



Original Article

# Serum thyroid autoantibodies are not associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in patients with Behcet's disease



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Received 20 May 2018

Available online 7 June 2018

## KEYWORDS

Behcet's disease;  
Iron deficiency;  
Folic acid deficiency;  
Hyperhomo-  
cysteinemia;  
Thyroglobulin  
antibody;  
Thyroid microsomal  
antibody

**Abstract** *Background/purpose:* Our previous study found that 13 of 63 recurrent aphthous stomatitis (RAS)/Behcet's disease (BD) patients have thyroglobulin antibody (TGA) positivity and/or thyroid microsomal antibody (TMA) positivity (TGA/TMA positivity) but without gastric parietal cell antibody positivity. This study mainly assessed whether the serum TGA/TMA positivity was significantly associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in TGA/TMA-positive RAS/BD patients.

*Materials and methods:* The mean blood hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine levels were measured and compared between 13 TGA/TMA-positive RAS/BD patients and 41 gastric and thyroid antibodies-negative RAS/BD patients (Abs-RAS/BD patients) or 126 healthy control subjects.

*Results:* We found no significant differences in the mean blood Hb, iron, vitamin B12, folic acid, and homocysteine levels as well as no significant differences in the frequencies of blood Hb and folic acid deficiencies and of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients. The 41 Abs-RAS/BD patients even had a significantly greater frequency of serum iron deficiency than the 13 TGA/TMA-positive RAS/BD patients. Moreover, although a significant greater frequency of anemia was demonstrated in 13

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TGA/TMA-positive RAS/BD patients than in 126 healthy control subjects, there were no significant differences in the mean serum iron, vitamin B12, folic acid, and homocysteine levels as well as no significant differences in the frequencies of serum iron and folic acid deficiencies and of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients and 126 healthy control subjects.

**Conclusion:** The serum TGA/TMA-positivity is not significantly associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in TGA/TMA-positive RAS/BD patients.

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## Introduction

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disease characterized by recurrent and painful ulcerations on the movable oral mucosae. Behcet's disease (BD) is a chronic, multisystemic, and inflammatory disorder. According to the criteria for diagnosis of BD proposed by the International Study Group for BD, the RAS is universally present in all BD patients (so-called RAS/BD patients in this study).<sup>1,2</sup>

Our previous study showed that 14.3%, 20.6%, and 20.6% of 63 BD patients have serum gastric parietal cell antibody (GPCA), thyroglobulin antibody (TGA), and thyroid microsomal autoantibody (TMA, also known as thyroid peroxidase antibody, TPO) positivities, respectively.<sup>3</sup> Moreover, we also demonstrated that 30.2%, 34.9%, 6.3%, 6.3%, and 14.3% of 63 BD patients have blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia, respectively.<sup>4</sup> The serum GPCA positivity, major-typed RAS, minor-typed RAS, BD itself, and the concomitant presence of atrophic glossitis (AG) in BD patients are found to be associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients.<sup>4–6</sup> However, we have not yet known whether the serum TGA positivity and/or TMA positivity (TGA/TMA positivity) plays a significant role in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the TGA/TMA-positive RAS/BD patients.

In our oral mucosal disease clinic or dental clinic, patients with RAS, AG, burning mouth syndrome, oral lichen planus are frequently encountered and patients with OSF or specific jaw bone lesions are sometimes seen.<sup>7–38</sup> For these particular groups of patients, complete blood count, serum iron, vitamin B12, folic acid, homocysteine, GPCA, TGA, and TMA levels are frequently examined to assess whether these patients have anemia, hematinic deficiencies, and serum GPCA, TGA, and TMA positivities.<sup>7–35</sup>

To assess the role of serum TGA/TMA positivity in the development of anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients, 13 TGA/TMA-positive RAS/BD patients without serum GPCA positivity, 41 gastric and thyroid autoantibodies-negative RAS/BD patients (Abs-RAS/BD patients), and 126 age- and sex-matched healthy control subjects were retrieved from our previous studies and included in this study.<sup>3–6</sup> The mean blood hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine levels in these RAS/BD patients and control subjects were measured and compared between 13 TGA/

TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients or 126 healthy control subjects to assess whether the serum TGA/TMA positivity was a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in TGA/TMA-positive RAS/BD patients.

