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The levels of vitamins and cytokines in serum of elderly patients with community-acquired pneumonia: A case–control study

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Abstract

Background and Aims: The community acquired pneumonia (CAP) is a common disease with high incidence rate and mortality among the elderly. This study aims to explore the association between vitamins, cytokines, and CAP infected by different pathogens in Handan First Hospital.

Methods: The subjects of this study were elderly patients with community-acquired pneumonia in Handan First Hospital and healthy elderly people in the community from February 2018 to December 2019. The study include 234 CAP patients and 180 healthy elderly people. The differences between the healthy and CAP groups were analyzed by blood routine test, urine routine test, Liquid chromatography tandem mass spectrometry (LC-MS/MS) detection of vitamin level, and Enzyme linked immunosorbent assay (ELISA) detection of cytokine level. Sputum culture was used to detect pathogens.

Results: According to our results, there was no significant difference in the contents of vitamin A, vitamin D, vitamin E, and vitamin C in serum between the healthy and CAP elderly. However, compared with the control group, the levels of serum cytokines of IL-2, IL-17, and TGF-β in the CAP patients were significantly increased. In addition, IL-17 was positively correlated with white blood cells, neutrophils, platelet/lymphocyte ratio and neutrophil/lymphocyte ratio of CAP patients, and negatively correlated with lymphocytes. The four pathogens with the highest positive rates were *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Mycoplasma pneumoniae* and *H. influenza*. In patients with *S. pneumoniae* infection, the IL-2, IL-17, and TGF-β levels were significantly higher than the levels in the control group; IL-17 was also significantly increased in the serum of patients infected with *M. pneumoniae*, *K. pneumoniae*, and *H. influenza*.

Conclusions: There is no direct association between vitamins in serum and community acquired pneumonia (CAP). However, the cytokines are closely related to the clinical indicators and pathogens of CAP patients, which can provide references for the diagnosis, prognosis and treatment of CAP patients.

KEYWORDS
community-acquired pneumonia, cytokines, pathogens, vitamins

1 | INTRODUCTION

The community acquired pneumonia (CAP) refers to inflammation of lung parenchyma (terminal airways, alveoli, and pulmonary interstitium) developed outside the hospital. Its incidence is related to the pathogenic microorganisms, physical and chemical factors, immune injury, allergy, drugs and other factors, which is one of the common infectious diseases among people over 65-year-old. The incidence rate of CAP varies with age. A study conducted in Guangzhou, Guangdong Province, China, from 2009 to 2012, shows that of 16 585 patients with CAP, 37.3% were children, 9.2% were adults between 26 and 45-year-old, and 28.7% were over 65-year-old.[1] In addition, incidence rate of CAP varies with age, region, and season.[2] One study in Barcelona, Spain, shows that the mortality rate could double with the increase of age, from 6% at 65-year-old to 15% at 90-year-old.[3] The clinical manifestations of pneumonia in the elderly are not typical, and missed diagnosis and misdiagnosis tend to occur. Moreover, the disease develops rapidly, so its early diagnosis and treatment are of great significance in clinical work. It is well known that vitamins can supplement nutrition, promote metabolism, and regulate the immune system for the elderly, but whether vitamins can be used to treat community-acquired pneumonia in the elderly is not clear.

The vitamins is an indispensable nutrient for regulating normal metabolism and maintaining physiological functions.[4] The available clinical data show that vitamins play a very important role in maintaining the health of the elderly. The deficiency of vitamins can increase the incidence of chronic diseases in the elderly, such as respiratory infection, pneumonia, hypertension, diabetes, and malignant tumors.[5–7] At present, there is no strong data to prove the correlation between vitamins and pneumonia of the elderly. Therefore, it is of great significance to understand the vitamin level of elderly patients with pneumonia, so as to provide guidance for nutritional intervention in the elderly and follow-up clinical treatment of the pneumonia patients.

If the pneumonia is caused by infection of various pathogens, the pathogen infection will cause the inflammatory pathological damage and immune function disorder, and at the same time, the anti-infection immune response of the body will be induced to produce a variety of cytokines.[8] Interleukin (IL) plays an important role in transmitting information, activating and regulating the immune cells, mediating the activation, proliferation and differentiation of T and B cells, and in the inflammatory response. Interleukin is related to CAP. It is reported that the higher the CAP severity score is, the higher the IL-27 level in the serum.[9] In addition, it was also found that the IL-10, IL-6, IL-17, tumor necrosis factor α (TNF-α), and transforming growth factor-β (TGF-β) of the community-acquired pneumonia patients were significantly higher than the levels of the normal control group, and were positively correlated with inflammatory response.[10–12] The serum factors could be effective biomarkers for diagnosis, prognosis, and treatment of the CAP patients.

This study aims to compare the levels of vitamins A, D, E, and C and the levels of immune cytokines IL-1, IL-2, IL-4, IL-17A, TGF-β, and IFN-γ in the serum of elderly patients with CAP and the healthy elderly. we wish to find the cytokines showing significant difference, so as to provide a basis for early diagnosis and early prevention of the elderly.

2 | MATERIALS AND METHODS

2.1 | Study design and the inclusion and exclusion criteria

The subjects of this study were elderly patients with community-acquired pneumonia in Handan First Hospital and healthy elderly people in the community from February 2018 to December 2019. For the control group, the healthy elderly in the control group are the people we obtained the informed consent of the healthy elderly in the process of free community clinic and then included. In this case–control study, the Inclusion criteria for all participants were aged ≥65-year-old and willing to accept blood vitamin tests. The exclusion criteria were supplementation history with vitamins A, D, E, and C in the past 3 months; have received steroid treatment or other drugs or supplements that affect the immune system or cytokines levels; For the control group, inclusion criteria: healthy people ≥65-year-old; the exclusion criteria are the same as above. The design flow chart of our study is presented in Figure 1. A total of 260 CAP patients and 200 healthy elderly people were recruited, excluding some cases and healthy people. Finally, 234 CAP patients and 180 healthy elderly people participated in the study. Serum extraction, no mandatory fasting. EDTA anticoagulant tube collected 5 mL of peripheral blood, and the collected serum was used to detect vitamins and cytokines, respectively. Sputum samples are collected to test for pathogens. All participants have signed informed consent. The study was approved by the Ethics Committee of Handan First Hospital (Ethics Approval No.: Medical Ethics Review Letter of the Ethics Committee of the First Hospital of Handan (2018) Medical Review No. 030).

