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(54) **BIOMARKERS FOR SEPSIS**

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(57) **ABSTRACT**

Biomarkers for sepsis and resulting mortality can be detected by assaying blood samples. Changes in the concentration of the biomarkers can be used to indicate sepsis, risk of sepsis, progression of sepsis, remission from sepsis, and risk of mortality. Changes can be evaluated relative to data sets, natural or synthetic or semisynthetic control samples, or patient samples collected at different time points. Some biomarkers' concentrations are elevated during disease and some are depressed. These are termed informative biomarkers. Some biomarkers are diagnostic in combination with others. Individual biomarkers may be weighted when used in combinations. Biomarkers can be assessed in individual, isolated or assays, in parallel assays, or in single-pot assays.

FIGURE 1.

Microarray

Slide

Subarray

Array 1, 2, 3 or 4
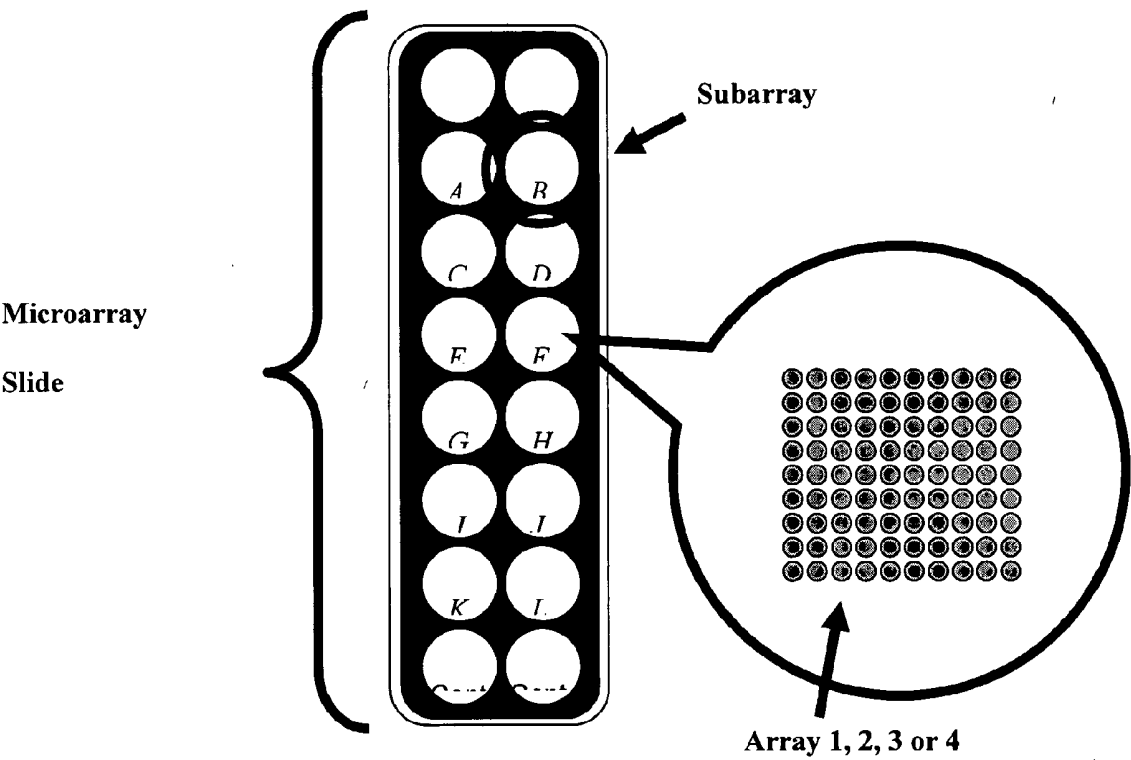
	GMSTEXPO			
GMSTEXPO	Frequency	Percent	Cumulative Frequency	Cumulative Percent
Mixed Gram	29	11.93	29	11.93
No Bacteria Expo	85	34.98	114	46.91
Pure Gram Neg	44	18.11	158	65.02
Pure Gram Pos	73	30.04	231	95.06
Unconfirm Gram	12	4.94	243	100

FIGURE 2

QDA Prevalence 0.3

Statistics	CutOff	Sensitivity	Specificity	PPV	NPV
Eot	7.06	0.92	0.7	0.57	0.95
IL-1b	1.71	0.95	0.66	0.55	0.97
MPIF-1	4.89	0.98	0.52	0.47	0.98
EGF	8.94	0.96	0.52	0.46	0.97
MCP-1	8.17	0.98	0.44	0.43	0.98
IL-2sRa	12.55	0.94	0.46	0.43	0.94
IL-1ra	10.28	0.96	0.45	0.43	0.97
TNF-R1	8.34	0.97	0.42	0.42	0.97
IL-6	3.19	0.97	0.42	0.42	0.97
ProteinC	13.89	0.98	0.41	0.41	0.98
MIF	10.08	0.95	0.43	0.41	0.95
ST2	7.54	0.97	0.39	0.4	0.97
MIP3b	7.75	0.97	0.38	0.4	0.97
OSM	7.77	0.9	0.41	0.39	0.9
MIP-1a	7.69	0.88	0.41	0.39	0.89
HCC4	10.6	0.97	0.34	0.39	0.96
ENA-78	9.74	0.94	0.37	0.39	0.93
PARC	10.16	0.94	0.3	0.37	0.92
MMP9	11.09	0.89	0.35	0.37	0.88
HVEM	10.53	0.87	0.35	0.37	0.86
P-Selectin	12.41	0.91	0.29	0.36	0.89
Leptin	8.29	0.92	0.29	0.36	0.9
BLC	5.14	0.96	0.26	0.36	0.93
IL-8	11.38	0.89	0.3	0.35	0.86
GROb	9.52	0.87	0.32	0.35	0.85
MIG	10.8	0.9	0.24	0.34	0.85
MCP-2	7.9	0.93	0.25	0.34	0.89
IL-15	6.33	0.88	0.26	0.34	0.83
M-CSF	9.12	0.9	0.22	0.33	0.84

LDA Prevalence 0.3

Statistics	CutOff	Sensitivity	Specificity	PPV	NPV
Eot	7	0.92	0.64	0.52	0.95
IL-1b	6.31	0.95	0.6	0.51	0.97
MPIF-1	8.44	0.98	0.51	0.46	0.99
EGF	8.94	0.96	0.52	0.46	0.97
TNF-R1	11.14	0.94	0.47	0.44	0.95
IL-1ra	10.38	0.96	0.44	0.43	0.96
ST2	7.45	0.98	0.42	0.42	0.98
MIF	10.19	0.94	0.44	0.42	0.95
MCP-1	10.73	0.97	0.44	0.42	0.97
IL-6	9.77	0.97	0.42	0.42	0.97
IL-2sRa	12.54	0.93	0.45	0.42	0.94
ProteinC	13.18	0.96	0.4	0.41	0.96
MIP3b	7.65	0.95	0.38	0.4	0.94
HCC4	10.92	0.96	0.38	0.4	0.96
ENA-78	9.94	0.94	0.4	0.4	0.94
PARC	11.93	0.92	0.38	0.39	0.92
OSM	7.73	0.91	0.39	0.39	0.91
MIP-1a	7.61	0.9	0.4	0.39	0.9
P-Selectin	12.59	0.91	0.32	0.32	0.9
GROb	9.16	0.91	0.33	0.37	0.9
MMP9	11.13	0.89	0.33	0.36	0.87
Leptin	9.32	0.9	0.3	0.36	0.88
HVEM	7.87	0.9	0.31	0.36	0.88
BLC	6.95	0.93	0.29	0.36	0.91
MIG	9.79	0.91	0.29	0.35	0.88
MCP-2	7.39	0.9	0.25	0.34	0.85
IL-8	11.76	0.86	0.29	0.34	0.83
IL-15	6.25	0.87	0.26	0.34	0.83
M-CSF	9.96	0.86	0.26	0.33	0.81

FIG. 3

FIGURE 4. Analyte aliases.

Array 1 analytes

	Analyte	Full Name	Aliases
1	ANG	Angiogenin	
2	BLC	B-lymphocyte chemoattractant	CXCL13, SCYB13, BCA-1
3	EGF	Epidermal growth factor	beta urogastrone, HMGF
4	ENA-78	Epithelial cell-derived neutrophil-activating peptide	CXCL5, SCYB5
5	Eot	Eotaxin	CCL11
6	Eot-2	Eotaxin-2	CCL24
7	Fas	Fibroblast-Associated	TNFRSF6, CD95, Apo-1
8	FGF-7	Fibroblast growth factor-7.	KGF-2
9	FGF-9	Fibroblast growth factor-9	GAF, HBGF-9
10	GDNF	Glial cell line derived neurotrophic factor	ATF, B49-derived neurotrophic factor
11	GM-CSF	Granulocyte macrophage colony stimulating factor	CSF-a, E-CSF
12	IL-1ra	Interleukin 1 receptor antagonist	IL-1F3, ILS, IL-1 inhibitor
13	IL-2 sRα	Interleukin 2 soluble receptor alpha	IL-2 Ra, CD25, IL-2R, p55
14	IL-3	Interleukin 3	BPA, E-CSF
15	IL-4	Interleukin 4	BCDF, BCGF
16	IL-5	Interleukin 5	BCDFm, BCDFm
17	IL-6	Interleukin 6	26-kDa protein, BSF-2
18	IL-7	Interleukin 7	LP-1; Lpo-1, Pre-B cell growth factor
19	IL-8	Interleukin 8	CXCL8, chemotaxin, GCP
20	IL-13	Interleukin 13	NC30, p600
21	IL-15	Interleukin 15	Interleukin T (IL-T)
22	MCP-2	Monocyte chemotactic protein 2	CCL8, HC14, SCYA8
23	MCP-3	Monocyte chemotactic protein 3	CCL7, MARC (mouse), FIC (mouse)
24	MIP-1α	Macrophage inflammatory protein 1 alpha	CCL3, GOS19, LD78 alpha
25	MPIF	Myeloid progenitor inhibitory factor 1	
26	OSM	Oncostatin M	OM; OncoM; ONC
27	PLGF	Placental growth factor	

Array 2 analytes

	Analyte	Full Name	Aliases
1	AR	Amphiregulin	AMR; AREG, CRDGF, KAF, SDGF
2	BDNF	Brain-derived neurotrophic factor	
3	Flt-3 Lig	fms-like tyrosine kinase-3 ligand	Flt-3/Flk-2 ligand, FL, Stk-1 ligand
4	GCP-2	Granulocyte chemotactic protein 2	CXCL6, CKA-3 (mouse), LIX (mouse, rat)
5	HCC4	Hemofiltrate CC chemokine 4	CCL16, ILINCK, interleukin 10 inducible chemokine, NCC4
6	I-309	I-309	CCL1, p500 (mouse), SCYA1
7	IL-1α	Interleukin 1 alpha	IL-1F1, BAF; BCAF, EP
8	IL-1β	Interleukin 1 beta	IL-1F2, catabolin, H1
9	IL-2	Interleukin 2	BF, costimulator
10	IL-17	Interleukin 17	CTLA-8
11	MCP-1	Monocyte chemotactic protein 1	CCL2, GDCF, HCII
12	M-CSF	Macrophage colony stimulating factor	CSA, CSF-1
13	MIG	Monokine induced by interferon gamma	CXCL9, SCYB9
14	MIP-1β	Macrophage inflammatory protein 1 beta	CCL4, ACT-2, G26
15	MIP-1α	Macrophage inflammatory protein 1 delta	LKN-1, CCL15, CC-2, HCC-2, MIP-5, NCC-3, SCYA15
16	NT-3	Neurotrophin 3	HDNF, NGF-2
17	NT-4	Neurotrophin 4	NT-4/5, NT-5
18	PARC	Pulmonary and activation-regulated chemokine	CCL18, AMAC-1, Dctactin
19	RANTES	Regulated upon activation, normal T expressed and presumably secreted	CCL5, EoCP-1, SCYA5
20	SCF	Stem cell factor	c-kit Ligand (KL), HLGF-1
21	Sgp130	Soluble glycoprotein 130	CD130, IL6ST
22	TARC	Thymus and activation regulated chemokine	CCL17, SCYA17
23	TNF-RI	Tumor necrosis factor receptor I	TNFRSF1A, CD120a, p55, p55TNFR
24	TNF-α	Tumor necrosis factor alpha	TNFSF2, cachectin, cytotoxin
25	TNF-β	Tumor necrosis factor beta	TNFSF1, differentiation-inducing factor, cytotoxin

	Analyte	Full Name	Aliases
26	VEGF	Vascular endothelial growth factor	VEGF-A, folliculo-stellate cell-derived growth factor, GD-VEGF, vascular endothelial cell proliferation factor, VPF, VAS

Array 3 analytes

	Analyte	Full Name	Aliases
1	BTC	Betacellulin	BCCIP
2	DR6	Death receptor 6	TNFRSF21
3	Fas Lig	Fibroblast-associated ligand	TNFSF6, CD178, Apo-1 ligand; APT1L, CD95L
4	FGF acid	Fibroblast growth factor acidic	FGF-1, a-ECGF, astroglial growth factor I
5	Fractalkine	Fractalkine	FKN; FK, CX3CL1, ABCD-3, CX3C membrane-anchored chemokine, NTN, SCYD1
6	GRO-β	Growth related oncogene beta	CXCL2, CINC-2a (rat), GRO-2
7	HCC-1	Hemofiltrate CC chemokine 1	CCL14, Chb-1, chemokine CC-1/CC-3
8	HGF	Hepatocyte growth factor	HPTA, Scatter Factor (SF)
9	HVEM	Herpes virus entry mediator	TNFRSF14, ATAR, HveA
10	ICAM-3	Intercellular adhesion molecule 3	CD50
11	IGFBP-2	Insulin-like growth factor binding protein 2	
12	IL-2 Rγ	Interleukin 2 receptor gamma	CD132, common gamma chain
13	IL-5 Rα	Interleukin 5 receptor alpha	CD125, CDw125
14	IL-9	Interleukin 9	MCGF, MEA (mouse)
15	Leptin	Leptin	Obesity (OB)
16	L-Selectin	Leukocyte selectin	CD62L, LAM-1, LECAM-1
17	MCP-4	Monocyte chemotactic protein 4	CCL13, CKb-10, NCC-1
18	MIP-3β	Macrophage inflammatory protein 3 beta	CCL19, CKb-11, Exodus-3
19	MMP-7 (total)	Matrix metalloproteinase 7	Matrilysin, Pump-1
20	MMP-9	Matrix metalloproteinase 9	2 kDa gelatinase, gelatinase B
21	PECAM-1	Platelet endothelial cell adhesion molecule-1	CD31
22	RANK	Receptor activator of NF-kappa-B	TNFRSF11A, ODAR, ODFR
23	SCF R	Stem cell factor receptor	CD117, c-kit
24	TIMP-1	Tissue inhibitors of metalloproteinases 1	
25	TRAIL R4	TNF-related apoptosis-inducing ligand receptor 4	TNFRSF10D, DcR2, TRUNDD
26	VEGF-R2	Vascular endothelial growth factor receptor 2	KDR, Flk-1 (mouse)
27	ST2	Interleukin 1 receptor 4	IL-1R4, DER4 (mouse)

Array 4 analytes

	Analyte	Full Name	Aliases
1	ALCAM	Activated leukocyte cell adhesion molecule	CD166, BEN (chicken), DM-GRASP (chicken)
2	β-NGF	beta-nerve growth factor	
3	CD27	Cluster of Differentiation 27	TNFRSF7, S152, T14
4	CTACK	Cutaneous T-cell attracting chemokine	CCL27, ALP, Eskin
5	CD30	Cluster of Differentiation 30	TNFRSF8, Ber-H2 antigen, Ki-1 antigen
6	Eot-3	Eotaxin-3	CCL26, MIP-4a, SCYA26
7	FGF-2	Fibroblast growth factor-2	FGF-basic, astroglial GF 2, cartilage-derived growth factor
8	FGF-4	Fibroblast growth factor-4	Hst
9	Follistatin	Follistatin	FS, activin binding protein, FSP
10	GRO-γ	Growth related oncogene gamma	CXCL3, CINC-2b(rat), GRO-3
11	ICAM-1	Intercellular adhesion molecule 1	CD54
12	IFN-γ	Interferon gamma	Antigen-Induced Interferon
13	IFN-ω	Interferon omega	Interferon alpha II (IFN-aII)
14	IGF-1R	Insulin-like growth factor I receptor	CD221
15	IGFBP-1	Insulin-like growth factor binding protein 1	34kD IGFBP, alpha pregnancy-associated endometrial globulin
16	IGFBP-3	Insulin-like growth factor binding protein 3	Binding Protein 29, BP-53
17	IGFBP-4	Insulin-like growth factor binding protein 4	Colon Cancer Cell Growth Inhibitor, HT29-IGFBP
18	IGF-II	Insulin-like growth factor II	growth-promoting activity for vascular endothelial cells, MSA
19	IL-1 sR1	Interleukin 1 soluble receptor I	CD121a, IL-1RI
20	IL-1 sRII	Interleukin 1 soluble receptor II	CDw121b, IL-1R2
21	IL-10 Rβ	Interleukin 10 receptor beta	CRF2-4, CRFB-4
22	IL-16	Interleukin 16	LCF
23	IL-2 Rβ	Interleukin 2 receptor beta	CD122, p75
24	I-TAC	Interferon gamma-inducible T cell alpha chemoattractant	CXCL11, B-R1, H174
25	Lptn	Lymphotactin	XCL1, Ltn, ATAC, SCM-1a
26	LT βR	lymphotoxin-beta receptor	TNFRSF3, TNFRIII, TNFRrp

	Analyte	Full Name	Aliases
27	M-CSF R	Macrophage colony stimulating factor receptor	CD115, c-fms, CSFR1
28	MIP-3α	Macrophage inflammatory protein 3 alpha	CCL20, Exodus-1, LARC
29	MMP-10	Matrix metalloproteinase 10	Stromelysin 2, Transin 2
30	PDGF Rα	Platelet-derived growth factor receptor alpha	CD140a
31	PF4	Stromal cell-derived factor beta	CXCL4, CTAP-3, Endothelial cell growth inhibitor, Platelet factor 4
32	sVAP-1	Soluble Vascular Adhesion Protein-1	
33	TGF-α	Transforming growth factor alpha	Sarcoma Growth Factor
34	TIMP-2	Tissue inhibitors of metalloproteinases 2	
35	TRAIL R1	TNF-related apoptosis-inducing ligand receptor 1	TNFRSF10A, DR4
36	VE-cadherin	Vascular Endothelial Cadherin	VECAD, CD144, 7B4, Cadherin-5
37	VEGF-D	Vascular endothelial growth factor-D	c-fos-induced growth factor (FIGF)

Array 5 analytes

	Analyte	Full Name	Aliases
1	4-1BB	4-1BB	TNFRSF9, CDw137, ILA CD137
2	ACE-2	Angiotensin I converting enzyme-2	
3	AFP	Alpha fetoprotein	HPAFP
4	AgRP	Agouti-related protein	ART
5	CD141	Thrombomodulin/CD141	Fetomodulin, TM
6	CD40	CD40	TNFRSF5, Bp50
7	CNTF Rα	Ciliary neurotrophic factor receptor alpha	
8	CRP	C-reactive protein	
9	D-Dimer	D-Dimer	
10	E-Selectin	E-selectin	CD62E, ELAM-1, LECAM-2
11	FGF-21	Fibroblast growth factor 21	
12	HCG	Human chorionic gonadotrophin	
13	IGFBP-6	Insulin-like Growth Factor Binding Protein 6	
14	IL-12 (p40)	Interleukin 12 p40	
15	IL-18	Interleukin 18	IGIF, IL-1gamma
16	LIF Rα	Leukemia inhibitory factor souble receptor alpha	gp190
17	MIF	Macrophage migration inhibitory factor	Glycosylation Inhibition Factor (GIF)
18	MMP-8 (total)	Matrix Metalloproteinase-8	Collagenase 2, Neutrophil Collagenase
19	NAP-2	Neutrophil Activating Peptide 2	CXCL7
20	Neutrophil elastase	Neutrophil elastase	
21	PAI-II	plasminogen activator inhibitor-II	PAI-2
22	Prolactin	Prolactin	PRL, Lactotropin, LTH
23	Protein C	Human Protein C	
24	Protein S	Human Protein S	
25	P-Selectin	P-Selectin	CD62P, GMP-140, PADGEM
26	TSH	Thyroid stimulating hormone	

FIGURE 5A
SlideMapName Pr te mics1.4

Analyte Name	Variabl	RunDay	DX						Pr > F
			Diff (1-2)		Sepsis		Sick		
			Mean	Std Dev	M an	Std Dev	Mean	Std D v	
BLC	visit1	d1	0.63	1.17	7.32	1.26	6.69	0.62	0.00041
		d2	0.82	1.18	6.95	1.24	6.13	0.86	0.000004
		d3	0.65	1.2	7.7	1.27	7.05	0.84	0.000404
EGF	visit1	d1	-2.2	1.3	7.89	1.34	10.09	1.1	<.000001
		d2	-2.27	1.31	7.4	1.23	9.67	1.63	<.000001
		d3	-2.31	1.24	8.13	1.27	10.44	1.1	<.000001
ENA-78	visit1	d1	-1.35	1.67	9.36	1.74	10.7	1.36	<.000001
		d2	-1.87	1.59	8.81	1.64	10.68	1.35	<.000001
		d3	-1.63	1.66	9.29	1.75	10.92	1.24	<.000001
Eot	visit1	d1	-0.56	0.53	6.78	0.47	7.34	0.71	<.000001
		d2	-0.87	0.62	6.32	0.54	7.19	0.9	<.000001
		d3	-0.82	0.56	6.79	0.55	7.61	0.62	<.000001
GDNF	visit1	d1	-0.23	0.39	7.34	0.39	7.56	0.43	0.000152
		d2	-0.26	0.46	6.84	0.44	7.1	0.53	0.000245
IL-15	visit1	d2	0.51	0.72	6.15	0.72	5.63	0.7	0.000002
		d3	0.24	0.64	6.6	0.66	6.36	0.5	0.011704
IL-1ra	visit1	d1	4.14	2.43	12.6	2.62	8.47	1.39	<.000001
		d2	4.27	2.59	12.25	2.75	7.97	1.76	<.000001
		d3	3.88	2.44	12.52	2.65	8.65	1.33	<.000001
IL-2sRa	visit1	d1	1.9	1.21	13.49	1.2	11.59	1.26	<.000001
		d2	1.84	1.35	13.32	1.36	11.49	1.29	<.000001
		d3	1.75	1.26	13.62	1.26	11.87	1.24	<.000001
IL-6	visit1	d1	3.61	2.53	11.7	2.73	8.09	1.4	<.000001
		d2	3.94	2.65	11.4	2.85	7.47	1.59	<.000001
		d3	3.49	2.4	11.79	2.58	8.31	1.42	<.000001
IL-8	visit1	d1	-0.84	1.64	11.33	1.68	12.17	1.47	0.00087
		d2	-1.02	1.84	11.08	1.9	12.1	1.56	0.000222
		d3	-0.89	1.8	11.55	1.88	12.44	1.44	0.001392
MCP-2	visit1	d1	1.01	1.68	8.18	1.82	7.16	0.9	0.00009
		d2	1	1.6	7.31	1.73	6.31	0.9	0.000033
		d3	0.67	1.62	8.06	1.74	7.4	0.91	0.007912
MIP-1a	visit1	d1	-0.44	0.64	7.68	0.61	8.12	0.73	0.000009
		d2	-0.58	0.63	7.11	0.59	7.68	0.77	<.000001
		d3	-0.41	0.58	7.34	0.59	7.75	0.54	0.000005

			DX						Pr > F
			Diff (1-2)		Sepsis		Sick		
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	
Analyte Name	Variabl	RunDay							
MPIF-1	visit1	d1	2.12	1.28	9.72	1.37	7.6	0.78	<.000001
		d2	2.17	1.32	9.18	1.4	7.02	0.9	<.000001
		d3	1.96	1.25	9.62	1.33	7.66	0.87	<.000001
OSM	visit1	d1	-0.66	0.64	7.8	0.65	8.46	0.63	<.000001
		d2	-0.56	0.66	7.41	0.57	7.98	0.95	<.000001

FIGURE 5B
SlideMapName Pr teomics2.3

Analyt Name	Variable	RunDay	DX Diff (1-2) Mean	DX Diff (1-2) Std Dev	DX Sepsis Mean	DX Sepsis Std Dev	DX Sick Mean	DX Sick Std Dev	Pr > F
AR	visit1	d1	0.25	0.59	7.36	0.62	7.11	0.46	0.005993
		d3	0.36	0.58	7.17	0.59	6.81	0.53	0.000114
HCC4	visit1	d1	-1.46	1.6	10.68	1.74	12.14	0.77	<.000001
		d2	-1.65	1.67	9.93	1.77	11.57	1.11	<.000001
		d3	-1.56	1.6	10.07	1.76	11.63	0.99	<.000001
IL-1a	visit1	d1	-0.24	0.56	6.68	0.57	6.92	0.51	0.003906
		d3	0.24	0.59	6.7	0.62	6.45	0.52	0.010592
IL-1b	visit1	d1	-1.56	0.81	5.77	0.71	7.34	1.14	<.000001
		d2	-1.37	0.92	5.25	0.84	6.62	1.19	<.000001
		d3	-1.12	0.76	6.37	0.61	7.5	1.08	<.000001
M-CSF	visit1	d1	0.36	0.83	10.37	0.87	10.01	0.66	0.00439
		d2	0.29	0.87	10.09	0.91	9.79	0.66	0.024124
MCP-1	visit1	d1	2.14	1.57	12.43	1.69	10.29	0.84	<.000001
		d2	1.97	1.78	11.75	1.95	9.78	0.73	<.000001
		d3	2.87	1.64	11.86	1.83	9	0.9	<.000001
MIG	visit1	d1	1.44	2.24	10.6	2.39	9.16	1.41	0.000023
		d2	1.39	2.27	10.47	2.41	9.08	1.56	0.000054
		d3	1.91	2.2	11.12	2.36	9.2	1.64	<.000001
PARC	visit1	d1	1.1	0.97	12.62	0.98	11.52	0.92	<.000001
		d2	0.78	0.97	12.84	0.97	12.06	0.94	<.000001
		d3	0.87	1.01	11.93	1.06	11.06	0.86	<.000001
Rantes	visit1	d2	-0.17	0.51	15.63	0.56	15.8	0.18	0.027227
		d3	0.31	0.42	15.35	0.47	15.04	0.2	0.000005
SCF	visit1	d2	0.23	0.74	7.23	0.74	7	0.7	0.033637
		d3	0.4	0.6	7.27	0.64	6.88	0.46	0.000057
TNF-R1	visit1	d1	1.79	1.02	12.46	1.07	10.68	0.79	<.000001
		d2	1.68	1.18	12.36	1.2	10.68	1.07	<.000001
		d3	1.47	1.26	11.23	1.27	9.76	1.26	<.000001
TNF-a	visit1	d2	0.37	0.64	7.56	0.66	7.19	0.56	0.000122
		d3	0.25	0.62	8.08	0.6	7.84	0.66	0.013088
VEGF	visit1	d1	0.23	0.59	8.47	0.62	8.24	0.48	0.010749
		d2	0.22	0.58	7.92	0.62	7.7	0.39	0.012154
sgp130	visit1	d1	-0.15	0.47	12.52	0.48	12.67	0.41	0.039511
		d3	-0.16	0.44	13.11	0.45	13.28	0.44	0.023336