## Materials and methods

### Subjects

This study included 13 (2 men and 11 women, age range 21–82 years, mean age  $58 \pm 14$  years) TGA/TMA-positive RAS/BD patients without the serum GPCA positivity.<sup>3</sup> For evaluation of the role of serum TGA/TMA positivity in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients, 41 Abs-RAS/BD patients and 126 age- and sex-matched healthy control subjects were retrieved from our previous studies and included in this study.<sup>3–6</sup> All the patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry, National Taiwan University Hospital from July 2007 to July 2017. The diagnoses of RAS and BD in our original 63 RAS/BD patients as well as their inclusion and exclusion criteria have been described in our previous studies.<sup>3–6</sup> Healthy control subjects had dental caries, pulpal disease, malocclusion, or missing of teeth but did not have any oral mucosal or systemic diseases.<sup>3–6</sup> In addition, none of the RAS/BD patients had taken any prescription medication for BD and RAS at least 3 months before entering the study.

The blood samples were drawn from our RAS/BD patients and healthy control subjects for measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations as well as serum GPCA, TGA, and TMA levels. All the RAS/BD patients and healthy control subjects signed the informed consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board at the National Taiwan University Hospital.

### Determination of complete blood count and serum iron, vitamin B12, folic acid and homocysteine concentrations

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by

the routine tests performed in the Department of Laboratory Medicine of National Taiwan University Hospital as described previously.<sup>7–11</sup> This study defined the Hb and hematinic deficiencies according to the World Health Organization (WHO) criteria. Thus, men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.<sup>39</sup> Patients with serum iron level < 60 µg/dL,<sup>31,40</sup> vitamin B12 level < 200 pg/mL<sup>41</sup> or folic acid level < 4 ng/mL<sup>42</sup> were defined as having iron, vitamin B12 or folic acid deficiency, respectively. Moreover, patients with the serum homocysteine level > 12.7 µM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having hyperhomocysteinemia.<sup>4–6</sup>

### Determination of serum GPCA, TGA, and TMA levels

The methods of determination of serum GPCA, TGA, and TMA levels in our RAS/BD patients and healthy control subjects have been described in our previous studies.<sup>12,14,15,18–21</sup>

### Statistical analysis

Comparisons of the mean corpuscular volume (MCV), the mean blood levels of Hb, iron, vitamin B12, folic acid, and homocysteine between 13 TGA/TMA-positive RAS/BD patients or 41 Abs-RAS/BD patients and 126 healthy control subjects as well as between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients were performed by Student's *t*-test. The differences in frequencies of blood Hb, iron, vitamin B12, and folic acid deficiencies and of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients or 41 Abs-RAS/BD patients and 126 healthy control subjects as well as between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients were compared by chi-square test or Fisher exact test, where appropriate. The result was considered to be significant if the *P*-value was less than 0.05.

### Results

The MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 13 TGA/TMA-positive RAS/BD patients, 41 Abs-RAS/BD patients, and 126 healthy control subjects are shown in Table 1. Because men and women usually had different normal blood Hb and iron levels, these two mean levels were calculated separately for men and women. We found significantly lower mean blood Hb levels (for men and women) in 13 TGA/TMA-positive RAS/BD patients than in 126 healthy control subjects (both *P*-values < 0.01, Table 1). However, there were no significant differences in the MCV and mean serum iron (for men and women), vitamin B12, folic acid, and homocysteine levels between 13 TGA/TMA-positive RAS/BD patients and 126 healthy control subjects. Moreover, no significant differences in the MCV and mean blood Hb (for men and women), iron (for men and women), vitamin B12, folic acid, and homocysteine levels were noted between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients, suggesting that the serum TGA/TMA-positivity does

not play a significant role in causing the anemia, hematinic deficiencies, and hyperhomocysteinemia in the serum TGA/TMA-positive RAS/BD patients (Table 1). In addition, the 41 Abs-RAS/BD patients do have significantly lower MCV, mean blood Hb (for men and women), iron (for men and women), folic acid, and homocysteine levels than 126 healthy control subjects (all *P*-values < 0.05, Table 1); these blood data have been published in our previous study.<sup>6</sup>

