Serum iron, prealbumin, D-dimer and prothrombin time are indicators of the diagnosis, severity and outcome of Salmonella enterica serovar Enteritidis infection

Xiaowei Li
Sixth Medical Center of PLA General Hospital https://orcid.org/0000-0003-3410-154X
Shaoxin Wang
Sixth Medical Center of PLA General Hospital
Lan Yu
Sixth Medical Center of PLA General Hospital
Xiaohui Wang
Sixth Medical Center of PLA General Hospital
Zhihui Yan
Sixth Medical Center of PLA General Hospital
Yan Zheng
Sixth Medical Center of PLA General Hospital
Yanjun Li
Sixth Medical Center of PLA General Hospital
Xiaona Liu
Sixth Medical Center of PLA General Hospital
Jia Rong
Sixth Medical Center of PLA General Hospital
Lihong Cui (luckycui861@sina.com)
Sixth Medical Center of PLA General Hospital

Research

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Abstract

Aims *Salmonella enterica* serovar Enteritidis (*S*. Enteritidis) infection is one of the main causes of foodborne illness among individuals in the military. In this study, we aimed to investigate indicators associated with the diagnosis, severity and outcome of *S*. Enteritidis infection, and to analyze the antimicrobial susceptibility of the isolated *S*. Enteritidis strains.

Methods Routine stool test, fecal occult blood tests (FOBTs), routine blood tests and blood biochemical analyses were completed in our clinical laboratory. The stool samples were inoculated on *Salmonella Shigella* (SS) agar plates, the single bacterial colony was identified with mass spectrometry (MS). The serum agglutination test was performed to identify the *Salmonella* serotype. Antimicrobial susceptibility testing was performed using the minimal inhibitory concentration (MIC) method with the VITEK 2 COMPACT analyzer.

Results Recently, 6 patients from one company visited our department complaining of fever, watery stool, abdominal pain and so on. The patients' white blood cell (WBC) counts (66.7%), neutrophil percentages (100%), C-reactive protein (CRP) levels (100%), prothrombin times (PTs, 100%) and serum D-dimer concentrations (83.3%) were higher or longer than normal, while serum iron (100%) and prealbumin (83.3%) were lower than normal when they were admitted. Stool cultures and serovar identification results indicated *S*. Enteritidis infection. The antimicrobial susceptibility results showed that the 6 isolated bacterial strains were sensitive to trimethoprim-sulfamethoxazole and levofloxacin, and resistant to ampicillin. All patients were administered levofloxacin accompanied by rifaximin and probiotics, and their symptoms and the abovementioned parameters were recovered. During the disease course, the prolonged PT, elevated D-dimer and decreased serum iron were positively correlated with severe symptoms and serum inflammatory indexes.

Conclusion Prolonged PT, elevated D-dimer and hypoferremia were associated with the diagnosis, severity and outcome of *S*. Enteritidis infection, and a decreased prealbumin level was associated with the diagnosis of *S*. Enteritidis infection. In patients complaining of acute fever, watery stool and abdominal pain, PT, D-dimer□prealbumin and serum iron should be monitored to attain an early diagnosis and initiate appropriate treatment for *S*. Enteritidis infection.

Background

Salmonella enterica serovar Enteritidis (*S*. Enteritidis) infection is one of leading infectious causes of foodborne illness worldwide[1]. Worldwide, 94 million patients with gastroenteritis are diagnosed with *Salmonella* infection, 155,000 of whom die every year[2, 3], and it has been a huge public health issue for both civilians and individuals in the military. Individuals in the military mostly work and live in special environments such as mountains, islands, and ships that are characterized by high humidity, high salinity, high temperatures, and low air pressure. They also suffer from stress due to fatigue, tension, lack of sleep and military-related issues; thus, they are more susceptible to *S*. Enteritidis infection than the general population. The characteristic manifestations of *Salmonella* infection include fever, chills, watery stool, nausea, vomiting, abdominal pain and so on[4], these symptoms can significantly reduce the combat effectiveness of soldiers.

In recent years, because of the abuse of antibiotics in both human beings and poultry, high proportions of *S*. Enteritidis isolates have been reported to be resistant to multiple antibiotics, resulting in increasingly challenging of *S*. Enteritidis treatment. The gold standards for *S*. Enteritidis infection diagnosis and antimicrobial susceptibility analysis rely on fecal/blood cultures, but these processes are time-consuming. Therefore, identifying other parameters correlated with the diagnosis, severity and outcome of *S*. Enteritidis and clarifying the antibiotic resistance status of *S*. Enteritidis are imperative. In recent years, because of the abuse of antibiotics, high proportions of *S*. Enteritidis isolates have been reported to be resistant to multiple antibiotics[5], resulting in increasingly challenging *S*. Enteritidis treatment. The gold standards for *S*. Enteritidis diagnosis and antimicrobial susceptibility analysis rely on fecal/blood cultures, but these processes are time-consuming. Therefore, identifying other parameters correlated with the diagnosis, severity and outcome of *S*. Enteritidis infection and clarifying the antibiotic resistance status of *S*. Enteritidis are imperative.

Iron is critical for both humans and invading pathogens[6], it is correlated with the oxygen transport, the tricarboxylic acid (TCA) cycle, lipid and sterol metabolism, mitochondrial respiration, chromatin remodeling, DNA replication and repair and so on[7]. The invading pathogens and the hosts compete for the use of iron[6]. In acute infectious diseases, including bacteria (such as *S*. Typhi[8]), fungi[7] (such as C.glabrata, C.albicans, A.fumigatus and C.neoformans) and virus (such as coronavirus disease 2019, COVID-19[9]), inflammatory factors, such as IL-6, could increase the expression of hepcidin to promote the degradation of the iron exporter ferroportin in duodenal enterocytes and macrophages[10–14], which could decrease the serum iron concentration by restricting iron uptake and macrophage iron release[15]. The dysregulation of iron release by macrophages could contribute to the survival of intracellular, macrophage-tropic bacteria[13], but could be detrimental to the extracellular pathogens[8, 16, 17]. However, its correlation with *S*. Enteritidis infection remains unknown.

Coagulation system disturbance is associated with various infectious diseases such as *S*. Typhi[18], Meningococcal[19], Staphylococcus[20], COVID-19[21] and HBV[22, 23]. *S*. Typhi can activate the coagulation system, and the consumption of coagulation factors could extend the prothrombin time (PT) and the activated partial thromboplastin time (APTT)[18]. D-dimer and PT are significant indicators of severe COVID-19 and a poor prognosis[24, 25]. However, the correlation between the function of the coagulation system and *S*. Enteritidis infection remains unclear.

