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Short Communication

Evaluation of cytokines as diagnostic and therapeutic indicators for recurrent aphthous stomatitis: A statistical study

Xuan Xiao [a,b†], Yiwen Deng [c,d†], Yuanyuan Long [c], Wei Liu [c,e**], Huan Shi [c,f*]

[a] *Department of Oral Mucosal Diseases, Shanghai Stomatological Hospital & School of Stomatology, Fudan University, Shanghai, China*
[b] *Shanghai Key Laboratory of Craniomaxillofacial Development and Diseases, Fudan University, Shanghai, China*
[c] *College of Stomatology, Shanghai Jiao Tong University, National Center for Stomatology, National Clinical Research Center for Oral Diseases, Shanghai Key Laboratory of Stomatology, Shanghai, China*
[d] *Department of Oral Mucosal Diseases, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[e] *Department of Oral and Maxillofacial–Head and Neck Oncology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[f] *Department of Oral Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

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Abstract Increasing evidence indicates that inflammatory cytokines are involved in the pathogenesis of recurrent aphthous stomatitis (RAS). A wide range of over ten cytokines in peripheral blood of RAS patients have been investigated in different studies. Yet, which of the ones are the most prominent indicators contributed for the process of RAS are uncertain. Herein, a total of 16 eligible case–control studies including 1051 cases of RAS and 616 health controls were summarized. The bubble chart analysis showed that the most prominent cytokines for RAS were interleukin (IL)-6 (646 cases, 308 controls), TNF-α (498 cases, 298 controls), and IL-2 (371 cases, 264 controls). On the other hand, 9 studies on cytokines as therapeutic indicators of RAS were identified. The effect of levamisole and thalidomide on cytokines mainly were

* Corresponding author. Department of Oral Surgery, Shanghai Ninth People's Hospital, Shanghai 200011, China.
** Corresponding author. Department of Oral and Maxillofacial–Head and Neck Oncology, Shanghai Ninth People's Hospital, Shanghai 200011, China.
E-mail addresses: liuweb@hotmail.com (W. Liu), shihuan1312@163.com (H. Shi).
† X. Xiao and Y. Deng contributed equally to this work.

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IL-6, TNF-α, and IL-8. Collectively, an optimum panel of IL-6, TNF-α, and IL-2 maybe serve as the potential significant indicators for RAS investigations.

Introduction

Recurrent aphthous stomatitis (RAS) is the most common oral mucosal disease, and can affect up to 25% of the general population.[1] The clinical symptoms of RAS involvement can severely influence the patients' speaking, swallowing, and eating.[1] Hence, RAS represents an important public health concern due to its high prevalence, associated pain, and impact on the quality of life of affected individuals. It is reported that some predisposing risk factors including genetic susceptibilities, local trauma, psychologic stress, viral and bacterial infections, hormonal disorders, nutritional deficiencies, hematological deficiencies, smoking, and immune-alteration factors have been proposed as causative agents.[1]

Although the precise etiology and pathogenesis of RAS remain unclear, evidence indicates that RAS occurs through epithelial destruction mediated by a cellular immune response.[2] Based on this principle, the aphthous process is accepted to be initiated by stimulation of the mucosal keratinocytes by unknown antigens, leading to T-lymphocyte stimulation and the secretion of various cytokines. Subsequently, the cytokines are believed to be released into peripheral blood, and then play a major role in T cell-mediated immunologic dysregulation of RAS.[1,2] Since interleukin (IL)-6 as a diagnostic and therapeutic indicator for RAS was studied by Chiang C.P. group from Taiwan in 2003,[3] over 10 cytokines were reported with a great variation in different studies on this disease.[4–17]

However, which of the ones are the most prominent indicators contributed for the process of RAS are uncertain, partly due to lack of the summary analysis on this issue. For instance, some investigators selected only 1 cytokine and some choose 10 cytokines in a study. Besides, the investigators in 2 studies utilized entirely different cytokines in the similar study design.[5,6] Therefore, we, to begin with, summarize these publications focused on peripheral blood cytokines in RAS patients; and then attempt to identify an optimum panel of cytokines by a visual analysis. This might provide the pertinent evidence for the investigators to choose rational cytokines into their study design and avoid potential unnecessary research.

