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Host and Bacterial Markers That Differ in Children with Cystitis and Pyelonephritis

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Abstract

Objective—To determine whether treatment for urinary tract infections (UTI) in children could be individualized using biomarkers for acute pyelonephritis.

Study design—We enrolled 61 children with febrile UTI, collected blood and urine samples and performed a renal scan within 2 weeks of diagnosis to identify those with pyelonephritis. Renal scans were interpreted centrally by 2 experts. We measured inflammatory proteins in blood and urine using LUMINEX or ELISA. We evaluated serum RNA expression using RNA sequencing in a subset of children. Finally, for children with *Escherichia coli* isolated from urine cultures, we performed PCR for 4 previously identified virulence genes.

Results—Urinary markers that best differentiated pyelonephritis from cystitis included CXCL1, CXCL9, CXCL12, CCL2, INF γ , and IL-15. Serum procalcitonin was the best serum marker for

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pyelonephritis. Genes in the interferon γ pathway were upregulated in serum of children with pyelonephritis. Presence of *E. coli* virulence genes did not correlate with pyelonephritis.

Conclusions—Immune response to pyelonephritis and cystitis differs quantitatively and qualitatively; this may be useful in differentiating these two conditions.

Keywords

Prediction rule; risk; UTI; diagnostic accuracy; calculator

In some children with urinary tract infection (UTI), infection is localized to the bladder (cystitis). In others, bacteria ascend from the bladder to the kidney causing acute pyelonephritis (APN). Because APN is required for the development of renal scars, identification of children with APN will allow identification of a high-risk subgroup of children with UTI. Compared with children with cystitis, children with APN may require a longer duration of antimicrobial treatment and/or more aggressive follow-up. However, because currently making a diagnosis of APN requires a scintigraphic imaging (Tc-99 dimercaptosuccinic acid [DMSA]), most children with febrile UTI are managed in the same way (with 10 days of antimicrobial therapy and a renal ultrasound) even though only approximately 50% have APN. If accurate biomarkers were available, a blood or urine specimen obtained at the time of diagnosis could be tools to individualize management of children with UTI. Because most children with UTI do not have a blood sample collected, urinary markers are especially attractive. Use of biomarkers at the time of presentation could potentially reduce use of unnecessary antimicrobials and imaging tests.

To date, although some promising markers have been identified in single studies, no markers have been validated for differentiating APN from cystitis that have both high sensitivity and specificity (Table 1). The most promising bacterial marker reported is the *E. coli pap* (pyelonephritis associated pili) gene.¹ Data on the role of urinary markers has been contradictory.

The objective of this pilot study was to identify potential host and/or bacterial markers that could differentiate children with APN from children with cystitis using both targeted (protein level analysis) and unbiased (RNA expression analysis) approaches.

Methods

From October 2010 to June 2015 we prospectively enrolled a convenience sample of febrile children 1 month to 10 years of age with presumed UTI presenting to one of 3 Emergency Departments (Children's Hospital of Pittsburgh, Children's National Medical Center, Hasbro Children's Hospital) or to an outpatient pediatric clinic affiliated with the Children's Hospital of Pittsburgh. We defined fever as having a documented temperature of $\geq 38.3^{\circ}\text{C}$ (101°F) within 24 hours of presentation. Exclusion criteria are listed in Figure 1. Of the children enrolled, we only offered a DMSA to those who had both pyuria (≥ 5 white blood cells [WBCs] per high powered field [hpf] or ≥ 10 WBC/mm³) and a positive urine culture, defined as $\geq 100,000$ CFU/mL of one or more uropathogens from a specimen collected by clean catch or $\geq 50,000$ CFU/mL of one or more uropathogens from a catheterized specimen.

All children in this report had a DMSA scan performed. All children were enrolled with their first UTI except one child who had a questionable previous UTI. This child was included in the primary analysis because the previous UTI was questionable, the likelihood of a scar from the a previous UTI is low (5 to 15%), and the child had symptoms and DMSA findings consistent with acute pyelonephritis. We performed sensitivity analysis excluding this child. The IRB at each respective site approved this study.

Reference standard for APN

We used the presence of photopenia without change in renal contours on a DMSA scan obtained within 14 days of the index UTI as the reference standard for diagnosing APN.^{2, 3} Children were injected with a 70 μ Ci/kg of DMSA. High-resolution magnified images of the kidney were obtained, including posterior and both right and left posterior oblique projections using a gamma-camera-computer system equipped with a high-resolution pinhole collimator, between 2-4 hours following injection. DMSA scans were evaluated by two reference nuclear medicine investigators who were unaware of any clinical information; disagreements in readings were resolved by discussion.