In this study, we isolated 6 strains of *Salmonella* from stool cultures, and serovar identification indicated *S*. Enteritidis. All 6 strains were sensitive to trimethoprim-sulfamethoxazole and levofloxacin, and resistant to ampicillin. Levofloxacin combined with rifaximin and probiotics could be effective in *S*. Enteritidis therapies. We also found that the white blood cell (WBC) count, neutrophil proportion, C-reactive protein (CRP), PT and D-dimer were elevated, while serum iron and prealbumin were decreased in *S*. Enteritidis-infected patients. Prolonged PT, elevated D-dimer and decreased serum iron were positively correlated with serious symptoms (high body temperature and high diarrhea frequency) and increased concentrations of serum inflammatory indexes (CRP, WBC count and neutrophil proportion). Thus, we concluded that prolonged PT, upregulated D-dimer and decreased serum iron were associated with the diagnosis, severity and outcome of *S*. Enteritidis infection, and decreased serum prealbumin was associated with the diagnosis of *S*. Enteritidis infection.

Methods

Blood sample collection and testing

Blood samples were collected from the patients every day by a skilled nurse according to the doctor's order; the first blood samples were collected before antibiotic treatment was initiated. Routine blood tests (Blood cell automated analyzer, XN-1500, Japan SYSMEX, Japan), blood biochemical analyses (Blood biochemical automated analyzer, Becman, American), serum CRP analyses, coagulation function analyses (Blood coagulation analyzer, ACL TPO 700, IL company, American), and serum folate analyses were conducted by our clinical laboratory. Serum hepcidin was detected by ELISA (ELISA Kit for Hepcidin, CEB979Hu, Cloud-Clone Corp, American) according to the manufacturer's instructions.

Faecal Sample Collecting And Test

Fecal samples were collected from the patients every day, and routine stool tests, FOBTs, stool *Clostridium difficile* (*C. difficile*) toxin and antigen detection, stool fungal detection by smear, stool bacterial cultures, and *Salmonella* serovar identification were conducted by our clinical laboratory.

The stool samples were inoculated on SS agar medium. After incubation at 35°C for 24 hours, the single bacterial colony was identified with MALDI TOF MS (Microflex series of Bruker, XJ-058, German). The Serum agglutination test was performed to identify the serotype with the diagnostic sera for *Salmonella* (NingBo, TianRun Bio-Parmaceutical, co.ltd, China), which could identify 60 kinds of *Salmonella* serovar. Antimicrobial susceptibility testing was performed using the MIC method with the VITEK 2 COMPACT analyzer (BioMerieux, France).

Bristol Stool Form Scale

The Bristol Stool Form Scale was used to analyze stool consistency. According to the form of the stool, human feces could be classified into 7 categories: type 1 (separate hard lumps); type 2 (sausage-like but lumpy); type 3 (sausage-like with cracks on the surface); type 4 (sausage- or snake-like, smooth and soft); type 5 (soft blobs with clear edges); type 6 (fluffy pieces with ragged edges, mushy stool); type 7 (watery, no solid pieces, entirely liquid).

Statistical Analyses

All statistical analyses were performed with SPSS 20.0. Pearson correlation coefficients were calculated to analyze correlations between different characteristics. The comparison of quantitative data between different groups was conducted with paired or unpaired Student's t-tests. A value of $p < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the patients infected with S. Enteritidis

Recently, 6 patients from one company visited our department, they all complained of fever (body temperatures ranged from 38.5°C to 39.2°C), watery stool (Bristol type 7, defection frequency ranged from 3 times/day to 10 times/day), nausea, abdominal pain, chills and fatigue; some reported vomiting (1 out of 6, 16.7%), lower back pain (2/6, 33.3%), headache (2/6, 33.3%) and knee pain (2/6, 33.3%) (Table 1). They were all males aged between 18 and 23 years.

Table 1
The clinicalpathological variables of the patients when they were admitted.

	Symptoms									
Patient	Temperature (℃)	Defection frequency (times/day)	Stool Bristol type	Nausea	Abdominal pain	Chills	Fatigue	Vomit	Lower back pain	Headache
Case 1	39.1	5	7	+	+	+	+	-	+	+
Case 2	38.8	3	7	+	+	+	+	+	-	-
Case 3	39	8	7	+	+	+	+	-	-	-
Case 4	39	4	7	+	+	+	+	-	-	-
Case 5	38	4	7	+	+	+	+	-	+	+
Case 6	38.8	10	7	+	+	+	+	-	-	-
	Blood routine test					**Coagulation function**				**Inflammatic**
Patient	WBC	Neutrophil	Lymphocyte	Hb	Platelet	PT	APTT	Fibrinogen	D-dimer	CRP
	(3.5–9.5*10^9/L)	(40–75%)	(20–50%)	(130–175 g/L)	(125–350 *10^9/L)	(9.4–12.5 s)	(25.1–36.5 s)	(2–4 g/L)	(0-299 mg/ml)	(0–5 mg/L)
Case 1	13.75	91.9	4.1	144	194	14.1	35.9	2.17	512	41.9
Case 2	8.37	86.7	9.2	153	172	16.5	27.7	2.21	428	7.2
Case 3	15.72	91.3	4.8	154	178	17.3	17.8	2.59	479	73.8
Case 4	11.54	88.5	6.3	153	161	13.2	31.8	2.06	422	14.3
Case 5	7.1	83.2	11.9	153	187	12.7	36.3	2.18	384	23.2
Case 6	10.03	78.4	14.5	163	179	13.1	34.6	2.21	296	8.3
	Liver function							**Renal function**		
Patient	ALT	AST	Albumin	Prealbumin	ALP	GGT	Total bile acid	Cr	BUN	Serum iron
	(9–50 U/L)	(8–40 U/L)	(40–55 g/L)	(250–400 g/L)	(40–150 U/L)	(11–50 U/L)	(<10 μmol/L)	(62–115 μmol/L)	(2.9–8.2 mmol/L)	(10.6–36.7 μmol/L
Case 1	15.6	21.8	48.6	218	63.3	15.2	1	89.6	4.5	1.3
Case 2	14.2	16.9	47.1	264	73.5	10.6	3.6	90.9	4.7	3.6
Case 3	8.5	16.6	49.1	219	95.5	20.9	1.3	101.1	5.9	3
Case 4	13.3	19.9	44.3	215	78	10	1	91.9	3.3	2.8
Case 5	9.9	16.1	46.5	241	102.3	11.9	2.5	78.2	3.7	4.8
Case 6	16.5	16.1	45.2	237	69.4	20	1.2	87.8	6.2	3.5

