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轻链检测在慢性肾脏病中的应用评估

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摘要 目的 评估慢性肾脏病(CKD)患者在不同分期下,血总轻链(sTLC)、尿总轻链(uTLC)及血游离轻链(sFLC)水平差异及其与肾功能指标的相关性;探讨各轻链指标在CKD患者分期中的预测价值。**方法** 回顾性分析292例CKD患者,排除浆细胞疾病、急性肾损伤、肿瘤性疾病等。根据估算肾小球滤过率(eGFR)将CKD患者分为CKD1~5期组。检测CKD患者sTLC、uTLC、sFLC及相应生化指标,比较各组指标间差异及相关性;采用受试者应用工作曲线(ROC曲线),以CKD1~2期合并为对照组,CKD3~5期合并为病例组,分析各轻链指标在CKD分期中的预测价值。**结果** CKD 1~5期多组间比较显示,sTLC κ、sTLC λ、sTLC κ/λ、sFLC κ/λ差异无统计学意义;而sFLC κ、sFLC λ、uTLC κ、uTLC λ差异有统计学意义($P<0.05$),且随着CKD分期期次的增高而增高。sFLC κ、sFLC λ与肌酐(Scr)、尿素氮(BUN)、eGFR的相关系数高于uTLC κ、uTLC λ($P<0.001$);sTLC κ、sTLC λ、sTLC κ/λ、sFLC κ/λ与肾功能指标相关系数低($P>0.05$)。sFLC κ、sFLC λ预测CKD3~5期病例组,最佳临界点为35.4、52.8 mg/L,AUC分别为0.916(0.883~0.949)、0.915(0.881~0.949),均高于uTLC κ、uTLC λ,AUC分别为0.811(0.754~0.869)、0.787(0.728~0.846)。**结论** 随着CKD分期期次的增加,sFLC、uTLC水平逐渐增加;sFLC、uTLC能够有效预测CKD3期以上患者,对CKD患者分层管理具有重要参考价值。

关键词 轻链;慢性肾脏病;受试者工作特征曲线
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轻链可与重链组装生成免疫球蛋白,根据其结构和恒定区抗原性差异分为κ和λ轻链,少量轻链可以游离状态释放入血,称为血游离轻链(serum free light chain, sFLC)。总轻链是指血或尿中结合和游离的轻链总和,总轻链及游离轻链常用于浆细胞疾病的辅助诊断及治疗监测。sFLC通过肾小球滤过而从血中快速清除,后被近端小管重吸收分解,轻链水平较为恒定。而日常工作中,常发现慢性肾脏病(chronic kidney disease, CKD)患者,轻链异常率较高。研究[1]指出CKD中,可观察到sFLC的积累。因此,排除单克隆sFLC对肾脏的损害外,多克隆的sFLC在CKD中也有重要价值。目前,研究多

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function indexes (TSH, T3, T4, TGAb, TPOAb) and thyroid tissue inflammatory factors (TNF-α, IL-10, IL-6, IL-12) levels were measured by ELISA; thyroid tissue pathological changes were observed by HE staining; thyroid tissue apoptosis was detected by TUNEL fluorescence staining; thyroid tissue expression of Fas, Fas-L was detected by immunohistochemistry; the expression of Fas, Fas-L, FADD and cleaved caspase-3 in thyroid tissue was determined by Western blot; Fas, Fas-L and FADD mRNA expression in thyroid tissue was detected by qRT-PCR. ***Results*** Compared with the control group, serum levels of TSH, TGAb, TPOAb, positive cell ratio, levels of TNF-α, IL-6 and expression of Fas, Fas-L, FADD and cleaved caspase-3 in thyroid tissue were significantly higher in the model group, and serum FT3 and FT4 levels and IL-10 and IL-12 levels in thyroid tissue significantly decreased ($P<0.01$). Compared with the model group, serum levels of TSH, TGAb, TPOAb, positive cell ratio, TNF-α and IL-6 contents and expression of Fas, Fas-L, FADD and cleaved caspase-3 in thyroid tissue were significantly lower in the metformin group, and serum FT3 and FT4 levels and IL-10 and IL-12 contents in thyroid tissue were significantly higher ($P<0.05$). ***Conclusion*** Metformin inhibits the progression of Hashimoto's thyroiditis, and its mechanism of action may be related to the inhibition of the Fas/Fas-L-mediated apoptotic pathway.

