Letter to the Editor

Formulation of an Early Warning Infectivity Score System for Adult Patients with Acute Bacterial Diarrhea*

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The aim of our study was to develop a scoring system to predict whether diarrhea is of a bacterial origin and whether the diarrheal patients constitute a potential source of infection to others. Adults with acute diarrhea (*n*=424) were enrolled in the study. Logistic regression and standard regression coefficients were used to formulate the Early Warning Infectivity Score System for Adults with Acute Bacterial Diarrhea (EWIS-ABD). Four risk factors were identified by logistic regression, including body temperature (*P*<0.01), abdominal pain (*P*<0.01), leukocyte count in stool (*P*<0.01), and unclean dietary history (*P*<0.01). EWIS-ABD was thus developed, in which the value ≥5 points was set as an indicator of bacterial diarrhea. The incidence of bacterial diarrhea increased along with the elevated score. EWIS-ABD was more specific for bacterial diarrhea than for viral diarrhea. The accuracy and reliability of EWIS-ABD was high by prospective validation in 478 patients with acute diarrhea.

Bacterium is the common cause of diarrhea worldwide which often poses a threat to human life and has implications for urgent epidemic control in the community. In China, about 75% of diarrhea cases are caused by bacteria, and dysentery bacillus and salmonella are the most common pathogens. Despite continued improvement of public health and patients' medical awareness, about 80% of infectious diarrhea cases are not determined[1]. Furthermore, microbiological diagnosis of infectious diarrhea would take several days using conventional techniques from stool samples collection to microbiological diagnosis due to samples shipping to an appropriate laboratory for testing and bacterial culture. Moreover, these assays are still not performed routinely in clinical practice in China. Therefore, the infectivity of most diarrheas cases cannot be determined promptly, resulting in prolonged hospitalization or death, or even in an outbreak of potential infection disease. In our former studies we identified infection risk factors for patients with acute fever and established a simple and practical criteria for Early Warning Infectivity Score System (EWIS) used in outpatient and emergency departments in order to identify infectious patients quickly and accurately[2], and we also identified risk factors for critically ill outpatients with acute fever and formulated a criteria for Adult Fever State Score (AFSS) to alert doctors at outpatient departments[3]. The EWIS-ABD in the current study was developed by using the two methods mentioned above which improved the discriminability of infectivity among patients with acute bacterial diarrhea, and this tool will guide doctors to diagnose and treat patients in an objective and prompt way.

A total of 424 adults (model group) with acute diarrhea (males, 253; females, 171; average age 39.67±18.94 years) who were hospitalized in the diarrhea ward, gastroenterology ward, and general ward of Beijing Friendship Hospital, Beijing Xuanwu Hospital, Beijing Haidian hospital, and Sino-Japanese Friendship Hospital between May 2006 and December 2008 were analyzed. Inclusion criteria were as follows: defecation frequency ≥3 times/day, changes in the characteristics of stool, age ≥18 years and duration of symptoms <1 week. Patients were excluded if the onset of diarrhea occurred 1 week or more than 1 week before admission to hospital.

Clinical data were collected using a unified case observation table, which included age, sex, diarrhea frequency/day, vomiting frequency/day, abdominal pain (related with diarrhea, mainly peripheral umbilicus or middle or lower abdominal pain), tenesmus, body temperature (the temperature taken for patient at their first admission to hospital), stool test [including color and characteristics of the stool,

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the number of erythrocytes and leukocytes in stool per high power (HP) field under the microscope], history of disease, unclean dietary history, contact history and transmission chain. All of these data were recorded at the initial visits. The stool test is a routine procedure in hospitals in China and is performed for almost all patients with diarrhea. Unclean dietary history was reported by the patients as prior consu-mption of rotten food, sea food, or raw\inadequately heated food, contaminated water, and so on.

