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MAJOR ARTICLE

Human Case Infected With *Babesia venatorum*: A 5-Year Follow-Up Study

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Background. Human babesiosis is a common zoonosis caused by *Babesia* and is attracting an increasing concern worldwide. The natural course of babesiosis infection and how the human immune system changes during the course of babesiosis infection are not clear.

Methods. We followed up 1 case infected with *Babesia venatorum* for 5 years. The patient was immune-intact and received no standard treatment. Clinical data were obtained from medical records. Microbiological tests, ribonucleic acid (RNA) sequence, and serum cytokines and chemokines were detected at different time points.

Results. The patient was confirmed as *B venatorum* infection based on his tick-bite history, clinical manifestations, and positive results of microbiological tests. The parasitemia of the patient persisted for approximately 2 months. With flu-like symptoms aggravating, most cytokines and chemokines in RNA and protein levels increased progressively and reached the peak when fever occurred; and their concentrations decreased to baseline during the same time as clearance of babesia parasites.

Conclusions. *Babesia venatorum* infection could take a mild self-limited course in immune-intact individuals. The natural changes of most cytokines and chemokines demonstrated very similar trends, which correlated with blood parasitemia and clinical manifestations. Cytokine profiles involving multiple inflammatory cytokines might be a good indicator of babesia infection.

Keywords. *Babesia venatorum*; babesiosis; cytokines and chemokines; follow-up study; natural course.

Human babesiosis, a worldwide, emerging, life-threatening zoonosis, caused by intraerythrocytic parasites of protozoan genus *Babesia*, is usually tick-borne, but it also can be acquired through blood transfusion or transplacentally [1]. The severity of clinical manifestations of babesiosis ranges from asymptomatic to fatal, depending on the immune states of humans and the babesia species infected [1]. Most cases of babesiosis experience subclinical infections, or nonspecific symptoms, such as fever, fatigue, sweating, chill, and headache [1–3]. Thus, the illness can be easily misdiagnosed or missed diagnosis, and antibiotics might be misused. A babesia carriage state can be established for 1 year or more [4], especially in cases who are not diagnosed or treated promptly [1]. The persistent babesiosis may impose a heavy health burden on infected cases and increase its transmission risk to the public [5].

Human babesiosis is attracting increasing concern around the world due to its expanding distribution and increasing prevalence [6]. In China, many cases of babesiosis have been diagnosed during the past few years [7], involving 48 cases infected with *Babesia venatorum* [8]. Previous studies have shown that *B venatorum* infection was primarily observed in Europe among patients with asplenia, and/or other immune deficits, who experience severe disease [6, 9–11]. Unlike the European patients, the 48 cases reported by our group revealed that *B venatorum* infection could occur in immune-intact population, with clinical manifestations varying from asymptomatic to severe [8]. It is the largest case series ever reported for this species of babesia [12], and it has important health implications in China.

China is the first reported babesiosis endemic site outside the United States. Because *B venatorum* is an emerging disease threat, active vigilance and a public health response are needed. In the present study, we followed up 1 confirmed case of *B venatorum* infection from the northeastern China for approximately 5 years. The objectives of the study are to (1) better understand the natural course of *Babesia* species eradication from infected humans, (2) explore potential indicators for clearance of the parasite, and (3) investigate how the immune system is activated and changes during the course of *B venatorum* infection.

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There is little previously published information on each of these 3 objectives.

METHODS

Patient

A follow-up study was conducted in the Mudanjiang Forestry Central Hospital in Heilongjiang province located in northeastern China. The patient visited the hospital in 2012 for his illness, and he had been followed up during the next 5 years. He was immune-intact, received no standard treatment, and had good compliance with follow-up investigations. A standardized questionnaire was used to collect the demographic characteristics, tick exposure, blood transfusion, and medical history of this patient. Clinical manifestations including symptoms and signs were obtained from medical records. This study was approved by the ethics committee of the Mudanjiang Forestry Central Hospital (Mudanjiang Forestry Central Hospital 2011-03), and written informed consent was obtained from the patient.

Biological Sample Collection

Peripheral venous blood samples were collected from the patient into two 5-mL tubes at different time points (see Supplementary Table 1). Ethylenediaminetetraacetic acid-anticoagulated blood was used for blood routine test, microbiological test, and ribonucleic acid (RNA) sequencing. The serum tube was coagulated at room temperature for 30 minutes and centrifuged at 800 ×*g* for 10 minutes. Subsequently, 0.5-mL separated serum was used for the blood biochemical test, and the remaining was stored at −40°C within 1 hour until cytokine determination [13].

Microbiological Test

Microbiological tests including *Babesia*-specific polymerase chain reaction (PCR) amplification and Giemsa-stained blood smear were used to confirm the *B venatorum* infection and to detect the possibility of infection persistence. As described in detail in our previous work [8], nested PCR-targeted full-length 18S rRNA gene of *Babesia* was conducted, and positive amplicon was sequenced for confirmation of *B venatorum* infection in a commercial company. A Giemsa-stained blood smear was observed with a light microscope for intraerythrocytic babesia organisms.