To make a definitive diagnosis, the blood tests were accomplished. The results of the inflammatory parameters showed that that the WBC counts in 4 patients (4/6, 66.7%) and the neutrophil proportions and CRP levels in all patients (6/6, 100%) were higher than normal (Table 1). Acute infection may result in multiorgan functional disturbance; thus, we investigated the parameters of liver function, renal function, myocardial enzymes, arterial blood gas analysis results, coagulation function and so on. The results showed that the patients' serum prealbumin levels (5/6, 83.3%) were lower than normal, and patients' PTs (6/6, 100%) and D-dimer (5/6, 83.3%) levels were significantly elevated. Moreover, the patients' alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin levels, APTTs, fibrinogen levels, renal function parameters (blood urea nitrogen [BUN], creatinine [Cr]), myocardial enzymes (TnI) and arterial blood gas analyses results (pH, PaO2, PaCO2) were normal (Table 1). Interestingly, we also found that the serum iron concentrations in the 6 patients were all lower than normal.

Ultrasonography and intestinal computed tomography (CT) examinations revealed intestinal mucosal edema in 3 patients (3/6, 50%), and ascites in 0 patient.

Routine stool examinations revealed red blood cells (RBCs) and WBCs in the stool of 2 patients (2/6, 33.3%); however, coccus to bacillus (C/B) ratio in all 6 patients could not be analyzed, because the bacterial loads were not sufficient (Table 2). In accordance with the routine fecal test results, fecal occult blood tests (FOBTs) were positive in 2 patients (2/6, 33.3%) (Table 2). The detection of *C. difficile* toxins and antigens in the stool were negative (Table 2). Stool culture on *Salmonella Shigella* (SS) agar plates were all positive, the single bacterial colonies were identified with mass spectrometry (MS) and the results indicated *Salmonella.* Serovar testing revealed all isolated strains were *S. enterica* serovar Enteritidis (*S.* Enteritidis) as detected by the Serum agglutination test (Table 2). Taken all, prolonged PT, elevated D-dimer, decreased serum iron and decreased serum prealbumin were associated with the diagnosis of *S.* Enteritidis infection.

Table 2
The fecal test results of the 6 patients when they were admitted.

	stool routine test (cells/per high-power field)		FOBT	C. difficile		Stool culture	Serovar
	WBC	RBC		antigen	toxin A&B	Salmonella	S.Enteritidis
Case 1	8–12	10–15	+	-	-	+	+
Case 2	0–1	0	-	-	-	+	+
Case 3	3–5	20–30	+	-	-	+	+
Case 4	0	0	-	-	-	+	+
Case 5	0	0	-	-	-	+	+
Case 6	0	0	-	-	-	+	+

The Kinetics Of The Patients'; Symptoms And Clinicopathological Variables

Antimicrobial susceptibility testing was performed using the MIC method with the VITEK 2 COMPACT analyzer. All the isolated *Salmonella* strains were sensitive to trimethoprim-sulfamethoxazole and levofloxacin and resistant to ampicillin.

Before fecal cultures and antibacterial susceptibility results were obtained, levofloxacin and *Bacillus licheniformis* capsules were administered according to experience, accompanied by body temperature control, fluid infusion and correction of electrolyte imbalance. According to the antibacterial susceptibility results, we added rifaximin and live combined *B. subtilis* and *Enterococcus faecium* enteric-coated capsules to destroy the *Salmonella* and to modulate the imbalance in the gut microbiota. During the disease course, the body temperatures of 5 patients returned normal on the second day of hospitalization, and the body temperatures of the other 2 patients were controlled on the third day (Fig. 1a). The defecation frequency and stool characteristics (Bristol type) kept decreasing to normal after admission (Fig. 1b). CRP levels increased on the second day and then returned to normal (Fig. 1c); WBC counts (Fig. 1d) and neutrophil percentages (Fig. 1e) continued to decrease after admission. Serum iron levels decreased on the second day and then increased (Fig. 1f). Serum prealbumin levels decreased in the first 3 days and then increased (Fig. 1g). PT increased on the second day and then decreased (Fig. 1h). D-dimer continued to decrease to normal after admission (Fig. 1i). The bowel wall was not detected to be thickening on the 8th day. After hospitalization, 3 additional fecal cultures (on the 3rd, 4th and 5th day respectively) were performed, and the results were all negative. Levofloxacin was withdrawn when a patient's body temperature remained normal for 3 days and the defecation frequency was less than 3 times per day. Patients were discharged on the 10th day.

Positive associations between decreased serum iron and the severity/outcome of S. **Enteritidis infection**

In this study, we found that serum iron levels in all patients was lower than normal, and patients with a higher body temperature, higher WBC count and higher neutrophil concentration had lower serum iron level, and with the remission of symptoms and inflammatory parameters, serum iron increased (Table 1 and Fig. 1). We then analyzed correlations between serum iron level and inflammatory parameters and found that the serum iron concentration was negatively associated with body temperature (Pearson correlation coefficient r= -0.458, p = 0.002, Fig. 2a), defecation frequency (Pearson r= -0.524, p < 0.001, Fig. 2b), CRP level (Pearson r= -0.604, p < 0.001, Fig. 2c), WBC count (Pearson r= -0.429, p = 0.005, Fig. 2d), neutrophil proportion (Pearson r= -0.658, p < 0.001, Fig. 2e) and PT (Pearson r= -0.531, p < 0.001, Fig. 2f).

Hepcidin is a cytokine synthesized by liver and plays a critical role in iron metabolism. We then investigated whether hepcidin is involved in iron down-regulation during *S.* Enteritidis infection. Our results showed that the patients' serum hepcidin levels (6/6, 100%) were higher than normal when they were admitted, they increased on the second day, and then decreased with symptom remission (Fig. 3a). Serum hepcidin was negatively correlated with serum iron (Pearson r= -0.889, p < 0.001, Fig. 3b). Above all, our results indicated that the decreased serum iron was associated with the severity and outcome of *S.* Enteritidis infection, and that the elevated serum hepcidin was associated with the decreased serum iron during S. Enteritidis infection.

