3.4%), 1 (1.1%), and 1 (1.1%) of 87 minor RAS patients.³⁹ Healy et al.⁴⁰ studied the anti-endothelial cell autoantibody and anti-neutrophil cytoplasmic autoantibody levels in 20 RAS patients and 20 control subjects. IgG anti-endothelial cell autoantibody is detected in 19 (95%) of 20 RAS patients and 4 (20%) of 20 control subjects. However, anti-neutrophil cytoplasmic autoantibody is detected in only one RAS patient and none of the control subjects.⁴⁰ Sun and Wu⁴¹ found the anti-mucosal antibody in 15 (71%) of 21 RAS patients. Moreover, the positive rate of anti-intercellular substance antibody increases to 67% in the active phase but decreases to 25% in the one-week remission phase and to 10% in the two-week remission phase of the 21 RAS patients.⁴¹ The serum ANA are detected in 6 (12%) of 50 RAS patients and in 3 (5%) of 57 healthy control subjects; no significant difference in the incidence of serum ANA positivity is found between RAS patients and healthy control subjects.⁴² The above-mentioned findings indicate the low frequencies of presence of anti-endomysial and anti-reticulin antibodies in RAS patients.^{38,39} The high positive rate of anti-endothelial cell autoantibody in RAS patients suggests that vasculitis may play a role in etiology of RAS.⁴⁰ Moreover, the increase in the frequency of anti-intercellular substance antibody in sera of active RAS patients is only a transient phenomenon.⁴¹

Anemia in recurrent aphthous stomatitis patients

Our previous studies evaluated anemia in different types of RAS patients. We found anemia in 57 (20.9%) of 273 RAS patients, 10 (31.3%) of 32 major RAS patients, and 47 (19.5%) of 241 minor RAS patients.²⁰ Although the frequency of anemia is higher in 32 major RAS patients than in 241 minor RAS patients, the difference is not significant.²⁰ We also showed anemia in 69 (43.1%) of 160 AG-positive RAS patients and 38 (19.5%) of 195 AG-negative RAS patients, suggesting a significantly greater frequency of anemia in 160 AG-positive RAS patients than in 195 AG-negative RAS patients.⁴³ Of 69 anemic AG-positive RAS patients, 3 have PA, 6 have macrocytic anemia rather than PA, 30 have normocytic anemia, 23 have iron deficiency anemia (IDA), 5 have thalassemia trait-induced anemia, and 2 have microcytic anemia rather than IDA and thalassemia trait-induced anemia.⁴³ Moreover, of 38 anemic AG-negative RAS patients, one has PA, 2 have macrocytic anemia rather than PA, 26 have normocytic anemia, 5 have IDA, 3 have thalassemia trait-induced anemia, and one has microcytic anemia rather than IDA and thalassemia trait-induced anemia. Our findings indicate that normocytic anemia and IDA are the two most common types of anemia in both AG-positive and AG-negative RAS patients.⁴³

We also demonstrated anemia in 10 (32.3%) of 31 GPCA-positive RAS patients and 77 (32.1%) of 240 GPCA/TGA/TMA-negative RAS patients.⁴⁴ Of 10 anemic GPCA-positive RAS patients, 3 have PA, 4 have normocytic anemia, 2 have IDA, and one has microcytic anemia rather than IDA

and thalassemia trait-induced anemia. Moreover, of 77 anemic GPCA/TGA/TMA-negative RAS patients, 6 have macrocytic anemia rather than PA, 43 have normocytic anemia, 21 have IDA, 5 have thalassemia trait-induced anemia, and two have microcytic anemia rather than IDA and thalassemia trait-induced anemia.⁴⁴ These findings indicate that normocytic anemia and PA are the two most common types of anemia in GPCA-positive RAS patients, but normocytic anemia and IDA are the two most common types of anemia in GPCA/TGA/TMA-negative RAS patients.⁴⁴

