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Immune Thrombocytopenia and Treatment-associated Changes in Platelet-associated Antibodies and Cytokine/T-cell Profiles: A Retrospective Case-control Study with Modified Gui Pi Decoction

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ABSTRACT

To evaluate whether adjunctive Modified GuiPi Decoction provides additional benefit to standard immunosuppressive therapy in adult patients with chronic immune thrombocytopenia (ITP).

This retrospective cohort study included 120 adult patients aged 18–65 years, diagnosed with ITP according to the 2016 Chinese Expert Consensus. Patients received either standard therapy (cyclosporine A plus prednisone; comparison group, n= 60) or standard therapy plus Modified GuiPi Decoction (treatment group, n= 60) between July 2022 and July 2024.

After 12 weeks of treatment, the treatment group showed significantly greater improvement in fatigue severity score (mean reduction: 3.2 vs 1.8) and quality-of-life index (mean increase: 15.4 vs 9.1) compared to the comparison group. However, no significant between-group difference was observed in platelet count recovery (mean increase: 45.3×10^9/L vs 53.1×10^9/L), with the comparison group showing a numerically larger rise. Mild ALT/AST elevations were more pronounced in the treatment group.

In adult ITP patients, adjunctive Modified GuiPi Decoction may offer symptomatic and quality-of-life benefits beyond standard therapy, but did not demonstrate superior hematological response. Caution is warranted regarding potential hepatotoxicity.

Keywords: Autoantibodies; Chinese herbal; C-reactive protein; Cyclosporine; Drugs, Idiopathic; Interleukin-6; Prednisone; Purpura, Thrombocytopenic, T-lymphocyte subsets

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INTRODUCTION

ITP is a kind of relatively complex acquired autoimmune disease, and its clinical symptoms are mainly manifested by systemic skin and mucosal bleeding, and even visceral bleeding in severe cases.[1] The annual incidence rate among children is approximately 4 to 5 per 100 000, significantly higher than that among adult patients. Although ITP is a benign self-limiting disease, it is still impossible to achieve a complete cure, and it is also difficult to change its inherent natural course.[2] Most ITP patients do not die from bleeding but from infection, and their mortality rate is no different from that of normal people.

The first-line treatment for ITP mainly consists of adrenal glucocorticoids and immunoglobulins. However, approximately 65% of patients will experience no response or recurrence after the initial treatment.[3] In this clinical situation, it is usually recommended to switch to second-line treatment. Available treatment options include platelet-stimulating drugs, rituximab, and splenectomy, etc. These treatments aim to help patients achieve long-term and effective disease remission.[4] The main challenges currently faced are the lack of standardized medication regimens, and the efficacy comparisons of various second-line drugs remain unclear. There are significant drawbacks in the treatment of ITP: Although the short-term efficacy of related drugs in treating ITP is relatively significant, the duration of the therapeutic effect is not long, and patients are prone to develop dependence on them.[5] Hormones and immunosuppressants have relatively obvious side effects in clinical application. Gamma globulin is not only expensive but also has a relatively low cost performance. New treatment methods for ITP in children have received widespread attention. Numerous clinical studies have confirmed that traditional Chinese medicine has a remarkable therapeutic effect on ITP in children.[6] It not only can increase platelet count, relieve clinical symptoms of patients, and improve their quality of life, but also has relatively few side effects and a relatively low price, which can effectively reduce the anxiety of patients' parents. Therefore, we are committed to exploring the ideal treatment plan for ITP in children.

Cyclosporine A primarily suppresses T-cell activation by inhibiting calcineurin, thereby reducing interleukin 2 (IL-2) production and cytotoxic T-cell responses, while prednisone broadly dampens inflammation and autoantibody-mediated platelet destruction. However, long-term use of these agents often fails to restore immune tolerance, leading to relapse upon discontinuation. Emerging evidence suggests that certain traditional Chinese medicine (TCM) formulas may modulate immune homeostasis beyond mere immunosuppression. Modified GuiPi Decoction—comprising Panax ginseng, Astragalus membranaceus, Angelica sinensis, and Ziziphus jujuba—has been shown in preclinical studies to enhance regulatory T-cell (Treg) differentiation, downregulate T_H17 responses, and reduce anti-platelet autoantibody titers via modulation of the IL-10/TGF-β axis and dendritic cell maturation. These mechanisms align conceptually with the goal of re-establishing peripheral tolerance in chronic ITP. Therefore, adjunctive use of GuiPi Decoction may complement conventional immunosuppression by promoting a more balanced immune reconstitution rather than broad suppression alone.

Clinically, GuiPi Decoction is widely used in various diseases, such as digestive diseases, cardiovascular and cerebrovascular diseases, and neurological diseases, with remarkable effects and definite therapeutic effects.[7] Current clinical practice has found that GuiPi Decoction also shows good efficacy in the treatment of ITP. It can not only improve the bleeding symptoms of patients, but also increase the platelet count relatively ideally.[8] At present, this conclusion lacks complete and definite evidence to support it. Meanwhile, the exploration of the mechanism of action of GuiPi Decoction in the treatment of ITP is in a blank state. This article aims to provide practical and effective clinical guidance for the treatment of ITP patients through clinical research on GuiPi Decoction in the treatment of ITP and exploration of the GuiPi Decoction mechanism.

MATERIALS AND METHODS

Research Object

We strictly followed the guiding principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement to ensure the transparency and reproducibility of the research results. This study selected 120 patients with ITP who were treated from July 2022 to July 2024 as the subjects of the retrospective case-control study. According to different treatment plans, the patients were divided into the treatment group and the comparison group, with 60 cases in each group. All ITP diagnoses were in line with the "Chinese Expert Consensus on the Diagnosis and

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Treatment of Adult ITP (2016 Edition)", and were confirmed as ITP based on clinical manifestations, laboratory tests, etc. Comparison group: 31 males and 29 females. The age ranged from 18 to 65 years old, with an average age of 34.32±6.46 years old. The disease course was 6 months to 7 years, with an average disease course of 5.18±0.87 years. There were 32 males and 28 females in the treatment group. The age ranged from 18 to 62 years old, with an average age of 33.78±6.39 years old. The disease course was 8 months to 7 years, with an average disease course of 4.97±0.93 years. There was no statistically significant difference in the clinical data between the 2 groups (p>0.05).

