



# Differences in cytokine production after stimulation of whole blood of septic patients with heat-killed *Pseudomonas aeruginosa* and *Staphylococcus aureus*



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## BACKGROUND

Health-care associated infections represent a serious medical problem. Infectious complications in hospitalized patients are associated with high morbidity and mortality, prolonged hospital stay, increased antibiotic resistance and economic burden. These infections mainly affect critically ill patients whose immune system is often compromised by exaggerated anti-inflammatory response and inability to respond appropriately to infectious agents. The aim of our experimental study was to determine the production of selected pro-inflammatory and anti-inflammatory cytokines and chemokines after *ex vivo* stimulation with heat-killed *Pseudomonas aeruginosa* or *Staphylococcus aureus*.

## METHODS

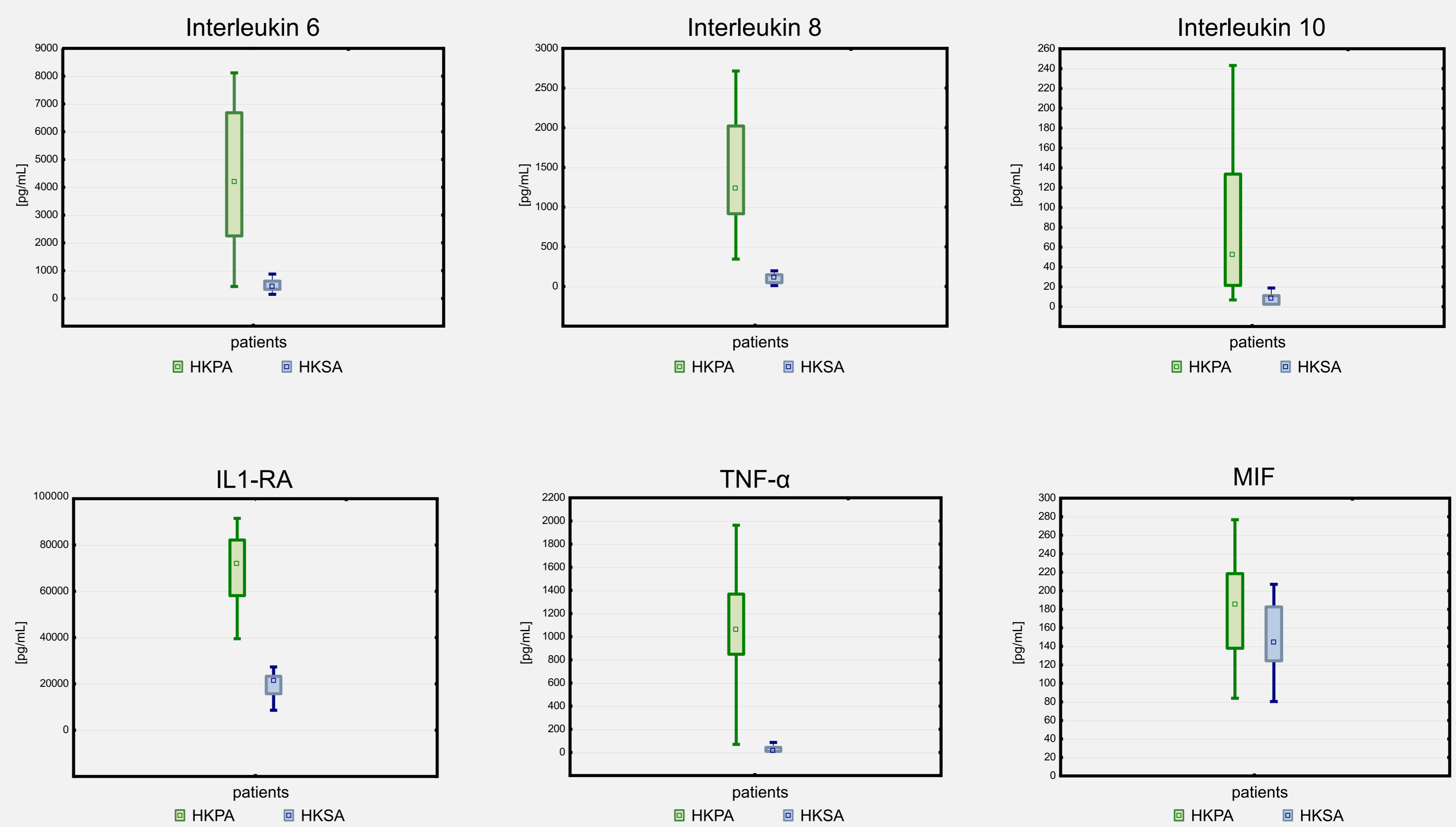
- Adult patients admitted to infectious diseases department with bacterial sepsis<sup>[1]</sup>.
- *Ex vivo* stimulation of whole blood with heat-killed bacteria.
- Experiments proceeded with *Pseudomonas aeruginosa* (Xen41) and *Staphylococcus aureus* (Xen81).
- Plasma cytokine and chemokine levels quantified by flow cytometry using multiplex bead-based immunoassay.
- Differences between groups of patients and healthy volunteers evaluated by Mann-Whitney U test.

