血清 NGAL 在大鼠肾脏缺血再灌注损伤不同时段的表达及其意义*

李香敏[1] 白光辉[2△] 梅 峰[2] 田 静[1] 马振华[1] 徐海琦[1]

(1 青海大学医学院 青海 西宁 810001;2 青海大学医学院附属医院肾病内科 青海 西宁 810001)

摘要 目的:观察大鼠肾脏缺血再灌注损伤不同时段血中性粒细胞明胶酶相关脂质运载蛋白(NGAL)的表达并探讨其在急性肾脏缺血再灌注损伤中的意义。方法:建立大鼠肾脏缺血再灌注损伤模型,将 50 只大鼠随机分为假手术组(S 组)和模型组(M 组),每组分为 5 个亚组,包括 2h、6h、12h、24h、48h,每亚组大鼠 5 只。观察各组血 NGAL,β2- 微球蛋白及血尿素氮,肌酐的变化。结果:M 组血 NGAL 于再灌注损伤后早期(2h)即开始升高,于 24h 达高峰,至 48h 仍高于正常(P<0.05);β2- 微球蛋白于 12h 升高至 48h 达高峰(P<0.01);尿素氮于 6h 升高于 48h 达高峰(P<0.01);而血肌酐则于 48h 才显著升高(P<0.05)。病理显示:M 组 2h 时可见受损肾小管上皮细胞肿胀,管腔扩张、刷状缘消失,至 6h 时少量上皮细胞脱落、变性甚至坏死,管腔内可见坏死脱落的细胞碎屑,蛋白管型出现,12h 时可见间质水肿压迫至管腔明显狭窄,于 24h、48h 可见蛋白管型显著增多。结论:血 NGAL 可作为肾脏缺血再灌注损伤早期敏感的生物标志物。

关键词 缺血再灌注;中性粒细胞明胶酶相关脂质运载蛋白;急性肾损伤

中图分类号:Q95-3,R692.5 **文献标识码**:A **文章编号**:1673-6273(2012)22-4231-05

The Expression and Significance of Serum NGAL during the Ischemia-Reperfusion Injury happened in Rat Kidney*

LI Xiang-min[1], BAI Guang-hui[2△], MEI Feng[2], TIAN Jing[1], MA Zhen-hua[1], XU Hai-qi[1]

(1 Medical College of Qinghai University, Xining, Xining, Qinghai, 810001, China;

2 Department of Nephrology, Affiliated Hospital of Medical College of Qinghai University, Xining, Qinghai, 810001, China)

ABSTRACT Objective: To observe the expression levels of neutrophil gelatinase-associated lipocalin (NGAL) in kidney ischemia-reperfusion injury (IRI) in rats and to discuss its function in acute kidney injury. Methods: Ischemia-reperfusion (IR) kidney injury rats model was established. Fifty male rats were divided into two groups randomly: sham group(group S), IR group(group M), which included 2h, 6h, 12h, 24h, 48h ssubgroups, each had 5 rats. Observed the changes of blood urea nitrogen (BUN), serum creatinine (SCr), NGAL and β2-microglobulin in each group. Results:In model group, NGAL levels were increased at an early time (2h) and peaked at the 24h samples (P<0.05), and still higher than the sham group by the 48h recovery period. The β2-microglobulin showed a significant elevation at 12h and peaked at the 48h after reperfusion(P<0.01). The expression of BUN was elevated at 6h and peak at the 48h subgroup. But the SCr was enhanced only at the 48h after injury. The pathology demonstrated that in model group there were a loss of brush border membranes, tubular dilation and tubular epithelial cell edema at the 2h subgroup, and at 6h there was a small quantity of epithelium fall off, degenerate, even necrosis, luminal debris, and the protein cast appearance. The lumens was narrowed obviously by the interstitial edema at the 12h. Then the protein cast increased gradually at the 24h and 48h. Conclusion: NGAL may represent an early, sensitive and specific serum biomarker for ischemic-reperfusion renal injury.