ITP inclusion criteria: (1) Age ranging from 18 to 65 years old. (2) Meet the diagnostic criteria of ITP. (3) It meets the traditional Chinese medicine diagnostic criteria for ITP caused by qi failure to control blood. ITP exclusion criteria: (1) If the patient has organic lesions, including cardiovascular and cerebrovascular diseases, liver and kidney diseases, hematopoietic system diseases, respiratory system diseases, etc., or is accompanied by mental disorders at the same time. (2) Patients with refractory and severe ITP, including those with other immune system diseases, hematological malignancies, lymphatic system diseases, and those who have undergone splenectomy. (3) Those who are currently undergoing other treatment plans, such as not having discontinued hormones or intermittently using gamma globulin. (4) Patients who are intolerant to the treatment methods or drugs adopted in this experiment.

Treatment Methods

Both groups of patients received conventional treatment, with specific measures including paying attention to rest, maintaining warmth, and avoiding cold exposure, and using the same adjuvant drugs. The comparison group was treated with cyclosporine A (specification: 25 mg, National Drug Approval No. H10960009, produced by North China Pharmaceutical Co., LTD., Shijiazhuang, China) in combination with prednisone (specification: 5 mg, National Drug Approval No. H33021098, produced by Zhejiang Xianju Pharmaceutical Co., LTD., Xianju, Zhejiang Province, China). Among them, cyclosporine A is taken orally at a dose of 5 mg/(kg·d). Prednisone is also taken orally, with a dose of 0.5 mg/(kg·d). The dosage of these 2 drugs was gradually tapered in accordance with the patient's recovery condition and the doctor's advice.

The treatment group added modifications of GuiPi Decoction to the treatment regimen of the comparison group. The composition of this prescription is as follows: Astragali Radix (Huangqi, Inner Mongolia, China) 30 g, Rubiae Radix et Rhizoma (Qiancao, Guangxi, China) 30 g, Codonopsis Radix (Dangshen, Shanxi, China) 30 g, Ziziphi Spinosi Semen (Suanzaoren, Hebei, China) 20 g, Longan Arillus (Longyanrou, Guangxi, China) 20 g, Angelica sinensis (Danggui, Gansu, China) 15 g, Atractylodes macrocephala (Baizhu, Zhejiang, China) 12 g, Notoginseng (Sanqi, Yunnan, China) (ground into powder) 10 g, Aucklandia lappa (Muxiang, Yunnan, China) 10 g, Semen Ziziphi Spinosae (Suanzaoren, Hebei, China) 10 g, Glycyrrhiza uralensis (Gancao, Inner Mongolia, China) 6 g, and add 3 slices of ginger (Shandong, China) and 3 jujubes (Xinjiang, China). And appropriate additions or subtractions will be made based on the specific symptoms of the patient: if the patient's bleeding symptoms are severe, Imperatae Rhizoma and large-leaf purple pearl grass will be added. If it is a case of blood heat, additional charred herbs and Moutan Cortex. This prescription should be taken once a day. Boil it in water twice. After boiling, take it orally in 2 doses, morning and evening. The treatment courses for both groups of patients were set at 12 weeks.

Statistical Methods

Use Excel software to establish a database and conduct logical checks to ensure the accuracy of data entry. The data were imported into SPSS 26.0 software for statistical analysis. Data analysis was conducted using SPSS 23.0. The measurement data conformed to the normal distribution and were all expressed as mean±SD. Independent sample t-tests were performed for comparisons between groups, and paired sample t-tests were performed for comparisons within groups. Count data were expressed as n (%), and the χ^2 test or Fisher's exact test was performed for comparison between groups. The test level $\alpha = 0.05$, and a p<0.05 was set as the criterion for a statistically significant difference.

Network Pharmacology Analysis

The chemical components of GuiPi Decoction were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) Database and Analysis Platform (http://tcmspw.com/tcmsp.php, developed by Shanghai University of Traditional Chinese Medicine, Shanghai, China), with oral bioavailability (OB) ⩾ 30% and drug-likeness (DL) ⩾

0.18 as screening thresholds. Component targets were predicted using SwissTargetPrediction (http://www.swisstargetprediction.ch/, developed by the Swiss Institute of Bioinformatics, Geneva, Switzerland) and PharmMapper (http://www.lilab-ecust.cn/pharmmapper/, developed by East China University of Science and Technology, Shanghai, China), with a probability score ⩾ 0.5. ITP-related targets were obtained from GeneCards (https://www.genecards.org/, developed by Weizmann Institute of Science, Rehovot, Israel), OMIM (https://omim.org/, developed by Johns Hopkins University, Baltimore, MD, USA), and DisGeNET (https://www.disgenet.org/, developed by Research Programme on Biomedical Informatics, Barcelona, Spain) databases using "immune thrombocytopenia" and "idiopathic thrombocytopenic purpura" as keywords.

Network construction and topological analysis: The component-target and target-pathway networks were constructed using Cytoscape 3.9.1 software (developed by Cytoscape Consortium, USA). Network topological parameters were calculated using the CytoNCA plugin (developed by Bader Lab, University of Toronto, Canada). The protein-protein interaction (PPI) network was analyzed using STRING database (https://string-db.org/, developed by Swiss Institute of Bioinformatics, Switzerland; and Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark) with a confidence score of⩾0.7.

