

## Quantitative Aspect of Constitutive Regulation by Leucocyte Subsets in Air Way Inflammation

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### Abstract

**Background:** The purpose of this report was to introduce CAM menu to regulated leucocyte subsets, lymphocyte/granulocyte ratio for allergic patient in case of Asthma, chronic airway inflammation. Within some reports, the percentage of leukocyte subset were different from each individual and age, sexuality and personality, namely constitution. Young age tend to be rich in lymphocyte subset but older age change to granulocyte rich type. So allergic patient may find a way for some CAM therapy out to the symptom in a sense for lymphocyte regulation.

**Method and Participant:** Many menu of CAM were tried to the normal subjects that could control the leucocyte subset. The menu were listed hot-spring hydrotherapy, light exercise, floor heating and some famous decoction of TCM including aroma. Leucocyte subset were traced before and after the CAM menu adjusting the time schedule in order to fit the circadian rhythm.

**Results:** A hot-spring hydrotherapy, light exercise, floor heating and some famous decoction of TCM could downly regulated to the lymphocyte subset. Under the same condition by CAM menu, the young generation down-regulated the number of both subset, but older generation up-regulated in both subset in simultaneous trials. Within a different type of constitution, granulocyte-rich type and lymphocyte-rich type of individual moderately regulated their constitution as to the neutral, in each generation.

### Conclusion:

- 1) The quantitative regulation of total leukocyte in number could be assessed by down-regulated, namely tailored scale for each constitution.
- 2) With each CAM proved to regulate the total number of leukocyte subsets, granulocyte and lymphocyte.
- 3) With each CAM proved down-regulated for granulocyte in number, but up-regulated for lymphocyte in adult and older age.

**Keywords:** Asthma; COPD; Chronic Respiratory Tract Infection; Chronic Cough; Bronchiolitis; Airway Inflammation; Leucocyte Subset; Lymphocyte; Hot-Spring Hydrotherapy; Light Exercise; Floor Heating; Blood Cell Supportive TCM

### Abbreviations

CAM: Complementary and Alternative Medicine, Beside the western medicine, there are many traditional medicine and/or health promoting menu all over the world; CD: Cluster of Differentiation. Each lymphocyte has name that expressed CD number, for example CD2, CD4, etc.; COPD: Chronic Obstructive Pulmonary Disease; DM: Diabetes Mellitus; Emotional Hormone: Adrenaline, Dopamine and Cortisone were selected as emotional hormones for this trial; FCM: Flow Cytometry; G-Rich Type: The individual that exhibit over 60% of granulocyte in peripheral blood, finding many in young gentleman; HCLH: Head Cooling and Leg Heating System; LCHH: Leg Cooling and Head Heating System; L-Rich Type: The individual that exhibit over 40% of lymphocyte in peripheral blood, finding lot in ladies and senile; QOL: Quality of Life; VAS: Visual Analog Scale

### Introduction

An allergic response is fundamentally normal physiological response to the not self substance, immune function. However, in case of Asthma is characterized by chronic airway inflammation, which is associated with bronchial hyper responsiveness, leading to variable airflow limitation and respiratory symptoms [1]. Airway epithelial cells play an important role in innate immune functions in the lungs. Airway epithelial cells also exhibit the characteristics of muco-ciliary cells and physically remove pathogens via a process known as the muco-ciliary movement, which involves the trapping of pathogens in the mucus produced in airways under inflammatory conditions and removing the mucus via the movement of cilia present on epithelial cells [2]. So a prophylactic consideration is very much important to figure out a therapeutic planning. For that purpose, constitution consideration is important for prophylactic aspect for allergic responses. The purpose of this report was introduce the CAM menu that enable to modify the constitution by some menu of CAM by moderate menu not for drastic manner. Abo reported that the leucocyte subset, lymphocyte/granulocyte ratio was different in each individual [3-5]. Then the purpose of this report was to present the importance to write digital word to clarify the condition of each physiological condition, even in an allergic disorder. A choice of health menu in conjunction with each constitution is in a quandary due to lack of information concerning these cross-interactions among general public and lack of information among the health professionals resulting with a potentially significant health scale. In other words, no tentative scale for evaluate the intense of each trial yet. Therefore we have been reported the best candidate for tailored scales were digitally representing lymphocyte/granulocyte ratio and the outcome of regulatory vector expressed as linear function, through hot spring hydro-therapy etc [6-9]. With these reports we reported that the immune system were closely related to the QOL that crosstalk with leukocyte in quantitatively and qualitatively [10-17].

