

doi: 10.13241/j.cnki.pmb.2019.04.031

## 脓毒症患者血清 TOLL 样受体 4、脂联素与炎症反应 和病情严重程度关系\*

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**摘要 目的:**探讨脓毒症患者血清 TOLL 样受体 4(TLR4)、脂联素(APN)与炎症反应和病情严重程度关系。**方法:**选取 2016 年 12 月到 2018 年 4 月期间在重庆市中医院接受治疗的脓毒症患者 60 例作为研究组,另选取同期本院健康体检者 60 例作为对照组。根据急性生理及慢性健康状况 II (APACHE II)评分将脓毒症患者分为高分组 17 例(APACHE II 评分 $\geq 20$ 分)和低分组 43 例(APACHE II 评分 $<20$ 分)。比较两组血清中的 TLR4、APN、降钙素原(PCT)、肿瘤坏死因子- $\alpha$ (TNF- $\alpha$ )、C 反应蛋白(CRP)水平,比较高分组和低分组患者血清中的 TLR4、APN 及炎症因子水平,分析脓毒症患者 TLR4、APN 的表达与炎症因子、APACHE II 评分的相关性。**结果:**研究组血清中的 TLR4、PCT、TNF- $\alpha$ 、CRP 水平均明显高于对照组,APN 水平明显低于对照组( $P<0.05$ )。高分组患者血清中的 TLR4、PCT、TNF- $\alpha$ 、CRP 水平明显高于低分组,APN 水平明显低于低分组( $P<0.05$ )。脓毒症患者 TLR4 的表达与 PCT、TNF- $\alpha$ 、CRP、APACHE II 评分呈正相关,APN 的表达与 PCT、TNF- $\alpha$ 、CRP、APACHE II 评分呈负相关( $P<0.05$ )。**结论:**脓毒症患者病情越严重,TLR4 水平越高,而 APN 水平越低,TLR4、APN 可能是通过调节炎症反应来影响脓毒症患者的疾病进展。

**关键词:**脓毒症;TOLL 样受体 4;脂联素;炎症反应;病情严重程度

中图分类号:R631.2 文献标识码:A 文章编号:1673-6273(2019)04-747-04

## Relationship between Serum TOLL-like Receptor 4, Adiponectin and Inflammation and Severity of Illness in Sepsis Patients\*

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**ABSTRACT Objective:** To investigate the relationship between the serum toll-like receptor 4 (TLR4), adiponectin (APN) and inflammation and severity of illness in sepsis patients. **Methods:** 60 patients with sepsis who were treated in Chongqing Traditional Chinese Medicine Hospital from December 2016 to April 2018 were selected as the study group, and 60 healthy persons were selected as the control group during the same period. According to the Acute Physiology and Chronic Health Status Scale II (APACHE II), sepsis patients were divided into high score group with 17 cases (APACHE II score  $\geq 20$ ) and low score group with 43 cases (APACHE II score  $<20$ ). The levels of serum TLR4, APN, procalcitonin (PCT), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and C-reactive protein (CRP) in the two groups were compared. The levels of serum TLR4, APN and inflammatory factors in patients with high and low groups were compared. The correlation between TLR4, APN expression and inflammatory factors, APACHE II scores in sepsis patients was analyzed. **Results:** The levels of serum TLR4, PCT, TNF- $\alpha$  and CRP in the study group were significantly higher than those in the control group, and the APN level was significantly lower than that in the control group ( $P<0.05$ ). The serum levels of TLR4, PCT, TNF- $\alpha$ , and CRP were significantly higher in high score group than those in low score group, and APN levels were significantly lower than that in low-score groups ( $P<0.05$ ). The expression of TLR4 in patients with sepsis was positively correlated with PCT, TNF- $\alpha$ , CRP and APACHE II scores, the expression of APN was negatively correlated with PCT, TNF- $\alpha$ , CRP and APACHE II scores ( $P<0.05$ ). **Conclusion:** TLR4 is highly expressed in serum of patients with sepsis, and APN is lowly expressed, TLR4 and APN may affect the disease progression in patients with sepsis by modulating the inflammatory response.

