Pro- and Synbiotics to Control Inflammation and Infection in Patients With Multiple Injuries

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Background: A recent randomized clinical trial of our group disclosed considerable reduction of the infective sequelae after administration of a synbiotic formula, namely Synbiotic 2000FORTE, in patients with multiple injuries, the latter being a preparation of four probiotics. The mechanism of action of synbiotics was studied.

Methods: A total of 72 patients with severe multiple injuries were allocated to a 15-day administration of either placebo or the synbiotic formula. The association of bloodstream infections, ventilator-associated pneumonia (VAP), serum levels of C-reactive protein (CRP), and endotoxins (LPS) were studied.

Results: Sepsis in the field of bacteremia occurred in 13 patients treated with placebo (36.1%) compared with 5 patients treated with Synbiotic 2000FORTE (13.9%, $p = 0.028$ between groups). The time to progression to primary bacteremia was longer among patients treated with Synbiotic 2000FORTE compared with placebo ($p = 0.0237$ between groups). Twelve (33.3%) and five (13.9%) placebo-treated and probiotic-treated patients, respectively, developed ventilator-associated pneumonia with *Acinetobacter baumannii* as a bacterial cause ($p = 0.047$ between groups). Treatment with synbiotics was accompanied by reduction of white blood cell counts and LPS and CRP levels in either patients who did or did not develop sepsis.

Conclusions: Synbiotics contained in the studied formula decrease significantly the risk for sepsis by bloodstream infections and the occurrence of VAP by *A. baumannii*. The mechanisms of action might involve direct immunomodulatory effect, prevention of bacterial translocation, or most likely a combination of both.

Key Words: Synbiotics, Bacteremia, Sepsis, Ventilator-associated pneumonia.

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Increasing evidence suggests that in the individual patient a high degree of inflammation is associated with a significantly increased risk of sepsis. The increased systemic inflammation precedes and seems to pave the way for infection.[1] The degree of systemic inflammation before and in the early stage of trauma seems to determine the outcome.[2] Patients with high degree of inflammation, such as patients with chronic diseases, are at higher risk to develop complications. Also, patients with high degree of inflammation in the early phase of trauma suffer a higher risk of development of septic manifestations in the subsequent course. As an example, a study in human liver transplantation demonstrated that patients with a sixfold or higher increase in tumor necrosis factor (TNF)-α and IL-6 at the late phase of the operation developed sepsis during subsequent postoperative days.[3]

Some specific lactic acid bacteria (LAB) have demonstrated unique abilities to induce cellular immunity, produce suppressive cytokines, and decrease antigen-specific IgE and IgG.[4–6] Prominent among these LAB species are *Lactobacillus plantarum*, *L. paracasei subsp. paracasei*, and to some extent also *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*. All these were chosen for their ability to suppress inflammation after extensive analysis of more than 350 LAB from the human gastrointestinal tract and 180 LAB obtained from growing plants.[7,8] A series of studies have proven the efficacy of these particular strains to suppress inflammation and reduce growth of pathogenic bacteria. Among more than 100 different LAB strains, *L. paracasei subsp. paracasei* was found to be the strongest inducer of Th1 and repressor of Th2 cytokines.[9] Studies of the ability of 50 different LAB to control 23 different *Clostridium difficile* strains found that only *L. paracasei* and *L. plantarum* are effective to eliminate *C. difficile* strains—more than half of the other tried LAB strains were totally ineffective.[10] A combination of these particular four LAB has also proven to be very effective to reduce the consequences of induced sepsis (cecal ligation and puncture) in animal studies: reduce inflammation, inhibit neutrophil accumulation in tissues, and prevent tissue destruction, both if supplied as pretreatment for 3 days[11] and as subcutaneous injection at initiation of trauma.[12]

Several clinical studies have also documented significant effects of these LAB. Among the observed effects are (1) reduction in perioperative infections in connection with liver transplantation (LAB treated 3% vs. controls 51%) as well as in the number of isolated pathogens (LAB treated 1 vs. controls 18)[13]; (2) reduction of infection rate in connection with pylorus-preserving pancreatoduodenectomy (LAB treated 12.5% vs. controls 40%) as well as in the number of isolated pathogens (5 vs. 20)[14]; (3) reduction in infection rate in severe acute pancreatitis (LAB treated 7% vs. controls 52%) as well as in cultivated pathogens (LAB treated 7 vs. controls 17)[15]; and (4) reduction in number of infections in