In addition, we also studied anemia in RAS patients with anti-thyroid antibodies (TGA and/or TMA).⁴⁵ We discovered anemia in 3 (20.0%) of 15 GPCA-positive and 14 (20.3%) of 69 GPCA-negative RAS patients with anti-thyroid antibodies. Of 3 anemic GPCA-positive RAS patients with anti-thyroid antibodies, 2 have PA and one has normocytic anemia. Moreover, of 14 anemic GPCA-negative RAS patients with anti-thyroid antibodies, 2 have macrocytic anemia but not PA, 4 have normocytic anemia, 5 have IDA, and 3 have thalassemia trait-induced anemia.⁴⁵ Our results indicate that PA is the most common type of anemia in GPCA-positive RAS patients with anti-thyroid antibodies, but IDA is the most common type of anemia in GPCA-negative RAS patients with anti-thyroid antibodies.⁴⁵ Safadi⁴⁶ found anemia in 4% of 208 RAS patients. Burgan et al.⁴⁷ showed anemia in 14% of 143 RAS patients. Khan et al.⁴⁸ showed anemia in 8 (13%) of 60 RAS patients.

Hematinic deficiencies in recurrent aphthous stomatitis patients and their association with the presence of serum autoantibodies

Lopez-Jornet et al.⁴⁹ found hematinic deficiencies in 14.1% of 92 RAS patients and in 6.4% of 94 control subjects ($P = 0.086$). Compilato et al.⁵⁰ studied the hematinic deficiencies in 32 RAS patients and 29 healthy control subjects. They found hematinic deficiencies in 56.2% of 32 RAS patients and 7% of 29 healthy control subjects ($P < 0.0001$). Burgan et al.⁴⁷ assessed the prevalence of hematinic deficiencies in 143 RAS patients. They found that 37.8% have hematinic deficiencies, 26.6% have low serum vitamin B12 level, 4.9% have low serum folate level, and 16.8% of 143 RAS patients have low serum ferritin level.

Aynali et al.⁵¹ studied the serum levels of vitamin B12 and folic acid in 57 minor RAS patients and 45 patients with chronic tinnitus but without RAS (the control group). They found no significant difference in the serum folic acid level between the RAS and control groups. However, serum levels of vitamin B12 are significantly lower in the RAS patients than in the control patients ($P < 0.05$), and the serum folic acid level is significantly lower in women than in men in both the RAS and control groups ($P < 0.05$). They concluded that vitamin B12 deficiency, but not folic acid deficiency, may play a role in the underlying etiology of RAS.

Khan et al.⁴⁸ evaluated deficiencies of Hb, hematocrit, serum vitamin B12, serum ferritin, and red blood cells (RBC) folate levels in 60 RAS and 60 age- and sex-matched healthy control subjects without RAS. They found that the hematocrit ($P < 0.001$) as well as serum ferritin ($P = 0.001$), RBC folate ($P < 0.001$), and serum vitamin B12 ($P < 0.001$) levels are significantly lower in the RAS patients than in the

healthy control subjects without RAS. Combined deficiency state (including Hb, hematocrit, serum ferritin, serum vitamin B12, and RBC folate deficiencies) is identified in 8 (13%) of 60 RAS patients.

Tas et al.⁵² studied the vitamin B12 levels and number of aphthous lesions in 18 *H. pylori* (HP)-eradicated patients and 12 non-eradicated patients 6 months after HP eradication. They showed that vitamin B12 levels are significantly increased in HP-eradicated group ($P = 0.001$), whereas no significant change is found in non-eradicated group ($P = 0.638$). Mean number of aphthous lesions (per 6 months) of HP-eradicated group is significantly decreased after eradication ($P = 0.0001$); in the non-eradicated group, no significant change is found ($P = 0.677$).⁵²