FIGURE 5C
SlideMapNam Pr teomics3.4

| Analyte Name | Variabl | RunDay | DX | | | | | | Pr > F |
| | | | Diff (1-2) | | Sepsis | | Sick | | |
			Mean	Std Dev	Mean	Std Dev	M an	Std Dev	
DR6	visit1	d1	0.39	0.52	9.47	0.56	9.08	0.33	0.000001
		d2	0.25	0.58	9.01	0.61	8.76	0.43	0.004055
GROb	visit1	d2	-0.57	1.1	8.38	1.17	8.95	0.76	0.000664
		d3	-0.74	1.19	9.42	1.26	10.17	0.84	0.000202
HCC1	visit1	d1	0.33	0.52	11.27	0.52	10.95	0.49	0.000045
		d2	0.61	0.48	11.08	0.51	10.47	0.31	<.000001
		d3	0.23	0.3	11.16	0.31	10.93	0.28	0.000002
HVEM	visit1	d1	0.87	0.77	8.45	0.84	7.58	0.43	<.000001
		d2	0.6	0.88	8.09	0.95	7.49	0.51	0.000009
		d3	0.36	0.84	8.19	0.87	7.83	0.69	0.007135
IGFBP2	visit1	d1	0.58	0.75	13.95	0.79	13.37	0.52	<.000001
		d2	1	0.7	13.66	0.72	12.65	0.63	<.000001
		d3	0.15	0.47	13.61	0.45	13.46	0.52	0.033055
Leptin	visit1	d1	-1.41	2.56	9.64	2.62	11.05	2.08	0.027099
		d2	-1.06	2.27	8.63	2.37	9.69	1.81	0.001948
		d3	-0.92	2.25	9.24	2.35	10.16	1.83	0.009683
MIP3b	visit1	d1	1.34	0.97	8.47	1.06	7.13	0.49	<.000001
		d2	1.09	1.03	8	1.11	6.91	0.58	<.000001
		d3	1.03	1.03	8.27	1.1	7.25	0.62	<.000001
MMP9	visit1	d1	1.18	1.07	11.72	1.07	10.54	1.1	<.000001
		d2	1.06	1.07	11.59	1.07	10.53	1.03	<.000001
		d3	0.86	1.01	11.62	0.97	10.76	1.16	<.000001
SCF R	visit1	d1	-0.21	0.6	9.21	0.63	9.42	0.46	0.02359
		d2	-0.3	0.61	8.88	0.65	9.18	0.39	0.0011
		d3	-0.29	0.52	9.16	0.53	9.44	0.48	0.000432
ST2	visit1	d1	1.75	1.24	8.6	1.34	6.85	0.67	<.000001
		d2	1.43	1.25	8	1.36	6.57	0.59	<.000001
		d3	1.49	1.32	8.37	1.44	6.88	0.59	<.000001
TIMP1	visit1	d1	1.15	0.52	13.44	0.53	12.3	0.5	<.000001
		d2	1.09	0.61	13.27	0.64	12.18	0.45	<.000001
		d3	0.53	0.41	13.17	0.4	12.64	0.45	<.000001

FIGURE 5D
SlideMapName Prot_mics4.5

Analyte Name	Variable	RunDay	DX						Pr > F
			Diff (1-2)		Sepsis		Sick		
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	
ALCAM	visit1	d1	-0.37	0.6	10.97	0.62	11.34	0.51	0.000045
		d2	-0.57	0.74	10.38	0.74	10.95	0.73	<.000001
FGF-4	visit1	d1	-0.5	0.42	7.63	0.42	8.14	0.44	<.000001
		d2	-0.55	0.47	7.71	0.48	8.26	0.43	<.000001
Follistatin	visit1	d1	0.36	0.86	9	0.92	8.64	0.58	0.005153
		d2	0.35	0.93	9.05	0.99	8.71	0.61	0.011832
		d3	0.46	0.81	8.9	0.84	8.43	0.7	0.000234
GRO-g	visit1	d1	-1.37	1.47	10.44	1.56	11.81	1.03	<.000001
		d2	-1.7	1.83	9.99	1.98	11.69	1.17	<.000001
		d3	-0.69	1.44	10.06	1.49	10.74	1.18	0.002013
I-TAC	visit1	d1	-2.25	1.66	8.21	1.63	10.47	1.79	<.000001
		d2	-1.79	1.52	8.17	1.47	9.96	1.71	<.000001
		d3	-1.61	1.61	8.2	1.57	9.81	1.78	<.000001
ICAM-1	visit1	d1	-0.28	0.66	12.31	0.61	12.59	0.84	0.004909
		d2	-0.33	0.71	11.91	0.73	12.24	0.66	0.002788
		d3	-0.18	0.46	12.91	0.45	13.09	0.48	0.008961
IFN-g	visit1	d1	-0.82	0.78	6.15	0.79	6.96	0.74	<.000001
		d2	-0.56	0.64	6.05	0.64	6.61	0.67	<.000001
		d3	-0.52	0.84	5.93	0.87	6.45	0.73	0.000093
IGF-II	visit1	d1	-1.14	0.91	11.25	0.97	12.4	0.55	<.000001
		d2	-1.17	0.92	10.85	0.99	12.02	0.53	<.000001
		d3	-1.09	0.84	11.94	0.91	13.04	0.44	<.000001
IGF-IR	visit1	d1	-0.52	0.47	9.07	0.46	9.59	0.51	<.000001
		d2	-0.26	0.46	8.6	0.47	8.87	0.45	0.000152
		d3	-0.24	0.32	8.84	0.33	9.08	0.29	0.000002
IGFBP-1	visit1	d1	2.52	1.19	14.79	1.07	12.27	1.61	<.000001
		d2	2.54	1.23	14.18	1.16	11.64	1.5	<.000001
		d3	2.68	1.16	14.62	1.03	11.93	1.63	<.000001
IGFBP-3	visit1	d1	-0.49	0.7	12.43	0.73	12.91	0.56	0.000004
		d2	-0.71	0.88	10.78	0.92	11.49	0.69	<.000001
		d3	-0.58	0.56	12.77	0.59	13.36	0.43	<.000001
IGFBP-4	visit1	d2	-0.28	0.69	10.67	0.72	10.95	0.53	0.006516
		d3	-0.18	0.47	11.59	0.46	11.78	0.5	0.010541
IL-10rb	visit1	d1	-0.31	0.64	8.59	0.65	8.9	0.58	0.001268
		d3	0.35	0.64	8.6	0.64	8.25	0.66	0.000563

| Analyte Name | Variable | RunDay | DX | | | | | | Pr > F |
| | | | Diff (1-2) | | Sepsis | | Sick | | |
			Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	
IL-16	visit1	d1	-1.34	0.88	6.79	0.82	8.12	1.09	<.000001
		d2	-0.77	0.75	6.61	0.72	7.38	0.87	<.000001
		d3	-1.28	0.87	6.57	0.77	7.85	1.2	<.000001
IL-1srII	visit1	d1	1.06	1.27	9.15	1.36	8.09	0.84	<.000001
		d2	0.73	1.26	8.97	1.35	8.24	0.79	0.000112
		d3	1.58	1.37	9.26	1.48	7.68	0.71	<.000001
IL-2rb	visit1	d2	-0.51	0.49	7.34	0.49	7.85	0.48	<.000001
LT bR	visit1	d1	0.63	0.88	9.76	0.89	9.12	0.82	0.000002
		d2	1.01	1.03	9.78	1.04	8.77	1	<.000001
		d3	1.28	1	9.61	1	8.32	0.97	<.000001
MIP-3a	visit1	d1	0.69	1.44	9.2	1.54	8.51	0.88	0.001427
		d2	0.95	1.49	9.22	1.61	8.27	0.87	0.000023
		d3	0.92	1.43	9.27	1.52	8.35	0.99	0.000028
PDGF-Ra	visit1	d2	-0.18	0.57	7.25	0.59	7.43	0.48	0.045536
		d3	0.31	0.56	7.51	0.56	7.19	0.55	0.000332
PF4	visit1	d1	-0.57	0.51	14.69	0.55	15.26	0.27	<.000001
		d3	-0.35	0.42	14.71	0.46	15.06	0.21	<.000001
VAP-1	visit1	d1	-0.24	0.61	13.57	0.65	13.81	0.39	0.008642
		d2	-0.38	0.6	13.47	0.63	13.85	0.42	0.000021
		d3	-0.65	0.54	13.35	0.58	14	0.34	<.000001
VE-cadherin	visit1	d1	-0.61	0.65	11.76	0.67	12.37	0.59	<.000001
		d2	-0.52	0.78	11.11	0.82	11.63	0.62	0.000012
		d3	-0.61	0.56	12.46	0.57	13.07	0.54	<.000001
VEGF-D	visit1	d2	-0.53	0.59	6.29	0.56	6.82	0.73	0.000002
		d3	-0.23	0.46	8.05	0.43	8.29	0.55	0.001004

FIGURE 5E

SlideMapName Proteomics5.3l

| Analyte Name | Variable | RunDay | DX | | | | | | Pr > F |
| | | | Diff (1-2) | | Sepsis | | Sick | | |
			Mean	Std D v	M an	Std D v	Mean	Std Dev	
AFP	visit1	d1	-1.06	0.95	8.57	0.97	9.63	0.85	<.000001
		d2	-0.97	0.95	8.59	0.96	9.57	0.88	<.000001
		d3	-1.03	0.97	8.3	1	9.33	0.83	<.000001
CD141	visit1	d1	0.4	0.9	9.9	0.94	9.5	0.72	0.002805
		d2	0.55	·0.85	9.29	0.87	8.74	0.78	0.00002
		d3	0.52	0.84	9.57	0.88	9.05	0.6	0.000045
CNTF Ra	visit1	d1	-0.15	0.51	7.19	0.54	7.35	0.38	0.043545
		d2	-0.46	0.5	7.25	0.49	7.71	0.57	<.000001
		d3	-0.34	0.54	7.08	0.52	7.42	0.59	0.000028
CRP	visit1	d1	-0.3	0.43	13.1	0.46	13.4	0.28	0.000003
		d2	-0.46	0.57	13.21	0.62	13.67	0.26	<.000001
		d3	-0.48	0.47	13.08	0.5	13.56	0.32	<.000001
E-Selectin	visit1	d1	1.15	1.23	12.46	1.29	11.31	0.94	<.000001
		d2	1.38	1.25	12.43	1.31	11.05	0.94	<.000001
		d3	1.32	1.21	12.5	1.29	11.18	0.8	<.000001
FGF21	visit1	d1	1.28	1.39	9.43	1.51	8.15	0.62	<.000001
		d2	1.27	1.41	9.69	1.52	8.43	0.8	<.000001
		d3	1.24	1.35	9.3	1.46	8.07	0.78	<.000001
HCG	visit1	d1	-0.32	0.82	6.52	0.72	6.84	1.15	0.009049
		d2	-0.28	0.76	6.66	0.66	6.93	1.1	0.015729
		d3	-0.43	0.78	6.64	0.7	7.07	1.04	0.000248
IGFBP-6	visit1	d2	0.31	0.44	12.85	0.47	12.54	0.3	0.000003
		d3	0.26	0.45	12.9	0.48	12.64	0.3	0.000137
IL-18	visit1	d1	0.48	1.03	10.7	1.07	10.22	0.81	0.002857
		d3	0.62	1	10.78	1.04	10.16	0.85	0.000053
LIF Ra	visit1	d2	-0.18	0.37	7.49	0.39	7.67	0.3	0.001838
		d3	-0.15	0.38	7.5	0.38	7.65	0.36	0.007441
MIF	visit1	d1	-1.39	1.25	9.36	1.28	10.75	1.08	<.000001
		d2	-1.24	1.16	9.32	1.21	10.57	0.89	<.000001
		d3	-1.09	1.16	9.06	1.21	10.15	0.88	<.000001
MMP-8	visit1	d1	1.85	1.26	14.26	1.11	12.41	1.75	<.000001
		d2	1.78	1.16	14.3	1.02	12.52	1.61	<.000001
		d3	1.91	1.2	14.07	1.09	12.16	1.58	<.000001

			DX						Pr > F
			Diff (1-2)		Sepsis		Sick		
			Mean	Std Dev	Mean	Std Dev	Mean	Std D v	
Analyt Name	Variable	RunDay							
Neut Elast	visit1	d2	0.32	0.55	12.52	0.55	12.2	0.57	0.00011
		d3	0.34	0.51	12.28	0.52	11.94	0.48	0.000012
P-Selectin	visit1	d1	-0.7	1.04	12.04	1.07	12.74	0.9	0.000009
		d2	-0.58	0.97	12.06	1.03	12.65	0.71	0.000069
		d3	-0.34	0.79	12.11	0.81	12.45	0.69	0.004137

Analyte Name	Variable	RunDay	DX						Pr > F
			Diff (1-2)		Sepsis		Sick		
			M an	Std Dev	Mean	Std Dev	Mean	Std Dev	
PAI-II	visit1	d1	-1.01	0.94	6.98	0.94	7.99	0.96	<.000001
		d2	-0.69	1.04	7.02	1.04	7.71	1.03	0.000011
ProteinC	visit1	d1	-0.39	0.49	12.87	0.49	13.27	0.49	<.000001
		d2	-0.17	0.42	12.75	0.45	12.92	0.24	0.007921
		d3	-0.16	0.43	12.63	0.47	12.8	0.23	0.011405
ProteinS	visit1	d1	0.26	0.4	12	0.38	11.74	0.44	0.00001
		d2	0.28	0.44	12	0.46	11.72	0.35	0.000033
		d3	0.1	0.3	11.85	0.32	11.75	0.21	0.030524
TSH	visit1	d1	-0.32	0.49	6.83	0.48	7.15	0.53	0.000014
		d2	-0.48	0.44	7.03	0.46	7.51	0.35	<.000001
		d3	-0.18	0.39	6.96	0.4	7.14	0.32	0.001454

FIGURE 6A

Analyt Name	THERAPY	Variabl	RunDay	SURVQUES						
				Aliv at Day 28		D ad at Day 28		Diff (1-2)		Pr > F
				Mean	Std Dev	Mean	Std D v	Mean	Std Dev	
BLC	PLACEBO	visit1	d1	7.05	1.01	7.65	1.44	-0.61	1.25	0.003882
			d2	6.74	1.04	7.23	1.35	-0.49	1.22	0.0089
			d3	7.34	1.09	8.05	1.36	-0.71	1.24	0.001368
		visit2	d1	7.07	1.13	7.54	1.39	-0.47	1.28	0.01435
			d2	6.68	1.13	7.15	1.25	-0.47	1.2	0.008795
			d3	7.36	1.12	7.85	1.34	-0.49	1.25	0.021496
EGF	PLACEBO	visit1	d1	8.12	1.4	7.52	1.12	0.61	1.26	0.012925
			d3	8.36	1.44	7.82	1.11	0.54	1.28	0.025237
Eot2	PLACEBO	visit1	d1	11.13	1.02	10.57	1.07	0.55	1.05	0.00116
			d2	10.77	0.98	10.27	1.13	0.5	1.06	0.005755
			d3	11.05	1.03	10.55	1.15	0.5	1.1	0.00602
		visit2	d1	11.09	1.23	10.68	1	0.41	1.11	0.021314
			d2	10.73	1.07	10.31	1.13	0.43	1.1	0.041176
			d3	11.03	1.13	10.59	1.09	0.45	1.11	0.023063
IL-15	PLACEBO	visit1	d1	6.22	0.68	6.52	0.61	-0.3	0.65	0.006281
			d2	5.93	0.64	6.26	0.72	-0.33	0.68	0.001209
			d3	6.47	0.63	6.69	0.64	-0.22	0.63	0.019567
		visit2	d1	6.25	0.62	6.64	0.69	-0.39	0.66	0.000391
			d2	5.81	0.78	6.38	0.76	-0.57	0.77	0.000004
			d3	6.33	0.62	6.74	0.79	-0.41	0.72	0.002256
IL-1ra	PLACEBO	visit1	d1	11.84	2.86	13.21	2.22	-1.37	2.54	0.00091
			d2	11.46	2.97	12.78	2.4	-1.33	2.68	0.001348
			d3	11.67	2.9	13.18	2.36	-1.51	2.63	0.000505
		visit2	d1	9.42	2.52	11.52	2.67	-2.1	2.61	0.000016
			d2	9.06	2.8	11.1	2.66	-2.04	2.72	0.000022
			d3	9.58	2.46	11.5	2.68	-1.92	2.58	0.000061
IL-2sRa	PLACEBO	visit1	d2	13.1	1.38	13.45	1.35	-0.35	1.36	0.034864
			d3	13.43	1.33	13.75	1.24	-0.31	1.28	0.044056
		visit2	d2	12.87	1.45	13.48	1.28	-0.61	1.36	0.001498
			d3	13.23	1.29	13.79	1.11	-0.57	1.19	0.001056
IL-6	PLACEBO	visit1	d1	10.93	2.92	12.03	2.51	-1.1	2.71	0.008352
			d2	10.55	3.17	11.94	2.47	-1.39	2.81	0.00127
			d3	10.98	2.92	12.39	2.3	-1.41	2.6	0.000781
		visit2	d1	9.09	2.19	11.03	2.33	-1.94	2.27	0.000007
			d2	8.68	2.31	10.6	2.51	-1.91	2.42	0.00001
			d3	9.28	2.01	11.05	2.4	-1.78	2.24	0.000056
IL-8	PLACEBO	visit1	d1	11	1.36	11.6	1.72	-0.6	1.56	0.013805
			d2	10.54	1.72	11.41	1.96	-0.87	1.86	0.002776
			d3	10.79	1.85	11.99	1.82	-1.2	1.83	0.000156
		visit2	d1	10.05	0.83	10.9	1.73	-0.85	1.39	0.000356
			d2	9.65	1.19	10.81	1.63	-1.15	1.45	0.000005
			d3	10.04	0.7	11.21	1.74	-1.17	1.38	0.000012
MCP-2	PLACEBO	visit1	d1	7.44	1.32	8.53	2.01	-1.09	1.73	0.000421
			d2	6.78	1.2	7.62	1.82	-0.85	1.56	0.00078
			d3	7.32	1.1	8.63	1.84	-1.31	1.54	0.000002
		visit2	d1	7.07	1.07	8.21	1.71	-1.13	1.44	0.000016
			d2	6.42	1	7.2	1.38	-0.79	1.22	0.000108
			d3	7	1.02	8.11	1.68	-1.1	1.42	0.000028
MCP-3	PLACEBO	visit1	d1	5.96	0.59	6.18	0.73	-0.22	0.67	0.034811
			d2	5.43	0.63	5.79	0.71	-0.35	0.67	0.001009
			d3	6	0.52	6.25	0.57	-0.25	0.55	0.014756
		visit2	d1	5.88	0.5	6.24	0.72	-0.37	0.63	0.00014
			d2	5.5	0.61	5.86	0.73	-0.36	0.68	0.000469
			d3	5.86	0.61	6.21	0.66	-0.35	0.64	0.006039

FIGURE 6B

Analyt Name	THERAPY	Variable	RunDay	SURVQUES						Pr > F
				Aliv at Day 28		D ad at Day 28		Diff (1-2)		
				M an	Std D v	Mean	Std D v	Mean	Std Dev	
AR	PLACEBO	visit1	d1	7.23	0.54	7.53	0.65	-0.3	0.6	0.000864
			d2	7.17	0.6	7.53	0.68	-0.36	0.64	0.000462
		visit2	d1	7.11	0.42	7.5	0.7	-0.39	0.59	0.000035
			d2	7.08	0.67	7.56	0.65	-0.48	0.66	0.000004
BDNF	PLACEBO	visit1	d1	12.8	1.19	11.99	1.48	0.81	1.36	0.000237
			d2	12.33	1.11	11.64	1.27	0.69	1.2	0.000612
			d3	12.8	1.42	12.1	1.24	0.7	1.31	0.012337
		visit2	d1	12.33	1.29	11.26	1.37	1.07	1.34	0.000002
			d2	11.81	1.29	10.87	1.3	0.94	1.3	0.000023
			d3	12.47	0.92	11.18	1.31	1.29	1.17	<.000001
GCP-2	PLACEBO	visit2	d1	6.66	0.7	6.94	0.73	-0.28	0.72	0.014131
			d2	6.89	0.8	7.32	0.89	-0.43	0.85	0.001527
HCC4	PLACEBO	visit1	d1	11.08	1.58	10.62	1.73	0.47	1.66	0.012208
			d2	10.34	1.69	9.96	1.73	0.38	1.71	0.043299
		visit2	d1	11.82	1.24	11.11	1.7	0.71	1.5	0.001485
			d2	10.97	1.76	10.4	1.63	0.57	1.69	0.022365
			d3	11.62	1.31	10.05	1.87	1.57	1.67	0.000288
I-309	PLACEBO	visit2	d1	6.48	0.51	6.69	0.54	-0.21	0.53	0.011069
			d2	6.85	0.77	7.33	0.79	-0.48	0.78	0.000069
IL-1b	PLACEBO	visit2	d1	5.62	0.52	5.92	0.58	-0.3	0.55	0.00116
			d2	4.88	0.7	5.52	0.92	-0.64	0.82	0.000025
M-CSF	PLACEBO	visit1	d1	10.13	0.7	10.42	0.93	-0.29	0.83	0.041699
			d2	9.75	0.76	10.24	1	-0.49	0.89	0.00166
MCP-1	PLACEBO	visit1	d1	12.03	1.64	12.63	1.79	-0.6	1.73	0.036096
			d2	11.1	1.99	12.15	2.03	-1.06	2.01	0.001137
		visit2	d1	11.23	1.29	12.37	1.65	-1.13	1.49	0.000073
			d2	10.29	1.33	11.67	1.76	-1.38	1.58	0.000004
			d3	10.62	1.55	11.78	1.76	-1.15	1.68	0.001709
MIG	PLACEBO	visit1	d1	10.07	2.17	10.92	2.31	-0.85	2.25	0.025287
			d2	9.87	2.07	10.8	2.4	-0.94	2.26	0.011627
		visit2	d1	9.42	1.81	10.66	2.21	-1.24	2.03	0.000573
			d2	8.94	1.91	10.58	2.34	-1.64	2.15	0.000043
NT4	PLACEBO	visit2	d1	6.5	0.55	6.8	0.63	-0.3	0.59	0.000959
			d2	6.77	0.8	7.31	0.89	-0.54	0.85	0.000064
Rantes	PLACEBO	visit1	d1	15.52	0.35	15.3	0.77	0.21	0.61	0.023814
			d2	15.76	0.4	15.49	0.74	0.27	0.61	0.005673
		visit2	d1	15.42	0.53	15.15	0.82	0.28	0.7	0.009634
			d2	15.62	0.67	15.27	0.88	0.35	0.79	0.005864
			d3	15.43	0.36	14.81	1.04	0.62	0.84	0.000331
SCF	PLACEBO									
		visit2	d1	6.95	0.53	7.23	0.57	-0.28	0.55	0.005563
			d2	7.15	0.75	7.44	0.79	-0.28	0.77	0.013957
TNF-R1	PLACEBO	visit1	d1	12.22	1.16	12.72	1	-0.5	1.08	0.02922
			d2	12.08	1.32	12.66	1.16	-0.57	1.24	0.014364
		visit2	d1	11.99	1.2	12.72	1.08	-0.73	1.14	0.001518
			d2	11.85	1.36	12.75	1.24	-0.9	1.3	0.000385

				SURVQUES						
				Alive at Day 28		Dead at Day 28		Diff (1-2)		
				M an	Std Dev	M an	Std D v	Mean	Std Dev	Pr > F
Analyte Name	THERAPY	Variabl	RunDay							
TNF-a	PLACEBO	visit1	d1	7.52	0.79	7.78	0.69	-0.26	0.74	0.03848
			d2	7.44	0.65	7.67	0.68	-0.23	0.67	0.030127
		visit2	d1	7.45	0.69	7.75	0.72	-0.3	0.71	0.008657
			d2	7.43	0.67	7.74	0.71	-0.31	0.69	0.0093

FIGURE 6C

| | | | | SURVQUES | | | | | | |
| | | | | Aliv at Day 28 | | D ad at Day 28 | | Diff (1-2) | | |
				Mean	Std D v	Mean	Std D v	M an	Std D v	Pr > F
Analyt Name	THERAPY	Variabl	RunDay							
HVEM	PLACEBO	visit2	d2	7.85	0.94	8.34	1.04	-0.5	0.99	0.009427
			d3	7.93	0.8	8.43	0.92	-0.5	0.87	0.004603
IGFBP2	PLACEBO	visit1	d2	13.55	0.66	13.88	0.7	-0.33	0.68	0.018424
			d3	13.49	0.38	13.78	0.49	-0.29	0.44	0.000884
		visit2	d2	13.57	0.7	13.94	.0.73	-0.37	0.72	0.007514
			d3	13.55	0.38	13.79	0.53	-0.24	0.47	0.004706
MIP3b	PLACEBO	visit2	d1	8.1	0.85	8.46	1.06	-0.36	0.96	0.04498
			d2	7.51	0.94	7.85	1.07	-0.34	1.01	0.042278
			d3	7.69	0.96	8.27	1.04	-0.58	1	0.001433
SCF R	PLACEBO	visit1	d1	9.39	0.51	9.08	0.68	0.31	0.6	0.018749
			d2	9.07	0.61	8.73	0.69	0.33	0.66	0.02022
			d3	9.31	0.46	9.04	0.51	0.27	0.49	0.013796
ST2	PLACEBO	visit2	d1	7.8	0.97	8.49	1.31	-0.69	1.16	0.001416
			d2	7.11	0.84	7.71	1.19	-0.6	1.04	0.0017
			d3	7.35	1.05	8.25	1.44	-0.9	1.28	0.000076