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abdominal cancer operations (LAB treated: 7%, placebo: 20%, and parenteral nutrition: 47%) (Han Chun Mao et al., personal communication). In the last-mentioned study, significant reductions in prealbumin, C-reactive protein (CRP), serum cholesterol, serum endotoxin, white cell blood count, and IgA were also observed. A randomized trial in 131 patients with multiple injuries compared the effects of glutamine, fermentable fiber, Nutricomp peptide, and the combination of the four LAB strains. The total number of infections were glutamine: 51%, fermentable fibers: 57%, Nutricomp peptide: 52%, and the composition of the four LAB: 14%.[16]

In a recent controlled study of our group, patients with multiple injuries were randomized to either placebo or Synbiotic 2000FORTE (Medipharm, Sweden), the latter being a preparation of four probiotics. More precisely, it contains a combination of 10^{11} colony-forming units (CFU) of each one of the following LAB: *P. pentoseceus* 5–33:3, *L. mesenteroides* 32–77:1, *L. paracasei* ssp. 19, and *L. plantarum* 2362 containing also inulin, betaglucan, pectin, and resistant starch as bioactive fibers. The rationale for the administration of a combination of four LAB was their proven efficacy in previous animal studies,[11,12] as stated earlier. Patients administered the symbiotic formula demonstrated a significantly reduced overall infection rate compared with placebo (63% vs. 90%, respectively, $p = 0.01$). This applied for infections of the lower respiratory tract (54% vs. 80%, respectively, $p = 0.03$), infections of the central lines (37% vs. 66%, $p = 0.02$), and infections of the urinary tract (17% vs. 43%, $p = 0.02$). As a consequence, the occurrence of septic phenomena was significantly reduced among Synbiotic 200FORTE-treated patients compared with placebo-treated patients (49% vs. 77%, respectively, $p = 0.02$), which led to reduced number of days on mechanical ventilation (mean, 16.7 days vs. mean, 29.7 days, respectively, $p = 0.001$) and reduced stay in the intensive care unit (ICU) (mean, 27.2 days vs. mean, 41.3 days, respectively, $p = 0.01$).[17]

Although the clinical efficacy seems to be overwhelmingly well documented, much remains until the mechanisms of actions by the LAB are fully clarified. This study analyzed the microbiological and laboratory findings of patients participating in the above mentioned trial,[17] aiming to provide evidence about the mechanism of action of Synbiotic 2000FORTE in the study population.

PATIENTS AND METHODS

This study is an analysis of the microbiological and laboratory findings of 72 patients participating in a double-blind, placebo-controlled, multicenter, randomized clinical trial.[17] The study was done in the five surgical ICUs of the Thessaloniki University's tertiary-care AHEPA Hospitals and the affiliated 424th Military Hospital. The protocol was approved by the Research and Ethics Committee of the University of Thessaloniki. Written informed consent was provided by first-degree relatives.

All participants were bearing severe multiple organ injuries necessitating emergency tracheal intubation and ventilation support and subsequent hospitalization in the ICU. Patients with any previous hospitalization over the last 60 days were excluded from the study. On admission to the ICU, they were randomly assigned to receive either placebo or the formula Synbiotic 2000FORTE. The stability of the symbiotic preparation was ascertained by the manufacturer. Both were provided in a form of 12-g sachets. The nutritional value of the symbiotic preparation was 10 calories contained in the bioactive fibers. The content was diluted in 100 mL of tap water and administered by a nasogastric tube or through gastrostomy once daily for 15 consecutive days post admission. Administration was performed by a study nurse who was responsible to ascertain that the whole prepared volume was given. Baseline demographic and clinical data for these patients have already been published.[17]

Patients were followed up for 28 days. On days 1, 4, 7, and 15, 6 mL of venous were sampled after venipuncture of a peripheral antecubital vein under aseptic conditions; 3 mL were collected into an ethyldiamine tetracetic acid-coated tube for the estimation of the white blood cell count (Beckman Coulter Co., Miami, FL); and the remaining 3 mL were collected into a pyrogen-free tube and centrifuged. CRP was estimated in serum in duplicate by a nephelometric assay (Behring, Berlin, Germany). The lowest limit of detection was 0.2 mg/dL.