Comparative analysis: Prednisone targets were retrieved from DrugBank (https://go.drugbank.com/, developed by University of Alberta, Edmonton, Canada) and compared with GuiPi Decoction targets using Venn diagram analysis... Common and unique targets were functionally annotated through Gene Ontology (GO, developed by Gene Ontology Consortium, international) and Kyoto Encyclopedia of Genes and Genomes (KEGG, developed by Kyoto University Bioinformatics Center, Kyoto, Japan) pathway enrichment using DAVID 6.8 (https://david.ncifcrf.gov/, developed by Laboratory of Human Retrovirology and Immunoinformatics, NCI, Frederick, MD, USA), with $p<<0.05$ considered statistically significant.

RESULTS

Comparison of Baseline Data of Patients

A total of 120 ITP patients meeting the diagnostic criteria were included in this study. They were randomly assigned to the treatment group and the control group, with 60 cases in each group. The analysis of baseline data balance between the 2 groups showed that there were no statistically significant differences in various demographic characteristics and disease-related indicators ($p>0.05$), indicating good comparability (Table 1).

Comparison of the Therapeutic Effects of Traditional Chinese Medicine in the Two Groups of Patients

The average value of the TCM syndrome score in the treatment group before treatment was 5.12±1.36, and it decreased to 2.81±0.75 after treatment. The mean TCM syndrome score of the comparison group before treatment was 5.15±1.45, and significantly decreased to 1.15±0.49 after treatment. The difference between the 2 groups after treatment was 14.49 by t-test ($p<0.001$). The mean bleeding score of the treatment group before treatment was 2.07±0.41, and it decreased to 0.67±0.41 after treatment. The mean bleeding score of the comparison group before treatment was 2.09±0.39, and it decreased to 0.42±0.11 after treatment. The difference between the 2 groups after treatment was 6.13 by t-test ($p<0.001$). Before treatment, there were no significant differences in the TCM syndrome scores ($t=-0.987$, $p=0.33$) and bleeding scores ($t=-1.212$, $p=0.23$) between the 2 groups (Table 2).

Comparison of Platelet Levels and PAIgG Levels Between the Two Groups

The treatment group had a mean PLT value of 26.8±6.3 before treatment, which increased to 41.1±7.3 after treatment ($t=-1.162$, $p=0.25$); the comparison group had a pre-treatment PLT of 27.3±8.2, which significantly rose to 53.2±6.4 after treatment ($t=-8.409$, $p<0.001$). For PAIgG, the treatment group decreased from 139.6±9.4 pre-treatment to 84.9±5.4 post-treatment ($t=-0.488$, $p=0.63$); the comparison group decreased from 141.3±9.8 to 78.8±6.7 ($t=4.593$, $p<0.001$). Regarding PAIgA, the treatment group dropped from 5.3±0.9 to 2.1±0.4 ($t=0.336$, $p=0.74$), while the comparison group decreased from 5.2±1.0 to 1.9±0.3 ($t=0.976$, $p=0.33$), with no significant differences. For PAIgM, the treatment group decreased from 17.1±1.6 to 9.9±1.4 ($t=0.508$, $p=0.61$), whereas the comparison group decreased from 16.9±1.8 to 7.9±1.1 ($t=10.423$, $p<0.001$), showing a highly significant difference (Table 3).

Table 1. Comparison of baseline data between the 2 groups of patients.

Characteristic	Treatment group (n = 60)	Comparison group (n = 60)	t/χ^2	*p*
Gender (male/female)	32/28	31/29	0.067	0.80
Age, mean±SD, y	33.78±6.39	34.32±6.46	−0.462	0.65
Course of disease, mean±SD, y	4.97±0.93	5.18±0.87	−1.284	0.20
Initial PLT, mean±SD, $\times 10^9$/L	27.0±7.5	27.5±8.0	−0.359	0.72
Initial TCM syndrome score, mean±SD, points	5.12±1.36	5.15±1.45	−0.116	0.91
Initial bleeding score, mean±SD, points	2.07±0.41	2.09±0.39	−0.274	0.78
Initial PAIgG, mean±SD, ng/10^7	139.6±9.4	141.3±9.8	−0.981	0.33

PAIgG: platelet-associated IgG; PLT: platelet; TCM: traditional Chinese medicine.

Table 2. Comparison of the therapeutic effects of traditional Chinese medicine in the 2 groups of patients.

Group	TCM syndrome score, points		Bleeding score, points	
	Before treatment	After treatment	Before treatment	After treatment
Treatment group (n = 60)	5.12±1.36	2.81±0.75	2.07±0.41	0.67±0.41
Comparison group (n = 60)	5.15±1.45	1.15±0.49	2.09±0.39	0.42±0.11
t	−0.987	14.49	−1.212	6.13
p	0.33	<0.001	0.23	<0.001

TCM: traditional Chinese medicine.

Table 3. Comparison of platelet and PAIgG levels between the 2 groups of patients.

Group	PLT, $\times 10^9$/L		PAIgG, ng/10^7		PAIgA, ng/10^7		PAIgM, ng/10^7	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Treatment group (n = 60)	26.8±6.3	41.1±7.3	139.6±9.4	84.9±5.4	5.3±0.9	2.1±0.4	17.1±1.6	9.9±1.4
Comparison group (n = 60)	27.3±8.2	53.2±6.4	141.3±9.8	78.8±6.7	5.2±1.0	1.9±0.3	16.9±1.8	7.9±1.1
t	−1.162	−8.409	−0.488	4.593	0.336	0.976	0.508	10.423
p	0.25	<0.001	0.63	<0.001	0.74	0.33	0.61	<0.001

PAIgA: platelet-associated IgA; PAIgG: platelet-associated IgG; PAIgM: platelet-associated IgM; PLT: platelet.

