

# Does East Meet West?—The Association between Oriental Tongue Inspection and Western Clinical Assays of White Blood Cell Subsets

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Received 27 May 2015; accepted 30 June 2015; published 3 July 2015

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## Abstract

The autonomic nervous system (ANS) controls white blood cell (WBC) subsets; therefore, the status of ANS can be assessed by assaying WBCs. However, this requires invasive blood sampling, time, cost, and training. Therefore, this study focused on a traditional technique, tongue inspection, which is a simpler method. The purpose of this study was to investigate whether there is an association between the traditional method of tongue inspection and clinical assay of WBC subsets. Twenty-one female alopecia areata patients were divided into two age-matched groups: 1) alopecia areata totalis (AT); and 2) alopecia areata multiplex (AM). Images of patient tongues were captured by a digital camera and categorized before blood sampling. Finally, patients were divided into five groups (normal, Yin+, Yang-, Yin- and Yang+) based on the Eight Principles of traditional Chinese medicine (TCM). Concurrently, venous blood was obtained for WBC subsets. The absolute numbers of WBCs and granulocytes of the AT group were higher than those of the AM group. The AT group was Yin+ but not Yang+, whereas the AM group was Yang+ but not Yin+. Thus, the AT group showed more elements of “cold” (Yin > Yang) compared with the AM group with elements of “hot” (Yin < Yang). Tongue inspection suggested a possibility of consistence with those

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**How to cite this paper:** Watanabe, M., Kainuma, E., Tomiyama, C., Oh, Z., Koshizawa, J. and Nagano, G. (2015) Does East Meet West?—The Association between Oriental Tongue Inspection and Western Clinical Assays of White Blood Cell Subsets. *Health*, 7, 801-808. <http://dx.doi.org/10.4236/health.2015.77094>

of WBCs although statistical significance was not obtained. Moreover, some Yin+ and Yang+ subjects showed some trend in similarities between tongue inspection and WBC subsets although this was not statistically significant. Therefore, traditional techniques (such as tongue inspection) acupuncture must be studied further to detect whether subtle effects are induced by acupuncture treatment. As this study is underpowered, a larger scale study including males is required in the future.

## Keywords

**Alopecia Areata Totalis, Alopecia Areata Multiplex, Tongue Inspection; White Blood Cell Subset, Autonomic Nervous System, Traditional Chinese Medicine (TCM)**

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## 1. Introduction

The autonomic nervous system (ANS) modulates the number and ratio of white blood cells (WBC) to maintain their balance [1] [2]. Abo and Fukuda advocated that WBC subsets provide useful information for patients and doctors [3]. They also reported that patients of dermatological diseases, such as atopic dermatitis, showed abnormal WBC subsets [3]. However, to examine WBC subsets, blood must be sampled, which is invasive to patients and requires time, cost, and training.

Therefore, this study focused on a traditional tongue inspection based on several reasons. First, it is considered a non-invasive, easy, and quick method. Second, it has been used for thousands of years and is considered a time-tested and standardized method in the clinical site of acupuncture treatment. Third, it is safe and does not cause infection or tissue damage.

Recently, tongue coating volume, a traditional clinical test item of the tongue inspection, was experimentally assessed by modern clinical techniques with regard to acetaldehyde concentration and reported that physiological acetaldehyde concentration in mouth air was associated with tongue coating volume [4]. Moreover, visual agreement analyses of tongue inspection was mathematically investigated although they could not judge whether a given “moderate” agreement coefficient is sufficient to quantify the reliability of the Eight Principles of traditional Chinese medicine (TCM) diagnostics or not [5].

It is known that the core of diagnosis in TCM is a pattern identification/syndrome differentiation and treatment with inspection and the Eight Principles [5]. They stand on “Yin-Yang” theory (an ancient philosophy as well as a medical theory in ancient China). It is a theory of the energy source of the universe (the ceaseless motion and continual variation of all things in the natural world). It has developed into the Eight Principles (Yang—excess, hot and superficial; Yin—deficit, cold and deep). For example, *Zhang jingyue* described this concept in (*Jingyue-quanshu*) (1624) [6].