### Subjects and Methods

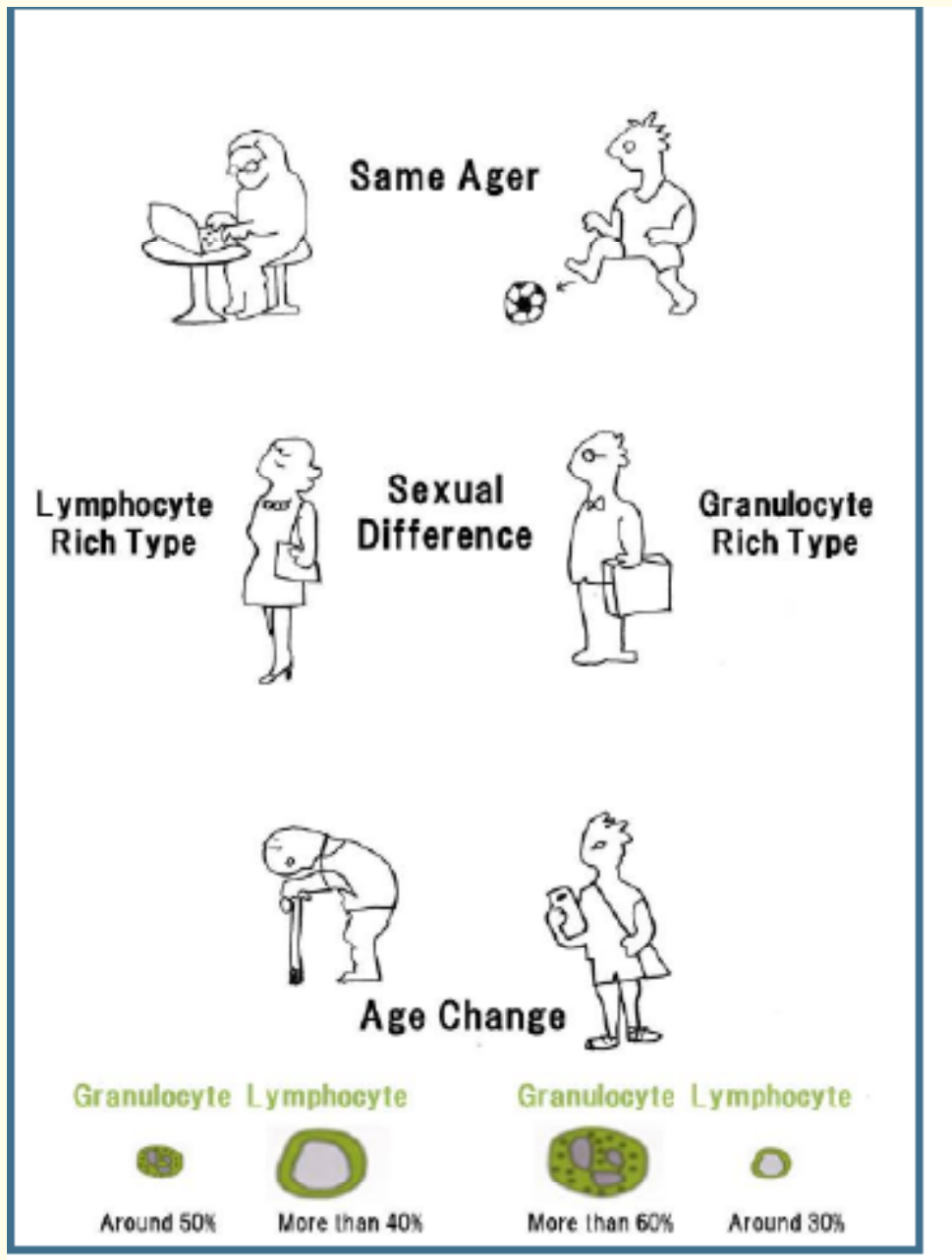
#### Subjects

We recruited 14 healthy attendants (mean age,  $19.5 \pm 10.2$  years, ranging 9 ~ 45 years old in both sexualities) and informed consented according to The Issue Ethics Committee of Kanazawa Medical University. The percentage of sexuality were 45.3% of female and 54.7% for male. Attendants were the university members of Medical University and the stuff and their offspring in the school of medicine. The attendants were divided in to two group and each group was kicked off the menu, the blood sample were collected at the same time of the first sampling. Further one or several weeks after a cooling down, the attendants were exchanged vice versa for the trial.