Ribonucleic Acid Specimens and Sequencing

Ribonucleic acid was isolated from the blood sample using miRNeasy Mini Kit (catalog number 217004, QIAGEN). The RNA concentration was measured using Qubit RNA Assay Kit in Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA). A total amount of 3 μg of RNA per sample was used as input material for RNA sample preparations. Sequencing libraries were generated using NEB Next Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) following the manufacturer's recommendations. The PCR products were purified (AMPure XP system), and the library quality was assessed on an Agilent Bioanalyzer 2100 system. The library preparations were sequenced on an Illumina Hiseq 4000 platform, and 150-base pair paired-end reads were generated.

Ribonucleic Acid-Sequence Data Analysis

Reference genome and gene model annotation files were downloaded from genome website browser Ensembl directly [14]. Indexes of reference genome were built using Bowtie v2.0.6 [15], and paired-end clean reads were aligned to reference genome using TopHat v2.0.9 [16]. Cufflinks v2.1.1 was used to count fragment numbers mapped of each gene [17]. Then, FPKM of each gene was calculated based on length of gene and fragments count mapped to this gene. Differential expression analysis was performed using DESeq2 R package (1.10.1) [18]. The resulting *P* values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted $P < .05$ found by DESeq2 were selected. Pathway analyses were performed using GSEA software and KOBAS web server based on REACTOME pathway database [19].

Determination of Cytokines and Chemokines

Circulating concentrations of cytokines and chemokines were determined. Serum levels of C-X-C motif chemokine ligand 2 (CXCL2), CXCL3, and C-C motif chemokine ligand 20 (CCL20) were measured by enzyme-linked immunosorbent assay kits according to manufacturer's instructions (Cytokine ELISA Kits; TBHealthcare, Foshan, Guangdong, China). Serum interleukin (IL)-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, CCL2, CCL3, CCL4, CCL5, CCL11, CXCL1, CXCL10, CXCL12, interferon (IFN)-γ, IFN-α, tumor necrosis factor (TNF)-α, TNF-β, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were analyzed using a Multiplex Luminex assay (ProcartaPlex kit; Thermo Fisher Scientific, Waltham, MA) per the manufacturer's protocols.

RESULTS

Case Presentation

The patient, a 24-year-old male who is a cook in a middle school, experienced symptoms for approximately 3 months and had follow-up visits over 4 and one half years. He sought a diagnosis in Mudanjiang Forestry Central Hospital on June 4, 2012, because of nausea, headache, dizziness, but no fever for 5 days. His peripheral venous blood was collected for microbiological tests because he recalled a tick bite on May 28. The patient admitted taking forestry activity routinely but had never received blood transfusion or splenectomy. He was immune-intact and has never had immune-suppressing drugs. On June 5, the patient was initially diagnosed for babesiosis through positive PCR assay and positive babesia parasites in erythrocytes (parasitemia <1%). Later, phylogenetic analysis revealed that the

sequence was identical to *B venatorum* reported in Europe [8, 9]. Based on his tick exposure, clinical manifestations, and positive results of microbiological tests, a diagnosis of *B venatorum* infection was confirmed. His physician recommended admission to the hospital and initiation of standard anti-babesia therapy, but the patient refused due to the high costs of this therapy. In these circumstances, a follow-up study and self-checks were arranged to monitor his infection (Figure 1).

After that, the patient experienced persistent nonspecific symptoms for approximately 3 weeks. On June 24, his symptoms had progressively worsened with no associated aggravating factors noted. Flu-like symptoms, involving headache, dizziness, nausea, nasal obstruction, runny nose, sore throat, and difficulty in swallowing, occurred but no fever. On his re-admission to hospital on June 28, the physician asked the patient to take his temperature daily. On July 7, the patient showed progressive symptoms with a fever of 37.5℃. Two days later, his symptoms reached the peak. On July 21, the first follow up of the patient, flu-like symptoms had been alleviated but still existed. Since then, his body status improved gradually. At his second follow up on August 11, blood smear and *B venatorum* PCR were negative and his clinical manifestations resolved. He remained blood smear and PCR negative without symptoms over the next 4 and one half years (Figure 1).

Clinical Examinations

The results of blood routine and blood biochemical tests are shown in Table 1. The level of lymphocyte percentage was higher than the normal range (20%–40%) during follow-up period except on August 11, whereas the percentage of intermediate cell was lower (normal range, 3%–10%) in several examinations. Mean platelet volume of the patient was always below the normal range (6–10 fL) until his last follow up, and the platelet hematocrit (PCT) was relatively low in his second and fourth follow ups (normal range, 0.08%–1%). On August 11, the concentration of platelet was 76×10^9/L, which was lower than the normal range ($85–303 \times 10^9$/L).