FIGURE 6D

| | | | | SURVQUES | | | | | | |
| | | | | Aliv at Day 28 | | D ad at Day 28 | | Diff (1-2) | | |
Analyt Name	THERAPY	Variable	RunDay	Mean	Std D v	Mean	Std D v	M an	Std D v	Pr > F
CD27	PLACEBO	visit2	d1	8.54	1.09	9.33	1.25	-0.79	1.18	0.000864
			d2	8.13	1.19	8.69	1.35	-0.56	1.27	0.029206
			d3	8.63	1.43	9.28	1.28	-0.66	1.35	0.016086
Follistatin	PLACEBO	visit2	d1	8.64	0.64	9.13	0.96	-0.49	0.82	0.001339
			d2	8.92	0.71	9.29	0.96	-0.37	0.85	0.02658
			d3	8.66	0.62	8.95	0.85	-0.28	0.75	0.028197
I-TAC	PLACEBO	visit2	d1	7.54	0.97	8.03	1.37	-0.49	1.19	0.009538
			d2	7.62	0.81	8.01	1.26	-0.38	1.07	0.027429
			d3	7.61	0.97	8.13	1.34	-0.52	1.18	0.007205
IFN-g	PLACEBO	visit1	d1	5.94	0.54	6.2	0.7	-0.26	0.63	0.014402
			d3	5.74	0.4	5.93	0.72	-0.19	0.59	0.03821
		visit2	d1	5.89	0.46	6.19	0.62	-0.3	0.55	0.000268
			d3	5.66	0.39	5.88	0.72	-0.22	0.59	0.004331
IGF-II	PLACEBO	visit1	d1	11.53	0.76	10.96	1.18	0.57	1.01	0.001911
			d2	11.15	0.72	10.59	1.15	0.57	0.98	0.001825
			d3	12.28	0.73	11.75	0.99	0.53	0.87	0.001572
		visit2	d1	11.81	0.71	11.29	0.86	0.53	0.79	0.000126
			d2	11.4	0.79	10.99	0.94	0.41	0.87	0.005136
			d3	12.59	0.68	11.97	0.93	0.62	0.82	0.000058
IGFBP-1	PLACEBO	visit1	d1	14.55	1.39	15.03	0.87	-0.48	1.14	0.028029
			d2	13.77	1.46	14.48	0.92	-0.71	1.2	0.00142
			d3	14.32	1.31	14.85	0.92	-0.54	1.13	0.012389
		visit2	d1	14.08	1.43	14.85	1.1	-0.77	1.26	0.000936
			d2	13.3	1.49	14.28	1.18	-0.98	1.33	0.00022
			d3	13.88	1.36	14.72	1.06	-0.84	1.21	0.000296
IGFBP-4	PLACEBO	visit2	d1	10.72	0.58	11.13	0.58	-0.41	0.58	0.000309
			d2	10.62	0.57	10.84	0.61	-0.22	0.59	0.030527
IL-10rb	PLACEBO	visit2	d1	8.48	0.67	8.79	0.59	-0.31	0.63	0.005013
			d2	8.1	0.62	8.36	0.7	-0.27	0.66	0.026761
IL-16	PLACEBO	visit2	d1	6.47	0.47	6.79	0.72	-0.32	0.61	0.007043
			d2	6.35	0.47	6.64	0.67	-0.29	0.59	0.005638
			d3	6.32	0.45	6.6	0.71	-0.28	0.6	0.006612
IL-1srII	PLACEBO	visit1	d1	8.68	1.15	9.4	1.48	-0.72	1.34	0.000408
			d2	8.57	0.92	9.13	1.49	-0.56	1.26	0.001622
			d3	8.74	1.17	9.52	1.57	-0.78	1.39	0.000348
		visit2	d1	8.86	1.27	9.72	1.67	-0.85	1.49	0.000122
			d2	8.8	1.01	9.64	1.65	-0.84	1.38	0.000051
			d3	8.92	1.34	9.97	1.68	-1.05	1.53	0.000027
LT bR	PLACEBO	visit1	d1	9.51	0.79	9.87	0.95	-0.37	0.88	0.010092
			d3	9.34	0.98	9.76	0.97	-0.42	0.98	0.008539
		visit2	d1	9.53	0.91	10.22	0.98	-0.69	0.95	0.000038
			d2	9.58	0.98	10.24	0.95	-0.66	0.97	0.000179
			d3	9.36	0.91	10.01	0.99	-0.65	0.96	0.000076
MIP-3a	PLACEBO	visit1	d1	8.79	1.22	9.47	1.69	-0.68	1.49	0.00294
			d2	8.84	1.23	9.42	1.67	-0.58	1.48	0.012387
			d3	8.9	1.24	9.44	1.51	-0.54	1.39	0.007054
		visit2	d1	8.12	0.68	9.03	1.4	-0.91	1.11	0.000001
			d2	8.2	0.78	9.02	1.37	-0.82	1.14	0.000024
			d3	8.3	0.74	8.99	1.3	-0.69	1.07	0.000055

FIGURE 6E
Slid MapNam Prot omics5.3l

Analyte Name	THERAPY	Variabl	RunDay	SURVQUES						Pr > F
				Aliv at Day 28		D ad at Day 28		Diff (1-2)		
				M an	Std Dev	Mean	Std D v	Mean	Std Dev	
4-1BB	PLACEBO	visit2	d2	7.01	0.53	7.22	0.65	-0.22	0.6	0.026256
			d3	6.76	0.4	6.97	0.48	-0.2	0.45	0.005293
AFP	PLACEBO	visit1	d1	8.84	1.16	8.36	0.88	0.48	1.02	0.016371
			d2	8.91	1.15	8.3	0.89	0.61	1.02	0.002057
			d3	8.6	1.21	8.09	0.8	0.51	1.01	0.009839
		visit2	d1	8.8	1.2	8.29	0.93	0.51	1.07	0.014505
			d2	8.87	1.2	8.44	0.84	0.44	1.03	0.034755
			d3	8.53	1.27	8.03	0.83	0.5	1.05	0.022268
AgRP	PLACEBO	visit2	d1	6.5	0.65	6.89	0.85	-0.39	0.76	0.002308
			d2	6.74	0.49	7.07	0.75	-0.32	0.64	0.001289
			d3	6.65	0.66	7.18	0.86	-0.53	0.77	0.00009
CD141	PLACEBO	visit2	d1	9.68	0.98	10.21	0.98	-0.53	0.98	0.003046
			d2	9.24	0.8	9.59	0.91	-0.35	0.86	0.010765
			d3	9.39	0.89	9.82	0.86	-0.43	0.88	0.00765
FGF21	PLACEBO	visit2	d1	8.86	1.28	9.52	1.64	-0.65	1.49	0.012856
			d2	9.17	1.31	9.91	1.55	-0.73	1.44	0.003844
			d3	8.62	1.29	9.29	1.53	-0.67	1.42	0.009077
IL-18	PLACEBO	visit1	d1	10.5	0.94	10.81	1.17	-0.32	1.06	0.024614
			d3	10.51	0.91	10.93	1.07	-0.42	1	0.005675
		visit2	d1	10.31	1	10.91	1.11	-0.6	1.06	0.000342
			d2	10.66	0.94	11.27	1.12	-0.61	1.04	0.0001
			d3	10.48	0.87	11.11	1.11	-0.63	1.01	0.000154
MIF	PLACEBO	visit1	d1	8.99	0.96	9.71	1.32	-0.72	1.17	0.000379
			d2	8.88	0.96	9.6	1.19	-0.73	1.09	0.000168
			d3	8.55	1.01	9.37	1.17	-0.82	1.1	0.000048
		visit2	d1	8.76	1.18	9.55	1.52	-0.79	1.37	0.00128
			d2	8.72	1.05	9.57	1.35	-0.85	1.22	0.000164
			d3	8.45	0.86	9.12	1.08	-0.67	0.99	0.000133
MMP-8	PLACEBO	visit2	d1	13.75	1.46	14.23	1.43	-0.49	1.44	0.017177
			d2	13.89	1.31	14.27	1.29	-0.39	1.3	0.026792
P-Selectin	PLACEBO	visit1	d2	12.3	0.85	11.84	1.2	0.46	1.05	0.013654
			d3	12.3	0.58	11.92	0.9	0.38	0.78	0.011523
PAI-II	PLACEBO	visit1	d1	6.78	0.52	7.22	1.23	-0.44	0.97	0.001098
			d2	6.76	0.71	7.15	1.37	-0.39	1.12	0.006891
			d3	7.71	1.11	8.68	1.61	-0.97	1.41	0.000014
		visit2	d1	6.73	0.74	7.44	1.43	-0.71	1.16	0.000124
			d2	6.74	0.8	7.4	1.32	-0.66	1.11	0.000165
			d3	7.72	1.23	8.69	1.48	-0.96	1.37	0.000006
ProteinS	PLACEBO	visit2	d1	11.93	0.47	12.09	0.41	-0.16	0.44	0.038102
			d3	11.73	0.36	11.89	0.29	-0.16	0.32	0.004756

FIGURE 7

P-values < 10^{-4}

FIGURE 8

56 ICU non
septic patients

235 sepsis
patients
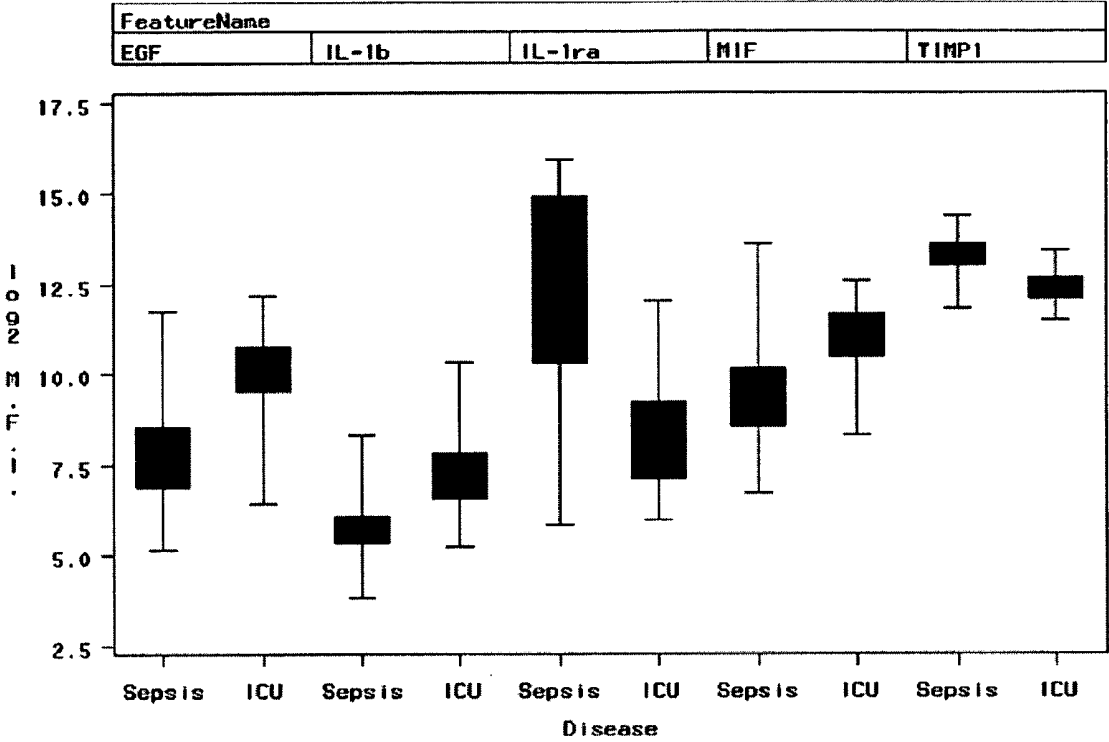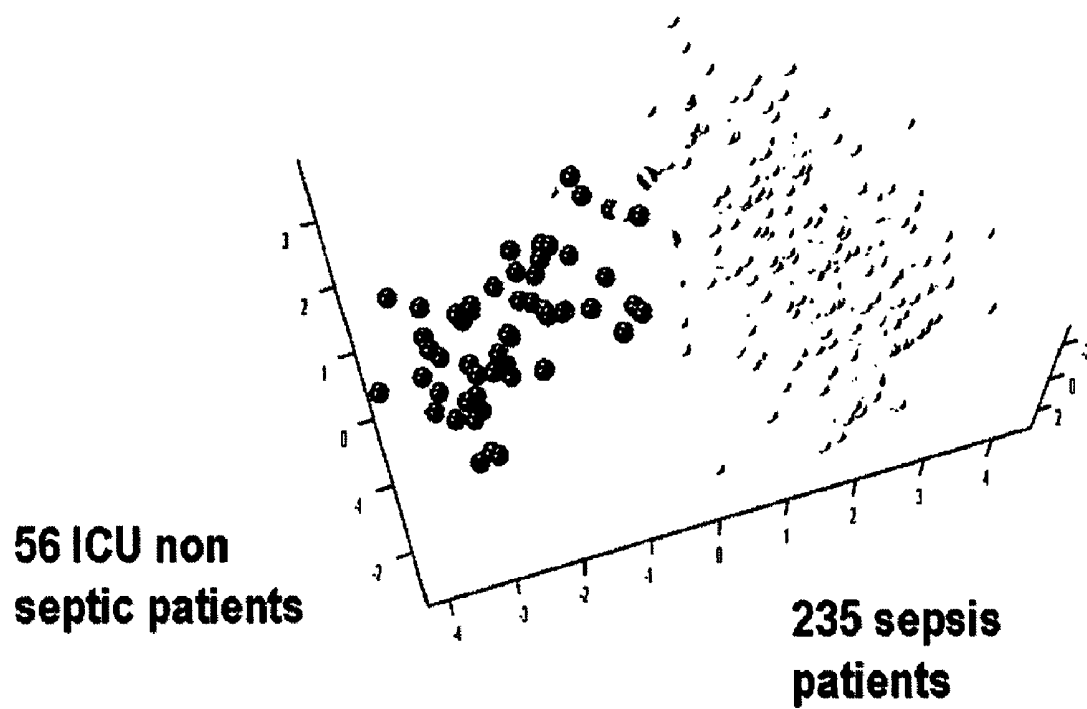
BIOMARKERS FOR SEPSIS

TECHNICAL FIELD OF THE INVENTION

[0001] This invention is related to the area of diagnosis. In particular, it relates to diagnosis, risk assessment, and monitoring of sepsis.

BACKGROUND OF THE INVENTION

[0002] Sepsis is the name given to infection when symptoms of inflammatory response are present. Of patients hospitalized in an intensive care unit (ICU) who have an infection, 82% have sepsis. Sepsis is defined as an infection-induced syndrome involving two or more of the following features of systemic inflammation: fever or hypothermia, leukocytosis or leukopenia, tachycardia, and tachypnea or a supranormal minute ventilation. Sepsis may be defined by the presence of any of the following ICD-9-CM codes: 038 (septicemia), 020.0 (septicemic), 790.7 (bacteremia), 117.9 (disseminated fungal infection), 112.5 (disseminated *Candida* infection), and 112.81 (disseminated fungal endocarditis).

[0003] Sepsis is diagnosed either by clinical criteria or by culture of microorganisms from the blood of patients suspected of having sepsis plus the presence of features of systemic inflammation. Culturing some microorganisms can be tedious and time-consuming, and may provide a high rate of false negatives. Bloodstream infection is diagnosed by identification of microorganisms in blood specimens from a patient suspected of having sepsis after 24 to 72 hours of laboratory culture. Currently, gram-positive bacteria account for 52% of cases of sepsis, gram-negative bacteria account for 38%, polymicrobial infections for 5%, anaerobes for 1%, and fungi for 5%. For each class of infection listed, there are several different types of microorganisms that can cause sepsis. The high rate of false negative microbiologic cultures leads frequently today to empiric treatment for sepsis in the absence of definitive diagnosis. Infection at many different sites can result in sepsis. The most common sites of infection in patients with sepsis are lung, gut, urinary tract, and primary blood stream site of infection. Since sepsis can be caused by many infection with microorganisms at many different sites, sepsis is a very heterogeneous disease. The heterogeneity of sepsis increases the difficulty in devising a diagnostic test

[0004] The number of patients with sepsis per year is increasing at 13.7% per year, and was 659,935 in 2000. The incidence of sepsis in the United States in 2000 was 240.4 cases per 100,000 population. Sepsis accounted for 1.3% of all hospitalizations in the U.S. from 1979 to 2000. During this period, there were 750 million hospitalizations in the U.S. and 10.5 million reported cases of sepsis.

[0005] Sepsis is the leading cause of death in critically ill patients, the second leading cause of death among patients in non-coronary intensive care units (ICUs), and the tenth leading cause of death overall in the United States. Overall mortality rates for sepsis are 18%. In-hospital deaths related to sepsis were 120,491 (43.9 per 100,000 population) in 2000.

[0006] Care of patients with sepsis is expensive and accounts for $17 billion annually in the United States alone. Sepsis is often lethal, killing 20 to 50 percent of severely affected patients. Furthermore, sepsis substantially reduces the quality of life of those who survive: only 56% of patients surviving sepsis are discharged home; 32% are discharged to other health care facilities (i.e., rehabilitation centers or other long-term care facilities), accruing additional costs of care.

[0007] Cost of care, morbidity and mortality related to sepsis are largely associated with delayed diagnosis and specific treatment of sepsis and the causal infection. Early diagnosis of sepsis is expected to result in decreased morbidity, mortality and cost of care. The average length of hospital stay in patients with sepsis is twelve days.

[0008] Severe sepsis is defined as sepsis associated with acute organ dysfunction. The proportion of patients with sepsis who had any organ failure is 34%, resulting in the identification of 256,033 cases of severe sepsis in 2000. Organ failure had a cumulative effect on mortality: approximately 15% of patients without organ failure died, whereas 70% of patients with 3 or more failing organs (classified as having severe sepsis and septic shock) died. Risk of death from sepsis increases with increasing severity of sepsis. Currently determination of the severity of sepsis and determination of whether, in a patient with sepsis, the sepsis is increasing or decreasing in severity, is based upon clinical events such as failing organs. Determination that, in a patient with sepsis, the sepsis is increasing in severity, may allow more intensive therapy to be given which may increase the likelihood of the patient surviving. The availability of a diagnostic test that would allow monitoring of patients with sepsis to determine whether the sepsis is increasing or decreasing in severity may allow early detection of deterioration and earlier intensification of therapy and less risk of death or disability.

[0009] Sepsis results either from community-acquired infections or hospital-acquired infections. Sepsis occurs in 1.3% of all U.S. hospitalizations. Hospital-acquired infections are a major source of sepsis, accounting for 65% of sepsis patients who are admitted to an intensive care unit. Sepsis is a major cause of admission to a hospital intensive care unit. 23-30% of patients admitted to an intensive care unit for longer than 24 hours will develop sepsis. Sepsis is a common complication of prolonged stay in an ICU. 8% of patients who remain in an ICU for longer than 24 hours will develop sepsis.

[0010] There is a need for screening diagnostic tests for sepsis and for tests to monitor sepsis severity with relatively few false negatives and high sensitivity and specificity.

SUMMARY OF THE INVENTION

[0011] In one embodiment of the invention a method of diagnosing sepsis in a human subject is provide. Concentration of at least one analyte in a test sample from a human subject is compared to concentration of the at least one analyte in a reference range that was determined for one or more control samples obtained from one or more human subjects not suffering from sepsis. The at least one analyte is selected from the group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2. Sepsis is diagnosed in the human subject if the concentration of the at least one analyte is elevated in the test sample relative to the reference range for the control samples.

[0012] According to another embodiment of the invention a method is provided for diagnosing sepsis in a human subject. Concentration of at least two analytes in a test sample from a human subject is compared to concentration of the at least two analytes in a reference range that was determined for one or more control samples obtained from one or more human subjects not suffering from sepsis. A first analyte of said two analytes is selected from a first group and a second analyte of said two analytes is selected from a second group. The first

group consists of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2. The second group consists of IL-6, sIL-2R, CD141, and MMP-9. Sepsis is diagnosed in the human subject if the concentration of at least one analyte in the first group and at least one analyte in the second group is elevated in the test sample relative to the reference range for the control samples.

[0013] According to still another embodiment of the invention a method of diagnosing sepsis in a human subject is provided. Concentration of at least one analyte in a test sample from said human subject is compared to concentration of the at least one analyte in a reference range that was determined for one or more control samples obtained from one or more human subjects not suffering from sepsis. The at least one analyte is selected from the group consisting of: EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. Sepsis is diagnosed in the human subject if the concentration of the at least one analyte is depressed in the test sample relative to the reference range for the control samples.

[0014] According to still another embodiment of the invention a method is provided of diagnosing sepsis in a human subject. Concentration in a test sample from a human subject of at least one analyte selected from a first group and at least one analyte selected from a second group is compared to concentration of said selected analytes in a reference range that was determined for one or more control samples obtained from one or more human subjects not suffering from sepsis. The first group consists of: EL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2; and the second group consists of EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. Sepsis is diagnosed in the human subject if the concentration of at least one analyte in the first group is elevated in the test sample relative to the control sample and the concentration of at least one analyte in the second group is depressed in the test sample relative to the reference range for the control samples.

[0015] Another aspect of the invention is still another method of diagnosing sepsis in a human subject. Concentration of at least one analyte in a test sample from a human subject is compared to concentration of the at least one analyte in a reference range that was determined for one or more control samples obtained from one or more human subjects not suffering from sepsis. The at least one analyte is selected from the group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, ST-2, and fragments or metabolites thereof. Sepsis is diagnosed in the human subject if the concentration of the at least one analyte is elevated in the test sample relative to the reference range for the control samples.

[0016] Yet another aspect of the invention is a method of diagnosing sepsis in a human subject at risk of developing sepsis. Concentration in a test sample from a human subject of at least one analyte selected from a first group and at least one analyte selected from a second group is compared to concentration of a reference range that was determined for one or more control samples. The first group consists of: IL-1Ra, MCP-1, MPT-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2. The second group consists of EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. Sepsis is diagnosed in the human subject if the con-

centration of at least one analyte in the first group is elevated in the test sample relative to the reference range or at least one analyte in the second group is depressed in the test sample relative to the reference range.

[0017] According to still another aspect of the invention a method is provided for diagnosis of deterioration or risk of progression to severe sepsis in a human subject suspected of having or having sepsis. The concentration of one or more analytes in a first sample obtained from a subject is determined. The one or more analytes is selected from a first group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2 or a second group consisting of EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. The concentration of said one or more analytes is also determined in a second sample obtained from said subject; the second sample is obtained at a time later than the first sample. The concentrations of analytes from said first and second samples are compared. An elevated concentration in the second sample relative to the first of an analyte selected from the first group or a depressed concentration in the second sample relative to the first of an analyte selected from the second group indicates deterioration or risk of progression to severe sepsis in the human subject.

[0018] According to still another aspect of the invention a method is provided for diagnosis of deterioration or risk of progression to severe sepsis in a human subject suspected of having or having sepsis. The concentration of one or more analytes in a sample obtained from a subject is determined. The one or more analytes is selected from a first group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2 or a second group consisting of EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. The concentration of said one or more analytes is compared with a reference range obtained from a one or more control samples obtained from control subjects who have sepsis but did not have severe sepsis. The concentrations of analytes from said patient and reference samples are compared. An elevated concentration in the patient sample relative to the control sample of an analyte selected from the first group or a depressed concentration in the patient sample relative to the control sample of an analyte selected from the second group indicates deterioration or risk of progression to severe sepsis in the test patient.

[0019] According to still another aspect of the invention a method is provided for diagnosing sepsis in an acutely ill human subject at risk of developing sepsis. Concentration of one or more analytes in a sample obtained from the subject is determined. The one or more analytes is selected from a first group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2; and a second group comsisting of: EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. Sepsis in the subject is diagnosed if the concentration of at least one analyte from the first group is elevated or the concentration of at least one analyte from the second group is depressed relative to the concentration of said analyte in a control sample or a reference range that was determined for one or more control samples.

[0020] Another embodiment of the invention is a method of diagnosis of improvement in a human subject suspected of having or having sepsis. The concentration of one or more

analytes in a first sample obtained from subject is determined. The one or more analytes is selected from a first group consisting of: IL-1Ra, MCP-1, MPIF-1, TNF-R1, MIG, BLC, HVEM, IL-15, MCP-2, M-CSF, MIP-3b, MMP-9, PARC, and ST-2 or a second group consisting of EGF, ENA-78, EOT, Gro-beta, IL-1b, Leptin, MIF, MIP-1a, OSM, Protein C, P-Selectin, and HCC4. The concentration of the one or more analytes is determined in a second sample obtained from said subject. The second sample is obtained at a time later than the time of obtaining the first sample. The concentrations of analytes from said first and second samples are compared. A depressed concentration in the second sample relative to the first of an analyte selected from the first group or an elevated concentration in the second sample relative to the first of an analyte selected from the second group indicates improvement of sepsis or the risk of sepsis in the human subject.

[0021] These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with reagents and methods for detection and diagnosis pertaining to sepsis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1. Schematic representation of a sample protein microarray slide with 16 subarrays. Subarrays refer to the 16 wells, or circular analysis sites, on the slide. Array refers to the antibody content printed in a well. Each microarray slide contains only one type of array.

[0023] FIG. 2. Causal organisms in the septic samples. "Mixed Gram" indicates a sample containing Gram positive and Gram negative bacteria, and possibly other microorganisms. "No Bacteria Expo" indicates exposure to one or more unknown organisms other than bacteria. "Unconfirmed Gram" indicates a sample comprising bacteria of unknown Gram stain Characteristics.

[0024] FIG. 3. Two sets of data for each analyte are shown. The first set is QDA (Quadratic Discriminant Analysis) at a prevalence of 0.3 sorted by PPV. This means that—at least 30% of patients admitted to an intensive care unit for longer than 24 hours will develop sepsis, as judged by changes in concentration of the analyte shown in the first column. The second set of data is LDA (Linear Discriminant Analysis) at a prevalence of 0.3. LDA is another method of statistical analysis. QDA and LDA analysis methods are described in [1] Krzanowski, W. J., Principles of Multivariate Analysis, Oxford University Press, Oxford, 1988; and [2] Seber, G. A. F., Multivariate Observations, Wiley, New York, 1984.

