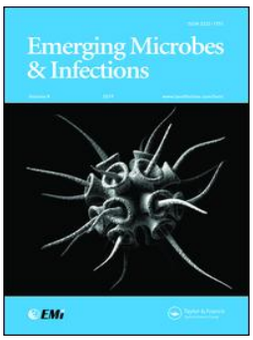

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Blood transcriptomics to characterize key biological pathways and identify biomarkers for predicting mortality in melioidosis

Running title: Biomarkers for mortality in melioidosis

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Abstract

Melioidosis is a tropical infectious disease caused by the Gram-negative bacillus, *Burkholderia pseudomallei* that is often lethal in many endemic areas. The objective of this study was to characterize the transcriptome in melioidosis patients and identify genes associated with outcome. RNA-seq was performed on whole blood RNA in a discovery set of 29 melioidosis patients and 3 healthy controls using Ion AmpliSeq Transcriptome. Transcriptomic profiles of patients who did not survive to 28 days were compared with patients who survived and healthy controls. RT-qPCR of 28 differentially expressed genes was performed in a validation set of 60 melioidosis patients and 20 healthy controls. In RNA-seq analysis, 65 genes were significantly up-regulated and 218 were down-regulated in non-survivors compared to survivors. Up-regulated genes were involved in myeloid leukocyte activation, Toll-like receptor cascades and reactive oxygen species metabolic processes. Down-regulated genes were hematopoietic cell lineage, adaptive immune system and lymphocyte activation pathways. RT-qPCR in the validation set of patients confirmed differential expression of a subset of genes. *IL1R2, GAS7, S100A9, IRAK3,* and *NFKBIA* were significantly higher in non-survivors compared with survivors ($P < 0.005$) and healthy controls ($P < 0.0001$). The AUROCC of these genes for mortality discrimination ranged from

0.80-0.88. In survivors, expression of *IL1R2*, *S100A9* and *IRAK3* genes decreased significantly over 28 days ($P < 0.05$). Whole blood transcriptomics characterizes the host response in melioidosis. Expression levels of specific genes are potential biomarkers to predict outcomes. These findings augment our understanding of this severe infection.

Keywords: RNA-sequencing, Transcriptomics, Melioidosis, Biomarkers, *Burkholderia pseudomallei*, Outcome, Immune response

Introduction

Melioidosis is a severe infectious disease caused by *Burkholderia pseudomallei*, a Gram-negative bacterium and biothreat agent [1]. The disease is highly endemic in the tropics, particularly in Southeast Asia and northern Australia but reported cases are increasing globally. Melioidosis carries a mortality rate of 40% or higher in many endemic regions where resources are limited. This poor outcome from melioidosis has remained unchanged for many years [2,3]. Melioidosis is associated with several host factors, but diabetes is the major risk [4,5]. Pneumonia and bacteremia are the most common manifestations of disease; infections of these systems are frequently associated with septic shock and contribute to high mortality [2].

A comprehensive understanding of the individual response to infection is necessary to develop effective and targeted therapies. Additionally, biomarkers that predict outcome may be useful to guide patient management. Evaluation of the entire transcriptome of cells offers both the possibilities of characterizing pathways activated in disease and identifying potential biomarkers. In murine melioidosis, blood transcriptomic profiling reveals the regulation of many immune pathways, which reflect severity of disease [6] and can be used to identify a potential marker of acute lung infection [7]. Transcriptomic changes have been reported in

human melioidosis during acute infection, highlighting the involvement of host immunity against infection [8]. Recent studies based on microarrays showed that blood transcriptional profiles can distinguish *B. pseudomallei* infection from sepsis caused by other microorganisms [9,10]. These studies suggest that these transcriptomic profiles may be useful in understanding the immune response during infection and serve as informative biomarkers of infection. RNA-sequencing (RNA-seq) is a unbiased approach and powerful tool to define the transcriptome [11]. However, to date, RNA-seq has not been used extensively to characterize human melioidosis. The aims of this study were to use RNA-seq (i) to analyze whole blood transcriptomic profiles of acute melioidosis patients to define biological pathways associated with death, and (ii) to identify host prognostic gene biomarkers that are associated with mortality.

Methods

Study design and patients

A prospective study of whole blood transcriptomic analyses in 97 individuals with melioidosis was conducted at seven hospitals in Northeast of Thailand: Udon Thani Hospital, Nakhon Phanom Hospital, Mukdahan Hospital, Roi Et Hospital, Buriram Hospital, Surin Hospial, and Sisaket Hospital. This study was part of a multi-centre study of patients aged ≥15 years who were culture-positive for *B. pseudomallei* from any type of clinical samples and admitted to the hospitals between January 2015 and December 2019. The inclusion and exclusion criteria were described previously [12]. *B. pseudomallei* were identified by biochemical tests and latex agglutination [13] at the microbiology laboratories of the hospitals and further confirmed by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF MS) as previously described [14]. Whole blood samples were

collected at the time of enrolment (within 24 hours of culture results, defined as day 0) and day 5, day 12, and day 28 after enrolment. Clinical information was obtained from the medical records. Mortality of patients was recorded at the hospitals or by phone calls for 28 days of follow up.

Twenty-three healthy individuals aged ≥ 18 years were recruited from Udon Thani Hospital and Mukdahan hospital as baseline controls for discovery and validation data sets. Inclusion and exclusion criteria for these controls were previously described [15].

This study was designed by the process of 3 data sets as follows: discovery set, validation set, and follow-up set as described in Supplementary Figure 1.

Ethical approval

The study was approved by the ethical committees of Faculty of Tropical Medicine, Mahidol University, Udon Thani Hospital, Nakhon Phanom Hospital, Mukdahan Hospital, Roi Et Hospital, Buriram Hospital, Surin Hospial, and Sisaket Hospital. Written informed consent was obtained from all participants or their representatives.

Sample collection

Three milliliters of whole blood were collected from melioidosis patients and healthy controls into Tempus^{TM} Blood RNA Tubes (Thermo Fisher Scientific) and stored at -20°C or -80°C at the hospitals. The frozen samples were transported on dry ice to the laboratory in Bangkok for RNA extraction.

RNA extraction

Total RNA was extracted from Tempus-stabilized blood using the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Life technologies). Total RNA concentration and

its purity were assessed by determining the A260/280 and A260/230 ratios, respectively on the NanoDrop Spectrophotometer (Thermo Fisher Scientific). RNA integrity number (RIN) was assessed with the Agilent RNA 6000 Pico kit on 2100 Bioanalyzer (Agilent Technologies). Genomic DNA contamination was checked by RT-qPCR using primers for the Peptidylprolyl isomerase A (*PPIA)* gene [16].

Library preparation for RNA-seq

Libraries were prepared from 50 ng of RNA per sample using Ion AmpliSeq™ Transcriptome Human Gene Expression Kit (Thermo Fisher Scientific). Targets of 20,802 genes were amplified with Ion AmpliSeq™ Transcriptome Human Gene Expression core panel (Life Technologies). The primer sequences were then digested, and DNA adaptors (Ion P1 Adaptor and Ion Xpress Barcode Adaptor, Life Technologies) were ligated to the targets. Adaptor ligated targets were purified using the Agencourt AMPure XP reagent (Beckman Coulter) and eluted into an amplification mix containing Platinum PCR SuperMix High Fidelity and Library Amplification Primer Mix (Life Technologies) for further amplification. Size-selection purification was performed using Agencourt AMPure XP reagent (Beckman Coulter). Amplicons were quantified using a Fragment Analyzer™ instrument with a DNF-474 High Sensitivity NGS Fragment Analysis Kit (Advanced Analytical Technologies, INC.). Samples were then pooled together with four samples per pool and performed an emulsion PCR on the Ion Chef System using the Ion PI Hi-Q Chef Kit (Life Technologies). The emulsion PCR samples were loaded on Ion PI v3 chips and sequenced on an Ion Proton System using an Ion PI Hi-Q Sequencing 200 Kit chemistry (Life Technologies) to obtain approximately 200 bp read length.

Transcriptomic data analysis

Sequencing data were generated using Torrent Suite Software version 5.4.0 with AmpliSeq RNA plugin (Thermo Fisher Scientific) and normalized using reads per million mapped reads (RPM) method. The normalized transcripts were analyzed using GeneSpring GX software version 14.9 (Agilent Technologies) to identify differentially expressed genes (DEGs) within the 10^{th} -100^{th} percentile. One-way ANOVA was used to compare DEGs among non-survivors, survivors, and healthy controls. Moderated t-test was used to compare DEGs between non-survivors and survivors. An adjusted *P* value < 0.05 was deemed significant (Benjamini-Hochberg correction method). Functional analysis was derived using Metascape tool (http://metascape.org). Area under the receiver operating characteristic curves (AUROCC) were plotted using GraphPad Prism version 6.0.

DEGs were initially selected for validation based on fold change ≥ 2 and adjusted *P* value ≤ 0.05 between non-survivors and survivors.

Quantitative reverse-transcriptase PCR (RT-qPCR)

Two-step RT-qPCR was used to quantitatively validate gene expression. Total RNA from whole blood was converted into cDNA using the iScript™ cDNA Synthesis Kit (Bio-Rad). The amplification was performed in duplicate in a total volume of 10 µl containing 5 µl of iTaq Universal SYBR Green (Bio-Rad), 2 µl of 4 ng cDNA, 0.4 µl of 10 mM forward primer, 0.4 µl of 10 mM reverse primer, 2.2 µl of distilled water. The cycle conditions were as follows: 1 cycle of 95°C for 30s followed by 40 cycles of 95°C for 10s and 60°C for 30s. After amplification, melting curve analysis was carried out from 65°C to 95°C. Primers were designed using NCBI PrimerBlast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). All primer pairs are listed in Supplementary Table 1. Peptidylprolyl isomerase A (*PPIA*), Human large ribosomal protein P0 (*RPLP0*), and Tata-box binding protein (*TBP*) were used as reference genes for calculating the relative expression levels of other genes [16] The

expression levels were calculated by using the $2^{-\Delta Ct}$ method, where ΔCt = mean Ct of target gene – mean Ct of the three reference genes.

Statistical analysis

Mann-Whitney or Kruskal-Wallis tests followed by Dunn's multiple comparison tests correction were used to test the difference in gene expressions among subject groups. Mean, median, interquartile range (IQR), standard deviation (SD), area under the receiver operating characteristic curve (AUROCC) values and 95% confidence intervals (CI) were assessed using Prism 6 (GraphPad Software). The classification accuracy of the 12 gene signature was determined using the randomForest machine learning R package (v. 4.16) [17] applied to the qRT-PCR data. The AUROCC curve was visualised using the pROC package (v. 1.10).

Results

Whole blood transcriptomic profiles of survivors and non-survivors

To identify genes associated with mortality, we performed whole blood transcriptomic analysis of a discovery set consisting of 29 Thai melioidosis patients, fourteen of whom survived and fifteen of whom died within 28 days, and 3 healthy controls. The clinical characteristics of the patients are shown in Table 1. The quality of 32 RNA samples were analyzed for integrity and read count/mapped read numbers. Overall average RNA integrity numbers (RIN) of 6.0-8.6, average OD ratios 260/280 > 1.8, 260/230 < 1, and average of 22 million reads with mapping rate of > 58% were achieved from each cDNA library. Out of 20,802 genes, 18,713 genes with expression values between 10^{th} - 100^{th} percentiles were further analyzed using one-way ANOVA and 5,189 genes were statistically

different among groups as shown in three dimensional principal component analysis (3D-PCA) plots (Figure 1).

Analysis of differentially expressed genes (DEGs) between non-survivors and survivors performed using the moderated t-test method identified 283 DEGs. Hierarchical cluster analysis of these genes was generated by GeneSpring (Figure 2). Whole blood of non-survivors presented more down-regulated genes compared to survivors (fold change ≥ 2). RNA-seq data of 65 up-regulated genes and 218 down-regulated genes with *P* value ≤ 0.05 and fold change ≥ 2 are shown in Supplementary Table 2. In comparison to melioidosis patients who survived, the fold changes of up-regulated genes in non-survivors ranged between 2.00 to 15.72 and *P* value = 1.70×10^{-3} to 5.47×10^{-9}. The fold change of down-regulated genes ranged between 2.00 to 9.42 and *P* value = 9.50×10^{-5} to 2.54×10^{-9}. The volcano plot in Figure 3 shows the distribution and relationship between fold change and *P* value of 65 up-regulated genes and 218 down-regulated genes in non-survivors in relation to survivors.

