Interruption of neutrophil extracellular traps formation dictates host defense and tubular HOXA5 stability to augment efficacy of anti-Fn14 therapy against septic AKI

Yin Ni<sup>1</sup>, Bang-Chuan Hu<sup>1</sup>, Guo-Hua Wu<sup>2</sup>, Zi-Qiang Shao<sup>1</sup>, Yang Zheng<sup>1</sup>, Run Zhang<sup>1</sup>, Jun Jin<sup>1</sup>, Jun Hong<sup>1</sup>, Xiang-Hong Yang<sup>1</sup>, Ren-Hua Sun<sup>1</sup>, Jin-Quan Liu<sup>1</sup>, Shi-Jing Mo<sup>1†</sup> <sup>1</sup>Department of Intensive Care Unit, Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College, Hangzhou 310014, Zhejiang, P.R.China <sup>2</sup>Zhejiang University School of Medicine, Zhejiang University, Hangzhou 310029, Zhejiang, P.R.China

Running title: Combined blockade of NETs and Fn14 for septic AKI therapy

## <sup>†</sup>Corresponding author:

\*Shi-Jing Mo, MD, PhD Department of Intensive Care Unit Zhejiang Provincial People's Hospital People's Hospital of Hangzhou Medical College Hangzhou, Zhejiang 310014 P.R. China Email : moshijing@hmc.edu.cn ORCID: https://orcid.org/0000-0002-1303-4766

## Supplemental Figure legends, Figures S1-15 and Table S1

Figure S1. Validation of septic AKI elicited by CLP, LIE and MDRS models. (A and B) Representative pictures (A) and quantification (B) of H&E staining in renal sections from CLP mice. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001, two-sided Student's *t*-test. Scale bar =  $50 \mu m$ . (C) Representative transmission electron microscopy (TEM) images examining mitochondrial morphology of kidney tubular epithelial cells in CLP mice. Scale bar =  $0.2 \mu m$ . (D and E) Serum creatinine (Scr, D) and blood urea nitrogen (BUN, E) levels in mice after CLP challenge. Data are expressed as mean  $\pm$  s.d. \*\*\*P < 0.001, two-sided Student's *t*-test. (F) Western blotting analyses testing levels of PAD4 and Fn14 protein expression in kidney tissues from isotype control and CLP mice. WB: western-blotting. (G and H) Representative pictures (G) and quantification (H) of H&E staining in renal sections from LIE mice. Data are expressed as mean  $\pm$  s.d. \*\*\*P < 0.001, two-sided Student's *t*-test. Scale bar  $= 50 \mu m$ . LIE: LPS-induced endotoxemia. (I) Representative TEM images testing mitochondrial morphology of kidney tubular epithelial cells in LIE mice. Scale bar = 0.2 µm. (J and K) Serum creatinine (Scr, J) and blood urea nitrogen (BUN, K) levels in mice with intraperitoneal LPS (10 mg/kg) injection. Data are expressed as mean  $\pm$ s.d. \*\*\*P < 0.001, two-sided Student's *t*-test. (L and M) Representative pictures (L) and quantification (M) of H&E staining in renal sections from MDRS mice. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001, two-sided Student's *t*-test. Scale bar = 50  $\mu$ m. MDRS: multidrug-resistant sepsis. (N) Representative TEM images detecting mitochondrial morphology of kidney tubular epithelial cells in MDRS mice. Scale bar  $= 0.2 \mu m$  (O and P) Serum creatinine (Scr, O) and blood urea nitrogen (BUN, P) levels in mice with intraperitoneal MDRSA infection. Data are expressed as mean  $\pm$ s.d. \*P < 0.05 and \*\*\*P < 0.001, two-sided Student's *t*-test.

Figure S2. Concurrence of NETs formation and Fn14 upregulation in septic AKI elicited by LIE and MDRS models as well as in patients with septic AKI. (A) Experimental scheme of immunohistofluorescence and immunohistochemistry analyses for NETs formation and Fn14 expression in LIE mice at 48 h. (B and C) Representative pictures (B) and quantification (C) of immunohistofluorescence and immunohistochemistry analyses comparing NETs formation and Fn14 expression in renal sections from LIE mice at the indicated time points. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001 versus 0 h, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm. (D) Experimental scheme of immunohistofluorescence and immunohistochemistry analyses for NETs formation and Fn14 expression in MDRS mice at 48 h. (E and F) Representative pictures (E) and quantification (F) of immunohistofluorescence and immunohistochemistry analyses comparing NETs formation and Fn14 expression in renal sections from MDRS mice at the indicated time points. Data are expressed as mean  $\pm$  s.d. \*\**P* < 0.01 and \*\*\**P* < 0.001 versus 0 h, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm. (G and H) ELISA assays comparing the serum concentration of NETs (G) and Fn14 (H) in 42 healthy controls (HC) and 93 patients with septic AKI (SA). Data are expressed as mean  $\pm$  s.d. of three independent experiments. (I) Functional association network of PAD4 with components of Fn14 signalling cascade using STRING database.