For the estimation of endotoxins (LPS), serum samples were diluted 1:10 in sterile and pyrogen-free water (BioWhitaker, Walkersville, MD) and incubated for 5 min at 70°C. The concentration of LPS was then measured by the kinetic QCL-1000 Limulus Amoebocyte Lysate assay (BioWhitaker, lower limit of detection 0.05 EU/mL) using a standard curve created by known concentrations of LPS of *Escherichia coli* serotype O111:B4. All determinations were performed in duplicate and the mean of two observations was applied.

On presentation of systemic inflammatory response syndrome, a complete diagnostic work-out was done comprising cultures of blood, quantitative urine cultures, and quantitative cultures of tracheobroncial secretions (TBS). Urine samples yielding a pathogen at a concentration $\geq 10^5$ CFU/mL were considered positive.[18] Cultures of TBS yielding a pathogen at a count $\geq 1 \times 10^6$ CFU/mL were considered positive.[19]

Coagulase-negative staphylococci were considered as a cause of bacteremia when they were isolated in parallel by a peripheral vein and the lumen of the central venous catheter or by at least two separate blood cultures drawn at different time intervals provided that their antibiogram was the same.[20]

Bloodstream infections were considered in all cases of positive blood cultures with clinical significance.[21] Primary bacteremia was defined as any case of bacteremia yielding an isolate that was not isolated from any other source and where extensive diagnostic work-out comprising lung X-ray and chest and abdominal computed tomography failed to disclose another source of infection.[20]

Systemic inflammatory response syndrome and sepsis were defined according to the American College for Chest Physicians/Society of Critical Care Medicine criteria.[22] The number of patients who presented with sepsis and CRP more than 35 mg/L was estimated in each group.

Ventilator-associated pneumonia (VAP) was diagnosed in patients presenting with all of the following: (a) new or persistent consolidation in lung X-ray, (b) purulent TBS, and

TABLE 1. Occurrence of Bloodstream Infections and of Primary Bacteremia in Relation to the Type of Pathogen Among 72 Patients with Multiple Injuries, 36 Receiving Placebo and 36 the Formula Synbiotic 2000FORTE

Isolated Microorganism	Placebo Group	Synbiotic 2000FORTE Group	*p*
	Number (%) of patients yielding a bloodstream infection by the respective pathogen		0.028
Coagulase-negative *Staphylococcus* spp.	5 (13.9)	0	
Acinetobacter baumannii	4 (11.1)	2 (5.6)	
Enterobacter aerogenes	2 (5.6)	0	
Pseudomonas aeruginosa	0	1 (2.8)	
Staphylococcus aureus	0	1 (2.8)	
Candida albicans	2 (5.6)	1 (2.8)	
	Number (%) of patients with primary bacteremia by the respective pathogen		0.038
Coagulase-negative *Staphylococcus* spp.	4 (11.1)	0	
Acinetobacter baumannii	1 (2.8)	0	
Enterobacter aerogenes	2 (5.6)	0	
Staphylococcus aureus	0	2 (5.6)	
Candida albicans	2 (5.6)	0	

(c) clinical pulmonary infection score (CPIS) more than 6 as assessed by Pugin et al.[23–25]

Results of WBCs and of CRP were expressed by their mean ± SD. Comparisons between groups over-time were done by one-way analysis of variance (ANOVA) with post-hoc Bonferroni corrections. Comparisons of qualitative characteristics between groups were done by the Fischer's exact test. Assessment of odds ratio (OR) and 95% confidence intervals (CI) for progression to sepsis in each group was preformed by Mantel and Haenzel's statistics. Time to detection of primary bacteremia was estimated separately for each group and drawn by Kaplan-Meier analysis. Comparisons were done by the log-rank test. Any value of p below 0.05 was considered significant.