We also found a significantly greater frequency of blood Hb deficiency in our 13 TGA/TMA-positive RAS/BD patients than in 126 healthy control subjects (*P* < 0.001) (Table 2). However, there were no significant differences in the frequencies of serum iron and folic acid deficiencies and of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients and 126 healthy control subjects. The frequency of iron deficiency was significantly greater in 41 Abs-RAS/BD patients than in 13 TGA/TMA-positive RAS/BD patients (*P* = 0.021). Moreover, 13 TGA/TMA-positive RAS/BD patients did not have significantly higher frequencies of blood Hb and folic acid deficiencies and of hyperhomocysteinemia than 41 Abs-RAS/BD patients, suggesting that the serum TGA/TMA-positivity does not play a significant role in causing the anemia, hematinic deficiencies, and hyperhomocysteinemia in the serum TGA/TMA-positive RAS/BD patients (Table 2). In addition, 41 Abs-RAS/BD patients do have significantly greater frequencies of blood Hb and iron deficiencies (both *P*-values < 0.001) than 126 healthy control subjects (Table 2); these blood data have been reported in our previous study.<sup>6</sup>

In this study, only 2 (15.4%) of 13 TGA/TMA-positive RAS/BD patients were diagnosed as having anemia according to the WHO criteria.<sup>39</sup> In addition to having Hb deficiency (men with Hb < 13 g/dL and women with Hb < 12 g/dL), macrocytic anemia was diagnosed as having MCV ≥ 100 fL,<sup>30</sup> normocytic anemia as having MCV between 80 and 99.9 fL,<sup>12–14</sup> microcytic anemia as having MCV < 80 fL,<sup>40</sup> and iron deficiency anemia as having MCV < 80 fL and iron < 60 µg/dL.<sup>31,39</sup> By these definitions, of 2 anemic TGA/TMA-positive RAS/BD patients, one had normocytic anemia and the other had microcytic anemia (Table 3). The anemia types of 14 anemic Abs-RAS/BD patients have been reported in our previous study;<sup>6</sup> of the 14 anemic Abs-RAS/BD patients, one had macrocytic anemia, 10 had normocytic anemia, and three had iron deficiency anemia.<sup>6</sup>

### Discussion

This study mainly evaluated whether the serum TGA/TMA positivity was a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the TGA/TMA-positive RAS/BD patients. The rationale for the design of this study was that if the TGA/TMA-positive RAS/BD patients had severer statuses of anemia, hematinic deficiencies, and hyperhomocysteinemia than the Abs-RAS/BD patients, then the serum TGA/TMA-positivity could be a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the TGA/TMA-positive RAS/BD patients. Our results found no significant differences in the mean blood Hb, iron, vitamin B12, folic acid, and homocysteine levels as well as no significant differences in the frequencies of blood Hb and folic acid deficiencies and

**Table 1** Comparisons of the mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid and homocysteine between any two of the three groups of 13 thyroglobulin antibody (TGA)-positive and/or thyroid microsomal antibody (TMA)-positive recurrent aphthous stomatitis (RAS)/Behcet's disease (BD) patients (TGA/TMA-positive RAS/BD patients), 41 gastric and thyroid autoantibodies-negative RAS/BD patients (Abs-RAS/BD patients), and 126 healthy control subjects.