Urine sample collection and processing for proteins

For urinary protein measurement, we used urine collected at the time of presentation in most instances; when this was not available we collected an additional sample using a perineal collection bag. All children had a urine culture and a UA that was collected at the time of presentation using catheterization or a clean catch; bags were used in a small minority for collection of urine protein if there was no urine left over at the clinical laboratory. We filtered urine samples (Millex filters, Millipore) and stored them in cryovials at -80°C . Processing generally occurred within 1 hour of collection. However, if a delay was anticipated, samples were stored in a specimen refrigerator until processing.

Processing of the uropathogen recovered from urine culture

In children with an infection caused by *E. coli*, we picked a representative colony and stored it in 25% glycerol at -80°C . We used these to test for papGIA2, *sfa*, *hly*, and *cnf-1* virulence alleles using the primers listed in the Appendix (available at www.jpeds.com). Details of the multiplex PCR reaction are also listed in the Appendix.

Blood sample collection and processing for proteins

We collected blood samples from children whose parents consented to phlebotomy. CRP was measured on an aliquot at the Children's Hospital of Pittsburgh Central laboratory using Siemens Dimension Vista 500 Intelligent Lab System. Another aliquot was spun at 2700 rpm for 10 minutes and plasma was subdivided into multiple cryovials for protein measurement (cytokines, neutrophil gelatinase-associated lipocalin [NGAL] and procalcitonin). Specimens were processed generally within 1 hour of collection and stored at -80°C and processed in batches.

Protein measurement

For cytokines in urine and blood we used Bio-Rad Pro Human Cytokine 27-plex and 21-plex LUMINEX plates. For neutrophil peptide 1 (HNP1), we used Hycult ELISA Kit (HK317). CRP was measured using Siemens Dimension Vista 500 Intelligent Lab System. We standardized urine protein levels by dividing by the values by the urine creatinine concentration, which was measured using R&D Creatinine Parameter Assay Kit (KGE005). We measured blood and urinary NGAL using BioPorto Rapid ELISA kit (KIT037). We measured serum procalcitonin using bioMerieux's miniVIDAS immunoanalyzer. On each plate, we included duplicate and control samples.

Statistical methods for protein data

We used logistic regression for binary variables (bacterial virulence), and t test for continuous variables (eg, protein levels). To assess the discriminative power, we constructed a receiver operating characteristic curve using pyelonephritis vs. cystitis status as the outcome and calculated the area under the curve (AUC). We also assessed whether ibuprofen use within 6 hours of presentation modified the relationship between biomarker levels and outcome using a test for interaction.

Serum RNA processing and sequencing methods

We used Applied Biosystems mini Tempus tubes to stabilize RNA. Total RNA libraries were generated using Illumina TruSeq Stranded Total RNA Sample Preparation Guide (Rev. E). First, we removed globin and ribosomal RNA using biotinylated, target-specific oligos combined with globin and rRNA removal beads. Following purification, RNA was fragmented into small pieces using divalent cations under elevated temperature. Cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers, followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. After ligation of the adapter, we added single 'A' base fragments to the cDNA fragments. We purified and enriched products with PCR to create the final cDNA library. We validated cDNA libraries using KAPA Biosystems primer premix kit with Illumina-compatible DNA primers and Qubit 2.0 fluorometer. We examined the quality of the RNA using Agilent Tapestation 2200. The cDNA libraries were pooled at a final concentration 1.8pM. Cluster generation and paired-read 75 bp sequencing was performed on Illumina NextSeq 500's. The technician was blinded to the results of the corresponding patient's DMSA scan.

RNA expression data preprocessing

We performed FastQC (FastQC v0.11.3)⁴ to assess the quality of data and used Trimmomatic⁵ (Trimmomatic-0.33) to trim reads with low quality using the default parameter setting. We used TopHat2 (TopHat v2.0.9)⁶ at the default parameters to align the reads to the reference (Homo Sapiens UCSC hg19, downloaded at https://support.illumina.com/sequencing/sequencing_software/igenome.html). The resulting bam files after alignment were converted to expression count data using HTseq.

