doi: 10.13241/j.cnki.pmb.2020.21.028

脓毒症患者血清 TNF-α 与 T 淋巴细胞亚群、凝血功能及预后的关系研究 *

陈俊杰[1] 仝瀚文[2] 陆俊杰[1] 单 祎[1] 葛志军[1△]

（1 苏州大学附属宜兴医院重症医学科 江苏 宜兴 214299；2 南京大学医学院附属鼓楼医院急诊医学科 江苏 南京 210008）

摘要 目的：探讨脓毒症患者血清肿瘤坏死因子 -α（TNF-α）与 T 淋巴细胞亚群、凝血功能及预后的关系。**方法：**选取 2017 年 4 月~2019 年 4 月期间我院收治的脓毒症患者 93 例作为脓毒症组，另选取同期来我院行健康体检的志愿者 90 例作为对照组，比较脓毒症组、对照组的 TNF-α、T 淋巴细胞亚群、凝血功能，根据 28d 后转归情况分为死亡组和存活组，比较死亡组和存活组 TNF-α、T 淋巴细胞亚群水平、凝血功能，采用 Pearson 相关分析分析 TNF-α 与 T 淋巴细胞亚群、凝血功能的相关性。**结果：**脓毒症组 TNF-α 高于对照组，凝血酶原时间（PT）、活化部分凝血活酶时间（APTT）长于对照组，而 $CD3^+$、$CD4^+$、$CD4^+/CD8^+$ 均低于对照组（$P<0.05$）；脓毒症组、对照组 $CD8^+$ 比较差异无统计学意义（$P>0.05$）。死亡组 TNF-α 高于存活组，PT、APTT 长于存活组，而 $CD3^+$、$CD4^+$、$CD4^+/CD8^+$ 均低于存活组（$P<0.05$）；死亡组、存活组 $CD8^+$ 比较差异无统计学意义（$P>0.05$）。Pearson 相关分析结果显示，脓毒症患者血清 TNF-α 与 PT、APTT 呈正相关，与 $CD3^+$、$CD4^+$、$CD4^+/CD8^+$ 呈负相关（$P<0.05$）；TNF-α 与 $CD8^+$ 无明显相关性（$P>0.05$）。**结论：**脓毒症患者血清 TNF-α、T 淋巴细胞亚群、凝血功能均存在异常变化，TNF-α 与患者的 T 淋巴细胞亚群、凝血功能和预后有关，检测 TNF-α 有助于评估脓毒症患者的病情和预后。

关键词：脓毒症；肿瘤坏死因子 -α；T 淋巴细胞亚群；凝血功能；预后；关系

中图分类号：R631.2 **文献标识码：**A **文章编号：**1673-6273（2020）21-4126-04

The Relationship between Serum TNF-α and T Lymphocyte Subsets, Coagulation Function and Prognosis in Sepsis Patients*

CHEN Jun-jie[1], TONG Han-wen[2], LU Jun-jie[1], SHAN Yi[1], GE Zhi-jun[1△]

(1 Department of Critical Medicine, Yixing Hospital Affiliated to Suzhou University, Yixing, Jiangsu, 214299, China;