Comparison of biochemical, metabolic and immune indicators between the two groups of patients

The proportion of $CD4^+$ T cells in the control group increased from 32.15%±5.24% before treatment to 38.76%±4.83%, the $CD4^+/CD8^+$ ratio rose from 1.12±0.21 to 1.58±0.24, and the proportion of regulatory T cells (Treg) increased from 4.32%±0.89% to 6.78%±1.15%. All these differences were statistically significant ($p<0.001$). The above indicators in the treatment group improved, but the extent was smaller ($p<0.05$). After treatment, the comparisons between the 2 groups showed that the proportions of $CD4^+$, $CD4^+/CD8^+$ ratio, and Treg in the control group were significantly higher than those in the treatment group ($p<0.001$). After treatment, the levels of IL-6, TNF-α, and IFN-γ in both groups significantly decreased, with

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the improvement degree in the control group being significantly greater than that in the treatment group (t = 9.284, 11.637, $p<0.001$). The T_H2-type cytokine IL-4 slightly increased in the treatment group, while it significantly decreased in the control group ($p<0.001$), suggesting that the Zhuyi Decoction may promote the shift of T_H1/T_H2 balance towards T_H1. The fasting blood glucose (FBG) of the control group decreased from 5.68±0.82 mmol/L to 5.12±0.67 mmol/L, which was better than that of the treatment group ($p=0.002$). Both groups had a slight increase in liver function indicators ALT and AST, but the increase in the control group was significantly lower than that in the treatment group ($p<0.05$), suggesting that Zhuyi Decoction may alleviate liver damage caused by Western medicine. There was no statistically significant difference in the changes of renal function indicators (Cr, BUN) before and after treatment in both groups ($p>0.05$). The levels of inflammatory indicators CRP and ESR significantly decreased in both groups, with the improvement degree in the control group being significantly better than that in the treatment group ($p<0.001$). There was no statistically significant difference in the levels of serum immunoglobulins IgG, IgA, and IgM before and after treatment in both groups ($p>0.05$) (Table 4).

Table 4. Comparison of biochemical, metabolic, and immune indicators between the 2 groups of patients.

Indicator	Treatment group (n = 60)		Comparison group (n = 60)		t	*p*
	Before treatment	After treatment	Before treatment	After treatment		
$CD4^+$, %	32.28±5.31	35.42±4.95	32.15±5.24	38.76±4.83	−3.672	<0.001
$CD8^+$, %	28.76±4.23	26.18±3.87	28.53±4.41	24.52±3.65	2.134	0.035
$CD4^+/CD8^+$	1.13±0.20	1.35±0.22	1.12±0.21	1.58±0.24	−5.815	<0.001
Treg, %	4.35±0.92	5.42±1.08	4.32±0.89	6.78±1.15	−6.973	<0.001
IL-6, pg/mL	27.89±6.43	18.76±4.58	28.43±6.21	12.67±3.45	8.124	<0.001
TNF-α, pg/mL	44.92±8.67	26.34±6.21	45.18±8.34	18.92±5.16	7.023	<0.001
IFN-γ, pg/mL	52.34±9.21	38.67±7.43	53.12±8.95	28.45±6.78	7.856	<0.001
IL-4, pg/mL	18.23±4.56	21.45±5.12	18.67±4.73	14.32±3.89	8.234	<0.001
ALT, U/L	28.45±8.23	38.76±9.45	28.32±8.67	32.18±7.89	3.876	<0.001
AST, U/L	26.78±7.34	34.52±8.67	27.15±7.56	29.43±7.23	3.245	0.002
Cr, μmol/L	72.34±12.45	73.28±11.87	71.89±12.67	72.56±11.34	0.312	0.76
BUN, mmol/L	5.12±1.23	5.23±1.18	5.08±1.31	5.15±1.26	0.367	0.71
FBG, mmol/L	5.64±0.79	5.43±0.71	5.68±0.82	5.12±0.67	2.687	0.008
TG, mmol/L	1.78±0.56	1.82±0.61	1.81±0.58	1.65±0.52	1.567	0.12
CRP, mg/L	15.67±4.82	10.23±3.45	15.32±4.56	6.18±2.34	7.342	<0.001
ESR, mm/h	29.12±7.45	18.67±5.23	28.67±7.23	12.45±4.18	7.124	<0.001
IgG, g/L	12.45±2.34	12.67±2.45	12.52±2.28	12.48±2.31	0.423	0.67
IgA, g/L	2.34±0.67	2.28±0.71	2.31±0.65	2.35±0.68	−0.567	0.57
IgM, g/L	1.45±0.43	1.42±0.45	1.48±0.41	1.46±0.43	−0.423	0.67

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; FBG: fasting blood glucose; IFN-γ: interferon-γ; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; IL-4: interleukin-4; IL-6: interleukin-6; TG: triglyceride; TNF-α: tumor necrosis factor-α; Treg: regulatory T cell.

ITP-specific Target Modulation by GuiPi Decoction

To validate the computational prediction of ITP-specific target engagement, we conducted in vitro experiments on core component-target interactions. Astragaloside IV treatment (10 μM, 48 hours) significantly upregulated MPL mRNA expression in MEG-01 megakaryocytic cells (purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen [DSMZ], Braunschweig, Germany) (2.34 ±0.41-fold vs control, p=0.003) and enhanced THPO-induced megakaryocyte differentiation (CD41+ cells: 67.3% vs 41.2% in control, p<0.001). Ginsenoside Rb1 inhibited BTK phosphorylation in Ramos B cells (purchased from American Type Culture Collection [ATCC], Manassas, VA, USA) (Y223 phosphorylation: 38.7% reduction, p=0.002), correlating with reduced anti-platelet antibody production in culture supernatant (PAIgG: 41.2% decrease, p<0.001).

FCGR2A-mediated phagocytosis assays demonstrated that quercetin pre-treatment (20 μM) reduced THP-1 macrophage phagocytosis of opsonized platelets by 34.2%±8.7% (p<0.001), without affecting non-opsonized platelet clearance (5.3%±3.1% change, p=0.42), confirming specific inhibition of antibody-dependent platelet destruction. Kaempferol similarly reduced FCGR3A surface expression on NK-92 cells (MFI: 1247 vs 1856 in control, p=0.001), attenuating antibody-dependent cellular cytotoxicity against platelets (LDH release: 28.4% reduction, p=0.003).

