Clinical Observations

Effect of Busui Shengxue Granule (补髓生血颗粒) on Chronic Aplastic Anemia Patients’ Hematopoietic Adhesion Molecule VLA-6/CD₄₉f and Its Ligand Laminin

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Objective: To observe the effect of Busui Shengxue Granule (Herbal granule for replenishing marrow to produce blood) on chronic aplastic anemia (CAA) patients’ integrin a 6 (VLA-6/CD₄₉f) and laminin (Ln).

Methods: Sixty-five patients were divided into experimental group and control group through random number table. There were 34 patients, 17 male and 17 female, aged 2–67, with a medianage of 30.2 ± 8.6, in the experimental group, including 17 patients of kidney-yin deficiency and 17 of kidney-yang deficiency, treated by Busui Shengxue Granule. There were 31 patients in the control group, 16 male and 15 female, aged 4–65, with a medianage of 31.2 ± 8.0; administered Zaizhang Shengxue Tablet (Herbal tablet for chronic aplastic anemia). Both groups were treated for six months and compared with 10 normal persons after the treatment. Flow cytometry was adopted to detect the change in the expression of VLA-6/CD₄₉f receptor in mononuclear cells of CAA patients and normal persons. Enzyme-linked immunosorbent assay was applied to detect the expression of peripheral serum Ln.

Results: CAA patients’ VLA-6/CD₄₉f was in the state of low expression and Ln in the state of high expression. After the treatment, both VLA-6/CD₄₉f and Ln were regulated to some extent and the change in the experimental group was better than that of the control group. Compared with the kidney-yin deficiency patients, those indices of kidney-yang deficiency patients were easier to correct.

Conclusion: The VLA-6/CD₄₉f and Ln expressions of CAA patients are abnormal. The treatment with Busui Shengxue Granule makes both of them improved.

Keywords: anemia; aplastic; adhesion molecule; integrin; laminin

In recent years, British Committee for Standards in Haematology defined Aplastic Anemia (AA) as the state of low medullary hyperplasia and peripheric pancytopenia, excluding marrow infiltration and retinal scleroprotein increase. It mainly means the trait acquired AA, not referring to the aplastic bone marrow after the chemotherapy and radiotherapy. Chronic aplastic anemia (CAA), characterized with slow and concealed onset, is a chronic disease. With effective treatment, it can be eased, however, it can be induced easily too. What’s more, few CAA patients may experience clinical deterioration and turn to acute aplastic anemia, namely SAA-II, which is a critical condition with a poor prognosis. Thus, it is significant to make deep studies on this disease. Cell adhesion molecules (CAM) are transmembrane glycoproteins which exist on the cell surface. The interaction between mediating cell and cell or cell and extracellular matrix may exert influence on cell’s proliferation, differentiation, supersession, transference and dynamic interaction between cells. This research takes myeloid element, stromal cell and peripheric serum in the hematopoietic microenvironment as the main study objects, observing the change of cell adhesion molecules’ receptors and corresponding ligands. The report is as follows.

METHODS

Case Selection

Objects: Sixty-five cases in this research were all hospitalized patients and outpatients during November 2004 to April 2007. They were divided into experimental group and control group through random number table, all group peoples sign the informed agreement. Of the 34 patients in the experimental group, 17 were kidney-yang deficiency and 17 kidney-yin deficiency, 17 were male and 17 female, aged 2–67, with a medianage of 30.2 ± 8.6. Of the 31 cases in the control group, 16 were male and 15 female, aged 4–65, with a medianage of 31.2 ± 8.0. There were 10 people in the normal control group, all healthy blood donors, 5 were male and 5 female, aged 12-65, with a medianage of 32.6±7.5.

Diagnostic Standard:

1. CAA Diagnostic Standard: All patients were primary CAA. Treatment and effect judgment were conducted according to Diagnosis and Therapeutic Effect Criterion of Hematosis.

1) Clinical manifestation: slow onset, slight anemia,
infection, and hemorrhage.

2) Hemogram: hemoglobin decreased slowly. Number of reticulocyte, leucocyte, neutrophil and platelet was more than that of acute aplastic anemia.