Materials and methods

In order to identify the relevant papers on cytokines in peripheral blood of RAS patients, literature searches were performed in Scopus, Web of Science, and PubMed. We searched electronic databases without any restriction following the Cochrane Collaboration and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Key search terms were: aphthous stomatitis, aphthous ulcerations, cytokine, interleukin, blood, plasma, serum, sera, and their equivalents. To identify more results, hand search (checking references of included primary articles and relevant reviews) performed manually. All the observational association studies about the distributional difference of peripheral blood cytokines between RAS patients and healthy controls published up to Jun 10, 2022 have been included in this study. Studies with not available full text and lack of peripheral blood cytokine data, other type of studies such as case reports or conference papers were excluded. Two independent authors (H.S. and W.L.) screened the titles, abstracts, and full text of all publications, and subsequently analyzed the case—control studies.

A descriptive analysis was performed on the parameters of included studies. The data on the number of RAS patients, the number of healthy controls, and the number of the studies with significant results was extracted to carried out a bubble chart analysis. A bubble chart used to visualize and interpret the performance of cytokines in RAS. The bubble chart for each cytokine shows the number of patients/healthy subjects and the number of the studies by the vertical/horizontal axis and bubble sizes. All the bubbles constitute a dashboard where the numbers of the subjects and studies in each theme bubble could be viewed by hovering over each data point. Excel Visual Basic for Applications (Microsoft 365, Seattle, WA, USA) was used to programme a module to plot the bubble chart.

Results

Cytokines as diagnostic indicators

A total of 72 potentially relevant papers was screened, and 56 were excluded with some reasons. As presented in Supplementary Figure S1, 16 eligible articles on over ten cytokines, such as IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, TNF-a, and IFN-γ, were identified for detailed evaluation from the search of the published literature (Table 1). These eligible case—control studies including 1051 cases of RAS and 616 health controls were identified. Serum was the most sample type used in 12 of 16 studies, followed by peripheral blood mononuclear cells and plasma. Enzyme-linked immunosorbent assay was the most method used in 10 of 16 studies to detect the quantitative concentration of cytokines. As shown in Fig. 1, the bubble chart analysis reveals that most prominent cytokines in peripheral blood of RAS patients were IL-6, TNF-α, and IL-2. IL-6 was reported by 8 studies including 646 cases of RAS and 308 controls. TNF-α was confirmed by 9 studies

Table 1 Characteristics of included studies on peripheral blood cytokines in the pathogenesis of patients with recurrent aphthous stomatitis.

Author, year	Country/Region	Cases			Control			Matched factors	Sample	Detection method	Cytokine detected
		n	Age (mean ± SD, y)	F/M	n	Age (mean ± SD, y)	F/M				
Shen et al., 2021[2]	China	127	43.7 ± 12.9	55/72	20	44.2 ± 13.3	10/10	Age, gender	Serum		
Han et al., 2021[4]	China	80	44.4 ± 6.1	40/40	80	44.3 ± 5.8	40/40	Age, gender	Plasma	ELISA	IL-1β, IL-2, TNF-α
Lu et al., 2020[5]	China	60	40.1 ± 2.8	30/30	60	40.3 ± 2.7	30/30	Age, gender	Plasma	ELISA	IL-6, IL-18
Mimura et al., 2017[6]	Brazil	45	NA	16/29	30	37.4	16/14	Age, gender, ethnicity	Serum	MAP Human Cytokine/ Chemokine Magnetic Bead	IL-4, IL-6, IL-10, IL-12p70, IL-17A, TNF-α
Ozyurt et al., 2014[7]	Turkey	24	34.4 ± 10.6	13/11	20	37.0 ± 11.1	11/9	Age, gender	Serum	ELISA	IL-1, IL-13, IL-17, IL-18, IFN-γ, α-enolase
Avci et al., 2014[8]	Turkey	25	NA	NA	25	NA	NA	Age, gender	Serum	ELISA	IL-2, IL-10, IL-12, TNF-a
Gupta et al., 2014[9]	India	30	27.8 ± 10.3	11/19	20	27.3 ± 9.1	8/12	Age, gender	Serum	ELISA	IL-8
Pekiner et al., 2012[10]	Turkey	30	37.0 ± 13.8	18/12	15	30.3 ± 9.0	6/9	Age, gender	Serum	Flow cytometry	IL-2, IL-6
Borra et al., 2009[11]	Brazil	17	31 ± 8.5	10/7	17	33 ± 10.6	10/7	Age, gender	Serum	ELISA	sCD14, TNF-a
Albanidou et al., 2007[12]	Greece	32	35.4 ± 12.0	18/14	40	31.7 ± 13.1	22/18	Age, gender	PBMC	ELISA	IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, IFN-γ
Sun et al., 2006[13]	China, Taiwan	146	39.4	80/66	54	NA	30/24	Gender	Serum	ELISA	TNF-α
Lin et al., 2005[14]	China, Taiwan	67	NA	NA	72	NA	NA	NA	Serum	ELISA	IL-2, IL-4, GM-CSF, sFas, FasL
Lewkowicz et al., 2005[15]	Poland	10	38.2 ± 13.0	4/6	12	NA	NA	Age, gender	PBMC	Flow cytometry	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TGF-β1, TNF-α, IFN-γ
Sun et al., 2004[16]	China, Taiwan	146	39.4	80/66	54	NA	30/24	Gender	Serum	Immulite assay	IL-6, IL-8
Aridogan et al., 2003[17]	Turkey	16	36.8 ± 13.1	8/8	20	33.2 ± 11.4	11/9	Age, gender	Serum	ELISA	IL-4, IL-10, IL-12, IL-13, TNF-α
Sun et al., 2003[3]	China, Taiwan	196	38	107/90	77	NA	44/33	Gender	Serum	Immulite assay	IL-6