Functional enrichment analysis of DEGs between survivors and non-survivors

In order to gain insight into the biological function of DEGs, the genes found significantly differential expressed (65 up-regulated and 218 down-regulated) between survivors and non-survivors were analyzed using the Metascape tool. The analysis was based on combined datasets for enrichment analysis, including gene ontology, KEGG pathways, reactome gene sets, canonical pathways, and CORUM complexes. The data in Figure 4 show that the significant DEGs were involved in functions of host immune response (n = 7), stress response (n = 6), cell development (n = 35), signaling transduction (n = 23), catabolic process (n = 16), and metabolic process (n = 24). The significant 65 up-regulated DEGs in non-survivors were involved in myeloid leukocyte activation (n = 14), Toll-like receptor cascades

(n = 8), and reactive oxygen species metabolic processes (n = 8) (Figure 4A) while the majority of 218 down-regulated genes set in non-survivors were hematopoietic cell lineage (n = 10), adaptive immune system (n = 24) and lymphocyte activation (n = 23) (Figure 4B). Gene names and details of each functional group are shown in Supplementary Table 3.

Pathway analysis of DEGs between melioidosis survivors and non-survivors

To gain better understanding of the underlying mechanisms of the 283 altered genes in non-survivors compared to survivors, we performed KEGG pathway analysis. Interestingly, KEGG identified six pathways in immunological response that were associated with 65 up-regulated genes (Supplementary Table 4). These included pathways of Toll-like receptor signalling, Th17 cell differentiation, MAPK, IL-17 signalling, FoxO signalling, HIF-1 signalling. Moreover, KEGG identified seven pathways in immunological response that were associated with 218 down-regulated genes. These included hematopoietic cell lineage, cell adhesion molecules (CAMs), intestinal immune network for IgA production, Th1 and Th2 cell differentiation, Th17 cell differentiation, antigen processing and presentation and B cell receptor signalling pathway.

RT-qPCR validation of DEGs to predict mortality in melioidosis

Twenty-eight DEGs were manually selected to confirm the expression by RT-qPCR in a validation set of 30 non-survivors, 30 survivors and 20 healthy controls. The DEGs were selected according to (i) their degree of alteration (fold changes and *P* value) (Supplementary Table 2) and (ii) their functions related with immunological responses (Supplementary Table 4). These DEGs included 20 up-regulated genes and 8 down-regulated. RT-qPCR results in the validation set confirmed significantly higher expression in non-survivors compared with survivors and healthy controls for 16 of the 20 up-regulated genes and 1 of the 8 down-

regulated genes, respectively (Figure 5 and Supplementary Table 5). RT-qPCR in the validation set confirmed significantly lower expression in non-survivors compared with survivors ($P = 0.016$) and healthy controls ($P < 0.0001$) for 1 of 8 down-regulated genes: *CD160*.

ROC assessment of gene expression as predictive markers for mortality

Receiver operating characteristic (ROC) curves were constructed based on the RT-qPCR results from the validation set of melioidosis patients to examine the classification accuracy of each DEG for distinguishing between non-survivors and survivors (Figure 6A-C). The highest area under the ROC (AUROCC) were obtained from the genes listed in Supplementary Table 6. Among these, *S100A9* showed the highest AUROCC value (0.88) followed by *IL1R2* (0.87) and *TLR4* (0.86). The down-regulated gene with the highest AUROCC was *CD160* (0.77). A combined signature of the expression of the 12 genes with best individual discriminatory ability was able to classify the non-survivors from the survivors in a Random Forest model (AUROCC 0.85, CI = 0.74 – 0.94), and completely discriminated the melioidosis patients from the healthy controls (Figure 6D).

Trajectory of gene expression profiles in survivors after enrolment

Five up-regulated DEGs (*S100A9, IL1R2, IRAK3, NFKBIA* and *GAS7*) were selected based on AUROCC ≥ 0.82 and whether the genes have secretory functions of proteins as they may be better suited to a point-of-care assay. Gene expression was measured by RT-qPCR in survivors (n = 8) at day 0, day 5, day 12, and day 28 to test whether expression decreases as patients recovered. The trend of gene expression at day 0, day 5, day 12, and day 28 were determined by calculating the fold change reduction. None of the five genes had major changes in expression at day 5 but *S100A9, IRAK3* and *IL1R2* subsequently had decreased

expression over time as patients recovered (Figure 7 and Supplementary Table 7). Expression of *S100A9, IRAK3, IL1R2* and *NFKBIA* significantly decreased at day 28 relative to day 5. Expression of *S100A9*, *IRAK3*, and *NFKBIA* in patients decreased at day 28 but did not reach to the expression level of healthy controls ($P < 0.0001$). However, expression of *IL1R2* and *GAS7* rapidly decreased to the same level of healthy controls and did not change further after day 12 ($P < 0.05$). The mean fold changes (day 28/day 5) for gene expression of 8 individual patients and 95% CI are shown in Supplementary Table 8.

Discussion

Our study demonstrated that the whole blood transcriptome of melioidosis patients who survived was distinguishable from non-survivors, with 283 DEGs significantly associated with mortality. The majority of these DEGs were related to the immune response, cellular functions and metabolism. Twenty-eight DEGs were selected by functional enrichment and pathway analyses and RT-qPCR of these genes in a validation cohort confirmed 16 up-regulated and 1 down-regulated gene associated with mortality. ROC analyses of the validation set identified the 15 most predictive genes. Subsequent RT-qPCR of four selected genes (*S100A9, IRAK3, IL1R2,* and *NFKBIA*) in surviving patients followed over time demonstrated a trajectory expression profile with decreased differential expression by day 12 and day 28 after enrolment.

Genes of melioidosis patients associated with death include *IL1R2, IRAK3, IL18RAP, MGAM, LPL, HGMB2, S100A9, GAS7, NFKBIA, TLR2, TLR4, MAPK14, GPR27, HIF1A,* and *ITGAM*. Many of these genes or their proteins have been reported in related studies. Elevation of *IL1R2* expression and soluble *IL1R2* concentrations are correlated with severity of *Escherichia coli* and *Staphylococcus aureus* infections [18]. Increased expression levels of the *IRAK3* gene are correlated with the development of acute lung injury in patients with

severe sepsis [19]. In melioidosis, Wiersinga et al. reported up-regulation of *IRAK3* is related to attenuated capacity of monocytes to respond to *B. pseudomallei* stimulation and this coincided with mortality [20]. In parallel to our study, a recent study reported that extracellular S100A8 and S100A9 (S100A8/A9), a Ca^{2+} sensor in cytoskeleton rearrangement and arachidonic acid metabolism, are the key mediators of sepsis secreted from neutrophils and monocytes during inflammation [21]. The S100A9 serve as damage associated molecular patterns and induce pro-inflammatory cytokine expression and secretion via toll-like receptor 4 (TLR4) activation [22,23]. Increasing evidence supports that *NFKBIA*-mediated inflammation is linked to susceptibility to infectious and inflammatory diseases [24-26]. A report demonstrated an up-regulation of *NFKBIA* expression in mouse macrophages in response to *B. pseudomallei* infection [27] and our data confirmed that increased *NFKBIA* expression is associated with fatality in melioidosis patients.

A recent study suggests that *HLA-DPA1* and *-DRB3* are under-expressed in whole blood of sepsis patients caused by *B. pseudomallei*, which distinguished melioidosis from sepsis caused by other organisms [9]. In addition, we found *HLA-DPB1* was down-regulated in non-survivors in our discovery cohort. Our data also revealed that non-survivors had reduced expression of *HLA-DPB1, HLA-DOA, HLA-DOB* and *HLA-DRA* representing MHC class II molecules, which are important for antigen presentation. Our results in melioidosis are similar to the results of other studies [28-30] suggesting that non-surviving patients with severe sepsis from melioidosis or other infections exhibit decreased MHC class II expression and that can contribute to persistent failure of T cell activation [31,32]. We did not observe the changes of these MHC class I at transcriptional levels. However, Dunachie et al. showed the presence of MHC class I genes, *HLA-B46* and *HLA-C*01* was associated with an increased mortality in an acute melioidosis cohort [8].

Enrichment analysis demonstrated a number of GO terms, including the up-regulation of myeloid leukocyte activation and down-regulation of lymphocyte activation in non-survivors compared with survivors. KEGG pathway analysis revealed many up-regulated genes involved in signal transduction pathways associated with severe melioidosis. Among these, TLRs are known to recognize *B. pseudomallei* LPS and initiate inflammation [33-36] and acute septic melioidosis patients had increased expression of many TLRs in leukocytes [34]. The activation of MAPK signaling and Th17 pathway in melioidosis patients have also been demonstrated in previous studies [37-39] [40]. Multiple signaling pathways were down-regulated in severe melioidosis suggesting that prolonged bacterial persistence exacerbates inflammatory responses that may lead to immune exhaustion, immune suppression, and poor outcome of the disease.

Expression of several genes, assayed on day 0, had high mortality discrimination, including *S100A9* and *IL1R2*. Notably, expression of these genes decreased significantly in surviving patients by day 12, suggesting that the gene expression tracks with clinical condition. Therefore, these genes and their encoded proteins could be considered as candidate biomarkers for predicting clinical outcomes in patients with melioidosis, and deserve further study in comparison to other clinical and biological prediction tools.

Strengths of our study were the multi-center design, prospective subject enrolment and sample collection, serial sampling over time in a subset of patients, and validation of selected findings. Some limitations are the relatively small number of samples in the discovery cohort, enrolment into our study only after the diagnosis of melioidosis was confirmed (rather than at the time of admission to hospital), and validation of only a subset of genes.

In conclusion, our findings provide new knowledge about transcriptional host responses in circulating leukocytes from hospitalized melioidosis patients and suggest several

candidate biomarkers for further study. These data are important to ongoing efforts to reduce the burden of this often severe infection.

Author contributions

JMC, TEW, and NC designed the study; TY, JSL, TK, MA, PE, WC, JMC, GL, TEW, and NC conducted the experiments; RP, TY, and TK acquired data; TY, RP, JSL, CE, TK, JMC, TEW, WC, and NC analyzed data; NC provided samples or reagents; TY, TK, MA, JMC, TEW and NC wrote the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Figure 1. Three-dimensional principal component analysis (3D-PCA) of differentially expressed genes among non-survivors and survivors and healthy controls. One point per subject in yellow, red, and light blue, represents groups of melioidosis patients who survived (n = 14) and did not survive (n = 15), and healthy controls (n = 3), respectively. Each axis shows percent variation explained by each group.

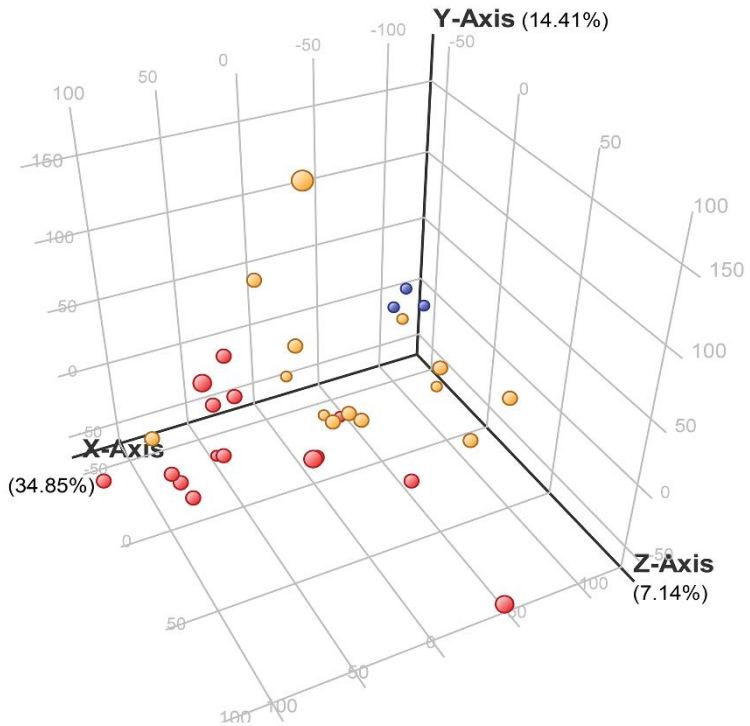


Figure 2. Hierarchical clustering analysis of 283 differentially expressed genes (DEGs) in whole blood of surviving and non-surviving melioidosis patients. High expression of genes is shown in green whereas low expression of genes is shown in red. Each column represents individual subjects and each row in the figure represents one altered gene that significantly expressed at $P \leq 0.05$ and fold change ≥ 2. Subjects from our study are melioidosis survivors (n = 14), melioidosis non-survivors (n = 15).