[0025] FIG. 4. Tables of alternative names (aliases) for analytes are shown segregated by array.

[0026] FIG. 5. SlideMapName Proteomics for sepsis.

[0027] FIG. 6. SlideMapName Proteomics for mortality.

[0028] FIG. 7. A sample strategy for multivariate analysis is shown.

[0029] FIG. 8. Results from the sample strategy for multivariate analysis shown in FIG. 7.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The present invention is based on rigorous statistical analyses. Statistical terms which can be used to describe the methods and assays include:

> diagnostic sensitivity=# of true positives (with Sepsis)/# of true positives+# of false negatives

[0031] (Diagnostic sensitivity is the probability that an individual with the disease is screened positive.)

> diagnostic specificity=# of true negatives (without Sepsis)/# of true negatives (with Sepsis)+# of false positives

[0032] (Diagnostic specificity is the probability that an individual without the disease is screened negative.)

> Positive Predictive Value=# of true positives/# of true positives+number of false positives

[0033] (Positive Predictive Value or PPV is the probability that an individual with a positive screening result has the disease.)

> Negative Predictive Value=# of true negatives/# of true negatives+# of false negatives

[0034] (Negative Predictive Value or NPV is the probability that an individual with a negative screening result does not have the disease.)

[0035] The Cut-Off value is the value for a standard, or control sample (No Sepsis). Higher values indicate elevation and lower values indicate depression; both can be diagnostic of a septic sample.

[0036] Sepsis is a very heterogeneous affliction. When looking at single analytes in a small number of patients differences in profile can sometimes be observed. According to this invention, differences in profile can be overcome by coordinated analysis of data from a plurality of analytes, rather than from single analytes. Thus, samples can optionally be scored on the basis of the concentrations of several analytes grouped together and weighted using some statistical measure which provides for greater statistical power and improved prediction rate over methods employing single analytes. For example, data from two, three, four, or more analytes are grouped together and analyzed in combination. This type of analysis, termed multivariate analysis, is known to those of skill in the art. In yet another aspect, this invention features a multivariate classifier that weights each analyte in the group optimally. The multivariate classifier takes into account the relative contribution or weighting of each of these analytes in the determination of diagnosis.

[0037] The Table shows diagnostic parameters determined by a sample multivariate analysis comprising 4 analytes.

Sensitivity	Specificity	PPV(0.1)	PPV(0.23)	PPV(0.3)	Correct Prediction Rate
1.00	0.98	0.86	0.94	0.96	0.99

[0038] For purposes of illustration only, and without limitation, a sample strategy for multivariate analysis is described below and sample results are shown in FIGS. 7 and 8.

[0039] Assuming that each group has a multivariate normal distribution, we develop a discriminant function or classification criterion using a measure of generalized squared distance. The classification criterion is based on the individual within-group covariance matrices; it also takes into account the prior probabilities of the classes. Each observation is placed in the class from which it has the smallest generalized squared distance. In addition we also compute the posterior probability of an observation belonging to each class.

[0040] The squared Mahalanobis distance from x to group t is

$$d_t^2(x)=(x-m_t)'V_t^{-1}(x-m_t)$$

where $V_t = S_t$ where S_t is the within-group t covariance matrice and m_t is the 5-dimensional vector containing variable means in group t.

[0041] The group-specific density estimate at x from group t is then given by

$$f_t(x) = (2\pi)^{-\frac{p}{2}} |V_t|^{-\frac{1}{2}} \exp(-0.5 d_t^2(x))$$

[0042] Using Bayes' theorem, the posterior probability of x belonging to group t is

$$p(t \mid x) = \frac{q_i f_i(x)}{\sum_u q_u f_u(x)}$$

where the summation is over all groups.

[0043] The generalized squared distance from x to group t is defined as

$$D_t^2(x) = d_t^2(x) + g_1(t) + g_2(t)$$

where

$$g_1(t) = \ln |S_t|$$

and

$$g_2 = -2 \ln(q_t)$$

where

[0044] q_i is the prior probability of membership in group t

[0045] The posterior probability of x belonging to group t is then equal to

$$p(t \mid x) = \frac{\exp(-0.5 D_t^2(x))}{\sum_u \exp(-0.5 D_u^2(x))}$$

[0046] The discriminant scores are $-0.5\,D_u^2(x)$. An observation is classified into group u if setting t=u produces the largest value of p(t|x) or the smallest value of $D_t^2(x)$.

[0047] Thus,

$$D_{ICU}(x) = -0.6931 - 0.5*(\Sigma(x - m_{icu}/S_{icu})^2 + 1.3270)$$

$$D_{sepsis}(x) = -0.6931 - 0.5*(\Sigma(x - m_{sepsis}/S_{sepsis})^2 + 1.3270)$$

Where

[0048] $m_{icu} = [10.0691\ 8.3308\ 10.9416\ 12.3883\ 7.0395]$

[0049] $S_{isu} =$

-1.1888	-0.5591	-0.6561	-0.0219	-0.2616
0	1.2814	0.2723	0.0944	0.0139
0	0	0.6571	-0.1357	0.4831
0	0	0	-0.3875	-0.0323
0	0	0	0	1.3280

And

[0050] $m_{sepsis} = [7.8059\ 12.4309\ 9.4300\ 13.2902\ 5.5798]$

[0051] $S_{sepsis} =$

1.1977	-0.3291	0.0043	0.1090	-0.1464
0	-2.6251	-0.4601	0.0202	-0.1352
0	0	1.1665	0.0734	-0.1312
0	0	0	0.4051	0.0419
0	0	0	0	-0.9892

[0052] Further, as shown in FIG. 7, samples can be grouped into septic or otherwise based on p-values alone using any of five different analytes. However, due to heterogeneity (error-bars), the strength of the diagnosis of individual samples may be weakened. Multivariate analysis of the same data using four analytes now makes it possible to diagnose individual samples with greater certitude (FIG. 8).

[0053] Those of skill in the art will be aware of several alternate methods to create a multivariate classifier that weights each analyte in the group optimally. Without limitation, these methods include supervised and unsupervised methods, including neural networks, self organizing maps, vector machine, perceptrons, clustering, multiple regression analysis, multiple linear discriminant analysis, principal component analysis and others as taught in Pattern Classification (2nd Edition) by Richard O. Duda, Peter E. Hart, David G. Stork ISBN: 0471056693. Each such multivariate classifier takes into account the relative contribution or weighting of each of these analytes in the determination of diagnosis.

[0054] Protein analytes can be detected in a sample by any means known in the art. Any immunological detection method known in the art can be used. Solid phase immunoassays are particularly useful for this purpose. Methods that apply the power of nucleic acid signal amplification to the detection of non-nucleic acid analytes can be employed for detecting, determining, and quantitating specific analytes in samples. See U.S. Pat. No. 6,531,283 which is incorporated herein by reference.

[0055] Multiple proteins can be analyzed, for example, by sandwich immunoassays on microarrays to which primary antibodies specific to the various proteins have been immobilized. First, the proteins or analytes, if present in the sample, are captured on the cognate spots on the array by incubation of the sample with the microarray under conditions favoring specific antigen-antibody interactions. Second, a rolling circle amplification (RCA) primer is associated with the various analytes using a secondary antibody that is specific for the analyte being detected and which is conjugated to the RCA primer or a hapten. In direct immunoassays, the secondary antibody is conjugated directly to the RCA primer. In indirect immunoassays, the secondary antibody is conjugated to a hapten, such as biotin and then incubated with a detector antibody conjugate or streptavidin conjugated with the RCA primer. Rolling circle replication primed by the primers results in production of a large amount of DNA at the site in the array where the proteins are immobilized. The amplified DNA serves as a readily detectable signal for the proteins.

[0056] Different proteins in the array can be distinguished in several ways. For example, the location of the amplified DNA can indicate the protein involved, if different proteins are immobilized at pre-determined locations in the array. Alternatively, each different protein can be associated with a

different rolling circle replication primer that in turn primes rolling circle replication of a different DNA circle. The result is distinctive amplified DNA for each different protein. The different amplified DNAs can be distinguished using any suitable sequence-based nucleic acid detection technique. Comparison of proteins or analytes found in two or more different samples can be performed using any means known in the art. For example, a first sample can be analyzed in one array and a second sample analyzed in a second array that is a replica of the first array. The intensity of a spot for each analyte at the first array can be compared with the intensity of the corresponding spot of the second array. The differences in the intensities of the spot between the first and second array determine if the concentration of the analyte is different in the two samples. If differences exist, they are recorded as elevated analyte or depressed analyte. Alternatively, the same analyte(s) from different samples can be associated with different primers which prime replication of different DNA circles to produce different amplified DNAs. In this manner, each of many analytes present in several samples can be quantitated.

[0057] A variety of different solid phase substrates can be used to quantitate or determine the concentration of an analyte. The choice of substrate can be readily made by the routineer, based on convenience, cost, skill, or other considerations. Useful substrates include, without limitation: beads, bottles, surfaces, substrates, fibers, wires, framed structures, tubes, filaments, plates, sheets, and wells. These substrates can be made from: polystyrene, polypropylene, polycarbonate, glass, plastic, metal, alloy, cellulose, cellulose derivatives, nylon, coated surfaces, acrylamide or its derivatives and polymers thereof, agarose, or latex, or combinations thereof. This list is illustrative rather than exhaustive.

[0058] Other methods of protein detection and measurement described in the art can be used as well. For example, a single antibody can be coupled to beads or to a well in a microwell plate, and quantitated by immunoassay. In this assay format, a single analyte can be detected in each assay. The assays can be repeated with antibodies to many analytes to arrive at essentially the same results as can be achieved using the methods of this invention. Bead assays can be multiplexed by employing a plurality of beads, each of which is uniquely labeled in some manner. For example each type of bead can contain a pre-selected amount of a fluorophore. Types of beads can be distinguished by determining the amount of fluorescence (and/or wavelength) emitted by a bead. Such fluorescently labeled beads are commercially available from Luminex Corporation (Austin, Tex.) and permit up to 100 analyte measurements simultaneously.

[0059] Proteins and other analytes can alternatively be measured by enzyme-linked immunosorbent assay (ELISA), which permits a single protein measurement per microwell, and can be scaled up to 384 or more measurements per plate. Non-immunological assays can also be used. Enzyme activity-based assays can achieve a high degree of sensitivity and can be used. Specific binding protein assays can be used where a protein is a member of a specific binding pair that has a high binding affinity (low dissociation constant). The other member of the specific binding pair may be a protein or a non-protein, such as a nucleic acid sequence which is specifically bound by a protein.

[0060] Samples for testing according to the invention can be derived from any readily available patient material. Typically this will be blood, serum, or plasma. Other body samples can be used as well, including urine, sputum, tears, saliva, cerebrospinal fluid, pleural fluid, or peritoneal fluid. Body samples can be fractionated prior to testing to improve sensitivity and reduce background. Any fractionation procedure known in the art can be used, so long as the desired analyte remains in the fraction which is used as a test sample.

[0061] Some analytes may be informative of sepsis or patient condition when considered in isolation. However, more typically a plurality of analytes will be tested and considered in determining a diagnosis. Two, three, four, five, or six analytes may be considered. In the determination of diagnosis, the contribution of each of these analytes may be weighted. In some cases a larger number of analytes may be tested, but only a subset may be sufficient to provide a diagnosis. It may be desirable in order to gain increased statistical power, to test even larger number of analytes, such as at least 10, 12, 14, or 16 analytes. It may also be desirable to utilize both one or more analytes which are elevated and one or more analytes which are depressed in the same assay.

[0062] Control samples can be derived from a healthy individual or individuals, or from an individual or individuals who are ill but who do not have sepsis. Patients who are ill can be critically ill, such as patients in the intensive care unit. These samples can be assayed individually or in pools. The data from individual controls can be pooled to provide a range of "normal" values, also known as a reference range. The data can be obtained at an earlier time. Thus controls need not be run in a side-by-side fashion with test samples. For some purposes, samples from a single individual taken at different times are compared to each other. In such cases there need not be, but may be, any control or normal sample evaluated. Control samples can also be synthetically produced, by mixing known quantities of particular analytes, either in an artificial or a natural body sample fluid.

[0063] Analytes can be tested directly, or derivatives of the analytes can be tested. The derivatives can be forms of the analyte which occur in the body, or forms which are produced, either spontaneously or by design, during sample processing. Examples of derivatives include proteolytic degradation products, phosphorylated products, acetylated products, myristoylated products, transaminated products, protein complexed products, and complex dissociated products. These are collectively termed "metabolites."

[0064] The inventors have developed tests that permit the early detection, risk assessment, and monitoring of patients who have or are susceptible to sepsis. The tests are based on the identification of biomarkers whose regulation is perturbed in septic patients. Concentrations of some of the biomarkers are elevated in sepsis and concentrations of others are depressed.

[0065] The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the claimed invention.

Example 1

Materials and Methods for the Experimental Results Described in Example 2

Microarray Manufacture

[0066] Glass slides were cleaned and derivatized with 3-cyanopropyltriethoxysilane. The slides were equipped with a

Teflon mask, which divided the slide into sixteen 0.65 cm diameter wells or circular analysis sites called subarrays (FIG. 11). Printing was accomplished with a Perkin-Elmer SpotArray Enterprise non-contact arrayer equipped with piezoelectric tips, which dispense a droplet (~350 pL) for each microarray spot. Antibodies were applied at a concentration of 0.5 mg/mL at defined positions.

[0067] Each chip was printed with sixteen copies of one type of array, either Array 1.1.1, 2.1.1, 3.1.1, or 4.1. A set of cytokines was printed with quadruplicate spots in each subarray. After printing, chips were inspected using light microscopy. If the percentage of missing spots observed was greater than 5%, then the batch failed and the slides were discarded immediately. For all print runs described herein, 100% of the antibody features and >95% of the biotin calibrators were printed. Microarray chips were validated in concert with a set of qualified reagents in two ways: First, mixtures of 1-3 different cytokines were prepared so as to provide a high intensity signal and applied to 14 wells of a chip (with each well being treated with a different mixture up to the total complement of detector antibodies) and two arrays were used as blank controls. The chips were developed and scanned and the resulting signals were compared to the positional map of the particular array. Second, a titration QC for all analytes of a specified array using known sample matrices was performed. Normal human serum and heparinized plasma were assayed neat or spiked with purified recombinant cytokines representing all analytes in the array. Spiked mixtures were then titrated down the subarrays of a slide from 9,000 pg/ml to 37 pg/mL of spiked cytokine concentrations along with two subarrays for each un-spiked control sample. The data was quantified and for every analyte in the array a titration curve was generated to examine feature intensity behavior as a function of concentration. Taken together, this data was used to confirm the activity of array features and reagent sets.

Samples

[0068] A total of 48 clinical samples were provided: 24 samples of 0.5 mL serum and 24 samples of 1 mL citrate plasma from 6 patients selected from the PROWESS trial, with 4 time points per patient (preinfusion, days 1, 4 and 7). These patients were not chosen on the basis of baseline disease severity. There were 2 groups (drug treated survivors and placebo treated survivors), 3 patients per group. The researchers were not blinded to sample identity.

[0069] In addition to these 48 clinical samples, 8 serum samples and 8 citrate plasma samples from individuals presumed healthy were purchased from Biological Specialty Corporation (Colmar, Pa.) to serve as normal controls.

[0070] Clinical serum and plasma samples were received on Mar. 19, 2003, thawed, centrifuged to remove particulate matter (15 min at 10,000×g for citrate plasma, and 15 min at 1300×g for other samples). The resulting supernatants were collected, divided into 5 aliquots (one for each array) and re-frozen at −80° C. Prior to testing, samples were thawed, Immunoglobulin Inhibiting Reagent (IIR, Bioreclamation Inc) was added to a final concentration 0.25 mg/mL, Heteroblock (Omega Biologicals) was added to samples at a concentration of 0.25 mg/mL, and Tween 20 was added to final concentration of 0.1%.

RCA Immunoassay

[0071] Prior to assay, the slides were removed from storage at room temperature in sealed containers and opened in a

humidity controlled chamber (45-55%). Slides were blocked with Seablock (Pierce Chemical Co.), diluted 1:1 with PBS for 1 h at 37° C. in a humidified chamber. Following removal of the blocking solution, they were washed twice with 1×PBS/0.5% Brij 35 prior to application of sample. On each slide, control serum (Jackson Immuno Research Laboratories) was applied to two subarrays, and a negative control with PBS buffer applied to two subarrays. The test samples were assayed on the remaining 12 subarrays. Twenty µL of the treated sample were then applied to each subarray. The basics of performing immunoassays with RCA signal amplification has been described (*Nat. Biotechol.* (2002) 20:359-65) and we are using SOPs dervyed from the protocols used in that study. Slides were scanned (GenePix 4000B, Axon Instruments Inc.) at 10 µm resolution with a laser setting of 100% and a PMT setting of 550 V. Mean pixel fluorescence values were quantified using the fixed circle method in GenePix Pro 4.0 (Axon Instruments). The fluorescence intensity of microarray spots was analyzed for each feature and sample, and the resulting mean intensity values were determined. Dose-response curves for selected cytokines were examined, ensuring that feature intensity is above background and exhibiting increasing intensity with increasing analyte concentration.

Example 2

Candidate Biomarkers for Sepsis

[0072] Analyte levels from sepsis patients were compared with normal individuals to identify candidate biomarkers for sepsis. Candidate biomarkers for sepsis were posited to be manifest as a difference in blood analyte levels between time zero sepsis patients and normal controls and that trended toward the normal control value at day 7 in the surviving sepsis patients, in whom sepsis was presumably resolving. For ease of comparison, data were plotted 4 different ways for both serum and citrate plasma samples.

[0073] Of 107 analytes analyzed, six (6%) exhibited interesting differences between the sepsis patients at time zero and normal controls, and that decreased over time, including IL-8, IL-6, IL2sRα, MMP-7, MIPF-1 and IGFBP-1. A brief description for each of the analyte is provided below.

[0074] Since the value of correlation coefficient is very sensitive to outliers and multimode distributions of data, our interpretation is very conservative.

[0075] IL-8: IL-8 levels were higher in sepsis patients relative to normal controls at the pre-infusion time point, in both the plasma and serum samples (FIG. 9). The level of IL-8 in sepsis patients declined to match levels in normal controls by day 7. Correlation analysis revealed significant correlation (correlation coefficient>0.8) between the serum IL-8 level in the sepsis group with each of MIP3β, IL-6, MCP-2, MCP-1, BLC (Table 1). Significant correlation was also observed between plasma IL-8 level and each of MCP-2, BLC, IL-2sRα, IL-6 in the citrate plasma samples (Table 1).

[0076] IL-6: Similar to IL-8, IL-6 levels were also higher in sepsis patients relative to normal controls at the pre-infusion time point, in both plasma and serum samples (FIG. 9) and levels declined to reach normal control levels by day 7. Correlation analysis revealed significant correlation (correlation coefficient>0.8) between the serum IL-6 level in the sepsis group with each of MIP-3β, MCP-1, IL-8 (Table 1). Signifi-

cant correlation was also observed between plasma IL-6 level and each of MIP-3β, MCP-1, IL-8 in the citrate plasma samples (Table 1).

[0077] IL-2sRα: Sepsis patients showed higher levels of IL-2sRα relative to the normal controls in both serum and plasma at all time-points (pre-infusion, days 1, 4 and 7). Although IL-2sRα levels declined in the sepsis patients with time, they still remained higher that the levels observed in normal controls at day 7. Correlation analysis revealed significant correlation (correlation coefficient>0.8) between the serum IL-2sRα level in the sepsis group with each of RANK, MMP-7, MIP-3β, DR6 (Table 1). Significant correlation was also observed between plasma IL-2sRα level and each of IL-8, MCP-1, MCP-2, BLC, HVEM in the citrate plasma samples (Table 1).

[0078] MMP-7: Serum and plasma levels of MMP-7 levels were higher in sepsis patients at all time-points examined. Interestingly, MMP-7 levels remained fairly stable as time progressed. Examination of the sepsis patients revealed considerable individual variation. Correlation analysis revealed significant correlation (correlation coefficient>0.8) between the serum MMP-7 level in the sepsis group with each of IL-2sRα, RANK, HVEM, DR6 (Table 1). Significant correlation was also observed between plasma MMP-7 level and HVEM in the citrate plasma samples (Table 1).

[0079] MPIF-1: MPIF-1 levels were higher in the sepsis patients relative to the normal controls in both serum and plasma, at all time-points examined (FIG. 9). As time progressed, levels of MP1F-1 declined in the sepsis groups to the levels observed in the normal controls. Correlation analysis did not reveal any significant correlation (correlation coefficient>0.8) between the serum MP1F-1 level and other analytes in the sepsis group in both serum and citrate plasma samples.

[0080] IGFBP-1: IGFBP-1 levels were higher in sepsis patients relative to the normal controls levels in both serum and plasma, at all the time-points examined (FIG. 9). As time progressed, levels of IGFBP-1 declined in the sepsis groups but not to the levels observed in the normal controls. As with MMP-7, sepsis patients exhibited considerable individual variation. Correlation analysis did not reveal any significant correlation (correlation coefficient>0.8) between the serum IGFBP-1 level and other analytes in the sepsis group in both serum and citrate plasma samples.

TABLE 1

Analytes that show correlation to IL-8, IL-6, IL2sRα, MMP-7, MIPF-1 and IGFBP-1 in either serum or citrate plasma samples.

Sample Type	Analyte#1	Analyte #2	Correlation Coefficient
Serum	IL-8	MIP3β	0.90887
		IL-6	0.87024
		MCP-2	0.8397
		MCP-1	0.83788
		BLC	0.8198
	IL-6	MIP3β	0.92821
		MCP-1	0.88214
		IL-8	0.87024
	IL-2sRα	RANK	0.87464
		MMP7	0.84191
		MIP3β	0.80768
		DR6	0.80667
	MMP-7	IL-2sRa	0.84191
		RANK	0.83404

TABLE 1-continued

Analytes that show correlation to IL-8, IL-6, IL2sRα, MMP-7, MIPF-1 and IGFBP-1 in either serum or citrate plasma samples.

Sample Type	Analyte#1	Analyte #2	Correlation Coefficient
		HVEM	0.82405
		DR6	0.80013
Plasma	IL-8	MCP-2	0.90452
		BLC	0.89497
		IL-2sRa	0.84953
		IL-6	0.82152
	IL-6	MIP3β	0.89956
		IL-8	0.82152
		MCP-1	0.81609
	IL-2sRα	IL-8	0.84953
		MCP-1	0.83809
		MCP-2	0.8159
		BLC	0.81149
		HVEM	0.80799
	MMP-7	HVEM	0.84646

Analytes with Differential Expression Levels in Sepsis Vs. Normal Controls

[0081] When sepsis samples were compared to normal serum at pre-infusion time-point, there were significant differences in the levels of several analytes (e.g., IL-8, IL-6, IL2sRα, MMP-7, MIPF-1 and IGFBP-1). Some of the observed changes in their levels were supported by the literature.

IL-6 (interleukin-6) and IL-8 (interleukin-8)

[0082] It is well known that the levels of IL-6 and IL-8 are increased in sepsis. In the present study, these cytokines were found to be elevated in sepsis samples at the pre-infusion time-point (compared to normal controls), and to gradually decrease with time, approaching normal levels at day 7. IL-6 and IL-8 are important pro-inflammatory cytokines that contribute to organ dysfunction in sepsis (Scand J Immunol 1998 48:509-14; Res Commun Chem Pathol Pharmacol 1994 84:291-300). Levels of IL-6 and IL-8 have also been shown to be associated with the severity and mortality of the disease (Eur J Haematol 1993 50:243-9; Nippon Geka Gakkai Zasshi 1996 97:1054-9). Therefore the high initial levels and reduction of IL-6 and IL-8 levels with time, observed in the present study, is in close agreement with the pro-inflammatory role of these cytokines described in the literature.

MMP-7 (Matrix Metalloproteinase 7)

[0083] Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. MMP-7 activity is upregulated in liver metastases of colorectal cancer (Clin Cancer Res 2002 8:144-8). Studies in mice suggest that MMP-7 regulates the activity of defensins in intestinal mucosa. Defensins represent an important component of host response to infection. No literature reports describing a direct association between MMP-7 levels and sepsis were found in the literature. However, other members of the MMP family of proteins have been shown to play a role in sepsis, e.g., MMP-9 (see MMP-9 discussion in next section).

IGFBP-1 (Insulin-Like Growth Factor Binding Protein 1)

[0084] Insulin-like growth factors I and II (IGF-I and -II) play an active role in cell proliferation. In biological fluids,

they are non-covalently bound to high-affinity binding proteins (IGFBPs), at least 6 species of which have been identified (Int J Cancer 1998 77:874-9). IGFBPs form high-affinity complexes with IGF-I and thereby either inhibit or potentiate its mitogenic and metabolic effects (Biochemistry 1999 38:6386-96). IGFBP-1 is a 28-kDa plasma protein that binds to IGF-I and IGF-II with high affinity (Endocrinology 2000 141:3156-64) and has been shown to inhibit or stimulate cellular responses to IGF in vitro (J Biol Chem 2001 276: 8740-5). IGFBP-1 was elevated in the blood as a result of sepsis (Endocrinology 2000 141:3156-64) and might, in part, be responsible for the observed wasting. IGFBP-1 is present at relatively low concentration in serum but its levels fluctuate acutely, suggesting regulation of IGF bioactivity in response to short-term metabolic changes. End organ failure, particularly of liver and kidney, significantly affects production and clearance rates of the circulating IGFBPs and may contribute to the catabolism frequently seen in these states and in chronic sepsis (Baillieres Clin Endocrinol Metab 1996 10:421-46). The liver is the principal site of IGFBP-1 synthesis, and it has been shown that pro-inflammatory cytokines can directly stimulate IGFBP-1 secretion in a human hepatoma cell line (Endocrinology 2000 141:3156-64). In mice, IL-1 alpha and TNF alpha regulate IGFBP-1 serum levels and hepatic mRNA abundance in vivo and in vitro (Horm Metab Res 1999 31:209-15). The increase in IGFBP-1 observed in sepsis may be also reproduced in vivo by IL-1β, TNF-α and dexamethasone, and in vitro by IL-1β, IL-6, and dexamethasone (Endocrinology 2001 142: 241-248). An early, short-lasting increase in IGFBP-1 (188.8+/−39% P<0.05, 3 h after LPS) was observed in sheep in response to endotoxin challenge, while no significant change was seen for either IGFBP-2, -3 or -4 (J Endocrinol 2000 164:361-9). After caecal ligation and puncture in rats, there was a reduction in both circulating and hepatic IGF-I mRNA levels associated with a specific and differential regulation of hepatic IGFBP-1, -2 and -3 mRNA levels (J Endocrinol 1996 151:287-92). IGFBP-1 was increased in the blood, liver, and muscle of septic rats (Am J Physiol 1996 270:E430-7). Critically ill, septic patients have high levels of IGFBP-1 (Horm Res 1993 40:87-91), which is in agreement with the results observed in the present study. Significant differences were found between nonsurvivors and survivors in the levels of IGFBP-1 (44.3 vs. 8.9 nmol/L) in children with meningococcal sepsis and the pediatric risk of mortality score correlated significantly with levels of IGFBP-1 (J Clin Endocrinol Metab 2002 87:3118-24).