The aim of the current study was to obtain evidence of the tongue inspection and then we investigated the association between oriental tongue inspection and western clinical assays of WBC subsets. We compared two types of patients: those with alopecia areata totalis (AT) and alopecia areata multiplex (AM), because we have previous experience of investigating the association between dermatological diseases and WBC subsets [3]. This study might indicate the validity of the tongue test versus WBC analysis to determine the status of the ANS.

## 2. Methods

### 2.1. Subjects

Twenty-one female patients with alopecia areata, who came to Nagano Clinic (Tokyo, Japan) during the period from October 2014 to April 2015 were investigated in this study (age range 29 to 67 years; mean  $45.0 \pm 0.9$  years). This study did not include any cancer patients and patients did not receive chemotherapy or irradiation therapy. Written informed consent was obtained from all subjects and the study was approved by the institutional review board of Nagano Clinic (Tokyo, Japan).

### 2.2. Tongue Inspection

Images of tongues were captured by a digital image camera (cx3, Ricoh, Tokyo, Japan) before blood sampling at

a fixed place in the medical examination room. Images were sent to experienced acupuncture therapists (>20 years experience) who were blinded to the patients. The acupuncture therapists categorized these images into a balanced group and unbalanced group. Then, the unbalanced group was divided into four groups based on the Eight Principles of TCM.

### 2.3. Venous Blood Sampling and WBC Subsets

Venous blood (2 mL) was obtained from the forearm median antebrachial vein for analysis. Leukocyte number and subsets were determined using a hemocytometer and the May-Grünwald Giemsa staining method (Showa Medical Science Corporation, Tokyo, Japan). Thus, the total number of WBCs was examined. The proportion of granulocytes (including neutrophils, basophils, and eosinophil) and lymphocytes was determined.

### 2.4. Statistical Analysis

The difference between the values of two groups (AT and AM) was determined by Student's t-test, Mann-Whitney's U-test and Welch's t-test. Results are presented as the mean  $\pm$  SD. P-values of < 0.05 were considered statistically significant, and all statistical tests were two-tailed.