2.2 | Liquid chromatography tandem mass spectrometry (LC-MS/MS)

LC-MS/MS was used to detect the contents of vitamin A, vitamin C, vitamin D, and vitamin E in serum.[13,14] The standard vitamin, mobile phase formic acid, and methanol were purchased from Merck. The LC-MS/MS platform connects the Shimadzu® Nexera X2 high performance liquid chromatograph (Shimadzu USA) with the AB Sciex® 6500 + triple quadrupole mass spectrometer (Sciex). The

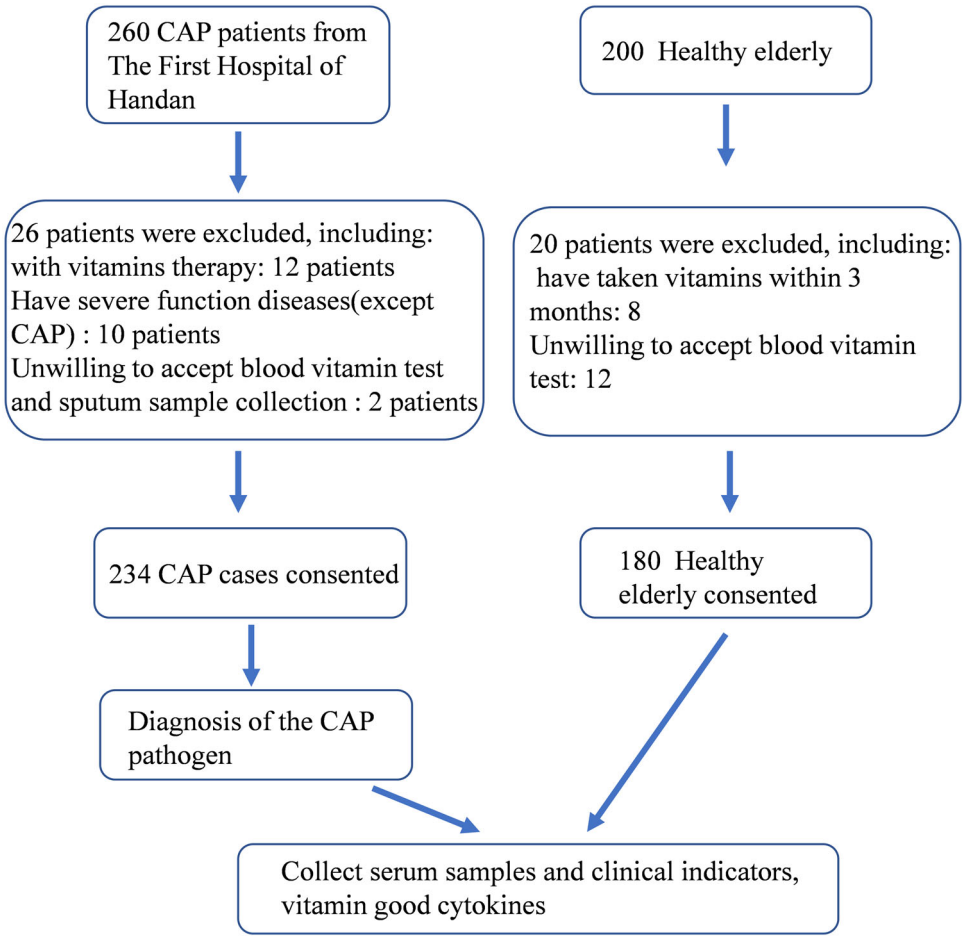

FIGURE 1 Flow chart of recruitment and follow-up study of the CAP patients and healthy elderly. CAP, community acquired pneumonia.

LC-MS/MS instrument is controlled using the Analyst® Software. Chromatographic conditions: chromatographic column: Poroshell 120 EC-C18 (4.6 × 50 mm) 2.7-Micron; mobile phase: liquid A: 0.5% formic acid water + 0.5 mol/L ammonium formate + water (1:1:8); liquid B: pure methanol. Under flow rate of 0.65 mL/min, gradient elution: for 0–0.5 min when mobile phases A:B = 20:80; for 0.5–2.1 min when mobile phases A:B = 20:80; for 2.1–4.6 min when mobile phases A:B = 4:96; for 4.6–6.8 min when mobile phases A:B = 0:100; for 6.8–9.2 min when mobile phases A:B = 15:85. Injection volume: 20 uL, column temperature: 40°C. Mass spectrum conditions: electrospray ion source (ESI), multireaction monitoring (MRM) scanning mode, ionization voltage (IS) set at 5500 V, and temperature (TEM) set at 350°C.

2.3 | Enzyme linked immunosorbent assay (ELISA)

The fasting blood sample (5 mL) was collected, 1 mL serum was obtain3e after centrifugation, and the blood sample was immediately stored in the refrigerator at −80°C. The ELISA kit used to test the inflammatory cytokines was purchased from R&D Systems. The cytokines and article numbers: IL-1 (DLB50), IL-2 (HS200), IL-4 (HS400), IL-17A (QK317), TGF-β (DY240-05), and IFN-γ (SIF50C).