Key words Hashimoto's thyroiditis; metformin; Fas/Fas-L pathway; cell apoptosis

关注 sFLC 与 CKD 分期及病死率的相关性[1-3]，而与血总轻链（serum total light chain，sTLC）、尿总轻链（urine total light chain，uTLC）的联合比较，以及在 CKD 分期预测性能差异少有文献提及。该研究旨在分析 CKD 患者各分期轻链水平的差异及其与肾功能指标的相关性及评估各轻链指标预测 CKD 分期的价值差异。

1 材料与方法

1.1 病例资料 回顾性分析安徽医科大学第一附属医院高新院区 2018 年 1—12 月期间住院的 CKD 患者 292 例，其中男性 169 例，女性 123 例，年龄 21～86 岁（中位年龄 52 岁），入选患者排除急性肾损伤、肿瘤性疾病、感染性疾病、免疫系统疾病，其中浆细胞疾病通过免疫分型检测结合临床诊断联合排除。将所有病例根据估算肾小球滤过率（estimated glomerular filtration rate，eGFR）分为 5 组：CKD1 期［eGFR ≥ 90 ml/（min · 1.73 m^2）］62 例，CKD2 期［eGFR 60～89 ml/（min · 1.73 m^2）］26 例，CKD3 期［eGFR 30～59 ml/（min · 1.73 m^2）］87 例，CKD4 期［eGFR 15～29 ml/（min · 1.73 m^2）］47 例，CKD5 期［eGFR < 15 ml/（min · 1.73 m^2）］70 例。以 CKD1～2 期合并为对照组，CKD3～5 期合并为病例组，分析各轻链指标在 CKD 分期预测价值。

1.2 仪器与试剂 轻链检测包括 sTLC、uTLC 及 sFLC，采用德国 Siemens BN Ⅱ SYSTEM 特殊蛋白分析仪测定；总蛋白（total protein，TP）、白蛋白（albumin，ALB）、血肌酐（serum creatinine，Scr）及尿素氮（blood urea nitrogen，BUN）采用美国 Beckman AU5800 全自动生化分析仪检测；免疫球蛋白免疫分型采用法国 sebia 毛细管电泳检测。所有患者采集清晨空腹肘部静脉血及晨尿进行检测。检测试剂、质控及校准均为仪器配套。eGFR 计算采用 CKD-EPI 4 种族公式中适合亚洲人群（Asia）[4] 的评估公式。研究方案通过安徽医科大学第一附属医院伦理委员会批准（编号：PJ2019-04-16）。

1.3 统计学处理 采用 SPSS 25.0 统计学软件进行数据处理，连续型变量通过 Kolmogorov-Smirnov 正态性检验检查数据正态性。正态分布资料以 $\bar{x} \pm s$ 表示，多组间比较采用方差分析；偏态分布资料用［$M(P_{25}, P_{75})$］表示，多组间比较采用 Kruskal-Wallis 检验。偏态分布指标间相关性分析采用 Spearman 相关性分析。应用受试者工作特征曲线（ROC 曲线）分析各指标预测 CKD 分期的性能。$P < 0.05$ 为差异有统计学意义。

2 结果

2.1 研究个体的临床及生物学特征 表 1 结果显示，CKD1～5 期组，多组间比较，sTLC κ、sTLC λ、sTLC κ/λ、sFLC κ/λ 差异无统计学意义（$P > 0.05$）；而 TP、ALB、Scr、BUN、eGFR、sFLC κ、sFLC λ、uTLC κ、uTLC λ、uTLC κ/λ 差异有统计学意义（$P < 0.05$）。随着 CKD 分期期次的增加，sFLC κ、sFLC λ、uTLC κ、uTLC λ 水平也逐渐增高。见图 1。