Total bilirubin (TBIL) and indirect bilirubin (IBIL) of the patient were higher than their upper limits (21 μmol/L for TBIL and 17 μmol/L for IBIL) since August 11. The level of direct bilirubin was 8 μmol/L and 7.6 μmol/L on September 18 and January 14, 2013, respectively, both higher than the upper limit (6.8 μmol/L). In addition, the concentration of prealbumin was significantly higher than normal (180–390 mg/L) on September 18. Other clinical indicators were basically within the normal range during follow ups.

Microbiological Test

On the day of admission (June 4), *Babesia*-specific 18S RNA PCR of the patient was positive, and parasitic inclusions in erythrocytes (parasitemia <1%) could be observed in Giemsa-stained blood smears (Figure 1 and Figure 2), confirming the infection of babesia. In the following tests from June 28 to July 21, PCR amplifications and microscopic examinations showed positive results, suggesting the persistence of infection. The parasitemia was present at a low level on July 21 (parasitemia <1%). Since August 11, PCR testing and blood smear turned negative, with 0% parasitemia, first indicating the clearance of *B venatorum*. Thereafter, the results of microbiological tests remained negative, showing no existence of babesia parasites. On January 10, 2015, an additional follow up determined that the patient was still PCR negative for *B venatrum* 3 years after initial infection. On February 8, 2017, we carried out the final follow up. The results of all microbiological tests were still negative, accompanied by no clinical

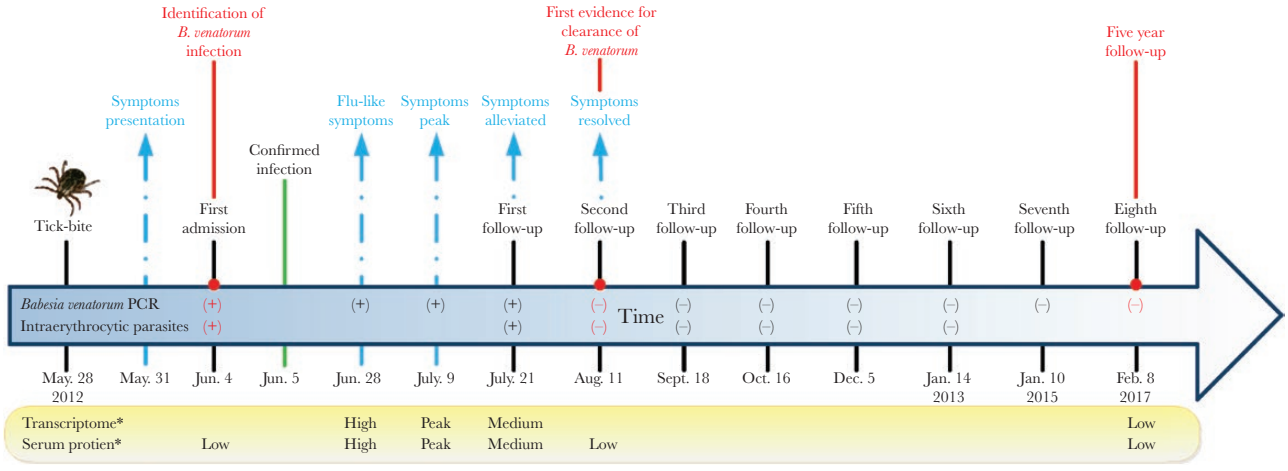

Figure 1. The course of the babesia infection and follow ups. The figure depicts the timeline of babesia infection and follow ups of this patient and the close relationship between clinical manifestations (above the time arrow), microbiological test (in the time arrow), and cytokine levels (below the time arrow). (+) positive; (–) negative; *, levels of most cytokines and chemokines.