Positive associations between the prolonged PT/elevated D-dimer and the severity /outcome of the S. **Enteritidis infection**

We then investigate the correlations between the coagulation system disturbance and the severity and outcome of *S.* Enteritidis infection. The patient with severe symptoms had a higher D-dimer and longer PT than the other patients, and with the remission of symptoms and inflammatory parameters, PTs and D-dimer levels were decreased (Table 1 and Fig. 1). PT was positively associated with body temperature (Pearson r = 0.447, p = 0.003, Fig. 4a), defection frequency (Pearson r = 0.566, p < 0.001, Fig. 4b), CRP level (Pearson r = 0.69, p < 0.001, Fig. 4c), WBC count (Pearson r = 0.544, p < 0.001, Fig. 4d) and neutrophil proportion (Pearson r = 0.612, p < 0.001, Fig. 4e); serum D-Dimer concentration was positively associated with body temperature (Pearson r = 0.823, p < 0.001, Fig. 4f), defection frequency (Pearson r = 0.693, p < 0.001, Fig. 4g), CRP level (Pearson r = 0.512, p = 0.001, Fig. 4h), WBC count (Pearson r = 0.752, p < 0.001, Fig. 4i) and neutrophil proportion (Pearson r = 0.884, p < 0.001, Fig. 4j). Taken together, the prolonged PT and elevated D-Dimer were correlated with the severity and outcome of the *S.* Enteritidis infection.

The Correlation Between Serum Prealbumin And Acute Inflammation Status

Serum prealbumin and albumin are indicators of liver synthesis function, and prealbumin may decrease earlier than albumin when liver function is damaged. As shown above, the decreased prealbumin was associated the diagnosis of *S.* Enteritidis infection. We then investigated the association between serum prealbumin and the severity and outcome of *S.* Enteritidis infection, and found that it was negatively correlated with CRP level (Pearson r= -0.678, p < 0.001, Fig. 5a) and PT (Pearson r= -0.353, p = 0.022, Fig. 5b); there was no significant correlations between serum prealbumin and body temperature (Pearson r =

0.208, $p = 0.187$, Fig. 5c), defection frequency (Pearson r= -0.138, $p = 0.383$, Fig. 5d), WBC count (Pearson r= -0.073, $p = 0.646$, Fig. 5e) and neutrophil proportion (Pearson r= -0.093, $p = 0.559$, Fig. 5f).

As the serum prealbumin continued to decrease in the first 3 days, and begun to increase at the 4th day, while other parameters of inflammation begun to restore at the 2th or 3th day. We then analyzed the correlation between serum prealbumin and inflammatory parameters of the first day, and found that although the correlation coefficients were larger than 0.2 or smaller than − 0.2, the p values were all larger than 0.05 (Fig. 5g-5 l), this could be reasoned by the small case number. Taken together, our results indicated that a decreased prealbumin was correlated with the diagnosis of the *S*. Enteritidis infection, but more solid proofs were needed to verify its association with the severity and outcome of *S*. Enteritidis infection.

The kinetics of serum homocysteine during S. Enteritidis infection

When the patients were admitted, all patients had symptoms of diarrhea, thus, a low-fat and low-protein diet was provided. A normal diet was reintroduced when a patient' defecation frequency was less than 3 times per day and a patient' fecal Bristol type returned to type 5. However, during the disease course in these 6 patients, we found that the patients' homocysteine levels continued to increase beginning on the 6th day of hospitalization, and 2 patients' homocysteine levels were higher than normal on the 7th day (Fig. 6a). The patients were aged from 18 to 23 years, their blood pressures were normal, and none had a family history of high blood pressure; therefore, other mechanisms responsible for the increased serum homocysteine may exist. Serum folate plays a critical role in homocysteine metabolism. Accompanied by methyltetrahydrofolic acid, homocysteine can be transformed into methionine and tetrahydrofolic acid by methylenetetrahydrofolate (MTHFR). We found that the patients' serum folate levels were either lower than normal or at the lower normal limit (7 nmol/L ~ 45.6 nmol/L) on the 8th day. We provided folate tablets (5 mg, QD) to the patients with high serum homocysteine and asked the other patients to improve their nutrition and consume more green vegetables for folic acid supplementation. Then, serum folate increased (Fig. 6b), while serum homocysteine decreased (Fig. 6a). Thus, we suggest that a normal diet should be reintroduced early.

Discussion

Salmonella infection is a foodborne disease, and meat products are the main sources of human salmonellosis. In this study, our epidemiological investigation revealed that all 6 patients had a history of consuming the same improperly stored, cold meals that were purchased online. Samples were collected and sent to the Center for Disease Control (CDC); the specific source needs further investigation.

To date, more than 2600 *Salmonella* serotypes have been reported, among which *S*. Enteritidis and *S*. Typhi are the most common[5]. *S*. Enteritidis infection is generally self-limiting, but patients can present with severe manifestations,and these patients should receive antibiotics therapy. In this study, the 6 patients suffered from fever, chills, watery stool, nausea and fatigue, which needed appropriate treatment. However, because of antibiotic abuse, high proportions of *Salmonella* isolates are resistant to tetracycline (53.9%), ciprofloxacin (47.2%), ampicillin (44.4%), nalidixic acid (42.7%), and trimethoprim-sulfamethoxazole (38.8%)[26]. In this study, all 6 isolates were sensitive to trimethoprim-sulfamethoxazole and levofloxacin and resistant to ampicillin. A combination of levofloxacin, rifaximin, and live combined *B. subtilis* and E. *faecium* enteric-coated capsules were administered, and the patients' symptoms began to improve on the second day, indicating that this treatment strategy was effective. Besides medicine treatment, we also recommend strengthening nutrition and reintroducing the normal diet early, however, the specific time to reintroduce the normal diet needs further investigation.

As stool culture and blood culture examinations are time-consuming, identifying other parameters correlated with *S*. Enteritidis infection is meaningful. In this study, we found that in addition to the elevated inflammatory indicators such as body temperature, CRP, WBC count and neutrophil cell proportion, the patients' serum iron and prealbumin levels were significantly decreased, while PT and D-dimer levels were elevated.