3) Hemomyelogram: a) Series 3 or 2 decreased. At least one part experienced undesirable proliferation. If proliferation was good, there would be the increased orthochromatic norm oblasts in erythrocytic series and obvious decrease of megakaryocytes. b) Non-hematopoietic cells and adipocytes in bone marrow increased.

4) Deterioration in clinical symptoms with hemogram and hemomyelogram similar to those of acute aplastic anemia was called heavy aplastic anemia II.

2. TCM syndrome differentiation diagnostic standard for experimental group: Based on the Guiding Principles of Clinical Researches on New Chinese Medicines, the experimental group was subdivided into kidney-yang deficiency and kidney-yin deficiency.

1) Kidney-yang Deficiency
Main symptoms: palpitation, dizziness, pale face and lips, general weakness, cold extremities, soreness and weakness in waist and knees. Secondary symptoms: sexual dysfunction, loose stools, no or slight bleeding, pale tongue, deep thready pulse. There were 17 such cases in the experimental group.

2) Kidney-yin Deficiency
Main symptoms: palpitation, dizziness, pale face and lips, general weakness, night sweating, hemorrhage. Secondary symptoms: low fever, feverish palms and soles, thirst with desire to drink water, dry stools, pale tongue or red tip of tongue, thready rapid pulse. There were 17 such cases in the experimental group.

Exclusion:
Pregnant, breast-feeding women; psychotic patients; those with incomplete clinical data; patients with insufficient treatment (less than 6 months); those not take medicine as required; quitters; those involved in other clinical research in recent three months; patients with uncontrollable hemorrhage or infections, other hemopathic or malignant disease.

Treatment
All patients stopped taking other CAA medicines for more than 2 weeks.

Busuí Shengxue Granule, composed of Shu Di Huang (Radix Rehmanniae Praeparata), Shan Yu Rou (Fructus Corni), Gou Qi Zi (Fructus Lycii), Yin Yang Huo (Herba Epimedii), Ba Ji Tian (Radix Morindae Officinalis), Lu Rong (Cornu Cervi Pantotrichum), Ren Shen (Radix Ginseng), Huang Qi (Radix Astragali), Dan Shen (Radix et Rhizoma Salviae Miltiorrhizae), Ji Xue Teng (Caulis Spatholobi), Bai Hua She She Cao (Herba Hedyotis Diffusae), and Zhu Ling (Polyporus), etc., produced by Liaoyuan Yadong Pharmaceutical Factory, Jilin Province, State Food and Drug Administration approval number Z22025856, 0.35 g/tablet, was applied to the control group, 5 tablets each time, 3 times daily.

Both groups were treated for 3 months as one course and the effect was judged after 2 courses and one year’s follow-up.

Reagents and Instruments
1. VLA-6/CD49f: PE staining Monoclonal antibody CD49f provided by USA BD Pharmingen Company; RCLB flow cytometry provided by USA BD Company FACSort®; test tube provided by Austria Greiner Company Vacuette®; anticoagulant EDTA K2; centrifuge provided by Zhengzhou Medical Equipment Plant.

2. Ln: Ln kit provided by Senxiong Biotechnology (Shanghai) Company; centrifuge: Zhengzhou Medical Equipment Plant; ELISA Clinibio 128, Epson USA.

Test Method
1. Detecting VLA-6/CD49f with Flow cytometry (FCM): Before and after treatment, 3 mL bone marrow drawn from the posterior or anterior superior iliac spine was put into a test tube with EDTA K2 in it. The specimen was processed and dyed within 6 hours after collection. The 100 μL bone marrow with EDTA K2 in it was put into each tube, 10 μL CD45 dyed by PerCP was added to each tube and then 10 μL McAb was added. The mixture should be shaken well and incubated in dark for 20–30 min in normal roomtemperature. RCLB 1 mL was added. Incubation in dark for 10 min in normal roomtemperature was carried out. After centrifugation for 5 min at 1000 rpm, the supernatant was removed. PBS was added for washing 2 times. The volume was expanded to 400–600 μL at the same time. Getting 10,000 cells per tube with flow cytometry. Analysis was done with the Cell Quest 3.0 software.

