MIAME CHECKLIST
WONG ET AL.

EXPERIMENT DESIGN

Goal of the experiment: To identify correlated genes, pathways and groups of patients with systemic inflammatory response syndrome and septic shock that is indicative of biologically important processes active in these patients.

Experiment description:

Background: We measured gene expression levels and profiles of children with systemic inflammatory response syndrome (SIRS) and septic shock as a means for discovering patient sub-groups and gene signatures that are active in disease-affected individuals and potentially in patients with poor outcomes.

Methods: Microarray and bioinformatics analyses of 123 microarray chips representing whole blood derived RNA from controls, children with SIRS, and children with septic shock.

Results: A discovery-based filtering approach was undertaken to identify genes whose expression levels were altered in patients with SIRS or septic shock. Clustering of these genes identified 3 Major and several minor sub-groups of patients with SIRS or septic shock. The three groups differed with respect to incidence of septic shock and trended toward differences in mortality. Statistical analyses demonstrated that 6,435 gene probes were differentially regulated between the three patient sub-groups (false discovery rate < 0.001%). Of these gene probes, 623 gene probes within 7 major gene ontologies accounted for the majority of group differentiation. Network analyses of these 623 gene probes demonstrated 5 major gene networks that were differentially expressed between the 3 groups. Statistical comparison of septic shock survivors and non-survivors identified one major gene network that was under expressed in a high fraction of the non-survivors and identified potential biomarkers for poor outcome.

Conclusions: This is the first genome-level demonstration of pediatric patient sub-groups with SIRS and septic shock. The sub-groups differ clinically and differentially express 5 major gene networks. We have identified gene signatures and potential biomarkers associated with poor outcome in children with septic shock. These data represent a major advancement in our genome-level understanding of pediatric SIRS and septic shock.

Key words: Septic shock, SIRS, pediatrics, outcome, infection, inflammation

Experimental factors: Normal children, children with SIRS, children with septic shock, and children with SIRS resolved.

Experimental design: Children < 10 years of age admitted to the pediatric intensive care unit and meeting the criteria for either SIRS or septic shock were eligible for the study. SIRS and septic shock were defined based on pediatric-specific criteria. We did not use separate categories of “sepsis” or “severe sepsis”. Patients meeting

criteria for “sepsis” or “severe sepsis” were placed in the categories of SIRS and septic shock, respectively, for study purposes. Control patients were recruited from the outpatient or inpatient departments of the participating institutions using the following exclusion criteria: a recent febrile illness (within 2 weeks), recent use of anti-inflammatory medications (within 2 weeks), or any history of chronic or acute disease associated with inflammation.

After obtaining informed consent, blood samples were obtained on Day 1 of the study, and when possible on Day 3 of the study. Blood samples were divided for RNA extraction and isolation of serum. Severity of illness was calculated based on the PRISM III score. Organ failure was defined based on pediatric-specific criteria. Annotated clinical and laboratory data were collected daily while in the intensive care unit. Study patients were placed in the study categories of SIRS or Septic Shock on Day 1 of the study. On Day 3 of the study, patients were classified as SIRS, Septic Shock, or SIRS resolved (no longer meeting criteria for SIRS). All study patients were followed for 28 days to determine mortality or survival. Clinical, laboratory, and biological data were entered and stored using a web-based data base developed locally.

Quality control steps: RNA quality was assessed by using the Agilent bioanalyzer (Agilent Technologies, Palo Alto, CA) and only those samples with 28S/18S ratios between 1.3 and 2 were subsequently used.

SAMPLES USED, EXTRACT PREPARATION, AND LABELING

Origin of biological samples: Whole blood from the above named patient categories.

Manipulation of biological samples: None

Experimental factor value: Day 1 samples = control, SIRS, or septic shock. Day 3 samples = SIRS, septic shock, or SIRS resolved.