Key words: Ischemia-reperfusion; NGAL; Acute kidney injury

Chinese Library Classification(CLC): Q95-3, R692.5 Document code: A

Article ID:1673-6273(2012)22-4231-05

前言

肾脏急性缺血再灌注损伤(ischemia-reperfusion injury, IRI)的发生发展是一个极其复杂的病理生理过程,其发病机制和早期诊断在本学科领域备受关注。本实验通过夹闭后开放双侧肾动脉建立大鼠急性缺血再灌注所致急性肾损伤(acute kidney injury, AKI)动物模型,模拟临床上创伤、休克等所致缺血性肾损伤,旨在探讨模型大鼠血清中性粒细胞明胶酶相关脂质运载蛋白(neutrophil gelatinase-associated lipocalin, NGAL)的表达及意义,为 AKI 患者的早期临床诊断提供一定的实验依据。研究提示 AKI 时血液和尿液中 NGAL 升高较早,而检测血液中的标记物,可以避免 AKI 时极度少尿所带来的问题,同时又能减

* 基金项目:校研究生基金资助

作者简介:李香敏(1982-),女,硕士研究生,研究方向:肾脏疾病

△通讯作者:白光辉,电话:0971-6162555,E-mail:qhbghy@163.com

(收稿日期:2011-12-04 接受日期:2011-12-30)

轻因治疗而使用利尿药等所带来的干扰。

1 材料与方法

1.1 实验动物和试剂

健康 SD 雄性大鼠 50 只，体重均在(200± 20)g 之间，普通型，由兰州大学医学院实验动物中心提供。适应性喂养 1 周，实验期间大鼠进食普通饲料，正常饮水。血清 NGAL、β-2 微球蛋白、尿素氮及肌酐试剂盒均购于南京建成生物工程研究所。

1.2 实验方法

将 50 只大鼠编号，随机分为 2 组：(1) 假手术组(S 组)：只游离双侧肾动脉后即关腹。(2) 模型组(M 组)：腹正中切口，钝性分离肾包膜，游离双侧肾动脉后用无创动脉夹夹闭，肾脏由红润立刻变为苍白，随后渐成暗红色。缺血 30min 后松开动脉夹恢复灌注。5min 内肾脏颜色由暗红色转为红润，表示再灌注成功，缝合切口。术中大鼠体温保持在 37℃ (将大鼠置于恒温手术台上)。

1.3 标本采集及测定

造模成功后于各时间点麻醉大鼠，经下腔静脉采血约 4mL 离心后留取上清冻存于 -70℃待测。取血后处死大鼠，取出肾脏，10％甲醛固定后经脱水、透明、石蜡包埋，切成 4μm 切片，作苏木精 - 伊红(HE)染色，光镜下观察肾小球和肾小管间质病理形态学改变。血 NGAL 及血 β2- 微球蛋白采用酶联免疫吸附试验(ELISA 法)测定，血尿素氮(BUN)测定采用二乙酰肟法，血肌酐(SCr)测定采用苦味酸法。各项实验过程均严格按照试剂盒说明书进行操作。

1.4 统计分析

采用 SPSS17.0 统计软件包，计量资料以($\bar{x}$± s)表示，多组间差异比较采用单因素方差分析，两组间差异比较用两个独立样本 t 检验。检验水准 α=0.05，P<0.05 有统计学意义。

2 结果

2.1 一般状态

S 组及 M 组大鼠均有不同程度的摄食减少，饮水量略有减少，精神尚好，活动良好，扎堆。尿量略有减少，色淡黄，体毛有光泽。模型 24h、48h 亚组可见眼周出血，而假手术组无此改变。

2.2 各指标检测结果

M 组 NGAL 于 2h 开始升高，于 24h 达高峰，至 48h 仍高于正常(P<0.05)；β2- 微球蛋白于 12h 升高至 48h 达高峰(P<0.01)；BUN 于 6h 升高于 48h 达高峰(P<0.01)；而血肌酐则于 48h 明显升高(P<0.05)。S 组各时间点各项指标比较差异无统计学意义(P>0.05)。结果见表 1、2 及图 1、2。

2.3 肾组织病理改变

2.3.1 肉眼观察 S 组肾脏外观正常，纵向剖开后颜色红润。M 组见肾脏呈暗红色，体积增大，部分表面可见散在小片状出血，纵向剖开后肾皮质颜色略白，髓质呈暗红色。