#### Leucocyte Regulating Menu as CAM

##### Hot Spring Hydrotherapy

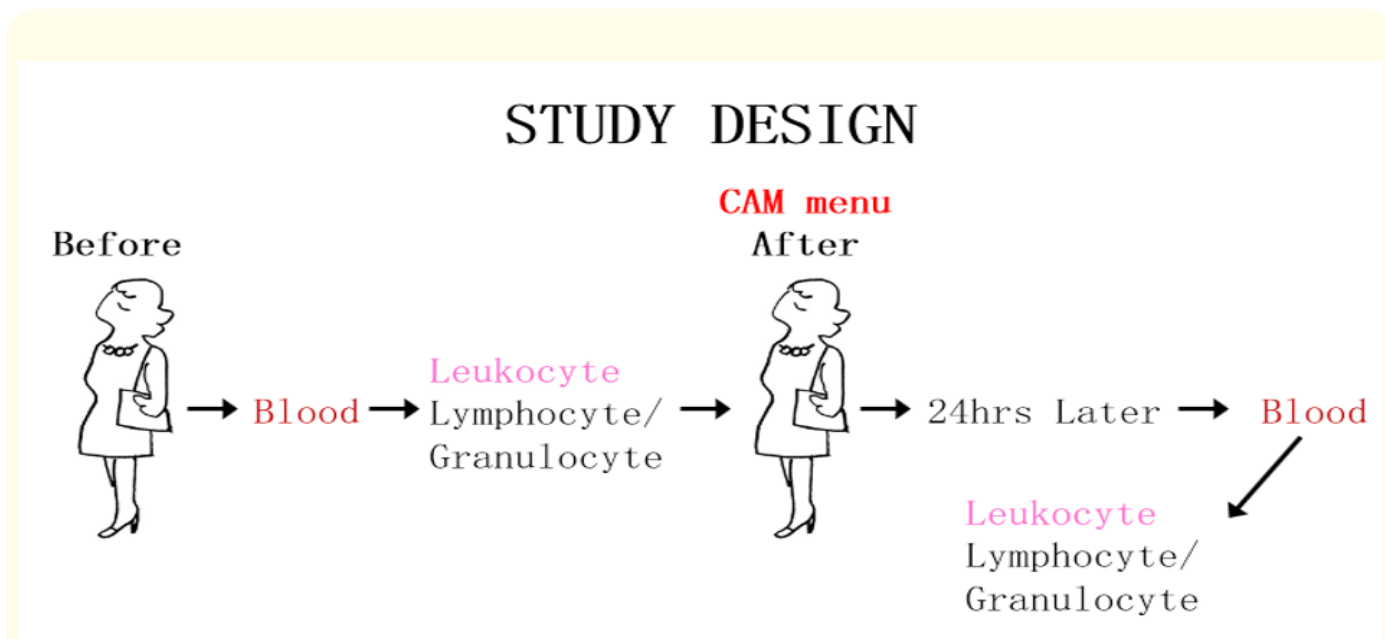
These recruited subjects attended in this study were after giving their informed and consented. We organized the trials by setting the control and experimental group at the same period and same saucers. We prepared peripheral blood from the 14 volunteers before and



**Figure 1:** Conceptual Representation of Human Constitution in Digitally.

There were two type of constitution, according to lymphocyte/Granulocyte ratio, namely Lymphocyte-rich type and Granulocyte rich type. Lymphocyte rich type contains over 40 % of lymphocyte and Granulocyte rich type has over 60% of granulocyte [2,3].

after this study at the same time on each day, in consideration of circadian rhythm of leukocyte [4]. The experimental menu started at ten o'clock for about hours, consecutive for 24 hours. Time interval of blood sampling between before and after the trial was approximately 24 hours. Estimation of the total leukocyte were performed for differential leukocyte counts, and granulocyte and lymphocyte ratio in the peripheral blood. The data were prepared by authorized institution of Ishikawa Prefecture Preventive Medicine Association. The total and differential leukocyte counts were assessed by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).



**Figure 2:** Experimental Schedule.

General experimental design to evaluate each CAM therapy. Time interval of blood sampling between before and after the trial was approximately 24 hours, adjusting circadian rhythm.

