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Original Article

Role of CD64$^+$ neutrophils in the diagnosis of infection for the patients with burns

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Abstract: Infection, particularly in the patients with major burns, is a serious threat to people's lives. New methods and biomarkers are needed to predict and prevent the infection occurs. The aim of the current study was to explore the trend of CD64$^+$ neutrophils in the peripheral blood of patients with burns injection. In this study, 86 patients with burns were divided into two groups, 44 cases in Infection Group and 42 in Control Group, depend on whether complicated infection or not. Here, we described a new biomarker, CD64$^+$ neutrophils, will be increased in the patients who has an infection and investigated the expression levels of CD64 in the neutrophils from different patients' peripheral blood samples with an immunostaining method and western blot. The quantity of WBCs and neutrophils were much more in the infection groups compared to the control group ($P < 0.001$), as well as the percentage of CD64$^+$ cells have the same change according to the flow cytometry analysis. The results of western blot and immunostaining showed that CD64 expression level was highly increased in the infection group. It is considered that CD64$^+$ cells in peripheral blood of patients with burns complicated by infection increased significantly, and so this study provided a new diagnostic biomarker for burns concurrent infection.

Keywords: Burns, CD64, infection, WBC

Introduction

Burns, especially severe burns always makes a contribution to a terrible outcome. 55 per 100,00 people of an annual all-injury mortality owned to the burns in the developed countries, or even higher in the developing ones [1-3].

A physiologic hypermetabolic response results from burns, which not only increased energy expenditure and protein consumption, but also lean muscle mass wasting and wound healing delayed [4, 5]. And in this period, it is characterized by organ dysfunction or body loss, which dues to the immunity of the body decreasing significantly, and thus results in infection.

Infection in the patients with burns has been the most serious factor contributing to the poor prognosis, or even threatening to the lives of the patients. It has been a dominated role for the past 30 years of leading to the death of patients with burns [6, 7]. It is widely concerned by the clinicians because of its characteristics, such as the high incidence, rapidly deterioration and occult symptoms. So whether could we diagnose early or carry on timely treatment for the burn patients complicated by infection becomes the key to improving the prognosis and reversing the situation.

Bacterial plays a critical role in the patients with burns complicated by infection [8, 9]. Usually, infection is diagnosed through common laboratory tests such as the white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), or C-reactive protein (CRP), but the clinical effect is unsatisfactory.

Table 1. General Information for the participants

		Infection Group	Control Group	Statistical Indicators	
				t	*P*
Total	n=86	n=44	n=42	--	--
Sex	Male	32	34	0.815	0.367
	Female	12	8		
Age	Years	70.66±15.57	65.84±12.28	1.589	0.116
Burn Area (%)		54.28±18.23	21.83±13.38	9.374	< 0.001
$CD64^+$ ($\times 10^9$)		1.60±0.063	0.97±0.08	6.307	< 0.001
WBCs ($\times 10^9$)		9.87±3.47	5.53±1.24	7.651	< 0.001

Note: The general information of participants is shown in the table. Data are shown as mean ± SD.

In recent years there were many researches put forward that CD64 receptor can be regarded as a good indicator for the early recognitions of infection, because of its early increasing 2 or 4 hours after infection happened. CD64 (FcgRI), one of the Fc receptors for immunoglobulins G, can be found in the macrophages, monocytes, and eosinophils, but only low expression on naïve neutrophils. When the neutrophils were activated by pathogens, the expression of CD64 increased rapidly. And CD64 has been proved to be a more valuable diagnostic indicator with better sensitivity and specificity [10-13], compared to the CRP or other common laboratory tests, due to its early and rapidly ascent [14-16].

To explore and analysis the diagnostic value of CD64 for the burns complicated by infection, this study was to detect the percentage of $CD64^+$ neutrophils in peripheral blood of healthy people.