2.4 | Identification of pathogenic bacteria

According to the identification method used in,[15] the sputum was treated; then, was inoculated into the blood agar medium, chocolate agar medium, and MacConkey agar medium. Next, the blood agar medium and chocolate agar medium of inoculated samples were incubated in a humid environment containing 5% CO_2 under 35°C, the MacConkey sample was incubated in a normal environment at 5°C. The isolated bacteria were preliminarily identified according to their colony morphology and Gram staining, and then the biochemical identification was carried out with an automatic bacteria identification instrument, which can identify the *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. The *Mycoplasma pneumoniae* culture medium was used for detection of the *M. pneumoniae*. If the color of the culture medium changed from red to yellow, the medium was positive; and if the color change was not obvious, it was negative. The culture medium was purchased from Thermo Fisher Scientific.

2.5 | Statistic analysis

All plots and analyses in this study were performed using the GraphPad Prism 8.0 software. The *t* test was used to compare the statistical differences between the two groups. χ^2 test is used to compare qualitative variables. The linear regression was employed for correlation analysis. The data were presented as the mean ± standard deviation (SD). We reported the results with a confidence interval of 95% and a $p < 0.05$ was considered significant.

3 | RESULTS

3.1 | Basic information

A total of 234 CAP patients and 180 healthy subjects participated in this study. Their demographic characteristics and clinical information are shown in Table 1. There were no significant differences between the CAP patients and the control group in gender distribution, age, smoking history, BMI, systolic blood pressure, and diastolic blood pressure. Among all the elderly included in the study, 68 had diabetes, and 38 of them had CAP. Therefore, it can be seen that the incidence rate of CAP is higher among the diabetes patients. The results of blood routine examination showed that compared with the control group, the white blood cell (WBC), neutrophil count, platelet-to-lymphocyte ratio (PLR), and neutrophil-to lymphocyte ration (NLR) of the CAP patients were significantly increased, while their lymphocyte number was decreased. At the same time, the liver function and renal function parameters of serum were measured. The results show that there were no significant differences between the CAP patients and controls in ALT, AST, BUN, creatinine, and uric acid.

TABLE 1 Demographic and clinical information of the CAP patients and the control group.

Variables	CAP ($n = 234$)	Control ($n = 180$)	*t*	*p* Value
Gender				
Male	54.3% (127)	52.2% (94)	2.118	0.65
Female	45.7% (107)	47.8% (86)	2.028	0.06
Smoking	20.5% (48)	22.2% (40)	1.715	0.09
BMI	23.5 ± 8.65	22.3 ± 7.52	1.507	0.13
Diabetes patients (68)	56% (38)	44% (30)	2.211	0.02
Systolic pressure (mmHg)	123.8 ± 18.8	124.62 ± 20.23	0.422	0.67
Diastolic pressure (mmHg)	75.4 ± 16.52	75.5 ± 18.2	0.058	0.95
WBC (10^9/L)	6.83 ± 3.34	5.84 ± 2.06	3.709	< 0.001
Neutrophil (10^9/L)	4.73 ± 2.44	3.95 ± 2.15	3.45	< 0.001
Lymphocyte (10^9/L)	1.83 ± 1.64	2.41 ± 1.35	3.321	0.001
PLR	180.23 ± 98.5	155.6 ± 96.3	2.554	0.01
NLR	2.47 ± 1.87	1.98 ± 1.42	3.123	0.002
MON	0.26 ± 0.24	0.23 ± 0.14	1.592	0.11
ALT (U/L)	21.2 ± 8.89	19.9 ± 6.2	1.751	0.08
AST (U/L)	24.8 ± 9.83	23.9 ± 6.8	1.1	0.27
Urea nitrogen (mmol/L)	5.21 ± 2.54	4.98 ± 2.62	0.897	0.37
Creatinine (μmol/L)	60.5 ± 15.3	62.85 ± 20.5	1.287	0.20
Uric acid (μmol/L)	265.64 ± 83.5	274.5 ± 68.8	1.196	0.23

Note: If $p < 0.05$, it was considered as statistically significant.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index, BMI = [weight (kg)/height (m)]^2; MON, monocyte-to-lymphocyte ration; NLR, neutrophil-to lymphocyte ration; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

3.2 | There was no significant difference between the healthy elderly people and the CAP patients in serum vitamin levels

To investigate whether the vitamin levels in the serum of old people are related to CAP, we detected the vitamin content in the elderly control group and the CAP group using the LC-MS/MS, and analyzed the differences between these two groups in vitamin levels. The results are as shown in Figure 2. The contents of vitamin A, vitamin C, vitamin D, and vitamin E in the control group were 2.38 ± 1.06 μ mol/L, 32.52 ± 6.45 μ mol/L, 1.38 ± 0.21 μ mol/L, and 28.76 ± 7.78 μ mol/L; the contents of vitamin A, vitamin C, vitamin D and vitamin E in the CAP group were 2.25 ± 1 μ mol/L, 30.68 ± 6.88 μ mol/L, 1.43 ± 0.24 μ mol/L, and 26.89 ± 5.54 μ mol/L. The difference significance analysis showed that the levels of vitamin A, vitamin D, vitamin C, and vitamin E in patients with CAP were not significantly different from those in the control group.

3.3 | IL-2, IL-17, and TGF-β levels in CAP patients were significantly increased

CAP is closely related to the inflammatory cytokines. To find out the differences of the levels of immune cytokines IL-1, IL-2, IL-4, IL-17A, TGF-β, and IFN-γ in the serum of the elderly control group and CAP group, we detected the contents of the above serum cytokines. The results are shown in Figure 3. Compared with the control group, the IL-2 ($p < 0.01$), IL-17 ($p < 0.001$), and TGF-β ($p < 0.01$) levels in CAP patients were significantly increased (Figure 3B,D,E). In the control group, the IL-2, IL-17, and TGF-β contents were 15.85 ± 10.24 pg/mL, 68.85 ± 18.54 pg/mL, and 49.5 ± 15.8 pg/mL, respectively; in the CAP group, the IL-2, IL-17, and TGF-β contents were increased to 19.6 ± 14.9 pg/mL, 77.24 ± 25.85 pg/mL, and 56.54 ± 28.85 pg/mL.