表 1 CKD 患者的临床及生物学特征［$M(P_{25}, P_{75})$］

项目	CKD 总体	CKD1 期	CKD2 期	CKD3 期	CKD4 期	CKD5 期	Z 值	P 值
N	292	62	26	87	47	70	-	-
性别（男/女）	169/123	22/40	17/9	57/30	29/18	44/26	-	-
年龄（岁）	52(43,64)	38(30,47)	54(45,64)	56(49,69)	54(49,66)	52(44,65)	64.50	<0.001
TP（g/L）	68.6(63.5,73.5)	71.2(66.5,73.8)	69.7(66.3,73.8)	69.1(63.6,74.7)	66.9(61.4,73.0)	66.1(60.4,72.1)	10.20	0.037
ALB（g/L）	40.5(36.0,43.3)	42.4(38.4,45.1)	42.4(38.6,44.0)	40.4(36.7,43.1)	40.2(34.9,41.8)	38.2(34.2,41.5)	23.70	<0.001
Scr（μmol/L）	165.2(88.5,359.1)	61.8(52.3,72.6)	101.2(82.0,114.8)	146.6(118.1,187.9)	281.0(237.7,347.9)	574.3(456.4,755.7)	242.00	<0.001
BUN（mmol/L）	10.18(6.39,16.73)	5.26(4.32,6.08)	6.35(5.34,8.59)	9.52(8.39,12.69)	14.62(11.06,17.78)	23.38(17.29,31.28)	202.60	<0.001
eGFR［ml/（min · 1.73 m^2）］	36(14,85)	116(104,126)	73(61,84)	42(33,54)	19(15,25)	9(6,12)	243.40	<0.001
sTLC κ（g/L）	2.24(1.84,2.68)	2.14(1.77,2.53)	2.50(2.11,2.92)	2.29(1.84,2.87)	2.24(1.88,2.68)	2.14(1.78,2.59)	8.80	0.068
sTLC λ（g/L）	1.37(1.12,1.66)	1.29(1.10,1.50)	1.46(1.27,1.63)	1.48(1.14,1.71)	1.30(1.04,1.59)	1.37(1.11,1.74)	7.60	0.109
sTLC κ/λ（$\bar{x} \pm s$）	1.66 ± 0.37	1.65 ± 0.37	1.74 ± 0.36	1.64 ± 0.38	1.74 ± 0.32	1.62 ± 0.38	1.08	0.369
sFLC κ（mg/L）	44.1(24.0,83.2)	17.9(13.5,24.2)	24.8(21.5,37.6)	39.9(27.5,52.0)	66.2(50.3,92.6)	105.0(83.5,150.0)	200.50	<0.001
sFLC λ（mg/L）	64.9(34.6,118.0)	25.8(19.7,34.5)	35.5(29.5,50.9)	57.3(40.5,77.7)	97.3(67.4,123.0)	143.0(119.5,217.5)	194.30	<0.001
sFLC κ/λ	0.69(0.59,0.79)	0.66(0.57,0.82)	0.70(0.60,0.81)	0.69(0.60,0.79)	0.70(0.61,0.79)	0.68(0.58,0.78)	0.75	0.946
uTLC κ（mg/L）	28.7(12.2,53.1)	10.5(7.0,16.6)	17.0(7.0,28.9)	25.6(11.0,45.0)	43.6(20.9,68.1)	54.2(37.5,69.1)	100.90	<0.001
uTLC λ（mg/L）	16.2(6.6,38.4)	6.6(3.8,11.7)	8.8(3.8,16.7)	13.0(5.9,25.5)	25.4(12.3,47.7)	41.8(29.2,54.8)	101.70	<0.001
uTLC κ/λ	1.67(1.32,1.97)	1.74(1.31,1.82)	1.82(1.60,1.95)	1.88(1.46,2.30)	1.68(1.46,2.00)	1.35(1.14,1.62)	39.00	<0.001