**Floor Heating**

**Regulating room temperature 180C by hot air on the ceiling**

Another member subjected in this study were after giving informed and consented. We figured out the purpose of this study by setting the control group at the same interval and same building. The room controlled by hot air heating by electric heater was 180C at the height 150 cm from the foot step floor. After 21 days of cooling down for all attendants, we started again for the same trial exchanging the room for trial. We collected peripheral blood from the same 11 volunteers before and after the trial, at the same time zone on the day, adjusting a circadian rhythm [4] of leukocyte.

### Assessment of lymphocyte subsets by FCM

In order to estimate a CD positive cell, the whole blood was collected from the subjects by blood collection tube containing an anticoagulant EDTA-2K. 100  $\mu$ l of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate (FITC)+CD4, FITC+CD8, FITC+CD16, FITC+CD19, FITC+CD57 (Becton Dickinson Co. U.S.A.), allo-phycoerythrin (APC)+CD8, and APC+CD57 (Beckman Coulter). After washing out with Phosphate Buffered Saline, the cell suspensions were fixed employing a X10 diluted Cell FIX (Becton Dickinson) and analyzed by flow cytometer system, FACS Caliber (Becton Dickinson) [18-20]. The negative controls were prepared PE+streptavidin and the isotype control antibodies to the CD antibodies. After incubating for 0.5 hr at 4°C, these samples were hemolyzed abundant RBC with a 10-times dilution FACS Lysing Solution (Becton Dickinson).

### Statistical Analysis

The statistical analysis along with the groups (before and after trial) for the test of significant difference were calculated by paired t-test and wilcoxon signed-ranks test. As for the examination of the correlation was calculated a spearman's correlation coefficient by rank test. Data are expressed as means  $\pm$  standard error of mean (SE). A P value < 0.05 was regard to be statistically significant.

## Results

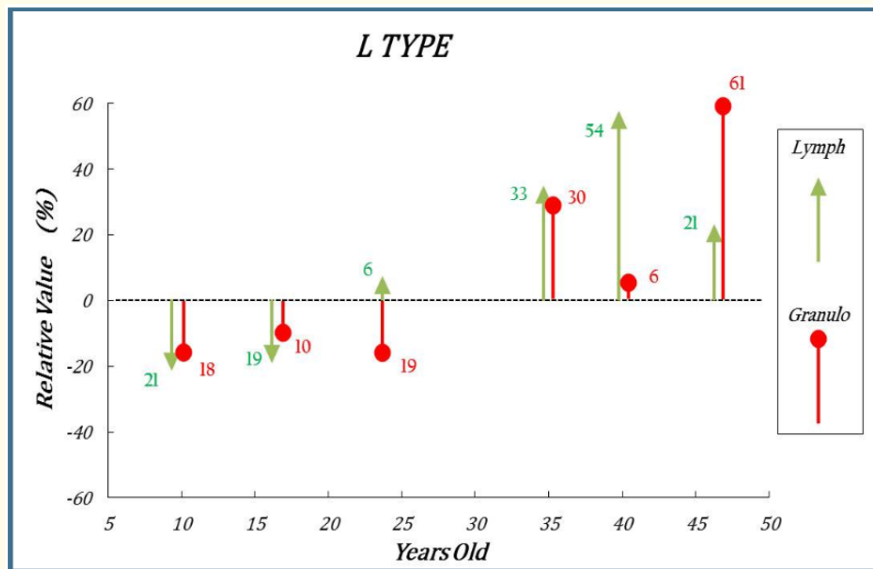
### Hot Spring Hydrotherapy

#### Total and differential leucocyte subset counts

The selected 14 healthy attendants (mean age, 19.5  $\pm$  10.2 years, ranging 9 ~ 45 years old in both sexualities) were informed and consented according to The Ethics Committee of Kanazawa Medical University. The total and differential leukocyte counts in the peripheral blood of 14 members tended to decrease after hot-spring hydrotherapy and the granulocyte counts significantly decreased especially in younger age, on the other hand increased in older age ( $p < 0.05$ ) (Figure 3 and 4). The reproducible result were obtained in the figure, indicating the turning point of vector was 40 years old, in both female and male.