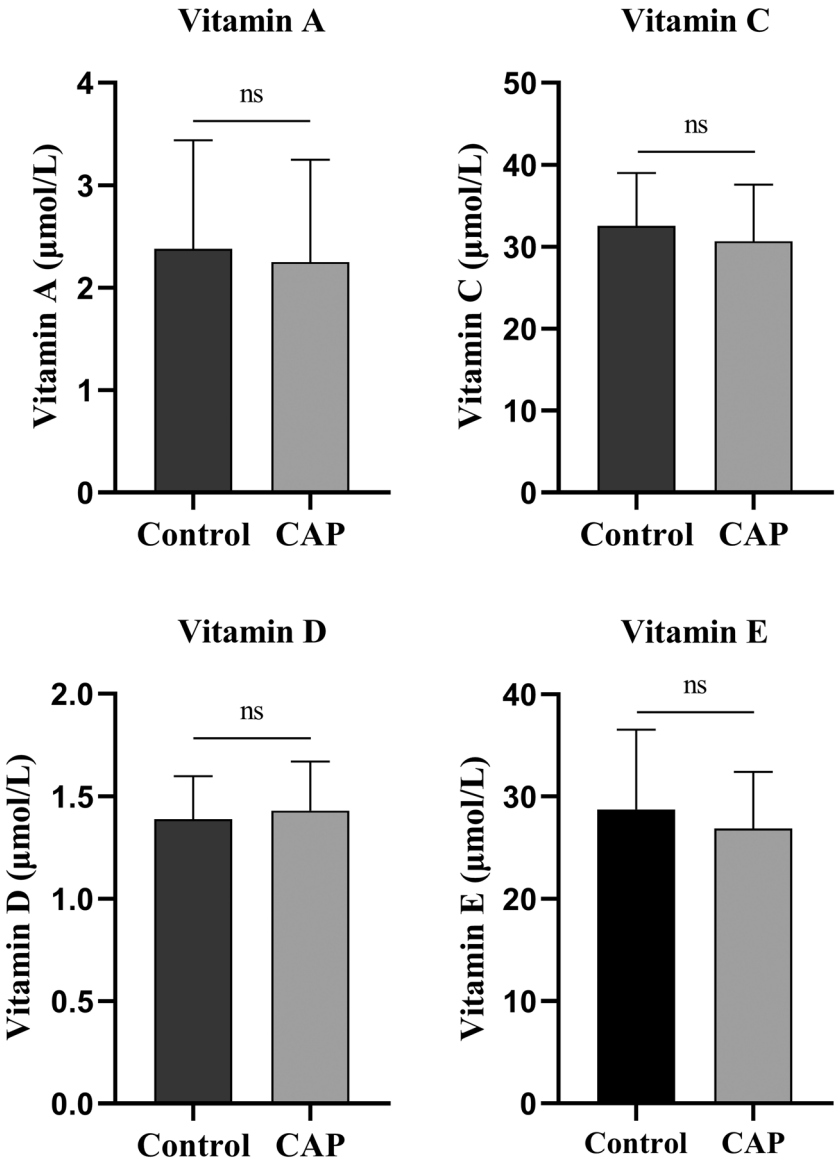

FIGURE 2 Serum vitamin levels in healthy elderly population and CAP patients difference between the elderly healthy people and the CAP patients with in the vitamin A, vitamin C, vitamin D, and vitamin E levels in serum. CAP, community acquired pneumonia; ns, no significance.

These two groups did not show significant differences in the IL-1, IL-4, and IFN-γ levels (as shown in Figure 3A,C,F). The above results indicate that the cytokines IL-17, IL-2, and TGF-β are closely related to the pathological process of CAP.

3.4 | Correlation analysis

The results of blood routine examination show that compared with the control group, the white blood cells, neutrophil count, PLR, and NLR of CAP patients were significantly increased, while the lymphocyte number was decreased. To find out whether these parameters are related to cytokines, we used linear regression to evaluate the correlation between the IL-17, TGF-β, and IL-2 levels in the serum of CAP patients and these parameters. The results show that serum IL-17 level was positively correlated with the WBC, neutrophils, PLR, and NLR of the CAP patients (as shown in Figure 4A,B,D,E), and negatively correlated with lymphocytes (Figure 4C). In addition, we also found that IL-17 was positively correlated with TGF-β (Figure 4F). However, IL-17 showed no significant correlation with the four vitamins. Although the TGF-β and IL-2 levels in the serum of the CAP patients were significantly increased, our study shows that they had no significant correlation with blood routine parameters and vitamins, as shown in the Appendix.