Materials and methods

Participants

A total of 86 patients with burns admitted to the Department of Burns and Plastic, the First Hospital of Guangxi Medical University, arranged from May 2014 to May 2015, were divided into 2 groups (Infection Group and Control Group) depended on whether they got infected, and the standards of Infection Group as follows: (1) The blood bacteriological culture of the patients was positive; (2) The blood bacteriological culture of the patients was negative but the wounds' were positive; (3) Low fever, body temperature is between (36.5~38.0)°C or there existed clinical symptoms and signs. Patients whose temperature were normal, or blood and wound bacterial cultures were both negative were excluded but enrolled in the control group. The participants were informed and signed written consent forms before the procedure. Moreover, the Medical Ethics Committee of Guangxi Medical University approved the study and the experiments have already been in accord with the ethical standards established by the institution.

Flow cytometry and cell staining

Samples were collected from the whole, but RBCs-depleted of the participants and stained with CD15-PE (San Diego, CA, USA) and CD64-FITC (San Diego, CA, USA), the cells were analyzed on a FACSCalibur cytometer (Beckman Coulter, Brea, CA, USA) and data were analyzed using Expo32 ADC (Beckman Coulter, Brea, CA, USA). After the cells were analyzed on a FACSCalibur cytometer then fixed with 4% formaldehyde solution (San Diego, CA, USA). For nuclear stain, we used 100 μL DAPI reagents (Invitrogen, US). All the samples were stained with DAPI for 3 min after centrifuging at speed of 1200 rpm. And then the samples were scanned and imaged with microscope (Nikon Eclipse DS-Ri1-80i) and a Nikon camera (DS-U3). The CD64-FITC labeling was in the green channel and imaged with 20 mms typical exposure time, the CD15-PE labeling was in the red channel and imaged with 200 mms typical exposure time, and the DAPI labeling was in the blue one and imaged with 300 mms typical exposure time.

Western blot

We lysed the PBMCs, isolated in the lymphocyte separation medium by density-gradient centrifugation, with radioimmunoprecipitation assay buffer (San Diego, CA, USA). Separated the protein and transferred to the PVDF membranes. Plumbed the membranes with relevant primary antibody and secondary antibodies respectively, after the membranes were blocked with milk.