RESULTS

The two groups did not differ in terms of age, disease severity, and comorbidities.[17] More precisely, mean age of placebo-treated patients was 55.9 years compared with 52.9 years of the Synbiotic 2000FORTE-treated patients ($p = 0.250$). Respective values for APACHE II score were 19.36 and 19.36 ($p = 0.630$) and for Glasgow Coma Scale 7.64 and 7.80 ($p = 0.600$). Abdominal trauma occurred among 50% of the placebo group and among 48.6% of the Synbiotic 2000FORTE group ($p = 0.900$); chest trauma occurred among 66.6% and 71.4% of them, respectively ($p = 0.670$); central nervous system injuries occurred among 60% and 57.1% of them, respectively ($p = 0.810$); and injuries of the extremities occurred among 23.3% and 22.9% of them, respectively ($p = 0.910$). The most common underlying disorder was diabetes mellitus type 2 who occurred among 16.7% and 20.0% of them, respectively ($p = 0.730$).

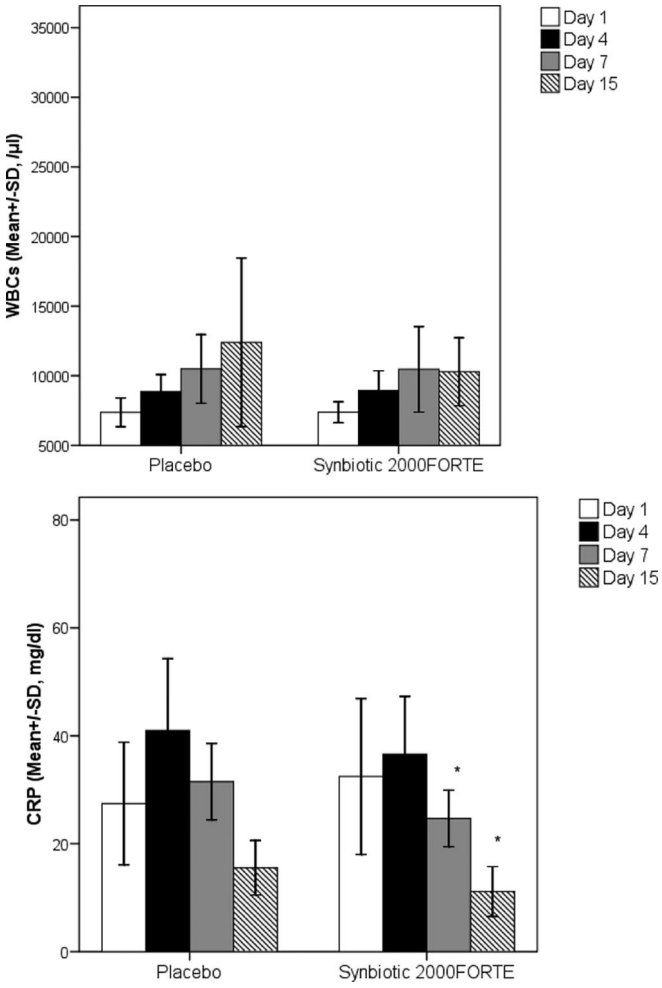

Figure 1. Comparative white blood cell counts (WBCs) and C-reactive protein (CRP) levels of patients who did not develop sepsis. Asterisks denote statistically significant differences between groups at the indicated time intervals.

From the 2nd postadmission day, all patients received isocaloric and isonitrogenous solutions infused continuously over 24 hours using a central venous catheter consisting of glucose, amino acids, lipids, vitamins, and trace elements. The total caloric intake goal for all patients was 1.3 times basal energy expenditure (calculated by the Harris-Benedict equation). Additionally, all patients received $0.5 g \cdot kg^{-1} \cdot d^{-1}$ alanyl-glutamine (Dipeptiven, Fresenius AG, Bad Homburg, Germany). From the 5th postadmission day, patients were also received an enteral pharmaconutrition supplement (Intestamin, Fresenius Kabi, Hellas), at a rate of 21 mL/h (equivalent to 500 mL/d or 250 kcal) using an endoscopic gastrostomy tube, for up to 10 days. Blood glucose level was monitored four times daily in each patient, and short-acting insulin was administered subcutaneously, if needed, to ascertain serum glucose levels between 110 and 180 mg/dL.