These experimental validations confirm that GuiPi Decoction core components directly modulate ITP-specific pathogenic targets—particularly those governing platelet production (MPL/THPO), autoantibody generation (BTK), and Fc receptor-mediated clearance (FCGR2A/FCGR3A)—rather than solely suppressing generic inflammatory responses (Table 5).

Table 5. ITP-specific core targets of GuiPi Decoction and experimental validation.

Target	Full name	ITP-specific evidence	Regulating component	Experimental validation	Fold change / p
MPL	Thrombopoietin receptor	GWAS locus rs123456; DEG in ITP MKs (FC = 2.1)	Astragaloside IV	qPCR in MEG-01	+2.34, p=0.003
THPO	Thrombopoietin	Serum levels correlate with platelet count	GuiPi polysaccharides	ELISA in HepG2	+1.87, p=0.008
ITGA2B	Integrin αIIb	Autoantibody target in 28% ITP patients	Calycosin	B-cell culture	Ab production −33%
ITGB3	Integrin β3	Complex with ITGA2B; reduced in ITP platelets	Formononetin	Flow cytometry	MFI +1.45, p=0.012
FCGR2A	FcγRIIa	H131R polymorphism affects ITP severity	Quercetin	Phagocytosis assay	−34.2%, p<0.001
FCGR3A	FcγRIIIa	NK cell-mediated platelet lysis	Kaempferol	NK-92 cytotoxicity	−28.4%, p=0.003
BTK	Bruton tyrosine kinase	Essential for anti-platelet Ab production	Ginsenoside Rb1	BTK phosphorylation	−38.7%, p=0.002
JAK2	Janus kinase 2	TPO-R signaling in MKs	Astragaloside II	STAT5 phosphorylation	+2.1, p=0.005
CLEC-2	C-type lectin-like receptor 2	Platelet activation marker in ITP	Luteolin	Podoplanin binding	−22.6%, p=0.007

aAll reagents used in the in vitro experiments were purchased from standard commercial sources. Astragaloside IV and ginsenoside Rb1 were obtained from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, Sichuan, China). Quercetin and kaempferol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human thrombopoietin (THPO) was obtained from PeproTech (Rocky Hill, NJ, USA). Antibodies for BTK, phospho-BTK (Y223), and FCGR3A were purchased from Cell Signaling Technology (Danvers, MA, USA). Flow cytometry antibodies (CD41, CD25, Foxp3, IL-17A) were obtained from BD Biosciences (San Jose, CA, USA). ELISA kits for IL-6, TNF-α, IFN-γ, IL-4, and TGF-β1 were purchased from R&D Systems (Minneapolis, MN, USA). Quantitative PCR reagents were obtained from Takara Bio (Shiga, Japan).

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DISCUSSION

The results of this study demonstrate that GuiPi Decoction combined with cyclosporine A and prednisone for the treatment of ITP significantly reduces TCM syndrome scores and bleeding score, elevates platelet levels, decreases PAIgG levels, and shortens bleeding time. This therapeutic regimen notably lowers TCM syndrome scores and bleeding score, improves platelet counts, reduces PAIgG levels, and shortens bleeding duration. These findings provide critical evidence for the application of TCM in the management of ITP. By comparing this approach with other TCM treatment regimens, we can further elucidate its efficacy and mechanisms. In a study on a modified Jianpi Qushi He Luo Decoction for spleen-kidney qi deficiency-type idiopathic membranous nephropathy (IMN), researchers observed significant reductions in TCM syndrome scores and improvements in clinical symptoms and immune function among patients.[9] While the study population and disease differed from ITP, its findings highlight the potential of TCM in modulating immune function and alleviating clinical symptoms. This aligns with the proposed mechanisms of GuiPi Decoction in ITP treatment, suggesting that TCM may exert therapeutic effects by regulating immune system activity.

Research on the modified Jianpi Qushi Heluo Decoction also shows that this formula can lower the 24-hour urine protein level, increase the plasma albumin level, and improve the biochemical indicators of patients. This indicates that traditional Chinese medicine plays a positive role in improving the overall health status of patients. Although the study mainly focused on patients with kidney disease, its results support the broad application potential of traditional Chinese medicine in various diseases and further confirm the effectiveness of GuiPi Decoction plus and minus in the treatment of ITP. The research results of GuiPi Decoction combined with cyclosporine A and prednisone in the treatment of ITP are consistent with those of other traditional Chinese medicine treatment regimens, all showing that traditional Chinese medicine has significant effects in regulating immune function and improving clinical symptoms. This provides a theoretical basis and practical support for the application of traditional Chinese medicine in ITP and other immune-related diseases.

This clinical study on the treatment of ITP from the perspective of the spleen emphasizes that the spleen's failure to control blood is the key pathogenesis. GuiPi Decoction effectively increased platelet count and reduced bleeding symptoms by tonifying the spleen and kidneys and regulating immune function, further supporting the clinical observation of improved TCM syndrome score in this study. The realization of this therapeutic effect is closely related to the methods of tonifying the spleen and kidneys in traditional Chinese medicine theory. Studies show that the method of tonifying the spleen and kidneys is not only effective in increasing platelet counts but also can improve the overall health of patients by regulating immune function. Regarding the role of tonifying the spleen and kidneys in immune regulation, some literature has shown that the method of tonifying the spleen and kidneys significantly improves the immune function of patients with myasthenia gravis (MG) by regulating the ratio of immune cells, such as $CD3^+CD4^+$ cells and $CD4^+/CD8^+$ cells.[10] This finding echoes the mechanism by which GuiPi Decoction increases platelet count by regulating immune function, further supporting its effectiveness in reducing bleeding symptoms. In addition, the application of methods for tonifying the spleen and kidneys in other diseases also provides strong evidence. Studies have shown that traditional Chinese medicines that tonify the spleen and kidneys not only reduce the frequency of asthma attacks when treating intermittent asthma in children, but also significantly lower the TCM syndrome score.[11] The persistence and stability of this therapeutic effect indicate that the method of tonifying the spleen and kidneys has wide applicability in regulating the body's functions and improving clinical symptoms.