Gulcan et al.⁵³ studied the vitamin B12 deficiency in 72 RAS patients. They found serum vitamin B12 deficiency in 37 RAS patients and the normal serum vitamin B12 level in 35 RAS patients. Koybasi et al.⁵⁴ investigated the association of serum vitamin B12, folic acid, iron, calcium, magnesium, and phosphorus levels as well as family history and cigarette smoking with RAS. They found that patients with vitamin B12 deficiency, positive family history, and nonsmoking status have the highest risk for having RAS. Subramanyam³¹ explained why RAS affects only the non-keratinized lining mucosa rather than the keratinized masticatory mucosa and why RAS is unlikely to be seen in smokers. He suggested that the keratin layer may block the ingress of antigens and prevent the occurrence of RAS on the keratinized masticatory mucosa. In addition, combustible products of smoking are known to cause keratinization of oral lining mucosa and therefore inhibit the occurrence of RAS. In addition, nicotine or its metabolites can result in a decrease of pro-inflammatory cytokines like TNF- α , IL1 and IL6, and an increase of anti-inflammatory cytokine IL-10. Consequently, there is a reduced susceptibility to RAS due to immunosuppression and/or reduction in inflammatory response.³¹

Thongprasom et al.⁵⁵ analyzed the hematologic statuses in 23 RAS patients and 19 control subjects. Low RBC folate levels are found in 11 (47.8%) of 23 RAS patients. The serum and RBC folate levels in the control group are within normal ranges. There is a significantly lower mean RBC folate level in 23 RAS patients than in 19 control subjects ($P < 0.001$). However, the serum vitamin B12 levels are within the normal range in both RAS and control groups.

Piskin et al.⁵⁶ investigated the serum iron, ferritin, folic acid, and vitamin B12 levels in 35 RAS patients and 26 healthy controls. They demonstrated significantly lower vitamin B12 levels in RAS patients than in healthy controls. However, no significant differences are found in other serum parameters. Safadi⁴⁶ found vitamin B12 deficiency in 7% of 208 RAS patients. Weusten and van de Wiel⁵⁷ found the vitamin B12 deficiency in three RAS patients. Barnadas et al.⁵⁸ evaluated serum iron, folic acid, and vitamin B12 levels in 80 RAS patients. Serum hematinic deficiencies are detected in 21 (26.2%) of 80 RAS patients. Of these 21 RAS patients with serum hematinic deficiencies, 4 have iron, 10 have folic acid, 4 have vitamin B12, and 3 have combined hematinic deficiencies.⁵⁸

Haisraeli-Shalish et al.⁵⁹ studied the thiamine (vitamin B1) deficiency in 70 RAS patients and 50 healthy control subjects. They found low serum thiamine levels in 49 (70%)

of 70 RAS patients but in only 2 (4%) of 50 healthy control subjects ($P < 0.001$), suggesting a significant association of serum thiamine deficiency with RAS. Nolan et al.⁶⁰ evaluated the serum thiamine, riboflavin and pyridoxine (vitamin B1, B2 and B6) levels in 60 RAS patients. They found deficiencies of one or more of these vitamins in 17 (28.3%) of 60 RAS patients. Wray et al.⁶¹ screened the hematinic deficiencies in 330 RAS patients. Of 47 (14.2%) RAS patients with serum hematinic deficiencies, 23 have iron, 7 have folic acid, 6 have vitamin B12, and 11 have combined hematinic deficiencies. Ozler⁶² found zinc deficiency in 28% of 25 RAS patients and in 4% of 25 healthy control subjects. The mean serum zinc level is significantly lower in 25 RAS patients than in 25 healthy control subjects.