2 Department of Emergency Medicine, Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008, China)

ABSTRACT Objective: To investigate the relationship between serum tumor necrosis factor-α (TNF-α) and T lymphocyte subsets, coagulation function and prognosis in sepsis patients. **Methods:** 93 patients with sepsis who were admitted to our hospital from April 2017 to April 2019 were selected as sepsis group, and 90 volunteers who received health examination in our hospital during the same period were selected as control group. TNF-α, T lymphocyte subsets and coagulation function of sepsis group and control group were compared, and T lymphocyte subsets and coagulation function of death group and survival group in sepsis patients were compared. According to the outcome after 28 days, the patients were divided into death group and survival group. The levels of TNF-α and T lymphocyte subsets and coagulation function were compared between death group and survival group. The correlation of TNF-α with T lymphocyte subsets and coagulation function were analyzed by Pearson correlation analysis. **Results:** TNF-α in sepsis group was higher than that in the control group. The prothrombin time (PT) and activated partial thromboplastin time (APTT) were longer than those in the control group, while $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ in the sepsis group were lower than those in the control group ($P<0.05$). There was no significant difference in $CD8^+$ between the sepsis group and the control group ($P>0.05$). TNF-α in the death group was higher than that in the survival group, PT and APTT in the death group were longer than those in the survival group, while $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ were lower than those in the survival group ($P<0.05$). There was no significant difference in $CD8^+$ between the death group and the survival group ($P>0.05$). Pearson correlation analysis showed that TNF-α were positively correlated with PT and APTT, negatively correlated with $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ ($P<0.05$), there was no significant correlation between TNF-α and $CD8^+$ ($P>0.05$). **Conclusion:** There are abnormal changes in TNF-α, T lymphocyte subsets and coagulation function in sepsis patients, TNF-α is associated with T lymphocyte subsets, coagulation function, and prognosis of patients, the detection of TNF-α is helpful to evaluate the condition and prognosis of sepsis patients.

Key words: Sepsis; Tumor necrosis factor-α; T lymphocyte subsets; Coagulation function; Prognosis; Relationship

* 基金项目：江苏省科技厅科技支撑计划项目（BE20140861）；宜兴市科技局社会发展项目（2018SF25）

作者简介：陈俊杰（1987-），男，硕士，主治医师，研究方向：重症医学，E-mail: yxph1325@163.com

△ 通讯作者：葛志军（1970-），男，博士，主任医师，研究方向：重症感染与免疫，E-mail: staff789@yxph.com

（收稿日期：2020-03-15 接受日期：2020-04-11）

Chinese Library Classification(CLC): R631.2 Document code: A
Article ID: 1673-6273(2020)21-4126-04

前言

脓毒症是重症监护病房中常见的危急重症，主要是由感染所引起的机体持续性、失控性炎性反应，可导致器官功能障碍或组织灌注不足[1,2]。现临床有关脓毒症的治疗尚无特异性方案，一直是重症医学面临的研究难点，若未能及时予以治疗，约有40%的脓毒症患者随着病情进展可发展至脓毒症性急性肾损伤，进而导致患者死亡，预后极差[3,4]，因此，对患者病情进行准确的预测及评估对其预后有重要意义。既往相关研究指出[5,6]，炎症因子被激活引发的炎症级联反应在脓毒症的病情进展中发挥重要作用。肿瘤坏死因子-α（Tumor necrosis factor-α，TNF-α）是由巨噬细胞分泌的炎性因子，正常情况下，TNF-α可抗感染、调节机体免疫应答、促进组织修复等，而当机体大量分泌时，可破坏机体免疫平衡，与其他炎性因子一起产生多种病理损伤[7,8]。有研究指出[9,10]，脓毒症可导致机体凝血功能障碍，而炎症因子的大量分泌和释放，又可进一步激活凝血系统，引起恶性循环。本文通过探讨脓毒症患者血清TNF-α与T淋巴细胞亚群、凝血功能及预后的关系，以期为临床脓毒症的防治提供参考。

1 资料和方法

1.1 一般资料

选取2017年4月~2019年4月期间我院收治的脓毒症患者93例作为脓毒症组，本次研究已获取我院伦理学委员会批准同意。纳入标准：（1）符合脓毒症3.0(Sepsis 3.0)诊断标准[11]；（2）临床资料完整者；（3）患者或其家属知情本研究且签署了同意书；（4）在抢救室存活时间>24h。排除标准：（1）存在免疫缺陷者或既往有免疫抑制剂治疗史者；（2）合并恶性肿瘤、精神疾病和传染性疾病者；（3）合并糖尿病、高血压、高血脂等基础性疾病者；（4）急性胰腺炎，但明确无感染者；（5）慢性疾病终末期者；（6）妊娠或哺乳期妇女；（7）合并心肝肾等脏器功能不全者。其中脓毒症组患者男53例，女40例，年龄29~63岁，平均（47.32±5.49）岁；肺部感染29例，腹腔感染23例，泌尿感染15例，其他部分感染26例。另选取同期来我院行健康体检的志愿者90例作为对照组，其中对照组男52例，女38例，年龄28~62岁，平均（47.51±6.03）岁。两组研究对象年龄、性别比例比较无差异（P>0.05），组间具有可比性。