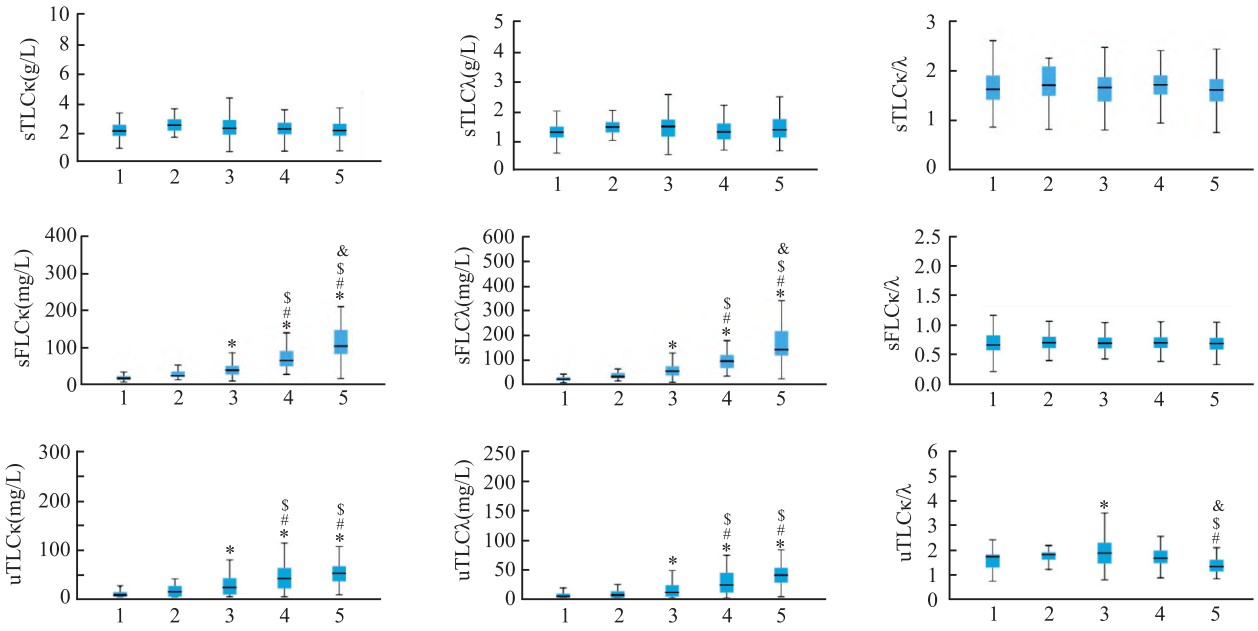

图 1 CKD 各分期血尿轻链及游离轻链水平箱线图

1:CKD1;2:CKD2;3:CKD3;4:CKD4;5:CKD5;与 CKD1 期比较:$^{*}P<0.05$;与 CKD2 期比较:$^{\#}P<0.05$;与 CKD3 期比较:$^{\$}P<0.05$;与 CKD4 期比较:$^{\&}P<0.05$

2.2 轻链结果与肾功能指标相关性分析 表 2 结果显示,尿轻链(uTLC κ、uTLC λ)、血游离轻链(sFLC κ、sFLC λ)与 Scr、BUN 呈正相关($P<0.001$),与 eGFR 负相关($P<0.001$)。且 sFLC κ、sFLC λ 与 Scr、BUN、eGFR 的相关性(sFLC κ:r_s = 0.839、0.799、−0.862;sFLC λ:r_s = 0.837、0.812、−0.862,$P<0.001$)优于 uTLC κ、uTLC λ(uTLC κ:r_s = 0.657、0.582、−0.662;uTLC λ:r_s = 0.665、0.592、−0.674,$P<0.001$)。总轻链(sTLC κ、sTLC λ)、sTLC κ/λ、sFLC κ/λ 与肾功能指标无显著相关性。

表 2 轻链与肾功能指标间相关性分析(r)

项目	Scr	BUN	eGFR
sTLC κ	−0.088	−0.069	0.060
sTLC λ	−0.026	−0.028	−0.005
sTLC κ/λ	−0.077	−0.052	0.083
sFLC κ	0.839	0.799	−0.862
sFLC λ	0.837	0.812	−0.862
sFLC κ/λ	−0.020	−0.061	0.028
uTLC κ	0.657	0.582	−0.662
uTLC λ	0.665	0.592	−0.674
uTLC κ/λ	−0.238	−0.227	0.246

2.3 ROC 曲线 以 CKD1 ~ 2 期合并作为对照组,sFLC κ、sFLC λ 预测 CKD3 ~ 5 期病例组,ROC 曲线分析最佳临界点为 35.4、52.8 mg/L,AUC 分别为 0.916(0.883 ~ 0.949)、0.915(0.881 ~ 0.949),敏感度分别为 0.825、0.799,特异度分别为 0.886、0.899。uTLC κ、uTLC λ 预测 CKD3 ~ 5 期病例组,最佳临界点为 26.3、15.1 mg/L,AUC 分别为 0.811(0.754 ~ 0.869)、0.787(0.728 ~ 0.846),敏感度分别为 0.698、0.651,特异度分别为 0.835、0.835。见表 3 和图 2。