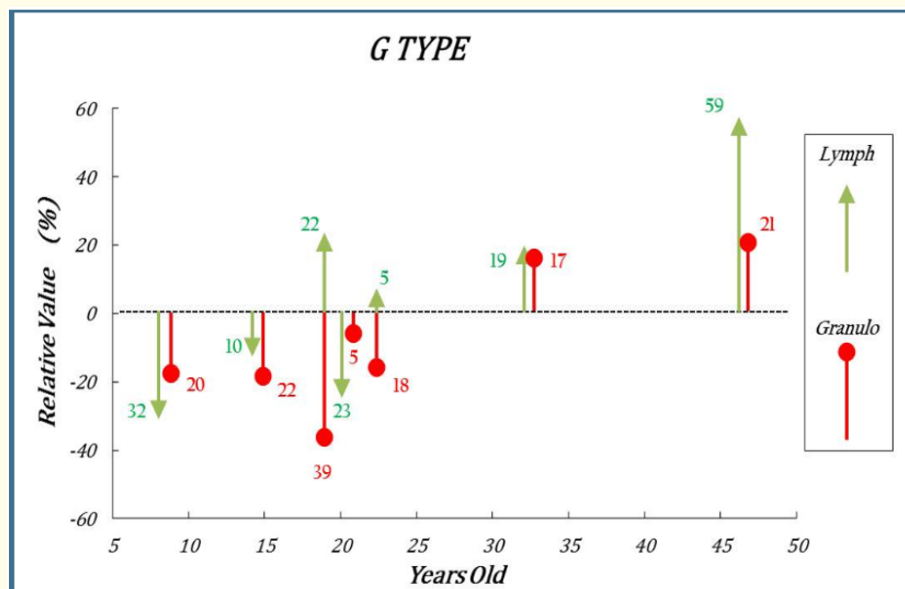
#### FCM analysis of $\beta_2$ -AR expression in lymphocyte subsets

In order to confirm the change in leukocyte was systematic regulation for each constitution or not, we set up to access receptor positive cell together with the emotional hormone reported in simultaneous report. The analysis of  $\beta_2$ -AR positive cells and CD+ cells by FCM was counted by gating in the lymphocytes area on the scattered gram. Nonspecific reaction of the PE fluorescence was found in the isotype control. So, the actual values of the AR positive cell numbers were estimated by subtracting the control one. The CD19 positive cells were observed nonspecific reactions which seems to response of the second antibody. The comparison of each lymphocyte subpopulations before and after hot spring hydrotherapy showed that the CD8 positive cell and CD56 positive cell counts tended to increase. However, the comparison of  $\beta_2$ -AR expressing cells was not seen the significant variation before and after hot-spring hydrotherapy. The mean % of  $\beta_2$ -AR expressing cells in the lymphocyte subsets were 18 - 19% in CD3 positive cells, 5% in CD4 positive cells, 57 - 63% in CD8 positive cells, and 93 - 95% in CD56 positive cells. We confirmed the correlation with the degree of change in adrenaline levels and that of change in  $\beta_2$ -AR positive cell counts of each subset or that in each CD-positive cell counts before and after hot-spring hydrotherapy. In the CD-positive cells, the relative value of change in adrenaline value was a positive relation with which in that of CD56 positive cells, CD8 positive cells, and CD3 positive cells; in particular a correlation with CD56 positive and CD8 positive cells was high. In  $\beta_2$ -AR expressing cells, the percentage of change in adrenaline levels was a positive correlation with the rate of change in the levels of  $\beta_2$ -AR positive CD56 positive cells. These results showed that the variation in adrenaline levels is correlation with CD56 positive cells.



**Figure 3:** Hot Spring Hydrotherapy, Result in L Type ; Lymphocyte Rich Type.

Both vector were indicated according to their age. Each individual was traced both for lymphocyte and granulocyte in number and the vector. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).