Iron metabolism disturbance has been widely reported in various infectious diseases. *S*. Typhi competes with humans for iron utilization by increasing the expression of hepcidin, which can restrict iron uptake and macrophage iron release, and the high serum hepcidin leads to hypoferremia[8]; COVID-19 infection resulted in hyperferritinemia due to cytokine storms, and serum iron transferrin saturation in intensive care unit (ICU) patients infected with COVID-19 was extremely reduced for the first 2 days and then increased from days 3 to 6[9, 27, 28]. In this study, we found that serum iron was significantly decreased in *S*. Enteritidis patients, and it seemed to be lower in patients with more serious symptoms; with the recovery of patients' symptoms, serum iron increased. Serum iron was negatively associated with body temperature, defecation frequency, CRP, WBC count, neutrophil proportion and PT. We also found that in the patients, serum hepcidin was elevated and that its concentration was negatively associated with serum iron. Thus, we hypothesized that *S*. Enteritidis infection downregulated serum iron by increasing serum hepcidin, and the decreased serum iron was correlated with the diagnosis, severity and outcome of *S*. Enteritidis infection.

Coagulation system activation is a characteristic parameter of acute infectious disease. Acute inflammation-mediated endothelial cell damage could result in the activation of the coagulation system, and the consumption of coagulation factors leads to disseminated intravascular coagulation (DIC)[29]. *S*. Typhi infection has been reported to activate the coagulation system, increase serum fibrinogen and D-dimer and prolong PT and APTT[18]. COVID-19 infection may lead to disturbances in the procoagulation, anticoagulation and fibrinolytic systems[30–34]; a high concentration of D-dimer and prolonged PT are associated with severe COVID-19 and a poor prognosis[24]. In this study, we found that RBCs were detected in the stool of 2 patients, PT was prolonged and D-dimer was elevated in all 6 patients, while APTTs and fibrinogen levels were normal. There was no obvious change in the APTT during the disease course (Additional file 1: Fig. 1a). Fibrinogen concentrations increased for the first 4 days and then decreased, but during the disease course, serum fibrinogen in only 1 patient was higher than normal (4 g/L) on days 4, 5 and 6 (Additional file 1: Fig. 1b). Prolonged PT and elevated D-dimer were positively correlated with more serious symptoms (high body temperature and high diarrhea frequency) and high concentrations of serum inflammatory indexes (CRP, WBC count and neutrophil proportion), thus, we reported that a prolonged PT and an increased D-dimer concentration were associated with the diagnosis, severity and outcome of *S*. Enteritidis infection.

Acute infection may cause a strike and suppress the function of multiple organs. Gamma-glutamyltransferase (GGT) and prealbumin were reported to have critical values in the diagnosis of COVID-19, and prealbumin and albumin were downregulated as the disease severity increased[25]. The decreased prealbumin is a marker of disease activity in cholerae infection[35]. In this study, we found that serum prealbumin in 5 patients was lower than normal when they were admitted, and it continued to decrease for the first 3 days. It finally began to increase on the 4th day of admission. However, analyzing the associations between serum prealbumin concentration and the index of the severity of the disease, there was no significant correlation, which may be explained by bowel wall edema-mediated intestinal absorption disorder and liver functional reserve. In all, we reported that decreased prealbumin was correlated with the diagnosis of *S*. Enteritidis infection, but more solid proofs are needed to verify its associations with the severity and the outcome of the *S*. Enteritidis infection.

Conclusion

In this study, we isolated 6 strains of *Salmonella* from stool cultures, and serovar testing indicated *S*. Enteritidis. All 6 strains were sensitive to trimethoprim-sulfamethoxazole and levofloxacin and resistant to ampicillin. Levofloxacin combined with rifaximin and probiotics could be an effective *S*. Enteritidis therapy. The prolonged PT, elevated D-dimer and decreased serum iron were associated with the diagnosis, severity and outcome of the *S*. Enteritidis infection, the decreased serum prealbumin was associated with the diagnosis of *S*. Enteritidis infection. In patients with symptoms of acute fever, watery stool, and abdominal pain, PT, D-dimer, serum iron and serum prealbumin should be monitored to attain an early diagnosis and initiate appropriate treatment for *S*. Enteritidis infection.

Abbreviations

S. Enteritidis: *Salmonella enterica* serovar Enteritidis; FOBTs: fecal occult blood tests; SS: *Salmonella Shigella*; MS: mass spectrometry; MIC: minimal inhibitory concentration; WBC: white blood cell; RBC: red blood cell; CRP: C-reactive protein; PT: prothrombin time; TCA: tricarboxylic acid; APTT: activated partial thromboplastin time; COVID-19: coronavirus disease 2019; *difficile: Clostridium difficile*; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: Gamma-glutamyltransferase; BUN: blood urea nitrogen; CR: creatinine; TnI: myocardial enzymes; CT: computed tomography; C/B: coccus to bacillus; CDC: Center for Disease Control.

Declarations

Ethics approval and consent to participate

The researchers collected the data after the project was certified from the Research Ethics Committee, the Sixth Medical Center of PLA General Hospital.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LHC designed the project, provided the funding, corrected the manuscript and approved the final version; XWL analyzed the data, accomplished the Elisa study, wrote the original draft; SXW, LY and XNL helped with the data collection and literature research; XHW, ZHY and ZY helped with the statistical analyses; YJL accomplished the main laboratory test; JR helped with the sample collection.