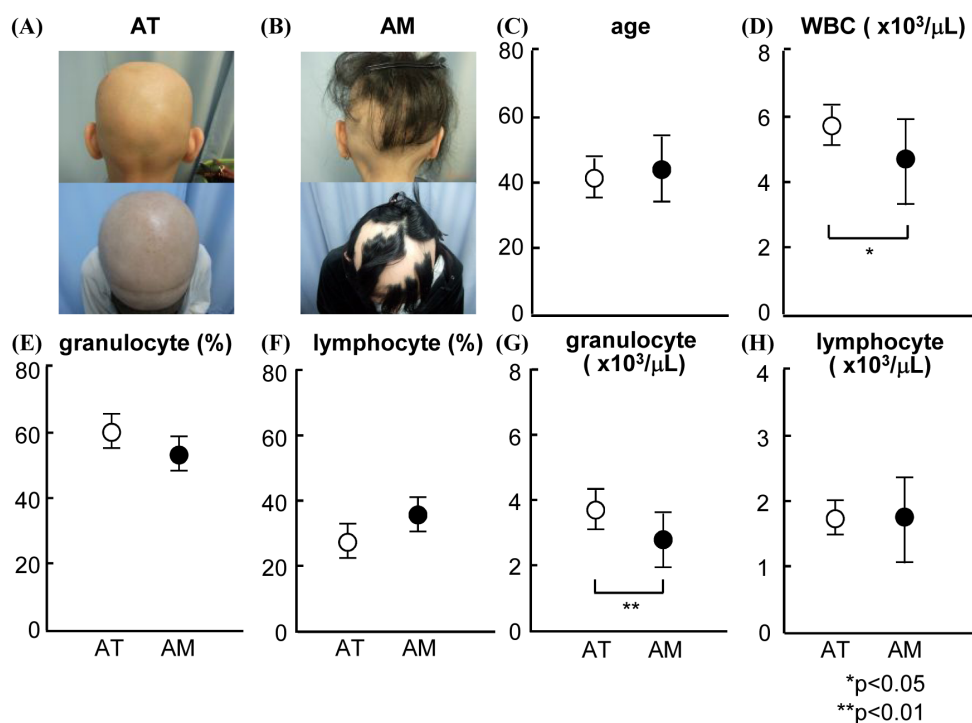
## 3. Results

### 3.1. Age of the AT and AM Groups

Twenty-one female subjects of alopecia areata were divided into two groups by a dermatologist with 35 years of experience. The mean age of the AT group was  $42 \pm 8$  years ( $n = 9$ ) and was  $46 \pm 10$  ( $n = 12$ ) years in the AM group. There was no statistical difference between the two groups (Figures 1(A)-(C)).

### 3.2. WBC Subset

Absolute numbers of WBCs of the two groups were compared (Figure 1(D)). WBC numbers were higher in the



**Figure 1.** Typical images of alopecia areata. (A) The alopecia areata totalis (AT); (B) alopecia areata multiplex (AM); (C)-(G) comparison of the two groups. The numbers of WBCs and granulocytes of the AT group were higher than the AM group. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

AT group compared with the AM group ( $p < 0.05$ ). The mean ratios of granulocytes and lymphocytes in the AT group was  $64\% \pm 5\%$  and  $31\% \pm 5\%$ , respectively, and were  $61\% \pm 6\%$  and  $33\% \pm 7\%$  in the AM group. There was no statistical difference in WBC subsets between the two groups (**Figure 1(E)** and **Figure 1(F)**).

The absolute number of granulocytes and lymphocytes were calculated as follows: (absolute number of WBCs in **Figure 1(D)**)  $\times$  (percentage of granulocytes or lymphocytes (**Figure 1(E)** and **Figure 1(F)** respectively)). Then the obtained numbers were statistically analyzed for each WBC subset (**Figure 1(G)** and **Figure 1(H)**).

The absolute number of granulocytes was higher in the AT group compared with the AM group ( $3982 \pm 588$  vs  $3131 \pm 836$ ;  $p < 0.01$ ). However, no difference was observed for lymphocytes between the two groups ( $1897 \pm 275$  vs  $1667 \pm 482$ ).

### 3.3. Tongue Inspection

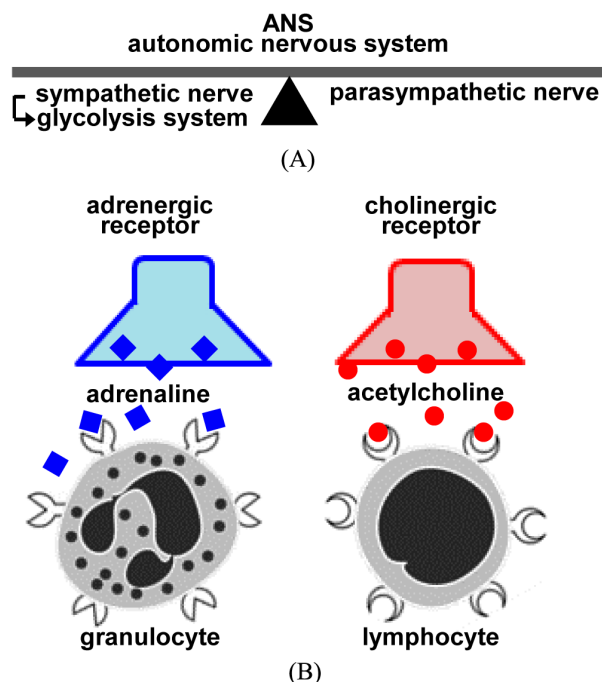
The tongue images were categorized into five groups: balanced [normal] group and four unbalanced groups: Yin+ (*Shihan*, excess coldness), Yang- (*Xuhan*, deficit heat), Yin- (*Xure*, deficit coldness) and Yang+ (*Shire*, excess heat) groups (**Figure 2**).

In the AT group the ratios of those groups were 11%, 56%, 0%, 33%, and 0% (normal, Yin+, Yang-, Yin- and Yang+), respectively. In the AM group they were 0%, 42%, 8%, 42% and 8% respectively (**Figure 3(A)** and **Figure 3(B)**).