表 3 轻链指标预测 CKD 分期的 ROC 曲线分析

项目	AUC (95% *CI*)	Cutoff 值	敏感性	特异性	*P* 值
sTLC κ	0.514(0.441 ~ 0.587)	2.635	0.291	0.785	0.722
sTLC λ	0.548(0.476 ~ 0.620)	1.495	0.407	0.734	0.216
sFLC κ	0.916(0.883 ~ 0.949)	35.350	0.825	0.886	<0.001
sFLC λ	0.915(0.881 ~ 0.949)	52.800	0.799	0.899	<0.001
uTLC κ	0.811(0.754 ~ 0.869)	26.250	0.698	0.835	<0.001
uTLC λ	0.787(0.728 ~ 0.846)	15.100	0.651	0.835	<0.001

3 讨论

轻链及游离轻链检测常用于浆细胞疾病的辅助诊断及治疗评估[5]。研究显示,sFLC 能够通过抑制自发凋亡来调节多形核白细胞的功能[6],也能降低中性粒细胞的趋化性和葡萄糖摄取[7]。多克隆 sFLC 在 CKD 患者中可出现累积,可能的机制包括多克隆产物增多、肾脏清除减少,或两种机制的共同作用,较高的sFLC水平与CKD患者较高的病死率

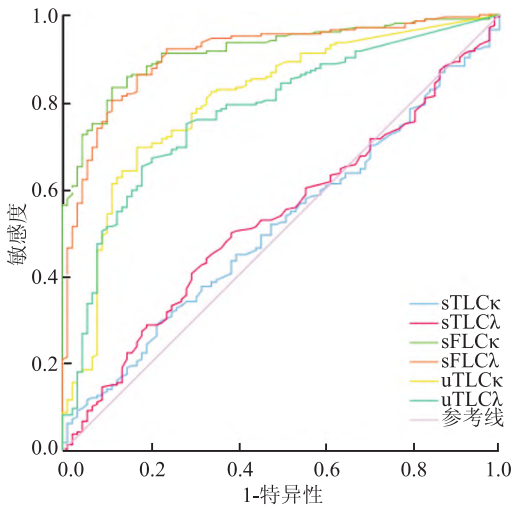

图 2 血尿轻链及游离轻链预测 CKD 分期的 ROC 曲线

和终末期肾病风险独立相关[8]。以往文献少有将血轻链、尿轻链及游离轻链联合分析,以及评估轻链指标在 CKD 分期预测价值差异。流行病学调查显示,每年中国 CKD 的发病率约为 5% 左右,而患病率已达 10.8%,接近发达国家水平[9],其防治已成为世界各国所面临的重要公共卫生问题[10]。该研究综合评估了血轻链、尿轻链及游离轻链在 CKD 中差异及在 CKD 分期预测中价值。

该研究显示,CKD1 ~5 期,随着 CKD 分期期次的增加,sFLC κ、sFLC λ 逐渐增高,与以往研究[1-2,11]结果近似。该研究也显示 uTLC κ、uTLC λ 也随着 CKD 分期期次的增加而增加,其可能的机制是完整的免疫球蛋白分子无法通过完整的肾小球滤过屏障,而游离的免疫球蛋白及轻链可通过肾小球滤过,并在肾小管重吸收,而随着 CKD 疾病进展,肾小球基底膜受损增加,尿液中轻链排出增加导致。然而,CKD1 ~5 期多组间比较,sTLC κ、sTLC λ 差异无统计学意义,推测其机制为血总轻链包括免疫球蛋白重链结合的轻链以及未结合的游离轻链,而结合状态的轻链浓度要远高于游离轻链,因此,总轻链血清浓度是由完整的免疫球蛋白分子浓度决定,其克隆性增殖(多克隆或单克隆性质)或减少会导致轻链的浓度升高或降低,故血总轻链与 CKD 分期相关性差。该研究中,CKD1 ~5 期多组间比较分析显示,sTLC κ/λ、sFLC κ/λ 差异均无统计学意义(P = 0.284、0.946)。