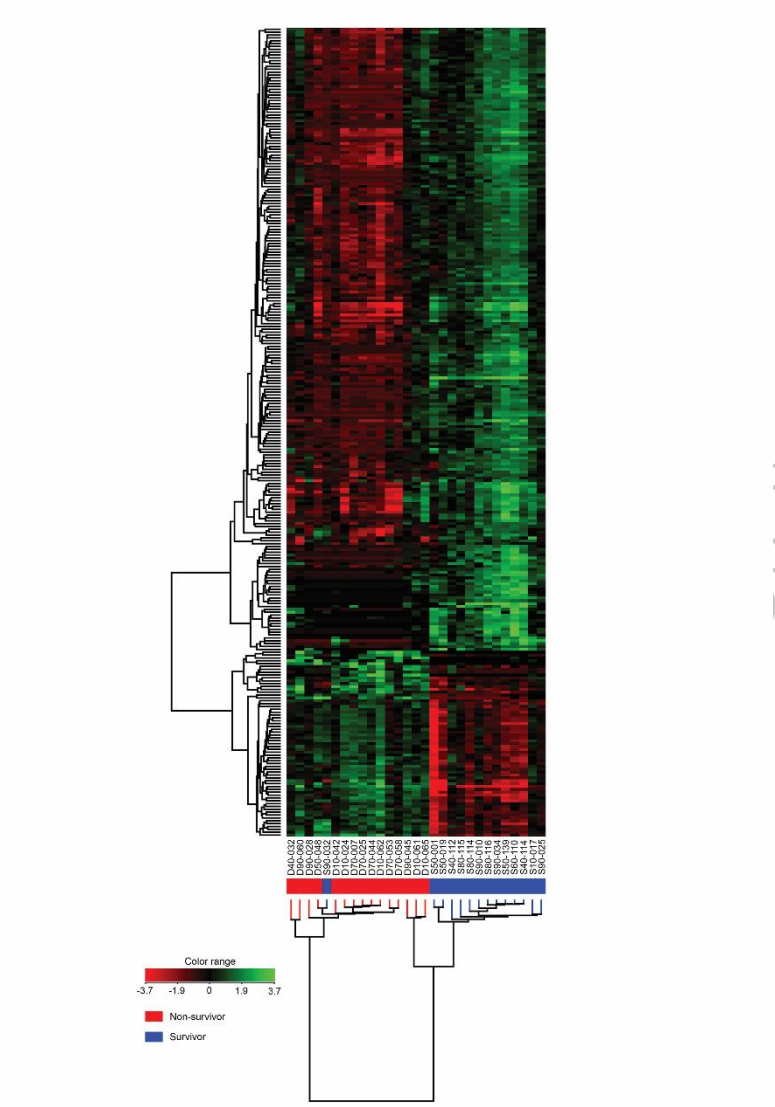


Figure 3. Differential expression analysis of survivors compared to non-survivors at the time of diagnosis (day 0). Gene expression profile of patients with melioidosis that survived after 28 days (n = 14) compared to patients that did not survive (n = 15). Color indicates statistically significant genes (adjusted *P* value ≤ 0.05, correction method = Benjamini-Hochberg) dark blue: down-regulated genes ≥ 2 fold change, dark red: up-regulated genes ≥ 2 fold change with grey corresponding to genes showing no expression change.

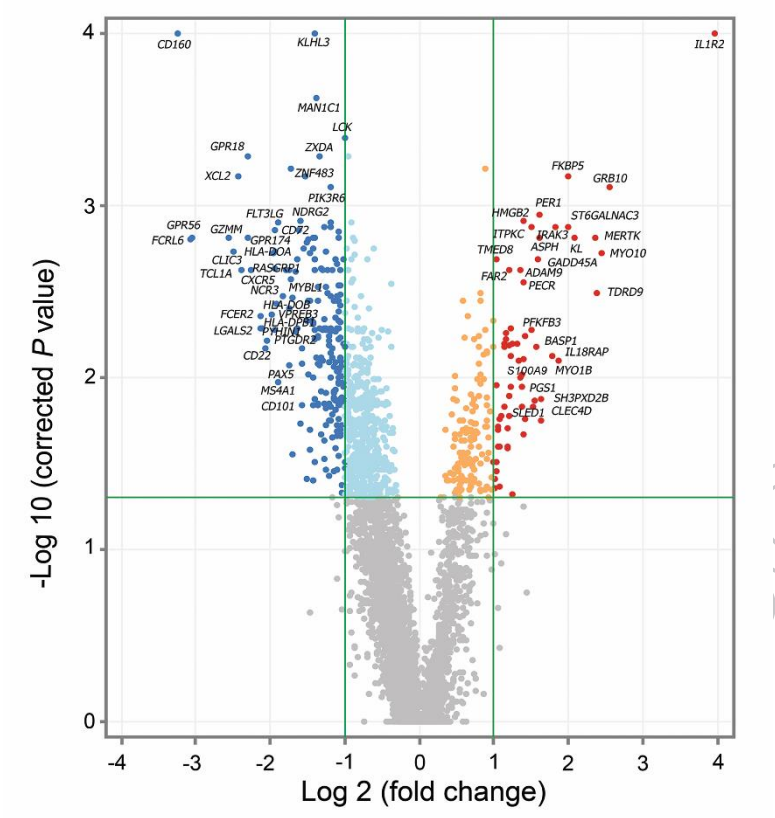


Figure 4. Functional enrichment analysis of DEGs in non-surviving melioidosis patients compared with patients that survived. (A) Top 20 enriched terms of 65 up-regulated genes in non-surviving melioidosis patients. **(B)** Top 20 enriched terms of 218 down-regulated genes in non-surviving melioidosis. Saturation of color corresponds to *P* values.

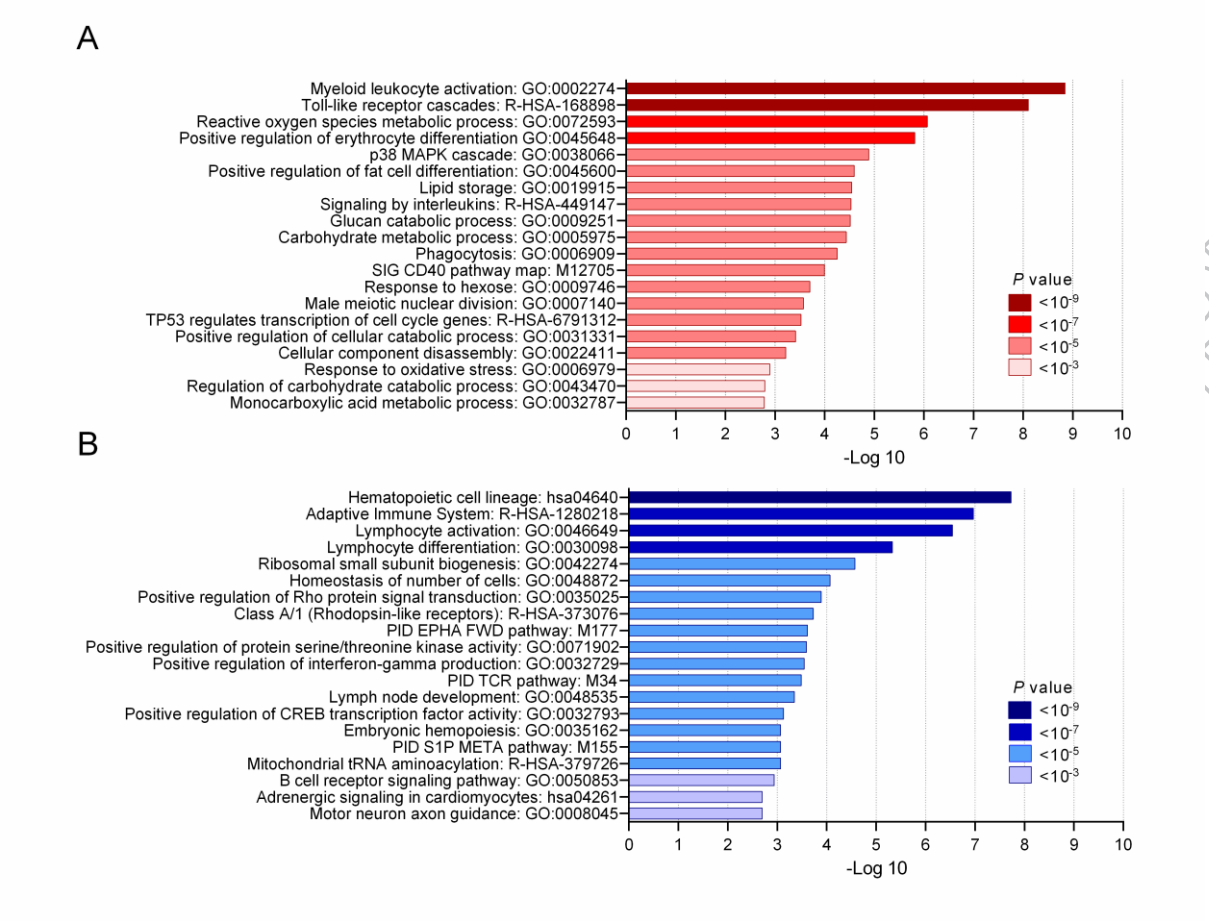


Figure 5. Validation of the differential expression analysis of 28 DEGs in whole blood from melioidosis patients. Genes that were found to be differentially expressed in patients with melioidosis that did not survive and survived were validated with real-time qPCR. The Kruskal-Wallis test was performed for comparing three groups. Subjects from our study were melioidosis survivors (n = 30), melioidosis non-survivors (n = 30), and healthy controls (n = 20).

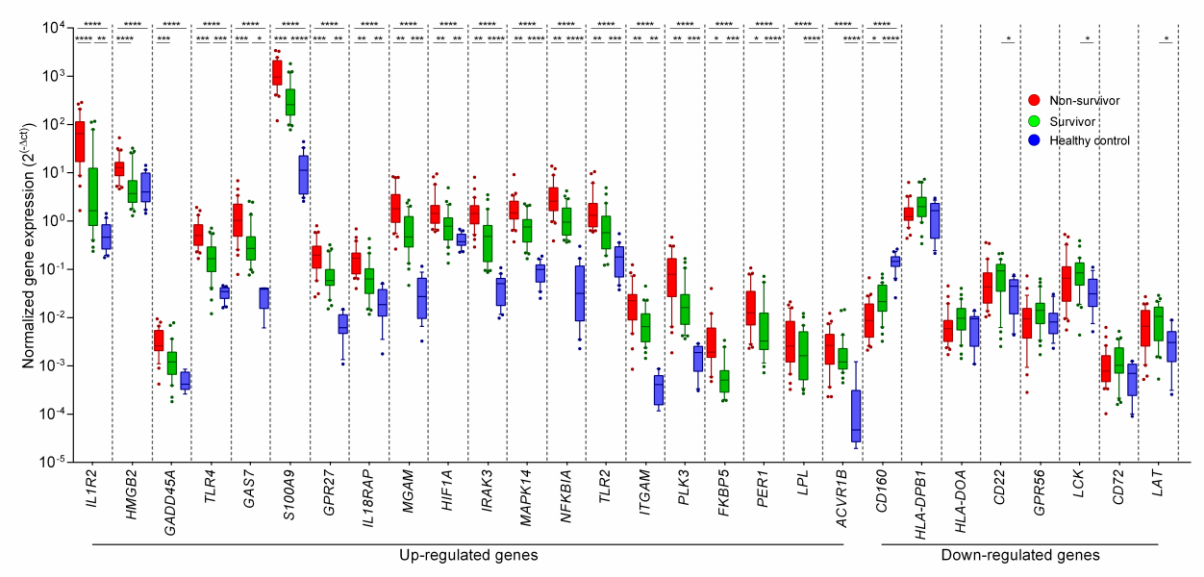


Figure 6. Area under the receiver operating characteristic curve (AUROCC) of DEGs in discrimination among non-survivors, survivors and healthy controls. (A) AUROCC of 10 DEGs between non-survivors versus survivors. **(B)** AUROCC of 10 DEGs between non-survivors versus healthy controls. **(C)** AUROCC of 10 DEGs between survivors versus healthy controls. (**D**). Random Forest model of a combined gene signature discriminates survivors and non-survivors**.** The 12 genes which individually discriminated clinical groups with AUROCC > 0.80 in qRT-PCR were combined to create a single model, which was used to classify the separation between survivors (S), non-survivors (NS) and healthy controls (HC) in the qRT-PCR dataset