Sepsis in the field of bacteremia occurred in 13 patients treated with placebo (36.1%) compared with 5 patients treated with Synbiotic 2000FORTE (13.9%, $p = 0.028$ between groups). Blood isolates for these infections are shown in Table 1.

WBCs and CRP values over consecutive follow-up for patients who did not develop any signs of sepsis are shown in Figure 1. WBCs did not differ between groups of treatment. CRP values were lower among Synbiotic 2000FORTE-treated patients compared with placebo-treated patients on days 7 and 15 ($p = 0.011$ and $p = 0.020$ of comparisons between groups, respectively).

Nine (25%) and two (5.6%) of placebo- and Synbiotic 2000FORTE-treated patients, respectively, developed primary bacteremia. Causing pathogens are shown in Table 1 ($p = 0.038$ between groups). The time to progression to primary bacteremia was longer among patients treated with Synbiotic 2000FORTE compared with placebo (Fig. 2, $p = 0.0237$ between groups).

Sixteen (44.4%) of placebo-treated and 15 (41.7%) of Synbiotic 2000FORTE-treated patients were presented with VAP (p = NS between groups, Table 2). In 12 (33.3%) and 5 (13.9%) of them, respectively, *A. baumannii* was identified as the bacterial cause of VAP ($p = 0.047$ between groups).

Comparative WBCs and serum CRP between groups of treatment separately for patients who developed primary bacteremia and for those who developed VAP by *A. baumannii* are shown in Figures 3 and 4. When considering patients who developed primary bacteremia, WBCs were significantly lower among Synbiotic 2000FORTE-treated compared with placebo-treated patients on day 15 ($p = 0.019$). In the same patients, CRP levels were significantly lower among Synbiotic 2000FORTE-treated compared with placebo-treated patients on day 7 ($p = 0.034$) and on day 15 ($p = 0.040$). Similar differences were counted when considering patients with VAP by *A. baumannii* on day 4 ($p = 0.015$), day 7 ($p = 0.020$), and day 15 ($p = 0.028$).

Eight among the placebo-treated patients presented with sepsis and CRP >35 mg/L (22.2%) compared with two of the Synbiotic 2000FORTE-treated patients who presented with sepsis and CRP >35 mg/L (5.6%, $p = 0.042$). Serum levels of LPS for the total of enrolled patients are shown in Figure 5. They were lower in the Synbiotic 2000FORTE-treated compared with placebo-treated patients on day 4 ($p = 0.027$) and on day 15 ($p = 0.014$).

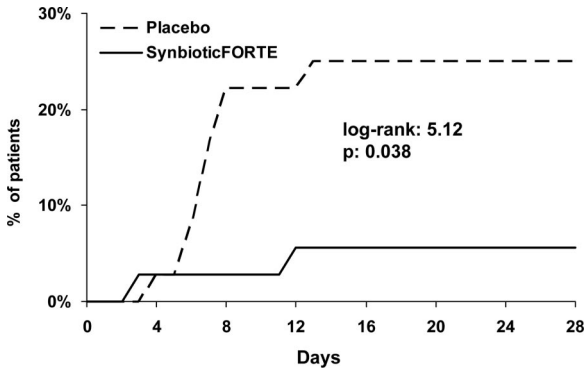

Figure 2. Comparative time to detection of primary bacteremia between patients receiving placebo and those receiving the Synbiotic 2000FORTE formula.

TABLE 2. Occurrence of Ventilator-Associated Pneumonia and of Positive Urine Cultures in Relation to the Type of Pathogen Among 72 patients with Multiple Injuries, 36 Receiving Placebo and 36 the Formula Synbiotic 2000FORTE

Isolated Microorganism	Placebo Group	Synbiotic 2000FORTE Group	*p*
	Number (%) of patients yielding a positive TBS culture by the respective pathogen		0.172
Acinetobacter baumannii	12 (33.3)	5 (13.9)	
Klebsiella pneumoniae	2 (5.6)	5 (13.9)	
Pseudomonas aeruginosa	2 (5.6)	3 (8.3)	
Candida albicans	0	2 (5.6)	
	Number (%) of patients with positive urine culture by the respective pathogen		0.210
Acinetobacter baumannii	7 (19.4)	6 (16.7)	
Klebsiella pneumoniae	2 (5.6)	0	
Pseudomonas aeruginosa	2 (5.6)	0	

Eleven and six of patients, respectively, had a positive urine culture ($p = 0.210$ between groups, Table 2).