2.3.2 HE 染色结果 S 组肾组织结构未见异常。M 组受损肾小管上皮细胞普遍水肿，管腔扩张、刷状缘消失，少量上皮细胞脱落、变性甚至坏死，管腔内可见坏死脱落的细胞碎屑；至 12h 时

表 1 各时间点 SCr 和 BUN 的变化 ($\bar{x}$± s，n=5)

Table 1 The changes of SCr and BUN at various time points ($\bar{x}$± s，n=5)

Time point	SCr (μmol/L)		BUN(mmol/L)	
	S	M	S	M
2h	91.58± 10.32	91.97± 6.64	8.95± 1.62	9.33± 2.12
6h	95.92± 12.38	97.29± 8.03	8.50± 1.33	16.08± 3.13▲
12h	86.00± 9.32	94.84± 5.31	7.69± 1.57	17.21± 1.51▲
24h	83.41± 8.89	100.29± 6.22	9.91± 2.36	18.80± 4.71▲
48h	83.95± 9.98	120.20± 16.64▲●	7.85± 1.22	23.45± 4.24▲●

Note: ▲P<0.01 Comparison between the sham group and model group at the same time point; ● P<0.05 Comparisons between subgroups in the group.

表 2 各时间点血 NGAL 和血 β2-MG 的变化特点($\bar{x}$± s，n=5)

Table 2 The changes of serum NGAL and β2-MG at various time points ($\bar{x}$± s，n=5)

Time point	NGAL (pg/ml)		β2-MG (ng/ml)	
	S	M	S	M
2h	67.98± 12.37	86.55± 5.91△	1.89± 0.47	2.04± 0.57
6h	75.27± 7.93	91.88± 5.41▲	2.15± 0.86	2.57± 0.68
12h	78.44± 6.05	97.58± 7.36△	2.48± 0.67	3.74± 0.60△
24h	70.77± 10.23	107.94± 6.22▲●	2.12± 0.72	3.78± 0.66▲
48h	76.49± 6.88	89.73± 6.69△	2.32± 0.73	4.77± 0.90▲●

Note: △P<0.05 Comparison between the sham group and model group at the same time point; ▲P<0.01 Comparison between the sham group and model group at the same time point; ● P<0.05 Comparisons between subgroups in the group.