Abbreviations: ELISA, enzyme-linked immunosorbent assay; F, female; M, male; NA, not available; PBMC, peripheral blood mononuclear cells; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

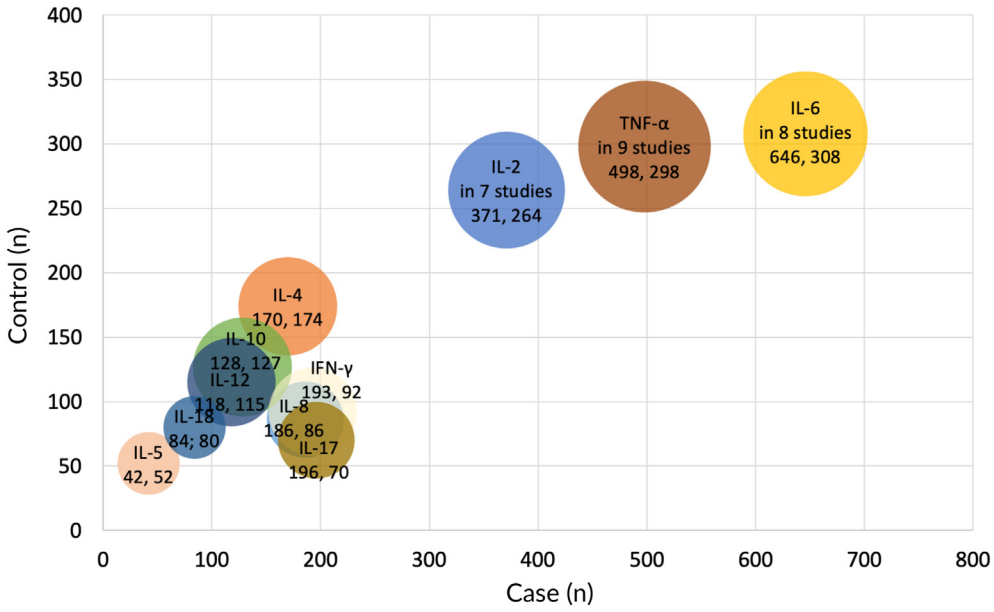

Figure 1 Bubble chart graphically represents the weighted value of the articles on recurrent aphthous stomatitis (RAS) by distinct cytokines. The number in the bubbles represents the sample size of case and control, respectively. The bubble diameter is proportionate to the number of articles included.

including 498 cases and 298 controls. IL-2 was verified by 7 studies including 371 cases and 264 controls.

Cytokines as therapeutic indicators

On the other hand, 9 studies focused on cytokines in peripheral blood as indicators for monitoring therapeutic response of RAS were identified (Table 2). There are 2 randomized controlled trials and 7 comparative studies on this issue. Among cytokines in peripheral blood with significant difference after treatment, the levels of IL-2, IL-4, IL-6, IL-8, IL-18, TNF-α, and IFN-γ were decreased after different systemic therapies, and the levels of IL-1, IL-6, and TNF-α were decreased after a topical treatment. Among these therapies, there were 5 studies on levamisole and thalidomide, which are highly effective at minimizing RAS symptoms and reducing the rate of ulcer recurrence. As systemic immunomodulators, levamisole and thalidomide are accepted to be second in line to systemic corticosteroids for RAS treatment. The effect of levamisole and thalidomide on blood cytokines mainly were IL-6, TNF-α, and IL-8.