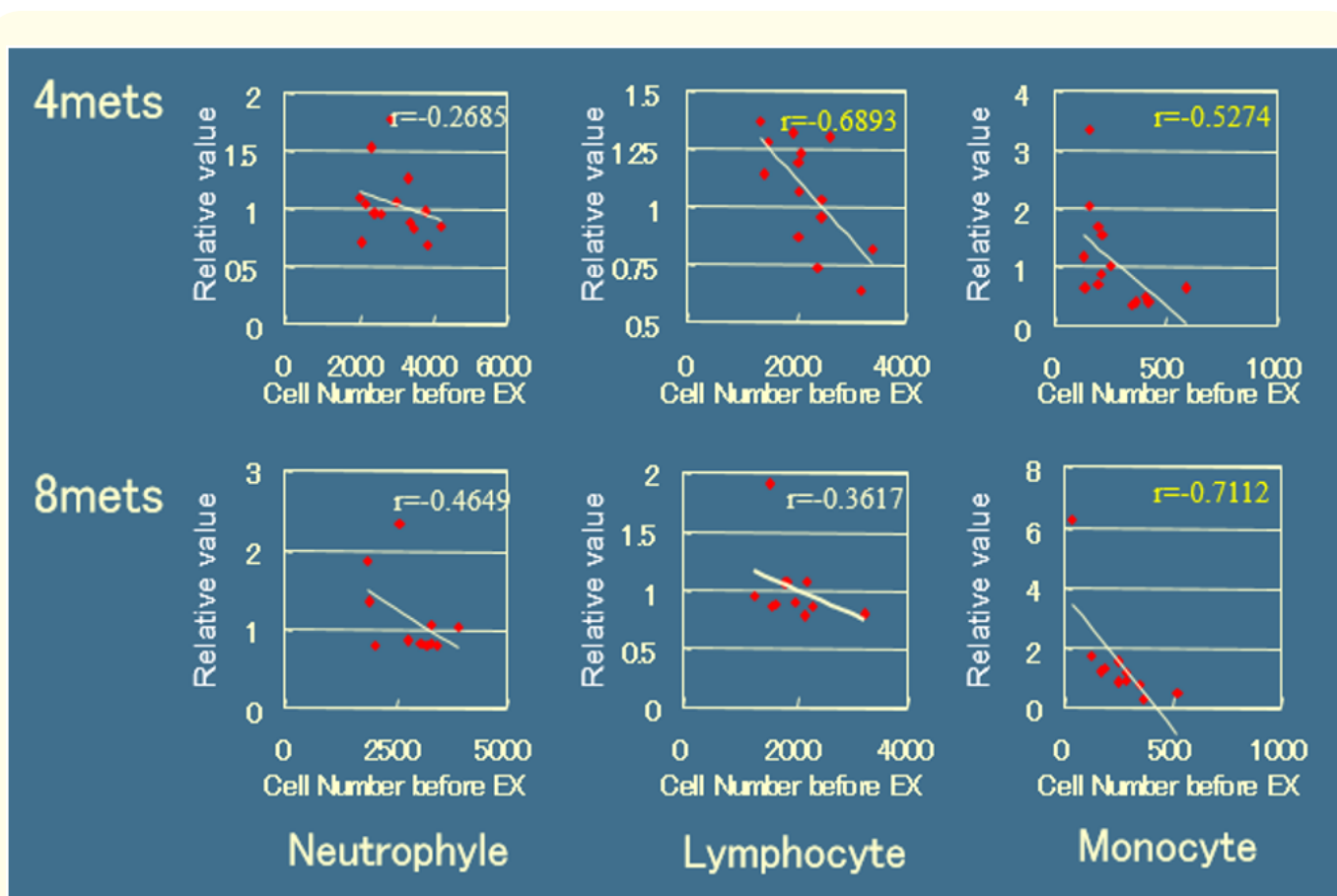


**Figure 4:** Hot Spring Hydrotherapy, Result in G Type; Granulocyte Rich Type.

Both vector were indicated according to their age. Each individual was traced both for lymphocyte and granulocyte in number and the vector. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).

## Light Excessive/Walking by 4 km/60 min and 4 km/30 min

The recruited 14 healthy attendants (mean age,  $19.5 \pm 10.2$  years, ranging 9 ~ 45 years old in both sexualities) were informed and consented according to The Ethics Committee of Kanazawa Medical University. We organized first for the same exercise except for the strength of light exercise was 4 Mets (4 km/hour) for the same kilometer of the same course. We collected peripheral blood from the same 14 volunteers before and after the exercise, at the same time on each day, in matching of circadian rhythm of leukocyte. After 21 days of cooling down for all attendants, we set up again for the same exercise except for the intense of exercise was 4 km/30 min for the same kilometer of the same rout (8 mets). We collected peripheral blood from the same 14 attendants before and after the menu, at the same time on each day, in a sense of circadian rhythm of leukocyte. The data from 4mets but not 8mets exercise was brought an ideal regulation of the leukocyte in number. But, more intense exercise 8mets could not bring such an effect, suggesting the linear slant of leukocyte number exhibited suitable impact for each individual about leukocyte regulation in quantitatively.



**Figure 5:** Light Exercise.

The results were plotted in the x-axis as in each value before the trial. The relative value (%) of post-trial was calculated with before and after and plotted in the y-axis. Total leukocytes, Leukocyte subset, granulocyte and lymphocyte were also traced in the figures. Regulative force was much more in 4 Mets rather than that in 8 Mets, clearly in the Lymphocyte section.