Technical protocols: Total RNA was extracted from whole blood samples using the PaxGene™ Blood RNA System (PreAnalytiX, Qiagen/Becton Dickson) according the manufacturer’s specifications. Labeling involved an in vitro transcription reaction using the ENZO BioArray HighYield RNA Transcript Labeling Kit (Affymetrix) according to manufacturer’s instructions.

External controls: Control Oligonucleotide B2 (Affymetrix), 20X Eukaryotic Hybridization Controls (1.5 pM bioB, 5 pM bioC, 25 pM bioD, 100 pM cre, Affymetrix).

Clinical and demographic data for all subjects.

	Controls	SIRS	Septic Shock[3]
Number of individual subjects[1]	15	28	46
Number of individual Microarray chips[2]	15	32	62
Mean Age (years) ± S.D.	3.1 ± 3.5	4.3 ± 3.3	4.1 ± 4.0
Mean PRISM Score ± S.D.	n/a	12.1 ± 7.9	18.5 ± 8.3
Gender (Male/Female)	8/7	17/11	27/19
Race	A.A./Black (6)	A.A./Black (8)	A.A./Black (11)
	Asian (4)	Pacific Islander (1)	American Indian (1)
	White (5)	Unreported (1)	Asian (3)
		White (18)	Unreported (3)
			White (28)

[1]Criteria for SIRS or septic shock were met on either study day 1 or 3. There were an additional 14 subjects included in the data set that were classified as "SIRS resolved" on study day 3.

[2]For the study categories of SIRS and septic shock there are a greater number of individual Microarray chips, than number of individual subjects, because not all of the individual subjects had Microarray data available on study days 1 and 3.

[3]Among the patients with septic shock there were 9 individual subjects that were non-survivors represented by 14 individual Microarray chips.

HYBRIDIZATION PROCEDURES AND PARAMETERS:

The protocol and conditions used during hybridization, blocking and washing: Create a hybridization cocktail for a single probe array that contains 0.05 μg/μL fragmented cRNA, 50 pM Control Oligonucleotide B2 (Affymetrix), 20X Eukaryotic Hybridization Controls (1.5 pM bioB, 5 pM bioC, 25 pM bioD, 100 pM cre) (Affymetrix), 0.1 mg/mL Herring Sperm DNA (Promega), 0.5 mg/mL Acetylated BSA (Invitrogen), and 1X Hybridization Buffer. Heat hybridization cocktail to 99°C for 5 minutes, to 45°C for 5 minutes, and spin at maximum speed in a microcentrifuge for 5 minutes. Fill probe array with 200 μL of 1X Hybridization Buffer. Incubate at 45°C for 10 minutes in the GeneChip Hybridization Oven 640 (Affymetrix) rotating at 60 rpm. Remove 1X Hybridization Buffer and fill probe array with 200 μL of the hybridization cocktail. Incubate at 45°C for 16 hrs in the Hybridization Oven rotating at 60 rpm.

MEASUREMENT DATA AND SPECIFICATIONS:

The image file was captured on an Affymetrix Gene Chip Scanner 3000 and initially processed with Microarray suite 5.0 (Affymetrix) to generate .CEL files that were subject to RMA normalization(Irizarry et al 2003) using GeneSpring software.

Standard Affymetrix internal control genes were used to check the quality of the assay quality by the signals of the 3' probe set to the 5' probe set of the internal control genes, GAPDH and B-actin, with acceptable 3' to 5' ratios between1-3. Prokaryotic Spike controls were used to determine the hybridization of target RNA to the array occurred properly.

GeneSpring 7.2 (Agilent technologies Inc. Palo Alto, California) was used to normalization, Clustering and filtering. The Raw CEL files were processed using the RMA (Robust Multichip Average) built in GeneSpring software. All the samples were then normalized to the median of the controls.

ARRAY DESIGN

Commercial Affymetrix Human Genome U133 Plus 2.0 Gene Chip (Affymetrix, Santa Clara, CA).