Table 1. Blood Routine and Blood Biochemical Data for the Babesiosis Patient

Date		2012							2013	2017
		June 4	July 9	July 21	August 11	September 18	October 16	December 5	January 14	February 8
Blood Routine Data										
WBC	10^9/L	5.5	4.7	4.6	4.0	4.9	4.5	4.6	5.3	5.5
RBC	10^{12}/L	5.10	5.10	5.20	4.50	5.70	5.10	5.50	4.80	5.97[a]
HGB	g/L	165	164	168	141	170	164	168	155	182[a]
HCT	%	43.8	47.6	49.1	42.9	54.0[a]	49.0	47.3	42.0	53.3[a]
MCH	pg	32.7	32.5	32.3	31.1	29.8	32.0	30.8	32.1	30.5
MCV	fL	86.7	94.3	94.4	94.5	94.7	95.7	86.6	87.0	89.3
MCHC	g/L	377[a]	345	342	329	315[b]	335	355	369[a]	341
PLT	10^9/L	167	196	159	76[b]	148	148	157	150	166
LYM%	%	44.7[a]	40.5[a]	47.0[a]	36.6	42.2[a]	45.7[a]	45.1[a]	40.2[a]	46.3[a]
MID%	%	2.3[b]	2.9[b]	3.1	3.3	2.4[b]	2.5[b]	3.2	2.3[b]	3.6
GRAN%	%	53.0	56.6	49.9[b]	60.1	55.4	51.8	51.7	57.5	50.1
RDW	%	12.8	12.6	12.1	11.7	12.9	12.1	11.7	11.7	11.4[b]
PCT	%	0.08	0.10	0.08	0.04[b]	0.08	0.07[b]	0.09	0.09	0.11
MPV	fL	5.1[b]	5.1[b]	5.1[b]	5.2[b]	5.1[b]	4.9[b]	5.6[b]	5.8[b]	6.9
PDW	fL	17.0	17.0	17.0	18.4[a]	17.1	16.3	17.5	16.2	18.3[a]
Blood Biochemical Data										
ALT	U/L	ND	ND	9.9	11.9	16.0	11.2	11.1	9.6	ND
AST	U/L	ND	ND	15.5	14.7	21.0	19.9	17.6	18.0	ND
AST/ALT		ND	ND	1.60	1.20	1.30	1.78	1.59	1.88	ND
TBA	μmol/L	ND	ND	8.99	1.19	1.09	0.20	1.60	0.80	ND
TBIL	μmol/L	ND	ND	18.6	25.7[a]	34.4[a]	29.4[a]	29.5[a]	28.6[a]	ND
DBIL	μmol/L	ND	ND	4.8	6.5	8.0[a]	6.8	5.0	7.6[a]	ND
IBIL	μmol/L	ND	ND	13.8	19.2[a]	26.4[a]	22.6[a]	24.5[a]	21.0[a]	ND
ADA	U/L	ND	ND	10.0	10.0	8.7	3.6	4.2	3.9	ND
ChE	KU/L	ND	ND	9.54	8.82	11.23	7.44	8.19	7.61	ND
PALB	mg/L	ND	ND	352.6	366.8	485.7[a]	321.1	269.1	252.2	ND
ALP	U/L	ND	ND	88	85	79	65	78	69	ND
GGT	U/L	ND	ND	48.0	16.3	17.4	17.0	17.0	16.0	ND
UA	μmol/L	ND	ND	350	334	339	313	311	327	ND
CRP	mg/dL	ND	ND	0.07	0.08	0.04	0.04	0.03	0.03	ND

Abbreviations: ADA, adenosine deaminase; ALP, alkaline phosphatase; ALT, alanine amiotransferase; AST, aspartate aminotransferase; ChE, cholinesterase; CRP, C-reactive protein; DBIL, direct bilirubin; GGT, γ-glutamyl transpeptadase; GRAN%, percentage of neutrophile granulocyte; HCT, hematocrit; HGB, hemoglobin; IBIL, indirect bilirubin; LYM%, lymphocyte percentage; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MID%, percentage of intermediate cell; MPV, mean platelet volume; ND, not determined; PALB, prealbumin; PCT, platelet hematocrit; PDW, platelet distribution width; PLT, platelet; RBC, red blood cells; RDW, red cell distribution width; TBA, total bile acid; TBIL, total bilirubin; UA, uric acid; WBC, white blood cells.

[a]Above the normal range.

[b]Below the normal range.

manifestations, demonstrating no relapsing of babesiosis in these years (Figure 1 and Figure 2).

Transcriptome Analysis

We detected the RNA transcriptome for cytokines and chemokines by high-throughput transcriptome analysis 4 times during acute illness and convalescence of this patient. To make it clearer, FPKM was shown in 4 separate graphs in Figure 3 according to their maximum numerical values (from high to low). After data analysis, the transcription of cytokines and chemokines showed similar trends. The levels of most cytokines including ILs (IL-6 and IL-1α) and chemokines (CXCL8, CXCL2, CXCL3, CCL2, and CCL20) were relatively high on June 28, 2012, when flu-like symptoms began, and then reached the peak on July 9, accompanied by the peak symptoms and fever. Since July 21, the levels of these cytokines decreased dramatically to low levels. For IL-1β, the level was highest on June 28 and showed a continuous decline in the following detections.

Cytokines and Chemokines in the Serum

The serum concentrations of cytokine and chemokine were monitored repeatedly. As shown in Figure 4, the changes of all indicators were shown in 4 separate graphs, which categorized by their maximum numerical values (from high to low). During his 6 follow-up investigations, the expression of most cytokines including ILs (IL-22, IL-2, IL-9, IL-5, IL-18, IL-27, IL-1α, IL-4, IL-21, IL-1β, IL-12p70, IL-13, IL-15, IL-17A), TNFs (TNF-β and TNF-α), chemokines (CCL5 and CXCL1), CSF (GM-CSF), and IFN-γ were of low levels on June 4, 2012, his first visit to the hospital. With the occurrence and aggravation of flu-like