### Acupuncture-Non-transcutaneous System

In practical consideration, wide variation of diagnosis and needling system are used such as meridian, reaction point, trigger point, skin impedance, anatomy, physiology, and so on. These subtype of Japanese acupuncture may due to its position of the medical systems in the world. This system rule out an infectious diseases for the adjustment of the physiological status of the host especially in older age, such in diabetes mellitus. Recently, more advanced acupuncture based on physiology and anatomy of the body has been develop traditionally, each therapy has its own character and efficacy for various syndrome. Through the years, local acupuncture was evaluated for its specific properties and with the advent of better transportation in our separated island, even remote springs in the mountains were visited for their specific medicinal effect. An ontogenetical and phylogenetical consideration of constitution, for the best condition. The major issues to be considered now is that there is no statistically significant difference between real TCM acupuncture member and those of sham acupuncture (minimal acupuncture) group. In the trials, non-transcutaneous acupuncture, Japanese type is characterized by sham needling to non-acupuncture points without de-qi impact. In the figure, within the same age and the sex, even in mankind can select out as G-rich type (granulocyte < 60%), and L-rich type (lymphocyte > 40%). On the other hand, as a stand point of sexuality, the ladies belong to L-rich type but the gentleman belongs to G-rich type. As for the age-related factor, G-rich type of gentleman tend to move from G-rich type to L-rich type within the same sex of senile.

### Floor Heating

#### Leukocyte Regulation after Working in Room, the Room Heating by Floor Heater

After lodging the housing, attendants enjoyed for the aim of trial. The trial for leukocyte regulation written by digital words had been first for floor heating in this report. The system had been developed in North-East part in China and Korean peninsula. The two major subsets, granulocyte and lymphocytes as digital scale for indicating their constitution in the floor heating system as a regulatory menu. As shown in figure 6, the groups was separated out for three, up-regulated individuals, down-regulated one and stood still. The correlation of change was demonstrated as a linear function. Figure 6 showed the best inclination also by floor heating, especially in cooling head and leg heating system. For example, in an individual with a low adrenaline level, the serum level were increased after the trial, while it decreased in another individual which was a higher adrenalin level. The correlation of regulation was expressed as a linear function and significant reverse correlation, suggesting ideal value of correlative index -0.5. The data obtained from floor heating was brought ideal regulation. As is in the leukocyte subset regulation, the ideal regulation of adrenaline and dopamine were found by floor heating system, cooling head and leg heating system but not in cooling leg and head heating system.

### Discussion

The data in this report exhibited that within 24 hours after each CAM, the leucocytes in peripheral blood could controlled significantly, not only in leukocyte total number but also lymphocyte and granulocyte subset ratio. The results indicated that these subsets could also reflect the immuno-competent cells in quantity and quality [21-26]. For example, in an individual with a low granulocyte in number, such number up-regulated after the menu, while it down-regulated in another individual with a higher cell counts. Our results also led us to believe that leukocyte subsets could be an interesting indicator for the efficacy of alternative medicinal therapy. Many ethnic medicine are in place to evaluate Western therapies that aim at healing the symptoms of the illness include complains. Abo reported that according to the lymphocyte subset content, there were two major type according to the number of the leucocyte subsets, namely, lymphocyte-rich type and granulocyte rich type. The lymphocyte rich type showed over 40%, on the other hand granulocyte rich type show over 60% of granulocyte. Each type exhibited different character even in the same age, sexuality and different age (Figure 1). This sorting was evident