Group	Hb (g/dL)		MCV (fL)	Iron (µg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (µM)
	Men	Women		Men	Women			
TGA/TMA-positive RAS/BD patients (n = 13)	13.8 ± 1.6 (n = 2)	12.6 ± 1.0 (n = 11)	88.5 ± 6.4	86.5 ± 23.3 (n = 2)	81.0 ± 27.2 (n = 11)	722.1 ± 220.9	11.9 ± 7.0	8.1 ± 1.4
P-value <sup>a</sup>	0.005	<0.001	0.096	0.344	0.077	0.382	0.231	0.504
P-value <sup>b</sup>	0.680	0.830	0.773	0.959	0.140	0.620	0.613	0.342
Abs-RAS/BD patients (n = 41) <sup>c</sup>	14.2 ± 1.2 (n = 12)	12.5 ± 1.4 (n = 29)	87.8 ± 7.9	85.3 ± 30.7 (n = 12)	65.2 ± 30.4 (n = 29)	687.6 ± 216.0	10.9 ± 5.9	7.4 ± 2.5
P-value <sup>a</sup>	<0.001	<0.001	0.004	0.045	<0.001	0.538	0.004	0.006
Healthy control subjects (n = 126) <sup>c</sup>	15.2 ± 0.6 (n = 36)	13.6 ± 0.7 (n = 90)	90.5 ± 3.8	102.6 ± 23.1 (n = 36)	98.4 ± 30.8 (n = 90)	661.8 ± 237.2	14.1 ± 6.2	8.5 ± 2.1

<sup>a</sup> Comparisons of means of parameters between 13 TGA/TMA-positive RAS/BD patients or 41 Abs-RAS/BD patients and 126 healthy control subjects by Student's *t*-test.

<sup>b</sup> Comparisons of means of parameters between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients by Student's *t*-test.

<sup>c</sup> The blood data of 41 bs-RAS/BD patients and 126 healthy control subjects were retrieved from our previous study.<sup>5</sup>

of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients and 41.

Abs-RAS/BD patients. In addition, the 41 Abs-RAS/BD patients even had a significantly greater frequency of serum iron deficiency than the 13 TGA/TMA-positive RAS/BD patients. Moreover, although a significantly lower mean blood Hb level and a significant greater frequency of Hb deficiency were demonstrated in 13 TGA/TMA-positive RAS/BD patients than in 126 healthy control subjects, there were no significant differences in the mean serum iron, vitamin B12, folic acid, and homocysteine levels as well as no significant differences in the frequencies of serum iron and folic acid deficiencies and of hyperhomocysteinemia between 13 TGA/TMA-positive RAS/BD patients and 126 healthy control subjects. These findings indicate that the serum TGA/TMA-positivity is not a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in TGA/TMA-positive RAS/BD patients.

It is interesting to know what significant factors can cause anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients. Our previous studies found that the serum GPCA is a major factor causing vitamin B12 deficiency and hyperhomocysteinemia in GPCA-positive RAS/BD patients.<sup>6</sup> Moreover, major-typed RAS, minor-typed RAS, and RAS/BD itself do play a significant role in causing anemia and hematinic deficiencies in RAS/BD patients including the GPCA-positive RAS/BD patients and Abs-RAS/BD patients.<sup>4-6</sup> Furthermore, we also discovered that the concomitant presence of AG in RAS/BD patients may result in significantly elevated frequencies of serum GPCA, TGA, and TMA positivities in RAS/BD patients.<sup>43</sup> Moreover, the AG-positive RAS/BD patients do have significantly higher frequencies of blood Hb, iron, vitamin B12, and folic acid deficiencies and of hyperhomocysteinemia than healthy control subjects and have significantly higher frequencies of Hb and vitamin B12 deficiencies than AG-negative RAS/BD patients.<sup>5</sup> Taking all our findings together, we further conclude that the serum GPCA, major-typed RAS, minor-typed RAS, RAS/BD itself, and the concomitant presence of AG in RAS/BD patients but not the serum TGA/TMA positivity are significant factors causing anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients.<sup>4-6,43</sup>