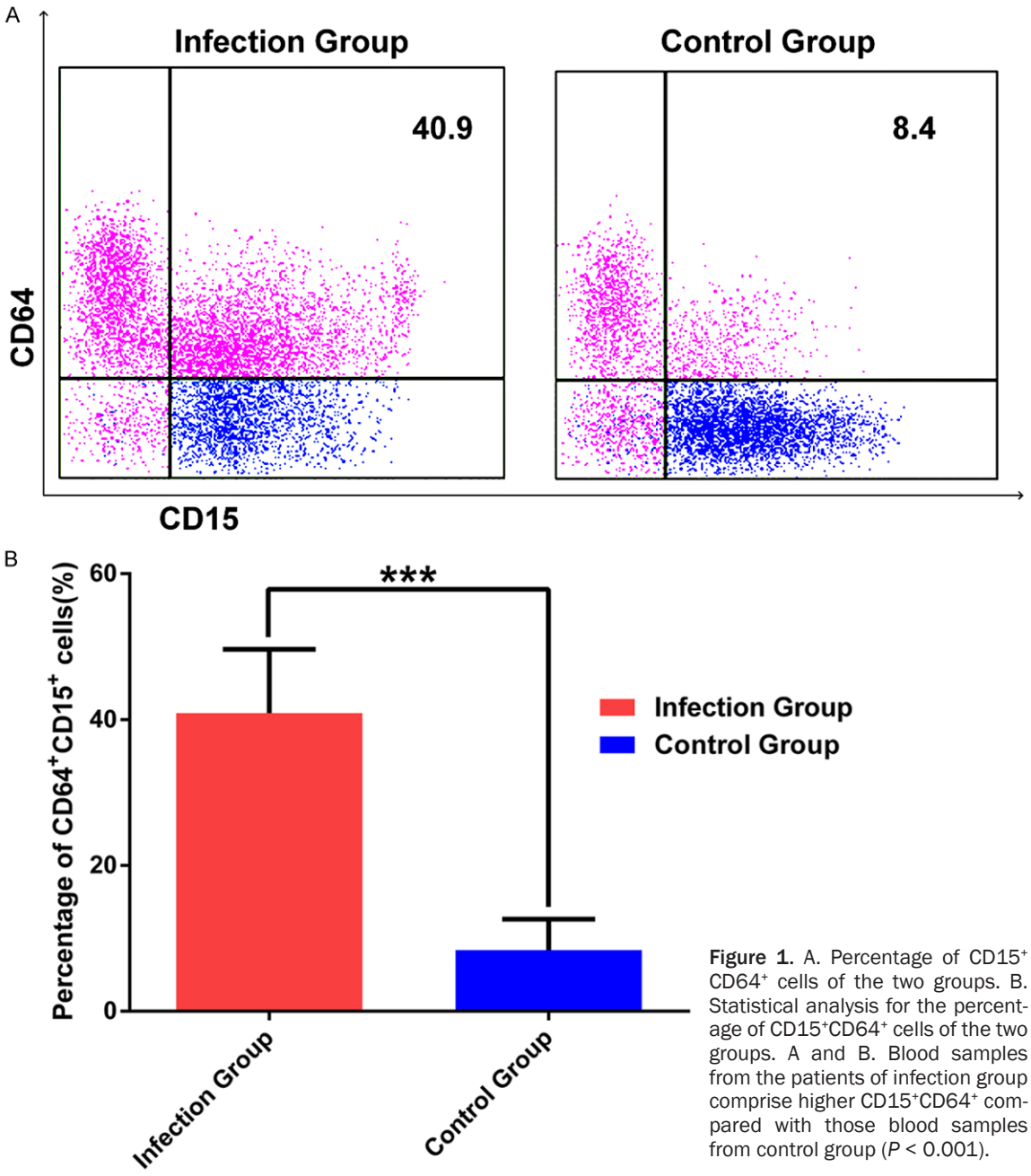

Figure 1. A. Percentage of $CD15^+$ $CD64^+$ cells of the two groups. B. Statistical analysis for the percentage of $CD15^+CD64^+$ cells of the two groups. A and B. Blood samples from the patients of infection group comprise higher $CD15^+CD64^+$ compared with those blood samples from control group ($P < 0.001$).

Statistical analysis

The following data was expressed as Means ± SD. Detection between the two groups on the means were analysis by student's *t*-test. Statistical analyses were performed using SPSS16.0 software and *P*-value < 0.05 (***P* < 0.01, ****P* < 0.001) was statistically significant.

Results

In this study, 86 cases were enrolled, 66 men and 20 women included, who were treated in the Department of Burns and Plastic. As the general information of the patients are presented in **Table 1**, both ages and burns area of the Infection Group [(70.66±15.57) Years, (54.28±18.23)%] were significantly older or higher than the Control Group (*P*-value < 0.05). The levels of WBCs and $CD64^+$ cells of the two groups were detected and the data was shown in **Table 1**. Generally, the values of WBCs and CD64, after being infected, would be elevated. The average values of $CD64^+$ and WBCs counts were found significantly different when compared between the two groups ($P < 0.05$). Both

