Vaccine 33 (2015) 1250–1255

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Characterization of immunological responses in patients with severe fever with thrombocytopenia syndrome: A cohort study in China

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ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 13 January 2015
Accepted 20 January 2015
Available online 30 January 2015

Keywords:
Severe fever with thrombocytopenia syndrome
Immunological response
Cohort study
Humoral immunity

ABSTRACT

Background: The immunological responses of patients with severe fever with thrombocytopenia syndrome (SFTS) remain largely unknown. We aim to study the magnitude and sustainability of host immune responses and their correlation with clinical, virological and hematological parameters.
Methods: A longitudinal cohort study was performed in a SFTS reference hospital. The sequential immunological evaluation was determined for SFTSV infected patients, including anti-SFTSV IgM, IgG antibodies and the lymphocyte subsets.
Results: Altogether 298 laboratory-confirmed SFTS cases were analyzed, from whom 55 patients were followed after convalescence. SFTSV specific IgM antibody could be detected at medium of 9 days, surged to peak levels by 4 weeks, and remained persistent until 6 months after disease onset. SFTSV specific IgG antibody could be detected at medium of 6 weeks; surged to peak levels by 6 months, and remained positive in most of the patients even at 3 years after infection. SFTS patients experienced obvious T cell, B cell and NK cells loss during the first week of infection, which was rapidly restored to normal levels. A significantly lower level of humoral immunity was identified concurrently from severe disease, especially in acute phase of the infection. These abnormalities can be used as a potential indicator in the prediction of an adverse clinical outcome.
Conclusions: Information gained from this study have clinical significance in enhancing our understanding of SFTS immunological characteristics and the disease pathogenesis.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a novel emerging infectious disease that was discovered during the 2009 outbreak of infections in the mid-East regions of China. The disease usually presents as fever, thrombocytopenia, and leukocytopenia, with an average case-fatality rate at about 12% and can be up to 30% in some areas [1]. The causative agent of SFTS was identified to be a novel bunyavirus named SFTS virus (SFTSV), which is a phlebovirus in the *Bunyaviridae* family. According to surveillance data released by China CDC, the geographic locations reporting incidence of SFTS has expanded to at least 15 provinces/municipalities in mainland China in 2012. The reported case number has increased remarkably from 571 in 2011 to 1476 in 2012 [2,3]. Recent case reports in South Korea and Japan have demonstrated its presence outside of China, thus indicating the public health significance of this emerging infectious disease [4,5].

Epidemiological and clinical characterizations of SFTS patients have been reported to some extent [3,6–8], but the immunological characteristic of SFTSV infection has remained largely unknown. Specific IgM antibody to SFTSV has been reported to appear on 7 days after the disease onset and can be detected in convalescence phase [1]. However, the long-term evolution of specific antibodies, including IgG and IgM to SFTSV remains uncharacterized. The kinetic of immune responses at different stages of the infection has not been determined, either. Other members of *Bunyaviridae* family, like Crimean–Congo hemorrhagic fever virus infection, can lead

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http://dx.doi.org/10.1016/j.vaccine.2015.01.051
0264-410X/

to abnormalities in lymphocyte counts and abnormal changes in the levels of lymphocyte subsets, which correlate with clinical outcomes [9]. For this emerging infectious disease, it remains unclear as to whether a robust or depressed immunological response is associated with more severe disease course after SFTSV infection.

Here we performed a prospective study on confirmed SFTSV infected cases to determine their immune response patterns over the entire hospitalization period and to correlate these parameters with clinical, virological and hematological parameters. Partial patients were followed up for a longest duration of three years post infection, in order to determine the persistence of antibodies that was elicited by SFTSV infection.

2. Methods

2.1. Patient recruitment and follow up studies

The study was performed in the SFTS designated hospital (The 154 Hospital) in Xinyang administrative district of Henan Province, China. SFTS patients who were hospitalized in the hospital during 2011–2013 were recruited based on the criteria of acute fever with thrombocytopenia and/or leukopenia as defined by the Chinese Ministry of Health [10]. The research protocol was approved by the human ethics committee of the hospital, and all participants provided written informed consent. On admission, all patients had sera samples collected and subjected to SFTSV RNA test by a molecular method as described previously [11]. Patients with a positive result were invited to participate in the prospective study. After written informed consent was obtained, sequential sera samples were collected at regular intervals (every three days) during the entire hospitalization period, which were subjected to IgM and IgG antibody titer and viral load quantification. A medical record review was performed in order to collect the information on the patients' demographic characteristics, physical examination, routine blood test, biochemical test and treatment regimens during the entire hospitalization period. The comorbidity was defined as patients presenting with one of the following: hypertension, diabetes, cancer, active hepatitis, cerebral infarction, et al. Multiple organ failure refers to the development of potentially reversible physiologic derangement involving two or more organ systems not involved in the disorder that resulted in ICU admission, and arising in the wake of a potentially life threatening physiologic insult. Severe cases were defined by the presence of hemorrhagic manifestations (epistaxis, hematemesis, and melena), or presence of one or more organ failure or encephalitis development.