可见间质水肿压迫致管腔明显狭窄，蛋白管型于 6h 出现，24h 及 48h 时显著增多。两组肾小球结构无明显差别（如图 3-14）。

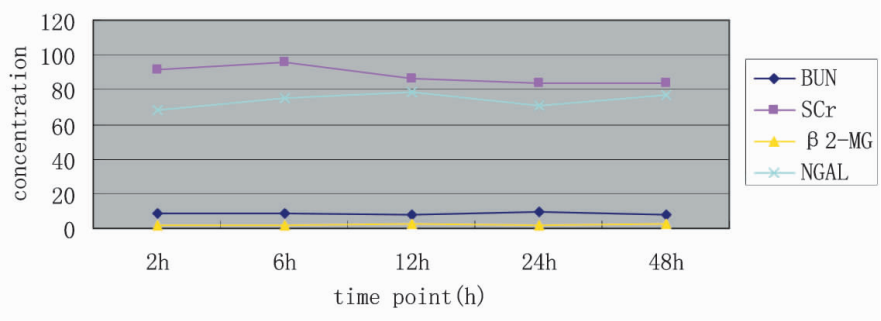

图 1 S 组各指标的动态变化

Fig.1 The changes of each index in group S

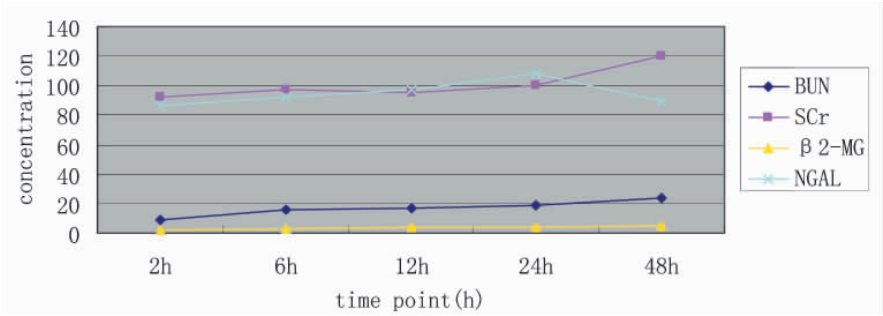

图 2 S 组各指标的动态变化

Fig.2 The changes of each index in group S

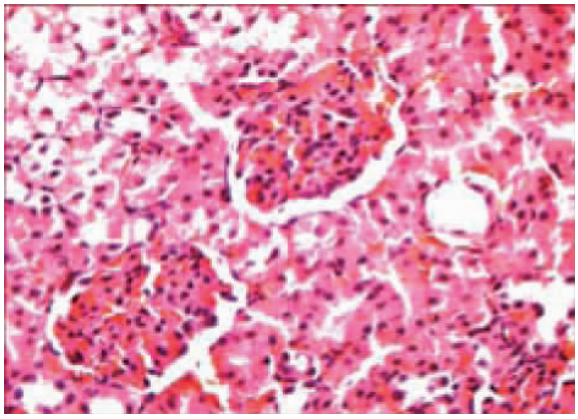

图 3 S 组肾小球结构正常（400×）

Fig.3 The glomerular structure was normal in group S (400×)

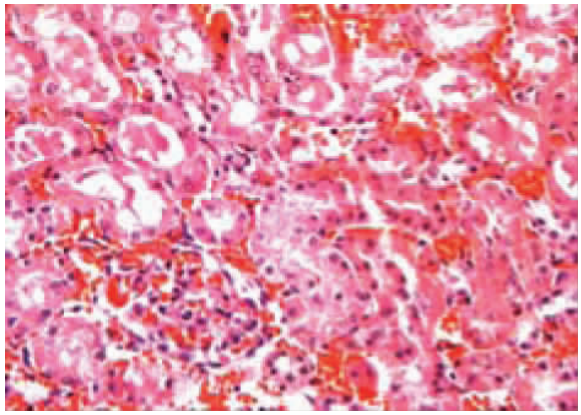

图 5 M2h 组肾小球充血明显（400×）

Fig.5 Observed glomerular hyperemia at 2h in model group (400×)

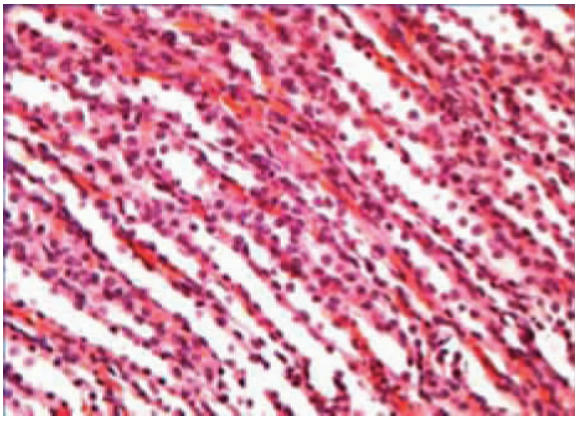

图 4 S 组肾小管结构正常（400×）

Fig.4 The tubular structure was normal in group S (400×)

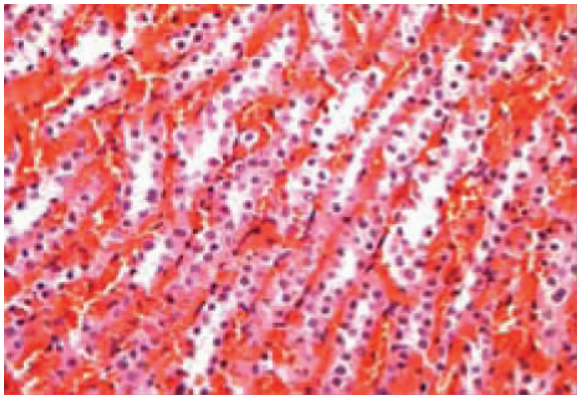

图 6 M2h 组肾小管上皮细胞普遍水肿，管腔扩张、刷状缘消失（400×）

Fig. 6 There were a loss of brush border membranes, tubular dilation and tubular epithelial cell edema at 2h in model group (400×)