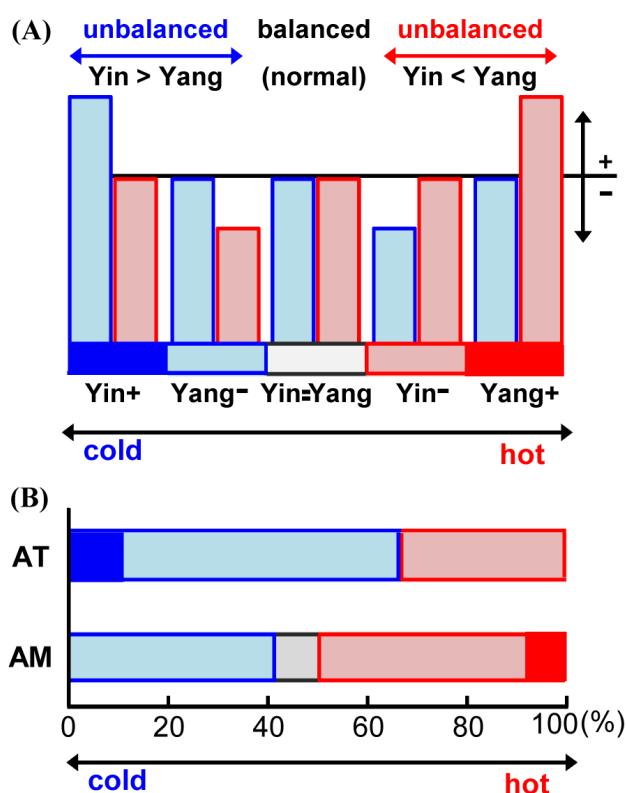
## 4. Discussion

### 4.1. The AT Group Showed More Sympathetic Nerve Dominance than the AM Group

The ANS contains two types of nerves: sympathetic nerves, which are dominant when we suffer stress and parasympathetic nerves that are dominant when we relax. They maintain our health by balancing each other [7] (**Figure 2(A)**). This balance is easily altered by small changes in our health. For example, the duration of sleep can affect this balance [8] [9]. Furthermore, long-lasting stress induces unbalanced internal conditions that might



**Figure 2.** ANS and WBCs. (A) ANS contains two types of nerves. Sympathetic nerves and parasympathetic nerves maintain health by maintaining a balance; (B) granulocytes express adrenergic receptors while lymphocytes express cholinergic receptors on the cell surface.



**Figure 3.** (A) Tongue images were categorized into five groups: a balanced [normal] group and four unbalanced groups: Yin+ (*Shihan*, excess coldness), Yang- (*Xuhan*, deficit heat), Yin- (*Xure*, deficit coldness) and Yang+ (*Shire*, excess heat) groups; (B) results of the tongue inspection. The AT group showed Yin+ (dark blue); however, it did not have Yang+ (vivid red). The AM group showed Yang+ but not Yin+. The AT group showed more elements of “cold” (Yin > Yang) compared to the AM group that contained elements of “hot” (Yin < Yang).

be involved in the onset of various diseases [10]. Because the ANS controls the distribution of WBCs, the status of ANS can be assessed by investigating WBC subsets [1]-[3].

Our results of WBC subsets showed that the sympathetic nerves were more dominant in the AT group compared to the AM group because the AT had higher numbers of granulocytes than the AM group (Figure 1(G)). Because the number of granulocytes was calculated based on the WBC numbers the difference in WBCs between two groups can be understood in the same way as granulocytes (Figure 1(D)).

#### 4.2. Difference in WBC Subsets and ANS Status Might Induce the Differences between the AT and AM Groups

Alopecia is the loss of hair. Patients with multiple areas of alopecia lesions are termed AM and patients who lose all the hair on the scalp are termed AT [11]. There are several theories to explain this condition, including the increase of natural killer (NK) cells in the peripheral blood lymphocytes of patients [12] [13].