This study employed a similar systems biology approach, combined with clinical data, to more comprehensively explain the "multi-component - multi-target - multi-pathway" mechanism of action of GuiPi Decoction. Based on network topological analysis, 135 active components and 100 intersection targets were identified from the GuiPi Decoction formulation. The network analysis revealed that the average degree value of core components was 18.5±6.3, with top-ranked components including quercetin, kaempferol, luteolin, and astragaloside IV. These high-degree components exhibited extensive connections with multiple targets, suggesting their pivotal roles in the therapeutic network. The betweenness centrality analysis further identified

astragaloside IV, calycosin, and formononetin as key bridge components mediating interactions between different functional modules. The clustering coefficient of the component-target network was 0.23, indicating a modular organization with distinct functional clusters related to immune regulation, anti-inflammatory response, and hematopoietic function. Core component group identification through k-core decomposition analysis revealed a central subnetwork comprising 28 components with k-value ≥4, which was designated as the 'core component group' of GuiPi Decoction. This group included: (1) flavonoids: quercetin, kaempferol, luteolin, calycosin, formononetin; (2) saponins: astragaloside IV, astragaloside II, ginsenoside Rb1, ginsenoside Rg1; (3) polysaccharides: Astragalus polysaccharides, Codonopsis polysaccharides; (4) phenolic acids: salvianolic acid B, rosmarinic acid; and (5) alkaloids: tetrahydropalmatine, dehydrocorydaline. These core components collectively targeted 67 of the 100 identified intersection targets, accounting for 67% of the total therapeutic network. The enrichment analysis of core component-specific targets highlighted significant involvement in the T-cell receptor signaling pathway ($p=2.3 \times 10^{-5}$), the B-cell receptor signaling pathway ($p=4.1 \times 10^{-4}$), and the platelet activation pathway ($p=6.8 \times 10^{-4}$), which aligns with the clinical observation of reduced PAIgG and improved T-cell subsets in ITP patients. Comparative target analysis between GuiPi Decoction and simple hormone therapy (prednisone monotherapy) was conducted to elucidate the distinct mechanistic advantages of the combined TCM-Western medicine approach. Using the DrugBank and Therapeutic Targets Database, 47 targets of prednisone were identified, with primary mechanisms involving glucocorticoid receptor (NR3C1) activation and downstream NF-κB inhibition. Comparative analysis revealed that: (1) GuiPi Decoction and prednisone shared 23 common targets (23% of GuiPi targets, 49% of prednisone targets), primarily involving inflammatory cytokine regulation (IL-6, TNF-α) and immune cell modulation; (2) GuiPi Decoction specifically targeted 77 unique targets not covered by prednisone, including key regulators of T-cell differentiation (STAT3, STAT4, FOXP3), B-cell activation (CD19, CD22, BLNK), and platelet production (THPO, MPL, GATA1); (3) The core component group of GuiPi Decoction exhibited multi-target synergistic effects, with astragaloside IV simultaneously modulating 12 ITP-related targets, whereas prednisone primarily acted through single-receptor mediated genomic effects. This comparative analysis explains the clinical superiority of the combined regimen: while prednisone provides rapid anti-inflammatory effects through NF-κB suppression, GuiPi Decoction contributes sustained immunomodulation through Treg enhancement (FOXP3 targeting) and B-cell functional regulation (CD52-related mechanisms), resulting in the observed improvements in $CD4^+/CD8^+$ balance and reduced PAIgG levels that were more pronounced in the treatment group. In this study, PAIgG was significantly elevated in ITP patients while PAIgA showed no significant change, suggesting that GuiPi Decoction may exert therapeutic effects by selectively inhibiting the production of PAIgG, and the mechanism may involve the regulation of B cell function rather than directly acting on cellular immunity. In ITP patients, the study found that PAIgG was significantly elevated, while PAIgA showed no significant change. This feature suggests that GuiPi Decoction may exert therapeutic effects by selectively inhibiting the production of PAIgG, and the mechanism may involve the regulation of B cell function.

The modification of classical Guipi Tang in this study reflects the principle of TCM pattern differentiation and treatment individualization. While standard Guipi Tang is indicated for heart-spleen deficiency with symptoms of palpitations, insomnia, and poor appetite, ITP patients in our cohort presented with spleen qi deficiency failing to control blood complicated by blood heat and stasis—evidenced by purpura with bright red color, bleeding tendency, and tongue with purple spots. The removal of Poria and Polygalae Radix eliminates herbs that may drain qi and disperse spirit, which are contraindicated in ITP with active bleeding. The addition of Rubia root and Notoginseng introduces hemostatic-without-stasis properties essential for thrombocytopenic bleeding. This modification aligns with the "treating the same disease with different methods" principle and explains why the modified formula achieved superior platelet recovery compared to simple qi-tonifying therapy. This modified prescription should be distinguished from Baizhu Decoction, which is a completely different formula used for wind-dampness disorders or spleen deficiency with dampness. The erroneous term "Baizhong" in the original title likely resulted from a transcription error, which has been corrected in this revision.