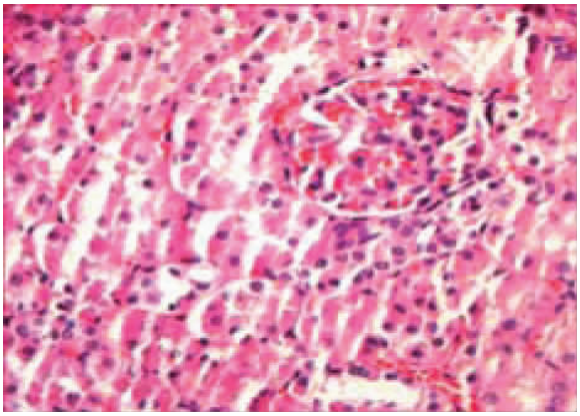

图 7 M6h 组肾小球仍有充血 (400×)

Fig.7 Observed glomerular hyperemia at 6h in model group (400×)

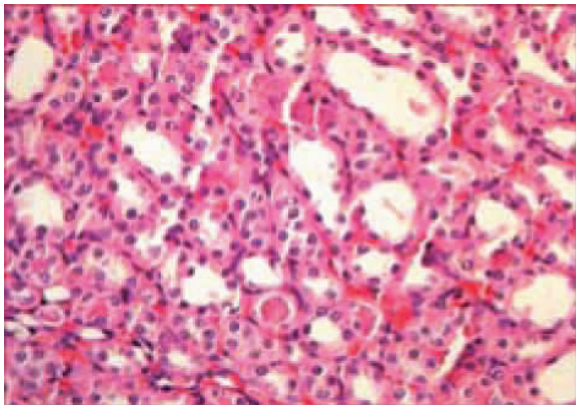

图 8 M6h 组少量上皮细胞脱落、变性甚至坏死,管腔内可见坏死脱落的细胞碎屑 ;出现蛋白管型 (400×)

Fig.8 There was a small quantity of epithelium fall off, degenerate, even necrosis, luminal debris, and the protein cast appearance at 6-h in model group (400×)

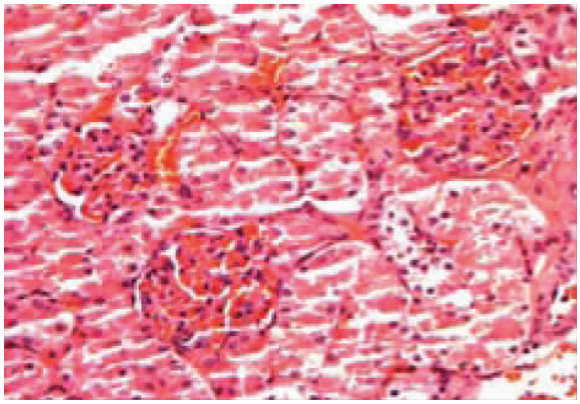

图 9 M12h 组肾小球充血仍较明显 (400×)

Fig.9 Observed glomerular hyperemia at 12h in model group (400×)

3 讨论

临床上由缺血再灌注损伤引起的 AKI 较为常见，其发病机制十分复杂。引起肾再灌注损伤的两个关键因素是细胞内钙超载和氧自由基的大量生成[1]。通过建立 IRI 模型,进而探讨 AKI 的相关机制及其早期诊断标志物已成为肾脏病领域研究的热点。

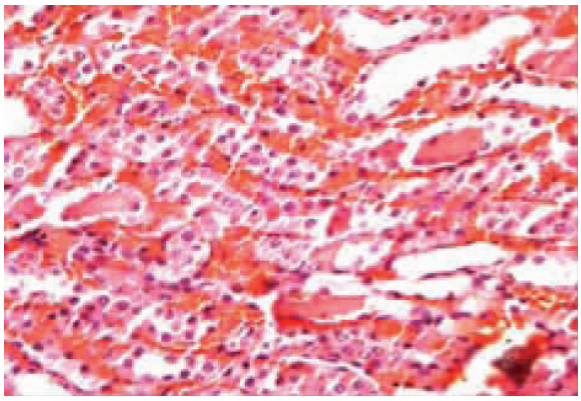

图 10 M12h 组间质水肿压迫致管腔明显狭窄 ;蛋白管型增多 (400×)

Fig.10 The lumens was narrowed obviously by the interstitial edema at 12h in model group (400×)

