Journal of Critical Care 42 (2017) 310–316

Contents lists available at ScienceDirect

Journal of Critical Care

journal homepage: www.jccjournal.org

Gut microbiota trajectory in patients with severe burn: A time series study

Xinying Wang, MD [a,*], Jianbo Yang, MD [a], Feng Tian, MD [a], Li Zhang, MD [a], Qiucheng Lei, MD [b], Tingting Jiang, MD [c], Jihong Zhou, MD [d], Siming Yuan, MD [d], Jun Wang, MD [d], Zhijian Feng, MD [d], Jieshou Li, Ph.D [a]

[a] Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing 210002, China
[b] Department of General Surgery, The First People's Hospital of Foshan, Sun Yat-Sen University, Foshan 528000, China
[c] Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai 200011, China
[d] Department of Burn, Jinling Hospital, Medical School of Nanjing University, Nanjing 210002, China

ARTICLE INFO

Keywords:
Gut microbiota
Severe burn
Microbiome biomarkers
Enteral nutrition

ABSTRACT

Purpose: This time series experiments aimed to investigate the dynamic change of gut microbiomes after severe burn and its association with enteral nutrition (EN).
Materials and methods: Seven severely burned patients who suffered from a severe metal dust explosion injury were recruited in this study. The dynamic changes of gut microbiome of fecal samples at six time points (1–3 days, 2, 3, 4, 5 and 6 weeks after severe burn) were detected using 16S ribosomal RNA pyrosequencing technology.
Results: Following the post-burn temporal order, gut microbiota dysbiosis was detected in the gut microbiome after severe burn, then it was gradually resolved. The bio-diversity of gut bacteria was initially decreased, and then returned to normal level. In addition, at the early stage (from 2 to 4 weeks), the majority of those patients' gut microbiome were opportunistic pathogen genus, *Enterococcus* and *Escherichia*; while at the end of this study, the majority was a beneficial genus, *Bacteroides*. EN can promote the recovery of gut microbiota, especially in EN well-tolerated patients.
Conclusions: Severe burn injury can cause a dramatic dysbiosis of gut microbiota. A trend of enriched beneficial bacteria and diminished opportunistic pathogen bacteria may serve as prognosis microbiome biomarkers of severe burn patients.

1. Introduction

Human gut microbiota plays crucial physiological roles, such as producing important metabolites, challenging and promoting the maturity of host immune system, and protecting the host from pathogen infection [1]. The composition and biodiversity of intestinal microbiomes can be affected by several agents, including diet, medication, infection and severe trauma. Alterations of gut microbiota composition can damage the steady state of mucosal immune response and adversely impact the intestinal epithelia barrier [2]. Thus, dysbiosis of gut microbiota was reported to be associated with many diseases, including inflammatory bowel disease, type 2 diabetes, colon cancer and obesity [3-5].

Severe burn is an intensive pathologic shock to human body, which may also affect the hemostasis of gut microbiota. Studies have demonstrated that burn injury increases the permeability of gut and contributes to bacterial translocation [6]. Also, for both mouse and human, gut microbiota dysbiosis can be triggered by burn injury, allowing the overgrowth of Gram-negative aerobic bacteria, which play a pivotal role in potentiating sepsis.

Although previous studies have demonstrated the association between severe burns with gut microbiota dysbiosis, few is focusing on the dynamic change of gut microbiomes and its association with enteral nutrition (EN) in severe burn patients [7]. Therefore, we carried out a time series study of seven severely burned patients to evaluate the serial changes of intestinal microbiota and its association with EN.

2. Material and methods

2.1. Patients and ethics statement

Seven severely burned patients who suffered from a severe metal dust explosion injury were recruited at Jinling Hospital. All the patients

* Corresponding author at: Xinying Wang, MD, Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, No. 305 East Zhongshan Road, Nanjing 210002, China.
E-mail addresses: wangxyjlh@126.com, dg1635084@smail.nju.edu.cn (X. Wang), yang_jianbo@126.com (J. Yang), tianfeng_nju@163.com (F. Tian), zlshe1107@163.com (L. Zhang), lqiuchenggd@163.com (Q. Lei), jiangtingting08med@163.com (T. Jiang), zhoujhjlh@126.com (J. Zhou), yuansmjlh@126.com (S. Yuan), wangj_jlh@126.com (J. Wang), fengzj_jlh@126.com (Z. Feng), lijsnjujlh@126.com (J. Li).

http://dx.doi.org/10.1016/j.jcrc.2017.08.020
0883-9441/

Table 1
Patient characteristics.

Subject	1	2	3	4	5	6	7
Age (years)	41	44	29	29	42	23	19
Sex (M/F)	M	F	M	M	M	F	M
Burn area (%)	95%	95%	95%	90%	70%	65%	75%
APACHE II score	18	18	25	13	17	12	9
SOFA score	7	7	11	6	13	2	3
BMI (kg/m^2)	21.6	19.5	22.5	16.7	22.7	23.4	26.8
Operation frequency, *n*	8	6	6	8	4	5	3
Duration of antibiotic treatment	130	57	62	79	44	57	22
Antibiotics							
Cefradine	Y	Y	N	N	N	N	Y
Ceftazidime	N	N	Y	Y	Y	Y	N
Etimicin	Y	Y	Y	Y	Y	Y	Y
Biapenem	N	Y	N	N	N	N	Y
Teicoplanin	Y	Y	Y	Y	Y	N	Y
Imipenem	Y	Y	Y	Y	Y	Y	Y
Cefoperazone sulbactam	Y	Y	Y	Y	Y	Y	Y
Vancomycin	Y	Y	Y	Y	Y	Y	N
Penicillin	N	N	N	N	Y	N	Y
Piperacillin tazobactam	N	N	N	N	Y	Y	Y
Gentamicin	Y	Y	Y	Y	Y	Y	N
EN starting day	3	2	3	2	2	2	2
Duration of EN	51	66	54	38	31	49	14
Duration of parenteral nutrition (d)	78	62	57	72	1	0	0
Pneumonia(Y/N)	Y	Y	Y	N	Y	N	N
Enteritis(Y/N)	Y	N	Y	N	N	N	Y
Bacteremia(Y/N)	N	Y	Y	Y	N	Y	Y
Sepsis (Y/N)	Y	Y	Y	Y	N	N	N
ICU stays (d)	67	67	63	72	53	50	21
Total hospital stays (d)	179	180	63	250	108	128	48
Amputation(Y/N)	N	N	Y	N	N	N	N
Survival(Y/N)	Y	Y	N	Y	Y	Y	Y

APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; BMI, Body mass index; EN, Enteral nutrition; ICU, Intensive care unit.

were undergone similar timely support and antibiotics treatment as well as similar nutrient supplement. Fecal samples were continuously collected at six time points (1–3 days, 2, 3, 4, 5 and 6 weeks) in this time series study. To study the alteration of the abundance of gut bacteria of severe burn patients, we divided six sample collection time points into initial stage (1–3 days), early stage (from two weeks to four weeks) and late stage (from five weeks to six weeks). Feeding intolerance was defined according to following symptoms during the study, including diarrhea, vomiting, gastric retention, and abdominal distension, as previously reported [8]. Written informed consents were obtained from all the severely burned patients.

2.2. The sample collection and DNA extraction

Fecal samples were frozen at −80 °C immediately and underwent DNA extraction by the standard methods at BGI laboratory (Beijing Genomic Institute, Shenzhen, China). After bacterial DNA isolation, sample analyses were performed, including concentration testing and sample integrity. Concentration was detected by fluorometer or microplate reader, and sample integrity was detected by 1% agarose gel electrophoresis (voltage, 150 V; electrophoresis time, 40 min) [9].

2.3. The Illumina Miseq 16S sequencing

Polymerase chain reaction (PCR) amplifications of the V4 region of bacterial 16S rRNA genes from fecal samples were performed, using fusion primer with dual index and adapters with a unique barcode sequence for each sample. Then 250 paired-end reads were generated with the Illumina MiSeq platform (MiSeq Reagent Kit; Illumina).

2.4. The data analysis

In order to obtain accurate and reliable results in subsequent bioinformatics analysis [12], the raw data were pre-processed to get clean data by in-house procedure as following: 1) Removal of reads whose average quality of "truncation of sequence reads" over a 30 bp sliding window are <20 (based on the Phred algorithm), and/or whose trimmed reads are <75% of their original length, as well as its paired read; 2) Removal of reads contaminated by adapter(default parameter: 15 bases overlapped by reads and adapter with maximal 3 bases mismatch allowed); 3) Removal of ambiguous reads as well as paired reads (N base); 4) Removal of reads with low complexity(default: reads with 10 consecutive same base).

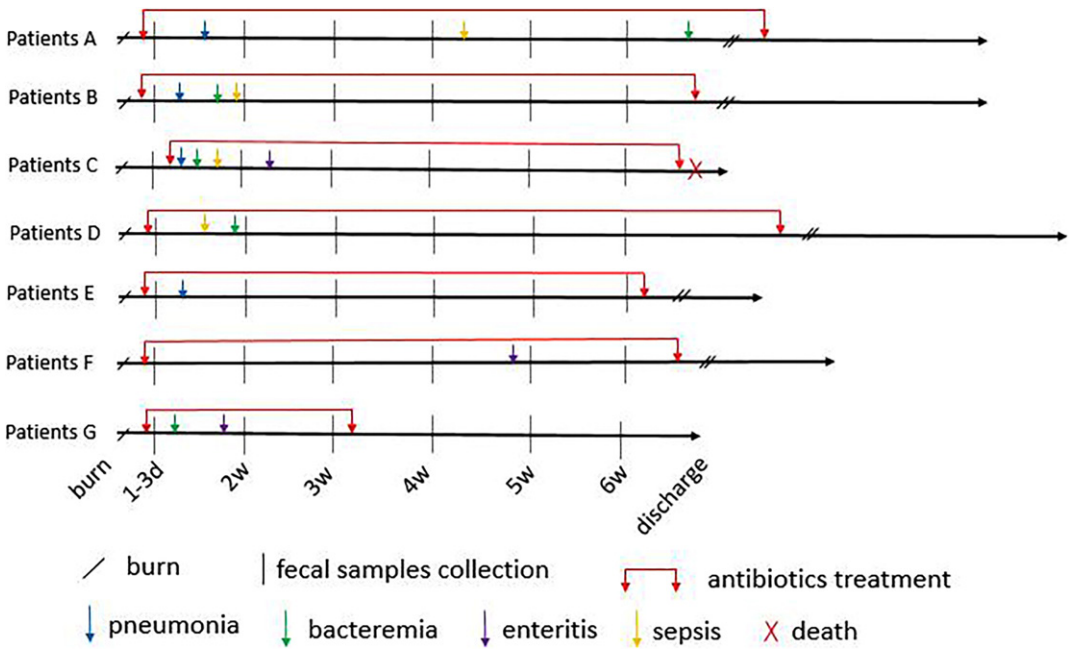

Fig. 1. Experimental details of the study. The timeline for each patients, starting from the time hospital admittance to ending upon discharge or death, included the times of fecal sample collection, antibiotics treatments and times of infection complications.