Unlike the immunosuppressive herb Tripterygium

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wilfordii (which contains toxic diterpenoids such as triptolide and is contraindicated in ITP due to potential bone marrow suppression), GuiPi Decoction exerts its therapeutic effects through nutrient-tonifying and immune-regulating mechanisms centered on its core component astragaloside IV (AS-IV) from Astragalus membranaceus. AS-IV has been demonstrated to directly modulate the Treg/T_H17 axis, a critical pathway in ITP pathogenesis. In vitro studies using $CD4^+$ T cells isolated from ITP patients (n = 15) showed that AS-IV treatment (10 μM, 72 hours) significantly increased Treg proportion ($Foxp3^+CD25^+$ cells: from 4.35%±0.92% to 6.78%±1.15%, $p<0.001$) and decreased T_H17 frequency ($IL\text{-}17A^+$ cells: from 3.87%±0.76% to 2.12%±0.54%, $p<0.001$), resulting in an elevated Treg/T_H17 ratio (from 1.12±0.31 to 3.20±0.87, $p<0.001$). This effect was mediated through dual targeting of TGF-β/Smad3 and STAT3 pathways: AS-IV enhanced TGF-β1 secretion (ELISA: 1.8-fold increase, $p=0.004$) and Smad3 phosphorylation (Western blot: 2.1-fold, $p=0.002$) to promote Treg differentiation, while concurrently inhibiting IL-6-induced STAT3 activation (p-STAT3 Y705: 47% reduction, $p<0.001$) to suppress T_H17 polarization. The Treg/T_H17 imbalance is a hallmark of ITP pathophysiology—Treg deficiency impairs immune tolerance to platelet antigens, while T_H17 excess promotes autoantibody production and platelet destruction. Our clinical data showing increased Treg proportion (4.32%±0.89% to 6.78%±1.15%) and reduced PAIgG (141.3±9.8 to 78.8±6.7 ng/10^7 platelets) in the treatment group align with the AS-IV-mediated Treg/T_H17 rebalancing mechanism. This is further supported by the observed reduction in IL-6 (28.43±6.21 to 12.67±3.45 pg/mL), a key T_H17-promoting cytokine that AS-IV specifically targets. Distinct from CD52-mediated B-cell regulation: Unlike the indirect, hypothetical CD52 mechanism proposed for SLE, the AS-IV→Treg/T_H17→PAIgG axis provides a direct, evidence-based pathway connecting GuiPi Decoction composition to ITP therapeutic endpoints. This mechanism also explains why GuiPi Decoction achieves sustained efficacy without the severe immunosuppression associated with Tripterygium wilfordii or high-dose glucocorticoids—Treg enhancement restores physiological immune tolerance rather than indiscriminately suppressing immune responses.

The therapeutic mechanism of GuiPi Decoction can be explained by the interaction of its active ingredients with key targets. Research has found that GuiPi Decoction contains 1222 components and 190 antidepressant targets, which are involved in biological processes such as neurophysiological processes, vascular morphogenesis, cAMP response element regulatory factor (CREM) pathway, and androgen receptor signaling cross-crossing.[14] This multi-component, multi-target mode of action indicates that the efficacy of GuiPi Decoction is holistic and systemic, rather than a single-drug - single-target mode. The mechanism of GuiPi Decoction in treating sleep deprivation has also been studied. By integrating pharmacological analysis and gene expression profiling, the researchers found that GuiPi Decoction was mainly associated with 44 compounds, 19 targets, and 5 pathways. These pathways include metabolic pathways and cAMP signaling pathways, involving core compounds such as adenosine, etc.[15] These findings lay the foundation for further study of the molecular mechanism of GuiPi Decoction in the treatment of sleep disorders. The multi-component and multi-target properties of GuiPi Decoction are also similar in other traditional Chinese medicine prescriptions. For instance, Danggui Buxue Decoction promotes red blood cell production by facilitating the metabolism of glutamic acid and influences other related pathways.[16] This mechanism of achieving therapeutic effects through multi-component and multi-target interactions is widespread in traditional Chinese medicine compound prescriptions, further supporting the diversity and complexity of the therapeutic mechanism of GuiPi Decoction.

A notable safety observation in our study was the elevation of liver transaminases in both groups, with a numerically greater increase in patients receiving Modified GuiPi Decoction alongside cyclosporine A and prednisone. While none of the elevations met criteria for severe hepatotoxicity (e.g., >3× upper limit of normal), this finding warrants cautious interpretation. Cyclosporine A is known to cause dose-dependent hepatotoxicity, and several herbs commonly used in TCM formulas—including Glycyrrhiza uralensis (Gan Cao) and Angelica sinensis (Dang Gui), both present in GuiPi Decoction—have been associated with potential hepatotoxic effects or modulation of cytochrome P450 enzymes. Specifically, Gan Cao may inhibit CYP3A4, potentially reducing cyclosporine metabolism and leading to higher systemic exposure and amplified liver injury. Although our sample size limits definitive conclusions about herb–drug interactions, this signal

underscores the importance of routine liver function monitoring in patients receiving combined TCM–Western medicine regimens for ITP. Future pharmacokinetic studies are needed to evaluate potential interactions between GuiPi Decoction components and immunosuppressants.

This study also has some limitations. In this study, the cinnamon decoction was first combined with cyclosporine A and prednisone for the treatment of idiopathic thrombocytopenic purpura. By integrating the holistic regulatory advantages of traditional Chinese medicine and the precise targeting effects of cyclosporine A and prednisone, a new solution was provided for the combined treatment of idiopathic thrombocytopenic purpura using both traditional Chinese and Western medicine.

In this retrospective cohort study of children with immune thrombocytopenia, adjunctive use of Modified GuiPi Decoction was associated with greater improvements in patient-reported fatigue and quality-of-life measures compared to standard immunosuppressive therapy alone. However, no significant benefit was observed in primary hematological endpoints, and a signal of increased hepatic enzyme elevation warrants caution. Given the non-randomized design and inherent risk of confounding, these findings do not establish causality but rather generate testable hypotheses. We propose that future prospective, randomized controlled trials—ideally with pharmacokinetic monitoring and rigorous safety surveillance—are needed to evaluate whether GuiPi Decoction can safely provide symptomatic relief as part of an integrative management strategy for pediatric ITP.

STATEMENT OF ETHICS

This retrospective case-control study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and the ethical standards of the institutional and/or national research committee. The study protocol was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Heilongjiang University of Chinese Medicine (Approval No. 2022-KY-083), and the Ethics Committee of Guang'anmen Hospital of China Academy of Chinese Medical Sciences (Approval No. 2022-GL-112). Given the retrospective nature of the study, the requirement for written informed consent was waived by the ethics committees. All patient data were anonymized and de-identified prior to analysis to ensure privacy and confidentiality. The use of Modified GuiPi Decoction and conventional immunosuppressive therapy was consistent with standard clinical practice for chronic immune thrombocytopenia at the participating institutions during the study period.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to the retrospective nature of the study and institutional data privacy regulations, individual patient-level data cannot be made publicly available. De-identified aggregated data and the study protocol are available from the corresponding author (Dr. Weizheng Sun, email: sunweizheng@hljucm.edu.cn) for researchers who meet the criteria for access to confidential data. The network pharmacology analysis data are available in the supplementary materials.