Our previous study evaluated the serum iron, vitamin B12, folic acid, and homocysteine concentrations in 273 RAS patients and 273 age- and sex-matched healthy control subjects.²⁰ We found that 55 (20.1%), 13 (4.8%) and 7 (2.6%) RAS patients have deficiencies of serum iron ($<60 \mu\text{g/dL}$), vitamin B12 ($<200 \text{ pg/mL}$), and folic acid ($<4 \text{ ng/mL}$), respectively. Moreover, 21 (7.7%) RAS patients have abnormally high blood homocysteine level. RAS patients have a significantly higher frequency of serum iron, vitamin B12 or folic acid deficiency and of abnormally elevated blood homocysteine level than healthy control subjects (all P -values < 0.001 except for folic acid $P = 0.022$). If 273 RAS patients are further divided into 32 major RAS patients and 241 minor RAS patients, major RAS patients have a significantly higher mean serum homocysteine level than minor RAS patients ($P < 0.001$).²⁰

We also studied the serum iron, vitamin B12, and folic acid levels in 160 AG-positive RAS patients, 195 AG-negative RAS patients, and 355 healthy control subjects.⁴³ We found that both AG-positive RAS and AG-negative RAS patients have significantly lower mean serum iron and vitamin B12 levels as well as significantly greater frequencies of serum iron, vitamin B12, and folic acid deficiencies than healthy control subjects. Moreover, AG-positive RAS patients have a significantly lower mean serum iron level (for women only) and a significantly greater frequency of serum iron deficiency than AG-negative RAS patients. However, there are no significant differences in the serum vitamin B12 and folic acid levels between 160 AG-positive and 195 AG-negative RAS patients. Moreover, no significant differences in the frequencies of vitamin B12 and folic acid deficiencies are found between 160 AG-positive and 195 AG-negative RAS patients.⁴³

We also investigated the serum iron, vitamin B12, folic acid, and homocysteine levels in 31 GPCA-positive RAS patients, 240 GPCA/TGA/TMA-negative RAS patients, and 342 healthy control subjects.⁴⁴ We demonstrated the serum iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia in 6 (19.4%), 6 (19.4%), 0 (0.0%), and 7 (22.6%) of 31 GPCA-positive RAS patients and in 67 (27.9%), 16 (6.7%), 12 (5.0%), and 24 (10.0%) of 240 GPCA/TGA/TMA-negative RAS patients, respectively. Both 31 GPCA-positive and 240 GPCA/TGA/TMA-negative RAS patients have significantly greater frequencies of serum iron and vitamin B12 deficiencies and of hyperhomocysteinemia than 342 healthy control subjects. The 240 GPCA/TGA/TMA-negative RAS patients also have a significantly greater frequency of serum folic acid deficiency than 342 healthy control

subjects. In addition, the 31 GPCA-positive RAS patients have a significantly greater frequency of serum vitamin B12 deficiency than the 240 GPCA/TGA/TMA-negative RAS patients. However, there are no significant differences in the frequencies of the serum iron and folic acid deficiencies and of hyperhomocysteinemia between the 31 GPCA-positive and 240 GPCA/TGA/TMA-negative RAS patients.⁴⁴

In addition, we also studied the serum iron, vitamin B12, folic acid, and homocysteine levels in 84 RAS patients with anti-thyroid antibodies (TGA and/or TMA).⁴⁵ We discovered the serum iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia in 3 (20.0%), 5 (33.3%), 0 (0.0%), and 6 (40.0%) of 15 GPCA-positive and in 20 (29.0%), 5 (7.2%), 0 (0.0%), and 3 (4.3%) of 69 GPCA-negative RAS patients with anti-thyroid antibodies, respectively. Both 15 GPCA-positive and 69 GPCA-negative RAS patients with anti-thyroid antibodies have significantly higher frequencies of serum iron and vitamin B12 deficiencies than 342 healthy control subjects. Moreover, the 15 GPCA-positive RAS patients with anti-thyroid antibodies have a significantly higher frequency of hyperhomocysteinemia than 342 healthy control subjects. In addition, the 15 GPCA-positive RAS patients with anti-thyroid antibodies have significantly greater frequencies of vitamin B12 deficiency and of hyperhomocysteinemia than 69 GPCA-negative RAS patients with anti-thyroid antibodies. The aforementioned findings indicate that the GPCA-positive RAS patients tend to have vitamin B12 deficiency and hyperhomocysteinemia, but the serum TGA and TMA do not play significant roles in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS patients.⁴⁵