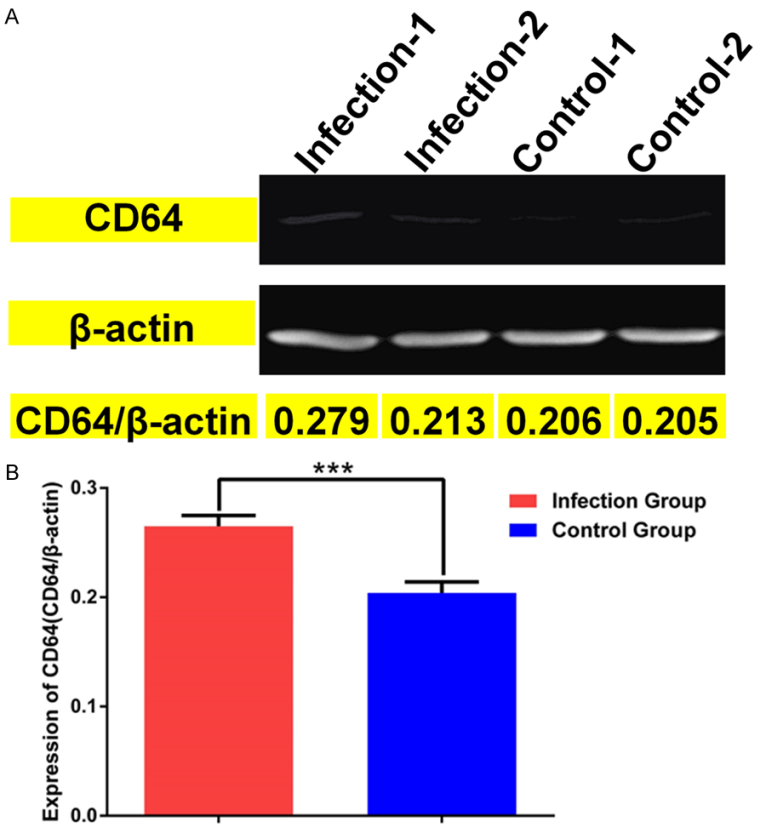

Figure 2. A. WB analysis of CD64 expression. B. Statistical analysis for the gray value of CD64 of the two groups. A and B. The WB analysis of CD64 expression in the blood samples from infection group (Infection-1 and 2) were significantly higher than the samples from control group (Control-1 and 2).

of the two indicators were significantly higher in the Infection Group than those in control group ($P < 0.001$).

As presented in **Figure 1A**, the mean value of $CD15^+CD64^+$ expression in the infection group has approached above 40%, far more huge compared with control group (about 8% average), and the percentage of $CD15^+CD64^+$ cells was greater than the control group as shown in the **Figure 1B** ($P < 0.001$). In the infection group and control group, the expression levels of CD64 were detected by western blot. Compared with control group, the same change was found in the result of WB as shown in the **Figure 2**, and the protein levels of CD64 in the blood samples from Ifection-1 and Infection-2 were increased ($P < 0.001$).

An immunostaining method was adopted to show the expression of CD64 clearly. The anti-CD64 (FTIC, the green channel) staining was used to distinguish the $CD64^{high}$ or $CD64^{low}$, and the staining with DAPI was performed to identify nucleated neutrophils. As shown in **Figure 3**, even though the positive rate of CD64 expression in the control group has reached 10%, the fluorescence intensity significantly is so much lower than the infection group according to the green channel, and only when the green fluorescence has reached certain strength can it be merged with red channel into a color of yellow around the cells.

Discussion

Infection is one of the common clinical diseases, but also one of the important factors led to the rapidly deterioration, or even death of patients in critical conditions. Especially in patients with burns, the incidence of infection and mortality remain a high level, and therefore early diagnosis and treatment of infection is very necessary [6, 17, 18].

Neutrophils play an essential role in defensing infectious agents, and they are one of key cellular components of innate immune system. The white blood cells (WBCs) of peripheral blood significantly increased when the body was stimulated by pathogens, so WBC test is one of the most important clinical indicators to diagnose infection. There are related research have reported that neutrophil CD64 is one of biomarkers for predicting infection [19, 20]. CD64 is one of the Fc receptors for IgG, but the only high-affinity receptors, showing low expression in physiological conditions. But when the body was infected by the pathogen, the expression of CD64 on the neutrophil significantly increased in 4-6 hours. At the same time a number of studies have suggested that the expression of CD64 is associated with the severity of the condition and prognosis [21], and the use of CD64 has been considered to be superior to the CRP [22-24].