1.2 方法

对照组于体检当天、脓毒症组于入院次日抽取其清晨空腹肘静脉血6 mL，室温下静置30 min，经离心半径12 cm，以3800 r/min的速率离心10 min，分离上清液，存于-30℃冰箱待测。采用酶联免疫吸附试验检测TNF-α水平，采用上海旭东海普药业有限公司生产的流式细胞仪检测T淋巴细胞亚群水平：$CD3^+$、$CD4^+$、$CD8^+$，并计算$CD4^+/CD8^+$。应用北京普朗公司生产的PUZS-300X全自动生化分析仪检测凝血功能指标：凝血酶原时间（Prothrombin time，PT）、活化部分凝血活酶时间（Activated partial thromboplastin time，APTT）。记录脓毒症组住院患者28d内转归情况，未满28d离院者采用电话随访进行记录，根据28d后转归情况分为死亡组（n=24）和存活组（n=69）。

1.3 观察指标

比较脓毒症组、对照组的血清TNF-α、T淋巴细胞亚群、凝血功能，比较脓毒症患者中死亡组和存活组TNF-α、T淋巴细胞亚群、凝血功能。

1.4 统计学方法

采用SPSS25.0统计学软件进行统计分析，计量资料采用均数±标准差（$\bar{x}\pm s$）描述，组间比较采用配对 检验；计数资料采用%表示，实施卡方检验，采用Pearson相关分析分析血清TNF-α与T淋巴细胞亚群、凝血功能的相关性，检验标准设置为α=0.05。

2 结果

2.1 脓毒症组、对照组的TNF-α、T淋巴细胞亚群、凝血功能比较

脓毒症组血清TNF-α高于对照组，PT、APTT长于对照组，而$CD3^+$、$CD4^+$、$CD4^+/CD8^+$均低于对照组（P<0.05）；两组研究对象$CD8^+$比较差异无统计学意义（P>0.05）；详见表1。

表1 脓毒症组、对照组的TNF-α、T淋巴细胞亚群、凝血功能比较（$\bar{x}\pm s$）

Table 1 Comparison of TNF-α, T lymphocyte subsets and coagulation function between sepsis group and control group（$\bar{x}\pm s$）

Groups	TNF-α(pg/mL)	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$	PT(s)	APTT(s)
Control group (n=90)	18.41±6.75	64.88±9.02	65.71±10.19	36.20±8.25	1.82±0.24	9.23±3.94	21.57±4.21
Sepsis group (n=93)	53.28±7.82	51.93±10.85	46.58±9.75	37.16±10.18	1.25±0.21	14.82±2.17	32.29±5.44
t	31.133	8.454	12.601	0.639	16.368	11.767	14.317
P	0.000	0.000	0.000	0.524	0.000	0.000	0.000

2.2 存活组、死亡组的TNF-α、T淋巴细胞亚群、凝血功能比较

死亡组TNF-α高于存活组，PT、APTT长于存活组，而$CD3^+$、$CD4^+$、$CD4^+/CD8^+$均低于存活组（P<0.05）；脓毒症不同预后患者的$CD8^+$比较差异无统计学意义（P>0.05）；详见表2。

2.3 脓毒症患者中TNF-α与T淋巴细胞亚群、凝血功能的相关性分析

经Pearson相关分析可知，脓毒症患者中TNF-α与PT、APTT呈正相关，与$CD3^+$、$CD4^+$、$CD4^+/CD8^+$呈负相关（P<0.