Figure S3. Validation of NETs blockade by SIVE and macrophages elimination by anti-CSF1R in CLP mice. (A) ELISA assays measuring the serum concentration of NETs in CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both at the indicated time points. (B and C) Representative pictures (B) and quantification (C) of CD68<sup>+</sup> staining in renal sections from CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both in the absence [-] or presence [+] of anti-CSF1R pretreatment. Data are expressed as mean  $\pm$  s.d. \*\**P* < 0.01 and \*\*\**P* < 0.001 versus [-], one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm.

**Figure S4. Inhibition of PAD4 synergizes with Fn14 mAb to protect mice against septic AKI.** (A) Kaplan-Meier curves analyzing survivals of mice with intraperitoneal administration of Cl-Amidine (50 mg/kg), ITEM-2 (0.5 mg) or both at the indicated times after CLP challenge. Log-rank t test was used to caculate the *P* value. (B and C) Representative pictures (B) and quantification (C) of H&E staining in kidney tissues from CLP mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm. (D and E) Serum creatinine (Scr, D) and blood urea nitrogen (BUN, E) levels in CLP mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. (F) ELISA assays assessing serum concentration of HMGB1 in CLP mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05, one-way ANOVA, post hoc comparisons, Tukey's test. (G) Kaplan-Meier curves analyzing survivals of Fn14-KO mice with intraperitoneal administration of SIVE (100 mg/kg) or Cl-Amidine (50 mg/kg) at the indicated times after CLP challenge. Log-rank t test was used to caculate the *P* value. (H and I) Representative pictures (H) and quantification (I) of H&E staining in kidney tissues from CLP Fn14-KO mice with intraperitoneal administration of SIVE or Cl-Amidine. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm.

## Figure S5. Elimination of circulating macrophages impairs the synergistic effects of combined NETs and Fn14 blockade to prevent septic AKI. (A and B) Representative pictures (A) and quantification (B) of CD68<sup>+</sup> staining in spleen sections from CLP mice with intraperitoneal administration of SIVE plus ITEM-2 in the absence or presence of clodronate liposome (CL) treatment. Data are expressed as mean $\pm$ s.d. \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 100 µm. (C) Kaplan-Meier curves examining survivals of mice with intraperitoneal administration of SIVE plus ITEM-2 in the absence or presence of clodronate liposome (CL) treatment at the indicated times after CLP challenge. Log-rank t test was used to caculate the *P* value. (D and E) Representative pictures (D) and quantification (E) of H&E staining in renal sections from CLP mice with intraperitoneal administration of SIVE plus ITEM-2 in the absence or presence of clodronate liposome (CL) treatment. Data are expressed as mean $\pm$ s.d. \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm. (F and G)

Serum creatinine (Scr, F) and blood urea nitrogen (BUN, G) levels of CLP mice with intraperitoneal administration of SIVE plus ITEM-2 in the absence or presence of clodronate liposome (CL) treatment. Data are expressed as mean  $\pm$  s.d. (H and I) ELISA assays detecting release of HMGB1 (H) and IL-1 $\beta$  (I) in CLP mice with intraperitoneal administration of SIVE plus ITEM-2 in the absence or presence of clodronate liposome (CL) treatment. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05, one-way ANOVA, post hoc comparisons, Tukey's test.