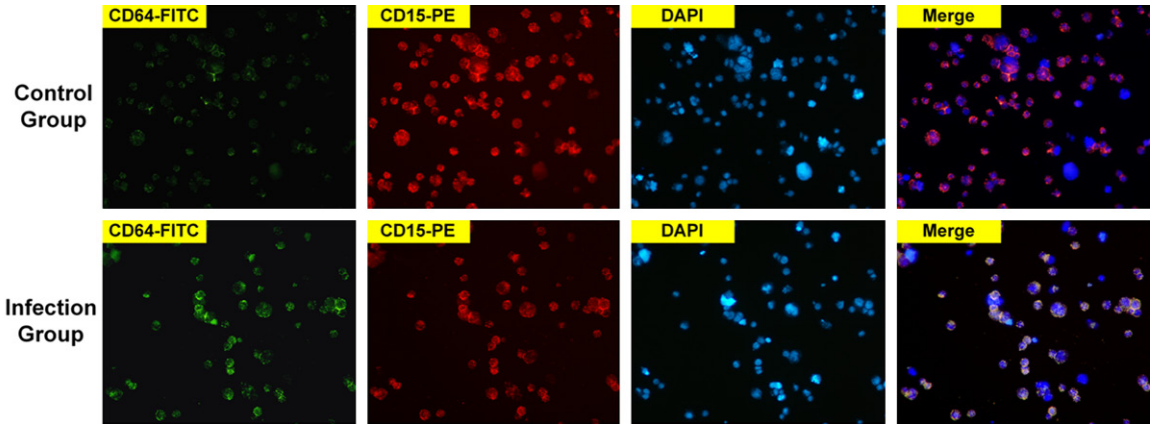

Figure 3. $CD15^{+}CD64^{+}$ cells identification. An immunostaining method was used to identify neutrophils. $CD15^{high}$ (PE, red channel) and $CD64^{high}$ (FITC, green channel) cells can be merged as yellow around the nuclear (DAPI, the blue channel). The expression level of CD64 was significantly higher in infection group compared to the control group.

In our study, 86 patients with burns were enrolled diagnosed with infectious disease or not. In the Infection Group we found that the average of ages was significantly older than the control group ($P < 0.05$), this is associated with the patients' immunity. The older a patient is the worse immunity he has in common. And burn area is another factor related to infection. When the wound exposed to the air, as the burn area increased, the risk of contacting with bacteria went up significantly, so the Infection Group with a larger burn area, had a higher incidence of infection.

Generally speaking, S. *aureus* and P. *aeruginosa* are the most common pathogen in the department of burns [25]. While in recent years, another pathogen named A. *baumannii* has become the prevailing factor of infection in burn units, with an increasing number [26]. It cannot be excluded that the wound on the patients with major burns may cause the infection. Even though the doctors have tried their best to prevent infection, specific guidelines for infection predicting and prevention haven't been defined. Therefore, in our study, when we isolated the neutrophils from the whole blood samples, the increased expression of CD64 was found in the infection group. This discovery may be a new way to forecast when the infection occurs in the near future.

The biomarker's cut-off which indicates inflammation, infection or trauma, should be raised, due to already strong immune response. So it is reasonable for this result that bacterial and viral infections upregulate CD64 expression in immune cells [27, 28], neutrophil CD64 is a superior biomarker for infection diagnosis.

In Conclusion, in our study consisting of 86 cases of burns, the role of CD64 in predicting infectious disease was found provide satisfied capability to the diagnosis of infection. In addition, early diagnosis of infection or potential development of burns complicated by infection must be determined with several biomarkers. Our findings indicate that the CD64 can help and provide superior capability to predict infection but cannot completely replace the other indicators. Overall, CD64 is a useful biomarker for assessing the infection risk of patients enrolled in the Department of Burns.

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Disclosure of conflict of interest

None.

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