05)；与 $CD8^+$ 无明显相关性(P>0.05)；详见表 3。

表 2 存活组、死亡组的 TNF-α、T 淋巴细胞亚群、凝血功能比较($\bar{x}\pm s$)

Table 2 Comparison of TNF-α, T lymphocyte subsets and coagulation function between survival group and death group($\bar{x}\pm s$)

Groups	TNF-α(pg/mL)	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$	PT(s)	APTT(s)
Survival group (n=69)	45.61±7.91	54.62±8.81	49.93±8.21	37.26±7.21	1.34±0.26	12.38±2.28	27.56±4.83
Death group (n=24)	75.33±8.26	44.20±7.93	36.95±7.36	36.87±8.63	1.00±0.31	21.84±2.31	45.89±5.53
t	24.061	8.185	10.963	0.319	7.287	27.018	23.035
P	0.000	0.000	0.000	0.750	0.000	0.000	0.000

表 3 脓毒症患者中 TNF-α 与 T 淋巴细胞亚群、凝血功能的相关性分析

Table 3 The correlation of TNF-α with T lymphocyte subsets and coagulation function in sepsis patients

Indexes	TNF-α	
	r	P
$CD3^+$	-0.553	0.000
$CD4^+$	-0.447	0.016
$CD8^+$	0.238	0.086
$CD4^+/CD8^+$	-0.503	0.003
PT	0.526	0.001
APTT	0.593	0.000

3 讨论

脓毒症是由多因素导致的严重并发症，在危急重症中发病率极高[12]。据相关报道统计[13]，我国每年可新增 300 万脓毒症患者，而在这之中约有三分之一的患者可导致死亡；而在美国每年有超过 75 万人发生脓毒症，病死率高达 20%[14]。可见，脓毒症的诊断、治疗及其预防已成为全球的难题与热点。既往研究认为[15,16]，脓毒症是一种全身性炎症反应，即当脓毒症患者在发病早期的时候，可表现为全身炎性反应，如 TNF-α 曾被普遍认为是导致患者多器官功能障碍和死亡的 " 核心因子 "。基于此理论，既往的脓毒症治疗主要采用单纯抗炎治疗，然后疗效并不十分突出，由于 TNF-α 通常在病因作用后的几分钟至 2h 内释放，半衰期较短，难以做到有效监测，因此，寻求有效的因子以期为脓毒症患者带来更为全面的诊疗信息具有积极的临床意义。伴随着研究的深入，不少学者认为脓毒症的发生、发展是机体促炎与抗炎机制失衡所致，且该失衡过程的发生与机体免疫功能紊乱关系密切[17-19]。此外，脓毒症患者长期处于全身性炎症状态下，可导致凝血系统被激活，引起凝血功能障碍，而凝血功能障碍又可进一步促进全身炎症反应的进展，造成恶性循环[20,21]。

本次研究结果显示，脓毒症组 TNF-α、PT、APTT 高于对照组，而 $CD3^+$、$CD4^+$、$CD4^+/CD8^+$ 均低于对照组，可见脓毒症患者长期处于炎性状态，且体内存在免疫功能紊乱、凝血功能障碍。TNF-α 是关键的促炎因子，在脓毒症感染后最早释放，进而激活细胞因子级联反应，引发炎症连锁反应，产生炎症 " 瀑布效应 "[22]。以往不少研究证实[23,24]，宿主的免疫功能状态在很大程度上决定着炎症反应的结局。T 淋巴细胞亚群水平可在一定程度上反映机体免疫状况，可在感染过程中发挥固有免疫作用。T 淋巴细胞亚群分为 $CD3^+$、$CD4^+$、$CD8^+$，其中 $CD3^+$ 表示总 T 淋巴细胞，$CD8^+$ 能够杀伤靶细胞，$CD4^+$ 能够促进 T 细胞亚群的成熟，当机体的免疫功能发生紊乱，$CD4^+/CD8^+$ 值下降[25,26]。脓毒症过度而持久的炎性反应可阻滞 T 淋巴细胞活化，引起 T 细胞低反应性、细胞因子产量降低甚至发生凋亡。由于脓毒症发展至最后阶段，可引起机体多系统功能受累，而这一生理病理过程中，凝血系统的活化和纤溶系统的受抑是多器官功能不全的中心生理病理环节[27,28]。PT、APTT 是反映机体凝血功能的有效指标，而凝血系统紊乱的主要机制可能在于：全身生理性抗凝血机制遭受破坏、组织因子介导的凝血酶使纤维蛋白原转化为纤维蛋白和血小板的活化；纤溶酶原活化抑制因子介导的纤溶系统被关闭[29,30]。此外，脓毒症患者中死亡组与存活组的上述免疫功能、凝血功能指标及 TNF-α 均存在组间差异，可见免疫功能、凝血功能异常程度可能与患者预后存在一定关系。进一步的 Pearson 相关分析可知，脓毒症患者中 TNF-α 与 PT、APTT 呈正相关，与 $CD3^+$、$CD4^+$、$CD4^+/CD8^+$ 呈负相关，可见 TNF-α 可能通过影响患者的免疫功能及凝血功能而影响脓毒症的发生及发展，这可能成为临床治疗脓毒症的新靶点。