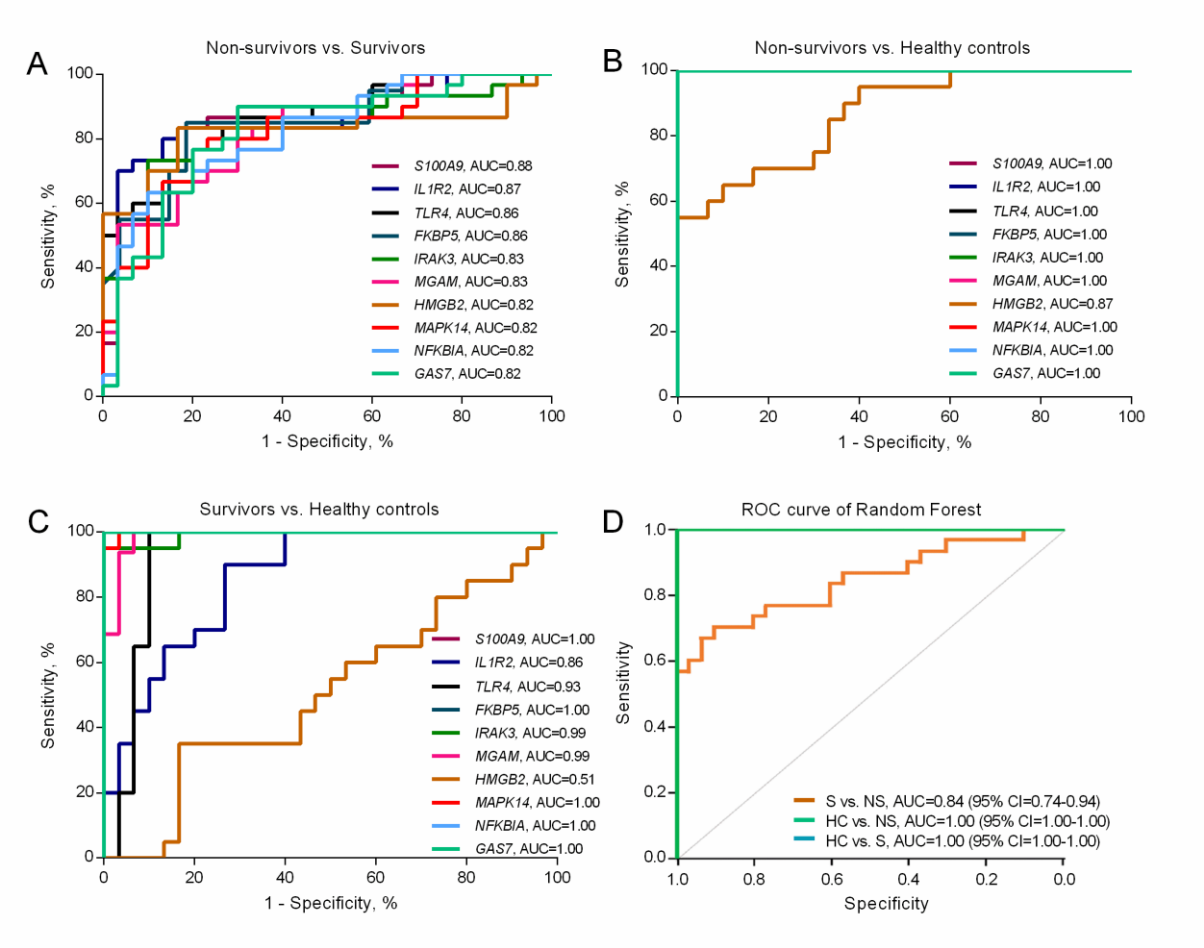


Figure 7. One month follow-up of *S100A9, IRAK3, IL1R2, GAP7,* and *NFKBIA* in surviving melioidosis patients over the course of illness. Whole blood samples from melioidosis survivors (n = 8) were collected at the various times from diagnosis (day 0, day 5, day 12, and day 28). The *P* values were calculated by Mann-Whitney test. Data of healthy individuals were plotted as the controls.

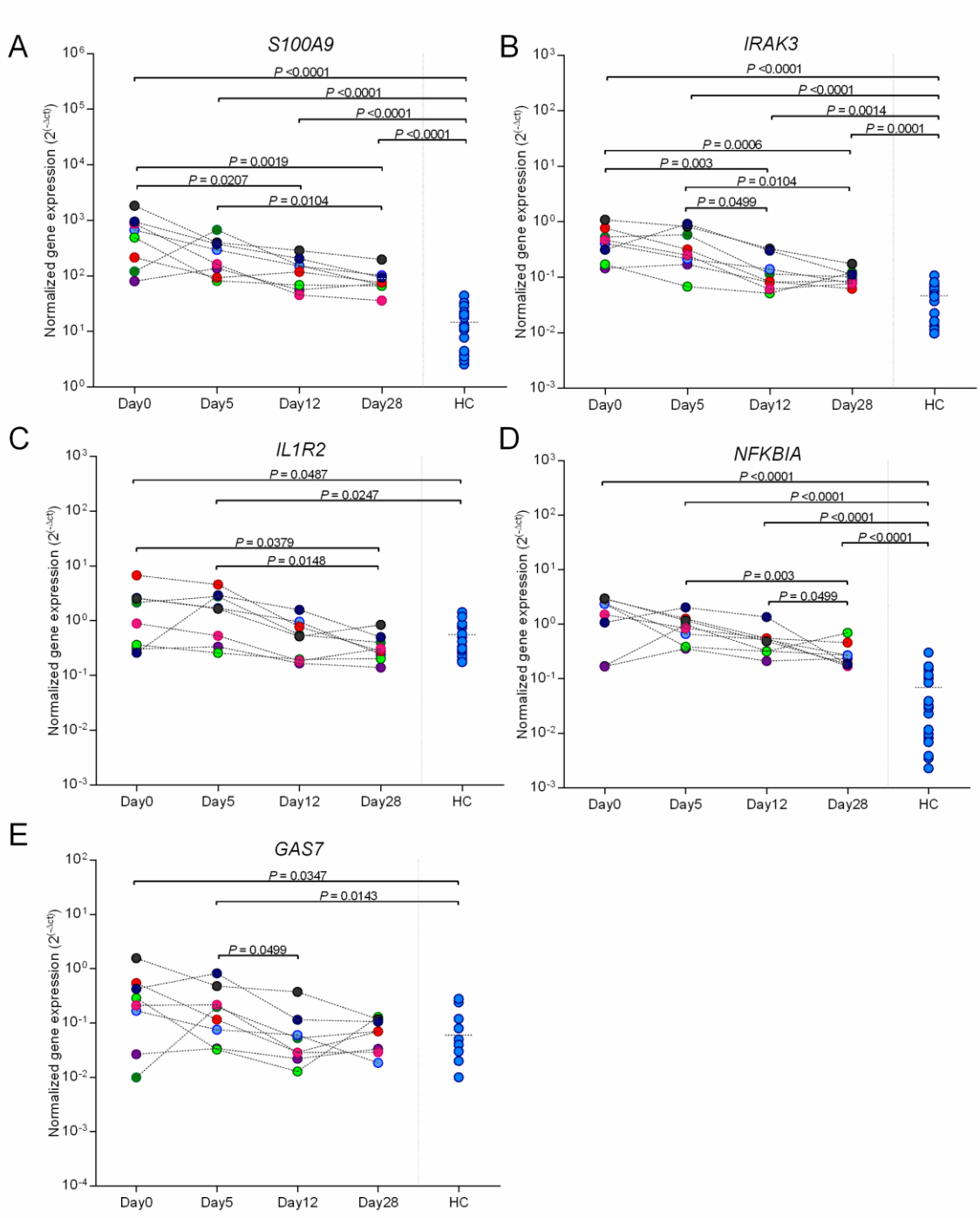
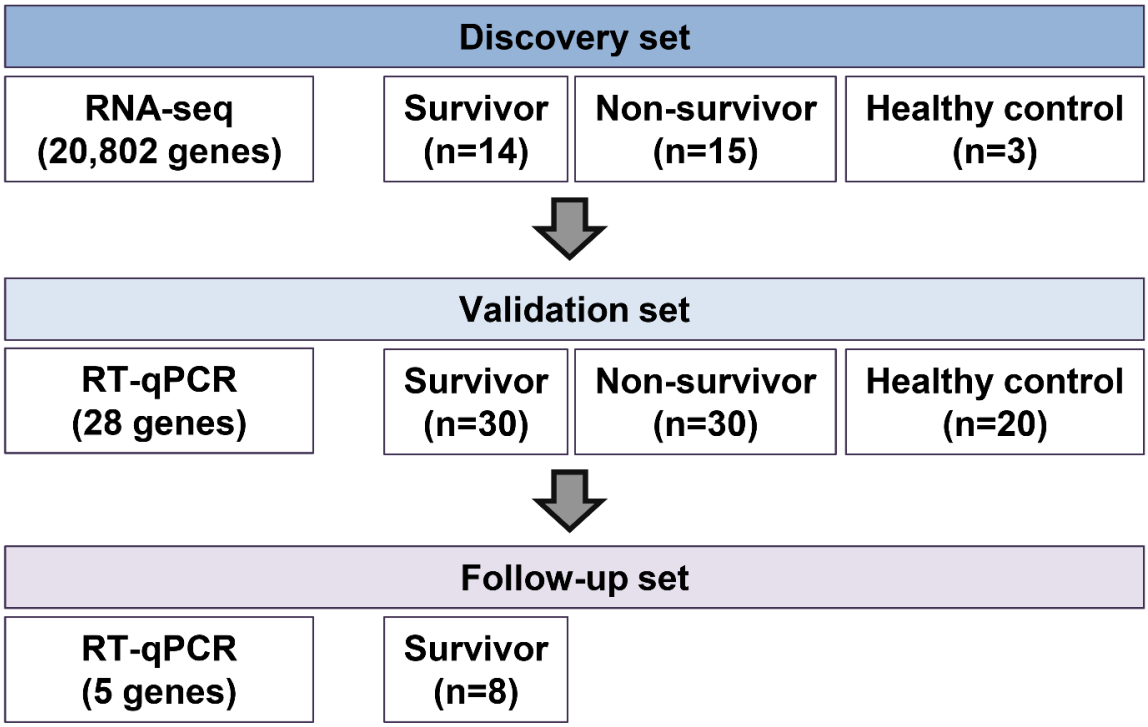


Table 1. Characteristics of melioidosis patients and healthy controls

Characteristics	Discovery cohort		Validation cohort		Follow-up cohort (n=8)	Healthy control (n=23)
	Non-survivors (n=15)	Survivors (n=14)	Non-survivors (n=30)	Survivors (n=30)		
Mean age in years (range)	57 (36-81)	51 (28-74)	62 (45-84)	60 (34-80)	50 (32-70)	43 (28-68)
Male (%)	11 (73%)	10 (71%)	26 (87%)	21 (70%)	8 (100%)	14 (61%)
Comorbidity						
Diabetes (%)	9 (60%)	7 (50%)	18 (60%)	17 (57%)	7 (88%)	-
Alcoholism (%)	3 (20%)	6 (43%)	7 (23%)	10 (33%)	3 (38%)	-
Kidney disease (%)	2 (13%)	1 (7%)	5 (17%)	3 (10%)	5 (63%)	-
Hypertension (%)	5 (33%)	2 (14%)	13 (43%)	7 (23%)	3 (38%)	-
Thalassemia (%)	-	2 (14%)	-	1 (3%)	-	-
Cancer (%)	2 (13%)	-	2 (7%)	1 (3%)	-	-
None (%)	3 (20%)	1 (7%)	4 (13%)	4 (13%)	-	23 (100%)
Clinical symptom						
Bacteremia (%)	14 (93%)	8 (57%)	28 (93%)	23 (77%)	7 (88%)	-
Fever						
<15 days (%)	14 (93%)	11 (79%)	28 (93%)	23 (77%)	7 (88%)	-
≥15 days (%)	1 (7%)	3 (21%)	2 (7%)	7 (23%)	1 (13%)	-

Supplementary Figure 1. Flow chart of the study.



Supplementary Table 1. Oligonucleotide primers used for quantitative RT-PCR (RT-qPCR).