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Figures

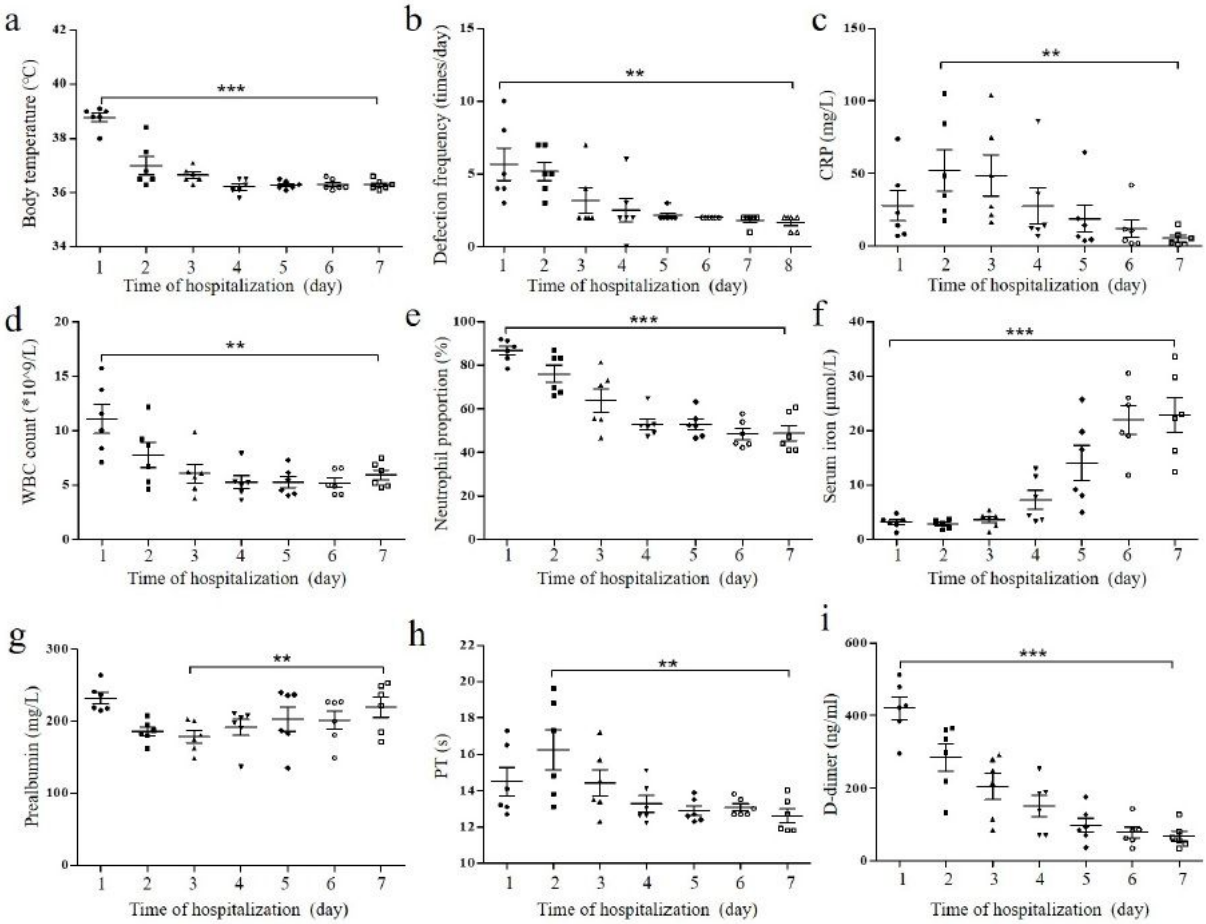

Figure 1

The kinetics of the patients' symptoms and clinicopathological variables. a Body temperatures, b Defection frequency, c CRP levels, d WBC counts, e: Neutrophil percentage, f Serum iron levels, g Prealbumin levels, h PT, i D-dimer levels were measured in the 6 patients infected with S. Enteritidis. **: $p<0.01$, ***: $p<0.001$.

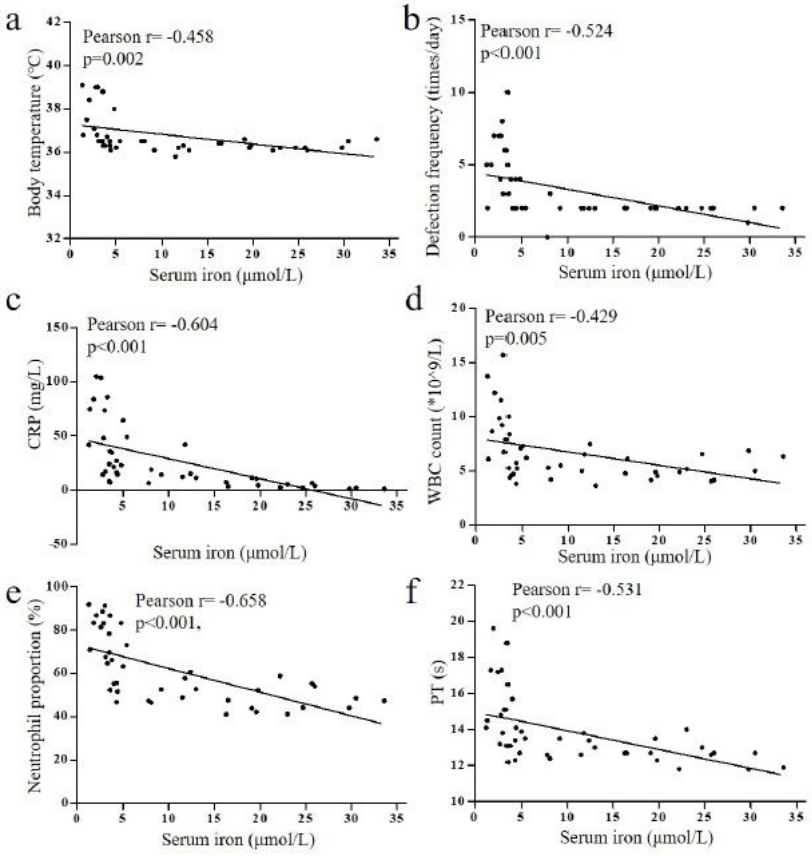

Figure 2

Correlations between serum iron levels and the severity/outcome of the S. Enteritidis infection. The correlation between a Serum iron levels and body temperatures, b Serum iron levels and defection frequency, c Serum iron levels and CRP levels, d Serum iron levels and WBC counts, e Serum iron levels and neutrophil percentage, f Serum iron levels and PT were analyzed. The data of each parameter in the first 7 days were included. The Pearson correlation coefficient and the p values are stated in the corresponding Figure.