2. Detecting Ln with ELISA
1) Preparation of Specimen: Draw 2 mL of venous blood from the CAA patients and the donor and put it into the test tube without pyrogen and endotoxin. Centrifugate for 20 min under the circumstance of 4 °C and 3,500 rpm
Take the supernatant and subpackage according to daily need. The supernatant should be in cryostorage at -20°C for research. Pay attention to removing the hemolysis or high blood lipid samples.

2. Operating Procedure: Take out the ELISA plate and add 50 μL standard into each blank micropore in accordance with the order; sign the number of the samples and add 50 μL sample into the blank micropore; add 100 μL enzyme-labelling solution to each pore; incubate at 37°C for 60 min; wash by plate cleaning instrument for 5 times and stew for 10–20 seconds each time; add 50 μL substrate A and B to each pore; incubate in dark for 15 min at 37°C; add 50 μL stop-solution to each pore; measure the light absorption value at a position of 450 nm; calculate the Ln content.

Statistical Analysis
Measurement data should be tested by using t test. The data should be represented as $\bar{X} \pm s$; inter block comparison should apply the pair t test; comparison between groups should employ the method of F test to do ANOVA; paired comparison is to be done by q test. Adopt SPSS 11.0 to analyze the result, take $P<0.05$ as having statistically significant difference.

RESULTS

VLA-6/CD49f Expression of Mononuclear Cells of Bone Marrow
The VLA-6/CD49f expression of the Experimental Group and Control Group before & after treatment (Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>34</td>
<td>23.25±5.71**</td>
<td>56.56±8.16**</td>
</tr>
<tr>
<td>Control</td>
<td>31</td>
<td>24.32±5.42**</td>
<td>46.49±9.33**</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>79.67±9.75</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: Compared with the normal group, *P<0.05, **P<0.01; Compared among the groups, \(\Delta\)P>0.05.

From the Table 1, it can be seen that, before the treatment, the mononuclear cells’ VLA-6/CD49f expression of the experimental group and control group is lower than that of the normal group (P<0.01) and there is no significant difference between the two groups (P>0.05); after the treatment, both of the groups experience restoration and there is a great difference between them (P<0.05); compared with itself before treatment, there is a great difference (P<0.05), however, compared with the normal group, there is still great difference.

The VLA-6/CD49f expression of kidney-yang deficiency and kidney-yin deficiency before & after treatment (Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney yang deficiency</td>
<td>17</td>
<td>25.30±5.81**</td>
<td>61.75±9.23**</td>
</tr>
<tr>
<td>Kidney yin deficiency</td>
<td>17</td>
<td>21.42±5.65**</td>
<td>49.37±7.43**</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>79.67±9.75</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: Compared with the normal group, *P<0.05, **P<0.01; Compared with itself before treatment, \(\Delta\)P<0.05, \(\Delta\Delta\)P<0.01, \(\Delta\)P>0.05; Compared among the groups, *P<0.05, **P<0.01, \(\Delta\)P>0.05.

From Table 2, it can be seen that, before the treatment, the mononuclear cells’ VLA-6/CD49f expression of kidney-yang deficiency and kidney-yin deficiency has no distinctive difference (P>0.05) and is lower than that of the normal group (P<0.01); after the treatment, both of the groups experience restoration of different degrees, compared with before treatment, there is a great difference (P<0.05), compared among the groups, there is a great difference (P<0.05), compared with the normal group, there is still a great difference.

Ln Expression of Peripheral Serum
The Ln expression of the experimental group and control group before & after treatment (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>34</td>
<td>38.31±9.73**</td>
<td>19.41±5.70**</td>
</tr>
<tr>
<td>Control</td>
<td>31</td>
<td>39.44±7.20**</td>
<td>23.72±6.90**</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>15.90±5.15</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: Compared with the normal group, *P<0.05, **P<0.01, \(\Delta\)P>0.05; Compared with itself before treatment, \(\Delta\)P<0.05, \(\Delta\Delta\)P<0.01, \(\Delta\)P>0.05; Compared among the groups, *P<0.05, **P<0.01, \(\Delta\)P>0.05.
It can be seen that the Ln expression between the experimental group and control group before the treatment has no distinctive difference ($P>0.05$) and is higher than that of the normal group, and there is a significant difference compared with the normal group ($P<0.01$); after the treatment, the Experimental group and Control group have a great difference ($P<0.01$), and there is no distinctive difference between the experimental group and normal group ($P>0.05$), but there is still a distinctive difference between the control group and normal group ($P<0.01$). Refer to the inter block comparison, both of the two groups have a great difference ($P<0.01$).