Our previous studies and this study found anemia in 19 (30.2%) of 63 BD, 13 (68.4%) of 19 major-typed RAS/BD, 6 (13.6%) of 44 minor-typed RAS/BD, 13 (43.3%) of 30 AG-positive RAS/BD, 6 (18.2%) of 33 AG-negative RAS/BD, 3 (33.3%) of 9 GPCA-positive RAS/BD, 14 (34.1%) of 41 Abs-RAS/BD, and 2 (15.4%) of 13 TGA/TMA-positive RAS/BD patients.<sup>4-6</sup> These findings suggest that in the subgroups of BD patients, major-typed RAS/BD patients have the highest frequency of anemia (68.4%) and minor-typed RAS/BD patients have the least frequency of anemia (13.6%).

For the iron deficiency in the subgroups of RAS/BD patients, the iron deficiency was noted in 22 (34.9%) of 63 BD, 12 (63.2%) of 19 major-typed RAS/BD, 10 (22.7%) of 44 minor-typed RAS/BD, 10 (33.3%) of 30 AG-positive RAS/BD, 12 (36.4%) of 33 AG-negative RAS/BD, 3 (33.3%) of 9 GPCA-positive RAS/BD, 18 (43.9%) of 41 Abs-RAS/BD, and 1 (7.7%) of 13 TGA/TMA-positive RAS/BD patients.<sup>4-6</sup> These

**Table 2** Comparisons of frequencies of blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and frequency of hyperhomocysteinemia between any two of the three groups of 13 thyroglobulin antibody (TGA)-positive and/or thyroid microsomaal antibody (TMA)-positive recurrent aphthous stomatitis (RAS)/Behcet's disease (BD) patients (TGA/TMA-positive RAS/BD patients), 41 gastric and thyroid autoantibodies-negative RAS/BD patients (Abs-RAS/BD patients), and 126 healthy control subjects.

	Hb deficiency (Men < 13 g/dL, Women < 12 g/dL)	Iron deficiency (<60 µg/dL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	High homocysteine level (>12.7 µM)
TGA/TMA-positive RAS/BD patients (n = 13)	2 (15.4%)	1 (7.7%)	0 (0%)	1 (7.7%)	0 (0%)
<i>P</i> -value <sup>a</sup>	0.001	0.161	ND	0.161	0.960
<i>P</i> -value <sup>b</sup>	0.301	0.021	ND	>0.999	>0.999
Abs-RAS/BD patients (n = 41) <sup>c</sup>	14 (34.1%)	18 (43.9%)	0 (0%)	2 (4.9%)	2 (4.9%)
<i>P</i> -value <sup>a</sup>	<0.001	<0.001	ND	0.095	0.845
Healthy control subjects (n = 126) <sup>c</sup>	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (4.0%)

<sup>a</sup> Comparisons of frequencies of parameters between 13 TGA/TMA-positive RAS/BD patients or 41 Abs-RAS/BD patients and 126 healthy control subjects by chi-square test.

<sup>b</sup> Comparisons of frequencies of parameters between 13 TGA/TMA-positive RAS/BD patients and 41 Abs-RAS/BD patients by chi-square test or Fisher exact test, where appropriate. ND = not done.

<sup>c</sup> The blood data of 41 Abs-RAS/BD patients and 126 healthy control subjects were retrieved from our previous study.<sup>6</sup>

findings suggest that in the subgroups of BD patients, major-typed RAS/BD patients have the highest frequency of iron deficiency (63.2%) and TGA/TMA-positive RAS/BD patients have the least frequency of iron deficiency (7.7%).

For the vitamin B12 deficiency in the subgroups of RAS/BD patients, vitamin B12 deficiency was noted in 4 (6.3%) of 63 BD, 2 (10.5%) of 19 major-typed RAS/BD, 2 (4.5%) of 44 minor-typed RAS/BD, 4 (13.3%) of 30 AG-positive RAS/BD, 0 (0%) of 33 AG-negative RAS/BD, 4 (44.4%) of 9 GPCA-positive.

