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Original Contributions

Prognostic significance of C5a2 on polymorphonuclear neutrophil and C5a2intra/C5a2 ratio level for early sepsis in an ED

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ABSTRACT

Introduction: The current study was designed to evaluate the expression of the second C5a receptor (C5a2) on polymorphonuclear neutrophil and in the cytoplasm of polymorphonuclear neutrophils (C5a2intra) in patients with sepsis in the emergency department (ED) for risk stratification and mortality.
Methods: Consecutive patients fulfilling the criteria for systemic inflammatory response syndrome (n = 357) were admitted to Beijing Chao-Yang Hospital ED between January 2015 and July 2015. They were enrolled to identify the expression of C5a2 and C5a2intra and categorized into the following 4 groups: systemic inflammatory response syndrome, sepsis, severe sepsis, and septic shock.
Results: We report that the surface C5a2 decreased and the C5a2intra/C5a2 ratio level increased with sepsis severity. As independent predictors of 28-day mortality, the areas under the receiver operating characteristic curves of combination of C5a2 or C5a2intra/C5a2 ratio level and the Mortality in ED Sepsis score were significantly higher than that of procalcitonin alone in predicting 28-day mortality in septic patients.
Conclusion: The C5a2 and the C5a2intra/C5a2 ratio levels are probably valuable for the risk stratification of sepsis and are associated with the mortality of early sepsis in the ED.

1. Introduction

In sepsis, the activated complement system results in quick inflammatory and adaptive immune responses to clear the infection [1,2]. The uncontrolled activation of the complement system results in excessive generation of anaphylatoxins, especially C5a [1]. The increasing C5a levels play a pivotal role in the chemotactic deactivation of polymorphonuclear neutrophils (PMNs), consecutively leading to the impaired responses of PMNs. The dysfunction of PMNs has been implicated in the pathogenesis of multiple organ failure of septic patients. The dysfunction of PMNs during sepsis is closely associated with a poor clinical outcome [3-5]. Moreover, the upregulated receptors of complement activation possibly allow PMNs to survive and reduce the development of sepsis. The reduced cellular receptor expression has also been strongly correlated with poor outcomes in patients with sepsis [6,7].

Notably, as a novel receptor, the second C5a receptor [C5a2 (Gpr77)] has been found on PMNs with abundant expression [8]. The C5a2 has a high affinity binding for C5a and C5a desArg [9]. Previous studies illustrated that the reduced expression of C5a2 was associated with the impaired responses of C and a poor outcome for in vivo and vitro experiments of patients with sepsis [5,10]. However, the expression of C5a2 and C5a2 in neutrophils (C5a2intra)/C5a2 ratio levels in early sepsis upon emergency department (ED) admission has not been defined before. This study aimed to investigate the clinical value of C5a2 and C5a2intra/C5a2 ratio levels in risk stratification and prognostic evaluation in early sepsis in ED.

2. Materials and methods

2.1. Patients

Between January 2015 and July 2015, patients who met the criteria for systemic inflammatory response syndrome (SIRS) [11,12] were consecutively enrolled in the ED of Beijing Chao-Yang Hospital which had an average 250 000 visits annually (Fig. 1). The exclusion criteria were (1) age <18 or >80 years, (2) immunocompromised individuals who underwent a peripheral stem cell transplant or had any type of autoimmune disease, (3) terminal stage of disease (ongoing malignant

Abbreviations: C5a2 (Gpr77), the second C5a receptor; PMNs, polymorphonuclear neutrophils; Neu, neutrophils; C5a2intra, the expression of the second receptor of C5a in neutrophils; ED, emergency deparment; SIRS, systemic inflammatory response syndrome; MEDS score, Mortality in Emergency Department Sepsis score; ROC curve, receiver operating characteristic curve; AUCs, the areas under the receiver operating characteristic curves; WBC, white blood cells; APACHE, acute physiology and chronic health evaluation; PCT, procalcitonin; IAI, intraabdominal infection; DKA, diabetic ketoacidosis; SSTI, skin/soft tissue infection; CI, confidence interval; MFI, mean fluorescence intensity.