Diarrhea was diagnosed by polymerase chain reaction and enzyme linked immunosorbent assays. (1) Detection of bacteria included the following steps: ① Choosing strains: strains of Enteropathogenic Escherichia coli (EPEC) CMCCB 44196, Enterotoxigenic *E.coli* (ETEC) CMCCB 44439, Enteroaggegative *E.coli* (EAEC) CMCCB 44404, nonpathogenic *E.coli*, CMCCB 44161 (negative control) and vibrio parahaemolyticus were purchased from the National Institute for the Control of Pharmaceutical and Biological Products. Strains of Shigella flexneri, Shigella sonnei, Shigella boydii, Shigella and salmonella were derived from strains stored in our laboratory; ② Primer design: thirteen pairs of primers were synthesized based on the published sequences; ③ DNA template preparation: a DNA extraction kit (QIAGEN) was used to extract bacterial DNA; ④ PCR amplification (DNA extracted from standard strains as a positive control, water as a negative control): PCR amplification was performed in a 25 μL reaction mixture containing 5 μL of 5× green buffer, 25 mmol/L $MgCl_2$ (final concentration, 2 mmol/L), 2.5 mmol/L dNTP mixture (final concentration, 0.2 mmol/L), 10 μmol/L of the corresponding primers (final concentration, 0.2 μmol/L), 1 U Taq DNA polymerase and 2 μL of the DNA template. The PCR pre-denatured at 94 °C for 7 min, then at 95 °C for 1 min, subsequently annealing for 1 min, followed by 30 cycles at 72 °C for 1 min and a final extension at 72 °C for 7 min. The resultant amplified samples were stored at 4 °C; ⑤ Detection of PCR products: The amplified DNA fragments were subjected to agarose gel electrophoresis (20 g/L agarose) in electrophoresis buffer (0.5 mg/L) and 1× TAE at a constant voltage of 6 V/cm for 40 min. A standard molecular marker was used for reference. The presence of bands indicates the expression of the target genes. Gels were photographed using a Gel Imager camera. ⑥ Sequencing of PCR products: the amplified gene-specific fragments were compared with the published standard strain sequence in the gene pool; ⑦Detection of specificity: all 13 primers were tested with DNA obtained from other related bacteria for cross-PCR amplification. Specificity of the primers was confirmed if the target genes were only amplified in the intended bacteria, rather than in other bacteria. (2) Detection of fecal norovirus and rotavirus: fecal norovirus and rotavirus were detected by enzyme-linked immunosorbent assays using the IDEIAIM norovirus kit and rotavirus kit obtained from DakoCytomation Co. (Denmark), in accordance with the manufacturer's instructions.

The χ^2-test and *t*-tests were used to identify the statistical significance ($P<0.05$) between bacterial diarrhea patients and non-infectious diarrhea patients. Logistic regression analysis was used to identify independent risk factors for infectivity, and the factors were scored based on the standard regression coefficient. These scores were used to develop a preliminary scoring system to identify infectious patients with acute bacterial diarrhea. The accuracy of the scoring system was assessed using the area under the receiver operating characteristic (ROC) curve. The kappa test was used to evaluate the reliability of the scoring system.

Among the 424 patients with acute diarrhea, 150 patients were affected with non-infectious diarrhea (absence of infectious pathogens, and patients without amebic dysentery or antibiotic-associated diarrhea, and patients with clear causes of diarrhea such as inflammatory bowel disease, tumor-associated diarrhea were included in the non-infectious group), 174 patients-with bacterial diarrhea (diagnosed by polymerase chain reaction) and 100 patients-with viral diarrhea (diagnosed by enzyme-linked immunosorbent assays) (For details of diagnosis see Table 1). The age of the patients with bacterial diarrhea was significantly less than that of the patients with non-infectious diarrhea (33.37±16.21 years versus 42.09±19.13 years, respectively; $P<0.01$). Of the 174 patients with bacterial diarrhea, 87.4% (152/174) had unclean dietary history or contact history, 45.4% (79/174) had fever, 20.1% (35/174) had vomiting symptoms, 24.7% (43/174) had tenesmus, 96.0% (167/174) had red blood cells in their stool, and 98.9% (172/174) had white blood cells in their stool. Of the 150 patients with non-infectious diarrhea, 30.7% (46/150) had unclean dietary history, 1.3% (2/150) had fever, 18.0% (27/150) had symptoms of vomiting, 3.3% (5/150) had red blood cells in their stool test and 2.7% (4/150) had white blood cell in their stool test.

Four independent risk factors for infectivity were identified using logistic regression model, and these factors were unclean dietary history, body temperature, abdominal pain and the presence of leukocytes in stool (Table 2). The standard regression coefficient (Table 2) was used as the weight for each parameter. The scoring system (EWIS-ABD) was formulated (Table 3) to identify the infectivity of patients with acute bacterial diarrhea.

Table 1. Causes of Diarrhea for 424 Diarrheal Patients

Cause of Ddiarrhea	Pathogen	Cases
Bacterial diarrhea	Bacillus dysenteriae	90
	Salmonella	52
	Vibrio parahaemolyticus	17
	Enteropathogenic *E.coli*	6
	Pathogenic *E.coli*	5
	Aggregate *E.coli*	1
Mixed infectious diarrhea	Pathogenic *E.coli* and salmonella	3
Viral diarrhea	Norovirus	92
	Rotavirus	8
Non-infectious diarrhea	Thyroid dysfunction	17
	Inflammatory bowel disease	32
	Colorectal cancer	19
	Eating diet pills or laxatives	28
	After cholecystectomy	6
	Carcinoid syndrome	2
	Irritable bowel syndrome	41
	Eosinophilic Gastroenteritis	1
	Malignant tumor after operation	4

Table 2. Risk Factors for Acute Bacterial Diarrhea by Logistics Regression Model

Items	B[1]	SE[2]	Wald	P	B′ [3]
Poor dietary hygiene	2.006	0.694	8.349	0.004	1.103
Abdominal pain	2.554	0.832	9.346	0.002	1.403
Body temperature	5.959	1.770	11.329	0.001	3.285
Presence of leukocytes in stool	7.077	1.178	36.088	0.000	3.902

Note. [1]Partial regression coefficient; [2]Standard error; [3]Standard regression coefficient.