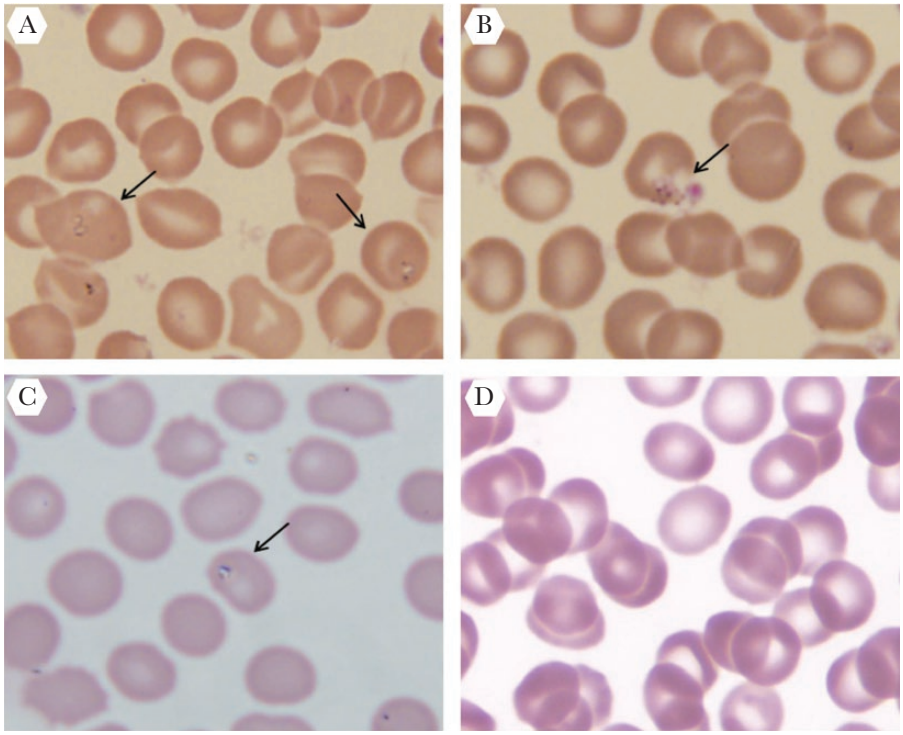

Figure 2. Giemsa-stained blood smear from the patient. Geimsa-stained blood smears revealed intraerythrocytic babesia on June 4 (A and B) and July 21 (C), but no babesia on August 11 (D).

symptoms, the concentrations increased and reached the peak on July 9. Since then, the levels started to decrease and remained low with a small fluctuation until the last detection. For CCL20, IL-6, CXCL2, and IL-10, the highest concentration was on June 28 and then decreased. Other cytokines we detected had no significant trends during follow ups.

DISCUSSION

Babesiosis is a tick-borne zoonosis, whose clinical manifestations vary from asymptomatic to even life-threatening [1]. Most cases infected with babesia manifest flu-like symptoms and could be easily confused with other diseases [3]. Physicians and the public are not well informed of babesiosis in most parts of China. Heilongjiang province, where the patient lives, is an endemic area of tick-borne diseases, including babesiosis. The dominant tick species are *Ixodes persulcatus* and *Haemaphysalis concina* [8], which are competent vectors for a wide variety of tick-borne infectious diseases [20, 21]. In this study, the patient developed symptoms 3 days after he noticed a tick bite. Fortunately, due to the widespread dissemination of information on tick-borne diseases from the local government, residents in northeastern China have improved awareness of tick-borne diseases and know they can receive help in their sentinel hospital. The patient was diagnosed with babesiosis soon after admission since he met the criteria for Babesia-confirmed case [8], including objective clinical evidence (fever), subjective clinical evidence (headache), and laboratory confirmatory criteria (positive PCR and sequence being identical to *B venatorum* deoxyribonucleic acid, and observation of typical pyriforms in the microscopic examination).

Previous studies have shown that the course of babesiosis, involving incubation period, duration of symptoms, and parasitemia may vary depending on babesia species [22, 23]. In our study, the patient was diagnosed with *B venatorum* infection on June 4, 2012. Since August 11, PCR testing and blood smear turned negative (0% parasitemia). The parasitemia of this patient persisted for approximately 2 months without any standard treatment. From May 31, the patient experienced persistent nonspecific symptoms for approximately 2 and one half months. The most severe symptoms occurred on July 9. The changes of most cytokines and chemokines demonstrated similar trends in this patient. The concentrations were low on June 4, and they increased to peak values on July 9. The elevated cytokines returned to low levels within 1 month. The changes were highly related to the symptom severity and blood parasitemia.

As a comparison, *Babesia microti* is the agent most frequently identified in the United States. Onset of symptoms usually occur within 1 to 4 weeks after the bite of a tick [1]. The symptoms usually last for 1 or 2 weeks, but fatigue and asymptomatic parasitemia may persist for a longer period of time [1, 24]. Compared with *B venatorum*, fever was more common among cases infected with *B microti* [8, 12]. Systemic elevation