**Key words:** Sepsis; TOLL-like receptor 4; Adiponectin; Inflammatory response; Disease condition

**Chinese Library Classification(CLC):** R631.2 **Document code:** A

**Article ID:** 1673-6273(2019)04-747-04

### 前言

脓毒症是由感染引发的全身炎症反应综合征,是严重感

\* 基金项目:重庆市卫计委医学科科研项目(201413021)

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(收稿日期:2018-06-06 接受日期:2018-06-30)

染、严重创伤、烧伤、休克、外科大手术的常见并发症,可导致患者多个器官出现功能障碍,患者预后差,病死率较高<sup>[1-3]</sup>。脓毒症患者体内的抗炎因子和致炎因子平衡明显失调,引起的炎症反应可能是导致患者病情加重的主要原因<sup>[4-6]</sup>。TOLL样受体4(toll-like receptor 4,TLR4)是一种跨膜受体蛋白,可介导非特异性免疫应答,进而导致多种炎症介质的释放,在脓毒症的疾病进展中起到重要的作用<sup>[7,8]</sup>。脂联素(Adiponectin,APN)一种内源性生物活性多肽,主要由脂肪细胞分泌,具有改善胰岛素抵抗、调节糖脂代谢的作用,其在糖尿病、高血脂症等多种代谢综合征疾病患者血清中异常表达<sup>[9,10]</sup>。近年来有研究显示<sup>[11,12]</sup>,APN具有抗炎作用,并且参与调节了动脉粥样硬化、溃疡性结肠炎等多种炎症性疾病的发展。目前关于TLR4和APN在脓毒症中的作用的相关研究较少,本研究旨在探讨脓毒症患者血清TLR4和APN的表达及其与炎症反应和病情严重程度的关系,现将研究结果整理如下。

## 1 资料与方法

### 1.1 一般资料

选取2016年12月到2018年4月期间在重庆市中医院接受治疗的脓症患者60例作为研究组,纳入标准:(1)所有患者均符合“国际脓毒症会议”中制定的诊断标准<sup>[13]</sup>;(2)临床资料完整者;(3)患者及其家属对本研究均知情同意。排除标准:(1)晚期恶性肿瘤者;(2)存在免疫系统疾病者;(3)存在器官功能衰竭者;(4)近一个月内使用过糖皮质激素者;(5)接受过器官移植术,并且在术后进行了免疫抑制剂治疗者。另选取同期本院健康体检者60例作为对照组。研究组男性36例,女性24例,年龄39-73岁,平均(58.65±8.69)岁,肺部感染18例,腹腔感染15例,泌尿感染12例,其他部位感染15例。对照组男性39例,女性21例,年龄36-71岁,平均(57.21±8.42)岁。两组研

究对象的性别、年龄比较无明显差异( $P>0.05$ ),均衡可比。本研究已通过我院伦理委员会批准。

### 1.2 研究方法

**1.2.1 血清指标的检测** 抽取所有研究对象的空腹静脉血5 mL,于室温下静置30 min,采用3000 r/min的离心速度进行10 min的离心运动,收集血清,将其置于-20℃的环境下保存待测。采用酶联免疫吸附法检测血清中的TLR4、APN、降钙素原(procalcitonin,PCT)、肿瘤坏死因子-α(tumor necrosis factor-α,TNF-α)、C反应蛋白(C reactive protein,CRP)水平,相关试剂盒均购于上海西唐生物技术公司,检测步骤严格遵循相关试剂盒的操作指南进行。

**1.2.2 脓症患者病情严重程度评估** 采用急性生理及慢性健康状况II(acute physiology and chronic health evaluation II,APACHE II)评分评估脓毒症患者的病情严重程度<sup>[14]</sup>,APACHE II主要由急性生理评分、慢性健康评分、年龄评分三部分组成,理论最高分值为71分,分数越高代表病情程度越重。根据APACHE II分值将脓症患者分为低分组(APACHE II评分<20分)和高分组(APACHE II评分≥20分),其中低分组43例,高分组17例。