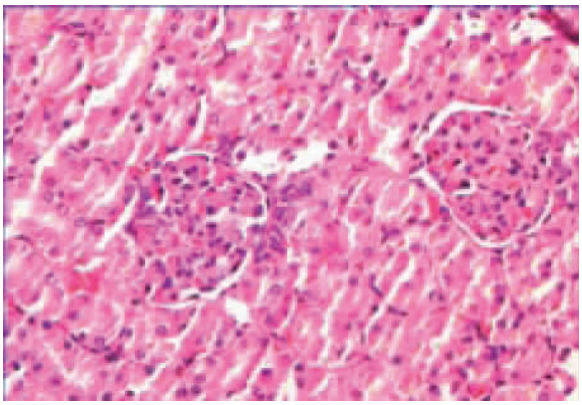

图 11 M24h 组肾小球充血减轻 (400×)

Fig.11 Observed glomerular hyperemia become lightening at 24-h in model group (400×)

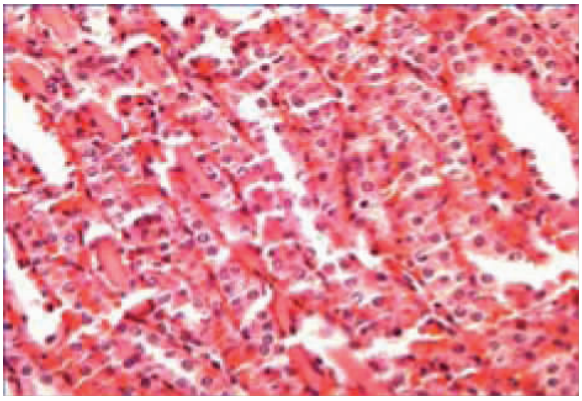

图 12 M24h 组蛋白管型明显增多 (400×)

Fig.12 The protein cast increased gradually at 24h in model group (400×)

通过对 AKI 早期应激反应的研究，进而发现了许多潜在的生物标记物[2-4],NGAL 和 IL-18 是其中研究的最为广泛的两个[5-11]。NGAL 是 1993 年在人中性粒细胞中发现的 lipocalin 家族的新成员,与中性粒细胞明胶酶共价结合[12-14]。存在于正常人体的肾脏、气管、肺、胃和结肠等组织,均呈低表达状态。研究表明 NGAL 是在肾近端肾小管缺血性损伤时出现的反应物,它可于肾损伤 2h 之内出现于尿液中[15-16]。蛋白质组学研究表明,在缺血性或肾毒性 AKI 动物模型的肾脏上皮细胞中,NGAL 是最早且最稳定表达的蛋白质之一,并易于在肾损伤发生不久

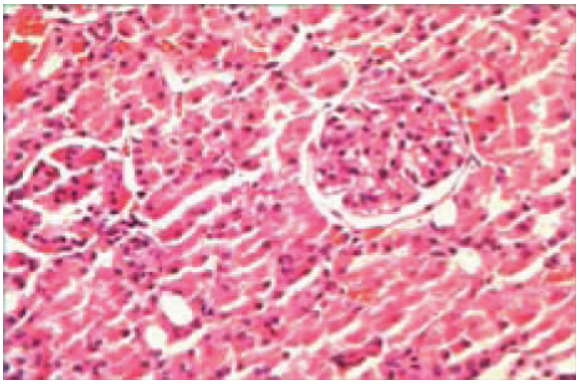

Fig.13 Observed no glomerular hyperemia at 24-h in model group (400×)

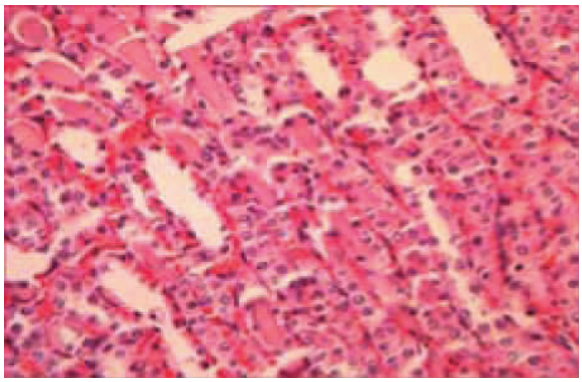

Fig.14 There was still much protein cast at 48-h in model group (400×)