Gene symbol	Gene description	Primer sequences (5'-3')	Amplicon size (bp)	TM (°C)	References
PPIA	Peptidylprolyl isomerase A	S_GCTGGACCCAACACAAATGG	86	59.68	[1]
		A_TTGCCAAACACCACATGCTT		59.17	
TBP	Tata-box binding protein	S_ATGGTGGGGAGCTGTGATGT	101	61.21	[1]
		A_AAACCAGGAAATAACTCTGGCTCA		60.20	
RPLP0	Human large ribosomal protein P0	S_GCTTCCTGGAGGGTGTCC	105	59.33	[1]
		A_GGACTCGTTTGTACCCGTTG		58.86	
TLR4	Toll like receptor 4	F_CAACCATTTGCCAGACACCA	143	58.96	This study
		R_ACGGGAAGCACAACCATCTA		59.02	
TLR2	Toll like receptor 2	F_TGCAAGCAGGATCCAAAGGA	111	59.59	This study
		R_CAAGACCCACACCATCCACA		59.89	
CD160	CD160 molecule	F_GCTTTGTAAGCCTTGTGCCA	119	59.33	This study
		R_CCTGTGCCCTGTTGCATTCT		60.90	
IL1R2	Interleukin 1 receptor type 2	F_TGTGCTGGCCCCACTTTC	101	60.20	[2]
		R_GCACAGTCAGACCATCTGCTTT		61.13	
S100A9	S100 calcium binding protein A9	F_TGGAGGACCTGGACACAAATG	109	59.93	[3]
		R_TCGTCACCCTCGTGCATCTT		61.53	
NFKBIA	NFKB inhibitor alpha	F_CTCCGAGACTTTCGAGGAAATAC	135	58.65	[4]
		R_GCCATTGAAGTTGGTAGCCTTCA		61.37	
HIF1A	Hypoxia inducible factor 1 subunit alpha	F_CATAAAGTCTGCAACATGGAAGGT	148	59.54	[5]
		R_ATTTGATGGGTGAGGAATGGGTT		60.25	
PLK3	Polo like kinase 3	F_TCACTGGGCTGTGTCATGTA	96	58.65	[6]
		R_GTGAACCTGCTTGATGCAG		56.92	
GADD45A	Growth arrest and DNA damage inducible alpha	F_AGAAGACCGAAAGCGACCC	131	59.71	This study
		R_GTTGATGTCGTTCTCGCAGC		59.91	
CD22	CD22 molecule	F_GCCAGAGCTTCTTTGTGAGG	182	58.84	[7]
		R_GGGAGGTCTCTGCATCTCTG		59.25	
HLA-DOA	Major histocompatibility complex, class II, DO alpha	F_TTTGCCCGCTTTGACCCGCA	118	65.99	This study
		R_TCACCCGTGGAGGCACGTTG		65.10	
LCK	LCK proto-oncogene, Src family tyrosine kinase	F_TGCCATTATCCCATAGTCCCA	95	58.29	This study
		R_GAGCCTTCGTAGGTAACCAGT		59.18	
LAT	Linker for activation of T cells	F_CTACCCACCTGTCACCTCCT	129	60.25	This study
		R_CTGTTGGCACCATCAGAATC		56.78	
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	F_CCTGGTGATGCTGGAAATG	105	56.26	This study
		R_GACTGTGCCTTCCACTCCA		59.25	
CD72	CD 72 molecule	F_CAGCTCCGCCTCAAGATAAC	177	58.42	This study
		R_TTGCAAGGTCTCCTTCGTCT		58.95	
IRAK3	Interleukin 1 receptor associated kinase 3	F_CAGCCAGTCTGAGGTTATGTTT	110	58.32	[8]
		R_TTGGGAACCAACTTTCTTCACA		58.30	
ITGAM	Integrin subunit alpha M	F_ATGCAGAAACAGGGATGGGA	71	59.00	This study
		R_GATAGCAGCGTGGAACCAAG		58.99	
KL	Klotho	F_ACTGGATCACCATCGACAACCC	192	62.32	This study
		R_CAATGGACACCTGACCTCCCT		61.46	
FKBP5	FKBP prolyl isomerase 5	F_GAGTTACATCCCCCATGCCAA	149	60.06	This study
		R_GGGGATTGTCGCTTCGTAGT		59.82	
IL18RAP	Interleukin 18 receptor accessory protein	F_CGTTCAGATACAAAAGCTGGCAGT	125	61.86	This study
		R_TCCCTTTCAGTTGGTCAAGGCT		61.83	
PER1	Period circadian regulator 1	F_GAGGACACTCCTGCGACCAG	192	62.22	This study
		R_TCCCCCATCAGCCCCTTCTA		61.91	
MGAM	Maltase-glucoamylase	F_CACCCTCCCTACATGCCACA	95	61.56	This study
		R_GAGCCGTCTGGGAGGATCTG		61.74	
HMGB2	High mobility group box 2	F_CCCTGGCCTATCCATTGGGG	176	62.09	This study
		R_CAGGGCCCTTCTTTCCTGCT		62.15	
GAS7	Growth arrest specific 7	F_TGCGACTACTTCTGGGCTGA	102	60.90	This study
		R_CTGCATTTGTTTGCCCTTCA		57.47	
MAPK14	Mitogen-activated protein kinase 14	F_GGGGCTGAGCTTTTGAAGAAA	180	59.04	This study
		R_GGCTTGGGCCGCTGTAATTC		62.00	
GPR27	G protein-coupled receptor 27	F_GCAAGATGTTCTACGCCGTCA	194	61.00	This study
		R_GTCCCTCAGCTCCCTGTTGAA		61.72	
LPL	Lipoprotein lipase	F_ACGGGCTCAGGAGCATTACC	142	61.97	This study
		R_GGCTCCAAGGCTGTATCCCA		61.64	
ACVR1B	Activin A receptor type 1B	F_CAGCAGAACCTTGGCGGTTTA	85	61.15	[9]
		R_GTTGGCAGATCCCAGAGGCTAC		62.70	

Supplementary Table 2. Differentially expressed genes in whole blood of melioidosis patients who were survived and died. The data show 65 up-regulated genes and 218 down-regulated genes in non-survivors.

Gene	Description	Fold change	*P* value	Regulation
IL1R2	Interleukin 1 receptor type 2	15.72	5.5E-09	up
GRB10	Growth factor receptor bound protein 10	5.88	9.0E-07	up
MYO10	Myosin X	5.48	7.6E-06	up
TDRD9	Tudor domain containing 9	5.25	2.2E-05	up
MERTK	MER proto-oncogene, tyrosine kinase	5.16	3.7E-06	up
KL	Klotho	4.27	3.8E-06	up
ST6GALNAC3	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	4.02	2.4E-06	up
FKBP5	FKBP prolyl isomerase 5	4.02	6.3E-07	up
MYO1B	Myosin IB	3.67	1.3E-04	up
IRAK3	Interleukin 1 receptor associated kinase 3	3.53	2.6E-06	up
IL18RAP	Interleukin 18 receptor accessory protein	3.43	1.2E-04	up
SH3PXD2B	SH3 and PX domains 2B	3.10	3.5E-04	up
CLEC4D	C-type lectin domain family 4 member D	3.10	6.3E-04	up
PER1	Period circadian regulator 1	3.06	1.4E-06	up
ASPH	Aspartate beta-hydroxylase	3.04	4.6E-06	up
GADD45A	Growth arrest and DNA damage inducible alpha	3.01	1.0E-05	up
BASP1	Brain abundant membrane attached signal protein 1	2.96	9.8E-05	up
PGS1	Phosphatidylglycerophosphate synthase 1	2.93	3.7E-04	up
SLED1	Proteoglycan 3, pro eosinophil major basic protein 2 pseudogene	2.87	4.6E-04	up
ITPKC	Inositol-trisphosphate 3-kinase C	2.86	2.6E-06	up
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	2.86	6.3E-05	up
SLC26A6	Solute carrier family 26 member 6	2.68	7.4E-05	up
SCN5A	Sodium voltage-gated channel alpha subunit 5	2.68	6.0E-04	up
PECR	Peroxisomal trans-2-enoyl-CoA reductase	2.66	1.8E-05	up
MGAM	Maltase-glucoamylase	2.65	9.5E-04	up
SLC2A3	Solute carrier family 2 member 3	2.64	1.3E-04	up
HMGB2	High mobility group box 2	2.64	1.6E-06	up
SYCP2	Synaptonemal complex protein 2	2.62	4.6E-04	up
SULT1B1	Sulfotransferase family 1B member 1	2.59	2.6E-04	up
S100A9	S100 calcium binding protein A9	2.59	1.9E-04	up
ADAM9	ADAM metallopeptidase domain 9	2.57	1.3E-05	up
GAS7	Growth arrest specific 7	2.55	2.1E-04	up
NFKBIA	NFKB inhibitor alpha	2.52	1.4E-04	up
ARMC12	Armadillo repeat containing 12	2.48	9.1E-05	up
TLR2	Toll like receptor 2	2.37	8.9E-05	up
CCNA1	Cyclin A1	2.37	3.4E-03	up
RALGAPA2	Ral GTPase activating protein catalytic alpha subunit 2	2.35	2.7E-04	up
RNF144B	Ring finger protein 144B	2.35	1.2E-04	up
KRT8	Keratin 8	2.33	5.3E-05	up
TLR4	Toll like receptor 4	2.32	3.2E-04	up
FAR2	Fatty acyl-CoA reductase 2	2.31	1.3E-05	up
GNG10	G protein subunit gamma 10	2.31	5.6E-04	up
KLF7	Kruppel like factor 7	2.30	9.3E-05	up
PLK3	Polo like kinase 3	2.29	7.8E-04	up
LHX4	LIM homeobox 4	2.29	1.2E-03	up
ZNF438	Zinc finger protein 438	2.27	1.2E-03	up

ACVR1B	Activin A receptor type 1B	2.25	6.9E-05	up
CEACAM4	Carcinoembryonic antigen related cell adhesion molecule 4	2.23	8.1E-05	up
DUSP1	Dual specificity phosphatase 1	2.22	9.8E-05	up
MAPK14	Mitogen-activated protein kinase 14	2.21	4.7E-04	up
TPK1	Thiamin pyrophosphokinase 1	2.20	8.8E-05	up
GPR27	G protein-coupled receptor 27	2.15	5.7E-04	up
DYSF	Dysferlin	2.12	2.8E-03	up
CCDC71L	Coiled-coil domain containing 71 like	2.11	6.1E-04	up
ALOX5	Arachidonate 5-lipoxygenase	2.10	1.2E-03	up
WDFY3	WD repeat and FYVE domain containing 3	2.08	1.2E-03	up
TLR8	Toll like receptor 8	2.08	8.1E-04	up
HIF1A	Hypoxia inducible factor 1 subunit alpha	2.07	7.4E-04	up
TCTEX1D1	Tctex1 domain containing 1	2.06	2.1E-03	up
PLIN5	Perilipin 5	2.05	1.7E-03	up
PPP1R3D	Protein phosphatase 1 regulatory subunit 3D	2.05	2.6E-04	up
TMED8	Transmembrane p24 trafficking protein family member 8	2.04	9.9E-06	up
LPL	Lipoprotein lipase	2.04	2.4E-03	up
PYGL	Glycogen phosphorylase L	2.01	2.9E-03	up
ITGAM	Integrin subunit alpha M	2.00	1.7E-03	up
CD160	CD160 molecule	9.42	2.5E-09	down
FCRL6	Fc receptor like 6	8.35	4.9E-06	down
ADGRG1	Adhesion G protein-coupled receptor G1	8.27	4.3E-06	down
GZMM	Granzyme M	5.90	4.6E-06	down
CLIC3	Chloride intracellular channel 3	5.65	7.1E-06	down
XCL2	X-C motif chemokine ligand 2	5.39	6.3E-07	down
TCL1A	T cell leukemia/lymphoma 1A	5.19	1.3E-05	down
GPR18	G protein-coupled receptor 18	4.96	2.6E-07	down
GPR174	G protein-coupled receptor 174	4.89	3.9E-06	down
CXCR5	C-X-C motif chemokine receptor 5	4.80	1.3E-05	down
FCER2	Fc fragment of IgE receptor II	4.36	4.0E-05	down
LGALS2	Galectin 2	4.34	5.3E-05	down
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	4.32	5.3E-05	down
CD22	CD22 molecule	4.20	1.0E-04	down
PTGDR2	Prostaglandin D2 receptor 2	4.10	8.4E-05	down
HLA-DOB	Major histocompatibility complex, class II, DO beta	3.93	3.9E-05	down
HLA-DOA	Major histocompatibility complex, class II, DO alpha	3.91	7.7E-06	down
PYHIN1	Pyrin and HIN domain family member 1	3.85	6.2E-05	down
RASGRP1	RAS guanyl releasing protein 1	3.85	6.8E-06	down
CD72	CD72 molecule	3.84	3.0E-06	down
NCR3	Natural cytotoxicity triggering receptor 3	3.83	1.2E-05	down
MYBL1	MYB proto-oncogene like 1	3.79	3.1E-05	down
MS4A1	Membrane spanning 4-domains A1	3.72	2.3E-04	down
FLT3LG	Fms related tyrosine kinase 3 ligand	3.70	1.9E-06	down
VPREB3	V-set pre-B cell surrogate light chain 3	3.56	2.5E-05	down
LPAR5	Lysophosphatidic acid receptor 5	3.43	1.3E-05	down
PIK3C2B	Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 beta	3.37	3.4E-05	down
SNX29P2	Sorting nexin 29 pseudogene 2	3.37	1.4E-05	down
PAX5	Paired box 5	3.33	1.6E-04	down
LBH	LBH regulator of WNT signaling pathway	3.31	1.7E-05	down
CYSLTR2	Cysteinyl leukotriene receptor 2	3.30	4.7E-07	down
FCRLA	Fc receptor like A	3.25	1.4E-03	down
ZNF683	Zinc finger protein 683	3.24	2.6E-05	down