### 1.3 统计学方法

采用SPSS20.0处理数据。计数资料以率(%)的形式表示,采用卡方检验,计量资料以( $\bar{x} \pm s$ )的形式表示,采用t检验,两指标间的相关性采用Pearson相关性分析,将 $\alpha=0.05$ 作为检验标准。

## 2 结果

### 2.1 对照组和研究组血清中TLR4、APN及炎症因子水平比较

研究组血清中的TLR4、PCT、TNF-α、CRP水平明显高于对照组,APN水平明显低于对照组( $P<0.05$ ),见表1。

表1 对照组和研究组血清中TLR4、APN及炎症因子水平比较( $\bar{x} \pm s$ )

Table 1 Comparison of serum levels of TLR4, APN and inflammatory factors in the control group and the study group( $\bar{x} \pm s$ )

| Groups        | n  | TLR4(ng/mL) | APN(mg/L) | PCT(ug/L) | TNF-α(ng/L) | CRP(ng/L)   |
|---------------|----|-------------|-----------|-----------|-------------|-------------|
| Control group | 60 | 0.84±0.06   | 7.66±2.63 | 0.28±0.11 | 20.61±5.26  | 8.26±1.53   |
| Study group   | 60 | 2.91±0.67   | 4.87±1.46 | 6.54±1.27 | 67.34±12.31 | 54.37±11.69 |
| t             |    | 23.836      | 7.184     | 38.038    | 27.039      | 37.194      |
| P             |    | 0.000       | 0.000     | 0.000     | 0.000       | 0.000       |

### 2.2 高分组与低分组血清中TLR4、APN及炎症因子水平比较

高于低分组,APN水平明显低于低分组( $P<0.05$ ),见表2。

高分组患者血清中的TLR4、PCT、TNF-α、CRP水平明显

表2 高分组与低分组血清中TLR4、APN及炎症因子水平比较( $\bar{x} \pm s$ )

Table 2 Comparison of serum levels of TLR4, APN and inflammatory factors in high and low score groups( $\bar{x} \pm s$ )

| Groups           | n  | TLR4(ng/mL) | APN(mg/L) | PCT(ug/L)  | TNF-α(ng/L) | CRP(ng/L)   |
|------------------|----|-------------|-----------|------------|-------------|-------------|
| High score group | 17 | 4.68±0.82   | 2.16±0.94 | 11.26±2.34 | 79.82±15.08 | 69.54±15.64 |
| Low score group  | 43 | 2.21±0.33   | 5.94±1.58 | 4.67±1.02  | 62.41±10.24 | 48.37±9.48  |
| t                |    | 21.645      | 15.926    | 19.997     | 7.398       | 8.966       |
| P                |    | 0.000       | 0.000     | 0.000      | 0.000       | 0.000       |

### 2.3 脓毒症患者 TLR4、APN 的表达与炎症因子水平、APACHE II 评分的相关性

经 Pearson 相关性分析显示, 脓毒症患者 TLR4 的表达与

PCT、TNF- $\alpha$ 、CRP、APACHE II 评分呈正相关( $P<0.05$ ), APN 的表达与 PCT、TNF- $\alpha$ 、CRP、APACHE II 评分呈负相关( $P<0.05$ ), 见表 3。

表 3 脓毒症患者 TLR4、APN 的表达与炎症因子水平、APACHE II 评分的相关性

Table 3 Correlation of TLR4 and APN expression with inflammatory factor level and APACHE II score in sepsis patients

| Indexes         | TLR4         | APN           |
|-----------------|--------------|---------------|
| PCT             | 0.368, 0.000 | -0.317, 0.000 |
| TNF- $\alpha$   | 0.326, 0.027 | -0.354, 0.013 |
| CRP             | 0.432, 0.000 | -0.439, 0.000 |
| APACHE II score | 0.521, 0.000 | -0.498, 0.000 |