IL-2sRα (Interleukin 2 Soluble Receptor Alpha)

[0085] The biological activity of IL-2 is mediated by binding to a high affinity receptor complex composed of IL-2 Rα, β, and γ. Alone, IL-2 Rα binds IL-2 with low affinity, but is unable to transduce a signal. A soluble form of IL-2 Rα appears in serum, concomitant with its increased expression on cells (Immunol Today 1993 14:264-70, FASEB J 1991 5:2567-74, Hybridoma 1985 4:91-102). The function of IL-2 Rα is unclear, since it would be expected to be a poor inhibitor of IL-2 because of its low binding affinity. In any case, increased levels of soluble EL-2 Rα in biological fluids reportedly correlate with increased T cell, B cell and immune system activation.

[0086] Elevated level of sIL-2R has been observed in sepsis patients in multiple studies (Br J Surg 2000 87:223-30, Intensive Care Med 1999 25:1402-6, J Infect Dis 1991 163:1145-8). An increase of soluble IL-2 receptor level was also observed in patients with systemic inflammatory response

syndrome (Thromb Res 1999 95:205-13). Furthermore, the level of sIL-2 receptor was found to be higher in non-surviving patients with septic shock than in surviving patients (J Infect 1998 37:241-7, Ann Clin Lab Sci 1996 26:426-32). The concentration of sIL-2R was found to have predictive value for identifying patients with gram-negative sepsis at risk for progression toward the syndrome of septic shock (J Crit. Care 1995 10:64-71). High concentrations of IL-6, IL-8 and sIL-2R predict organ failure in patients with community-acquired septic shock, and the combination of these markers may provide the means to identify sepsis patients who will develop organ failure (Clin Sci 1999 97:529-38). Furthermore, a study suggests that soluble interleukin-2 receptor levels can be used to facilitate the diagnosis of sepsis in premature infants where sepsis diagnosis is difficult because newborn infants often suffer from bacterial and viral infections without presenting typical symptoms (J Pediatr 1995 126:982-5). Therefore literature reports corroborate the increase of sIL-2R levels observed in the present study.

MPIF-1 (Myeloid Progenitor Inhibitory Factor 1)

[0087] MPIF-1 is a member of the CC cytokine family that displays chemotactic activity on resting T lymphocytes and monocytes, and a minimal but significant activity on neutrophils. This cytokine is also a potent suppressor of bone marrow low proliferative potential colony-forming cells, a committed progenitor that gives rise to granulocyte and monocyte lineages (J Exp Med 1997 185:1163-72). The MPIF-1 signal transduction pathway appears to include binding to CCR1, transduction by G proteins, effector function by phospholipase C, protein kinase C, calcium flux, PLA2, and cytoskeletal remodeling (J Immunol 1999 162:435-44). No literature reports describing a direct association between MPIF-1 levels and sepsis were found in the literature.

[0088] In summary, changes in the levels of some cytokines observed in the present study between sepsis patients and normal controls are in agreement with findings reported in the literature.

Example 3

Materials and Methods for Results Described in Example 4

Microarray Manufacture

[0089] Glass slides were cleaned and derivatized with 3-cyanopropyltriethoxysilane. The slides were equipped with a Teflon mask, which divided the slide into sixteen 0.65 cm diameter wells or circular analysis sites called subarrays (FIG. 14). Printing was accomplished with a Perkin-Elmer SpotArray Enterprise non-contact arrayer equipped with piezoelectric tips, which dispense a droplet (~350 pL) for each microarray spot. Antibodies were applied at a concentration of 0.5 mg/mL at defined positions.

[0090] Each chip was printed with sixteen copies of one type of array, i.e. Array 5. A set of cytokines was printed with quadruplicate spots in each subarray. After printing, chips were inspected using light microscopy. If the percentage of missing spots observed was greater than 5%, then the batch failed and the slides were discarded immediately. For all print runs described herein, 100% of the antibody features and >95% of the biotin calibrators were printed. Microarray chips were validated in concert with a set of qualified reagents in two ways: First, mixtures of 1-3 different cytokines were

prepared so as to provide a high intensity signal and applied to 14 wells of a chip (with each well being treated with a different mixture up to the total complement of detector antibodies) and two arrays were used as blank controls. The chips were developed and scanned and the resulting signals were compared to the positional map of the particular array. Second, a titration QC for all analytes of a specified array using known sample matrices was performed. Normal human serum and heparinized plasma were assayed neat or spiked with purified recombinant cytokines representing all analytes in the array. Spiked mixtures were then titrated down the subarrays of a slide from 9,000 pg/ml to 37 pg/mL of spiked cytokine concentrations along with two subarrays for each un-spiked control sample. The data was quantified and for every analyte in the array a titration curve was generated to examine feature intensity behavior as a function of concentration. Taken together, this data was used to confirm the activity of array features and reagent sets.

Samples

[0091] A total of 48 clinical samples were provided: 24 samples of 0.5 mL serum and 24 samples of 1 mL citrate plasma from 6 patients selected from the PROWESS trial, with 4 time points per patient (preinfusion, days 1, 4 and 7). These patients were not chosen on the basis of baseline disease severity. There were 2 groups (drug treated survivors and placebo treated survivors), 3 patients per group. The researchers were not blinded to sample identity.

[0092] In addition to clinical samples, 8 serum samples and 8 citrate plasma samples from individuals presumed healthy were purchased from Biological Specialty Corporation (Colmar, Pa.) to serve as normal controls.

[0093] Clinical serum and plasma samples were thawed, centrifuged to remove particulate matter (15 min at 10,000×g for citrate plasma, and 15 min at 1300×g for other samples). The resulting supernatants were collected, divided into 5 aliquots (one for each array) and re-frozen at –80° C. Prior to testing, samples were thawed, Immunoglobulin Inhibiting Reagent (IIR, Bioreclamation Inc) was added to a final concentration 0.25 mg/mL, Heteroblock (Omega Biologicals) was added to samples at a concentration of 0.25 mg/mL, and Tween 20 was added to final concentration of 0.1%.

RCA Immunoassay

[0094] Prior to assay, the slides were removed from storage at room temperature in sealed containers and opened in a humidity-controlled chamber (45-55%). Slides were blocked with Seablock (Pierce Chemical Co.), diluted 1:1 with PBS for 1 h at 37° C. in a humidified chamber. Following removal of the blocking solution, they were washed twice with 1×PBS/0.5% Brij 35 prior to application of sample. On each slide, control serum (Jackson Immuno Research Laboratories) was applied to two subarrays, and a negative control with PBS buffer applied to two subarrays. The test samples were assayed on the remaining 12 subarrays. Twenty μL of the treated sample were then applied to each subarray. The basics of performing immunoassays with RCA signal amplification has been described (*Nat. Biotechol.* (2002) 20:359-65) and we are using SOPs derived from the protocols used in that study. Slides were scanned (GenePix 4000B, Axon Instruments Inc.) at 10 μm resolution with a laser setting of 100% and a PMT setting of 550 V. Mean pixel fluorescence values were quantified using the fixed circle method in GenePix Pro

4.0 (Axon Instruments). The fluorescence intensity of microarray spots was analyzed for each feature and sample, and the resulting mean intensity values were determined. Dose-response curves for selected cytokines were examined, ensuring that feature intensity is above background and exhibiting increasing intensity with increasing analyte concentration.

Example 4

Candidate Biomarkers for Sepsis

[0095] Analyte levels from the 6 sepsis patients were compared with normal individuals to identify candidate biomarkers for sepsis. Biomarkers for sepsis were identified based on analyte differences observed between sepsis and normal at time zero.

[0096] Coagulation markers: Several of the analytes measured on array 5 are of potential relevance to the anticoagulation. They include protein C (the precursor of activated protein C), protein S (which acts in combination with protein C to inactivate Factor Va), D Dimer (which is a measure of intravascular coagulation), and CD141 (which binds thrombin and cleaves protein C to yield activated protein C). Analysis of D Dimer DD5 and DD6 in plasma and protein S in serum was complicated by grossly elevated values that required a sepsis sample dilution of 1:50 to bring values into the dynamic range of the assay. A difference was observed between the sepsis group and the normal controls in serum values of D Dimer DD5 and DD6, but this finding is of uncertain significance since determination of D Dimer in serum lacks clinical meaning [data not shown]. Protein C showed a small decrease in level in sepsis patient sera, but no difference in sepsis plasma. The only coagulation marker to exhibit a clear difference between the groups was CD141, which was increased in serum of septic patients at all time points. Unlike sepsis biomarker candidates identified in the previous study, CD141 did not trend toward normal values by day 7 in sepsis-surviving patients, when sepsis was presumably resolving, indicating CD141 to represent a more chronic index of sepsis or disease.

[0097] Other analytes exhibiting notable differences between sepsis patients and normal controls included MIF, MMP-8, IL-18, AgRP, and CRP. A brief description of each profile is provided below.

[0098] AgRP, MMP8, IL18, MIF: AgRP, MMP8, MIF and IL18 levels were higher in sepsis patients than in normal controls in both plasma and serum samples (FIG. 12). The differences were more pronounced in serum than in plasma, and observed at all time points. MMP8 showed the greatest magnitude of difference between sepsis and normal controls (–25-fold). In fact, this difference was even greater than measurable in neat samples since many sepsis sample values for MMP8 were saturated. It should be noted that MMP9 exhibited a similar profile to MMP8.

[0099] CRP: CRP comparisons were complicated by the fact that levels were so high in sepsis patients that values were artefactually depressed in sepsis patients. A 1:625 dilution was necessary to bring CRP values within the dynamic range of the assay in sepsis samples whereas values were within the dynamic range in neat normal control samples (data not shown). It should be noted that 1:50 sample dilutions were run only with sepsis samples and only in singlicate in the pre-run in the present study. In longitudinal study 2, 1:50 sample dilutions will be performed for all samples and run in tripli-

cate on array 5 to allow direct comparisons of CRP and other analytes at or near the upper limit of quantitation.

[0100] Correlation analysis revealed significant correlation (coefficient>0.8) between the serum or plasma levels of these analytes and other analytes in these samples. Since correlation coefficient values are very sensitive to outliers and multimode distributions of data, however, interpretation needs to be very conservative.

TABLE 2

Analytes correlating with AgRP, IL18, MMP8, MIF, CD141
& Protein C in serum & citrated plasma. Negative correlations
indicate trends in the opposite directions.

Index analyte	Plasma Correlate		Serum Correlate	
Protein C	NAP-2	0.85078	0.52675	
	IGFBP-6	0.68619	0.55952	
	Neut Elast	0.68161	0.73483	
	ProteinS	0.57674	0.52831	
	CD141	0.53515	0.49269	
CD141	IGFBP-3	0.77435	0.62038	
	IL-1ra	0.70672	0.63177	
	MMP7	0.65748	0.6273	
	AgRP	0.69181	0.58788	
	ALCAM	0.7317	DR6	0.62635
	IGFBP-1	0.66008	L Selectin	0.61656
AgRP	MMP7	0.87709	0.8346	
	IL-2sRa	0.80326	0.71104	
	RANK	0.72941	0.72298	
	HVEM	0.81839	0.74574	
	I-309	0.73521	0.66089	
	IGFBP-1	0.74057	ENA-78	−0.72375
	IL-18	0.72091	DR6	0.68636
	MIG	0.7056	IL-1a	0.64342
	SCF R	−0.70543	IL-7	−0.6202
MMP-8	CRP	−0.89196	−0.71546	
	IL-10rb	0.8444	ENA-78	−0.74543
	GRO-g	0.81551	MMP7	0.7175
	TRAIL R1	0.80501	MIP-1d	0.70679
	FGF-4	0.79541	IL-2sRa	0.68527
	GROb	0.78096	IL-18	0.61728
	PDGF-Ra	0.77071	HCC1	0.60376
	IL-1srII	0.76559	RANK	0.5916
	Lymphotactin	0.74288	HVEM	0.56404
MIF	MMP-8	0.72123	D-Dimer⑦	0.74156
	TIMP1	0.69271	D-Dimer⑦	0.6582
	TRAIL R4	0.66063	D-Dimer⑦	0.63237
	BDNF	0.64952	PAI-II	0.59991
	GRO-g	0.63311	LIF Ra	0.59456
	ICAM-1	0.63247	IL-2	0.59085
	MMP9	0.61095	IL-17	0.58761
IL-18	HVEM	0.8612	0.88672	
	MMP7	0.79136	0.80958	
	M-CSF	0.75023	0.80279	
	TNF-a	0.72586	0.71039	
	GCP-2	0.72236	HCC1	0.82688
	MIG	0.72185	IL-2sRa	0.82065
	AgRP	0.72091	RANK	0.73954
	CD27	0.71855	ICAM3	0.72965
	TNF-b	0.71703	4-1BB	0.72739

⑦ indicates text missing or illegible when filed

Example 5

Analytes with Differential Expression Levels in Sepsis Vs. Normal Controls

[0101] When sepsis samples were compared to normal serum at pre-infusion time-point, there were significant differences in the levels of several analytes (e.g., CD141, AgRP, MMP-8, MIF, IL-18). Many of the observed changes in their levels were supported by the literature.

[0102] Coagulation markers: Array 5 measured several coagulation markers of potential relevance in sepsis. These include, protein C (precursor of activated Protein C), protein S (inactivates Factor Va), D-Dimer, and CD141. CD141 was the only coagulation marker that exhibited a clear difference between sepsis patients and normal controls.

[0103] CD141: CD141 also called thrombomodulin or fetomodulin belongs to C-type lectin family. It is a single chain, type I membrane glycoprotein (575 amino acids) with an approximately 500 amino acid extracellular region consisting of several domains, which include six EGF-like (epidermal growth factor) repeats which function in thrombin/protein C binding and a serine/threonine-rich region which is important for optimal anticoagulant function (Blood 1990 75:329). It is expressed on a number of cell types including endothelial cells, keratinocytes, megakaryocytes, platelets, monocytes, neutrophils, smooth muscle cells and synovial lining cells. CD141 is critical for the activation of protein C and initiation of the protein C anticoagulant effect. CD141 is a cofactor for the thrombin-mediated generation of APC (FASEB J 1995 9:946). APC then acts to degrade the cofactors Va and VIIIa on platelets and endothelial cells, thereby decreasing the production of thrombin, which is the central effector protease in clotting. Thrombin activates platelets, converts fibrinogen to fibrin and promotes fibrin cross-linking by activating factor XIII, leading to clot formation.

[0104] Elevated levels of CD141 have been reported in several studies on sepsis and sepsis-related organ failure (Am J Hematol, 199, 38:281-87; Surg Today, 1995, 25:585-90). In a study involving pediatric sepsis patients, CD141 levels were 1.5 to 3 times greater than healthy control patients (Crit. Care Med 1998, 25: 933-8). Mice carrying CD141 gene mutation, that disrupts the CD141-dependent activation of protein C, exhibit a hypercoagulable state and an increased susceptibility to thrombosis and sepsis (Arterioscler Thromb Vasc Biol 2001 September; 21(9):1531-7). There are also reports of people with mutation in the CD141 gene presenting with thromoembolic disease (Blood 1995 Jan. 15; 85(2):330-6). Therefore the elevated levels of CD141 observed in the present study in sepsis is in complete agreement with the literature.

[0105] Other analytes that exhibited differential expression between sepsis patients and normal controls:

[0106] AgRP: Agouti-Related Protein (AgRP), first identified by Ollman et al (Science 278: 135-138, 1997), is a potent antagonist of MC3-R (melanocortin-3 receptor) and MC4-R (melanocortin-4 receptor) and is an integral component in the metabolic processes that regulate feeding behavior and body weight. Present in the hypothalamus, AgRP levels are, elevated in obesity (Katsuki et al, 2001). The mature human protein is a single polypeptide chain of 112 amino acid residues, consisting of an N-terminal acidic region and a unique C-terminal cysteine-rich domain (Biochemistry 1998 Sep. 1; 37(35):12172-7). LPS has been shown to stimulate expression of pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α in the hypthalamus and stimulate the hypothalamus-pituitary-adrenal (HPA) axis (Ann. N.Y. Acad. Sci., 2000, 917:169-74, Psychoneuroendocrinology, 2003, 28:481-500). It has been shown that mice deficient for corticotrophin releasing hormone (CTH), are unable to control systemic IL-6 levels in an induced inflammatory situation, suggesting the role of HPA regulating the inflammatory process (J. Clin. Invest., 2001, 108:1159-66). Alpha-MSH (alpha-melanocyte stimulating hormone) has been shown to attenuate the stimulatory effects

of IL-1β on the HPA. AgRP is an alpha-MSH antagonist. In primate experiments, intracerebroventricular administration of AgRP along with IL-1β significantly enhanced ACTH (adrenocorticotropic hormone) levels over administration of IL-1β alone. ACTH is an important monitor of HPA activation. These studies suggest that, in addition to regulating appetite, AgRP may play a role in neuroendocrine regulation and specifically that AgRP may interact with alpha-MSH to modulate neuroendocrine responses to inflammation (Endocrinology 2003 144(5):1736-41). Despite its role in obesity, higher levels of AgRP have been seen in brain tissue of patients who died of prolonged illness than in obese subjects (J. Clin. Endocrinol. Metab., 2002, 87:927-37). In the present study, AgRP levels are consistently higher in sepsis patients as compared to non-septic controls. These sepsis patients are severely ill with very high levels of pro-inflammatory cytokines in their circulation (as shown in Phase I report). It is therefore reasonable to expect a significant influence of these pro-inflammatory cytokines on the HPA. Given the high levels of pro-inflammatory cytokines in these patients, the increased AgRP levels may reflect the loss of HPA-homeostasis in the sepsis patients associated with the severity of the disease. Interestingly, in this regard AgRP levels on day 4 and day 7 correlated well with day 0 APACHE II scores.

[0107] There is an increasing body of knowledge that neuroendocrine and immune systems communicate bi-directionally. This interface is mediated by cytokines acting as auto/paracrine or endocrine factors in regulating pituitary development, cell proliferation, hormone secretion and feed back control of HPA axis (Endocrinology, 2002 143:1571-4; Psychoneuroendocrinology, 2002, 26:761-88). It is interesting to note that three cytokine/hormones related to the HPA seem to be modulated in the present study namely, AgRP, Leptin (Stage I report) and Prolactin (see below).

[0108] MMP-8. MMP-8 is a member of the matrix metalloproteinase family, which are zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix. It is stored as a latent proenzyme in specific granules of polymorphonuclear leukocytes. Matrix metalloproteinases are usually not expressed constitutively, and their expression is regulated by a variety of physiologic and pharmacologic signals, including cytokines and growth factors, bacterial endotoxin, phorbol esters, phagocytosable material, and hormones. Additionally, the activity of MMPs is regulated post-translationally through the activation of the proenzymes and the inhibition of active enzymes by endogenous inhibitors, α2-microglobulins and tissue inhibitors of metalloproteinases. What is the role of MMPs in sepsis? In early phase of endotoxinaemia or bacteraemia, the bone marrow is activated, which leads to leukocytosis. Neutrophils secrete a considerable amount of latent MMPs, including neutrophil procollagenase (MMP-8) and progelatinase B (MMP-9). It has been hypothesized that MMP-8 and MMP-9 may contribute to sepsis through the degradation of endothelial basement membranes, resulting in vascular leakage and shock (Acad Geneeskd Belg 2001; 63(6):531-8). Furthermore, in recent years, strong evidence has suggested a member of the MMP family to play a role in sepsis: plasma MMP-9 concentrations and monocyte MMP-9 mRNA levels were found be to significantly higher in non-surviving patients with septic shock than in surviving patients and normal controls (Am J Med Sci 1998 316:355-60) and MMP-9 deficient mice were resistant to endotoxin shock (Eur J Immunol 2002 32:2163-71). In rats MMP inhibitors have been shown to specifically suppress

TNF-α secretion following LPS stimulation (Shock, 1997, 7:427) suggesting a possible role of MMP's in inflammatory response. Our observation that plasma and serum levels of MMP-8 were elevated in sepsis patients as compared to normal controls is in agreement with the literature, and, together with the differences in MMP-7 and MMP-9 in sepsis in the previous report, suggest an important role for this protein family in the pathophysiology of sepsis.

[0109] MIF: Macrophage migration inhibitory factor is a pleiotrophic cytokine secreted by macrophages, T cells and pituitary gland during inflammatory responses (Human Cytokines. Handbook for Basic and Clinical Research, p. 222-56; FASEB J. 199610:1607-13). It is a 147 amino acid, 15.6 Kd protein. MIF is a unique cytokine in that it can function as an enzyme (EMBO J, 1998, 13:3534-41). MIF has been found to be a critical mediator of septic shock. The corticotrophic cells of the anterior pituitary contain large stores of pre-formed MIF in secretory granules, which are released into the circulation by stress or infectious stimuli such as LPS. Injection of MIF potentiates LPS-induced death in animals, and the administration of anti-MIF antibodies fully protects animals from lethal endotoxemia. MIF is also the first protein to be identified that is secreted directly from immune cells upon glucocorticoid stimulation. Once released, MIF overrides the anti-inflammatory effects of steroids and thus appears to act physiologically as a unique, glucocorticoid-induced counter-regulatory hormone. Neutralization of MIF in animal models of additional inflammatory diseases such as arthritis, glomerulonephritis, and acute lung injury has pronounced therapeutic effects. (Curr Opin Pharmacol. 2001 December; 1(6):662-8.)

[0110] MIF regulates TLR-4 expression, the signal-transducing molecule of the LPS-receptor complex, by modulating transcription factor PU.1. This has been demonstrated by studying mouse macrophage cell lines transfected with antisense MIF messenger RNA. Thereby, MIF enhances the production of inflammatory cytokines such as TNF-α, and facilitates the initiation of host defense response (Nature 2001, 414:920). Experiments with MIF knock-out mice show that these mice are resistant to high doses of LPS but still retain their ability to clear bacteria indicating MIF to be an interesting target for treatment of sepsis (J. Exp. Med. 1999, 189: 341-46). Anti-MIF antibody protected TNFα knockout mice from developing lethal peritonitis induced by cecal ligation and puncture, providing evidence of an intrinsic contribution of MIF to pathogenesis of sepsis (Nat. Med. 2000, 6:164-70). MIF has been shown to be elevated in sepsis. In a study with sepsis patients, severe sepsis patients demonstrated four to five times higher median MIF levels than in healthy controls (Intensive Care Med., 2001, 27:1412-5). In another study, high MIF levels were closely linked with poor outcome in patients with systemic inflammatory response syndrome (SIRS) (Intensive Care Med, 2001, 27:1187-93). In agreement with these reports we found MIF levels consistently higher in the sepsis patients at all time-points as compared to healthy controls.

[0111] IL-18: IL18 is an important pro-inflammatory cytokine that belongs to the IL-1 superfamily. It is a 24 kDa, non-glycosylated polypeptide with a structure similar to IL-1. IL-18 is synthesized as a bio-inactive propeptide that undergoes proteolytic cleavage by either ICE (interleukin-1 beta converting enzyme) or another caspase to generate a mature, bioactive, 18 kDa molecule (J. Immunol. 1996, 156:4274). IL-18 is an important regulator of IFN-γ production by

immune cells. One of the most important consequences of IFN-γ secretion is the activation of macrophages. This is achieved through the induction of reactive oxygen intermediates and nitrogen monoxide (NO), which activate a variety of anti-bacterial, anti-tumor and anti-viral responses (Adv. Immunol., 1996, 62:61) In addition, IFN-γ contributes to endothelial cell activation, Th1 cell development, and upregulation of MHC expression on both professional APCs and non-APCs. This makes the regulation of IFN-gamma an extremely important step in the overall scheme of an inflammatory response (J. Immunol., 1996, 157:1350). Another important function of IL18 is the regulation of functionally distinct subsets of T-helper cells required for cell-mediated immune responses (Ann. Rev. Immunolo. 19: 423-74 2001).

[0112] A number of reports have shown elevated levels of IL-18 in sepsis patients. In a study to predict adverse outcome in post-operative sepsis, levels of IL-12 and IL-18 were evaluated. IL-18 levels were significantly increased during the course of lethal sepsis. Logistic regression analysis of IL-18 values measured on days 1 or 2 of sepsis revealed high serum values, suggesting that IL-18 represents an early predictive factor for adverse outcome of sepsis (Shock, 2002, 18:301-5). In another similar study IL-18 levels correlated significantly with APACHE II scores and other proinflammatory cytokines in sepsis patients, suggesting that levels of IL-18 are a good reflection of severity of disease (J. Med., 2000, 31:15-20). Our results are in good agreement with the literature indicating that IL-18 is indeed a good candidate biomarker for sepsis.

[0113] Overview of LIF:

[0114] Leukemia inhibitory factor (LIP), originally characterized by its ability to induce differentiation in myeloid cells of a murine leukemia cell line, has many biological actions which parallel those of IL-1, IL-6 and tumor necrosis factor-alpha. LIF is a pleiotropic cytokine expressed by multiple tissue types. The LIF receptor shares a common gp130 receptor subunit with the IL-6 cytokine superfamily. LIF signaling is mediated mainly by JAK-STAT (janus-kinase-signal transducer and activator of transcription) pathways and is abrogated by the SOCS (suppressor-of cytokine signaling) and PIAS (protein inhibitors of activated STAT) proteins. In addition to classic hematopoietic and neuronal actions, LIF plays a critical role in several endocrine functions including the utero-placental unit, the hypothalamo-pituitary-adrenal axis, bone cell metabolism, energy homeostasis, and hormonally responsive tumors. Local and systemic LIF serve to integrate multiple developmental and functional cell signals, culminating in maintaining appropriate hormonal and metabolic homeostasis. LIF thus functions as a critical molecular interface between the neuroimmune and endocrine systems.