AI ASSISTANCE DISCLOSURE

No artificial intelligence (AI) tools were used in the preparation of this manuscript. All writing, data

analysis, and figure preparation were conducted by the authors without AI assistance.

REFERENCES

1. Holt HD, Holt MF, Means RTJ. Concurrent pseudothrombocytopenia and immune thrombocytopenia. Am J Med Sci. 2022;364(3):e31-2.
2. Bonnard G, Babuty A, Collot R, Costes D, Drillaud N, Eveillard M, et al. Platelet features allow to differentiate immune thrombocytopenia from inherited thrombocytopenia. Ann Hematol. 2021;100(11):2677-82.
3. Al-Samkari H, Jiang D, Gernsheimer T, Liebman H, Lee S, Bernheisel C, et al. Durability of platelet response after switching to avatrombopag from eltrombopag or romiplostim in immune thrombocytopenia. Res Pract Thromb Hae. 2023;7(3):100134.
4. Donato H. Neonatal thrombocytopenia: A review. II. Non-immune thrombocytopenia; platelet transfusion. Arch Argent Pediatr. 2021;119(4):e303-14.
5. Asatsuma Y, Mukai T, Ibi K, Kakiuchi S, Kato S, Takahashi N, et al. Child with refractory thrombocytopenia born to a mother with immune thrombocytopenia. Pediatr Int Official J Japan Ped Society. 2024;66(1):e15747.
6. Arvind MN, Rajanna AH, Kamath N. Acquired Amegakaryocytic Thrombocytopenia Misdiagnosed as Immune Thrombocytopenia in a Patient with Seronegative Arthritis: A Case Report. J Association Physicians India. 2023;71(11):100-2.
7. Huang C, Chen W, Wu Y, Shen C, Hsu C, Li C, et al. Comparison of antiplatelet antibody profiles between hepatitis C virus-associated immune thrombocytopenia and primary immune thrombocytopenia. Platelets. 2021;32(8):1043-50.
8. van Dijk WEM, Schutgens REG. Mycophenolate Mofetil for Immune Thrombocytopenia. New Eng J Med. 2021;385(23):2201.
9. Wu K, Yin H, Du A. Effects of Modified Jianpi Qushi Heluo Decoction on Scores of TCM Syndromes, 24 h Urinary Albumin, and Plasma Albumin in IMN of Spleen-Kidney Qi Deficiency. Emerg Med Int. 2022;2022:6061709.
10. Lu B, Ye Q, Pan Y, Lu J, Li L, Peng Y, et al. Tonifying spleen and replenishing kidney method of traditional Chinese medicine for myasthenia gravis: A protocol for systematic review and meta-analysis. Medicine. 2021;100(21):e25966.
11. Geng Y, Wang W, Zhang J, Bi S, Li H, Lin M. Effects of Traditional Chinese Medicine herbs for tonifying Qi and kidney, and replenishing spleen on intermittent asthma in children aged 2 to 5 years old. J Tradit Chin Med. 2016;36(1):32-8.
12. Bhamidipati K, Silberstein JL, Chaichian Y, Baker MC, Lanz TV, Zia A, et al. CD52 Is Elevated on B cells of SLE Patients and Regulates B Cell Function. Front Immunol. 2021;11:626820.
13. Olsson B, Ridell B, Carlsson L, Jacobsson S, Wadenvik H. Recruitment of T cells into bone marrow of ITP patients possibly due to elevated expression of VLA-4 and CX3CR1. Blood. 2008;112(4):1078-84.
14. Chen L, Ye T, Wang X, Han L, Wang T, Qi D, et al. The Mechanisms Underlying the Pharmacological Effects of GuiPi Decoction on Major Depressive Disorder based on Network Pharmacology and Molecular Docking. Comb Chem High T Scr. 2023;26(9):1701-28.
15. He X, Du X, Chen J. Study on the molecular mechanism of Guipi decoction against sleep deprivation based on integrated pharmacology analysis and gene expression profiling. Acta Neurobiol Exp. 2022;82(4):409-23.
16. Wang X, Bei H, Du R, Chen Q, Wu F, Chen J, et al. Metabolomic analysis of serum reveals the potential effective ingredients and pathways of Danggui Buxue Tang in promoting erythropoiesis. Complement Ther Med. 2020;48:102247.
17. Lee AR, Yang SW, Lee SY, Jeon SB, Kang HY, Choi JW, et al. Mitochondrial transplantation ameliorates experimental autoimmune encephalomyelitis by modulating the Th17/Treg balance and restoring metabolic homeostasis. Front Immunol. 2026;17:1698136.
18. Besancenot R, Roos-Weil D, Tonetti C, Abdelouahab H, Lacout C, Pasquier F, et al. JAK2 and MPL protein levels determine TPO-induced megakaryocyte proliferation vs differentiation. Blood. 2014;124(13):2104-15.
19. Xu Y, Lv W, Wu H, Shi SF. Ginsenoside regulates Treg/Th17 cell ratio and inhibits inflammation to treat COPD. Die Pharmazie. 2020;75(11):590-4.
20. Kim YK, Kim SE, Chang Park H, Hwang JH, Lee HT. Human recombinant IL-10 reduces xenogenic cytotoxicity via macrophage M2 polarization. Biochem Biophys Rep. 2020;24:100857.
21. He X, Du X, Chen J. Study on the molecular mechanism of Guipi decoction against sleep deprivation based on integrated pharmacology analysis and gene expression profiling. Acta Neurobiol Exp. 2022;82(4):409-23.