The Ln expression of kidney-yang deficiency and kidney-yin deficiency before & after treatment (Table 4)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney-yang deficiency</td>
<td>17</td>
<td>36.04±9.41**</td>
<td>17.14±4.45*</td>
</tr>
<tr>
<td>Kidney-yin deficiency</td>
<td>17</td>
<td>40.57±9.79**</td>
<td>21.68±6.18*</td>
</tr>
<tr>
<td>Normal group</td>
<td>10</td>
<td>15.90±5.15</td>
<td></td>
</tr>
</tbody>
</table>

It can be seen that in the between-group comparison the Ln expression of the kidney-yang deficiency group and kidney-yin deficiency group before treatment has no significant difference ($P>0.05$) and is higher than that of the normal group, and there is a significant difference compared with the normal group ($P<0.01$); after the treatment, the kidney-yang deficiency group and kidney-yin deficiency group have a great difference ($P<0.05$), and there is no significant difference between the kidney-yang deficiency group and normal group ($P>0.05$), but there is still a significant difference between the kidney-yin deficiency group and normal group ($P<0.05$). Refer to the inter block comparison, both of the two groups have a great difference ($P<0.01$).

**DISCUSSION**

Busui Shengxue Granule is the product of the seminar’s long-term exploration, specific to its pathological change that CAA is closely related to kidney deficiency, the seminar summarizes years of clinical experience, consults the modern pharmacology, takes tonifying the kidney as the treating principle, uses Chinese medicines tonifying the kidney as well as those promoting blood circulation by removing blood stasis and clearing away heat and toxic material, successfully accomplishes the production of Busui Shengxue Granule.

Busui Shengxue Granule is effective in reinforcing the kidney yang, producing marrow, and nourishing the blood. As for the components, Shu Di Huang can tonify the kidney and replenish essence, nourish yin and blood; Ba Ji Tian is effective in tonifying the kidney and supporting yang, nourishing marrow and blood; Hong Shen has the effect of reinforcing vital energy; Ji Xue Teng can promote blood circulation by removing blood stasis, which is in accordance with the old saying “pathogen usually intruding into collateral in protracted disease”. The Chinese herbalist doctors hold that the key cause of CAA is kidney deficiency and the treatment should take tonifying the kidney as the principle. However, based on the theory of “renal yin and renal yang” and “interdependence between yin and yang”, the prescription should contain not only medicines nourishing yang but also those nourishing yin. This can make yin and yang equilibrated, so consequently promote hemogenesis. Tonifying the kidney can ease the symptom of kidney deficiency. What’s more, it can improve and adjust the balance and coordination of neuro-endocrine-immune network fundamentally, and then promote the ability of “hematopoiesis”.

Generally, CAM molecule can be divided into three parts: extracellular part is the N-terminal, which is generally big, with a sugar chain and responsible for distinguishing and combining with its ligand; transmembrane part is mostly single pass; cytoplasmic part is generally small and can link with the skeleton component under the plasmalemma to conduct signals, or has protein tyrosine kinase activity and phosphorylation sites.

Action mechanism of CAM has three modes: mutual recognition and binding between homophilic cell adhesion molecules on the surfaces of two flanking cells (homophilic adhesion); mutual recognition and binding between heterophilic cell adhesion molecules on the surfaces of two flanking cells (heterophilic adhesion); mutual recognition and binding between homophilic cell adhesion molecules on the surfaces of two flanking cells through the extracellular linkers.

With the development of biology and immunology, it is found that adhesion molecule is related to anything happened in our bodies. Consequently, adhesion molecule has been a research focus.