The area under the ROC curve (AUC) for EWIS-ABD was 0.975 (Figure 1) (95% CI: 0.962-0.987) at a cut-off value of 5, and the standard error was 0.006. A score of ≥5 indicated the presence of bacterial diarrhea (specificity, 90.0%; sensitivity, 96.6%). Therefore, a score of ≥5 would identify 96.6% of patients with bacteria diarrhea. Only 10% of patients with non-infectious diarrhea and viral diarrhea would score ≥5. The positive predict value was 87.0% and the negative predict value was 97.40%. The kappa value, which represents the consistency between the EWIS-ABD score and the clinical diagnostic criterion of bacterial diarrhea was 0.851, and the EWIS-ABD was found to have high accuracy and reliability (P<0.001).

Table 3. The Scoring System-EWIS-ABD

Risk Factors	Score				
	0	1	2	3	4
Poor dietary hygiene	–	+			
Abdominal pain	–	+			
Body temperature (°C)	≤37.3			≥37.4	
Presence of leukocytes in stool	–				+

Note. '+' means Yes, and '-' means No, for example, if the patient had the history of poor dietary hygiene, it will be recorded as 1 point, otherwise it will be recorded as 0 point.

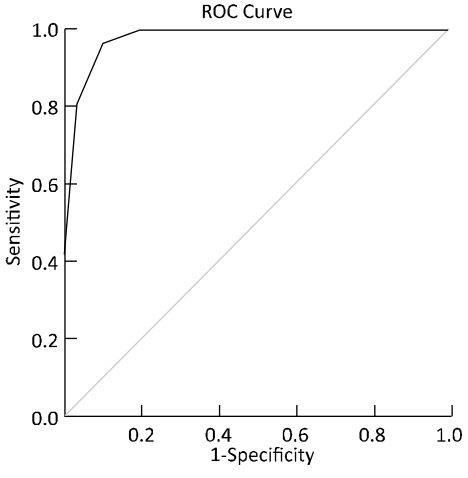

Figure 1. The AUC of EWIS-ABD for bacterial diarrhea was 0.975. Cut-off value =5, specificity was 90.0%, sensitivity was 96.6%, Kappa value was 0.851.

The most common scoring systems are APACHEII[4], APACHEIII[5], SAPSII[6], SOFA[7], and EWS[8]. However, these systems were originally developed to predict the severity and mortality of patients in an intensive care unit (ICU). EWS is a tool that combines clinical parameters into a single scoring system that can be used to identify early signs of deterioration and assess the prognosis of patients. Therefore, similar to these early warning systems, in this study we used an objective scoring method to identify the infectivity of adults with acute bacterial diarrhea. A predictive/diagnostic system should be cheap and easy for use with quick and high sensitive and specific results. Our EWIS-ABD is one of the predictive system that provides an evidence-based approach for early identification of the infectivity for adult patients with acute bacterial diarrhea. The factors included in the scoring system were unclean dietary history, abdominal pain, body temperature, and presence of leukocytes in stool, which are simple to determine and are available when a patient visits doctors in an emergency or outpatient department.

The EWIS-ABD scoring system was also used for 150 non-infectious cases and 100 viral diarrhea cases to assess the identification power of EWIS-ABD for viral diarrhea diseases. The AUC of EWIS-ABD for viral diarrhea was 0.755, lower than that of EWIS-ABD for bacterial diarrhea (AUC=0.992), and the kappa value was 0.438 lower than that of EWIS-ABD identifying bacterial diarrhea (the kappa value=0.932). These results clearly indicated that EWIS-ABD had a better identification power to bacterial diarrhea than viral diarrhea.

In order to test the predictive capability of the EWIS-ABD for bacterial diarrhea, a total of 478 cases (test group) with acute diarrhea (males, 261; females, 217; average age 43.36±15.82 years) hospitalized in the diarrhea ward, gastroenterology ward and general wards of the Beijing Tiantan Hospital, Beijing Chaoyang Hospital and Peking University Third Hospital between January 2011 and June 2011 were analyzed by EWIS-ABD. Inclusion criteria and the diagnostic methods were the same as the model group. Differences of age distribution, gender ratio, as well as the constituent ratio of bacterial diarrhea, viral diarrhea and non-infectious diarrhea were analyzed between these two groups and no significant difference (P>0.05) was shown. The AUC of the EWIS-ABD for the test group to identify bacterial diarrhea was 0.934, with specificity 83.9%, sensitivity 87.0%, as well as kappa value 0.709 (P<0.01).

In conclusion, our scoring system is an easy, convenient and fast tool to identify the infectious status of patients with acute bacterial diarrhea, and the parameters used for the scoring system are easy to obtain. The scoring system can be used in general hospitals or in primary hospitals to quickly determine the infectivity of patients with high accuracy. However, further studies are needed to improve its accuracy and reliability and to test whether it can be used for children. Besides, a scoring system with high specificity and sensitivity to identify the infectivity of patients with viral diarrhea warrants further study in the future.

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