C reactive protein and prealbumin as markers of disease activity in shigellosis

W A Khan, M A Salam, M L Bennish

Abstract

To evaluate serum C reactive protein (CRP) and prealbumin concentrations as markers of disease activity in shigellosis this study serially measured serum concentrations of CRP and prealbumin in 39 patients infected with *Shigella* spp, and a comparison group of 10 patients infected with *Vibrio cholerae* serotype 01. On admission, patients with shigellosis had significantly higher median concentrations of CRP (109 v 5 mg/l; $p < 0.01$) and significantly lower median concentrations of prealbumin (16 v 23 mg/dl; $p < 0.01$) than did patients with cholera. Among *Shigella* spp infected patients, CRP concentrations were significantly lower, and prealbumin concentrations significantly higher, on study days 3 and 5 when compared with admission values. Among *Shigella* spp infected patients, those in whom treatment failed had higher admission CRP concentrations than those in whom treatment was successful ($p = 0.142$). An admission CRP concentration ≥ 110 mg/l had a 70% sensitivity and a 61% specificity in predicting failure of treatment among patients infected with *Shigella* spp; the predictive value of a positive and negative test was 14% and 96% respectively. In summary, acute shigellosis elicits an acute phase response, the magnitude of which predicts clinical outcome.

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Keywords: *Shigella* spp, dysentery, bacillary, *Shigella dysenteriae* type 1, acute phase reactants, C reactive protein, prealbumin.

Shigellosis is a tissue invasive infection that elicits a pronounced inflammatory response at the site of primary infection in the colon.¹⁻⁴ Although *Shigella* spp infections are usually associated with fever and peripheral blood leucocytosis,⁵ information on other acute phase reactants during disease and recovery is limited.

Previous studies have found an inverse correlation between total serum protein concentration and the risk of death.⁶ It is possible that measurement of specific serum proteins, instead of determination of total serum protein, may provide an even more sensitive measure of the clinical course of *Shigella* spp infections. Determining changes in serum proteins could also provide insight into the nutritional disorders that occur during shigellosis. The nutritional consequences of

shigellosis include an acute depression in serum protein concentrations, and growth interruption in children,^{7,8} effects that can be partly ameliorated by feeding a high protein diet.^{9,10}

C reactive protein (CRP) is an acute phase protein that is present in very low concentrations in the serum of healthy subjects, but increases considerably and rapidly in response to an inflammatory stimulus.¹¹ It is commonly used as a screening test to detect bacterial infection,^{12,13} and has been used to monitor the clinical response to treatment in patients with ulcerative colitis,¹⁴ a disease that shares many clinical and pathological similarities with shigellosis.

Prealbumin, like CRP, is a serum protein with a short half life of about two days.¹⁵ Unlike CRP, serum concentrations of prealbumin are responsive to nutrient, and especially to energy, intake.^{16,17} Concentrations of prealbumin decline during acute infection, especially in association with anorexia, and usually rise after termination of the inflammatory process and renewed feeding.

As a measure of the systemic response to *Shigella* spp infection, CRP and prealbumin concentrations were determined in 39 adults acutely ill with shigellosis, and were compared with concentrations in 10 patients with diarrhoea caused by *Vibrio cholerae* serotype 01.

Methods

Study patients

This study was conducted at the Diarrhoea Treatment Centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Patients included in this study were participating in a clinical trial comparing the efficacy of short course (one or two dose) ciprofloxacin treatment of *Shigella* spp infection with a standard five day, 10 dose regimen.¹⁸ The enrolment criteria for entry into the clinical trial have been reported earlier.¹⁸ Briefly, all patients enrolled in the study were men, between 18-60 years of age, had symptoms of dysentery for < 96 hours, and had *Shigella* spp isolated from a stool or rectal swab sample obtained on admission to the study. All patients included in the study stayed in hospital for six days, and clinical outcomes were evaluated using predetermined criteria.¹⁸ Treatment was considered to have failed, if on study day 5 patients had more than six stools, one or more watery or dysenteric stools, or were febrile (oral temperature $> 37.8^{\circ}\text{C}$). In addition, treatment was also considered to have failed in patients who had no improvement in their symptoms on

Clinical Sciences
Division, International
Centre for Diarrhoeal
Disease Research,
Dhaka, Bangladesh
W A Khan
M A Salam

Departments of
Pediatrics and
Medicine, New
England Medical
Center, Tufts
University School of
Medicine, Boston,
USA
M L Bennish

Correspondence to:
Dr W A Khan, ICDDR,B,
GPO Box 128, Dhaka 1000,
Bangladesh.