Ten patients treated with placebo died (27.8%) compared with five patients treated with Synbiotic 2000FORTE (13.9%, $p = 0.123$). All deaths were caused by multiple organ dysfunction syndrome.

DISCUSSION

Synbiotic 2000FORTE is a formula of four LAB probiotics that was administered in a randomized clinical trial in a population of critically ill patients with multiple injuries. Results of that trial disclosed clinical benefit assessed by a significant reduction of the occurrence of infection during follow-up.[17] Microbiology and laboratory data of patients who participated in the latter trial were analyzed in an attempt to provide a rationale for the mechanism of action of the synbiotic formula.

Analysis revealed a considerable benefit of Synbiotic 2000FORTE administration on the advent of bloodstream infections. The risk of sepsis due to bacteremia was significantly decreased along with the occurrence of primary bacteremia. The time to primary bacteremia was considerably prolonged (Fig. 2), an effect which could have been even greater if the time of administration of Synbiotic 2000FORTE had been prolonged.

Probiotic regimens are a novel concept in the management of infectious diseases. Treatment of acute infectious diarrhea and antibiotic-associated diarrhea are their main indications whereas they have also been proposed as treatment adjuvants in Crohn's disease and ulcerative colitis as well as in pouchitis and diverticulitis.[26] Two randomized trials have recently been published on the effectiveness of Synbiotic 2000FORTE. The first enrolled 66 patients

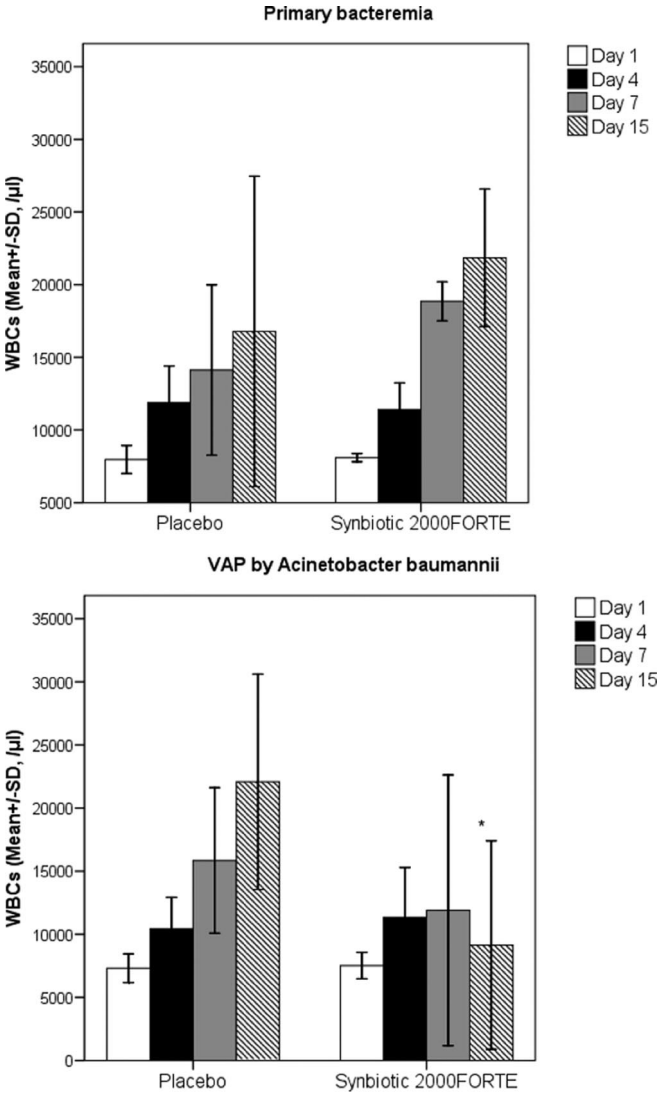

Figure 3. Comparative white blood cell counts (WBCs) of patients with primary bacteremia and with ventilator-associated pneumonia (VAP) by *Acinetobacter baumannii*. Asterisks denote statistically significant differences between groups at the indicated time intervals.