轻链结果与肾功能指标相关性分析结果表明,血游离轻链(sFLC κ、sFLC λ)、尿轻链(uTLC κ、uTLC λ)与 Scr、BUN 呈正相关(P < 0.001),与 eGFR 负相关(P < 0.001),并且血游离轻链与肾功能指标相关性要高于尿轻链结果。sTLC κ/λ、sFLC κ/λ 与肾功能指标相关性差异无统计学意义(P > 0.05)。虽然尿轻链比值 uTLC κ/λ 与肾功能指标呈弱相关(r = −0.238、−0.227、0.246,P < 0.001),但相关性系数较小,在 CKD 分期预测价值有限,可见轻链比值在 CKD 分期中价值不显著。当多克隆免疫球蛋白增多和(或)肾脏功能受损时,血清中的 κ 和 λ 型轻链会同时增加,因此 κ/λ 不变,而肿瘤性浆细胞会过量产生一种单克隆免疫球蛋白轻链,而同时骨髓对于另一种轻链的生成不变,因此 κ/λ 会明显异常。而尿液中 κ/λ 值因为非肿瘤细胞产生的轻链量很低不能持续地通过肾单位,而不如血清可靠。

CKD 是各种原因引起的肾脏结构和功能障碍引起肾小球滤过率下降,其中 CKD1 ~2 期患者,eGFR 正常或轻度下降,而 CKD3 期患者,eGFR 轻到中度下降,是临床干预及进行相关治疗的关键节点。故该研究以 CKD1 ~2 期作为对照组,CKD3 ~5 期为病例组,来比较各指标在 CKD 分期中的预测价值,sFLC κ、sFLC λ 最佳临界点为 35.4、52.8 mg/L,AUC 分别为 0.916、0.915,要高于 uTLC κ、uTLC λ(AUC:0.811、0.787),其最佳临界点 26.3、15.1 mg/L。以上研究表明 sFLC κ、sFLC λ 在 CKD 分期中的预测价值高于 uTLC κ、uTLC λ。因此,当 sFLC κ > 35.4 mg/L、sFLC λ > 52.8 mg/L 时,相当于 CKD 进展至 CKD3 期,应当给予相应的临床干预。

综上所述,随着 CKD 分期期次的增加,sFLC、uTLC 水平逐渐增加;sFLC、uTLC 能够有效预测出 CKD3 期以上患者,对 CKD 患者分层管理具有重要参考价值。sFLC 在肾功能相关性及 CKD 分期预测价值上均高于 uTLC。sTLC、各轻链比值与肾功能指标相关性差,在 CKD 分期预测方面价值有限。

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Evaluation of the clinical application of light chain detection in chronic kidney disease

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Abstract ***Objective*** To evaluate the differences of serum total light chain (sTLC), urine total light chain (uTLC) and serum free light chain (sFLC) in different stages of chronic kidney disease (CKD) and their correlation with renal function indexes. To investigate the predictive value of light chain indexes in CKD staging. ***Methods*** 292 patients with CKD were analyzed retrospectively, and plasma cell diseases, acute kidney injury and tumor diseases were excluded. According to the estimated glomerular filtration rate (eGFR), CKD patients were divided into five groups from CKD 1 stage to CKD 5 stage. The levels of sTLC, uTLC, sFLC and corresponding biochemical indexes of CKD patients were detected, and the differences and correlations among the indexes of each group were compared. The receiver operating curve (ROC curve) was used to analyze the predictive value of each light chain index in CKD stage, with CKD1 - 2 stage combined as control group and CKD3 - 5 stage combined as case group. ***Results*** There was no significant difference in sTLC κ, sTLC λ, sTLC κ/λ and sFLC κ/λ among CKD1 - 5 stage ($P > 0.05$). There were significant differences between sFLC κ, sFLC λ and uTLC κ, uTLC λ among CKD1-5 stage ($P < 0.05$), which increased with the increase of CKD staging. The correlation between sFLC κ, sFLC λ and serum creatinine (Scr), blood urea nitrogen (BUN), eGFR were better than uTLC κ, uTLC λ ($P < 0.001$). The sTLC κ, sTLC λ, sTLC κ/λ and sFLC κ/λ had no correlation with renal function indexes ($P > 0.05$). The best critical points of sFLC κ and sFLC λ for predicting CKD3 - 5 stage were 35.4 mg/L and 52.8 mg/L, and AUC was 0.916 (0.883 - 0.949) and 0.915 (0.881 - 0.949), which were higher than uTLC κ and uTLC λ, AUC was 0.811 (0.754 - 0.869) and 0.787 (0.728 - 0.846), respectively. ***Conclusion*** With the increase of CKD staging, the levels of sFLC and uTLC gradually increase. The sFLC and uTLC can effectively predict patients with CKD3 and above, which has an important reference value in stratified management of patients with CKD.

Key words light chain; chronic kidney disease; receiver operating characteristic curve