CRIP2	Cysteine rich protein 2	3.14	6.6E-05	down
ERBB2	Erb-b2 receptor tyrosine kinase 2	3.13	1.5E-05	down
LDOC1	LDOC1 regulator of NFKB signaling	3.12	5.7E-05	down
TRABD2A	TraB domain containing 2A	3.11	9.6E-06	down
HABP4	Hyaluronan binding protein 4	3.05	2.8E-06	down
NDRG2	NDRG family member 2	3.02	1.7E-06	down
HLA-DRA	Major histocompatibility complex, class II, DR alpha	3.01	7.0E-04	down
BTLA	B and T lymphocyte associated	2.99	1.5E-04	down
PCBP4	Poly(rC) binding protein 4	2.97	1.0E-04	down
CD101	CD101 molecule	2.97	4.3E-04	down
CROCC	Ciliary rootlet coiled-coil, rootletin	2.94	6.3E-06	down
ZNF483	Zinc finger protein 483	2.91	6.8E-07	down
AK5	Adenylate kinase 5	2.86	5.4E-06	down
CA5B	Carbonic anhydrase 5B	2.85	4.4E-05	down
CD79A	CD79a molecule	2.83	2.4E-03	down
CD200	CD200 molecule	2.81	2.9E-05	down
PVRIG	PVR related immunoglobulin domain containing	2.80	4.8E-06	down
CYP4V2	Cytochrome P450 family 4 subfamily V member 2	2.79	1.9E-04	down
CHI3L2	Chitinase 3 like 2	2.77	8.3E-04	down
BLK	BLK proto-oncogene, Src family tyrosine kinase	2.77	1.3E-03	down
MLLT3	MLLT3 super elongation complex subunit	2.75	7.4E-06	down
APBA2	Amyloid beta precursor protein binding family A member 2	2.74	2.8E-05	down
FBXL16	F-box and leucine rich repeat protein 16	2.72	4.4E-05	down
TMEM229B	Transmembrane protein 229B	2.70	4.5E-04	down
LAT	Linker for activation of T cells	2.69	6.4E-06	down
NMUR1	Neuromedin U receptor 1	2.68	4.7E-05	down
CASS4	Cas scaffold protein family member 4	2.68	2.5E-03	down
SFMBT2	Scm like with four mbt domains 2	2.67	2.1E-04	down
AGMAT	Agmatinase	2.67	4.8E-05	down
ZXDB	Zinc finger X-linked duplicated B	2.66	4.1E-06	down
GPR68	G protein-coupled receptor 68	2.66	4.4E-06	down
HIVEP3	HIVEP zinc finger 3	2.65	3.5E-04	down
RHOF	Ras homolog family member F, filopodia associated	2.65	9.8E-06	down
ATP1A3	ATPase Na+/K+ transporting subunit alpha 3	2.64	9.0E-06	down
ADRB2	Adrenoceptor beta 2	2.64	6.2E-05	down
DOCK10	Dedicator of cytokinesis 10	2.63	8.0E-05	down
KLHL3	Kelch like family member 3	2.63	9.1E-09	down
CCN3	Cellular communication network factor 3	2.63	3.6E-04	down
APOL3	Apolipoprotein L3	2.63	1.6E-03	down
PLEKHO1	Pleckstrin homology domain containing O1	2.63	1.4E-05	down
MAN1C1	Mannosidase alpha class 1C member 1	2.59	7.2E-08	down
RHOBTB2	Rho related BTB domain containing 2	2.59	1.3E-04	down
LTA	Lymphotoxin alpha	2.58	1.5E-04	down
USP28	Ubiquitin specific peptidase 28	2.58	7.7E-05	down
CCDC88C	Coiled-coil domain containing 88C	2.58	7.3E-05	down
LDLRAD4	Low density lipoprotein receptor class A domain containing 4	2.56	2.0E-05	down
ZDHHC14	Zinc finger DHHC-type containing 14	2.56	2.9E-05	down
UTP20	UTP20 small subunit processome component	2.55	4.2E-04	down
NOL6	Nucleolar protein 6	2.55	4.4E-04	down
DNPEP	Aspartyl aminopeptidase	2.53	1.6E-04	down
ZXDA	Zinc finger X-linked duplicated A	2.53	3.2E-07	down
GSE1	Gse1 coiled-coil protein	2.51	5.7E-05	down
MRPL4	Mitochondrial ribosomal protein L4	2.51	9.3E-04	down

EFNB1	Ephrin B1	2.50	1.4E-04	down
EXOG	Exo/endonuclease G	2.50	1.2E-04	down
CEP290	Centrosomal protein 290	2.48	1.1E-05	down
ZFPM1	Zinc finger protein, FOG family member 1	2.48	2.9E-04	down
RPS6KA5	Ribosomal protein S6 kinase A5	2.45	6.0E-05	down
ARRB1	Arrestin beta 1	2.44	1.4E-05	down
OBSCN	Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF	2.43	1.4E-04	down
PPP1R13B	Protein phosphatase 1 regulatory subunit 13B	2.43	5.2E-05	down
CTSO	Cathepsin O	2.42	4.3E-05	down
TMEM263	Transmembrane protein 263	2.42	4.2E-04	down
S1PR5	Sphingosine-1-phosphate receptor 5	2.42	1.5E-03	down
LINC00926	Long intergenic non-protein coding RNA 926	2.42	1.1E-03	down
NIPA1	NIPA magnesium transporter 1	2.40	2.6E-06	down
GPR162	G protein-coupled receptor 162	2.39	5.5E-05	down
NOP14	NOP14 nucleolar protein	2.39	6.1E-05	down
VCL	Vinculin	2.39	2.0E-03	down
SMYD2	SET and MYND domain containing 2	2.38	6.9E-06	down
RRP7A	Ribosomal RNA processing 7 homolog A	2.38	7.3E-04	down
PRKX	Protein kinase X-linked	2.37	3.0E-04	down
CHIC1	Cysteine rich hydrophobic domain 1	2.37	5.4E-05	down
SH2D3A	SH2 domain containing 3A	2.37	4.9E-04	down
SNURF	SNRPN upstream reading frame	2.36	2.0E-05	down
LTB	Lymphotoxin beta	2.35	2.2E-05	down
ZNF548	Zinc finger protein 548	2.33	1.4E-05	down
POGLUT3	Protein O-glucosyltransferase 3	2.33	8.2E-05	down
ZNF853	Zinc finger protein 853	2.32	7.2E-05	down
CACNA2D2	Calcium voltage-gated channel auxiliary subunit alpha2delta 2	2.31	4.2E-04	down
SNPH	Syntaphilin	2.31	9.4E-05	down
PKIA	cAMP-dependent protein kinase inhibitor alpha	2.31	1.4E-04	down
TPPP3	Tubulin polymerization promoting protein family member 3	2.30	2.3E-03	down
NOM1	Nucleolar protein with MIF4G domain 1	2.30	6.3E-04	down
SLC9A7	Solute carrier family 9 member A7	2.29	1.1E-04	down
PATZ1	POZ/BTB and AT hook containing zinc finger 1	2.29	2.5E-05	down
REXO4	REX4 homolog, 3'-5' exonuclease	2.28	6.7E-05	down
PRSS23	Serine protease 23	2.28	2.1E-04	down
SLC4A4	Solute carrier family 4 member 4	2.28	6.2E-05	down
CEP126	Centrosomal protein 126	2.27	2.3E-06	down
RPUSD2	RNA pseudouridine synthase domain containing 2	2.27	6.3E-04	down
PIK3R6	Phosphoinositide-3-kinase regulatory subunit 6	2.27	8.8E-07	down
MSANTD2	Myb/SANT DNA binding domain containing 2	2.27	5.1E-05	down
TPCN1	Two pore segment channel 1	2.27	5.6E-05	down
ZNF571	Zinc finger protein 571	2.27	1.9E-06	down
CCR4	C-C motif chemokine receptor 4	2.26	4.8E-04	down
PABPC3	Poly(A) binding protein cytoplasmic 3	2.25	1.0E-04	down
PEA15	Proliferation and apoptosis adaptor protein 15	2.25	5.5E-04	down
ICOSLG	Inducible T cell costimulator ligand	2.24	1.0E-03	down
LOC389906	Zinc finger protein 839 pseudogene	2.24	1.2E-04	down
CFAP36	Cilia and flagella associated protein 36	2.24	1.3E-05	down
EARS2	Glutamyl-tRNA synthetase 2, mitochondrial	2.23	3.0E-04	down
EPHA4	EPH receptor A4	2.22	3.6E-04	down
IGFBP3	Insulin like growth factor binding protein 3	2.22	3.4E-04	down
IL11RA	Interleukin 11 receptor subunit alpha	2.21	2.7E-05	down
LMTK3	Lemur tyrosine kinase 3	2.20	3.0E-04	down
ICAM2	Intercellular adhesion molecule 2	2.20	1.6E-04	down