[0115] Soluble LIF Ra has been shown to inhibit the binding of LIF to its cellular receptor, suggesting that its function may be to attenuate unwanted systemic effects of LIF.

[0116] LIF and Sepsis:

[0117] LIF levels are elevated in sepsis and correlate with shock and poor prognosis. Lipopolysaccharide (LPS) administration induces hypothalamic and pituitary LIF expression in vivo, which is associated with the acute rise in circulating adrenocorticotrophic hormone (ACTH) levels (Endocrine. 1997 December; 7(3):325-30.).

[0118] Circulating leukemia inhibitory factor levels correlate with disease severity in meningococcemia. LIF partici-

pates in the host response to infection, and it may contribute to the pathogenesis of septic shock. (J Infect Dis. 1994 November; 170(5):1224-8.).

[0119] Passive immunization of mice against LIF (D factor) blocks lethality and cytokine release during endotoxemia. (J Exp Med. 1993 Sep. 1; 178(3): 1085-90.).

[0120] During day 1 of septic shock, peak plasma levels of G-CSF, interleukin (IL)-6, and leukemia inhibitory factor (LIF), but not GM-CSF, were greater than in sepsis or shock alone (p<0.001), and were correlated among themselves (rs=0.44-0.77; p<0.02) and with the APACHE II score (rs=0. 25-0.40; p=0.03 to 0.18). Plasma levels of G-CSF, IL-6, and LIF are greatly elevated in critical illness, including septic shock, and are correlated with one another and with the severity of illness (Crit. Care Med. 2000 July; 28(7):2344-54.). Patients with rapid onset of septic shock demonstrated very high but transient tumor necrosis factor, interleukin (IL)-6, IL-8, and leukemia inhibitory factor concentrations (Crit. Care Med. 1997 March; 25(3):405-12.).

[0121] LIF, a pleiotropic cytokine with many biologic effects overlapping with those of IL-6, has been implicated in the pathogenesis of sepsis. LIF levels increased 2 h after E. coli challenge, and reached maximum values at 4 and 8 h after a sublethal (4.4+/−1.6 ng/ml) or lethal (40.9+/−3.8 ng/ml) dose, respectively. Circulating LIF correlated with plasma levels of IL-6, both after E. coli challenge (Spearman Rank coefficient of correlation (r)=0.849, p<0.001), as well as upon TNF-alpha injection (r=0.863, p<0.001). TNF-alpha is an intermediate factor in concerted release of LIF and IL-6 in vivo, and indicate that the enhanced elaboration of these cytokines may predict disease outcome in severe sepsis. (J. Immunol. 1996 Jun. 1; 156(11):4401-7.).

[0122] LIF protects against experimental lethal *Escherichia coli* septic shock in mice. This protective effect resembled endotoxin tolerance and was characterized by suppression of E. coli-induced serum tumor necrosis factor concentration (P<0.05), reduction in the number of viable bacteria (P<0.05), and prevention of

[0123] Sepsis-induced tissue injury. Systemic LIF production is part of the host response to both endotoxin and sepsis-induced tissue injury. (Proc Natl Acad Sci USA. 1995 Feb. 28; 92(5):1337-41.)

[0124] Leukemia inhibitory factor levels are elevated in septic shock and various inflammatory body fluids. Serum LIF concentrations were transiently elevated (2-200 ng/ml) in six subjects with meningococcal or Gram-negative septic shock. Moderately elevated LIF concentrations (>10 ng/ml) were detected in cerebrospinal fluid from subjects with bacterial meningitis, in effusions associated with pneumonia and peritonitis, and in amniotic fluid from a woman with chorio-amnionitis. (J Clin Invest. 1992 November; 90(5):2031-7.).

Example 6

Biomarker Discovery

[0125] The major goals of this longitudinal study were to confirm and extend the findings of reported in the prior longitudinal study (Examples 2 and 4) in a second patient cohort and to perform a first examination for candidate biomarkers for death in sepsis.

Candidate Markers for Sepsis

[0126] Identification of sepsis biomarkers was performed using the same criteria defined in Examples 2 and 4, by

identifying analyte profiles that revealed differences between the sepsis patient group and a group of normal controls at pre-infusion. The candidate sepsis biomarker profile of interest should show a marked difference in analyte level between 12 sepsis patients and 8 normal controls during acute sepsis prior to therapy (time zero). Analysis of variance (ANOVA) was used to identify significant differences between normal and sepsis patients at pre-infusion using GLM procedure of SAS. Because this analysis is limited by sample size, caution is required in interpreting p-values. With a small number of patients, there is increased potential for underestimation of p-values.

[0127] Only analytes that fulfilled the following criteria were evaluated as possible sepsis markers: (1) at least one of the group means (either sepsis group or normal controls) should be greater than 10 when MFI is expressed in log 2 scale (>1024 MFI in the linear scale), (2) there should be at least a two-fold difference between the group means, and (3) the p-value for the difference should less than 0.05. The first two criteria served as filters to ensure that (1) the data were within a fluorescent intensity range where meaningful measurements could be made, and (2) the differences were large enough so that differences due to random events in the small sample set were likely to be excluded.

[0128] 63 out of 133 analytes (35 of 133 serum analytes and 48 of 133 plasma analytes) showed greater than 2-fold changes in intensity between sepsis patients and normal controls. 25% (20/83) of analytes exhibited greater than 2-fold difference in both serum and plasma matrices. Half of those (9/18) that exhibited more than a 5-fold difference in either plasma or serum also showed at least a 2-fold difference in the other matrix. Most notable among the latter were IL-6 and ST-2, because of large differences in both serum and plasma. However, several analytes exhibiting largest differences did so in only one matrix [leptin (serum only), MIP-3α, (plasma only), IL-2sRα, (serum only), IGFBP-1 (plasma only) and IL-18 (plasma only)]. IGFBP-1 is notable because in the sepsis survivors IGFBP-1 levels fell over time, whereas in the cohort that died, levels remained high. Thus, plasma IGFBP-1 level may be useful both in diagnosis of sepsis and prediction of sepsis death, albeit group sizes in the present study were very small. Discordance between serum and plasma underscores the benefit of measurements in both matrices for identification of biomarkers for sepsis.

[0129] The largest change was a 26-fold higher level of IL-6 in sepsis patients. CRP, an important acute response marker, was artifactually lower in sepsis patients than normal controls due to saturating levels of analyte in the undiluted sample. This saturation artifact was reduced upon dilution of the sample.

[0130] 10 of 11 candidate sepsis biomarkers identified in the prior longitudinal study were concordant with the results obtained in this study. With the exception of AgRP, the other 10 cytokines that were previously identified as potential sepsis biomarkers also demonstrated significant differences in between sepsis patients and normal controls in Study 2. They were CD141, IL-18, IL-2sRα, IL-6, IL-8 [plasma], MIF [plasma], MMP-8 [plasma], MMP-7, MPIF (plasma) and IGFBP-1 (plasma) (Table 3). A number of other analytes including ST2 (25 fold), FGF-21 (9.5 fold), Leptin (9 fold), MCP-1 (8 fold), MIP-3β and TNF-R1 (7 fold) showed greater than >5 fold difference between sepsis patients and normal controls in this study alone. This study may have been more sensitive for identification of sepsis biomarkers than the prior

study because there were more patients in this study, and sepsis was, on average, more severe, in the patients in this study.

TABLE 3

Concordance of Study 2 sepsis biomarkers with Study 1

Analyte Name	Matrix	Study1	Study2
AgRP	Serum	↑	—
CD141	Serum	↑	↑
CD141	Plasma	—	↑
IL-18	Serum	↑	↑
IL-18	Plasma	—	↑
IL-2 sRα	Serum	↑	↑
IL-2 sRα	Plasma	↑	↑
IL-6	Serum	↑	↑
IL-6	Plasma	↑	↑
IL-8	Serum	↑	—
IL-8	Plasma	↑	↑
MIF	Plasma	↑	↑
MMP-8	Plasma	↑	↑
MPIF-1	Serum	↑	—
MPIF-1	Plasma	↑	↑
IGFBP-1	Serum	↑	—
IGFBP-1	Plasma	↑	↑
MMP-7	Serum	↑	↑
MMP-7	Plasma	↑	↑

Legend:
'—' no difference;
'↑' elevated in sepsis patients,
'↓' decreased in sepsis patients

[0131] When sepsis samples were compared to normal serum at the pre-infusion time-point, there were significant differences in the levels of several analytes (e.g., IL-6, ST2, FGF-21, IL2sRα, MCP-1, MIP-3β, TNF-R1, Leptin, MMP-7, and MMP-8). Some of the observed changes in their levels were supported by the literature. IL-6, IL-2sRα and MMP-7 had previously been identified as candidate sepsis biomarkers in our prior study. These analytes are briefly discussed below.

IL-6 (Interleukin-6)

[0132] It is known that levels of IL-6 are increased in sepsis. In the present study, IL-6 was found to be elevated in sepsis samples at study enrollment (compared to normal controls), and to gradually decrease with time, approaching normal levels at day 7. IL-6 is an important pro-inflammatory cytokine that contributes to organ dysfunction in sepsis (Scand J Immunol 1998 48:509-14; Res Commun Chem Pathol Pharmacol 1994 84:291-300). Levels of IL-6 have also been shown to be associated with severity and mortality in sepsis (Eur J Haematol 1993 50:243-9; Nippon Geka Gakkai Zasshi 1996 97:1054-9). Therefore the high initial levels and reduction with time, observed in the present study, is in agreement with the literature.

ST2 (Suppression of Tumorigenicity 2; Interleukin 1 Receptor 4)

[0133] ST2/ST2L, a member of the IL-1R gene family, is expressed by fibroblasts, mast cells, and Th2 cells (J. Immunol. 2001; 166:6633-9). It exists in both membrane-bound (ST2L) and soluble forms (ST2). Although ST2/ST2L has immunoregulatory properties, its ligand, cellular targets, and mode of action are unknown. In the present study, ST2 was elevated in sepsis patients. This is a novel finding. It has been reported that ST2 binds to primary bone marrow-derived macrophages (BMM) and that this binding was enhanced by

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treatment with LPS (J. Immunol. 2001; 166:6633-9). ST2 treatment of BMMs inhibited production of the LPS-induced proinflammatory cytokines IL-6, IL-12, and TNF-alpha but did not alter IL-10 or NO production. Treatment of BMMs with ST2 down-regulated expression of Toll-like receptors-4 and -1 but induced nuclear translocation of NF-kappaB. Administration of ST2 in vivo after LPS challenge significantly reduced LPS-mediated mortality and serum levels of IL-6, IL-12, and TNF-alpha (J. Immunol. 2001; 166:6633-9). Conversely, blockade of endogenous ST2 through administration of anti-ST2 Ab exacerbated the toxic effects of LPS. Thus, ST2 has anti-inflammatory properties in sepsis by acting directly on macrophages. Increased levels of ST2 in sepsis patients observed in this study may represent a protective mechanism against the effects of LPS-induced proinflammatory cytokines. ST2 may represent a candidate for sepsis therapy.

FGF-21 (Fibroblast Growth Factor-21)

[0134] Little is known about FGF-21. It is most similar (approximately 35% amino acid identity) to FGF-19 and has a hydrophobic amino terminus (approximately 30 amino acids), with a typical signal sequence. FGF-21 mRNA was most abundantly expressed in the liver, and also in the thymus at lower levels. In the present study, FGF-21 was found to be elevated in sepsis patients. No literature reports describing a direct link between FGF-21 and sepsis were identified.

IL-2sRα (Interleukin 2 Soluble Receptor Alpha)

[0135] The biological activity of IL-2 is mediated by binding to a high affinity receptor complex composed of IL-2 Rα, β, and γ. By itself, IL-2 Rα binds IL-2 with low affinity, but is unable to transduce a signal. A soluble form of IL-2 Rα appears in serum, concomitant with its increased expression on cells (Immunol Today 1993 14:264-70, FASEB J 1991 5:2567-74, Hybridoma 1985 4:91-102). The function of IL-2 Rα is unclear, since it would be expected to be a poor inhibitor of IL-2 because of its low binding affinity. In any case, increased levels of soluble IL-2 Rα in biological fluids reportedly correlate with increased T cell, B cell and immune system activation.

[0136] Elevated level of sIL-2R has been observed in sepsis patients in multiple studies (Br J Surg 2000 87:223-30, Intensive Care Med 1999 25:1402-6, J Infect Dis 1991 163:1145-8). An increase of soluble IL-2 receptor level was also observed in patients with systemic inflammatory response syndrome (Thromb Res 1999 95:205-13). Furthermore, the level of sIL-2 receptor was found to be higher in non-surviving patients with septic shock than in surviving patients (J Infect 1998 37:241-7, Ann Clin Lab Sci 1996 26:426-32). The concentration of sIL-2R was found to have predictive value for identifying patients with gram-negative sepsis at risk for progression toward the syndrome of septic shock (J Crit. Care 1995 10:64-71). High concentrations of IL-6, IL-8 and sIL-2R predict organ failure in patients with community-acquired septic shock, and the combination of these markers may provide the means to identify sepsis patients who will develop organ failure (Clin Sci 1999 97:529-38). Furthermore, a study suggests that soluble interleukin-2 receptor levels can be used to facilitate the diagnosis of sepsis in premature infants where sepsis diagnosis is difficult because newborn infants often

suffer from bacterial and viral infections without presenting typical symptoms (J Pediatr 1995 126:982-5). Therefore literature reports corroborate the increase of sIL-2R levels observed in the present study.

MCP-1 (Monocyte Chemoattractant Protein-1)

[0137] MCP-1 belongs to a superfamily of chemokines that have a leading role in the early chemotaxic events during inflammation (J Comp Neurol. 2001 434:461-77). In the present study, MCP-1 was found to be elevated in sepsis patients. This was a novel finding. It has been shown that MCP-1 protects mice in lethal endotoxemia (J Clin Invest. 1997 99:2832-6). Intraperitoneal administration of LPS to CD-1 mice induced a substantial increase in MCP-1 in plasma, lung, and liver. Immunization of mice with rabbit antimurine MCP-1 antiserum 2 h before endotoxin administration resulted in an increase in LPS-induced mortality from 10% to 65% and was associated with increases in TNF-alpha and IL-12 levels (J Clin Invest. 1997 99:2832-6). Conversely, the administration of recombinant murine MCP-1 intraperitoneally protected mice from endotoxin-induced death, and resulted in an increase in IL-10 levels and a decrease in IL-12 levels (J Clin Invest. 1997 99:2832-6). These findings suggest that MCP-1 has protective effects in murine endotoxemia. Similarly, it has been demonstrated that endogenous MCP-1 protects mice in a model of acute septic peritonitis (J. Immunol. 1999 163:6148-54). MCP-1 appears to influence the cytokine balance in tissues in favor of anti-inflammatory and immune-enhancing cytokines, probably protecting the host from tissue/organ damage during sepsis (Exp Mol. Pathol. 2000 68:77-84). Human peritoneum rapidly reacts to abdominal surgery with increased production of MCP-1 (Shock. 2000 14:91-4). The increased levels of MCP-1 in sepsis patients, observed in this study, may represent a protective mechanism against the effects of sepsis.

MIP-3β (Macrophage-Inflammatory Protein-3 Beta)

[0138] MIP-3β is a member of the CC-chemokine family. In the present study, MIP-3β was found to be elevated in sepsis patients. MIP-3β is expressed in bone marrow after induction with inflammatory cytokines and LPS, and may play a role in trafficking of macrophage progenitors in and out of the bone marrow in inflammatory conditions (J. Immunol. 1998 161:2580-5). Addition of human MIP-3β beta to cultures of human PBMCs that have been activated with LPS resulted in a significant enhancement of IL-10 production (J. Immunol. 1999 163:4715-20). This effect was concentration-dependent. MIP-3β addition also inhibited IL-12 p40 and TNF-alpha production by monocytes and IFN-gamma production by T cells, possibly via IL-10 induction (J. Immunol. 1999 163:4715-20). These MIP-3β effects may represent a protective mechanism in sepsis. The increased levels of MIP-3β in sepsis patients, observed in the present study, is a novel finding.

TNF-R1 (Tumor Necrosis Factor Receptor 1)

[0139] TNF-α is a major mediator of apoptosis, inflammation and immunity, and it has been implicated in the patho-

genesis of a wide spectrum of human diseases, including sepsis. TNF-alpha exerts its biologic effects through two distinct cell surface receptors, TNF-R1 and TNF-R2. The extracellular domain of TNF-alpha receptors inhibits TNF-α effects (Immunology. 1992 76:20-3). In the present study, TNF-R1 was found to be elevated in sepsis patients. Both soluble TNF receptor proteins (sTNF-R1 and sTNF-R2) have been described to influence the post-traumatic inflammatory response and organ dysfunction (Acta Anaesthesiol Scand. 2001 45:364-70). TNF alpha and soluble TNF receptor levels were elevated in trauma patients compared to healthy persons. Severe trauma led to enhanced sTNF-R1 levels on scene and on hospital admission. Development of systemic inflammatory response syndrome (SIRS) along with elevated sTNF-R1 began on scene and was present on admission. In trauma patients, early post-traumatic multiple organ dysfunction syndrome (MODS) and SIRS coincided with increased levels of TNF alpha and TNF receptor proteins, revealing different, time-dependent changes (Acta Anaesthesiol Scand. 2001 45:364-70). Transgenic mice expressing high levels of soluble TNF-R1 fusion protein are protected from lethal septic shock (Eur J. Immunol. 1995 25:2401-7). In children with severe meningococcaemia, the levels of sTNF-RI, -RII and TNF-alpha were markedly increased and all three correlated with the disease outcome. At admission, in patients with fatal outcome, the ratios TNF-alpha/sTNF-RI and -RII were higher than in survivors (Immunology. 1992 76:20-3). The increased levels of TNF-R1 in sepsis patients may represent a protective mechanism against the effects of proinflammatory cytokines.

Leptin

[0140] Leptin, an adipocyte-derived signaling factor, is a member of the IL-6 cytokine family. In addition to regulating food intake and energy expenditure, leptin also plays an important role in hematopoiesis. Leptin increases the proliferation of hematopoietic stem cell populations, and the proliferative effects seem to be at the level of a multilineage progenitor, as shown by increased myelopoiesis, erythropoiesis and lymphopoiesis (Curr Biol 1996 6:1170-80).

[0141] Both leptin and IL-6 are hypersecreted in acute critical illness such as sepsis. Leptin inhibits, whereas IL-6 stimulates, the hypothalamic-pituitary-adrenal axis (Horm Metab Res 1998 30:726-9). Patients with sepsis or septic shock were found to have leptin concentrations 2.3- and 4.2-fold greater, respectively, than a control group (J Infect Dis 1999 180:908-11). In a study looking at the serum leptin levels in 55 children with severe sepsis, the increase in leptin levels was higher in non-survivor patients than in survivors (Acta Paediatr 2002 91:626-31).

[0142] In the present study, leptin was found to be elevated in sepsis patients, which is in agreement with literature.

Matrix Metalloproteinase-8 (MMP-8)

[0143] Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic devel-

opment, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Matrix metalloproteinases are usually not expressed constitutively, and their expression is regulated by a variety of physiologic and pharmacologic signals, including cytokines and growth factors, bacterial endotoxin, phorbol esters, phagocytosable material, and hormones. Additionally, the activity of MMPs is regulated post-translationally through the activation of the proenzymes and the inhibition of active enzymes by endogenous inhibitors, α2-microglobulins and tissue inhibitors of metalloproteinases. Neutrophils secrete a considerable amount of MMPs, including neutrophil procollagenase (MMP-8). It has been hypothesized that MMP-8 may contribute to sepsis through the degradation of endothelial basement membranes, resulting in vascular leakage and shock (Acad Geneeskd Belg 2001; 63(6):531-8). Furthermore, in rats MMP inhibitors have been shown to specifically suppress TNF-α secretion following LPS stimulation (Shock, 1997, 7:427) suggesting a possible role of MMP's in inflammatory response.

Matrix Metalloproteinase-7 (MMP-7)

[0144] MMP-7 activity is upregulated in liver metastases of colorectal cancer (Clin Cancer Res 2002 8:144-8). Studies in mice suggest that MMP-7 regulates the activity of defensins in intestinal mucosa. Defensins represent an important component of host response to infection. No literature reports describing a direct association between MMP-7 levels and sepsis were found in the literature.

Candidate Biomarkers for Mortality in Sepsis

[0145] The profile of interest for candidate mortality markers in sepsis was a difference in analyte levels between the placebo survivors (3 patients) and placebo non-survivors (3 patients) that occurred in the days immediately preceding death. The time-period between day 0 and day 4 is of the greatest interest in this analysis, because this was the period during which most sepsis patients died. It is also important to qualify this analysis by recognizing that 3 patients per group is insufficient for the true significance of differential expression to be calculated. Given these caveats, ANOVA was used to estimate the ratio of specific to random effects, and to generate a list of analytes ranked by effect size that exhibit significant differences between the placebo survivors and placebo non-survivors. As previously described, only cytokines that fulfilled the following criteria were evaluated: (1) at least one of the group means (either sepsis group or normal controls) should be greater than 10 when MFI is expressed in log 2 scale (>1024 MFI in the linear scale) and (2) there should be at least a two-fold difference between the group means.

[0146] 16 of 133 serum analytes and 3 of 133 plasma analytes exhibited a greater than 2-fold death difference in the day(s) immediately preceding death (Table 4). Analytes showing greatest difference prior to death (>3 fold change) in the analysis performed with the non-normalized data: Follistatin (15 fold), Gro-γ (11.46 fold), MIG (9.5 fold), BDNF (5.8 fold), Neutrophil elastase (3.5 fold), IL-18 (3.47) and MIP-3β (3.2 fold).

TABLE 4

Candidate biomarkers for mortality in sepsis from non-normalized data: Analytes exhibiting >2 fold
differences between placebo survivors (3 patients) and placebo non-survivors (3 patients).

Array	Cytokine	Matrix	Dilution	Time-point	Alive	Dead	Fold	F	MS	p-value
Array4	Follistatin	SERUM	Neat	3.00	8.40	12.31	15.02	1692.70	11.46	<0.01
Array4	GRO-g	SERUM	Neat	2.00	12.71	9.19	11.46	28.78	9.28	0.03
Array2	MIG	SERUM	Neat	0.00	10.60	13.90	9.85	4.77	16.34	0.09
Array2	BDNF	SERUM	Neat	2.00	13.47	10.92	5.85	32.87	9.74	<0.01
Array5	Neut Elast	SERUM	Diluted	0.00	13.44	15.28	3.58	6.68	5.81	0.05
Array5	IL-18	SERUM	Neat	0.00	11.87	13.66	3.47	6.74	4.82	0.06
Array3	MIP3β	SERUM	Neat	3.00	11.41	9.72	3.24	194.39	3.45	<0.01
Array4	Follistatin	SERUM	Neat	0.00	9.69	11.21	2.87	167.84	3.47	<0.01
Array2	Flt3Lig	SERUM	Neat	3.00	11.71	10.22	2.81	7.34	2.66	0.07
Array4	Follistatin	SERUM	Neat	1.00	8.91	10.26	2.54	12.47	1.35	0.07
Array5	IGFBP-6	SERUM	Diluted	3.00	12.89	14.13	2.35	39.92	1.83	0.01
Array2	HCC4	SERUM	Neat	0.00	14.01	15.21	2.31	26.91	2.19	0.01
Array5	ProteinS	SERUM	Diluted	0.00	13.46	14.39	1.91	5.49	1.48	0.07
Array5	D-Dimer DD6	PLASMA	Neat	0.00	12.73	11.81	1.89	21.08	1.26	0.01
Array2	HCC4	PLASMA	0.00	0.00	14.47	15.38	1.88	13.28	1.24	0.02
Array2	MIP-1b	SERUM	0.00	2.00	10.73	9.83	1.87	5.03	1.22	0.09
Array5	MIF	PLASMA	1.00	0.00	9.66	10.51	1.80	5.78	1.08	0.07
Array2	SCF	SERUM	0.00	0.00	9.74	10.58	1.79	6.53	1.07	0.06
Array5	ProteinC	SERUM	0.00	1.00	12.01	12.85	1.79	6.32	1.05	0.07

See datafile 'AnovaLSMEANSEventByTherapyS2abs.xls'.

[0147] Some of the analytes in Table 4 are likely to show significant differences because of differences at the pre-infusion time-point. The data was therefore adjusted for baseline levels and ANOVA was performed on normalized data from serum samples. Plasma samples were not analyzed since samples from day 1, 2 and 3 samples were not available. Results are shown in Table 5.

correlation between Protein C levels and survival. A complete set of results and analysis variance of placebo survivors vs. placebo non-survivors are provided in the appended excel files: 'AnovaLSMEANSEventByTherapyS2abs.xls' for non-normalized data and 'AnovaLSMEANSEventByTherapyS2ratio.xls' for normalized data.

TABLE 5

Candidate biomarkers for mortality in sepsis from normalized data: Analytes exhibiting >1.6
fold differences between placebo survivors (3 patients) and non-survivors (3 patient).