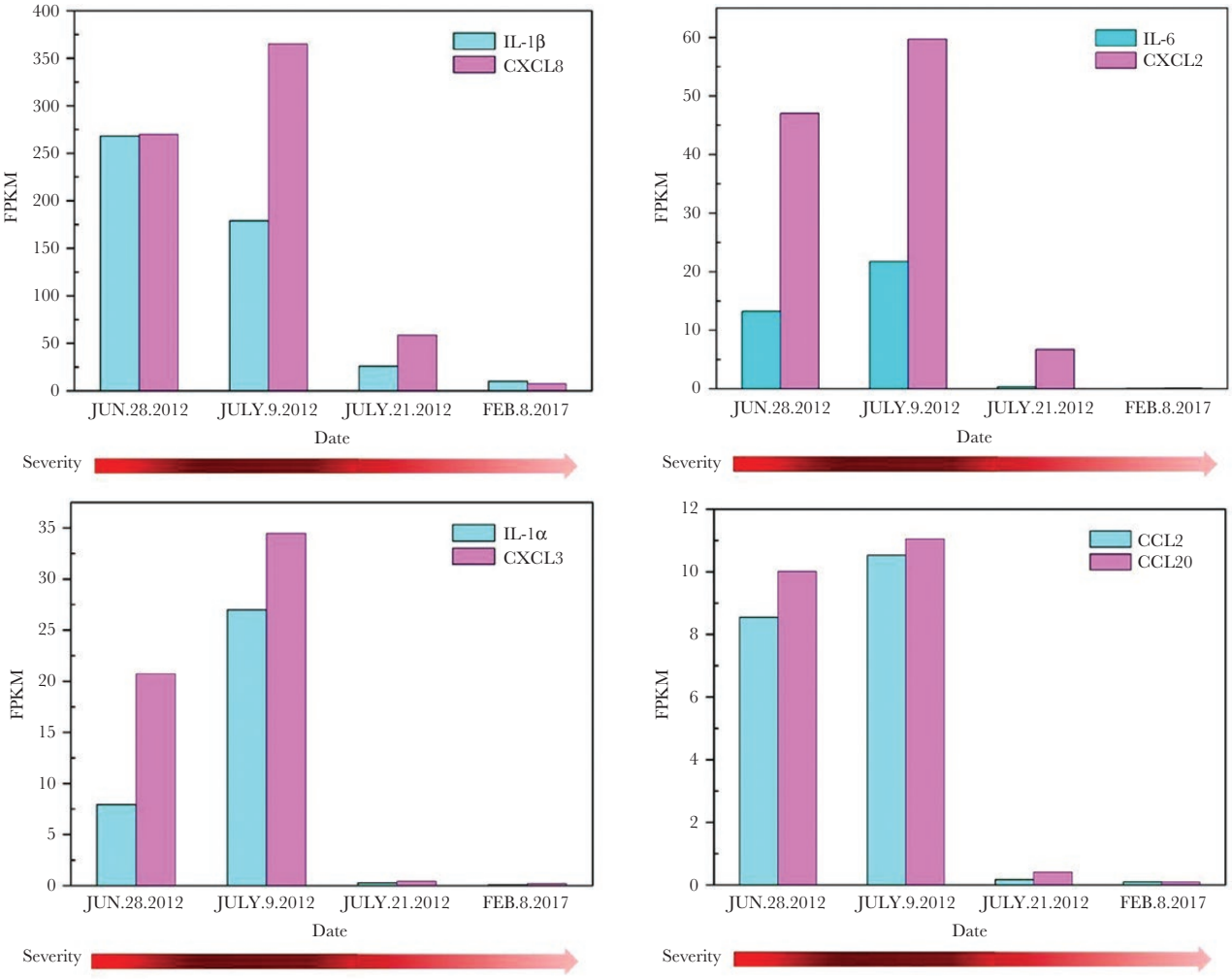

Figure 3. Change in ribonucleic acid (RNA) transcriptome for cytokines and chemokines in the case during acute illness and convalescence. FPKM was shown in 4 separate graphs by their maximum numerical values (from high to low). The RNA proinflammatory cytokine and chemokine transcription increased as symptoms worsened and decreased as symptoms improved, with return to very low level within 2 months of symptom resolution. Bar at the bottom of the figure depicts severity of symptoms: the darker the bar, the greater the severity of symptoms. CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; IL, interleukin.

of cytokine levels, such as TNF-α, IFN-γ, and IL-2, occurred during the acute phase of infection and returned to normal after 1-month treatment [25]. *Babesia divergens* is more prevalent in Europe. Most symptomatic patients become ill 1 to 3 weeks after the infecting tick bite [22]. Infections caused by this babesia species usually tend to be more severe than those caused by *B venatorum* and *B microti* [23]. *Babesia divergens* babesiosis usually occurs in immunocompromised individuals, which is similar to *B venatorum* in Europe [26]. Patients often experience a fulminant illness with severe complications, and even death when parasitemia is low, requiring hospital admission in a short time.

