Replicability of Differentially Expressed Genes Versus Biological Pathways Biomarkers in Diagnosing Sepsis

Kelsey Winkeler[1][a] and Carly A. Bobak[2][b]
[1]*Department of Psychology, Virginia Polytechnic Institute and State University Blacksburg, VA 24060, U.S.A.*
[2]*Biomedical Data Science, Dartmouth College, Hanover, NH 03755, U.S.A.*

Keywords: Differentially Expressed Genes, Sepsis, Replicability, Biological Pathways.

Abstract: It is generally believed that biological pathways representing curated gene sets are not only more interpretable, but also more replicable and reproducible than gene signatures. With the falling costs of next generation sequencing, we are approaching a point where the cost fully sequencing the transcriptome is competitive with quantifying a targeted gene expression signature which opens up the possibility of pathway signatures for infectious disease. In this work, we evaluated if pathway based signatures are really more reproducible than gene signatures (improvement between 0.83 and over 1 million fold), and amend a meta-analysis framework known for generating highly reproducible gene signatures to instead produce pathway signatures (AUC improves from 0.854 to 0.964 and 0.556 to 0.677 between gene and pathway signatures in independent validation data). We conclude that pathway based signatures show clinical promise for the diagnosis of infectious disease, and there is a growing need for methods considering such signatures.

1 INTRODUCTION

Reproducibility and replicability, wherein discriminatory biological features are consistently associated with a phenotype both within the same dataset and across new datasets, is a major challenge to using differentially expressed genes (DEGs) for diagnoses (Crow et al., 2019; Sweeney et al., 2015). Most approaches take minimal biological information into account, struggle to remain consistent across samples and platforms, and make data difficult to interpret biologically. (Tan, 2003; Zhang et al., 2008) Recent efforts have been made to combine gene expression with knowledge of biological pathways and function using Gene Ontology (GO) terms and other annotated pathways. These methods have the advantage of being less complex and more biologically interpretable than traditional analysis of DEGs, emphasizing networks of related genes over individual genes. (Khatri et al., 2012; Zhang et al., 2009)

There has been some successes: Zarringhalam et al. predicted kidney transplant rejection and response to Infliximab in ulcerative colitis (Zarringhalam et al., 2014), and Pradines et al. found improved reproducibility within and between datasets in several diseases (Pradines et al., 2020). Such approached may be useful even in studies with small sample sizes. (Lim et al., 2015) While prior attempts at diagnostics aim to reduce biomarker quantity to make any resulting assay more cost-effective to produce, the falling cost of transcriptome-wide sequencing makes these proposed pathway signatures possible.(Alpern et al., 2019; Mayday et al., 2019; Sholder et al., 2020)

Sepsis is a particularly important potential application, as it can present similarly to the non-infectious systemic inflammatory response syndrome (SIRS) and lacks a rapid, gold-standard diagnostic. Beginning treatment within the first 'golden hour' is integral for reducing mortality in severe sepsis; however, inappropriate and overuse of antibiotics in hospitals continues to increase rates of MRSA and other antibiotic-resistant microbes. (Ferrer et al., 2014; van Zanten, 2014; Cohen et al., 2015; Sweeney and Khatri, 2017) Attempts to create a sepsis diagnostic DEG signature include the sepsis meta score (Sweeney et al., 2015), *FA1M3:PLAC8* ratio (Scicluna et al., 2015), and the SeptiCyte lab (McHugh et al., 2015), the three of which were compared using 39 public data sets in Sweeney and Khatri in 2017(Sweeney and Khatri, 2017) and demonstrated a similar ability to discriminate between patients with and without sepsis.

[a] https://orcid.org/0000-0002-9452-9428
[b] https://orcid.org/0000-0001-8631-4753

Winkeler, K. and Bobak, C.
Replicability of Differentially Expressed Genes Versus Biological Pathways Biomarkers in Diagnosing Sepsis.
DOI: 10.5220/0010976300003123
In *Proceedings of the 15th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2022) - Volume 3: BIOINFORMATICS*, pages 160-167
ISBN: 978-989-758-552-4; ISSN: 2184-4305

In this work, we extend the current research comparing pathway biomarkers to DEGs by specifically focusing on the implications of pathway biomarkers in diagnosis pipelines. Specifically, we consider comparing the overall rank of important DEGs to the rank of important pathways across multiple datasets collected on multiple gene expression array platforms, and evaluate how a pathway based approach could be amended to a meta-analysis framework to further improve replicability of diagnostic biomarkers.