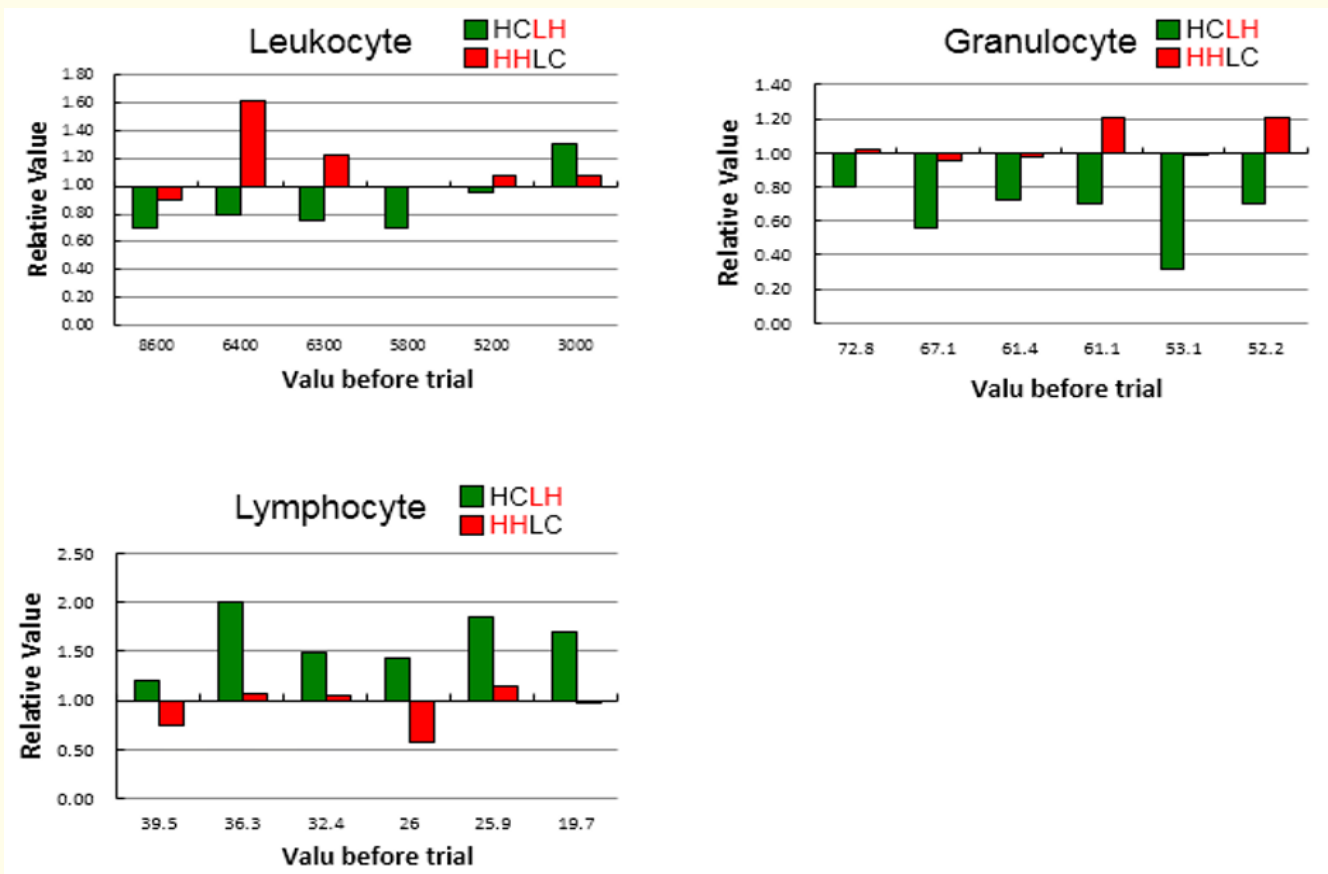


Figure 6: Flower Heating.

We tried to express the effect of peripheral total leukocyte number by individual level (HCLH: Head Cooling and Leg Heating system, LCHH: Leg Cooling and Head Heating system).

## Conclusion

We have measured the number of leukocyte subsets, granulocyte and lymphocyte ratio after different mode of CAM. The conclusions were as following:

- 1) The quantitative regulation of total leukocyte in number could be assessed by down-regulated, namely tailored scale for each constitution.
- 2) With each CAM proved to regulate the total number of leukocyte subsets, granulocyte and lymphocyte
- 3) With each CAM proved down-regulated for granulocyte in number, but up-regulated for lymphocyte in adult and older ager.

- 4) With each CAM proved to regulate the total number of leukocyte subsets, granulocyte and lymphocyte
- 5) With each CAM proved down-regulated for granulocyte in number, but up-regulated for lymphocyte in adult and older age.
- 6) The quantitative regulation of total leukocyte in number could be assessed by down-regulated, namely tailored scale for each constitution.
- 7) With each CAM proved to regulate the total number of leukocyte subsets, granulocyte and lymphocyte
- 8) With each CAM proved down-regulated for granulocyte in number, but up-regulated for lymphocyte in adult and older age.
- 9) With each CAM proved down-regulated for lymphocyte as well as granulocyte in number, especially in young age.

### Conflict of Interests

No conflict of interest hit in this trial.

### Acknowledgement

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