Discussion

Increasing evidence indicates that T cell-mediated immune responses are involved in the pathogenesis of RAS, in which cytokines secreted by T helper (Th) cells have been shown to play a major role.[1,2] Thus, research investigating the correlation between inflammation-related cytokines and RAS activity is important for elucidating the pathogenesis of this disease. It is proposed that T-cell mediated immune responses may contribute to a loss of immunological tolerance in the oral mucosa, favoring the occurrence of inflammatory reactions, and the appearance of oral ulcerations.[2] The overall Th cell-related cytokine networks consist of Th1 (IL-1β, IL-2, IL-12p70, TNF-α, and IFN-γ), Th2 (IL-4, IL-5, IL-6, and IL-10), Th17 (IL-17), and cell-death signals (Fas and FasL). A wide range of over ten cytokines in peripheral blood of RAS patients have been investigated in different studies.

Currently, the evaluation of RAS disease severity and therapeutic response mainly rely on the subjective indicators, such as clinical presentation, score of disease activity, and visual analog scale for symptoms. Admittedly, the objective laboratory indicators like cytokines are needed to be investigated and established in clinical practice. To begin with, increasing evidence indicates that inflammatory cytokines are involved in the pathogenesis of RAS. Increased levels of cytokines in peripheral blood of RAS patients compared to control was observed before treatment. Next, decreased levels of blood cytokines in RAS patients was observed after treatment, as expected. Thus, detection of blood cytokines has the potential in monitoring therapeutic response of RAS.[18,19]

Although blood cytokines may be promising minimal invasive biomarkers for monitoring therapeutic response of RAS, there still existed some obvious limitations. First, limited studies reported blood cytokines in RAS treatment, and a great mass of the studies had small sample size. Secondly, different therapies should be prescribed based on the patient's severity of RAS and personal circumstances. The level of the cytokine in RAS patients received different therapies might be varied based on personal inflammatory microenvironment. Thirdly, it is a challenge to make narrowing down of accurate cytokines because the results of the previous studies are varied. For instance, various cytokines adopted diverse cutoff values for the aberrant levels of quantification.

Table 2 Characteristics of included studies on peripheral blood cytokines in the treatment of patients with recurrent aphthous stomatitis.

Author, year	Country /Region	Study design	Case (n)	Treatment	Sample	Detection method	Cytokine detected	Cytokine with significant difference after treatment
Elamrousy et al., 2021[18]	Egypt	Randomized controlled	20	Topical camel whey protein	Serum	Flow cytometry	IL-1, IL-6, TNF-α	IL-1, IL-6, TNF-α
Han et al., 2021[4]	China	Comparative study	80	Not reported	Plasma	ELISA	IL-1β, IL-2, TNF-α	IL-1β, IL-2, TNF-α
Lu et al., 2020[5]	China	Comparative study	60	Systemic thalidomide	Plasma	ELISA	IL-6, IL-18	IL-6, IL-18
Mimura et al., 2017[6]	Brazil	Randomized controlled	22	Systemic symbiotic	Serum	MAP Human Cytokine/ Chemokine Magnetic Bead	IL-4, IL-6, IL-10, IL-12p70, IL-17A, TNF-α	IL-4, TNF-α
Gupta et al., 2014[9]	India	Comparative study	30	Systemic levamisole	Serum	ELISA	IL-8	IL-8
Sun et al., 2006[13]	China, Taiwan	Comparative study	55	Systemic levamisole	Serum	ELISA	TNF-α	TNF-α
Sun et al., 2005[19]	China, Taiwan	Comparative study	19	Systemic Tien-Hsien liquid	Serum	ELISA	IL-2, IL-6, IL-10, TNF-α, IFN-γ	IL-2, IL-6, TNF-α, IFN-γ
Sun et al., 2004[16]	China, Taiwan	Comparative study	82	Systemic levamisole	Serum	Immulite assay	IL-6, IL-8	IL-6, IL-8
Sun et al., 2003[3]	China, Taiwan	Comparative study	45	Systemic levamisole and Chinese medicinal herbs	Serum	Immulite assay	IL-6	IL-6

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

To obtain an optimum proposal panel of cytokines, we use a bubble chart analysis method to summarize the most common cytokines using the existing literature on this issue. Based on the data analysis, we propose that a panel of IL-6, TNF-α, and IL-2 maybe serve as the most prominent indicators for RAS investigations. They may be used as molecular markers to monitor the treatment response during the aphthous active period, and predict the recurrence of RAS during the aphthous stationary phase. This panel serves as a reference for investigators to select the appropriate cytokines in their studies.

In summary, the panel of IL-6, TNF-α, and IL-2 as significant indicators for RAS patients are required to evaluate and verify in more studies with a larger sample size. Furthermore, more clinical trials with a large sample size should be carried out to further confirm the roles of the various cytokines during different therapies in diagnosis and monitoring of RAS.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2022.10.013.

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