## RESULTS

Bacterial sepsis group	n = 10
Age (mean ± SD)	51.5 ± 17.8
Sex, males (%)	5 (50%)
Hospital days (mean ± SD)	13.9 ± 11.4
Site of infection	
Urinary tract infection	5
Pneumonia	2
Skin and soft tissue infection	2
Osteomyelitis	1
Etiology	
Gram-negative	5
Gram-positive	3
Not identified <sup>#</sup>	2
Microbiology results	
<i>Escherichia coli</i>	5
<i>Streptococcus pyogenes</i>	1
<i>Streptococcus pneumoniae</i>	1
<i>Staphylococcus aureus</i>	1
Positive hemocultivation	2

Control group	n = 9
Age (mean ± SD)	47.4 ± 17.7
Sex, males (%)	5 (56%)

SD, standard deviation; <sup>#</sup>sepsis determined by Sepsis-2 criteria



IL1-RA, interleukin 1 receptor antagonist; TNF-α, tumor necrosis factor α; MIF, migration inhibitory factor; HKPA, heat-killed *Pseudomonas aeruginosa*; HKSA, heat-killed *Staphylococcus aureus*.

	Before stimulation		HKPA		HKSA		blank	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Interleukin 6 (pg/mL)	447.60 (± 872.53)	19.31 (± 6.31)	4294.91 (± 2490.24)	2749.13 (± 5.13)	712.53 (± 895.23)	150.10 (± 2.10)	584.58 (± 997.50)	23.57 (± 4.57)
Interleukin 8 (pg/mL)	19.52 (± 18.63)	5.00 (± 5.00)	1381.71 (± 779.16)	589.68 (± 5.68)	104.70 (± 59.56)	110.89 (± 0.89)	47.66 (± 45.82)	16.90 (± 4.90)
Interleukin 10 (pg/mL)	7.18 (± 5.74)	5.02 (± 6.02)	95.94 (± 105.22)	66.17 (± 4.17)	7.99 (± 5.38)	6.86 (± 7.86)	7.43 (± 4.87)	5.07 (± 7.07)
IL1-RA (pg/mL)	3568.85 (± 3451.93)	33.00 (± 0.00)	71621.83 (± 29228.89)	19409.71 (± 3.71)	20528.02 (± 8486.84)	3487.22 (± 8.22)	10951.68 (± 7963.97)	33.00 (± 0.00)
IFN-γ (pg/mL)	81.52 (± 120.34)	11.00 (± 0.00)	81.65 (± 119.06)	14.33 (± 5.33)	83.98 (± 129.09)	11.00 (± 0.00)	96.27 (± 155.08)	11.00 (± 0.00)
TNF-α (pg/mL)	8.16 (± 3.29)	7.86 (± 2.86)	1013.14 (± 585.55)	250.98 (± 2.98)	26.73 (± 26.31)	26.11 (± 4.11)	6.98 (± 0.00)	6.00 (± 9.00)
MIF (pg/mL)	67.88 (± 27.11)	49.34 (± 9.34)	181.95 (± 55.88)	129.90 (± 7.90)	145.63 (± 37.58)	113.82 (± 8.82)	169.04 (± 46.37)	120.75 (± 1.75)
MCP-1 (pg/mL)	55.11 (± 40.11)	50.30 (± 5.30)	35.69 (± 32.90)	32.80 (± 4.80)	38.05 (± 31.84)	29.36 (± 8.36)	37.56 (± 35.04)	38.84 (± 1.84)

Plasma concentration reported as mean (± standard deviation); HKPA, heat-killed *Pseudomonas aeruginosa*; HKSA, heat-killed *Staphylococcus aureus*; IL1-RA, interleukin 1 receptor antagonist; TNF-α, tumor necrosis factor; MIF, migration inhibitory factor; MIP-1β, macrophage inflammatory protein 1β; MCP-1, monocyte chemoattractant protein 1. Whole blood was *ex vivo* stimulated with heat-killed bacteria or buffer (blank) for 6 hours at 37°C.

## CONCLUSION

In our study, we observed more pronounced immunogenic potential of heat-killed *Pseudomonas aeruginosa* in comparison to *Staphylococcus aureus*. Significant elevation of anti-inflammatory cytokines after stimulation with heat-killed *P. aeruginosa* suggests that *P. aeruginosa* may actively create an immunosuppressive niche leading to subsequent secondary infection.

## ACKNOWLEDGMENTS

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## REFERENCES

<sup>[1]</sup>Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003; 31:1250-6