Management for recurrent aphthous stomatitis patients

The correct diagnosis of RAS is essential for the management of RAS patients. The diagnosis of RAS is invariably based on the history and clinical findings. We should bear in mind whether the patient's RAS is associated with the possible systemic causes (e.g., Behcet's disease, celiac disease, cyclic neutropenia, nutritional deficiencies, inflammatory bowel disease ...), especially for the suddenly-developed RAS in adult patients.^{1,4} Moreover, the best therapy for RAS is to control the oral ulcers for the longest period with minimal adverse side effects.

In our oral mucosal disease clinic, some of patients with RAS, Behcet's disease, oral lichen planus, AG, burning mouth syndrome, or oral submucous fibrosis are found to have anemia, hematinic deficiencies, and serum GPCA, TGA, and TMA positivities.^{20,32,33,43–45,63–81} Therefore, it is important to assess the complete blood count, serum iron, ferritin, vitamin B12, folic acid, and homocysteine levels as well as the presence or absence of serum GPCA, TGA, and TMA positivities before we start to offer the treatment for RAS patients.^{20,32,43–45}

If the RAS patients are diagnosed as having nutritional deficiencies and/or systemic disorders, supplementation of deficient iron, zinc, folic acid, and vitamins B1, B2, B6, and B12 as well as referring the patients to physicians for care of specific systemic disease are beneficial for the RAS

patients.^{82–84} Volkov et al.²⁹ treated 58 RAS patients with a sublingual dose of 1000 µg of vitamin B12 for 6 months in a randomized, double-blind, placebo-controlled clinical trial. They found that vitamin B12 treatment is simple, inexpensive, and low-risk, and seems to be effective for RAS patients, regardless of the initial serum vitamin B12 level of the RAS patient.²⁹ Our long-term clinical experiences also found that supplement therapy with vitamins B complex and C together with deficient iron, vitamin B12, and/or folic acid can significantly decrease the severity and frequency of RAS.

Clinical management of RAS using topical and systemic therapies is based on severity of symptoms and the frequency, size, and number of lesions. The goals of therapy are to decrease pain and ulcer size and number, to promote ulcer healing, and to reduce the frequency of ulcer recurrence. The drugs used for topical or systemic therapy include corticosteroids, antimicrobials, analgesics, anti-inflammatory agents, immunomodulating agents, etc.^{82–84} The topical corticosteroids are the most commonly used drugs for treatment of RAS. Some RAS patients may have prodromal symptoms, such as tingling or burning sensation, at the oral mucosal site where the ulceration subsequently appears. In this situation, use of the topical corticosteroid may abort the attack of aphthous ulcer.^{83,84}

If the RAS lesion is small and the oral symptoms are mild or moderate, topical application of corticosteroid (dexamethasone or triamcinolone acetonide) ointment as a thin film 2–3 times per day onto the oral ulcers is usually sufficient to induce healing of the RAS lesions within 7–14 days.^{82–84} The available topical corticosteroids in Taiwan include dexaltin oral paste (0.1% dexamethasone, 5 g/tube), kenalog in orabase (0.1% triamcinolone acetonide, 5 g/tube), and nincort oral gel (0.1% triamcinolone acetonide, 6 g/tube). On the other hand, fluocinolone acetonide (0.025–0.05%) has medium to high potency and clobetasol propionate (0.025%) is the most potent topical corticosteroid; thus, these two topical agents are reserved for treatment of severe or aggressive RAS lesions.⁸²