3.5 | Analysis of cytokine difference in patients infected by different pathogens

Pneumonia caused by various pathogens induces the body's anti-infection immune response to produce a variety of cytokines, to further explore the correlation between cytokines and pathogens, we analyzed the pathogen. In this study, the positive rates of

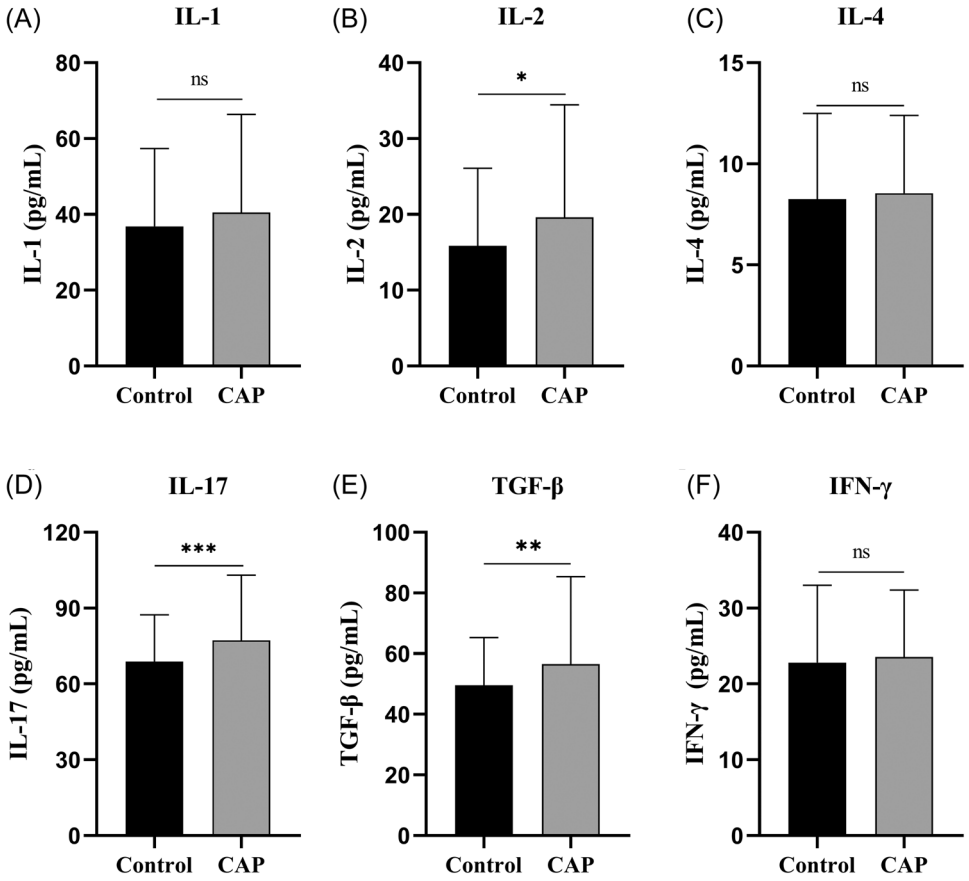

FIGURE 3 Immune cytokines levels in the serum of healthy elderly people and elderly patients with pneumonia (A) the difference between the CAP patients and the control group in the IL-1 level; (B) the difference between the CAP patients and the control group in the IL-2 level; (C) the difference between the CAP patients and the control group in the IL-4 level; (D) the difference between the CAP patients and the control group in the IL-17 level; (E) the difference between the CAP patients and the control group in the TGF-β level; (F) the difference between the CAP patients and the control group in the IFN-γ level. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. ns, no significance.

S. pneumoniae, *K. pneumoniae*, *M. pneumoniae*, and *H. influenzae* in 234 CAP patients in Handan First Hospital were high, which were 22.2% (52 cases), 20.9% (49 cases), 16.2% (38 cases), and 14.1% (33 cases), respectively, The positive rates of *S. aureus* and *Pseudomonas aeruginosa* were low. There were eight cases of mixed infection: two cases of *Pseudomonas aeruginosa* and *S. pneumoniae*, three cases of *S. pneumoniae* and *K. pneumoniae*, 1 case of *K. pneumoniae* and *H. influenzae*, and two cases of *S. pneumoniae* and *M. pneumoniae*. The mixed infection rate was 3.4%. See Table 2 for specific information. Moreover, these common pathogens were not detected in 11.1% (26 cases) of the patients, which may be caused by other pathogens.

Different pathogens generated different cytokines, which may be associated to the pathogenesis of different pathogens. Therefore, we analyzed the horizontal differences of cytokines IL-2, IL-17, and TGF-β in the serum of the patients affected with the four pathogens with the highest positive rates presented in Table 2. The results show that the IL-2, IL-17, and TGF-β levels of patients with *S. pneumoniae* infection were significantly higher than the levels of the control group (as shown in Figure 5A); the IL-17 and TGF-β levels of patients with *K. pneumoniae* were significantly higher than the levels of the control group (as shown in Figure 5B); for patients infected with *M. pneumoniae* and *H. influenza*, only their IL-17 level was significantly higher than that of the control group (as shown in Figure 5C,D). To sum up, the inflammation or other response triggered in the CAP patients infected by different pathogenic microorganisms are different, and the cytokines involve complex mechanism of action during the pathological process of CAP, which requires more research for verification.

4 | DISCUSSION

CAP is a common respiratory disease among the elderly. With the aging of population, environmental degradation, antibiotic abuse, and the influence from other factors, the incidence rate of CAP among the elderly is growing year by year. The elderly CAP patients often also have malnutrition, decreased immune function, changes in organ structure and function, and basic diseases. In our study, there was no significant difference between the CAP patients and the control group in gender, age, smoking history, BMI, systolic blood pressure, and diastolic blood pressure. However, we found that the incidence rate of CAP was higher among the diabetes patients, which is