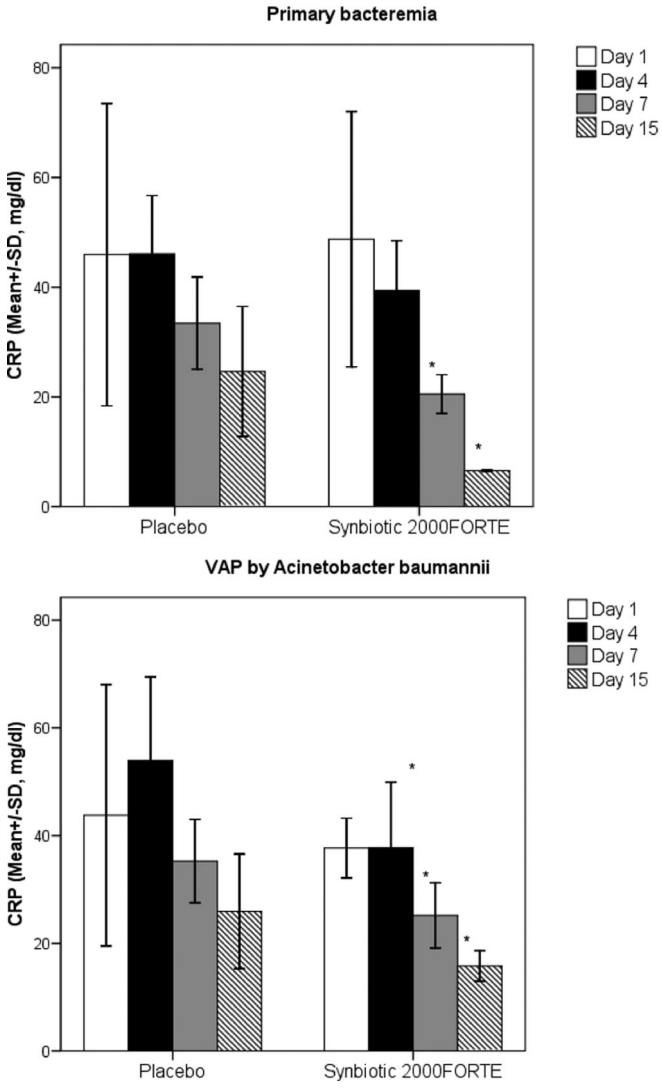

Figure 4. Comparative serum levels of C-reactive protein (CRP) of patients with primary bacteremia and with ventilator-associated pneumonia (VAP) by *Acinetobacter baumannii*. Asterisks denote statistically significant differences between groups at the indicated time intervals.

undergoing liver transplantation[13] and the second 80 patients undergoing pylorus-preserving pancreato-duodenectomy.[14] Administration of Synbiotic 2000FORTE reduced considerably the occurrence of postoperative infective complications. These results are in general agreement with those of our trial.[17] A recent meta-analysis of eight randomized trials of the efficacy of enterally administered prebiotics, probiotics, and synbiotics in adult critically ill patients has been published.[27] Studied outcomes were advent of pneumonia, in-hospital mortality, and stay in the ICU. Analysis failed to disclose any benefit of the administered formulas. However, two points should be mentioned: (a) the efficacy of the administered formulas on the incidence of bloodstreams infections was not studied; and (b) trials with prebiotics, probiotics, and synbiotics were encountered together.

Based on the presented findings, a dual mechanism of action of synbiotics may be proposed; the first comprises an immunomodulatory effect on gut-associated lymphoid tissue and the second involves stabilization of the normal intestinal flora leading to prevention of bacterial transolocation. In patients who did not develop signs of sepsis, CRP levels were reduced after several days of enteral feeding with synbiotics (Fig. 1), a phenomenon consistent with a probable immunomodulatory effect. A similar finding in both white blood cell counts and CRP levels was also prominent in patients who developed sepsis either as a result of bloodstream infection or of VAP (Figs. 3 and 4).