Our previous study indicated that under sympathetic nerve dominance, the number, ratio and function of NK cells were enhanced [14] [15]. This is explained by two points. First, sympathetic nerves elevate the level of adrenaline [14]-[16] and second, NK cells express adrenaline receptors [17]-[19]. Interestingly, granulocytes also mainly express adrenergic receptors (lymphocytes express cholinergic receptors on the cell surface) [20] and that granulocytes are stimulated by sympathetic nerves (lymphocytes are stimulated by parasympathetic nerves) (Figure 2). Thus, the prominent differences between the number of WBCs and granulocytes between two groups might indicate patients of those two groups have different ANS balances.

##### 4.2.1. Tongue Inspection and the Potential Increase of Life Energy (ATP)

The most interesting result was found in the tongue inspection. The AT group showed Yin+ (dark blue), but it

did not have Yang+ (vivid red). The AM group showed the opposite. The AT group showed more elements of “cold” (Yin > Yang) compared with the AM group that showed more elements of “hot” (Yin < Yang).

This may be explained in two ways. The first explanation is from modern medicine. Under sympathetic nerve dominance, a fight-or-flight response is induced. At this time, the immediate action of instantaneous force in the white muscles is required supported by quick increases of ATP produced via glycolysis [21]. ATP is often called “life energy” or “energy flow” [22]. Therefore, increased ATP via the glycolysis system might be associated with the *Qi* of TCM. However, further research is required to support this hypothesis.

#### 4.2.2. Life Energy (*Qi*) Propels *Xue* to Maintain Head Hair

A second explanation is based in TCM. *Qi* is known to have various physiological functions, such as warming or propelling. Heat in TCM is not the same as physiological body temperature. However, it is understood that *Qi* has a warming function. The difference between the AT group (cold) and the AM group (hot) in our results may meet this traditional explanation (Figure 3(A) and Figure 3(B)).

Moreover, *Qi* has a propelling function and it is useful to propel *Xue* (the philosophical image of blood in TCM) to flow through the whole body. *Xue* functions to nourish and moisten the body. In “Compendium of Materia Medica (*Bencao wangmu*)”, a classical traditional Chinese doctor, described how “head hair was recognized as a fullness of *Xue*”. Therefore, a deficiency of *Xue* might induce the loss of hair. Therefore, *Qi*, which propels *Xue*, was considered necessary to maintain head hair. Thus, the results of the tongue inspection may be explained in two ways.

#### 4.3. Does East Meet West?

Recently we reported that repetitive manual acupuncture increased markers of innate immunity in mice subjected to restraint stress [23]. This result might agree with the description in TCM, “*Huangdi Neijing*”, because, anger stress might influence the liver.

In addition, we found that acupuncture stimuli were effective only within the range of normal homeostasis [23]. Therefore, in general, statistical differences in the results of alternative medicine (such as acupuncture stimuli) might not be as prominent as those of conventional drugs (such as anti-inflammatory agents, fever reducers, or diabetes drugs). For example, our results of the tongue inspection suggested a certain degree of consistency with those of WBC analysis. Moreover, some subjects in the Yin+ and Yang+ groups showed clearer results in the tongue inspection compared to the WBC subsets although no statistical differences were observed (data not shown). Therefore, traditional techniques such as tongue inspection and acupuncture must be studied to detect subtle effects induced by acupuncture treatment. Thus, a comprehensive approach is needed at the bedside because the tongue inspection only one part of the “four inspections” in TCM (inspection, listening/smelling, inquiring and palpation).

Our study has several limitations. This study may have been underpowered to detect small differences, and further research with larger scale studies including males is required. Moreover, we should obtain samples from healthy subjects to compare with those of the AT and AM groups. Additionally, body temperature and pulse rates obtained by western methods should be assessed as we studied the effect of an ancient type of acupuncture needle [24]-[26]. If we could obtain those data in larger samples, multiple logistic regression analysis could be used to investigate the relationship between the tongue inspection and clinical assays of WBC subsets to contribute to medical advances in the future.

#### Acknowledgements

The authors wish to thank all medical staff and patients who participated in this study.

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