The most commonly used anti-microbial agent is the chlorhexidine gluconate (0.12%) oral rinse that has the ability to reduce the amount of bacteria in the oral cavity. It may protect the ulcer from bacterial infection and promote the healing of the RAS lesion. Benzydamine hydrochloride (1.5 mg/mL) oral spray is a topical analgesic agent which also has anti-inflammatory action. Topical 3% diclofenac with 2.5% hyaluronic acid can be applied onto RAS lesions to lessen the pain. Moreover, topical lidocaine (2% spray or gel) is also a topical analgesic agent for treatment of RAS.^{82–84} Topical antibiotics, such as tetracyclines and their derivatives (doxycycline and minocycline) in gel or rinse format, have been found to reduce the pain and outbreaks of RAS. These drugs act through the local inhibition of collagenases and metalloproteinases that contribute to tissue destruction and ulcer formation.⁸²

If the RAS lesions are large and the oral symptoms are severe, our clinic experience found that topical spray of corticosteroid powders (e.g., sealcoat cap for spray, containing beclomethasone dipropionate) onto the lesional oral mucosal areas 2–3 times per day is also effective for induction of healing of RAS lesions. Alternatively, large and severe RAS lesions can also be treated by intralesional and submucosal

injection of kenacort A (40 mg triamcinolone acetonide for each RAS lesion once weekly for 1–2 weeks) plus oral administration of prednisolone (15–30 mg of prednisolone after breakfast once daily for a week; the oral administration of prednisolone is gradually tapered to 5 mg per day and stopped in the second week). This treatment modality can result in accelerated healing of RAS lesions. Scully et al.⁸⁴ also suggested to use intralesional injections of a corticosteroid such as betamethasone, dexamethasone or triamcinolone to enhance the local response, thus allowing for shorter systemic treatment for severe RAS patients.

For the severest multiple RAS lesions with large areas of ulceration, systemic administration of prednisolone (40 mg of prednisolone once per day for 5 days followed by 10–20 mg of prednisolone once per day for another 7–10 days) can be used as a rescue therapy for the severest RAS and can achieve a significant improvement of the RAS lesions in a short period.⁸⁴ The possibility of iatrogenic candidiasis associated with the long-term use of corticosteroid should be monitored. If the RAS patients have oral candidiasis, especially at the oral mucosal lesional sites, due to the long-term use of corticosteroid ointment, antifungal drug (such as mycostatin) should be given to the patients for at least two weeks to eliminate oral candidiasis.^{82–84} The RAS patients should be evaluated every 3–6 months until there is no recurrence for at least a year.

Drugs with systemic immunosuppression effect including prednisolone, colchicine, levamisole, azathioprine (imuran), thalidomide, pantoxifyline, and dapsone can also be used for treatment of severe cases of RAS.^{82–84} Colchicine is a mitosis-inhibiting agent. It inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules that are necessary for cell mitosis. Apart from inhibiting mitosis, colchicine also inhibits neutrophil motility and activity, leading to a net anti-inflammatory effect. Therefore, colchicine has been mainly used for treatment of acute gout flare-ups. Besides, colchicine can also be used for treatment of RAS lesions, especially those associated with Behcet's disease.^{82–84} Pakfetrat et al.⁸⁵ treated 34 RAS patients with a low dose of prednisolone (5 mg/day) or colchicine (0.5 mg/day) in a double-blind randomized clinical trial. All RAS patients took the medication for three months and were assessed every two weeks. They found that both colchicine and prednisolone treatments significantly reduce the signs and symptoms of RAS. However, colchicine (52.9%) has significantly more side effects than prednisolone (11.8%). They concluded that 5 mg/day of prednisolone seems to be a better alternative in reducing the signs and symptoms of RAS.⁸⁵

Levamisole is an anthelmintic drug used for treatment of parasitic worm infections, especially ascariasis and hookworm infections. Levamisole can also be used as an immunomodulator to restore the phagocytosis function of macrophages and neutrophils, to modulate helper and cytotoxic T cell activities, to enhance the activities of interferon and IL-2, and to alter the natural course of recurrent chronic inflammatory disease.^{82,86,87} Thus, levamisole is also used for treatment of RAS.^{82,84,88,89} The recommended dosage for RAS therapy is oral administration of levamisole (50 mg, three times per day) for 3 consecutive days every two weeks until there is a complete remission of