Statistical analyses for gene expression data

We used edgeR⁷ to detect differentially expressed (DE) genes. We further investigated the significance level of DE genes with $P < .05$ and fold change >1.5 (regardless of direction) using the permutation test. We also conducted Pathway analysis using KEGG, BIOCARTA, GO and REACTOME databases from MsigDB (<http://software.broadinstitute.org/gsea/msigdb/collections.jsp#C2>) treating all genes after filtering as background. We excluded pathways with less than 5 genes or more than 200 genes. We also performed modular analysis using modules proposed by Chaussabel.⁸ We obtained q-values using the Benjamini-Hochberg correction.⁹

Results

Figure 1 describes the flow of patients into the study. We noted no differences in mean age, sex, race, duration of fever, maximum temperature, or method of urine collection among the 61 children included in this report and the 22 children who were excluded because they did not have DMSA scan results. Table II describes the demographic characteristics of 39 children with cystitis and 22 children with APN; differences in clinical characteristics between the two groups were not significant. Mean age of children included was 3.5 years. *E. coli* was the uropathogen isolated from all but one child. Mean creatinine level in the two groups did not differ significantly ($p=.78$), nor did the number of children receiving ibuprofen within 6 hours of diagnosis ($p=.60$). Bag urine was used in 3 children for collection of urinary proteins.

Protein markers

Urinary proteins that best discriminated APN from cystitis (Table 3) were chemoattractants for neutrophils (CXCL1, CCL2) chemoattractants for monocytes (CCL2, CXCL12), proteins in the interferon γ pathway (INF γ , CXCL9), or proteins involved with T-cell response (IL-15, CXCL9, CXCL12). Division by urine creatinine had little effect on the p values or the AUC of urinary markers; accordingly, we present only raw values in the tables included in this report. Of note, results were similar when we excluded the child with a possible previous UTI (see tables in the Appendix).

Serum procalcitonin had the best overall ability to discriminate between cystitis and APN (AUC = .82); urinary IL-15 levels, however, offered nearly the same predictive power (AUC = .76). Ibuprofen use did not modify the relationship between biomarker values and outcome except for procalcitonin; the difference in procalcitonin levels between the pyelonephritis and cystitis groups was significantly ($p=.035$) larger in children that had received ibuprofen compared with children who had not received ibuprofen within 6 hours of diagnosis.

RNA expression data

19 children with cystitis and 16 with APN were included in this analysis (Figure 1). Per base sequence quality was high (Phred score generally >30) and the average alignment rate was 89.7%. Of the 23,710 unique genes isolated, 463 were differentially expressed ($p<0.05$ and fold change >1.5). The false discovery rate via the permutation test was 0.093. The expression levels of the DE transcripts can be visualized in Figure 2 (available at

www.jpeds.com) and the list of the top DE genes is shown in Table 1. The marker with the highest q value, IFI27 (interferon alpha inducible protein 27) is expressed in both the bladder and kidney¹⁰ and is involved in cytokine signaling and in the type I interferon-mediated signaling in response to infection. One study found it was useful in differentiating patients with primary kidney disease from controls.¹¹ The result of the Modular and Pathway analyses is shown in Table 4; in both interferon response differed significantly in children with cystitis and pyelonephritis.

E. coli virulence factors

Of virulence factors investigated (hly, cnf_1, pap, sfa) none were linked to APN; AUC for all were <0.6.

Discussion

In this pilot study, we identified urinary and serum protein markers that appear promising in differentiating cystitis from APN. Proteins identified were largely chemoattractants (CCL2, CXCL1, CXCL9, CXCL12), involved in the interferon γ pathway, or involved in immune response to bacteria (IL-15, IL-2 receptor α , procalcitonin). These findings suggest that the immune response to APN and cystitis differs quantitatively and qualitatively, and that these differences might be useful in differentiating these two conditions.

Of the 10 markers that differed most significantly between the two groups, 8 were urinary markers. Although none of these urinary markers matched the predictive capability of serum procalcitonin, it is possible that a combination of urinary markers (e.g., combining a marker with high sensitivity with one that has a high specificity) may outperform serum procalcitonin. We did not have the sample size needed to assess this in this pilot study, but this could be a promising approach to investigate in future studies.

Procalcitonin was the single marker with the highest overall accuracy. The sensitivity was slightly higher in this study than in a previous meta-analysis.¹² Nevertheless, because it requires a blood draw and because its specificity is only 74%, the search for novel urinary markers appears justified. The cutoff that maximized accuracy in this study was 0.5, which is the one recommended by the manufacturer. Procalcitonin discriminated children with pyelonephritis from cystitis better in children who had received ibuprofen within 6 hours of presentation; this likely reflects the higher severity of illness in children who received ibuprofen rather than any direct effect of ibuprofen on procalcitonin.