Figure S6. Effects of combined NETs and Fn14 blockade on F4/80<sup>+</sup> macrophages, CD4<sup>+</sup>/FOXP3<sup>+</sup> regulatory, CD3<sup>+</sup> total or CD8<sup>+</sup> cytotoxic T cells and CD115<sup>+</sup> monocytes. (A and B) FACS analyses (A) and histogram (B) assessing percentage of F4/80<sup>+</sup> macrophages in Ly6G<sup>-</sup>CD45<sup>+</sup> live cells (LCs) from kidney tissues of CLP mice with intraperitoneal administration of Cl-Amidine with or without ITEM-2 administration. Data are expressed as mean  $\pm$  s.d. \*\*P < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test. (C and D) Representative imags (C) and quantification (D) of CD4<sup>+</sup>/FOXP3<sup>+</sup> regulatory T cells in renal sections from CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50  $\mu$ m. (E and F) Representative imags (E) and quantification (F) of CD3<sup>+</sup> T cells in renal sections from CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. Scale bar = 50 µm. (G and H) Representative imags (G) and quantification (H) of CD8<sup>+</sup> T cells in renal sections from CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. Scale bar = 50  $\mu$ m. (I and J) Representative imags (I) and quantification (J) of CD115<sup>+</sup> monocytes in renal sections from CLP mice with intraperitoneal administration of SIVE, ITEM-2 or both in the presence or absence of clodronate liposome (CL) treatment. Data are expressed as mean  $\pm$  s.d. \*\**P* < 0.01 and \*\*\**P* < 0.001, two-sided Student's *t*-test. Scale bar =  $50 \mu m$ .

## Figure S7. NETs interruption endows macrophages with anti-tubulotoxic

properties. (A) Experimental scheme depicting adoptive transfer of renal F4/80<sup>+</sup> macrophages from CLP mice receiving PBS, SIVE monotherapy or SIVE plus ITEM-2 combination therapy to CLP mice receiving anti-CSF1R pretreatment. (B and C) Representative pictures (B) and quantification (C) of H&E staining in renal sections from CLP mice with adoptive transfer of renal F4/80<sup>+</sup> macrophages from CLP mice receiving PBS, SIVE monotherapy or SIVE plus ITEM-2 combination therapy in the presence or absence of ITEM-2 administration. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm.

Figure S8. Genetic ablation of PAD4 abolishes NETs formation and genetic NETs interruption in combination with Fn14 mAb inhibits HMGB1 and IL-1 $\beta$ production dependent of macrophages. (A) ELISA assays testing the serum concentration of NETs in PAD4<sup>*fl/fl*</sup> and *CMV-Cre*; PAD4<sup>*fl/fl*</sup> mice with or without intraperitoneal administration of ITEM-2 upon CLP challenge. (B and C) ELISA assays detecting serum concentration of HMGB1 (B) and IL-1 $\beta$  (C) in *CMV-Cre*; PAD4<sup>*fl/fl*</sup> mice with intraperitoneal administration of ITEM-2 in the presence or absence of anti-CSF1R treatment upon CLP challenge. Data are expressed as mean ± s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test.

Figure S9. NETs blockade transcriptionally upregulates tubular cell-intrinsic Fn14 in a DNMT3a-dispensable manner. (A) Western-blotting analyses detecting Fn14 protein levels in WT and PAD4<sup>-/-</sup> RPTCs in the presence or absence of PAD4 sgRNA (sg.PAD4) transfection. (B) Western-blotting analyses determining Fn14 protein levels in the LPS-primed HK-2 cells with Cl-Amidine treatment followed by withdrawal at the indicated times. (C) Western-blotting analyses assessing DR5 and RANK protein levels in the LPS-primed HK-2 cells with SIVE plus Cl-Amidine cotreatment. (D) RT-qPCR analyses comparing levels of DR5 and RANK mRNA expression in the LPS-primed HK-2 cells with SIVE plus Cl-Amidine totreatment. (D) RT-qPCR analyses comparing levels of DR5 and RANK mRNA calculate the *P* value. (E) Western-blotting analyses detecting DNMT3a protein levels in WT and PAD4<sup>-/-</sup> RPTCs. (F) RT-qPCR analyses measuring levels of DNMT3a mRNA expression in WT and PAD4<sup>-/-</sup> RPTCs. Data are expressed as mean  $\pm$  s.d. Unpaired, two-tailed Student's *t* test was used to calculate the *P* value. (G) Western-blotting analyses evaluating DNMT3a protein levels in the LPS-primed HK-2 cells transfected with control siRNA (si.Ctrl) or DNMT3a siRNA (si.DNMT3a). (H) Luciferase assays of the LPS-primed HK-2 cells expressing full-length Fn14 promoter reporter constructs with control siRNA (si.Ctrl) or DNMT3a siRNA (si.DNMT3a) transfection in the presence or absence of SIVE plus Cl-Amidine cotreatment. Experiments were performed three times and data are expressed as mean  $\pm$  s.d. One-way ANOVA with Tukey's test post hoc comparisons was used to calculate the *P* value.