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study day 3. Treatment was considered to have been successful, if on study day 5 a patient had $\leq$ six total stools, no watery or dysenteric stools, and was afebrile.

Serum CRP and prealbumin concentrations were determined on 39 (33%) of the 118 patients enrolled in the original study. These 39 patients included 10 patients who were infected with *S dysenteriae* type 1 and in whom treatment was successful; 10 patients infected with *S dysenteriae* type 1 in whom treatment failed; and 19 patients who were infected with *Shigella* spp other than *S dysenteriae* type 1 and in whom treatment was successful. CRP and prealbumin concentrations were also measured in 10 patients who had diarrhoea caused by *V cholerae* serotype 01.

Nine of 10 patients infected with *S dysenteriae* type 1 who failed treatment had received short course (single or two dose) ciprofloxacin therapy. Seven of these nine patients ultimately received five days of treatment with ciprofloxacin starting on study day 4 (three patients); study day 5 (one patient); and study day 6 (three patients). Six of 10 patients who were infected with *S dysenteriae* type 1 and in whom treatment was clinically successful received short course therapy, and only one (because of failure of eradication of *Shigella* spp from stool) patient subsequently received a full five day course of ciprofloxacin beginning on study day 4. Of 19 patients infected with *Shigella* spp other than *S dysenteriae* type 1, 15 received short course treatment, none failed treatment, and none required antimicrobial treatment in addition to the short course treatment they had initially received.

Study samples

For patients infected with *Shigella* spp, blood samples for determination of serum CRP and prealbumin concentrations were obtained on four occasions: on admission to the study, on study days 3 and 5, and at a follow up visit approximately seven days after discharge from the hospital (14 days after admission). Blood samples were obtained from the *V cholerae* infected patients on two occasions: on admission to the study and at a follow up visit approximately 10 days after admission.

Laboratory methods

For determination of CRP and prealbumin concentrations blood was collected into sterile tubes, kept at room temperature for an hour, and then centrifuged at 4000 revolutions per minute for 10 minutes. The serum was separated and stored at -70°C until the assays were performed. For determination of CRP concentrations a turbidimetry technique using a Cobas-Bio discrete analyser (Hoffmann-La Roche, Basle, Switzerland) and monospecific rabbit antihuman CRP immunoglobulin (Dakopatts, Glostrup, Denmark) were used. For determination of prealbumin concentrations a single radial immunodiffusion technique using plates containing monospecific antisera was used (Hoechst-Behring, Marburg, Germany). The lowest limit of detection for CRP using this method was 4.8 mg/l and for prealbumin 2.5 mg/dl in our laboratory. CRP values less than 4.8 were recorded as 0.

Statistical methods

Data were entered into a personal computer using StatPac Gold (version 3.2, Walonick Associates, Minneapolis, MN); and were analysed with Statistical Package for Social Sciences, PC⁺ version 4.0 (SPSS, Chicago, IL) and Epi Info version 5.0 (USD, Stone Mountain, GA). Differences in means between the four groups were tested for significance using the analysis of variance (ANOVA) test, or the Kruskal-Wallis test if the findings were not normally distributed. When differences between the groups were significant on the ANOVA test, differences between individual groups were tested for significance using Scheffe's procedure. If the Kruskal-Wallis test was significant, individual groups were then compared using the Mann-Whitney U test. Differences in categorical variables were tested for significance using the χ^2 test, or Fisher's exact test if the predicted size of any cell was five or less. Sensitivity, specificity, predictive value of a positive test, and predictive value of a negative test were determined using standard methods.¹⁹ The values used for determining sensitivity and specificity were selected by first drawing receiver operator curves, and then

TABLE 1 Admission characteristics of the four patient groups

Characteristic	Patient group			
	<i>S dysenteriae</i> type 1 treatment successful (n=10)	<i>S dysenteriae</i> type 1 treatment failed (n=10)	Other <i>Shigella</i> spp treatment successful (n=19)	<i>V cholerae</i> 01 (n=10)
Age (y)*	27 (5)	30 (9)	32 (9)	38 (8)
Duration of illness (h) (median, range)†	57 (25–72)	67 (34–72)	42 (6–86)	14 (9–31)
Temperature $>37.8^{\circ}\text{C}$ (n) (%)‡	3 (30)	5 (50)	8 (42)	0
Weight (kg)	50 (9)	52 (7)	49 (8)	45 (8)
Moderate or severe dehydration (n) (%)§	0	0	0	10 (100)
Other enteric pathogens (n) (%)				
<i>Salmonella</i> spp	0	0	1 (5)	0
<i>Aeromonas</i> spp	2 (18)	0	1 (5)	0
Admission PCV %	43 (4)	44 (6)	45 (4)	60 (4)
Admission blood leucocyte count $\times 10^2/\text{mm}^3$	104 (39)	111 (38)	113 (25)	Not done
Stool microscopic examination (n) (%)				
>50 leucocytes/high powered microscopic field§	10 (100)	10 (100)	18 (95)	1 (10)
>50 erythrocytes/high powered microscopic field§	9 (90)	10 (100)	17 (90)	0