Array	Cytokine	Dilution	Time-point	Therapy	Alive	Dead	Diff	Fold	MS	F	p-value
Array5	Neut Elast	Diluted	3.00	PLACEBO	0.50	-2.64	3.14	8.81	11.82	7.60	0.07
Array5	MMP-8	Diluted	3.00	PLACEBO	0.38	-2.56	2.93	7.65	10.33	5.59	0.10
Array4	Follistatin	Neat	3.00	PLACEBO	-1.29	1.14	2.43	5.40	4.44	202.59	<0.01
Array2	Flt3Lig	Neat	3.00	PLACEBO	0.81	-1.15	1.96	3.88	4.60	17.59	0.02
Array2	BDNF	Neat	2.00	PLACEBO	-0.51	-2.46	1.94	3.85	5.67	7.20	0.06
Array4	IL-1srII	Neat	1.00	PLACEBO	-1.18	0.32	1.50	2.82	1.68	161.83	0.01
Array2	MIG	Neat	3.00	PLACEBO	0.27	-1.06	1.33	2.51	2.11	131.40	<0.01
Array2	Flt3Lig	Neat	2.00	PLACEBO	0.48	-0.78	1.26	2.39	2.38	38.54	<0.01
Array4	IL-10rb	Neat	3.00	PLACEBO	-1.19	-0.09	1.10	2.14	0.90	17.00	0.05
Array5	CD40	Diluted	3.00	PLACEBO	0.32	-0.53	0.85	1.80	0.87	11.40	0.04
Array2	PARC	Neat	3.00	PLACEBO	0.40	-0.39	0.79	1.73	0.75	6.61	0.08
Array2	PARC	Neat	2.00	PLACEBO	0.30	-0.43	0.73	1.66	0.80	7.61	0.05
Array2	Flt3Lig	Neat	1.00	PLACEBO	0.18	-0.55	0.73	1.66	0.80	39.86	<0.01
Array5	ProteinC	Diluted	2.00	PLACEBO	0.21	-0.50	0.71	1.64	0.76	4.78	0.09
Array5	ProteinS	Neat	3.00	PLACEBO	-0.21	-0.90	0.69	1.61	0.57	40.49	0.01
Array5	TSH	Neat	2.00	PLACEBO	-0.37	0.32	0.68	1.61	0.70	7.51	0.05

See datafile 'AnovaLSMEANSEventByTherapyS2ratio.xls'.

[0148] Several analytes showed significant differences between placebo survivors and non-survivors, including neutrophil elastase, MMP-8, follistatin, MIG and BDNF. Neutrophil elastase (9 fold, day 3), MMP-8 (8 fold, day 4) and Follistatin (8 fold, day 3) showed a >5 fold change between placebo survivors and placebo non-survivors. Interestingly, Protein C rate of intensity changes were reduced 1.6-fold in patients who died as compared to survivors, suggesting a

Neutrophil Elastase

[0149] Severe inflammatory responses after surgery, trauma, and infection result in multiple organ dysfunction. Activated neutrophils are thought to be important in the pathogenesis of these responses acting via secretion of neutrophil elastase (NE), the dominant serine proteinase secreted from activated neutrophils (Inflammation. 1994 18:337-47). Neutrophil elastase is stored in azurophil granules along with other serine proteinases. As a result of its capacity to effi-

ciently degrade extracellular matrix, NE has been implicated in a variety of destructive diseases (Nat. Med. 1998 4:615-8). In the present study, NE was found to be specifically elevated in placebo-treated sepsis patients that did not survive. This is a novel finding.

[0150] It has been shown that a recombinant human Kunitz-type proteinase inhibitor (R-020) was effective in significantly improving survival after induction of lethal peritonitis in rats (Inflammation. 1994 18:337-47). It has been suggested that various serine proteinases are implicated in the pathogenesis of neutrophil-related multiple organ failure and that recombinant human Kunitz-type proteinase inhibitors might be effective in the treatment of organ dysfunction. Similarly, in a hamster model of acute lung inflammation, analysis of BAL fluid revealed that after LPS administration the total cell number and neutrophil number, albumin concentration, and elastase-like activity were significantly lower in animals injected with an inhibitor of neutrophil elastase (ONO-5046. Na) (Eur Respir J. 1995 8:1293-9). Histological examination of the lungs of the animals treated with LPS alone showed intra-alveolar haemorrhages and inflammatory cell infiltration after LPS administration, whereas the lungs of the ONO-5046.Na-treated animals were only sparsely infiltrated by inflammatory cells. On the other hand, it has been demonstrated that mice lacking neutrophil elastase reveal impaired host defense against Gram negative bacterial sepsis (Nat. Med. 1998 4:615-8). NE−/− mice are more susceptible than their normal littermates to sepsis and death following intraperitoneal infection with Gram negative (*Klebsiella pneumoniae* and *Escherichia coli*) but not Gram positive (*Staphylococcus aureus*) bacteria. Neutrophils migrated normally to sites of infection in the absence of NE, but NE was required for maximal intracellular killing of Gram negative bacteria by neutrophils. Therefore, the levels of NE need to be precisely regulated and deviations from the optimal range could lead to severe damage and death.

Follistatin

[0151] Follistatin (FS) is the specific binding protein of activin, a growth and differentiation factor of many cell types. In the present study, follistatin was found to be elevated in placebo-treated sepsis patients that did not survive. It has been shown that the serum concentration of follistatin is elevated in patients with septicemia, consistent with a potential role of follistatin in the systemic inflammatory response (Eur J. Endocrinol. 2003 148:559-64; Clin Endocrinol (Oxf). 1998 48:413-7). It has been demonstrated that activin A and follistatin are involved in the systemic inflammatory response (Mol Cell Endocrinol. 2001 180:155-62). Activin A is released early in the cascade of circulatory cytokines during systemic inflammatory episodes. Release of activin A into the circulation occurred in sheep within 1 hour of injection of lipopolysaccharide (Endocrinology. 2000 141:1905-8). This rapid peak in activin A preceded the release of the key inflammatory cytokines, TNF-alpha and IL-6. Follistatin release into the circulation occurred some 4 hours after the peak of activin A and continued out to 24 hours from lipopolysaccharide treatment. Although the exact role of follistatin in sepsis and its outcome has not yet been revealed, the involvement of FS in systemic inflammation has been demonstrated by the changes in follistatin levels.

MMP-8

[0152] When normalized data was evaluated, a rapid decline in MMP-8 levels was observed in sepsis patients who

died (Table 5). MMP-8 has previously been discussed. The rapid decline of MMP-8 in sepsis non-survivors was an unexpected and novel finding.

MIG (Monokine Induced by Interferon Gamma)

[0153] MIG belongs to the family of chemotactic cytokines known as CXC-chemokines. The MIG receptor is CXCR3, a receptor also shared by two other chemokines, I-TAC and IP-10. The synthesis of MIG is specifically induced in macrophages and in other cells by IFN-gamma, but not by IFN-alpha or bacterial lipopolysaccharides. It is thought that MIG is involved in modulating the growth, motility, and activation state of cells participating in inflammatory reactions. MIG is a chemoattractant for stimulated but not resting T-cells, and is not active on neutrophils or monocytes. As is the case with MMP-8, MIG levels also decline rapidly in sepsis patients who died.

BDNF

[0154] Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in neuronal survival and plasticity that binds to high-affinity receptors named TrkB. In the present study, BDNF was found to be decreased in placebo-treated sepsis patients that did not survive. It has been shown that LPS enhanced the production/secretion of BDNF from microglia (J. Neurochem. 2002 80:697-705; J Neurosci Res. 2001 65:322-31). LPS stimulation caused a marked increase in BDNF mRNA expression (J Neurosci Res. 1997 50:1023-9). LPS also elevated the expression of BDNF in mouse splenocytes and B cells (J. Neuroimmunol. 2000 103:112-21). The increased BDNF levels following LPS stimulation indicate a potential involvement of BDNF in systemic inflammation.

Correlation of APACHE II Scores with Cytokine Levels at Pre-Infusion Time-Point

[0155] Identifying cytokines that appear to represent surrogates for APACHE II scores is of interest since greatest benefit of certain drug treatments is evident in a high APACHE II score population. In addition, APACHE II scores may be used to determine the severity of sepsis in a patient suspected of having sepsis at a given time. APACHE II scores are determined subjectively based upon a number of clinical signs and symptoms. APACHE II scores may vary somewhat when determined by different physicians. An objective test that both identifies sepsis in hospitalized patients and can substitute for APACHE II score as an approved indication may be beneficial in assisting physicians select patients for certain drug therapy or identify patients with sepsis who are deteriorating or improving. If pre-infusion time-point cytokine levels correlate well with APACHE II scores, these cytokines may be useful as replacements or supplements to APACHE II scores. With this in mind, linear correlation of cytokine levels at pre-infusion time-point and APACHE II scores was performed in a manner similar to analysis done for study 1. A good correlation in this small patient subset suggests that these cytokines may have value as surrogate markers for APACHE II scores. For a simple bivariate correlation, Rho squared is qualitatively equal to $R2$ in linear regression analysis. The Pearson coefficient of correlation is very sensitive to outliers and non-linear relationships between variables, so the quality of the data as regards both of these aspects needs to be considered. The results of the analysis are shown in Table 6. Several analytes including AFP, CTACK and Eot-3 correlated well with baseline APACHE II scores.

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With the exception of CTACK, analytes that correlated with APACHE II scores in study 1 did not exhibit high correlation with baseline APACHE II scores in Study 2.

[0156] Ten analytes (6 of the 133 serum analytes and 5 of the 133 plasma analytes) correlated with baseline APACHE II score at pre-infusion point with a correlation coefficient of >0.6. Complete results of the correlation analysis are appended.

TABLE 6

Correlation analysis of analyte levels and baseline
APACHE II at pre-infusion time point (cytokines
with correlation coefficient >0.6 are shown.

Cytokine	Dilution	SAMPLETYPE	rho
AFP	Neat	PLASMA	0.739085
AFP	Neat	SERUM	0.682285
CTACK	Neat	SERUM	0.659666
Eot-3	Neat	SERUM	0.617859
FGF-basic	Neat	SERUM	0.615801
IGFBP-4	Neat	SERUM	0.679348
IGFBP2	Neat	PLASMA	0.646765
sgp130	Neat	PLASMA	0.659828
sgp130	Neat	SERUM	0.664349
AFP	Diluted	PLASMA	0.779448
AFP	Diluted	SERUM	0.726881
E-Selectin	Diluted	SERUM	0.602813
IL-18	Diluted	PLASMA	0.618898

Example 7

A Larger Longitudinal Study

[0157] Serum samples for 127 patients were provided for this study. There were 58 survivors and 69 non-survivors. Samples were run at a 1:5 dilution on five arrays. In addition, the study included 57 samples taken from acutely ill patients in an Intensive Care Unit (ICU) with non-sepsis illnesses. All samples were serum. In studies 1 and 2 shown in Examples 2, 4, and 6, all samples were run undiluted on protein arrays. For the current study, all samples were diluted five-fold. The five-fold dilution resulted in somewhat decreased signals for all analytes and increased background on array 3.

[0158] All samples were diluted 1:5 and run a total of three times on each array, on consecutive days. Each of the different runs was treated as a separate, complete dataset for analysis. Analyses of variance were completed for each day, and the results were compared. Only analytes exhibiting differences

between groups that fulfilled the following significance criteria were included in this report: (1) p value for the observed difference between groups should be less than 0.05 (2) there should be a significant change identified in at least two of the 3 replicates, (3) the change should be concordant (log$_2$ ratio either positive or negative) between replicates, and (4) the size of the observed difference should be greater than the variability observed in the blank control feature on the array, to eliminate false positives attributable to random variation.

[0159] Candidate Markers for Sepsis

[0160] Sepsis biomarkers in Study 3 were identified using criteria slightly different than those which were used in Longitudinal Studies 1 and 2. In the previous two sepsis studies, analyte levels in sepsis patients at pre-infusion were compared to analyte levels in healthy individuals to identify analytes elevated in sepsis. These studies identified markers that were not necessarily specific to sepsis since elevated expression levels may have represented acute phase reactants. In an effort to identify markers specific for sepsis, the present study contained comparisons between the sepsis patient group at pre-infusion and a group of equally ill patients that had been admitted to the intensive care unit with a variety of illnesses but who did not have sepsis. The use of sick controls was expected to yield markers with greater specificity for sepsis by controlling for differential expression in acute illness. The sepsis biomarker profile sought in the present study was a significant difference in analyte level between the sepsis patient group during acute sepsis prior to therapy (pre-infusion) and the sick control group. Analysis of variance (ANOVA) was used to identify significant differences between sick and sepsis patients using the GLM procedure of SAS. Differences between the two groups were not considered relevant if they showed less variability than the blank control feature on each array. Results are shown in Table 7.

[0161] 29 of 83 analytes demonstrated significant differences in mean fluorescence intensity between the sepsis patient group at pre-infusion and sick controls (Table 7). 17 of the 29 had more than a two-fold difference in mean fluorescence intensity over all three assay repeats. IL-1Ra (16×) and IL-6 (12×) showed the greatest elevated levels of expression in sepsis patients as compared to sick controls. MCP-1 (5×), MPIF-1 (4×), IL-2sRa (3.5×) TNF-R1(3×), MIG (2.5×) and ST-2 (2.5×) were also substantially elevated in sepsis patients. Interestingly, other analytes showed decreased expression in the sepsis group as compared to sick controls. For instance, EGF levels were fourfold lower in the sepsis group, and ENA-78 and HCC4 were almost 3-fold lower.

TABLE 7

Study 3 biomarkers for sepsis on Arrays 1-3: 483 sepsis and 57 ill control samples
were run a total of three times on each array, on consecutive days (replicate).

| Analyte | | log2 ratio | | Sepsis | | Sick | | |
| | | (Sepsis/Sick) | | Mean | | Mean | | |
Name	Replicate	Mean	Std Dev	log2 MFI	Std Dev	log2 MFI	Std Dev	p-value
BLC	run 1	0.63	1.17	7.32	1.26	6.69	0.62	0.00041
	run 2	0.82	1.18	6.95	1.24	6.13	0.86	0.000004
	run 3	0.65	1.2	7.7	1.27	7.05	0.84	0.000404
EGF	run 1	−2.2	1.3	7.89	1.34	10.09	1.1	<.000001
	run 2	−2.27	1.31	7.4	1.23	9.67	1.63	<.000001
	run 3	−2.31	1.24	8.13	1.27	10.44	1.1	<.000001

TABLE 7-continued

Study 3 biomarkers for sepsis on Arrays 1-3: 483 sepsis and 57 ill control samples
were run a total of three times on each array, on consecutive days (replicate).

		log2 ratio (Sepsis/Sick)		Sepsis Mean		Sick Mean		
Analyte Name	Replicate	Mean	Std Dev	log2 MFI	Std Dev	log2 MFI	Std Dev	p-value
ENA-78	run 1	−1.35	1.67	9.36	1.74	10.7	1.36	<.000001
	run 2	−1.87	1.59	8.81	1.64	10.68	1.35	<.000001
	run 3	−1.63	1.66	9.29	1.75	10.92	1.24	<.000001
Eot	run 2	−0.87	0.62	6.32	0.54	7.19	0.9	<.000001
	run 3	−0.82	0.56	6.79	0.55	7.61	0.62	<.000001
GROb	run 2	−0.57	1.1	8.38	1.17	8.95	0.76	0.000664
	run 3	−0.74	1.19	9.42	1.26	10.17	0.84	0.000202
HCC4	run 1	−1.46	1.6	10.68	1.74	12.14	0.77	<.000001
	run 2	−1.65	1.67	9.93	1.77	11.57	1.11	<.000001
	run 3	−1.56	1.6	10.07	1.76	11.63	0.99	<.000001
HVEM	run 2	0.6	0.88	8.09	0.95	7.49	0.51	0.000009
	run 3	0.36	0.84	8.19	0.87	7.83	0.69	0.007135
IL-15	run 2	0.51	0.72	6.15	0.72	5.63	0.7	0.000002
	run 3	0.24	0.64	6.6	0.66	6.36	0.5	0.011704
IL-1b	run 1	−1.56	0.81	5.77	0.71	7.34	1.14	<.000001
	run 2	−1.37	0.92	5.25	0.84	6.62	1.19	<.000001
	run 3	−1.12	0.76	6.37	0.61	7.5	1.08	<.000001
IL-1ra	run 1	4.14	2.43	12.6	2.62	8.47	1.39	<.000001
	run 2	4.27	2.59	12.25	2.75	7.97	1.76	<.000001
	run 3	3.88	2.44	12.52	2.65	8.65	1.33	<.000001
IL-2sRa	run 1	1.9	1.21	13.49	1.2	11.59	1.26	<.000001
	run 2	1.84	1.35	13.32	1.36	11.49	1.29	<.000001
	run 3	1.75	1.26	13.62	1.26	11.87	1.24	<.000001
IL-6	run 1	3.61	2.53	11.7	2.73	8.09	1.4	<.000001
	run 2	3.94	2.65	11.4	2.85	7.47	1.59	<.000001
	run 3	3.49	2.4	11.79	2.58	8.31	1.42	<.000001
IL-8	run 1	−0.84	1.64	11.33	1.68	12.17	1.47	0.00087
	run 2	−1.02	1.84	11.08	1.9	12.1	1.56	0.000222
	run 3	−0.89	1.8	11.55	1.88	12.44	1.44	0.001392
Leptin	run 1	−1.41	2.56	9.64	2.62	11.05	2.08	0.027099
	run 2	−1.06	2.27	8.63	2.37	9.69	1.81	0.001948
	run 3	−0.92	2.25	9.24	2.35	10.16	1.83	0.009683
MCP-1	run 1	2.14	1.57	12.43	1.69	10.29	0.84	<.000001
	run 2	1.97	1.78	11.75	1.95	9.78	0.73	<.000001
	run 3	2.87	1.64	11.86	1.83	9	0.9	<.000001
MCP-2	run 1	1.01	1.68	8.18	1.82	7.16	0.9	0.00009
	run 2	1	1.6	7.31	1.73	6.31	0.9	0.000033
	run 3	0.67	1.62	8.06	1.74	7.4	0.91	0.007912
M-CSF	run 1	0.36	0.83	10.37	0.87	10.01	0.66	0.00439
	run 2	0.29	0.87	10.09	0.91	9.79	0.66	0.024124
MIG	run 1	1.44	2.24	10.6	2.39	9.16	1.41	0.000023
	run 2	1.39	2.27	10.47	2.41	9.08	1.56	0.000054
	run 3	1.91	2.2	11.12	2.36	9.2	1.64	<0.000001
MIP-1a	run 1	−0.44	0.64	7.68	0.61	8.12	0.73	0.000009
	run 2	−0.58	0.63	7.11	0.59	7.68	0.77	<0.000001
	run 3	−0.41	0.58	7.34	0.59	7.75	0.54	0.000005
MIP3b	run 1	1.34	0.97	8.47	1.06	7.13	0.49	<.000001
	run 2	1.09	1.03	8	1.11	6.91	0.58	<.000001
	run 3	1.03	1.03	8.27	1.1	7.25	0.62	<.000001
MMP9	run 1	1.18	1.07	11.72	1.07	10.54	1.1	<.000001
	run 2	1.06	1.07	11.59	1.07	10.53	1.03	<.000001
	run 3	0.86	1.01	11.62	0.97	10.76	1.16	<.000001
MPIF-1	run 1	2.12	1.28	9.72	1.37	7.6	0.78	<.000001
	run 2	2.17	1.32	9.18	1.4	7.02	0.9	<.000001
	run 3	1.96	1.25	9.62	1.33	7.66	0.87	<.000001
OSM	run 1	−0.66	0.64	7.8	0.65	8.46	0.63	<.000001
	run 2	−0.56	0.66	7.41	0.57	7.98	0.95	<.000001
PARC	run 1	1.1	0.97	12.62	0.98	11.52	0.92	<.000001
	run 2	0.78	0.97	12.84	0.97	12.06	0.94	<.000001
	run 3	0.87	1.01	11.93	1.06	11.06	0.86	<.000001
ST2	run 1	1.75	1.24	8.6	1.34	6.85	0.67	<.000001
	run 2	1.43	1.25	8	1.36	6.57	0.59	<.000001
	run 3	1.49	1.32	8.37	1.44	6.88	0.59	<.000001

TABLE 7-continued

Study 3 biomarkers for sepsis on Arrays 1-3: 483 sepsis and 57 ill control samples
were run a total of three times on each array, on consecutive days (replicate).

Analyte		log2 ratio (Sepsis/Sick)		Sepsis Mean		Sick Mean		
Name	Replicate	Mean	Std Dev	log2 MFI	Std Dev	log2 MFI	Std Dev	p-value
TNF-R1	run 1	1.79	1.02	12.46	1.07	10.68	0.79	<.000001
	run 2	1.68	1.18	12.36	1.2	10.68	1.07	<.000001
	run 3	1.47	1.26	11.23	1.27	9.76	1.26	<.000001

Mean log2 fluorescence intensity values for sepsis patients and sick patients are shown. The difference between the sepsis and sick group values were calculated (log2 ratio (sepsis/sick)), with the accompanying p-value for the difference. Each of the different runs was treated as a separate, complete dataset. Identical analyses were completed for each day, and the results are shown for each analysis. Only analytes that fulfilled the following criteria were included: (1) p value for the difference less than 0.05 (2) a significant change observed in at least two of the replicates, (3) the change should be concordant (log₂ ratio either positive or negative) between days, and (4) the size of the observed difference should be greater than the variability of the blank control feature on the array, to eliminate false positives attributable to random variation.

[0162] Several analytes (P-Selectin, Protein C and MIF) from array 5 demonstrated excellent correlation and low variability when comparing replicates, so little or no change is expected for these analytes in the final curated dataset. Data was base 2 log transformed for all calculations.

[0163] P-Selectin, Protein C and MIF all show significant (p-value$<10^{-6}$) differences between ICU control and Sepsis patients. Expression levels of analytes were more than 1.4 fold lower in sepsis (Table 8).

TABLE 8

P-Selectin, Protein C and MIF are all decreased
in sepsis as compared to sick controls.

Analyte	Replicate	Log ratio (sepsis/sick)		Sepsis		Sick		
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	p-value
MIF	1.00	−1.5	1.26	9.56	1.31	11.06	1.05	<.000001
	2.00	−1.52	1.24	9.3	1.3	10.82	0.96	<.000001
P-Selectin	1.00	−0.8	0.93	12.27	0.97	13.06	0.75	<.000001
	2.00	−0.8	0.99	12.12	1.04	12.92	0.74	<.000001
ProteinC	1.00	−0.42	0.47	13.09	0.5	13.51	0.28	<.000001
	2.00	−0.49	0.4	12.81	0.43	13.29	0.26	<.000001

[0164] There were at least 4 major differences between Study 3 and the previous studies that might have been anticipated to impact sepsis biomarker findings. First, studies 1 and 2 were very small, with only 6 and 12 sepsis patients, respectively, whereas the current study contained 240 sepsis patients. Utilization of a greater number of patients may be expected to increase the power of the study to identify sepsis markers. Second, in Study 3 the average APACHE II score was above 25, indicating that patients in Study 3 had more severe sepsis than in studies 1 and 2, where APACHE scores for some of the patients were relatively low. The third difference was that sepsis markers were identified in Study 1 and Study 2 in comparisons between healthy controls and sepsis patients. In Study 3, comparisons were made between ill, non-septic (ICU admitted) patients and sepsis patients.

Fourth, Studies 1 and 2 used plasma and serum samples whereas Study 3 used 1:5 diluted serum only. A comparison of the three studies (Table 9) showed that many sepsis markers such as IL-6, IL-2sRa, and MPIF-1 were common to all three studies, while others (MMP-7) fell away.

TABLE 9

Concordance of Study 3 sepsis serum
biomarkers with Study 1 and 2.

Analyte Name	Study1	Study2	Study3Prelim
AgRP	↑	—	N/A
CD141	↑	↑	N/A
IL-18	↑	↑	N/A
IL-2 sRα	↑	↑	↑

TABLE 9-continued

Concordance of Study 3 sepsis serum
biomarkers with Study 1 and 2.

Analyte Name	Study1	Study2	Study3Prelim
IL-6	↑	↑	↑
IL-8	↑	—	↓
MPIF-1	↑	—	↑
IGFBP-1	↑	—	N/A
MMP-7	↑	↑	—

Sepsis biomarkers were found in serum in Study 1 and 2 by comparing sepsis and healthy patients at pre-infusion. Study 3 compared sepsis patients with individuals admitted to the ICU for non-sepsis related injuries or illnesses. Analytes highlighted in gray are elevated in sepsis above levels seen for both normal and sick individuals. Data have not yet been obtained for analytes marked N/A, these will be discussed in the final report.
Legend:
'—' no difference;
'↑' elevated in sepsis patients,
'↓' decreased in sepsis patients

[0165] Several additional sepsis markers were identified in Study 3 (Table 10), in addition to the markers shown above.

TABLE 10

Sepsis specific markers identified for the first time in Study 3

Analyte Name	Level in Sepsis
BLC	↑
EGF	↓
ENA-78	↓
EOT	↓
Gro-beta	↓
HCC4	↓
HVEM	↑
IL-15	↑
IL-1b	↓
IL-1ra	↑
IL-8	↓
Leptin	↓
MCP-1	↑
MCP-2	↑
M-CSF	↑
MIG	↑
MIF	↓
MIP-1a	↓
MIP-3b	↑
MMP-9	↑
OSM	↓
PARC	↑
Protein C	↓

TABLE 10-continued

Sepsis specific markers identified for the first time in Study 3

Analyte Name	Level in Sepsis
P-selectin	↓
ST-2	↑
TNF-R1	↑

Sepsis markers were identified in study 3 by comparing analyte levels in sepsis patients with levels in individuals admitted to the ICU for non-sepsis related injuries or illnesses. This comparison screens out markers that are common to multiple diagnoses and focuses on markers that are specific to sepsis. Analytes with a 2.5-fold or greater difference are highlighted in gray.
Legend:
'—' no difference;
'↑' elevated in sepsis patients,
'↓' decreased in sepsis patients compared to ICU admitted sick controls.

[0166] Candidate Biomarkers for Mortality in Sepsis
[0167] Biomarkers for sepsis mortality in Study 3 were identified using the following criteria:
[0168] In order to avoid the influence of drug treatment, only placebo-treated patients were considered.
[0169] Samples in this study were taken either at preinfusion (visit 1) or 24 hours after infusion of placebo (visit 2). As a result, predictive mortality biomarkers could be identified using samples taken at both time points, so both were included in the analysis.
[0170] In an effort to identify biomarkers specific for mortality associated with sepsis and not other conditions, only analytes that were already identified as biomarkers for sepsis in the sepsis/sick analysis above were considered as potential biomarkers for sepsis mortality.
[0171] P-value for a given candidate biomarker should be <0.05 when sepsis survivor patients were compared to sepsis non-survivors.
[0172] Differences between the two groups were not considered meaningful if they showed less variability than the blank control feature on each array.
[0173] Analysis of variance (ANOVA) was used to identify significant differences between survivors and non-survivors at pre-infusion and day 1 using the GLM procedure of SAS. Results of the analysis are shown in Table 11.
[0174] 19 out of 83 analytes demonstrated significant differences in mean fluorescence intensity between the sepsis survivors and non-survivors (Tables 11 and 12). 6 of the 19 had more than a two-fold difference in mean fluorescence intensity in at least one visit: IL-1Ra, IL-6, IL-8, MCP-1, MCP-2, and MIG. All of these analytes showed increased expression in the sepsis non-survivors as compared to sepsis survivors.