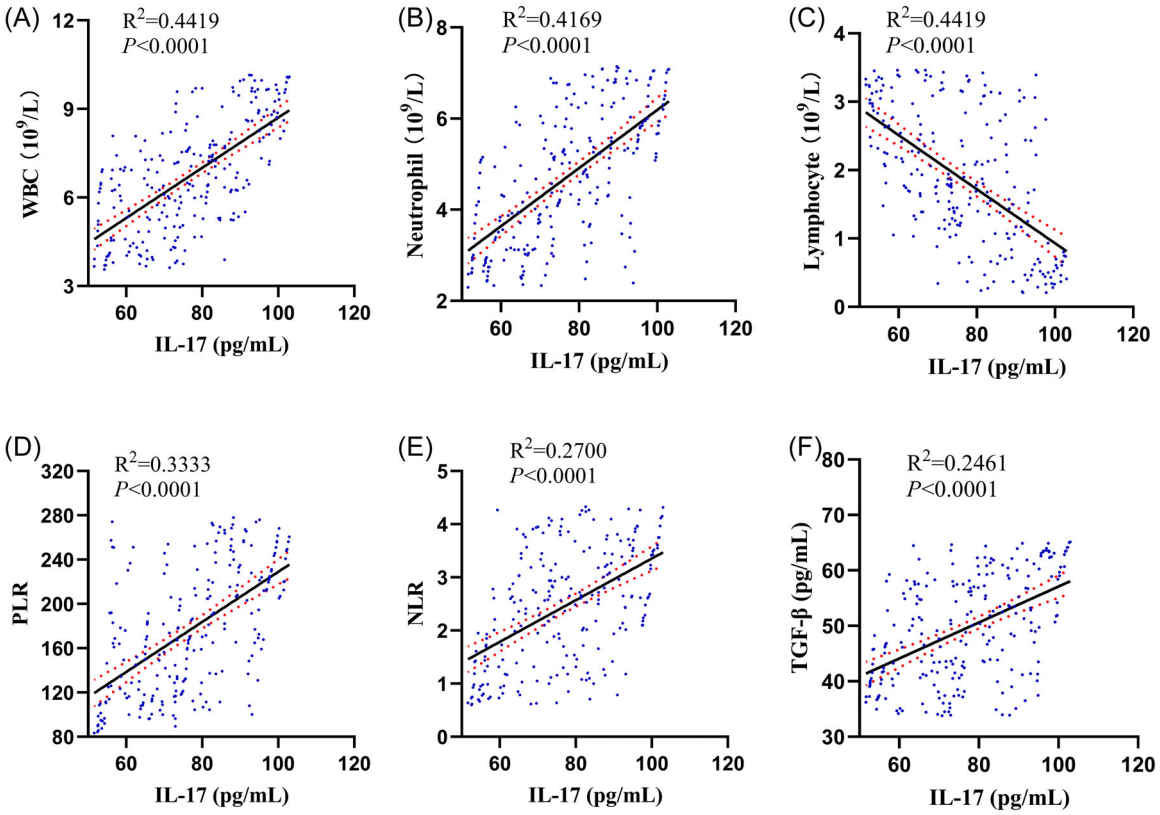

FIGURE 4 Correlation analysis. (A) Correlation analysis between IL-17 and WBC; (B) correlation analysis between IL-17 and neutrophil; (C) correlation analysis between IL-17 and lymphocyte; (D) correlation analysis between IL-17 and PLR; (E) correlation analysis between IL-17 and NLR; (F) correlation analysis between IL-17 and TGF- β. MON, monocyte-to-lymphocyte ration; NLR, neutrophil-to lymphocyte ration; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

TABLE 2 Detection of pathogens in the CAP patients.

	CAP (*n* = 234)
Streptococcus pneumoniae	22.2% (52)
Staphylococcus aureus	7.7% (18)
Klebsiella pneumoniae	20.9% (49)
Mycoplasma pneumoniae	16.2% (38)
Haemophilus influenzae	14.1% (33)
Pseudomonas aeruginosa	4.3% (10)
Mixed infection	3.4% (8)
Other	11.1% (26)

Abbreviation: CAP, community acquired pneumonia.

consistent with the findings.[16] Vitamins participate in human metabolism, which are the essential nutrients to maintain normal life activities. It is reported that vitamins are related to the severity of CAP.[17,18] Dongxi Lu and colleagues reported that the risk of respiratory tract infection is increased for patients with vitamin deficiency.[18] However, other studies also show that there was no significant correlation between vitamins and CAP.[19–21] Our study demonstrates that there were no significant differences between the control group and the CAP group in the vitamin A, vitamin D, vitamin E, and vitamin C contents in serum.

Many studies have reported that CAP is closely related to the inflammatory cytokines, and the imbalance of cytokine levels could lead to inflammatory responses of varying degrees.[22–24] We found that the IL-2, IL-17, and TGF-β levels of the CAP patients were significantly increased, especially IL-17, which is one of the important cytokines. IL-17 plays a key role in a variety of lung diseases, including pulmonary fibrosis, emphysema, acute lung injury, and pulmonary hypertension. The TGF-β Level can be used as a useful parameter to predict the prognosis of patients with severe CAP.[25] In addition, we found that the serum IL-17 level was positively correlated with WBC, neutrophils, PLR, and NLR of the CAP patients, while negatively correlated with lymphocytes, which is consistent with research by Jiale Wang of Anhui Medical University in Hefei.[22] IL-17 was positively correlated with TGF-β, but showed no significant correlation with IL-2. Although the TGF-β and IL-2 contents in the serum of CAP patients were significantly increased, but our study shows that there was no significant correlation between them and the blood routine parameters. IL-17, TGF-β, and IL-2 all showed no correlation with vitamins. Cytokines can be potential biomarkers for diagnosis and treatment of CAP.[26]