RAS/BD, 0 (0%) of 41 Abs-RAS/BD, and 0 (0%) of 13 TGA/TMA-positive RAS/BD patients.<sup>4-6</sup> These findings suggest that in the subgroups of BD patients, GPCA-positive RAS/BD patients have the highest frequency of vitamin B12 deficiency (44.4%) and AG-negative, gastric and thyroid autoantibodies-negative, and TGA/TMA-positive RAS/BD patients have the least frequency of vitamin B12 deficiency (0%).

For the folic acid deficiency in the subgroups of RAS/BD patients, the folic acid deficiency was noted in 4 (6.3%) of 63 BD, 2 (10.5%) of 19 major-typed RAS/BD, 2 (4.5%) of 44 minor-typed RAS/BD, 2 (6.7%) of 30 AG-positive RAS/BD, 2 (6.1%) of 33 AG-negative RAS/BD, 1 (11.1%) of 9 GPCA-

positive RAS/BD, 2 (4.9%) of 41 Abs-RAS/BD, and 1 (7.7%) of 13 TGA/TMA-positive RAS/BD patients.<sup>4-6</sup> These findings suggest that in the subgroups of BD patients, GPCA-positive RAS/BD patients have the highest frequency of folic acid deficiency (11.1%) and minor-typed RAS/BD patients have the least frequency of folic acid deficiency (4.5%).

For the hyperhomocysteinemia in the subgroups of RAS/BD patients, the hyperhomocysteinemia was noted in 9 (14.3%) of 63 BD, 4 (21.1%) of 19 major-typed RAS/BD, 5 (11.4%) of 44 minor-typed RAS/BD, 6 (20.0%) of 30 AG-positive RAS/BD, 3 (9.1%) of 33 AG-negative RAS/BD, 7 (77.8%) of 9 GPCA-positive RAS/BD, 2 (4.9%) of 41 Abs-RAS/BD, and 0 (0%) of 13 TGA/TMA-positive RAS/BD patients.<sup>4-6</sup> These findings suggest that in the subgroups of BD patients, GPCA-positive RAS/BD patients have the highest frequency of hyperhomocysteinemia (77.8%) and TGA/TMA-positive RAS/BD patients have the least frequency of hyperhomocysteinemia (0%).

After analyses of the frequencies of anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients and in different subgroups of RAS/BD patients, we further conclude that the major-typed RAS may play a significant role in causing anemia and iron deficiency in

**Table 3** Anemia types of 2 of 13 thyroglobulin antibody (TGA)-positive and/or thyroid microsomaal antibody (TMA)-positive recurrent aphthous stomatitis (RAS)/Behcet's disease (BD) patients (TGA/TMA-positive RAS/BD patients).

Anemia type	Patient number (%)				
	Patient number (%)	MCV	Vitamin B12 deficiency (<200 pg/mL)	Iron deficiency (<60 µg/dL)	Folic acid deficiency (<4 ng/mL)
<b>TGA/TMA-positive RAS/BD patients (n = 13)</b>					
Normocytic anemia	1 (50.0)	80–99.9 fl	0 (0.0)	0 (0.0)	0 (0.0)
Microcytic anemia	1 (50.0)	<80 fl	0 (0.0)	0 (0.0)	0 (0.0)
Total	2 (100.0)		0 (0.0)	0 (0.0)	0 (0.0)

RAS/BD patients and the serum GPCA positivity is the major factor causing vitamin B12 deficiency and hyperhomocysteinemia in RAS/BD patients. It needs further studies to elucidate what factors are most important for causing folic acid deficiency in RAS/BD patients. However, the serum TGA/TMA-positivity is not significantly associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in RAS/BD patients.

## Conflicts of interest

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

## Acknowledgements

This study was supported by the grants (No. 102-2314-B-002-125-MY3 and No. 105-2314-B-002-075-MY2) of Ministry of Science and Technology, ROC.

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