LINC00299	Long intergenic non-protein coding RNA 299	2.20	2.1E-03	down
NARS2	Asparaginyl-tRNA synthetase 2, mitochondrial	2.19	1.3E-03	down
ZC3H8	Zinc finger CCCH-type containing 8	2.17	9.6E-06	down
ARHGEF19	Rho guanine nucleotide exchange factor 19	2.17	4.1E-05	down
KIF5C	Kinesin family member 5C	2.17	5.1E-04	down
GPA33	Glycoprotein A33	2.17	2.8E-04	down
LOC100505549	Uncharacterized LOC100505549	2.17	3.9E-04	down
CCDC102A	Coiled-coil domain containing 102A	2.17	6.6E-05	down
FAM227B	Family with sequence similarity 227 member B	2.16	1.4E-04	down
SETD6	SET domain containing 6, protein lysine methyltransferase	2.15	5.5E-05	down
ZNF573	Zinc finger protein 573	2.15	2.4E-05	down
GALNT12	Polypeptide N-acetylgalactosaminyltransferase 12	2.15	1.1E-05	down
RANGAP1	Ran GTPase activating protein 1	2.15	7.3E-04	down
PTER	Phosphotriesterase related	2.14	3.8E-04	down
L3MBTL2	L3MBTL histone methyl-lysine binding protein 2	2.14	9.6E-04	down
KIAA1328	KIAA1328	2.14	1.8E-04	down
STK39	Serine/threonine kinase 39	2.13	2.9E-05	down
GFI1B	Growth factor independent 1B transcriptional repressor	2.13	8.8E-04	down
FAM120C	Family with sequence similarity 120C	2.13	2.5E-05	down
LAS1L	LAS1 like, ribosome biogenesis factor	2.13	2.0E-03	down
GSPT2	G1 to S phase transition 2	2.13	2.8E-05	down
ZNF485	Zinc finger protein 485	2.13	3.2E-06	down
ITGA6	Integrin subunit alpha 6	2.12	3.6E-05	down
FAM50B	Family with sequence similarity 50 member B	2.12	2.5E-04	down
SMPD3	Sphingomyelin phosphodiesterase 3	2.12	1.7E-04	down
PDZD4	PDZ domain containing 4	2.12	4.5E-04	down
TCEAL3	Transcription elongation factor A like 3	2.12	4.2E-04	down
CAMKMT	Calmodulin-lysine N-methyltransferase	2.12	1.9E-05	down
TRMT10B	tRNA methyltransferase 10B	2.12	5.3E-05	down
MDC1	Mediator of DNA damage checkpoint 1	2.12	1.4E-03	down
ADGRL1	Adhesion G protein-coupled receptor L1	2.12	8.0E-05	down
SGPP1	Sphingosine-1-phosphate phosphatase 1	2.11	2.0E-04	down
MAK16	MAK16 homolog	2.11	2.3E-04	down
RPS27	Ribosomal protein S27	2.11	5.7E-05	down
PDLIM2	PDZ and LIM domain 2	2.11	3.7E-06	down
KMT2A	Lysine methyltransferase 2A	2.11	4.4E-05	down
UBE2Q2	Ubiquitin conjugating enzyme E2 Q2	2.10	6.1E-04	down
POU6F1	POU class 6 homeobox 1	2.10	2.4E-04	down
TRANK1	Tetratricopeptide repeat and ankyrin repeat containing 1	2.10	2.4E-04	down
GIMAP6	GTPase, IMAP family member 6	2.10	8.3E-04	down
BEX2	Brain expressed X-linked 2	2.10	1.2E-04	down
DDX24	DEAD-box helicase 24	2.09	3.5E-04	down
KNOP1	Lysine rich nucleolar protein 1	2.09	1.4E-04	down
UNK	Unk zinc finger	2.09	1.9E-05	down
PARP16	Poly(ADP-ribose) polymerase family member 16	2.09	2.0E-04	down
FAM53B	Family with sequence similarity 53 member B	2.08	9.0E-04	down
CMC1	C-X9-C motif containing 1	2.08	7.5E-05	down
TTC12	Tetratricopeptide repeat domain 12	2.08	4.6E-05	down
ZNF527	Zinc finger protein 527	2.07	3.2E-05	down
NLE1	Notchless homolog 1	2.07	9.9E-04	down
DENND2D	DENN domain containing 2D	2.07	1.3E-05	down

CCDC92	Coiled-coil domain containing 92	2.07	1.7E-04	down
PAIP2B	Poly(A) binding protein interacting protein 2B	2.07	2.4E-05	down
PAXX	PAXX non-homologous end joining factor	2.07	2.0E-04	down
NLRP1	NLR family pyrin domain containing 1	2.06	1.6E-04	down
GNAO1	G protein subunit alpha o1	2.05	2.7E-03	down
ZNF354C	Zinc finger protein 354C	2.05	3.3E-04	down
DYRK2	Dual specificity tyrosine phosphorylation regulated kinase 2	2.05	3.4E-04	down
SLC25A26	Solute carrier family 25 member 26	2.05	7.2E-04	down
PDGFD	Platelet derived growth factor D	2.05	4.0E-04	down
PIGM	Phosphatidylinositol glycan anchor biosynthesis class M	2.04	3.3E-03	down
USP46	Ubiquitin specific peptidase 46	2.04	4.3E-04	down
TRIM44	Tripartite motif containing 44	2.03	4.0E-04	down
HEATR1	HEAT repeat containing 1	2.03	7.8E-04	down
IPO4	Importin 4	2.03	2.0E-04	down
SOGA1	Suppressor of glucose, autophagy associated 1	2.02	9.1E-05	down
MFSD6	Major facilitator superfamily domain containing 6	2.02	3.7E-04	down
CCDC28B	Coiled-coil domain containing 28B	2.02	1.4E-04	down
KLHL42	Kelch like family member 42	2.02	1.4E-04	down
THTPA	Thiamine triphosphatase	2.02	2.3E-04	down
AKT3	AKT serine/threonine kinase 3	2.02	8.9E-06	down
TMEM99	Transmembrane protein 99 (putative)	2.01	1.4E-04	down
HHLA3	HERV-H LTR-associating 3	2.01	2.7E-04	down
RPL32	Ribosomal protein L32	2.01	3.5E-04	down
SARS2	Seryl-tRNA synthetase 2, mitochondrial	2.00	1.7E-03	down
LCK	LCK proto-oncogene, Src family tyrosine kinase	2.00	1.6E-07	down
CUL1	Cullin 1	2.00	1.9E-03	down
TMEM42	Transmembrane protein 42	2.00	9.5E-05	down

Supplementary Table 3. Enriched functional analysis of 65 up-regulated and 218 down-regulated genes in non-survivors. The biological functions were analysed using MetaScape.

Up-regulation			
Term	**Accession**	**No. of genes**	**Gene**
GO:0002274	Myeloid leukocyte activation	14	*ALOX5, MAPK14, ITGAM, PYGL, S100A9, SLC2A3, TLR2, TLR4, DYSF, ADAM9, IL18RAP, MGAM, TLR8, CLEC4D*
R-HSA-168898	Toll-like Receptor Cascades	8	*MAPK14, ITGAM, NFKBIA, S100A9, TLR2, TLR4, IRAK3, TLR8*
GO:0072593	Reactive oxygen species metabolic process	8	*MAPK14, GADD45A, HIF1A, ITGAM, TLR2, TLR4, SH3PXD2B, PLIN5*

GO:0045648	Positive regulation of erythrocyte differentiation	4	*ACVR1B, MAPK14, HIF1A, HMGB2*
GO:0038066	p38MAPK cascade	4	*MAPK14, GADD45A, DUSP1, PER1*
GO:0045600	Positive regulation of fat cell differentiation	4	*MAPK14, LPL, CCDC71L, SH3PXD2B*
GO:0019915	Lipid storage	4	*LPL, NFKBIA, DYSF, PLIN5*
R-HSA-449147	Signaling by Interleukins	8	*ALOX5, MAPK14, HIF1A, ITGAM, NFKBIA, IL1R2, IL18RAP, IRAK3*
GO:0009251	Glucan catabolic process	3	*PPP1R3D, PYGL, MGAM*
GO:0005975	Carbohydrate metabolic process	9	*MAPK14, HIF1A, PFKFB3, PPP1R3D, PYGL, SLC2A3, DYSF, MGAM, KL*
GO:0006909	Phagocytosis	7	*CEACAM4, ITGAM, MYO10, TLR2, TLR4, DYSF, MERTK*
M12705	SIG CD40PATHWAYMAP	3	*MAPK14, DUSP1, NFKBIA*
GO:0009746	Response to hexose	5	*GPR27, HIF1A, LPL, KLF7, SLC26A6*
GO:0007140	Male meiotic nuclear division	3	*CCNA1, SYCP2, TDRD9*
R-HSA-6791312	TP53 regulating transcription of cell cycle genes	3	*PLK3, GADD45A, CCNA1*
GO:0031331	Positive regulation of cellular catabolic process	6	*PLK3, HIF1A, PFKFB3, ADAM9, RNF144B, PLIN5*
GO:0022411	Cellular component disassembly	7	*ASPH,PLK3, HIF1A, HMGB2, ITGAM, IRAK3, SH3PXD2B*
GO:0006979	Response to oxidative stress	6	*PLK3, DUSP1, HIF1A, TLR4, ADAM9, IL18RAP*
GO:0043470	Regulation of carbohydrate catabolic process	3	*HIF1A, PFKFB3, PPP1R3D*

GO:0032787	Monocarboxylic acid metabolic process	7	*ALOX5, MAPK14, HIF1A, LPL, PFKFB3, PECR, PLIN5*
Down regulation			
Term	**Accession**	**No. of genes**	**Gene**
hsa04640	Hematopoietic cell lineage	10	*MS4A1, CD22, FCER2, FLT3LG, HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, IL11RA, ITGA6*
R-HSA-1280218	Adaptive Immune System	24	*BLK, CD22, CD79A, CTSO, HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, ICAM2, LCK,CD200,CUL1,CD101,AKT3,RASGRP1,CD160,ICOSLG,KLHL3,LAT,KLHL42,UBE2Q2,FBXL16,BTLA,NCR3*
GO:0046649	Lymphocyte activation	23	*CXCR5, MS4A1, CD22, CD79A, EFNB1, ERBB2, FLT3LG, GPR18, HLA-DOA, HLA-DPB1, LCK, RASGRP1, CD160, ICOSLG, PATZ1, LAT, DOCK10, ZC3H8, PIK3R6, BTLA, ZFPM1, ZNF683, NCR3*
GO:0030098	Lymphocyte differentiation	14	*MS4A1, CD79A, ERBB2, FLT3LG, GPR18, HLA-DOA, LCK, RASGRP1, PATZ1, DOCK10, ZC3H8, PIK3R6, ZFPM1, ZNF683*
GO:0042274	Ribosomal small subunit biogenesis	6	*RPS27, NOP14, UTP20, RRP7A, HEATR1, NOM1*
GO:0048872	Homeostasis of number of cells	10	*CCR4, FLT3LG, CCN3, AKT3, LAT, NLE1, DOCK10, ZC3H8, GPR174, ZFPM1*
GO:0035025	Positive regulation of Rho protein signal transduction	4	*ARRB1, GPR18, ADGRG1, GPR174*
R-HSA-373076	Class A/1 (Rhodopsin-like receptors)	11	*ADRB2, CXCR5, CCR4, GPR18, XCL2, GPR68, NMUR1, PTGDR2, S1PR5, CYSLTR2, LPAR5*
M177	PID EPHA FWDPATHWAY	4	*BLK, EPHA4, LCK, PIK3R6*

GO:0071902	Positive regulation of protein serine/threonine kinase activity	11	*ADRB2, ARRB1, EPHA4, ERBB2, TCL1A, PEA15, RASGRP1, STK39, PARP16, PDGFD, PIK3R6*
GO:0032729	Positive regulation of interferon-gamma production	5	*HLA-DPB1, LTA, RASGRP1, CD160, ZFPM1*
M34	PID TCR PATHWAY	5	*HLA-DRA, LCK, RASGRP1, LAT, STK39*
GO:0048535	Lymph node development	3	*CXCR5, LTA, LTB*
GO:0032793	Positive regulation of CREB transcription factor activity	3	*CD200, RPS6KA5, LPAR5*
R-HSA-379726	Mitochondrial tRNA aminoacylation	3	*SARS2, NARS2, EARS2*
M155	PID S1P META PATHWAY	3	*GNAO1, S1PR5, SGPP1*
GO:0035162	Embryonic hemopoiesis	3	*FLT3LG, KMT2A, ZFPM1*
GO:0050853	B cell receptor signaling pathway	6	*BLK, MS4A1, CD22, CD79A, LCK, PAX5*
hsa04261	Adrenergic signaling in cardiomyocytes	6	*ADRB2, ATP1A3, RPS6KA5, CACNA2D2, AKT3, PIK3R6*
GO:0008045	Motor neuron axon guidance	3	*EPHA4, ERBB2, KIF5C*

Supplementary Table 4. Summary of KEGG pathways of up-regulated and down-regulated genes in whole blood of non-survivors compared with survivors.

Regulation	Term	KEGG pathway	Log10 (P)	Log10 (Q)	Number of gene	Genes symbols
Up	hsa04620	Toll-like receptor	-5.08	-2.55	5/104	*MAPK14, NFKBIA, TLR2, TLR4, TLR8*

		signaling pathway				
	hsa04659	Th17 cell differentiation	-2.54	-0.62	3/107	*MAPK14, HIF1A, NFKBIA*
	hsa04010	MAPK signaling pathway	-2.33	-0.46	4/255	*MAPK14, GADD45A, DUSP1, IL1R2*
	hsa04657	IL-17 signaling pathway	-2.71	-0.74	3/93	*MAPK14, NFKBIA, S100A9*
	hsa04068	FoxO signaling pathway	-2.28	-0.42	3/132	*PLK3, MAPK14, GADD45A*
	hsa04066	HIF-1 signaling pathway	-2.61	-0.67	3/101	*HIF1A, PFKFB3, TLR4*
Down	hsa04640	Hematopoietic cell lineage	-7.73	-3.42	10/97	*MS4A1, CD22, FCER2, FLT3LG, HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, IL11RA, ITGA6*
	hsa04514	Cell adhesion molecules (CAMs)	-4.27	-1.19	8/145	*CD22, HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, ICAM2, ITGA6, ICOSLG*
	hsa04672	Intestinal immune network for IgA production	-4.10	-1.11	5/49	*HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, ICOSLG*
	hsa04658	Th1 and Th2 cell differentiation	-3.72	-0.92	6/92	*HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, LCK, LAT*
	hsa04659	Th17 cell differentiation	-3.37	-0.73	6/107	*HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA, LCK, LAT*
	hsa04612	Antigen processing and presentation	-2.28	-0.09	4/77	*HLA-DOA, HLA-DOB, HLA-DPB1, HLA-DRA*
	hsa04662	B cell receptor signaling pathway	-2.41	-0.19	4/71	*CD22, CD72, CD79A, AKT3*

P, *P* value; Q, *P* value adjusted using the Benjamini-Hochberg procedure; hsa, *Homo sapient*

Supplementary Table 5. *P* values of Dunn's multiple comparisons test of differentially expressed genes in whole blood among groups of non-survivors, survivors, and healthy controls.