Figure S10. PAD4 deficiency transcriptionally upregulates Fn14 via stabilizing HOXA5. (A-F) Western-blotting analyses examining SPIB (A), HOXA5 (B), RXRA (C), ESRRB (D), RORA 1 (E) and CREB1 (F) protein levels in the LPS-primed HK-2 cells with control siRNA (si.Ctrl), SPIB siRNA (si.SPIB), HOXA5 siRNA (si.HOXA5), RXRA siRNA (si.RXRA), ESRRB siRNA (si.ESRBB), RORA 1 siRNA (si. RORA 1) or CREB1 siRNA (si.CREB1) transfection. (G) Western-blotting analyses determining HOXA5 protein abundance in the LPS-primed HK-2 cells transfected with HOXA5 shRNA (sh.HOXA5) in the presence or absence of Flag-tagged wild-type HOXA5 re-expression. (H) Western-blotting analyses detecting HOXA1 protein abundance in the LPS-primed HK-2 cells transfected with HOXA5 shRNA (sh.HOXA1). (I and J) Western-blotting analyses testing HOXA5 protein levels in WT and PAD4<sup>-/-</sup> RPTCs. (K) Trypan blue assay evaluating survival of WT and PAD4<sup>-/-</sup> RPTCs in the presence or absence of HOXA5 shRNA (sh.HOXA5) transfection at the indicated time points. Experiments were performed three times and data are expressed as mean  $\pm$  s.d. (L) Western-blotting analyses comparing Fn14 protein levels in WT and PAD4-/- RPTCs in the presence or absence of HOXA5 shRNA (sh.HOXA5) transfection. (M) ChIP analysis for HOXA5 and IgG binding to

Fn14 gene promoter in WT and PAD4<sup>-/-</sup> RPTCs. Enrichment of promoter region was normalized by input and data are expressed as mean ± s.d. of at least four experiments. Unpaired, two-tailed Student's *t* test was used to calculate the *P* value. (N) Western-blotting analyses detecting HOXA5 and HOXA1 protein abundance in WT and PAD4<sup>-/-</sup> RPTCs in the presence or absence of PAD4 sgRNA (sg.PAD4) transfection. (O) CHX pulse-chase experiments determining the turnover of HOXA5 protein in WT and PAD4<sup>-/-</sup> RPTCs.

Figure S11. Combination of NETs and Fn14 blockade improves renal functions in endotoxemic sepsis. (A) Blood urea nitrogen (BUN) levels of LIE mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test. (B) ELISA assay detecting release of IL-1 $\beta$  in LIE mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test.

Figure S12. Combination of NETs and Fn14 blockade improves survivals of mice and protects them against AKI in endotoxemic sepsis. (A and B) Representative images (A) and quantification (B) for H&E staining in renal sections from LIE mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm. (C) Serum creatinine (Scr) levels of LIE mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. One-way ANOVA with Tukey's test post hoc comparisons was used to calculate the *P* value.

Figure S13. Combination of NETs and Fn14 blockade improves renal functions in multidrug-resistant sepsis. (A and B) Serum creatinine (Scr, A) and blood urea nitrogen (BUN, B) levels of MDRS mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test. (C and D) ELISA assays detecting release of HMGB1 (C) and IL-1 $\beta$  (D) in MDRS mice with intraperitoneal administration of SIVE, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \**P* < 0.05 and \*\**P* < 0.01, one-way ANOVA, post hoc comparisons, Tukey's test.

Figure S14. Combination of NETs and Fn14 blockade improves survivals of mice and protects them against AKI in multidrug-resistant sepsis. (A) Kaplan-Meier curves analyzing survivals of MDRS mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both at the indicated times. Log-rank t test was used to caculate the *P* value. (B-D) Representative images (B) and quantification for H&E (C) and NGAL (D) staining in renal sections from MDRS mice with intraperitoneal administration of Cl-Amidine, ITEM-2 or both. Data are expressed as mean  $\pm$  s.d. \*\**P* < 0.01 and \*\*\**P* < 0.001, one-way ANOVA, post hoc comparisons, Tukey's test. Scale bar = 50 µm.

Figure S15. Schematic diagram illustrating the synergistic effects and mechanisms of combined NETs and Fn14 blockade against septic AKI in murine models of LIE, CLP and MDRS.





