综上所述，脓毒症患者中血清 TNF-α、T 淋巴细胞亚群、凝血功能均存在异常变化，TNF-α 与患者的 T 淋巴细胞亚群、凝血功能和预后有关，监测 TNF-α 有助于评估脓毒症患者的病情和预后。

参 考 文 献(References)

[1] Arcagok BC, Karabulut B. Platelet to Lymphocyte Ratio in Neonates:

A Predictor of Early onset Neonatal Sepsis [J]. Mediterr J Hematol Infect Dis, 2019, 11(1): e2019055

[2] 王丽丽, 赵鸿雁, 金鑫, 等. 连续性血液净化治疗对脓毒症患者炎性因子及免疫功能的影响[J].现代生物医学进展, 2017, 17(25): 4876-4879, 4935

[3] Kovach CP, Fletcher GS, Rudd KE, et al. Comparative prognostic accuracy of sepsis scores for hospital mortality in adults with suspected infection in non-ICU and ICU at an academic public hospital[J]. PLoS One, 2019, 14(9): e0222563

[4] Peltan ID, Liu VX. More Than We Bargained For: The "Dominating" Cost Effectiveness of Sepsis Quality Improvement? [J]. Crit Care Med, 2019, 47(10): 1464-1467

[5] Tu H, Lai X, Li J, et al. Interleukin-26 is overexpressed in human sepsis and contributes to inflammation, organ injury, and mortality in murine sepsis[J]. Crit Care, 2019, 23(1): 290

[6] He F, Zhang C, Huang Q. Long noncoding RNA nuclear enriched abundant transcript 1/miRNA-124 axis correlates with increased disease risk, elevated inflammation, deteriorative disease condition, and predicts decreased survival of sepsis [J]. Medicine (Baltimore), 2019, 98(32): e16470

[7] Wu H, Liu J, Li W, et al. LncRNA-HOTAIR promotes TNF-α production in cardiomyocytes of LPS-induced sepsis mice by activating NF-κB pathway[J]. Biochem Biophys Res Commun, 2016, 471(1): 240-246

[8] Lv S, Han M, Yi R, et al. Anti-TNF-α therapy for patients with sepsis: a systematic meta-analysis[J]. Int J Clin Pract, 2014, 68(4): 520-528

[9] Chou KT, Cheng SC, Huang SF, et al. Impact of Intermittent Hypoxia on Sepsis Outcomes in a Murine Model[J]. Sci Rep, 2019, 9(1): 12900

[10] Iba T, Umemura Y, Watanabe E, et al. Diagnosis of sepsis-induced disseminated intravascular coagulation and coagulopathy [J]. Acute Med Surg, 2019, 6(3): 223-232

[11] 宋麦芬, 张羽, 郭玉红, 等. Sepsis3.0 对 ICU 脓毒症患者诊断及预后评估的验证[J].中国中西医结合急救杂志, 2017, 24(1): 6-9

[12] Zhang Z, Zhang G, Goyal H, et al. Identification of subclasses of sepsis that showed different clinical outcomes and responses to amount of fluid resuscitation: a latent profile analysis [J]. Crit Care, 2018, 22(1): 347