### 3 讨论

脓毒症是急诊内科常见的危急重症, 是引发多器官功能障碍综合征的重要原因。脓毒症患者病情发展迅速, 一旦进展为严重脓毒症或脓毒症休克, 患者的预后极差, 病死率高, 因此及时诊断患者病情, 针对性的制定治疗方案具有重要的临床意义<sup>[15,16]</sup>。炎症反应是机体面对损伤性伤害时做出的一种防御性措施, 适度的炎症反应对人体是有益的, 然而机体在受到严重创伤、烧伤、外科大手术等强烈刺激时会释放线粒体损伤相关模式分子, 进而通过激活中性粒细胞、促分裂素原活化蛋白激酶信号通路等方式启动炎症反应<sup>[17-19]</sup>。除此之外, 细菌内毒素、脂多糖(lipopolysaccharide, LPS)与脂多糖结合蛋白(lipopolysaccharide binding protein, LBP)进行结合, 形成免疫复合物, 该免疫复合物通过与单核、巨噬细胞表面的 TOLL 样受体(toll-like receptor, TLR)结合从而促进大量炎症介质的转录和释放, 加重机体的炎症反应, 促进脓毒症快速发展<sup>[20,21]</sup>。由此可见炎症反应在脓毒症的发生、发展中起到重要的作用, 因此抑制炎症反应是治疗脓毒症的重中之重。

TLR 是一类模式识别受体, 可识别病原体的脂类特殊结构, 如 TLR2 可识别革兰阳性菌细胞壁成分, TLR4 可识别革兰阴性菌的 LPS, 目前 TLR4 是研究较多的 TLR<sup>[22,23]</sup>。APN 是脂肪细胞分泌的一种活性蛋白, 大量存在于血液循环中, 是机体的脂质代谢和血糖稳态的调控网络中的重要调节因子<sup>[24,25]</sup>。本研究中脓毒症患者 TLR4 水平较高, APN 水平较低, 且均与病情严重程度有关。PCT、TNF- $\alpha$ 、CRP 均是常见的炎症因子, 其中 PCT 是细菌、真菌感染的敏感性指标, TNF- $\alpha$  是炎症反应的重要调节因子, CRP 是炎症反应的急性时相蛋白<sup>[26]</sup>。本研究结果显示, 研究组血清中的 PCT、TNF- $\alpha$ 、CRP 水平均明显高于对照组, 高分组患者血清中的 PCT、TNF- $\alpha$ 、CRP 水平均明显高于低分组( $P<0.05$ ), 这说明脓毒症患者体内存在明显的炎症反应, 且病情越严重炎症反应越剧烈。进一步研究发现, 脓毒症患者 TLR4 的表达与炎症因子水平、APACHE II 评分呈正相关, APN 的表达与炎症因子水平、APACHE II 评分呈负相关( $P<0.05$ )。细菌感染引发的脓毒症中以革兰阴性菌感染最为常见<sup>[27]</sup>, 革兰阴性菌细胞壁的毒性成份 LPS 可先与 LBP 结合, 之后再与细胞表面的可溶性分化抗原簇 14 (Cluster of differentiation 14, CD14) 结合, 形成的 LPS-LBP-CD14 复合物可活化 TLR4

信号传导通路, 并通过激活核因子 KB 来促进大量炎症因子分泌<sup>[28]</sup>, 因此 TLR4 高表达可促进炎症因子的分泌, 加重机体的炎症反应。APN 可通过抗氧化、保护内皮细胞功能等途径来抑制机体的炎症反应, 此外还有研究显示<sup>[29]</sup>, APN 可抑制 TNF- $\alpha$  的分泌。徐亚杰等人的研究显示<sup>[30]</sup>, 脂联素可降低 LPS 诱导的 RAW264.7 单核巨噬细胞株的炎症反应, 说明 APN 对脓毒症患者体内炎症反应的抑制作用。由此可见, TLR4、APN 可能是通过调节脓毒症患者体内的炎症反应来影响疾病进展, 这为临床诊断、评估、治疗脓毒症提供了新的思路。

综上所述, TLR4 在脓毒症患者血清中呈高表达, APN 呈低表达, 且 TLR4 与炎症因子的表达水平呈正相关, APN 与炎症因子的表达水平呈负相关, TLR4、APN 可预测和判断脓毒症患者的病情严重程度。

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