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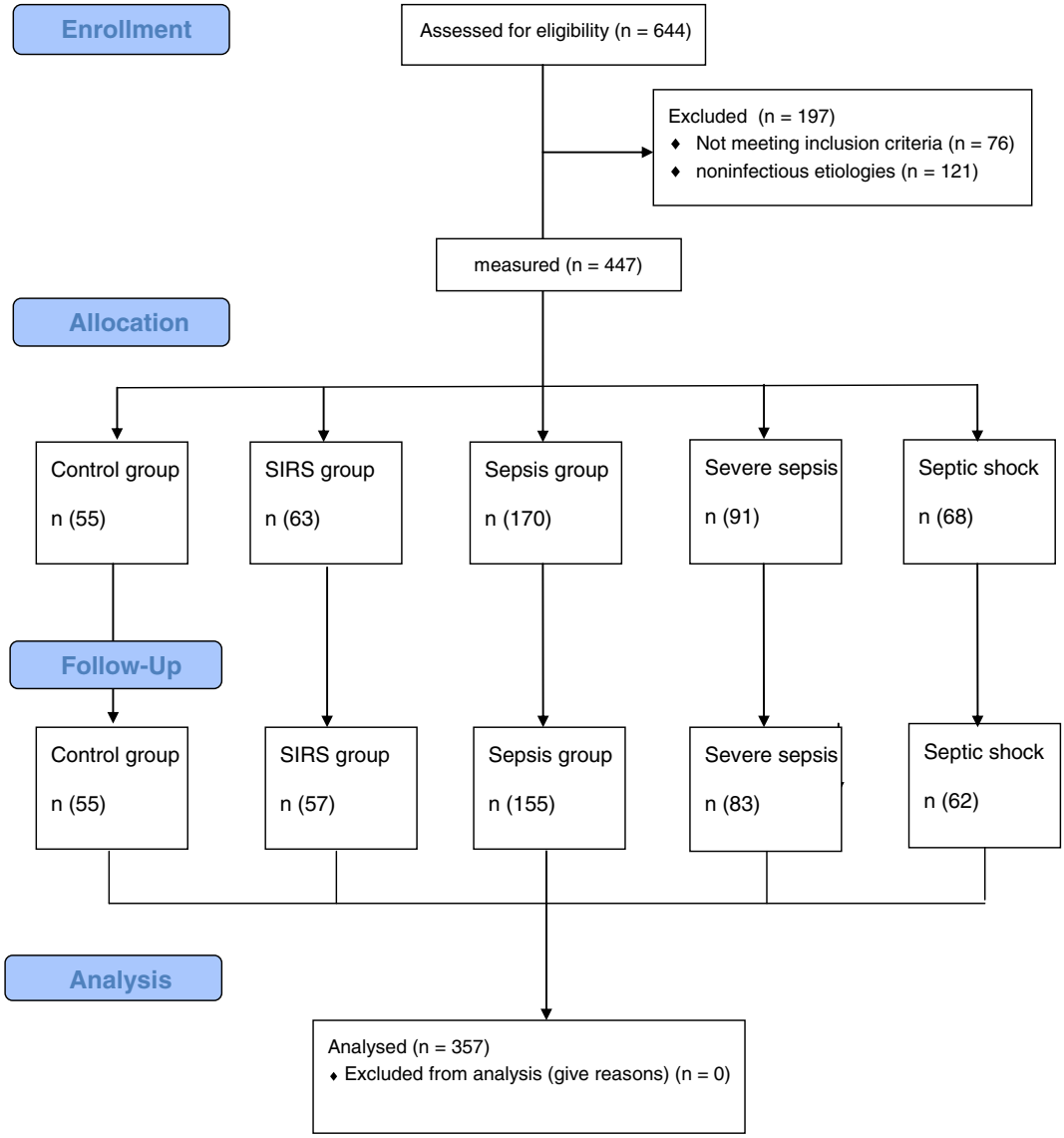

Fig. 1. A Consolidated Standards Of Reporting Trials style flow diagram for study. The C5a2, C5a2intra, and C5a2intra/C5a2 ratio levels of septic patients in an ED.

cancer of any type, acquired immunodeficiency syndrome, or end-stage liver or renal disease), (4) individuals undergoing chemotherapy, therapy with immunosuppressive or immunopotentiating agents, or steroid-based therapy (>10 mg/d for >15 days), and (5) lack of informed consent by the patients or relatives.

Based on the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference and surviving sepsis campaign guidelines [11,12], a total of 375 patients were categorized into the following subgroups: SIRS (n = 57), sepsis (n = 155), severe sepsis (n = 83), and septic shock (n = 62). The control group included 55 healthy blood donors with no history or clinical evidence of acute or chronic disease. They were similar in age and sex to the experimental groups.

2.2. Blood samples

Most of the blood samples were obtained from blood drawn for routine diagnostic procedures. Informed consent was provided by the patients or healthy controls before blood collection. Experiments were approved by the Human Ethics Committee of Beijing Chao-Yang Hospital affiliated with the Capital Medical University (Beijing, China).

2.3. Reagents and antibodies

Labeled antibodies for flow cytometry were used against the following antigens: anti–CD45-APC was from BD Biosciences (San Diego, CA). Anti–C5L2/C5a2-PE and anti–C5L2/C5a2-FITC were from BioLegend (San Diego, CA). The corresponding isotype and fluorophore controls were obtained from the same companies. Lysing Buffer and the BD Intrasure Kit were purchased from BioLegend (San Diego, CA). An enzyme-linked immunosorbent assay (Human C5a ELISA Kit II) was purchased from BD Biosciences (San Diego, CA).

2.4. Flow cytometry for C5a2 expression and ELISA for C5a concentration

As for flow cytometry, all whole blood samples (100 μL) were immediately processed for multiple staining with 5 μL anti–CD45-APC, anti–C5L2/C5a2-PE, and anti–C5L2/C5a2-FITC [13]. After 20 minutes incubation in the dark at 4°C, 2 mL of lysis buffer (BD Pharm Lyse; BD Biosciences, San Diego, CA) was added to each sample liquid at least 10 minutes in the dark at 4°C. Intracellular permeabilization was performed for intracellular staining with anti-C5a2. All flow cytometry data were acquired within 2 hours after collection because of the better stability of the markers and lower intraassay variance (Leukogate;

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