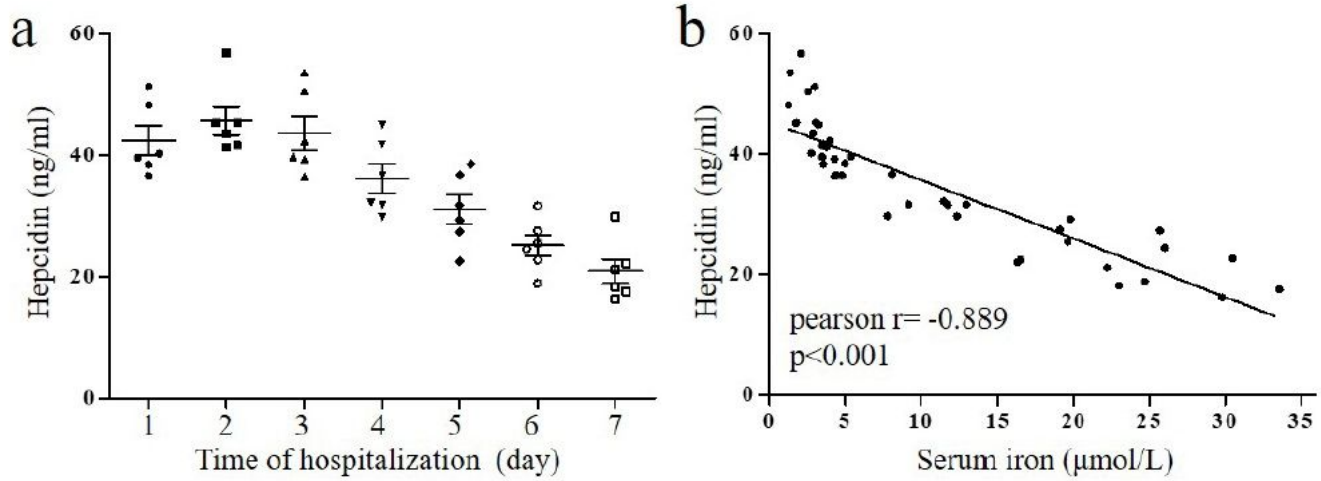

Figure 3

The elevated serum hepcidin was associated with the decreased serum iron. a The kinetics of the patients' serum hepcidin levels were measured in the 6 patients infected with S. Enteritidis. ***: p<0.001. b The correlation between serum iron levels and hepcidin levels in the first 7 days were analyzed. The Pearson correlation coefficient and the p values are stated in the corresponding Figure.

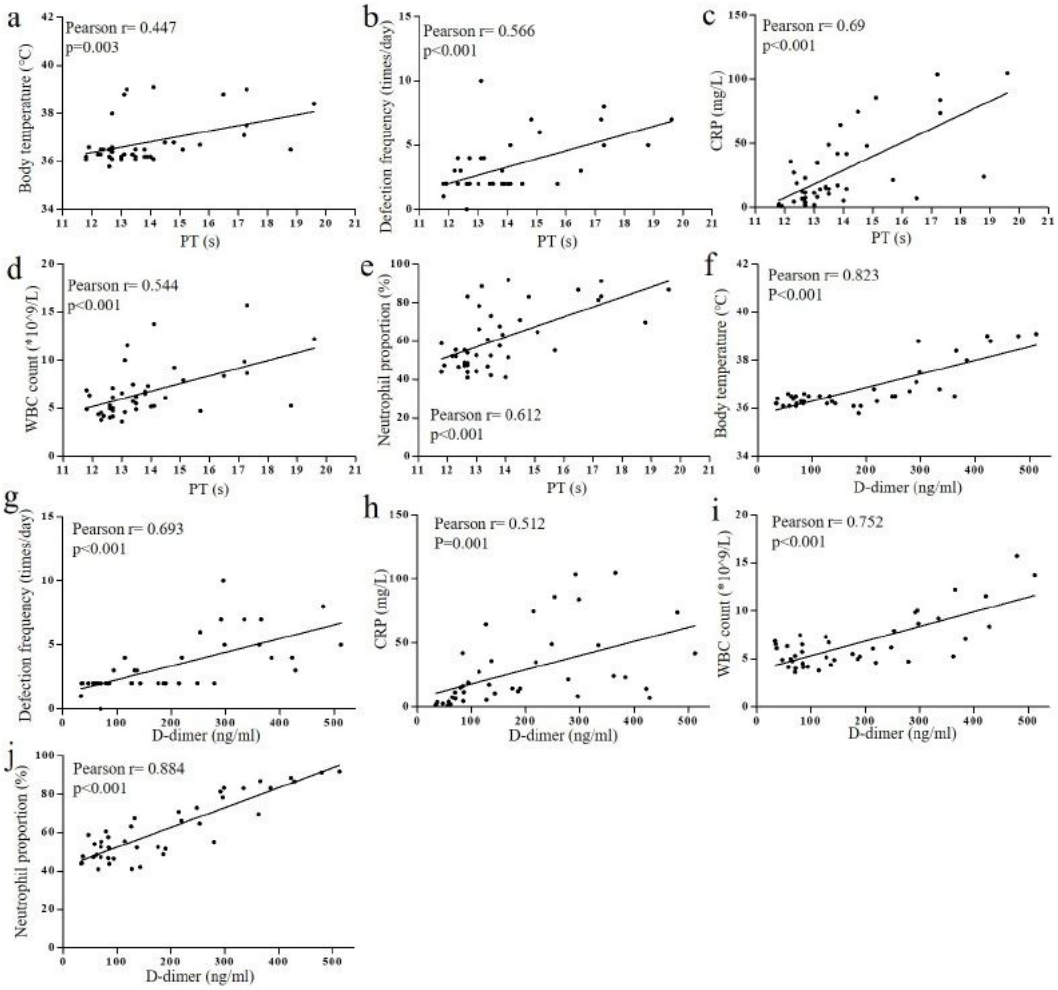

Figure 4

Correlations between PT/D-dimer and the severity/outcome of the S. Enteritidis infection. The correlation between a PT and body temperatures, b PT and defection frequency, c PT and CRP levels, d PT and WBC counts, e PT and neutrophil percentage, f D-dimer levels and body temperatures, g D-dimer levels and defection frequency, h D-dimer levels and CRP levels, i D-dimer levels and WBC counts, j D-dimer levels and neutrophil percentage were analyzed. The data of each parameter in the first 7 days were included. The Pearson correlation coefficient and the p values are stated in the corresponding figure.