In the preliminary study, we took the myeloid element and stroma cell in the hemopoietic microenvironment as our main study objects and try to observe the change of adhesion molecules and hematopoietic regulatory factors on its surface. The research result shows that adhesion behavior of CAA’s hemopoietic stem cells, hemopoietic progenitors, as well as the adhesion molecules and hematopoietic regulatory factors are abnormal. It is also found that, in the hemopoietic microenvironment, besides the hematopoietic cells and
stromal cells, the abnormality of adhesion molecules also happen to other cells, such as T, B lymphocyte, and granular leukocytes. The abnormality of these cells' adhesion molecules also has an important influence on the nosogenesis of aplastic anemia immunity.

The existing documents and research results show that the compound formulas, which take kidney tonifying and homogenesis as the core, could take effect in CAA treatment from different aspects, such as regulating the immunity, promoting the hematopoietic cell proliferation, and improving the hemopoietic microenvironment, etc. Then, is there a mutual mechanism for the multi-target effect — which affects the expression of the adhesion molecule, sequentially exerts influence on the immune-ology mechanism, hematopoietic microenvironment damage mechanism, and even the apoptosis mechanism? This needs further study.

Pathologic and Physiological Properties of VLA-6/CD49f
VLA-6/CD49f has A and B, two allotypes. The former one is expressed at thymus cells, T cells, monocytes, and platelets. The latter is expressed at brain, ovary and kidney. Both of them are expressed at thymus cells, T cells, monocytes, and platelets. The VLA-6/CD49f expression of activated T cells and memory T cell is increased. VLA-6/CD49f’s ligand is Ln. Ln, combined with VLA-6/CD49f on the T cell, provides the costimulatory signal for T cell’s activation and proliferation. Ln, combined with VLA-6/CD49f, also participates in fetation. Protein kinase C (PKC) activator PMA could stimulate CD49f subunit to be phosphorylated.

It is clear now, hematopoietic stem / progenitor cell homing is a multi-step process, in which its traversing of endothelium is dependent on some homing-related molecules on the surfaces of hematopoietic stem / progenitor cells, especially the expression level of two members of β1 integrin family — CD49e and CD49f.

Effect of Reinforcing Kidney Medicines on VLA-6/CD49f
In this research, the authors found that the VLA-6/CD49f expression level of bone marrow mononuclear cell of CAA patients was significantly lower than that of the normal group (P<0.01). After the treatment with Busui Shengxue Granule, the expression recovered to some extent, but still didn’t reach the normal level (P<0.05). The healing effect on treatment group was better than on control group.

And there was no great difference between the kidney-yang deficiency type and kidney-yin deficiency type (P>0.05). The results suggest that: 1) the expression of adhesion molecule VLA-6/CD49f of CAA patient’s bone marrow mononuclear cell is in a low state; 2) Busui Shengxue Granule could raise the expression level of VLA-6/CD49f and the increase is larger than that of the control group; 3) Busui Shengxue Granule’s effect on Kidney-yang deficiency patients is better than on Kidney-yin deficiency patients (P<0.05). 4) After the treatment with Busui Shengxue Granule, the expression level of VLA-6/CD49f is still different from that of the normal group. This may be caused by small dosage and short medication time. Thus, we speculate that the decline of VLA-6/CD49f expression lowers the ability of hematopoietic stem / progenitor cell migration and homing, and consequently causes CAA.

Pathologic and Physiological Properties of Ln
Ln, one of the main components of cell basement membrane, belongs to the non-collagenous glycoprotein of extracellular matrix and is distributed in the stratum lucidum of basilar membrane. Ln, IV-C, and chondroitin sulfate keep the completeness of basilar membrane.6,7 Laminin is a heterotrimer composed of three polypeptide chains, α, β, γ. Till now, five α chains (α1–α5), three β chains (β1–β3) and two γ chains (γ1, γ2) have been proved. By spin projection and negative stain electron micrograph, it is shown that laminin is a asymmetric cross, composed of a long arm and three similar short arms. Generally, cell doesn’t combine with collagen type IV or proteoglycan directly, but anchors on the basilemma through laminin. There are at least two different receptor binding sites in laminin: the combining site for collagen type IV and combining with the integrin on the cytomembrane through its own R-G-D sequence.