Babesiosis could induce mild-to-moderate hemolytic anemia [1]. In our previous study, anemia was detected in 22% of confirmed cases of *B venatorum* infection, and elevated TBIL was shown in 29% of the patients admitted to hospital (2 of 7) [8]. This patient demonstrated a persistent increase of IBIL and TBIL, indicating the destruction of erythrocytes and hemolysis. Similar to malaria, babesiosis is one of the classic examples of direct erythrocyte parasitization. Babesia species can invade erythrocytes, induce alterations to cell membrane, and initiate a cycle of cell lysis and further parasitization [27]. The mechanism of hemolysis in babesiosis remains unclear. Previous studies have shown that the hemolysis may be either extravascular or intravascular [28]. Both mechanical damage and metabolic activity of parasites can change the membrane of invaded erythrocytes [27]. Structural alterations may include protrusions, inclusions, and perforations [28]. In addition, autoimmune response contribute to the hemolysis in nonparasitized erythrocytes, which might explain why the degree of hemolysis often exceeds the degree of parasitemia and persists after the clearance of parasites in our study [28, 29]. Another possible reason for hemolysis in babesiosis is considered to be chronic hypersplenism [1, 27]. Intact spleen plays a key role in clearing

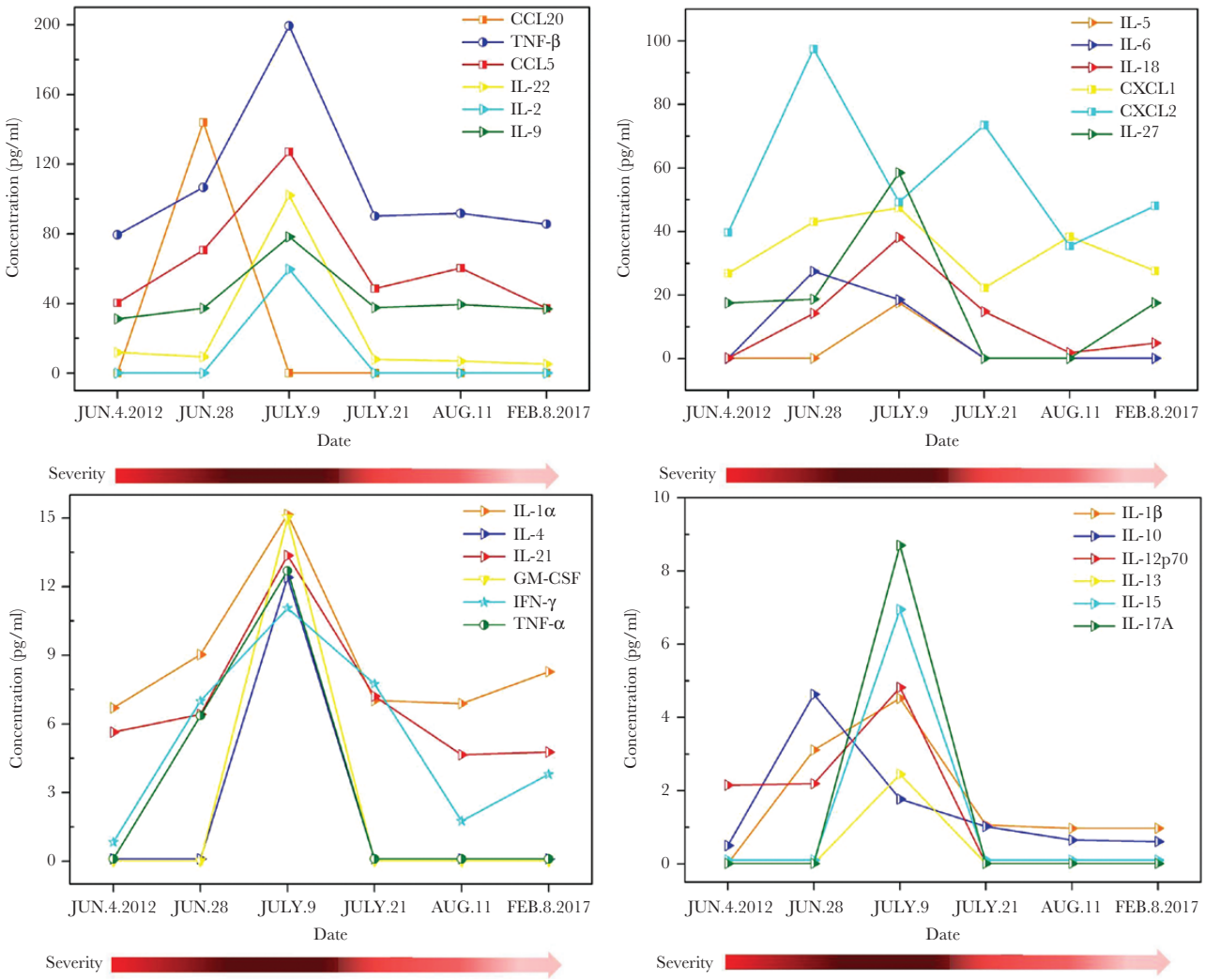

Figure 4. Proinflammatory cytokine and chemokine serum concentrations correlate with symptom severity and return to low levels after symptom resolution. The proinflammatory cytokine and chemokine serum concentrations were shown in 4 separate graphs, categorized by maximum numerical values (from high to low). The levels of cytokines increased as symptoms worsened and decreased as symptoms resolved. The bar at the bottom of figure depicts severity of symptoms: the darker the bar, the greater the severity of symptoms. Right triangle represents interleukins (IL); circle represents tumor necrosis factors (TNF); square represents chemokines; star represents interferons (IFN); and down triangle represents colony-stimulating factors. CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor.

babesia parasites. Erythrocytes with structural altered membrane can be phagocytosed and destroyed by macrophages in the splenic cords [27]. However, due to the lack of blood biochemical data, we cannot fully understand the changes of bilirubin during the course of babesiosis. The specific mechanism for its change remains to be investigated in the future. In the present study, there were no significant changes of other erythrocyte indicators that involved hemoglobin (HGB) and hematocrit (HCT). A possible reason for the normal and stable HGB concentration may be due to the chronic compensation of the bone marrow in a minority of immune-intact patients [27].