We found higher levels of interferon γ in the urine samples of children with APN compared with the urine of those with cystitis (Table 3). Serum RNA expression also showed an upregulation of interferon response, and in particular, interferon γ , in children with APN compared with children with cystitis (q values of 5.4 E-14 and .0004, respectively in modular analysis). Expression of IFI27 (interferon alpha inducible protein 27) gene was 18-fold higher (Q=.07) in APN compared with cystitis, and this gene is involved in interferon γ pathway and in cytokine signaling. Interferon γ is expressed in the bladder, kidney¹⁰ and in neutrophils¹³ and is involved in cytokine signaling in response to UTI.^{14–18} Specifically it is involved in the recruitment of macrophages,^{13,19} in IL-6 release by epithelial cells,¹⁸ in the

upregulation of TLR2 and 4 during infection. Interferon γ knockout mice were deficient in their ability to clear *E. coli* UTI after intraurethral challenge.¹⁷ Studies in humans have shown that differences in interferon γ levels could help differentiate patients with severe kidney infections from children with mild infection,²⁰ and patients with primary kidney disease from controls.¹¹ Interferon γ administration to rabbits with experimental pyelonephritis prolonged their survival.¹⁵ In summary, our results, in conjunction with findings from other studies to date, suggest that interferon γ is important in the pathophysiology of APN and its measurement may help differentiate the two conditions. Interferon γ can be easily measured using available ELISA kits from both blood and urine.

Urinary IL-15 was markedly different in the two groups. IL-15 is involved in natural killer (NK) and T cell proliferation. Recently, IL-15 was found to be released by uroepithelial cells in response to *E. coli*.²¹ NK cells are an important innate immune response to UTI,^{22, 23} and resident NK cells in the kidney are involved in ischemic injury.²⁴ Perhaps, IL-15 is involved in the regulation of NK cell immune response in the kidney; it appears to be a promising urinary marker for APN.

Our data also suggest that neutrophil response differs between APN and cystitis. We found differences in serum RNA expression ($Q=0.00057$ for neutrophil module) and in urinary CXCL1, a potent neutrophil chemoattractant. A potential explanation is that at the time of clinical presentation, in children with cystitis recruitment of neutrophils is already decreasing whereas in children with APN neutrophils are still being recruited to the site of infection. One study in adults found higher levels of CXCL1 in adults with recurrent UTI compared with those who did not suffer from recurrences ($p=0.054$).²⁵ CXCL1 also has been higher in patients with febrile UTI who were bacteremic vs. those who were not.²⁶

Large differences in potent T-cell chemokines (CXCL2 and 9), consistent with recent findings by others,²⁷ suggest that T cell recruitment differs in children with APN and cystitis and that adaptive immunity may be more important for APN as compared with cystitis. Knockout mice with $\gamma\delta$ -T cell deficiencies were more susceptible to UTI.¹⁷ Thumbikat found that T-cell response was important in eradicating UTI in a murine model of UTI.²⁸ Several studies in both animals and humans have shown that an adaptive immune response fails to occur in those with cystitis alone.^{29–31} As such, use of urinary CCL2 and CCL9 may be a promising means of differentiating the two conditions.

NGAL differed in children with APN and cystitis. Although the function of NGAL is not completely understood, it is released from neutrophils (and perhaps to a smaller degree from renal cells) under conditions of “stress”. NGAL is involved in innate immunity against bacterial infection by sequestering iron required for bacterial growth. However, differences between NGAL levels in the two groups were less striking than the other markers described above.

Unlike previous studies (Table 1), we did not find significant differences in levels of IL-8, IL-6, IL1 β , MIF, or *E. coli pap* gene between the two groups of children. The level of α -defensin 1 (also known as human α defensin 1), one of the better studied antimicrobial peptides, and one which has been shown to differ in children with and without UTI,³² did

not differ in the 2 groups we compared. Differences found in previous studies might be explained by differences in study design (many previous studies include afebrile children or used a case-control design) which are detailed in Table 1.

This study had a number of limitations. Our sample size was small; therefore, we did not have the ability to examine potentially important subgroup differences. Cytokine levels could have been influenced by viral co-infection, which we did not investigate. Strengths of our study were the exclusion of children without fever, avoidance of bag-collected urine samples for diagnosis of UTI and use of two experienced radiologists to interpret DMSA scans.