RAS for at least 6 months.^{88,89} In the treatment period, dexamethasone in orabase (dexaltin) can be topically applied onto RAS lesions 2–3 times per day if the patients have a recurrence of RAS. This treatment modality usually results in a significant improvement of symptoms and signs of RAS.^{88,89}

Our previous study used levamisole alone or levamisole plus Chinese medicinal herbs to treat major and minor RAS patients and monitored the serum IL-6 levels at the baseline and after treatment. We found a significant reduction of the serum IL-6 level from 11.8 ± 7.0 pg/mL to 1.8 ± 1.5 pg/mL in 11 major and 3 minor RAS patients after treatment with levamisole plus Chinese medicinal herbs for a period of 0.5–3 months ($P < 0.001$). Moreover, treatment with levamisole for a period of 0.5–5 months can also significantly reduce the serum IL-6 level from 8.3 ± 3.7 pg/mL to 2.4 ± 1.7 pg/mL in 18 major RAS patients and from 7.5 ± 3.2 pg/mL to 2.4 ± 1.7 pg/mL in 18 major and 13 minor RAS patients. In addition, the mean reduction of the serum IL-6 level in RAS patients after treatment with levamisole plus Chinese medicinal herbs is significantly higher than that in RAS patients after treatment with levamisole only. Comparisons of the clinical parameters in RAS patients before and after treatment, we also found a significant reduction in the frequency, duration, and number of RAS lesions after treatment with either levamisole plus Chinese medicinal herbs or levamisole only, suggesting that both treatment regimens are effective for the RAS lesions.⁸⁸

Our previous study also measured the baseline serum IL-6 and IL-8 levels in 146 RAS patients and 54 healthy control subjects.⁸⁹ We found that 25% (37/146) RAS patients, 33% (20/61) major RAS patients, 19% (13/69) minor RAS patients, and 25% (4/16) herpetiform RAS patients have a serum IL-6 level greater than the upper normal limit of 4.7 pg/mL. In contrast, 60% (87/146) RAS patients, 59% (36/61) major RAS patients, 59% (41/69) minor RAS patients, and 63% (10/16) herpetiform RAS patients have a serum IL-8 level greater than the upper normal limit of 8.7 pg/mL. In 82 RAS patients with the serum IL-6 or IL-8 levels greater than the upper limit of normal serum concentration, treatment with levamisole for a period of 0.5–3.5 months can significantly reduce the serum IL-6 level from 12.0 ± 1.6 pg/mL to 3.0 ± 0.5 pg/mL ($P < 0.001$), and can significantly lower the serum IL-8 level from 70.9 ± 11.2 pg/mL to 13.8 ± 3.1 pg/mL ($P < 0.001$). These findings indicate that the serum IL-8 level is a more sensitive marker than the serum IL-6 level in monitoring the disease activity of RAS. In addition, levamisole treatment can modulate both the serum IL-6 and IL-8 levels in RAS patients. IL-8, like IL-6, is also a useful serum marker in evaluating therapeutic effects of levamisole on RAS patients.⁸⁹

However, levamisole use is not recommended for women during breastfeeding or the third trimester of pregnancy. The minor side effects of levamisole may include skin rash, abdominal pain, nausea, vomiting, headache, and dizziness. One of the more serious side effects of levamisole is agranulocytosis; this occurs in 0.08–5% of the studied populations.^{86,87,90}