Some studies have suggested that systemic inflammatory response and "cytokine storm" might be the possible mechanism of babesia infection pathobiology [30, 31]. Cytokines including IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF-α have been reported to be associated with stimulating immunity against human babesiosis [25, 30, 32–34]. To better understand how the immune system is activated and changes during the course of babesiosis, we evaluated the levels of cytokines and chemokines on different time points, which correlated with onset phase (June 4, 2012), acute phase (June 28 and July 9), remission phase (July 21 and August 11), and convalescent phase (February 8, 2017) of babesia infection. For this patient, the serum concentrations of some cytokines and chemokines were significantly higher than normal [35], including IL-1α, IL-4, IL-10, IL-22, IL-27, and TNF-β, especially in the acute phase of babesiosis (Supplementary Table 2). However, most cytokines and chemokines were consistently within normal range

compared with reference values. The possible reasons may be due to the relatively low parasitemia (<1%) and mild clinical manifestations of this patient. Although the levels of cytokines in serum were not too high, the natural changes of most cytokines and chemokines were significant and revealed similar trends in this patient. In the first few weeks, the levels of cytokines and chemokines of this patient were low, accompanied by mild clinical manifestations. This might be because the light parasite burden within the bloodstream and the host immune response had not yet developed [31]. In the acute phase, concentrations of most cytokines increased dramatically and reached the peak when fever occurred, in both RNA and protein levels, indicating the activation of host immunopathologic responses with the increasing pathogen load. Then, the levels decreased progressively in the remission phase and to very low levels in the convalescent phase. Considering that individual factors, such as age, sex, ethnicity, adiposity status, and smoking, could affect the circulating levels of cytokines [36], we used self-control to adjust these factors through repeated investigations. Our findings demonstrated that the serum concentrations of cytokines and chemokines correlate with symptom severity. In addition, through transcriptome analysis and extensive serum protein detection, we found that some cytokines that have never been reported before, such as IL-13, IL-15, IL-17A, CCL2, CCL5, CCL20, CXCL1, CXCL8, CXCL2, CXCL3, and GM-CSF, were involved in the course of babesiosis. The role of these cytokines in the clearance of babesia needs to be further investigated.

In clinical practice, it is difficult to determine *B venatorum* infection due to the lack of specific signs or symptoms, lack of commercial *B venatorum*-specific PCR product, and lack of well trained, competent microscopists. It is quite necessary to explore some alternative indicators of this infection, especially in cases with low parasitemia. Our study suggested that excessive production of inflammatory cytokines might induce severe clinical manifestations in the natural course of babesiosis infection. Serum levels of multiple cytokines have positive correlations with parasite numbers. It is suggested that cytokine profiles involving multiple cytokines could possibly be unique for different infections and might be a better indicator of babesia infection in clinical settings.

This study has the following limitation. We are unable to fully understand the natural course and human immune response of babesiosis by 1 single case. However, to our knowledge, this is the first follow-up study of *B venatorum* patient without treatment. Although this is only 1 case, we could clearly see the natural course of babesiosis and the role of inflammatory cytokines on the protozoa clearance and thus give the suggestion to use cytokines profiles as indicators of babesia parasites clearance. This report provides new information on the human immune response to *B venatorum* illness from disease onset to convalescence and provides evidence that further investigation of the immune response, medical management, and preventive measures for *B venatorum* infection in a large sample size study would be useful.

CONCLUSIONS

China is the only country where *B venatorum* infection is endemic around the world. For further control, authorities in high-risk areas should strengthen information dissemination on this illness to the public; physicians should be well trained for early diagnosis and proper treatment; and surveillance and investigations should be enhanced to better understand the mechanisms and hazards for *B venatorum* infection. There is no doubt that the prevention of babesiosis, in particular, *B venatorum* infection, will continue to be a challenging issue, until an efficient and effective detection method with acceptable cost benefit is widely applied.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. H. W., J. J., and W. C. contributed to conception and design of the study. L. Z. and R. J. wrote the first draft of the manuscript. Y. Z., Q. H., Y. C., C. B., and J. S. recruited the patient and gathered the data. N. J., N. N., B. J., Y. S., T. Y., T. L., H. L., and R. W. did the laboratory tests. X. L. and H. W. performed the data analysis. All authors contributed to manuscript revision and read and approved the submitted version.

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