The preliminary evidence provided here, if supported by future studies, could support a role for measurement of urinary interferon γ , CCL2, CXCL1, CXCL9, CXCL1, IL-15, IL-2 receptor α in differentiating cystitis from APN in febrile children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations:

UTI	urinary tract infection
APN	acute pyelonephritis
DMSA	dimercaptosuccinic acid
PCR	polymerase chain reaction

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Figure 1.
Flowsheet

Figure 2.

Heatmap showing relative expression. Green, red and black denote low, high, and median expression levels, respectively. Each row represents a gene and each column represent a patient. The black and red bars on top denote the pyelonephritis group and cystitis groups, respectively.

Table 1.

Studies on accuracy of markers for acute pyelonephritis

Study, Year	Best Marker identified ^a	N with UTI	Sensitivity, specificity	Limitations ^b
Urinary markers				
Ghasemi, 2016 ³³	U-NGAL	89	67%, 98%	F
Arambaši, 2016 ³⁴	U-NGAL	94	88%, 79%	B, F
Yim, 2014 ³⁵	U-NGAL	73	75%, 74%	None
Lee, 2015 ³⁶	U-NGAL/Cr	33	61%, 87%	D
Lertdumrongluk, 2015 ³⁷	U-HBP	32	100%, 100%	D, F
Otukesh, 2009 ³⁸	U-MIF	33	92%, 100%	A, C, D
Rodriguez, 2008 ³⁹	U-IL6	35	39%, 94%	C, D
Sheu, 2007 ⁴⁰	U-IL18	75	88%, 79%	A
Serum markers				
Tekin, 2015 ⁴¹	S-MPV	94	81%, 86%	C
Shaikh, 2015 ¹²	S-PCT	434 ^c	86%, 74%	B, C, E
Leroy, 2013 ⁴²	S-PCT	1011 ^c	71%, 72%	B
Shaikh, 2015 ¹²	S-CRP	1638 ^c	94%, 39%	B, C, E
Shaikh, 2015 ¹²	S-ESR	1737 ^c	87%, 48%	B, C, E
Sim, 2015 ⁴³	S-NGAL	123	89%, 71%	C
Seo, 2014 ⁴⁴	S-NGAL	47	75%, 78%	A, D
Mahyar, 2013 ⁴⁵	S-IL8	87	81%, 28%	C
Sheu, 2006 ⁴⁶	S-IL6	78	88%, 83%	A
Fretzayas, 2000 ⁴⁷	S-Ea1Pi	140	96%, 50%	A, C
<i>E. colivirulence genes</i>				
Jantaush, 1992 ¹	<i>E. coli pap</i> gene	59	68%, 0%	None

^aU=urine, S= serum/plasma, Cr=creatinine^bA=Case-control design, B= Used bags to collect urine in some children, C= fever not required for inclusion or unclear inclusion, D= Sample size <50; E=Meta-analysis without individual patient data, F= Defined acute pyelonephritis clinically, did not require DMSA for all children, or DMSA interpretation not standard (i.e., not categorized as normal vs. abnormal)^cMeta-analysis

Table 2.

Demographic and clinical characteristics of the 61 children with interpretable DMSA scans

	Cystitis (N=39)	Pyelonephritis (N=22)	P value
	Number (%)	Number (%)	
Age (months)			.44
1-11	16 (41.0)	7 (31.8)	
12-23	4 (10.3)	4 (18.2)	
24-59	3 (7.7)	4 (18.2)	
60	16 (41.0)	7 (31.8)	
Sex			.08
Male	6 (15.5)	0	
Female	33 (84.6)	22 (100)	
Race			.06
White	22 (56.4)	13 (59.1)	
Black	15 (38.5)	4 (18.2)	
Other	2 (5.1)	5 (22.7)	
Duration of Fever			.35
48 hours	13 (33.3)	11 (50.0)	
< 48 hours	25 (64.1)	11 (50.0)	
Unknown	1 (2.6)	0	
Maximum Reported Temperature			.55
39° C	24 (61.5)	16 (72.7)	
< 39° C	15 (38.5)	6 (27.3)	
Method of collection			.75
Catheterization	20 (51.3)	13 (59.1)	
Clean Catch	19 (48.7)	9 (40.9)	
Ibuprofen given within 6 hours of presentation			.60
Yes	11 (30.8)	9 (40.9)	
No	27 (69.2)	13 (59.1)	

Table 3.