TABLE 11

Study 3 biomarkers for sepsis mortality on Arrays 1-3: Comparisons
were made between survivors and nonsurvivors for samples taken at
visit 1 (preinfusion) or visit 2 (24 hours after infusion).

Analyte			Alive at Day 28		Dead at Day 28		log2 ratio		
			Mean		Mean		(Alive/Dead)		
Name	Visit	Replicate	log2 MFI	Std Dev	log2 MFI	Std Dev	Mean	Std Dev	p-value
BLC	visit1	run1	7.06	0.99	7.63	1.39	−0.57	1.21	0.0043
		run2	6.74	1.03	7.21	1.32	−0.47	1.19	0.0079
		run3	7.33	1.08	8.06	1.34	−0.73	1.23	0.0009
	visit2	run1	7.06	1.1	7.51	1.34	−0.45	1.23	0.0125
		run2	6.66	1.11	7.13	1.22	−0.47	1.17	0.0051
		run3	7.29	1.11	7.84	1.31	−0.55	1.23	0.0103

TABLE 11-continued

Study 3 biomarkers for sepsis mortality on Arrays 1-3: Comparisons
were made between survivors and nonsurvivors for samples taken at
visit 1 (preinfusion) or visit 2 (24 hours after infusion).

| Analyte | | | Alive at Day 28 | | Dead at Day 28 | | log2 ratio | | |
| | | | Mean | | Mean | | (Alive/Dead) | | |
Name	Visit	Replicate	log2 MFI	Std Dev	log2 MFI	Std Dev	Mean	Std Dev	p-value
EGF	visit1	run1	8.08	1.39	7.51	1.1	0.57	1.25	0.0089
		run2	7.53	1.17	7.13	1.12	0.4	1.14	0.0479
		run3	8.28	1.48	7.82	1.07	0.46	1.28	0.0333
Eot2	visit1	run1	11.06	1.02	10.55	1.08	0.51	1.05	0.0013
		run2	10.76	0.95	10.26	1.12	0.5	1.04	0.0042
		run3	11.02	1.02	10.54	1.13	0.49	1.08	0.0041
	visit2	run1	11.04	1.2	10.63	0.98	0.41	1.09	0.0128
		run2	10.72	1.05	10.26	1.11	0.46	1.08	0.0268
		run3	10.99	1.11	10.51	1.15	0.48	1.13	0.0142
HCC4	visit1	run1	11.06	1.54	10.65	1.71	0.42	1.63	0.014
		run2	10.34	1.64	10.01	1.69	0.34	1.67	0.0482
		run3	10.73	1.59	10	1.84	0.73	1.75	0.0495
	visit2	run1	11.79	1.2	11.06	1.72	0.73	1.5	0.0011
		run2	10.96	1.71	10.36	1.63	0.61	1.66	0.0158
		run3	11.64	1.3	10.07	1.83	1.57	1.65	0.0001
HVEM	visit2	run1	8.33	0.74	8.61	0.82	-0.28	0.78	0.0464
		run2	7.83	0.92	8.4	1.01	-0.56	0.97	0.0016
		run3	7.93	0.78	8.46	0.9	-0.53	0.85	0.0012
IL-1b	visit2	run1	5.6	0.52	5.93	0.57	-0.33	0.55	0.0003
		run2	4.87	0.7	5.51	0.88	-0.64	0.8	9E-06
		run3	6.24	0.52	6.45	0.55	-0.21	0.54	0.0327
IL-1ra	visit1	run1	11.93	2.79	13.33	2.16	-1.4	2.48	0.0003
		run2	11.6	2.93	12.93	2.37	-1.33	2.64	0.0006
		run3	11.84	2.89	13.29	2.31	-1.45	2.59	0.0003
	visit2	run1	9.4	2.46	11.56	2.62	-2.17	2.55	2E-06
		run2	9.08	2.71	11.11	2.6	-2.03	2.65	5E-06
		run3	9.57	2.38	11.53	2.62	-1.96	2.52	1E-05
IL-2sRa	visit1	run2	13.16	1.38	13.53	1.33	-0.37	1.35	0.0197
		run3	13.53	1.33	13.8	1.21	-0.27	1.27	0.0473
	visit2	run1	13.18	1.16	13.46	1.44	-0.29	1.32	0.0482
		run2	12.9	1.44	13.55	1.26	-0.65	1.35	0.0004
		run3	13.28	1.29	13.83	1.09	-0.56	1.18	0.0006
IL-6	visit1	run1	11.02	2.83	12.14	2.46	-1.13	2.65	0.0043
		run2	10.64	3.08	12.05	2.44	-1.41	2.75	0.0006
		run3	11.08	2.86	12.45	2.25	-1.37	2.55	0.0006
	visit2	run1	9.1	2.11	11.03	2.32	-1.93	2.23	2E-06
		run2	8.68	2.24	10.59	2.52	-1.91	2.4	3E-06
		run3	9.25	1.95	11.04	2.43	-1.79	2.23	3E-05
IL-8	visit1	run1	10.94	1.4	11.63	1.66	-0.7	1.54	0.0038
		run2	10.6	1.67	11.44	1.91	-0.84	1.8	0.0019
		run3	10.84	1.81	12.02	1.77	-1.18	1.79	1E-04
	visit2	run1	10.06	0.8	10.89	1.66	-0.83	1.34	0.0002
		run2	9.71	1.16	10.77	1.59	-1.06	1.41	4E-06
		run3	10.06	0.67	11.19	1.68	-1.13	1.33	5E-06
MCP-1	visit1	run1	12.07	1.6	12.73	1.76	-0.66	1.68	0.0164
		run2	11.15	1.92	12.23	1.99	-1.08	1.96	0.0004
	visit2	run1	11.25	1.26	12.39	1.61	-1.13	1.46	3E-05
		run2	10.31	1.3	11.67	1.75	-1.35	1.56	1E-06
		run3	10.69	1.52	11.68	1.74	-0.99	1.66	0.0025
MCP-2	visit1	run1	7.51	1.38	8.64	2.03	-1.13	1.76	0.0002
		run2	6.83	1.23	7.73	1.89	-0.9	1.61	0.0004
		run3	7.28	1.08	8.68	1.87	-1.4	1.55	<.000001
	visit2	run1	7.09	1.06	8.26	1.73	-1.17	1.46	5E-06
		run2	6.44	0.98	7.23	1.4	-0.8	1.23	5E-05
		run3	6.95	1	8.15	1.68	-1.2	1.41	3E-06
M-CSF	visit1	run1	10.14	0.68	10.43	0.96	-0.29	0.84	0.032
		run2	9.79	0.76	10.28	1.02	-0.5	0.91	0.0013
MIG	visit1	run1	10.08	2.11	11.04	2.31	-0.96	2.22	0.0069
		run2	9.86	2.04	10.93	2.41	-1.07	2.25	0.0027
	visit2	run1	9.36	1.79	10.72	2.21	-1.36	2.02	9E-05
		run2	8.88	1.86	10.65	2.34	-1.77	2.13	4E-06
		run3	10.15	1.81	11.01	2.29	-0.86	2.11	0.049
MIP3b	visit2	run1	8.06	0.85	8.45	1.03	-0.38	0.94	0.0222
		run2	7.52	0.95	7.87	1.05	-0.35	1	0.0189
		run3	7.67	0.94	8.25	1.03	-0.58	0.99	0.0005

TABLE 11-continued

Study 3 biomarkers for sepsis mortality on Arrays 1-3: Comparisons
were made between survivors and nonsurvivors for samples taken at
visit 1 (preinfusion) or visit 2 (24 hours after infusion).

Analyte			Alive at Day 28		Dead at Day 28		log2 ratio (Alive/Dead)		
			Mean		Mean				
Name	Visit	Replicate	log2 MFI	Std Dev	log2 MFI	Std Dev	Mean	Std Dev	p-value
ST2	visit2	run1	7.7	0.99	8.47	1.28	−0.77	1.15	0.0002
		run2	7.06	0.83	7.67	1.15	−0.61	1.01	0.0007
		run3	7.36	1.02	8.19	1.4	−0.83	1.25	7E−05
TNF-R1	visit1	run1	12.25	1.15	12.79	0.99	−0.54	1.07	0.0097
		run2	12.13	1.33	12.74	1.15	−0.61	1.24	0.0048
	visit2	run1	12	1.19	12.78	1.06	−0.78	1.12	0.0003
		run2	11.87	1.35	12.8	1.22	−0.93	1.28	9E−05

All samples were run a total of three times on each array, on consecutive days (replicate). Mean log2 fluorescence intensity
values for placebo treated sepsis survivors and nonsurvivors are shown. The difference between the sepsis and sick values were
calculated (log2 ratio (alive/dead)), with the accompanying p-value for the difference. Each of the different runs was treated
as a separate, complete dataset. Identical analyses were completed for each day, and the results are shown for each analysis.
Only analytes that fulfilled the following criteria were considered: (1) p value for the difference should be less than 0.05 (2)
there should be a significant change identified for at least two of the days, (3) the change should be concordant (log2 ratio either
positive or negative) between days, and (4) the size of the observed difference should be greater than the variability of the blank
control feature on the array, to eliminate false positives attributable to random variation.

[0175] On the selected array 5 analyte data, significant (p-value<0.001) differences in MIF levels were found between survivors and non-survivors at visit1 and visit2. Expression levels of MIF were ~1.7 fold lower in survivors at baseline (Table 12). Expression levels of P-Selectin were decreased (p-value<0.04) by 1.2 fold in survivors (Table 12).

TABLE 12

Array 5 mortality biomarkers

Analyte	THERAPY	Variable	Replicate	Alive at Day 28		Dead at Day 28		Diff (1-2)		
				Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	p-value
MIF	PLACEBO	visit1	1.00	9.2	1.01	9.96	1.26	−0.75	1.15	0.000137
			2.00	8.94	1.07	9.6	1.2	−0.66	1.14	0.000288
		visit2	1.00	8.98	1.14	9.76	1.57	−0.78	1.39	0.001274
			2.00	8.77	1.06	9.57	1.46	−0.81	1.29	0.000349
P-Selectin	PLACEBO	Logratio	1.00	−0.29	0.61	0.01	0.93	−0.3	0.8	0.036262
			2.00	−0.28	0.66	0.07	0.96	−0.35	0.83	0.019712

(p-value < 0.05)

[0176] As mentioned above, there were at least three major differences (number of patients, average APACHE scores and control populations) between Study 3 and the previous studies that were expected to impact mortality biomarker findings. Nevertheless, a comparison of Studies 2 and 3 (no mortality biomarkers could be identified in Study 1 due to experimental design) showed that some sepsis mortality markers such as MIG, MIP-3b, and HCC-4 were common to both studies (Table 13).

TABLE 13

Concordance of Study 3 sepsis mortality serum
biomarkers with Study 2 (non-normalized data).

Analyte Name	Study2	Study3Prelim
BDNF	↓	—
Flt3Lig	↓	—
Follistatin	↑	N/A

TABLE 13-continued

Concordance of Study 3 sepsis mortality serum
biomarkers with Study 2 (non-normalized data).

Analyte Name	Study2	Study3Prelim
Gro-g	↓	N/A
HCC-4	↑	↓
IGFBP-6	↑	N/A
IL-18	↑	N/A
MIG	↑	↑
MIP-1b	↓	—
MIP-3b	↓*	↑*
Neutr. elastase	↑	N/A

TABLE 13-continued

Concordance of Study 3 sepsis mortality serum
biomarkers with Study 2 (non-normalized data).

Analyte Name	Study2	Study3Prelim
Protein C	↑	—
Protein S	↑	N/A
SCF	↑	—

Data have not yet been obtained for analytes marked N/A, these will be discussed in the final report.
Legend:
'—' no difference;
'↑' elevated in non-survivors,
'↓' decreased in non-survivors
*different time point

[0177] Other sepsis markers were identified in Study 3 (Table 14), in addition to the markers shown above.

TABLE 14

Sepsis mortality markers identified only in Study 3.

Analyte Name	Levels in non-survivors
BLC	↑
EGF	↓
Eot2	↓
HVEM	↑
IL-1b	↑
IL-1ra	↑
IL-2sRa	↑
IL-6	↑
IL-8	↑
MCP-1	↑
MCP-2	↑
M-CSF	↑
MIF	↑
P-selectin	↑
ST-2	↑
TNF-R1	↑

Legend:
'—' no difference;
'↑' elevated in non-survivors,
'↓' decreased in non-survivors

[0178] Cytokines play an essential role in the coordination of the cellular and humoral responses in systemic inflammation. In the present study, three array 5 analytes, MIF, P-selectin and protein C, were found to be differentially expressed when sepsis patients and ICU controls were compared and are discussed in more detail here. MIF and P-selectin were identified also as potential biomarkers for mortality.

[0179] In the present study, macrophage migration inhibitory factor (MIF) was identified as a potential biomarker for sepsis and mortality. MIF has been shown to carry out important functions as a mediator of the immune system. Constitutively expressed by a broad spectrum of cells and tissues, including monocytes and macrophages, MIF is rapidly released after exposure to microbial products and pro-inflammatory mediators, and in response to stress. After it is released, MIF induces pro-inflammatory biological responses that act as a regulator of immune responses. MIF activates the extracellular signal-regulated kinase 1 (ERK1)/ERK2-mitogen-activated protein kinase pathway, inhibits the activity of JUN activation domain-binding protein 1 (JAB1), upregulates the expression of Toll-like receptor 4 to promote the recognition of endotoxin-expressing bacterial pathogens, sustains pro-inflammatory function by inhibiting p53-depen-

dent apoptosis of macrophages and counter-regulates the immunosuppressive effects of glucocorticoids on immune cells (Nature Reviews Immunology 2003 3, 791-800). As a pro-inflammatory mediator, MIF has been shown to be implicated in the pathogenesis of severe sepsis and septic shock, acute respiratory distress syndrome, and several other inflammatory and autoimmune diseases, including rheumatoid arthritis, glomerulonephritis and inflammatory bowel diseases. MIF has been shown to be a critical mediator of septic shock. High concentrations of MIF were detected in the peritoneal exudate fluid and in the systemic circulation of mice with bacterial peritonitis (Nat. Med. 2000 6:164-70). Anti-MIF antibody protected TNF-α knockout mice from lethal peritonitis induced by cecal ligation and puncture (CLP), providing evidence of an intrinsic contribution of MIF to the pathogenesis of sepsis. Anti-MIF antibody also protected normal mice from lethal peritonitis induced by both CLP and E. coli, even when treatment was started up to 8 h after CLP. Conversely, co-injection of recombinant MIF and E. coli markedly increased the lethality of peritonitis. Finally, high concentrations of MIF were detected in the plasma of patients with severe sepsis or septic shock (Nat. Med. 2000 6:164-70). These studies define a critical part for MIF in the pathogenesis of septic shock and identify a new target for therapeutic intervention. Furthermore, it has been shown that activated protein C (APC) inhibits MIF and TNF production in monocytes (Eur Cytokine Netw. 2000 11:407-13). Inhibition of MIF release by APC was found to be independent of TNF, as stimulation of MIF release by LPS was unaltered in the presence of anti-TNF antibodies. These results suggest that the anti-inflammatory properties of APC are mediated via inhibition of the release of the pro-inflammatory cytokines MIF and TNF. Given its crucial role as a regulator of sepsis, pharmacological or immunological modulation of MIF activity might offer new treatment opportunities for the management of systemic inflammation.

[0180] Another analyte that was identified as a potential biomarker for sepsis was protein C(PC). In the coagulation cascade, PC is activated by the thrombin-thrombomodulin-complex and inactivates factors V a and VIM. Additionally, activated protein C has a profibrinolytic effect. The relevance of protein C to the pathogenesis of sepsis is well known.

[0181] P-selectin was identified in the present study as a potential biomarker for sepsis and mortality. P-selectin is a protein found in the alpha-granules of platelets and the Weibel-Palade bodies of endothelial cells. On cell activation it is expressed on the cell surface and also secreted into plasma (Thromb Haemost. 1997 77:1081-5). P-selectin in the plasma membrane surface is exposed and serves as a cell adhesion receptor to interact with other cell receptors, including PSGL-1 and GPM (Thromb Haemost. 2001 86:214-21). P-selectin upregulates tissue factor in monocytes and leads to leukocyte accumulation in areas of vascular injury associated with thrombosis and inflammation. High levels of soluble P-selectin were found in patients with sepsis (398+/−203 ng/mL) compared to healthy controls (122+/−38 ng/mL). Similarly, the concentration of P-selectin per platelet was higher in patients with sepsis (4.8+/−4.3 fg/platelet) than in the control group (0.6+/−0.2 fg/platelet) (Thromb Haemost. 1997 77:1081-5). P-selectin expression was increased in various organs within the peritoneum of mice exposed to polymicrobial sepsis (Am J Physiol Gastrointest Liver Physiol. 2001 280:G291-7). The plasma concentrations of soluble P-selectin, platelet factor-4 (PF-4) and beta-thromboglobulin (be-

taTG) in patients with septic shock were significantly higher than those in healthy control subjects (p<0.001), suggesting that these mediators could be associated with septic shock (Inflamm Res. 1999 48:171-5). Interestingly, in whole blood APC was found to inhibit recombinant tissue factor-induced platelet activation, measured by the amount of P-selectin (Thromb Res. 1997 87:197-204). The anticoagulant effect of APC is believed to be based mainly on the inhibition of thrombin generation. This is also supported by in vitro studies using human plasma in which APC, after activation by a thrombin-thrombomodulin complex (Thr+Tm) formed during coagulation via the extrinsic pathway, caused a delay in thrombin generation and a reduction of the maximum thrombin concentration (Thromb Haemost. 1993 70:423-6). The generation of APC by the thrombin-thrombomodulin complex is stimulated by platelet factor-4 (PF-4), which interacts with both the thrombomodulin's glycosaminoglycan and the protein C Gla domain to enhance markedly the affinity of the thrombin-thrombomodulin complex for protein C (J Biol. Chem. 1994 269:25549-56). By this mechanism, PF-4 may play an important role in the physiologic regulation of clotting. PF-4 is a novel analyte measured on the latest version of MSI array 4. Very preliminary analysis indicates that it also demonstrates differential expression in the present study.

Example 8

Summary of Final Array 4 Dataset

[0182] 480 sepsis and 57 ICU control patient samples were assayed in triplicate on MSI Array 4, which contains 37 analytes.

[0183] 515 samples passed in triplicate, 21 samples passed in duplicate and 1 samples passed in singlicate (Table 15). More than 99% of samples passed in duplicate or triplicate, exceeding the 85% minimum acceptable pass rate, indicative of successful completion of Array 4 data generation.

TABLE 15

Statistics For Passed Replicates		
Passed replicates	Number of Samples	Percentage (%)
0	0	0
1	1	0.19
2	21	3.91
3	515	95.9
Total	537	100

Data Processing and Experimental Set Up

[0184] Three replicates for each sample were run on 3 consecutive days on three different microarray lots. A four-point concentration titration curve ("anchor points", Type=CurveC in attached dataset) was run on every slide for normalization and quality control purposes. The four-point concentration titration curve comprised four wells on each slide that were interrogated with a standard buffer supplemented with 12, 37, 333 and 1000 pg/mL of each array 1 analyte. In addition, expanded 16- or 8-point concentration curves (full-titration, Type=CurveD) were run on up to 9 slides.

[0185] Individual sample values were normalized using linear regression of that slide's anchor points within a day to reduce slide to slide imprecision. Day-to-day imprecision

was reduced using regression-based normalization of day-to-day averages of anchor points. Between-day variability (CV) was 32%, on average, for samples, 15% for the anchor points, and 28% for the full-titration wells, respectively (Table 16). These values are consistent with standard platform performance.

TABLE 16

Day-to-day Variability On A Linear Scale (CV in %)				
Sample Type	<CV>	N	Std Dev	Median CV
Sample	32.18	19869	19.07	29.01
Anchor Point	15.55	152	12.01	11.01
Full Titration	28.55	608	14.87	26.78

Data File Descriptions

[0186] Slidemapname: the name of the array. The current data related to version 5 of array 4 (Proteomics 4.5).

[0187] AnalyteName: the name of the measured analyte. BLANK is a negative control feature.

[0188] MSIBarcode: sample barcode per MSI operation.

[0189] Run day: the consecutive day number in which the samples were run. For example, d1 is day 1.

[0190] MFI: mean fluorescence intensity, on a linear scale.

[0191] Client Sample_ID, PatientNumber, PatIni, Client-Description have been provided by the client.

[0192] Note: Samples with ICUControl prefix in Client Sample_ID field represent non-sepsis, critically ill, hospitalized control patients provided by MSI.

[0193] DX: diagnosis (sepsis or sick for ICU controls).

[0194] Type: Source of data. Sample for Sepsis and ICU controls, CurveC for anchor points and CurveDB for titrations.

[0195] Conc: the concentration of analytes in pg/ml.

Summary of Final Array 5 Dataset

[0196] 480 sepsis and 57 ICU control patient samples were assayed in triplicate on MSI Array 5, which contains 27 analytes.

[0197] 507 samples passed in triplicate, 30 samples passed in duplicate (Table 17). One hundred percent of samples passed in duplicate or triplicate, exceeding the 85% minimum acceptable pass rate, indicative of successful completion of Array 5.

TABLE 17

Statistics For Passed Replicates		
Passed replicates	Number of Samples	Percentage (%)
0	0	0
1	0	0
2	30	5.59
3	507	94.41
Total	537	100

Data Processing and Experimental Set Up

[0198] Three replicates for each sample were run on 3 consecutive days on three different microarray lots. A four-point concentration titration curve ("anchor points",

Type=CurveC in attached dataset) was run on every slide for normalization and quality control purposes. The four-point concentration titration curve comprised four wells on each slide that were interrogated with a standard buffer supplemented with 12, 37, 333 and 1000 pg/mL of each array 1 analyte. In addition, expanded 16- or 8-point concentration curves (full-titration, Type=CurveD) were run on up to 9 slides.

[0199] Individual sample values were normalized using linear regression of that slide's anchor points within a day to reduce slide to slide imprecision. Day-to-day imprecision was reduced using regression-based normalization of day-to-day averages of anchor points. Between-day variability (CV) was 25%, on average, for samples, 11% for the anchor points, and 19% for the full-titration wells, respectively (Table 18). These values are consistent with standard platform performance.

TABLE 18

Day-to-day Variability On A Linear Scale (CV in %)				
Sample Type	<CV>	N	Std Dev	Median CV
Sample	25.37	14909	15.62	22.69
Anchor Point	11.13	112	6.78	8.95
Full Titration	19.79	448	16.90	16.53

Data File Descriptions

[0200] Slidemapname: the name of the array. The current data related to version 3I of array 5 (Proteomics 5.3I).

[0201] AnalyteName: the name of the measured analyte. BLANK is a negative control feature.

[0202] MSIBarcode: sample barcode per MSI operation.

[0203] Run day: the consecutive day number in which the samples were run. For example, d1 is day 1.

[0204] MFI: mean fluorescence intensity, on a linear scale.

[0205] Client Sample_ID, PatientNumber, PatIni, Client-Description have been provided by the client.

[0206] Note: Samples with ICUControl prefix in Client Sample_ID field represent non-sepsis, critically ill, hospitalized control patients provided by MSI.

[0207] DX: diagnosis (sepsis or sick for ICU controls).
[0208] Type: Source of data. Sample for Sepsis and ICU controls, CurveC for anchor points and CurveDB for titrations.
[0209] Conc: the concentration of analytes in pg/ml.

What is claimed is:

1. A method of diagnosing severe sepsis in a human subject, comprising:

determining a concentration of at least one analyte in a fluid test sample from said human subject;

comparing the concentration of each said analyte(s) to a corresponding reference concentration selected to indicate the presence or absence of severe sepsis, wherein said reference concentration is determined using one or more control samples obtained from one or more human subjects not suffering from sepsis, provided that at least one of said analyte(s) is myeloid progenitor inhibitory factor-1 ("MPIF-1"), wherein an elevation in concentration of said analyte(s) in the test sample of about two fold relative to the reference concentration is indicative of the presence of severe sepsis in the human; and

diagnosing severe sepsis in the human subject using the result(s) of said comparing step.

2. The method of claim 1 wherein said samples comprise blood.

3. The method of claim 1 wherein said samples comprise serum.

4. The method of claim 1 wherein said samples comprise plasma.

5. The method of claim 1 wherein further provided that at least one analyte is interleukin 1 receptor antagonist ("IL-1 Ra").

6. The method of claim 1 wherein further provided that at least one analyte is monocyte chemotactic protein-1 ("MCP-1").

7. The method of claim 1 wherein further provided that at least one analyte is tumor necrosis factor receptor-1 ("TNF-R1").

8. The method of claim 1 wherein severe sepsis is diagnosed if the concentrations of at least two of said analytes in said test sample are elevated about two fold relative to the corresponding reference concentrations.

9. The method of claim 1 wherein an elevation in concentration of MPIF-1 in the test sample of about four fold relative to the reference concentration is indicative of the presence of severe sepsis in said human.

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专利名称(译)	败血症的生物标志物		
公开(公告)号	US20100304981A1	公开(公告)日	2010-12-02
申请号	US12/775382	申请日	2010-05-06
申请(专利权)人(译)	BIOSITE INCORPORATED		
当前申请(专利权)人(译)	了Alere圣迭戈，INC.		
[标]发明人	KINGSMORE STEPHEN F LEJNINE SERGUEI J DRISCOLL MARK TCHEMEV VELIZAR		
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IPC分类号	C40B30/00 G01N33/53 G01N33/68 G01N33/537 G01N33/543		
CPC分类号	G01N33/6893 G01N2800/26		
其他公开文献	US8029982		
外部链接	Espacenet USPTO		

摘要(译)

可以通过测定血液样品来检测败血症的生物标志物和所导致的死亡率。生物标志物浓度的变化可用于指示败血症，败血症的风险，败血症的进展，脓毒症的缓解和死亡风险。可以相对于数据集，天然或合成或半合成对照样品或在不同时间点收集的患者样品评估变化。一些生物标志物的浓度在疾病期间升高，一些生物标志物降低。这些被称为信息生物标记物。一些生物标志物与其他生物标志物一起诊断。当组合使用时，可以对各个生物标志物进行加权。可以在个体，分离或测定中，在平行测定中或在单罐测定中评估生物标志物。

FIGURE 1.