Values are mean (SD). * $p=0.035$ by ANOVA. Group 1 compared with group 4, $p<0.05$ by Scheffe's procedure. † $p<0.001$ by Kruskal-Wallis test. Group 1, 2, and 3 compared with group 4, $p<0.001$ by Mann-Whitney U test. ‡ $p=0.02$ by Fisher's exact test, all *Shigella* spp compared with *V cholerae*. § $p<0.001$ by Fisher's exact test, all *Shigella* spp compared with *V cholerae*. || $p<0.001$ by ANOVA. Group 1, 2, and 3 compared with group 4, $p<0.05$ by Scheffe's procedure.

TABLE II Sensitivity, specificity, and positive and negative predictive values of selected variables for determining failure of antimicrobial treatment in patients with shigellosis

Variable	Sensitivity	Specificity	Predictive value of a positive test	Predictive value of a negative test
≥10 stools in the 24 hours before admission	80	65	18	97
CRP ≥110 mg/l	70	61	14	96
Temperature on admission ≥38.4°C	40	79	15	93
Band count ≥4% of total leucocytes	20	93	22	93
Study day 1 temperature ≥38.2°C	70	41	10	94
Prealbumin ≤25 mg/dl	20	89	15	92
Blood leucocytes ≥10 000/mm ³	70	38	10	93
Blood neutrophil proportion ≥70% of total leucocytes	40	45	6	89

Values are percentages.

selecting the test value that gave the largest sum when sensitivity and specificity were added. For calculating predictive values, the results from the sample population were extrapolated to the total study population, and the calculations performed using these extrapolated values.

This study was approved by the scientific and ethical review committees of the ICDDR,B.

Results

Table I shows the characteristics of the patients in the four groups on admission to the study.

All 10 patients with cholera, but none of the patients with shigellosis, were moderately or severely dehydrated. Patients with cholera had a significantly shorter illness duration, a higher blood packed cell volume, fewer erythrocytes and leucocytes in their stools, and less often had fever than did patients infected with *Shigella* spp.

Figure 1 shows the median serum CRP concentrations by patient group and study day. Patients infected with *V. cholerae* had significantly ($p < 0.01$) lower admission CRP concentrations than did the three groups of patients infected with *Shigella* spp. In all three groups of patients infected with *Shigella* spp, median CRP concentrations were significantly higher on admission and study day 3 than when

Figure 1: Median CRP concentration by patient group and study day. Admission CRP concentrations were significantly ($p < 0.05$) lower in the cholera group when compared with each of the other three groups. CRP concentrations were significantly higher on study days 3 and 5 ($p < 0.05$) in *S. dysenteriae* type 1 infected patients in whom treatment subsequently failed than in the patients infected with *Shigella* spp other than *S. dysenteriae* type 1.

measured on study day 5 and at a follow up visit. On admission median CRP concentrations were higher in *S. dysenteriae* type 1 infected patients in whom initial treatment failed, than in the other two groups of patients infected with *Shigella* spp. Of the 10 patients infected with *V. cholerae*, three had CRP concentrations above 4.8 mg/l on admission, with the highest being 70.4 mg/l. At follow up, all 10 patients had a CRP concentration less than 4.8 mg/l.

Figure 2 shows the median serum prealbumin concentrations by patient group and study. On admission, the median prealbumin concentration in the 10 patients infected with *V. cholerae* was significantly ($p < 0.05$) higher than in either of the two groups of patients infected with *S. dysenteriae* type 1. When compared with admission concentrations, median prealbumin concentrations were significantly higher on study days 3 and 5, and at follow up, in all three groups of patients infected with *Shigella* spp. Median prealbumin concentrations were within the normal range in all three *Shigella* spp infected groups on study day 5 and at the time of follow up examination. Median concentrations of prealbumin in *V. cholerae* infected patients did not differ between admission and follow up.