Univariate association of protein markers with pyelonephritis by increasing p value (limited to markers with p<0.05)

Biomarker (alternate name)	Tissue	No. (cystitis/pyelonephritis)	Mean level (SD) in cystitis (pg/mL)	Mean level (SD) in pyelonephritis (pg/mL)	P value	AUC	Sensitivity	Specificity
IL-15	Urine	35/21	5.58 (8.05)	17.70 (14.94)	0.00194	0.76	0.76	0.69
CCL2 (MCP1)	Urine	35/21	492.13 (545.20)	1045.11 (701.50)	0.00389	0.74	0.76	0.74
CXCL9 (MIG)	Urine	35/21	510.85 (541.68)	1306.49 (1174.52)	0.00722	0.73	0.86	0.63
CXCL1 (GRO α)	Urine	35/21	885.98 (828.41)	1915.19 (1506.20)	0.00762	0.76	0.76	0.69
Hepatocyte Growth Factor	Urine	35/21	1177.91 (795.48)	1726.77 (859.30)	0.02228	0.71	0.81	0.71
CXCL12 (SDF1)	Urine	35/21	128.39 (51.87)	165.78 (64.30)	0.03005	0.67	0.71	0.60
Interferon γ	Urine	35/21	33.61 (25.42)	54.59 (38.05)	0.03207	0.68	0.52	0.89
IL-2 receptor α	Urine	35/21	1481.89 (1068.19)	2271.18 (1423.78)	0.03507	0.68	0.95	0.40
TRAIL	Serum	28/17	102.59 (139.04)	42.56 (41.20)	0.03997	0.66	0.94	0.36
Procalcitonin ^a	Serum	27/16	630.37 (1520.16)	5250.00 (8205.46)	0.04085	0.82	0.81	0.74
Hepatocyte Growth Factor	Serum	28/17	367.61 (347.12)	679.61 (552.79)	0.04725	0.69	0.59	0.89
NGAL ^b	Urine	35/21	468906.91 (470675.71)	770414.28 (575400.85)	0.05000	0.70	0.71	0.69

^aUsed a cutoff of 500 pg/mL, which is also the one recommended by the manufacturer

^bUsed a cutoff of and 428000 pg/mL for NGAL

Table 4.

Modules (top panel) and pathways (bottom panel) upregulated in the serum of children with pyelonephritis;^a

	P value	Q value	No. of DE genes in module/pathway	Percent upregulated
Significant modules sorted by p value				
2.3 Erythrocytes_Red_Anemia_Globin_Hemoglobin ^b	6.73E-18	1.61E-16	23	100%
3.1 ISRE_Influenza_Antiviral_IFN_γ_IFN_α_Interferon ^c	4.52E-15	5.42E-14	20	95%
2.2 Granulocytes_Neutrophils_Defense_Myeloid_Marrow ^d	7.11E-05	0.00056915	7	71%
Significant pathways sorted by p value				
Interferon alpha beta signaling (Reactome) ^e	1.01E-09	1.57E-06	13	100%
Interferon signaling (Reactome) ^f	4.87E-07	0.0004	17	88%

DE genes = differentially expressed genes

^a Only modules/pathways with $Q < .05$ are shown

^b DE genes in module: BCL2L1/HBA1/MYL4/ANK1/SNCA/BAG1/FBXO7/GMPR/HMBS/MKRN1/HBM/ASCC2/E2F2/ADIPOR1/EPB49/CARM1/HAGH/SLC4A1/UBB/SELENBP1/SLC6A8/KLF1/FAM146C

^c DE genes in module: IFIT3/MX2/OAS3/HERC5/MX1/AGRN/OAS2/RSAD2/IFI44L/EIF2AK2/OAS1/NT5C3/LY6E/LGALS3BP/OASL/CXCL10/ANKRD22/PPM1K/PGAP1/HERC6

^d DE genes in module: HEMGN/BNIP3L/CSDA/RETN/HP/BPGM/MYL4

^e DE genes in pathway: IFI27/IFI16/IFIT1/IFIT3/ISG15/ISG20/MX1/MX2/OAS1/OAS2/OAS3/OASL/USP18

^f DE genes in pathway: EIF2AK2/FCGR1A/HERC5/IFI27/IFI6/IFIT1/IFIT3/ISG15/ISG20/MX1/MX2/NEDD4/OAS1/OAS2/OAS3/OASL/USP18