的血液和尿液标本中检测出来[17]。本研究显示，M 组 NGAL 于再灌注损伤后早期（2h）即开始升高，明显早于血肌酐及血 β2-微球蛋白的升高。在缺血性肾损伤动物模型中，NGAL 主要表达于处于增殖和再生中的增殖细胞核抗原（proliferating cell nuclear antigen, PCNA）- 阳性的近端肾小管上皮细胞中，提示了其在修复过程中发挥作用[18-19]。研究发现，单侧肾缺血后直接取同侧肾静脉血液显示肾脏合成的 NGAL 并未有效的进入血循环，而在同侧输尿管中却大量出现[20]。而在 AKI 时可出现远距离器官尤其是肝和肺 NGAL mRNA 的表达显著增高，过量表达的 NGAL 释放入血循环，再加上 GFR 下降使 NGAL 清除减少从而增加了血循环中的蓄积[21]。因此，血 NGAL 可作为 AKI 的早期诊断指标。

综上所述，AKI 时血清 NGAL 是一个较为敏感且具有预测性的早期标志物。因此，AKI 时同时找出尿和血中的标志物是非常重要的也是最终目标。进一步研究 NGAL 在 AKI 发病中的作用及其机制，将深化人们对 AKI 的认识，为 AKI 的早期诊断及治疗提供新的思路。

参考文献（References）

[1] 金慧铭，王建枝.病理生理学[M]. 北京：人民卫生出版社，2008：255
Jin Hui-ming, Wang Jian-zhi. Physiopathology [M]. Beijing: The People's Medical Publishing House, 2008:255(In Chinese)

[2] Devarajan P. Proteomics for biomarker discovery in acute kidney injury[J]. Semin Nephrol, 2007, 27:637-651

[3] Devarajan P. Proteomics for the investigation of acute kidney injury [J]. Contrib Nephrol, 2008, 160:1-16

[4] Devarajan P, Parikh C, Barasch J. Case 31-2007: a man with abdominal pain and elevated creatinine[J]. N Engl J Med, 2008, 358(3):312

[5] Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation[J]. Am J Transplant, 2006,6(7):1639-1645

[6] Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery[J]. Lancet, 2005,365(9466):1231-1238

[7] Parikh CR, Abraham E, Ancukiewicz M, et al. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit[J]. J Am Soc Nephrol, 2005,16:3046-3052

[8] Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery [J]. Kidney Int, 2006,70:199-203

[9] Wagener G, Jan M, Kim M, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery[J]. Anesthesiology, 2006,105:485-491

[10] Washburn KK, Zappitelli M, Arikan AA, et al. Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children [J]. Nephrol Dial Transplant, 2007,23:566-572

[11] Zappitelli M, Washburn KK, Arikan AA, et al. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: A prospective cohort study [J]. Crit Care, 2007,11:R84

[12] Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans[J]. Genomics, 1997, 45: 17-23

[13] Cowland JB, Sorensen OE, Schested M, et al. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1β but not by TNF-α[J]. J Immunol, 2003, 171: 9366-0366

[14] Mishra J, Mori K, Ma Q, et al. Amelioration of ischemic acute renal injury by neutrophil gelatinase-associated lipocalin [J]. J Am Soc Nephrol, 2004, 15: 3073-3082

[15] Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease [J]. Clin Lab Invest Suppl, 2008, 241: 89-94

[16] Mishra J, Mori K, Ma Q, et al. (2004) Neutrophil gelatinase-associated lipocalin (NGAL): a novel urinary biomarker for cisplatin nephrotoxicity[J]. Am J Nephrol, 2004, 24:307-315

[17] Mori K, Lee HT, Rapoport D, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kid ney from ischemia-reperfusion injury[J]. J Clin Invest, 2005, 115 (3) :610-621

[18] Mishra J, Ma Q, Prada A, et al. Identification of NGAL as a novel urinary biomarker for ischemic injury[J]. J Am Soc Nephrol, 2003, 4: 2534-2543

[19] Devarajan P, Mishra J, Supavekin S, et al. Gene expression in early ischemic renal injury: Clues towards pathogenesis, biomarker discovery and novel therapeutics[J]. Mol Genet Metab, 2003, 80: 365-376

[20] Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin [J]. J Am Soc Nephrol, 2007, 18: 407-413

[21] Grigoryev DN, Liu M, Hassoun HT, et al. The local and systemic inflammatory transcriptome after acute kidney injury [J]. J Am Soc Nephrol, 2008, 19: 547-558