[13] 李志华, 刘宣, 葛勤敏, 等. 脓毒症诊断的生物学标志物的研究进展[J].现代生物医学进展, 2016, 16(7): 1358-1362

[14] 侯有华. 脓毒症的诊断与治疗新进展 [J]. 安徽医学, 2011, 32(8): 1205-1207

[15] Fujii E, Fujino K, Eguchi Y. An evaluation of clinical inflammatory and coagulation markers in patients with sepsis: a pilot study [J]. Acute Med Surg, 2019, 6(2): 158-164

[16] Wang Y, Fu X, Yu B, et al. Long non-coding RNA THRIL predicts increased acute respiratory distress syndrome risk and positively correlates with disease severity, inflammation, and mortality in sepsis patients[J]. J Clin Lab Anal, 2019, 33(6): e22882

[17] Li P, Zhao R, Fan K, et al. Regulation of dendritic cell function improves survival in experimental sepsis through immune chaperone [J]. Innate Immun, 2019, 25(4): 235-243

[18] Tong Y, Ku X, Wu C, et al. Data-independent acquisition-based quantitative proteomic analysis reveals differences in host immune response of peripheral blood mononuclear cells to sepsis [J]. Scand J Immunol, 2019, 89(4): e12748

[19] Kumar V. Natural killer cells in sepsis: Underprivileged innate immune cells[J]. Eur J Cell Biol, 2019, 98(2-4): 81-93

[20] Popescu NI, Silasi R, Keshari RS, et al. Peptidoglycan induces disseminated intravascular coagulation in baboons through activation of both coagulation pathways[J]. Blood, 2018, 132(8): 849-860

[21] Busch D, Kapoor A, Rademann P, et al. Delayed activation of PPAR-β/δ improves long-term survival in mouse sepsis: effects on organ inflammation and coagulation [J]. Innate Immun, 2018, 24(4): 262-273

[22] Pischke SE, Hestenes S, Johansen HT, et al. Sepsis causes right ventricular myocardial inflammation independent of pulmonary hypertension in a porcine sepsis model [J]. PLoS One, 2019, 14(6): e0218624

[23] Hensley MK, Deng JC. Acute on Chronic Liver Failure and Immune Dysfunction: A Mimic of Sepsis [J]. Semin Respir Crit Care Med, 2018, 39(5): 588-597

[24] Shankar Hari M, Summers C. Major surgery and the immune system: from pathophysiology to treatment [J]. Curr Opin Crit Car, 2018, 24 (6): 588-593

[25] Rodriguez-Rosales YA, Kox M, van Rijssen E, et al. Long-Term Effects of Experimental Human Endotoxemia on Immune Cell Function: Similarities and Differences With Sepsis [J]. Shock, 2019, 51(6): 678-689

[26] Komic A, Martinez-Quinones P, McCarthy CG, et al. Increase in soluble protein oligomers triggers the innate immune system promoting inflammation and vascular dysfunction in the pathogenesis of sepsis[J]. Clin Sci (Lond), 2018, 132(13): 1433-1438

[27] Schuler A, Wulf DA, Lu Y, et al. The Impact of Acute Organ Dysfunction on Long-Term Survival in Sepsis [J]. Crit Care Med, 2018, 46(6): 843-849

[28] Samuels JM, Moore HB, Moore EE. Coagulopathy in Severe Sepsis: Interconnectivity of Coagulation and the Immune System [J]. Surg Infect (Larchmt), 2018, 19(2): 208-215

[29] Sakurai K, Miyashita T, Okazaki M, et al. Role for Neutrophil Extracellular Traps (NETs) and Platelet Aggregation in Early Sepsis-induced Hepatic Dysfunction[J]. In Vivo, 2017, 31(6): 1051-1058

[30] Scărlătescu E, Lancé MD, White NJ, et al. Effects of malignancy on blood coagulation in septic intensive care patients [J]. Blood Coagul Fibrinolysis, 2018, 29(1): 92-96