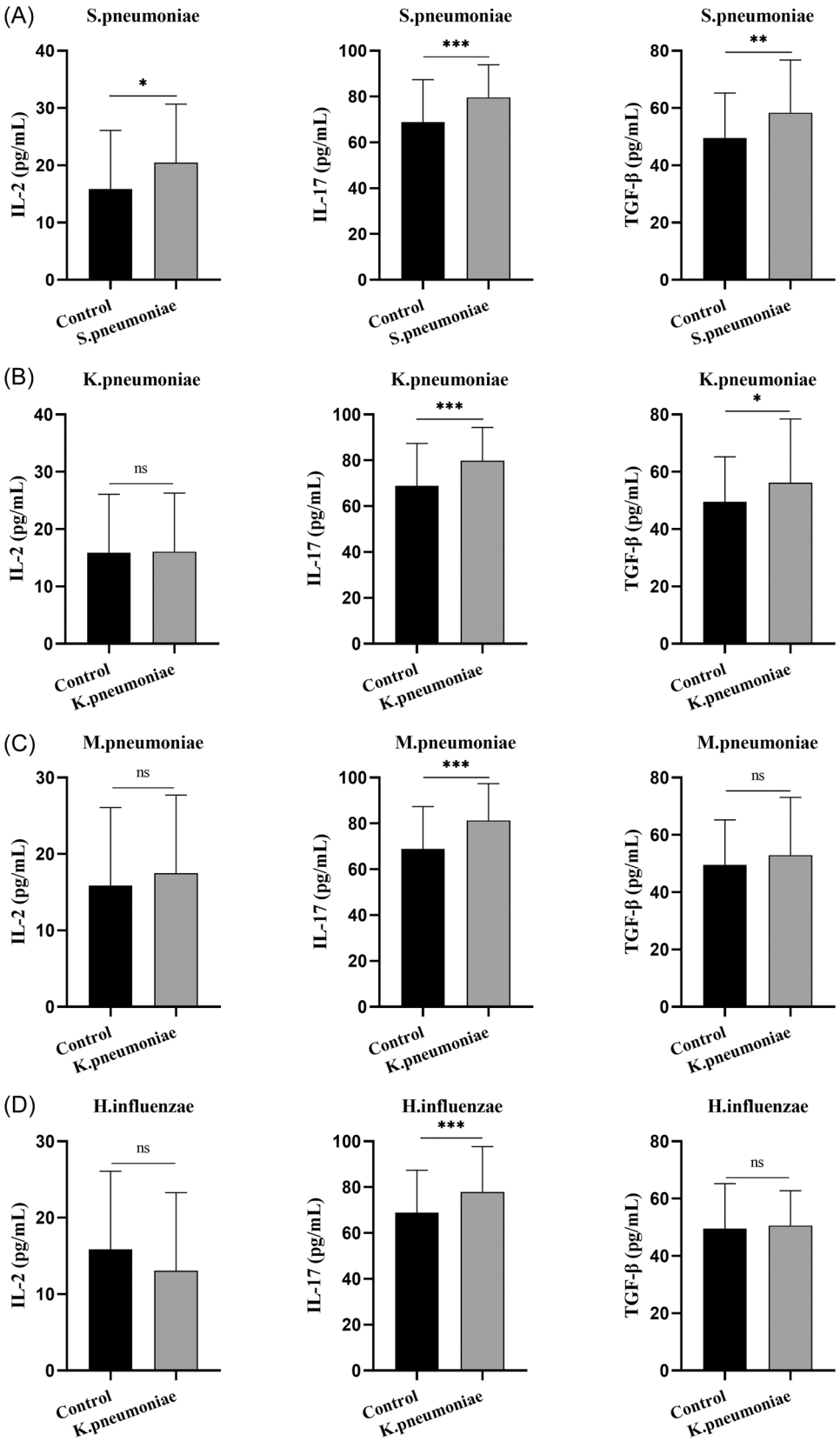

FIGURE 5 Serum cytokine levels of patients with different pathogens (A) horizontal differences between the patients with *Streptococcus pneumoniae* infection and control group in the IL-2, IL-17, and TGF-β levels; (B) horizontal differences between the patients with *Klebsiella pneumoniae* infection and control group in the IL-2, IL-17, and TGF-β levels; (C) horizontal differences between the patients with *Mycoplasma pneumoniae* infection and control group in the IL-2, IL-17, and TGF-β levels; (D) horizontal differences between the patients with *Haemophilus influenzae* infection and control group in the IL-2, IL-17, and TGF-β levels. $^{*}p < 0.05$; $^{**}p < 0.05$; $^{***}p < 0.001$. ns, no significace.

The pathogens of CAP vary according to different countries, regions, age groups and patients with different basic diseases. In our study, among the 234 patients with community-acquired pneumonia treated in the First Hospital of Handan City, the four pathogens with the highest positive rates were *S. pneumoniae*, *K. pneumoniae*, *M. pneumoniae*, and *H. influenza*, respectively. The positive rates of *S. aureus* and *Pseudomonas aeruginosa* were lower. An epidemic study on CAP conducted in China[27] shows that *M. pneumoniae* and *S. pneumoniae* are the most common pathogens of adult CAP in China, and *H. influenzae*, *K. pneumoniae*, and *S. aureus* are also common pathogens. *S. pneumoniae* is the most common pathogen isolated from the European CAP patients. However, there is also a significant difference in the incidence rates of CAP caused by this pathogen. Other common pathogens include *H. influenzae*, Gram negative Enterobacter, respiratory viruses and *M. pneumoniae*.[28] *S. pneumoniae*, *H. influenzae* and respiratory viruses are more common in elderly CAP patients ≥65-year-old.[29] Therefore, we analyzed the differences of the serum cytokines IL-2, IL-17, and TGF-β in the patients infected with the four pathogens with the highest positive rates. We found that the IL-2, IL-17, and TGF-β levels of patients with *S. pneumoniae* infection were significantly higher than the levels of the control group; the IL-17 and TGF-β levels of patients with *K. pneumoniae* were significantly higher than the levels of the control group; for patients infected with *M. pneumoniae* and *H. influenza*, only their IL-17 level was significantly higher than that of the control group. The level of IL-17 in the serum of patients infected with *S. pneumoniae* and *H. influenzae* is higher, which may mainly act on neutrophils and *H. influenzae* with *S. pneumoniae* and *S. pneumoniae*. Activation of neutrophils and release of a large number of cytokines involved in the development of inflammation are related, and neutrophils activation is positively correlated with IL-17.[30] The inflammatory cytokines produced by different pathogens are also different, which may be related to the pathogenic mechanism of different pathogens. This study provides a theoretical basis for the study of the mechanism of inflammation in community-acquired pneumonia.