Table II shows the utility of different measures in predicting clinical outcome. A CRP concentration of ≥110 mg/l had a sensitivity of 70% and a specificity of 61% in identifying patients in whom treatment would ultimately fail. This CRP concentration was a better predictor of outcome than total blood leucocyte count, total premature leucocytes, maximum temperature on the first study day, and admission prealbumin concentration. A history of 10 or more stools in the 24 hours before admission was, however, as sensitive and specific as CRP in identifying patients in whom treatment would fail. Because failure of antimicrobial treatment was comparatively uncommon, the positive predictive value of a serum CRP concentration ≥110 mg/l was only 14%.

Discussion

In comparison with patients infected with *V. cholerae*, patients infected with *Shigella* spp had considerably increased serum concentrations of CRP, and considerably diminished serum concentrations of prealbumin. These findings are consistent with the pathogenesis of *V. cholerae* and *Shigella* spp infections; the first is largely confined to the intestinal lumen, and the second invades the colonic mucosa. That patients infected with *S. dysenteriae* type 1 had higher concentrations of CRP than patients infected with other *Shigella* spp is also consistent with both clinical and experimental studies that have shown that of all the *Shigella* spp serotype, infection with *S. dysenteriae* type 1 elicits the most severe inflammation.²⁰

Among patients infected with *Shigella* spp, the highest CRP concentrations were seen in patients in whom initial treatment failed. Serum CRP concentrations were a better

Figure 2: Median prealbumin concentrations by patient group and study day. Admission prealbumin concentrations were significantly ($p < 0.05$) higher in patients infected with *V. cholerae* than in both groups of patients infected with *S. dysenteriae* type 1. Patients infected with *Shigella* spp other than *S. dysenteriae* type 1 had significantly higher ($p < 0.05$) prealbumin concentrations on day 3, 5, and at follow up visit than did patients infected with *S. dysenteriae* type 1 in whom treatment was successful. At follow up visit patients infected with *S. dysenteriae* type 1 in whom treatment was successful had significantly ($p < 0.05$) lower prealbumin concentrations than did patients infected with *S. dysenteriae* type 1 in whom initial treatment failed. Seven of 10 *S. dysenteriae* type 1 patients in whom initial treatment failed received alternate treatment before returning for their follow up visit.

predictor of outcome than total blood leucocyte count, maximum fever on the first hospital day, or the blood neutrophil proportion of total leucocytes.

Three of 10 *V. cholerae* infected patients had increased CRP concentrations on admission. Although *V. cholerae* is characteristically thought not to elicit an inflammatory response in the gut, studies have found that peripheral blood leucocyte counts are increased, and that some cholera patients are febrile during their illness.²¹ A previous study of 22 patients also showed increased CRP concentration in patients with cholera.²² The mechanism by which *V. cholerae* infection elicits this increase in CRP concentrations remains unknown.

The diminished prealbumin concentrations in patients with shigellosis in this study are consistent with previous epidemiological findings that dysentery has a profound effect on nutrition and growth,⁹ and could be the result of a number of different pathological processes. Firstly, anorexia is a common feature of shigellosis and prealbumin concentrations are known to decline rapidly after energy or protein deprivation. Secondly, the acute inflammatory response is known to down regulate synthesis of prealbumin and albumin. Lastly, patients with shigellosis have been shown to have a protein losing enteropathy,²³ and the diminished prealbumin concentrations may in part be caused by the loss of this protein into the gut. During hospital course prealbumin concentrations in *S. dysenteriae* type 1 infected patients, irrespective of outcome, were lower than in patients infected with other *Shigella* spp. *S. dysenteriae* type 1 infection is known to cause more severe colitis and greater gut protein loss than infections caused by other *Shigella* spp.^{2,23} The return of prealbumin concentrations to normal when patients returned for a follow up visit suggests that with appropriate antimicrobial treatment and nutritional support, such as these patients received, the impact of shigellosis on nutrition can be ameliorated.^{7,8} In contrast with the patients with shigellosis, prealbumin concentrations were normal in the patients with cholera. This finding is consistent with the finding that watery diarrhoea has less of an impact on nutritional status than dysentery.⁹

The findings of this study re-emphasise the

need to consider, and treat, shigellosis as a clinical entity that is different from watery diarrhoea caused by *V. cholerae* or other enteropathogens. In contrast with cholera, patients with shigellosis have a systemic disease that persists after rehydration. While fluid replacement in cholera patients is the crucial element in their treatment, patients with shigellosis clearly require additional nutritional support, and perhaps control of the inflammatory response, for early return to health.

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