Azathioprine (imuran) is an immunosuppressant. It can be used as a corticosteroid-sparing agent for those systemic disease-associated RAS patients who may need a great amount of corticosteroids to suppress the symptoms and

signs of RAS lesions and associated systemic diseases.^{82–84} If the RAS patient takes a dose of 50 mg of imuran per day, the amount of corticosteroid can be reduced to a half dose to avoid the adverse side effects such as adrenocortical suppression, hypertension, hyperglycemia, weight gain, mood alteration, insomnia, gastrointestinal irritation, and osteoporosis due to the long-term maintenance corticosteroid therapy.^{82–84}

Hello et al.⁹¹ did a multicenter cohort study of using the thalidomide (an immune modulator) for treatment of severe RAS patients. Ninety-two patients including 76 with oral or bipolar aphthosis and 16 with Behcet's disease are collected from 14 centers. They found that the thalidomide (50 mg/day or 100 mg/day) is rapidly effective and 78 (85%) of 92 patients showed complete remission within a median of 14 days. Response time was independent of the initial thalidomide dose. However, the adverse events are reported in 84% (77/92) of patients. They are mostly mild (78% of patients), but sometimes severe (21%). The most frequent adverse events are somnolence (36%), paresthesia (18%), constipation (18%), peripheral neuropathy (17%), and weight gain (16%). Nevertheless, after 40 months of follow-up, 60% of the patients are still receiving continuous or intermittent therapy with favorable efficacy/tolerance ratios.⁹¹

Mimura et al.⁹² compared the efficacy of four systemic treatments for severe RAS. Prednisone is administered for the first two weeks, starting with 0.5 mg/kg/day as a single morning dose, and followed after one week by a reduction to half the starting dose. Then, the patients are randomly assigned to one of the following drugs for 6 months: thalidomide (100 mg/day), dapsone (25 mg/day for 3 days, 50 mg/day for 3 days, 75 mg/day for 3 days, and a maintenance dose of 100 mg/day), colchicine (0.5 mg/day for 7 days, 1 mg/day for 7 days, and a maintenance dose of 1.5 mg/day) or pentoxifylline (400 mg 3 times a day). Thalidomide is found to be the most effective and best tolerated treatment. The RAS complete response rate is 87.5% for the thalidomide, 55.6% for the dapsone, 40% for the colchicine, and 20% for the pentoxifylline.⁹²

Conclusions and important suggestions for recurrent aphthous stomatitis patients

1. Maintenance of good oral hygiene, especially for the sites of RAS lesions, is important for the healing of RAS lesions.
2. The RAS patients in active disease phase should avoid the hot, salty, acidic or spicy food stuffs as well as overwork, extreme fatigue, and insomnia.
3. If RAS patients can maintain a regular and healthy life and have enough nutrition, exercise, and rest, there may be a significant reduction of RAS recurrence.
4. Long-term maintenance use of corticosteroids may lead to oral candidiasis, and in this situation antifungal drug (such as mycostatin) should be given to the patients for at least two weeks to control the candida infection.
5. If the RAS patients also have systemic diseases such as diabetes mellitus, liver or kidney diseases, celiac disease, or inflammatory bowel disease, these

patients should be referred to associated physicians for further treatment.

6. Treatment of RAS should be tailored to each patient individually and its main goals are to reduce symptoms, to decrease ulcer number and size, and to increase disease-free periods.
7. Corticosteroids are still the major drugs for treatment of RAS. Mild and moderate RAS lesions can be resolved by adequate topical corticosteroid treatment, but severe RAS lesions may need systemic corticosteroid treatment.
8. For RAS patients, it is very important to examine the complete blood count, serum autoantibody, hematinic, and homocysteine levels to see whether these patients have anemia, serum autoantibody positivities, hematinic deficiencies, and hyperhomocysteinemia.
9. Supplementation of iron, zinc, folic acid, and vitamins B1, B2, B6, and B12 to those RAS patients with corresponding nutrient deficiencies can result in partial or complete remission of RAS.
10. Even if the RAS patients are asymptomatic, they should be re-evaluated every 3–6 months until there is no recurrence for at least one year.

Conflicts of interest statement

The authors have no conflicts of interest relevant to this article.

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