Results of a recent experimental study have proposed prevention of bacterial translocation as a mechanism of action of probiotics. More precisely, feeding of rats with a mixture

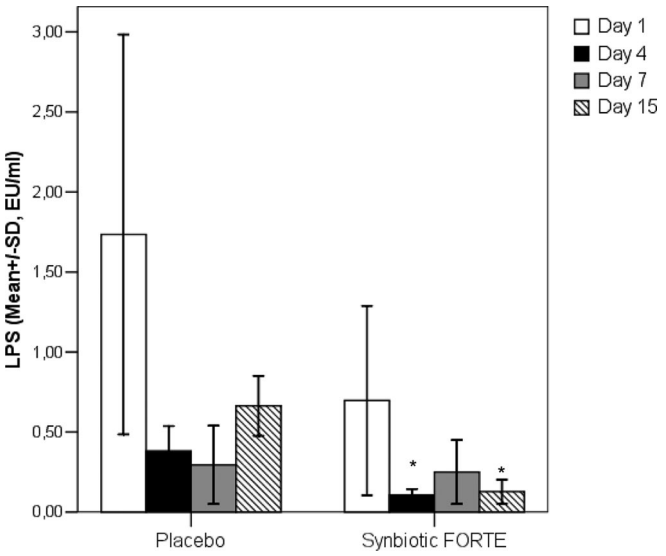

Figure 5. Comparison of serum levels of endotoxins (LPS) between patients receiving placebo and those receiving the Synbiotic 2000FORTE formula. Asterisks denote statistically significant differences between groups at the indicated time intervals.

of *L. acidophilus*, *L. helveticus*, and *Bifidobacterium* spp. significantly reduced serum levels of endotoxemia after induction of pancreatitis.[28] Three main findings of this study support prevention of bacterial translocation as the mechanism of action of Synbiotic 2000FORTE: (a) the reduction of the rate of primary bacteremia; (b) the reduction of the rate of VAP by *A. baumannii* (Table 2); and (c) the reduction of the level of endotoxemia (Fig. 5). Primary bacteremia signifies the lack of availability of any documented source of primary infection. Coagulase-negative staphylococci and enterobacteriaceae were the main pathogens in the study population (Table 1). In experimental studies in rats subjected to intraperitoneal challenge by zymozan or by installation of pneumoperitoneum as well as in clinical studies of our group aiming at the occurrence of bacterial translocation after major intrabdominal surgery, these types of bacteria were isolated from cultures of mesenterial lymph nodes.[29–31] These observations support the hypothesis that primary bacteremia detected in patients with multiple injuries of this study resulted from translocation from the gut. Results of experimental studies have also implicated the lungs receiving mesenterial lymph through the thoracic duct as a site for translocating bacteria.[32,33] The latter mechanism may explain the decreased frequency of VAP by *A. baumannii* after administration of synbiotics in this study population. The high epidemiology of *A. baumannii* as a causative pathogen for VAP seems to be a specific trait of ICUs in Greece, as already described in a recently published trial.[25]

Tight adhesion of probiotics to the apical surface of the intestinal epithelium may be considered as the underlying mode of action leading to prevention of bacterial translocation.[34] Administration was safe as it was not connected with any episode of infection by any of the species contained in the ingested formula. A former trial in 28 critically ill children disclosed safety after ingestion of *L. casei*.[35]

The presented analysis revealed that the beneficiary effect of the administration of Synbiotic 2000FORTE in critically ill patients with multiple injuries in decreasing the occurrence of postinjury infections was linked to the reduction of primary bacteremia and of VAP by *A. baumannii*. This was further accompanied by decrease of the risk of sepsis attributed to bloodstream infections. The effects of the administration of Synbiotic 2000FORTE on the incidence of primary bacteremia and of VAP are consistent with the reduction of stay in the ICU and with the earlier weaning from mechanical ventilation seen in the these patients compared with placebo.[17] The presented findings implicate prevention of bacterial translocation and a direct immunomodulatory effect as the most probable mechanisms of action of this synbiotic formula.

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