Early in 1980, Terranova found that Ln had the effect of enhancing intercellular adhesion. With the deepening study on laminin, people found its biological function was extremely wide. It has been proved that, through the interaction with cells, laminin could control directly or indirectly activity of cells, such as adhesion or transfer, differentiation and polarization, proliferation or apoptosis and gene expression.

Ln is the ligand of VLA-6/CD49f, the subfamily adhesion molecule of integrin VLA. As the transmembrane glycoprotein receptor, the extracellular domain of VLA-6/CD49f participates in the combination with extracellular matrix ligand and the intracellular domain, linking with intracellular signaling systems and skeleton components, mediates the outward transmit of intracellular signal and inward transmit of extracellular signal.10

In some diseases, Laminin content in the local tissues or body fluid may have some corresponding changes.11 Some research shows that many tumor patients’ serum Ln rises significantly.12 ZHOU, et al,13 adopting the radioimmunoassay, assayed the serum Ln of 63 lymphoma patients and 28 normal persons and consequently found that the Ln level decreased with the ease of disease, rose with the relapse. This could reflect the change of disease state to some extent.
It is found in some researches that myelodysplastic syndrome and acute and chronic leukemia have the condition of Ln rising abnormally and won’t be influenced by the healing effect of chemo-treatment and chronic leukemia’s state. Some other researches show that laminin’s antisense RNA could influence the expression of E-cadherin, the signaling molecule in Transforming Growth Factor (TGF)-β1, and Focal Adhesion Kinase (FAK). WANG, et al, adopting the method of suppression subtractive hybridization, dissociate the splenic cells’ differentially expressed genes of CAA mice which are treated with Jianzhong Decoction (建中汤) of fourteen ingredients and the control mice. The results show that the CAA mice’s gene expression of laminin receptor reduce and Jianzhong Decoction of fourteen ingredients could raise its expression, which may promote the signal transmission between cells. WANG, et al,16 adopting the method of suppression

**Effect of Reinforcing-kidney Herbal Medicines on Ln**

In this research, the authors found that CAA patients’ peripheral serum Ln expression was apparently higher than the normal group’s. Compared with the normal group, the difference was significant (P<0.01). After the treatment, the Ln expression of most patients in the experimental group recovered to the normal level and there was no great difference between these two groups (P>0.05). The marked difference between the control group and the normal group still existed (P<0.01). There was a significant difference between the experimental group and the control group (P<0.01). The same situation appeared between the healing effect of the Kidney-yang deficiency and Kidney-yin deficiency patients (P<0.05). The results suggest that 1) CAA patient’s peripheral serum Ln expression is higher than normal person’s; 2) Busui Shengxue Granule could lower the peripheral serum Ln expression to normal, which is better than the control group; 3) Busui Shengxue Granule’s effect on the Kidney-yang deficiency patients’ peripheral serum Ln expression is different from its effect on the Kidney-yin deficiency patients.

In the very research, the peripheral blood’s Ln, treated by the Chinese medicine, gets close to the normal level. If this result can get a further validation, it will provide our researching for the best effect target of CAA treatment with Chinese medicine with scientific basis. Moreover, different types of CAA patients who use the reinforcing kidney medicines have difference in their Ln. The Ln expression of Kidney-yang deficiency patients reached the level of the normal control group, however, compared with the normal control group, the Ln expression of Kidney-yin deficiency patients still has a difference. For this reason, it seems Ln can be the objective material basis of different clinical effect for different patients.

The authors’ research shows that, as Ln’s receptor, VLA-6/CD49 expression of CAA patient is declining. From this, it can be speculated that VLA-6/CD49’s reduction causes the increase of free Ln in the peripheral blood, which won’t combine with CD49. Ln expression of peripheral blood can reflect the state of aplastic anemia. After the treatment with Chinese medicine, patient’s condition is improved and Ln expression gets close to normal. From another perspective, the Chinese medicine’s regulation on Ln indirectly exerts influence on the expression of TGF-β1, the negative hematopoietic regulator. It lowers TGF-β1’s expression and relieves TGF-β1’s repression on hematopoiesis.

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