Gene ID	Regulation	*P* value		
		NS *vs* S	NS *vs* HC	S *vs* HC
IL1R2	Up	< 0.0001	< 0.0001	0.0032
HMGB2	Up	< 0.0001	0.0001	> 0.9999
GADD45A	Up	0.0002	< 0.0001	0.1096
TLR4	Up	0.0004	< 0.0001	0.0004

GAS7	Up	0.0004	< 0.0001	0.0105
S100A9	Up	0.0005	< 0.0001	< 0.0001
GPR27	Up	0.0009	< 0.0001	0.0018
IL18RAP	Up	0.0013	< 0.0001	0.0033
MGAM	Up	0.0015	< 0.0001	0.0002
HIF1A	Up	0.0020	< 0.0001	0.0091
IRAK3	Up	0.0023	< 0.0001	< 0.0001
MAPK14	Up	0.0038	< 0.0001	< 0.0001
NFKBIA	Up	0.0040	< 0.0001	< 0.0001
TLR2	Up	0.0047	< 0.0001	0.0007
ITGAM	Up	0.0054	< 0.0001	0.0017
PLK3	Up	0.0100	< 0.0001	0.0010
FKBP5	Up	0.0110	< 0.0001	0.0002
CD160	Down	0.0159	< 0.0001	< 0.0001
PER1	Up	0.0383	< 0.0001	< 0.0001
HLA-DPB1	Down	0.0579	> 0.9999	0.0966
HLA-DOA	Down	0.1274	> 0.9999	0.7229
CD22	Down	0.1986	0.6618	0.0239
GPR56	Down	0.3047	> 0.9999	0.3343
LPL	Up	0.5096	< 0.0001	< 0.0001
ACVR1B	Up	0.4826	< 0.0001	< 0.0001
LCK	Down	0.6225	0.2826	0.0222
CD72	Down	0.6719	> 0.9999	0.3161
LAT	Down	0.8859	0.0793	0.0093

NS, non-survivors; S, survivors; HC, healthy controls

Supplementary Table 6. Area under the receiver operating characteristic curves (AUROCC) of 28 DEGs in discrimination between non-survivors and survivors.

Note: NS = Non-survivors, S = Survivors, and HC = Healthy controls.

Gene ID	Regulation	AUROCC (95% CI)					
		NS *vs* S		NS *vs* HC		S *vs* HC	
S100A9	Up	0.88	(0.79-0.97)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
IL1R2	Up	0.87	(0.78-0.96)	1.00	(1.00-1.00)	0.86	(0.76-0.96)
TLR4	Up	0.86	(0.77-0.95)	1.00	(1.00-1.00)	0.93	(0.84-1.01)
FKBP5	Up	0.85	(0.74-0.96)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
IRAK3	Up	0.83	(0.73-0.94)	1.00	(1.00-1.00)	0.99	(0.97-1.01)
MGAM	Up	0.83	(0.73-0.93)	1.00	(1.00-1.00)	0.99	(0.96-1.01)
HMGB2	Up	0.82	(0.71-0.94)	0.87	(0.77-0.97)	0.51	(0.34-0.67)
MAPK14	Up	0.82	(0.72-0.93)	1.00	(1.00-1.00)	1.00	(0.99-1.00)
NFKBIA	Up	0.82	(0.72-0.93)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
GAS7	Up	0.82	(0.71-0.93)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
GADD45A	Up	0.82	(0.70-0.93)	1.00	(0.95-1.02)	0.82	(0.69-0.96)
GPR27	Up	0.82	(0.71-0.93)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
IL18RAP	Up	0.80	(0.68-0.91)	0.99	(0.98-1.01)	0.83	(0.72-0.94)
CD160	Down	0.77	(0.65-0.89)	0.99	(0.98-1.01)	0.98	(0.93-0.02)
ITGAM	Up	0.77	(0.65-0.89)	1.00	(0.98-1.01)	1.00	(1.00-1.00)
TLR2	Up	0.77	(0.65-0.89)	1.00	(1.00-1.00)	0.85	(0.75-0.96)
HIF1A	Up	0.76	(0.64-0.88)	0.99	(0.98-1.01)	0.76	(0.63-0.89)
PLK3	Up	0.76	(0.63-0.89)	0.98	(0.95-1.02)	1.00	(1.00-1.00)
PER1	Up	0.75	(0.63-0.88)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
HLA-DPB1	Down	0.68	(0.54-0.82)	0.51	(0.32-0.70)	0.68	(0.53-0.83)
HLA-DOA	Down	0.66	(0.51-0.80)	0.53	(0.31-0.74)	0.61	(0.41-0.80)
LPL	Up	0.64	(0.50-0.78)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
ACVR1B	Up	0.64	(0.49-0.78)	1.00	(1.00-1.00)	1.00	(1.00-1.00)
CD22	Down	0.64	(0.49-0.78)	0.62	(0.43-0.80)	0.76	(0.62-0.90)
GPR56	Down	0.62	(0.47-0.77)	0.52	(0.34-0.70)	0.67	(0.49-0.85)
LCK	Down	0.59	(0.44-0.74)	0.65	(0.48-0.81)	0.76	(0.62-0.90)
CD72	Down	0.59	(0.44-0.73)	0.58	(0.38-0.78)	0.69	(0.52-0.86)
LAT	Down	0.58	(0.44-0.73)	0.74	(0.59-0.90)	0.81	(0.67-0.94)

Supplementary Table 7. *P* values of Mann-Whitney test of differentially expressed genes in melioidosis patients at different time points.

Gene ID	Median (IQR)				*P* values					
	Day 0	Day 5	Day 12	Day 28	Day 0 *vs* Day 5	Day 0 *vs* Day 12	Day 0 vs Day 28	Day 5 vs Day 12	Day 5 *vs* Day 28	Day 12 *vs* Day 28

GAS7	0.25 (0.06-0.52)	0.16 (0.04-0.41)	0.04 (0.02-0.10)	0.07 (0.03-0.11)	0.70	0.13	0.08	0.05	0.10	0.43
NFKBIA	1.94 (0.40-2.78)	0.92 (0.46-1.24)	0.51 (0.32-0.55)	0.25 (0.18-0.41)	0.23	0.13	0.08	0.08	< 0.01	0.05
IL1R2	1.52 (0.32-2.59)	1.68 (0.39-2.88)	0.54 (0.19-0.91)	0.30 (0.21-0.48)	0.85	0.13	0.04	0.07	0.01	0.43
S100A9	582.2 (144.30 - 927.20)	229.3 (103.80 - 393.10)	134.4 (59.45 - 191.80)	74.68 (66.92 - 99.89)	0.23	0.02	< 0.01	0.10	0.01	0.32
IRAK3	0.44 (0.21-0.71)	0.28 (0.18-0.76)	0.10 (0.07-0.26)	0.10 (0.08-0.12)	0.78	< 0.01	< 0.001	0.05	0.01	0.78

Supplementary Table 8. Temporal changes in gene expression of melioidosis patients relative to day 0, day 5, and day 12.

Gene ID	Gene expression fold change of 8 individual patients Day 5/Day 0								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	19.84	1.28	0.45	0.11	0.21	0.31	1.02	1.92	3.14 (-1.56 -7.84)
NFKBIA	5.71	2.13	0.28	0.16	0.43	0.39	0.56	1.88	1.44 (0.14-2.74)
IL1R2	1.10	0.66	0.71	0.68	0.64	0.60	11.20	1.29	1.29 (-0.44-4.66)
S100A9	5.59	1.71	0.44	0.17	0.43	0.22	0.19	0.40	1.14 (-0.16-2.44)
IRAK3	1.10	1.17	0.53	0.40	0.41	0.75	0.53	2.93	0.98 (0.40-1.56)

Gene ID	Gene expression fold change of 8 individual patients Day 12/Day 0								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	5.31	0.83	0.35	0.04	0.05	0.24	0.14	0.27	0.90 (-0.34-2.15)
NFKBIA	1.88	1.26	0.22	0.13	0.19	0.16	0.36	1.26	0.69 (0.21-1.16)
IL1R2	0.26	0.54	0.37	0.54	0.11	0.20	0.21	6.05	1.04 (-0.37-2.44)
S100A9	1.28	0.70	0.22	0.14	0.55	0.16	0.05	0.22	0.41 (0.13-0.70)
IRAK3	0.22	0.56	0.34	0.30	0.11	0.30	0.13	0.97	0.37 (0.17-0.56)

Supplementary Table 8. Temporal changes in gene expression of melioidosis patients relative to day 0, day 5, and day 12 (Cont.)

Gene ID	Gene expression fold change of 8 individual patients Day 28/Day 0								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	7.08	1.26	0.11	0.45	0.13	0.07	0.14	0.25	1.86 (-0.49-2.86)
NFKBIA	1.57	1.41	0.11	0.29	0.16	0.06	0.11	0.17	0.49 (0.52-0.92)
IL1R2	0.19	0.46	0.09	0.56	0.04	0.33	0.35	1.93	0.49 (0.07-0.91)
S100A9	0.56	0.91	0.15	0.14	0.35	0.11	0.04	0.10	0.30 (0.09-0.50)
IRAK3	0.19	0.59	0.19	0.72	0.08	0.16	0.17	0.36	0.31 (0.15-0.47)

Gene ID	Gene expression fold change of 8 individual patients Day 12/Day 5								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	0.27	0.65	0.79	0.39	0.25	0.78	0.13	0.14	0.43 (0.23-0.62)
NFKBIA	0.33	0.59	0.79	0.82	0.44	0.42	0.65	0.67	0.59 (0.47-0.71)
IL1R2	0.20	0.50	0.56	0.76	0.17	0.31	0.35	0.54	0.42 (0.28-0.56)
S100A9	0.23	0.41	0.51	0.84	1.27	0.72	0.28	0.54	0.60 (0.37-0.84)
IRAK3	0.20	0.48	0.65	0.75	0.26	0.40	0.24	0.33	0.41 (0.28-0.55)

Supplementary Table 8. Temporal changes in gene expression of melioidosis patients relative to day 0, day 5, and day 12 (Cont.)

Gene ID	Gene expression fold change of 8 individual patients Day 28/Day 5								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	0.36	0.98	0.24	3.99	0.61	0.25	0.13	0.13	0.84 (-0.07-1.74)
NFKBIA	0.28	0.66	0.41	1.80	0.37	0.16	0.20	0.09	0.50 (0.11-0.88)
IL1R2	0.14	0.42	0.14	0.79	0.06	0.51	0.58	0.17	0.35 (0.17-0.53)
S100A9	0.10	0.53	0.35	0.82	0.82	0.49	0.22	0.25	0.45 (0.26-0.63)
IRAK3	0.17	0.50	0.36	1.80	0.20	0.21	0.31	0.12	0.49 (0.07-0.84)

Gene ID	Gene expression fold change of 8 individual patients Day 28/Day 12								Mean fold change (95% CI)
	50-076	50-080	50-081	50-091	50-092	50-208	50-209	50-211	
GAS7	1.33	1.52	0.31	10.10	2.42	0.31	1.01	0.93	2.24 (-0.01-4.49)
NFKBIA	0.84	1.12	0.51	2.20	0.83	0.38	0.31	0.13	0.79 (0.34-1.24)
IL1R2	0.73	0.84	0.25	1.03	0.36	1.63	1.67	0.32	0.85 (0.46-1.24)
S100A9	0.44	1.30	0.68	0.97	0.64	0.68	0.79	0.46	0.75 (0.55-0.94)
IRAK3	0.88	1.05	0.56	2.38	0.74	0.53	1.27	0.37	0.97 (0.53-1.42)

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