However, our study still has its limitations due to the small sample size and by including patients from only one hospital, and we will obtain a larger sample volume and carry out multicenter study in the future. In addition, we need to verify our conclusion and mechanism based on further research based on experiment. We only tested the quantities of cytokines in the serum, and we may expand the detection of f cytokines in the body in the next step.

5 | CONCLUSION

Serum levels of vitamin A, vitamin D, vitamin E, and vitamin C in the elderly were not directly related to community-acquired pneumonia infection, but cytokine levels were closely related to clinical indicators and pathogenic bacteria in CAP patients. Our results can provide a reference for the diagnosis, prognosis and treatment of community-acquired pneumonia in the elderly, and also provide a theoretical basis for further research on CAP.

AUTHOR CONTRIBUTIONS

Yongfa Jing: Conceptualization; data curation; methodology; writing—original draft. **Lingling Chen:** Conceptualization; supervision; project administration; writing—review and editing. **Linna Geng:** Data curation; formal analysis; validation. **Zhaofei Shan:** Software; supervision; validation. **Juling Yang:** Methodology; investigation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

TRANSPARENCY STATEMENT

The lead author Lingling Chen affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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APPENDIX

Correlation analysis of TGF-β and IL-2 with blood routine parameters and vitamins.

Figure A1

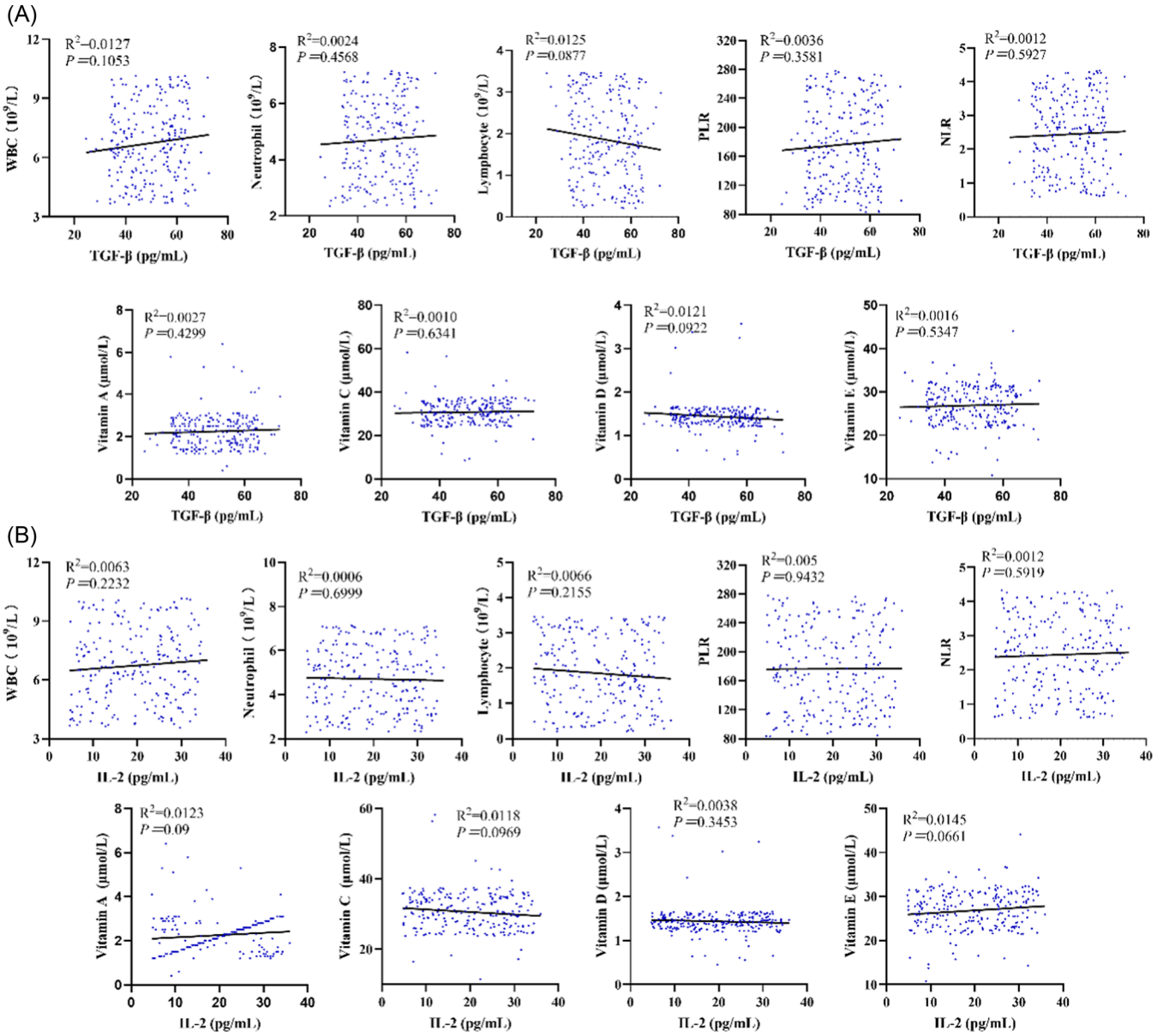

FIGURE A1 (A) Correlation analysis between TGF-β and blood routine parameters and vitamins. (B) Correlation analysis between IL-2 and blood routine parameters and vitamins.

Correlation analysis of IL-17 with vitamins.