С В 3.2 8 *P* = 0.0054 \* \* \* Score of tubulotoxicity P = 0.5597 2.8-2.4-2.0-1.6-1.2-0.8-0.4-7-6-5-4-3-2-1-0-Creatinine (mg/dl) • Ci-Anidine HEMA Crambine TEMP 0.0-







Patients	Age	Gender	SOFA	KIDIGO	Patients	Age	Gender	SOFA	KIDIGO
number			score	stage	number			score	stage
1	67	Male	N/A	N/A	40	38	Female	N/A	N/A
2	67	Male	N/A	N/A	41	32	Male	N/A	N/A
3	66	Male	N/A	N/A	42	31	Female	N/A	N/A
4	65	Male	N/A	N/A	1	52	Female	24	
5	65	Female	N/A	N/A	2	52	Female	24	III
6	63	Female	N/A	N/A	3	49	Male	21	III
7	61	Male	N/A	N/A	4	48	Male	18	III
8	61	Male	N/A	N/A	5	47	Male	17	III
9	61	Male	N/A	N/A	6	47	Male	16	III
10	60	Female	N/A	N/A	7	47	Male	16	III
11	60	Male	N/A	N/A	8	47	Male	15	III
12	59	Male	N/A	N/A	9	46	Male	15	III
13	59	Male	N/A	N/A	10	46	Male	14	III
14	56	Female	N/A	N/A	11	46	Female	13	III
15	55	Female	N/A	N/A	12	46	Male	12	III
16	54	Male	N/A	N/A	13	45	Female	11	
17	53	Male	N/A	N/A	14	45	Female	11	III
18	53	Female	N/A	N/A	15	45	Female	11	III
19	53	Male	N/A	N/A	16	45	Female	10	III
20	53	Male	N/A	N/A	17	45	Male	10	III
21	52	Male	N/A	N/A	18	45	Male	9	III
22	52	Male	N/A	N/A	19	45	Female	8	III
23	51	Male	N/A	N/A	20	40	Male	8	
24	51	Female	N/A	N/A	21	38	Male	7	III
25	51	Male	N/A	N/A	22	81	Male	17	II
26	50	Female	N/A	N/A	23	80	Male	16	II
27	49	Male	N/A	N/A	24	77	Male	15	II
28	48	Female	N/A	N/A	25	77	Male	14	II
29	46	Male	N/A	N/A	26	77	Male	14	II
30	45	Male	N/A	N/A	27	77	Female	13	II
31	44	Female	N/A	N/A	28	75	Female	12	II
32	43	Female	N/A	N/A	29	73	Male	12	II
33	43	Male	N/A	N/A	30	71	Male	10	II
34	42	Male	N/A	N/A	31	69	Male	10	II
35	42	Female	N/A	N/A	32	68	Female	10	II
36	42	Male	N/A	N/A	33	66	Male	9	II
37	42	Female	N/A	N/A	34	66	Male	9	II
38	41	Female	N/A	N/A	35	65	Male	9	II
39	40	Female	N/A	N/A	36	63	Female	9	II

Table S1 Patient information for serum NETs and Fn14 measurement

37	62	Female	8		67	60	Female	4	I
38	61	Male	8	II	68	59	Female	4	I
39	60	Male	7	II	69	59	Male	4	I
40	60	Male	7	II	70	58	Male	4	I
41	60	Male	7	II	71	58	Male	3	I
42	60	Female	7	II	72	57	Male	3	I
43	59	Male	7	II	73	56	Female	3	I
44	56	Male	6	II	74	55	Male	3	I
45	56	Male	6	II	75	54	Male	3	I
46	54	Female	6	II	76	53	Male	3	l
47	53	Female	5	II	77	53	Female	2	ĺ
48	52	Female	5	II	78	52	Female	2	I
49	52	Female	5	II	79	52	Male	2	ļ
50	51	Male	5	II	80	51	Male	2	I
51	51	Male	5	II	81	50	Male	2	I
52	50	Male	5	II	82	50	Male	2	I
53	46	Male	4	II	83	48	Male	2	ļ
54	46	Female	4	II	84	48	Male	2	I
55	43	Male	4	II	85	47	Male	2	I
56	43	Male	3	II	86	45	Male	2	I
57	35	Female	2	II	87	43	Male	2	I
58	75	Male	7	I	88	41	Male	2	I
59	68	Male	6	I	89	39	Male	2	I
60	66	Female	6	I	90	36	Male	2	I
61	63	Female	6	I	91	35	Male	2	I
62	62	Female	5	I	92	30	Male	2	I
63	61	Male	5	I	93	29	Male	2	ļ
64	60	Male	4	I	Note: The plasma of 42 healthy volunteers and				
65	60	Male	4	I	93 patients clinically diagnosed with septic AKI				
66	60	Male	4	I	were collected for neutrophilelastase (NE) and				
					Fn14 measurement.				