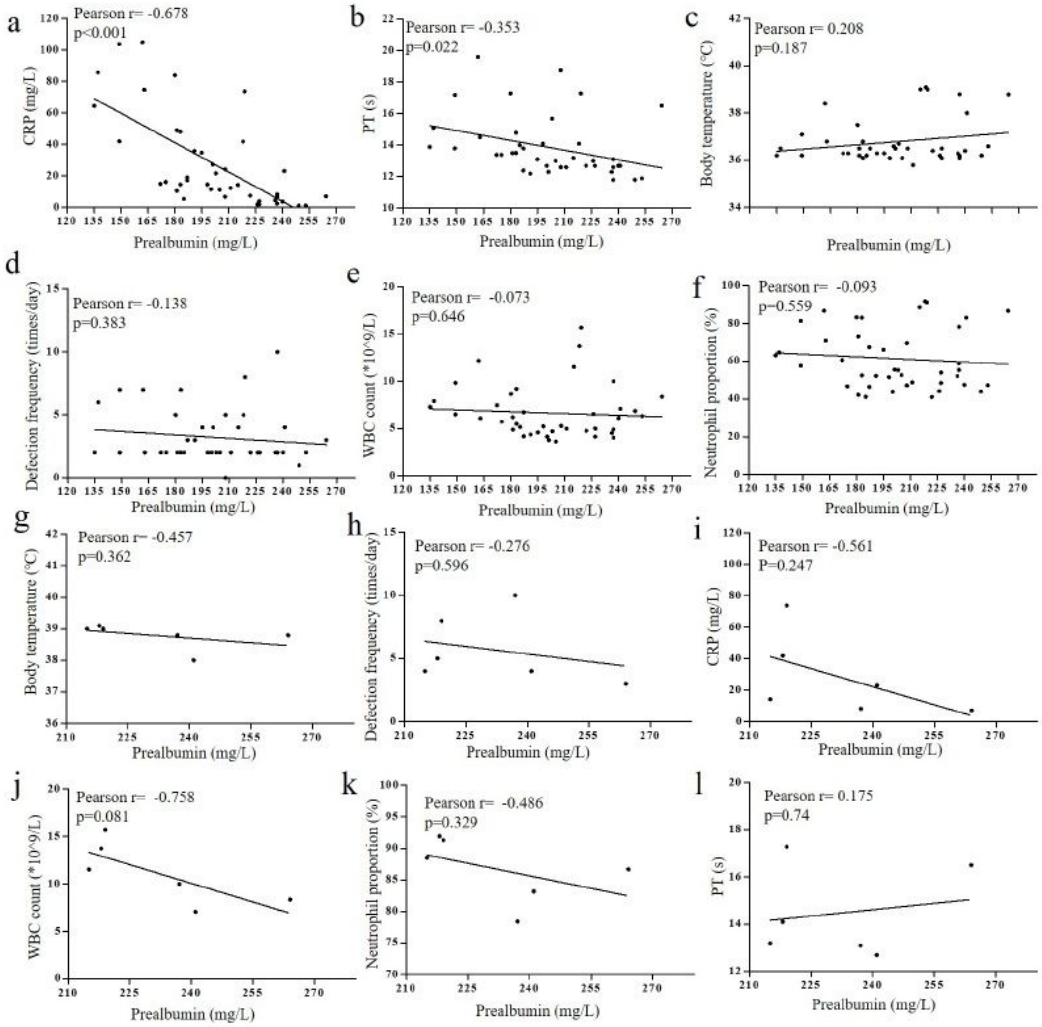

Figure 5

Correlations between serum prealbumin and the acute inflammation status. The correlation between a Serum prealbumin levels and CRP levels, b Serum prealbumin levels and PT, c Serum prealbumin levels and body temperatures, d Serum prealbumin levels and defection frequency, e Serum prealbumin levels and WBC counts, f Serum prealbumin levels and Neutrophil percentage were analyzed. Fig. 5 a-f contained the data in the first 7 days. The correlation between g Serum prealbumin levels and body temperatures, h Serum prealbumin levels and defection frequency, i Serum prealbumin levels and CRP levels, j Serum prealbumin levels and WBC counts, k Serum prealbumin levels and neutrophil percentage, l Serum prealbumin levels and PT were analyzed. Fig. 5 g-l contained the data on the first 1 day. The Pearson correlation coefficient and the p values are stated in the corresponding figure.

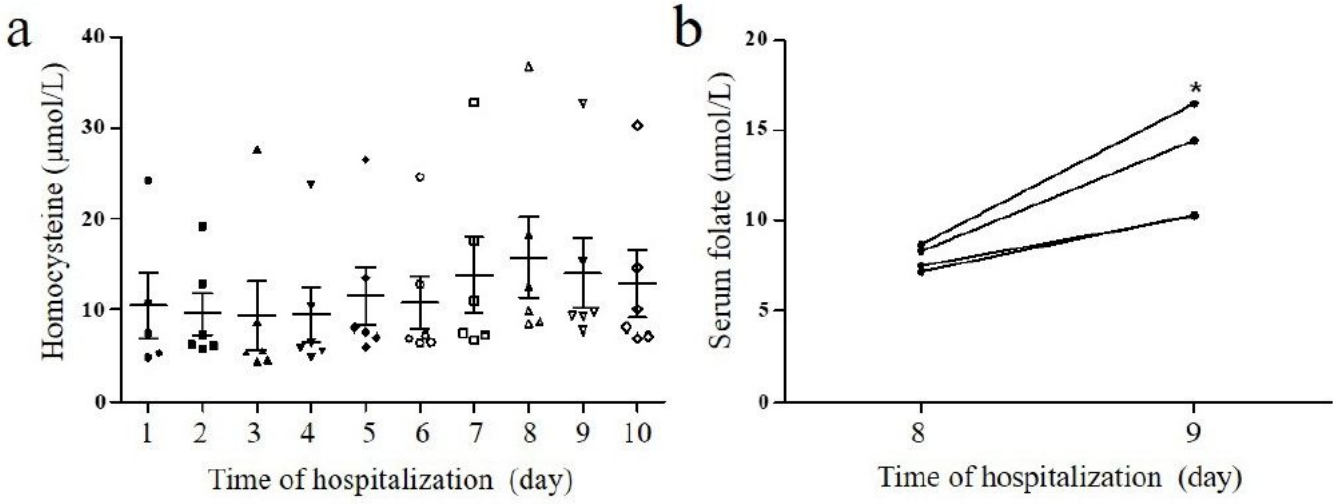

Figure 6

The kinetics of patients' serum homocysteine during S. Enteritidis infection. a Serum homocysteine were measured in the 6 patients infected with S. Enteritidis during the disease course. b Serum homocysteine on the 8th and 9th days were measured in the patients. **: $p<0.01$. Note: Fig. 6B didn't conclude the folate of the two patients who were administered folate plate, their folate on the 8th day was 5.8 and 7.3 nmol/L respectively, and their serum folate on the 9th day were larger than the upper limit that our machine could measure (54.6 nmol/L).

Supplementary Files

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