After discharge from the hospital, those patients who agreed to participate in the long-term follow up were contacted to revisit the hospital at regular intervals, i.e., weekly until 1 month post disease onset (M1), then every 3 months until M6, and thereafter followed every 6 months until the end of the study. At each visit, blood samples were collected for IgM, IgG antibody titer quantification and routine blood tests.

From the cohort, we selected patients who agreed to have additionally 10-ml EDTA anti-coagulated blood samples collected. The samples were transferred to our laboratory within 6 h of collection for PBMC separation, which was used for lymphocytes and lymphocyte subsets evaluation. The patient inclusion flow diagram was provided in Figure S1.

2.2. Laboratory tests

IgM and IgG antibodies were tested and the titers were quantified by the enzyme-linked immunosorbent assay (ELISA) using the recombinant nucleoprotein of SFTSV [12]. Virus loads were determined using quantitative RT-PCR targeting the viral S gene segment as described previously (detailed in the supplemental materials) [11]. In brief, the copy number of viral cDNA in copies/ml sera samples was determined by comparison with a serially diluted plasmid standard of known concentration. The high viral load was defined if one patient's maximum viral load exceeded the median value of all patients during the hospital process. Lymphocytes and lymphocyte subsets were quantified by flow cytometry [13]. Detailed information on these laboratory tests are provided in the supplementary material.

2.3. Statistical analysis

Descriptive statistics were calculated for all variables; continuous variables were summarized as means and standard deviations or median and range, and categorical variables were summarized as frequencies and proportions. The antibody reciprocal titers were log-transformed to allow for comparison of geometric mean reciprocal titers (GMRTs) across groups. To determine the difference between groups, independent t test, chi-square test, Fisher exact, or non-parametric test was used where appropriate. Biologically plausible variables with $P < 0.10$ in the univariate analysis were entered into multivariate logistic regression model by stepwise method. IgM and IgG antibody titers were computed using nonlinear generalized estimating equations (GEE) [14] to determine the effect from considered variables on IgM and IgG antibody production and decay. Odds ratios (ORs) and their 95% confidence intervals (CIs) were estimated using maximum likelihood methods. The IgG antibody decay rate was derived from the negative slopes of the regression lines for antibody concentrations versus time in days. Half-life ($t_{1/2}$) was calculated as the time required for antibody concentrations to decrease by 50% from the initial value. The differences of lymphocyte subsets were compared by the rank and general linear model between different subjects groups. A two-sided P value of less than 0.05 was considered to be statistically significant. All analyses were performed using SAS software (Version 9.1.3, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Basic information

A total of 472 hospitalized SFTS cases with positive RNA detection were identified during the study period. Among the 350 patients who agreed to participate in the prospective study, 52 did not have appropriate specimens collected because of early death, failure to follow up, or inappropriate timing of serum collection. Altogether, sera from 298 patients were successfully collected and analyzed. The median age of these patients was 59 years (20–82 years), with a male-to-female ratio of 0.7:1. A total of 34 (11.4%) patients were defined with severe disease based on development of multiple organ failure (MOF), among whom 21 died. Altogether, 55 patients participated in the long term follow up after hospital discharge, from whom 438 samples were collected for a median duration of 140 days (range 32–1293 days) post infection. Compared with all the recruited patients, those who participated in the long term observation had lower mortality rate and younger age (Supplemental Table 1). Three (5.9%), four (7.8%) and one (2.0%) patients experienced thrombocytopenia, leukocytopenia and elevated LDH, respectively, which was observed on M6 after disease onset.

3.2. Serological response

The IgM and IgG responses in SFTS patients over time are presented at 17 time points post infection. Based on the GMRT estimation, the IgM antibody level peaked at week 4 (GMRT, 216.1,