2 METHODS

All analyses were performed in R version 4.0.3. (R Core Team, 2017)

2.1 Data Sources

Following Sweeney et al.(Sweeney and Khatri, 2017), we downloaded publicly available human gene expression microarray datasets from the NIH Gene Expression Omnibus (GEO). (Barrett et al., 2012; Sutherland et al., 2011; Dolinay et al., 2012; Parnell et al., 2012; McHugh et al., 2015; Kim et al., 2021) Cases included blood samples from adult patients with severe infection or sepsis, taken on day 1 of presentation and pre-treatment. Controls were limited to hospitalization patients without infectious diagnoses. A summary of the included datasets is shown in Table 1. Of the discovered datasets, two were withheld for diagnostic validation while three were used for discovery purposes.

2.2 Processing and Biomarker Analysis

Normalized, publicly available datasets were downloaded using the 'MetaIntegrator' package in R.(Haynes et al., 2016) Probe IDs were matched to HUGO gene symbol; where multiple probes matched to the same symbol, the median expression value was used. (Tweedie et al., 2021) Differentially expressed genes (DEGs) were identified using the 'limma' package in R. (Ritchie et al., 2015)

We conducted pathway analysis using two methods. First, we conducted Gene Set Enrichment Analysis (GSEA) using the results from the DEGs described above. (Mootha et al., 2003; Subramanian et al., 2005) Genes were first ranked using $\text{sign}(foldchange) \times (-\log_{10}(p\text{-}value))$ as described previously (Chen et al., 2007). Ranked lists were imported into the GSEA 4.1.0 and enriched or depleted pathways were identified from Gene Ontology: Biological Processes (GO:BP),(Ashburner et al., 2000) Reactome,(Wu and Haw, 2017) BioCyc Genome Database Collection (Karp et al., 2019) and Wikipathways(Martens et al., 2021). Only pathways between 5 and 500 genes were considered.

Second, we used single sample Gene Set Enrichment Analysis (ssGSEA) to reduce the dimension of each gene expression matrix to a pathway expression matrix using the 'GSVA' package in R (Hänzelmann et al., 2013). Following above, we only mapped to pathways between 5 and 500 genes. We then repeated the 'limma' analysis described to identify DEGs using pathways as biomarker signatures in lieu of genes.

2.3 Replicability of Ranked Lists

The results from the DEGs, GSEA, and ssGSEA were ranked according in each discovery dataset. To compare ranked lists, we used Rank Biased Overlap (RBO). RBO has the benefit of being appropriate for ranked lists, particularly lists which may contain different numbers of elements and is often used in comparing search results (Webber et al., 2010).

2.4 Meta Analysis and Diagnostic Score

Following Sweeney et al. (Sweeney et al., 2015; Sweeney and Khatri, 2017), we used the meta integrator package (Haynes et al., 2016) on the gene expression matrices and ssGSEA pathway matrices to conduct a random effects meta analysis of possible sepsis biomarkers in the discovery datasets. Gene/pathway biomarkers that had a summary FDR p-value < 0.01, heterogeneity p-value < 0.05, or were not significant in all three discovery datasets were filtered out from further analysis. We first considered the 'top' biomarker results by taking the 10 biomarkers with the highest magnitude in summary effect size. We also constructed a diagnostic score using the greedy forward based search defined in Sweeney et al., wherein the biomarker with the most discriminatory power is added first, and then subsequent biomarkers are added based on their improvement to area under the receiving operator characteristics (AUROC) curves until AUROC no longer improves. This score is used to construct AUROC curves in both the discovery and validation datasets to compare how gene or pathway biomarker signatures perform in discriminating sepsis cases across multiple datasets (Sweeney et al., 2015; Sweeney and Khatri, 2017; Haynes et al., 2016).

Table 1: The experimental design for the datasets included in the reproducibility analysis.

	Split	Control definition	Case Definition	n	Sample
GSE28750	Validation	24h after 'major surgery'	community-acquired sepsis	21	Blood
GSE32707	Discovery	MICU with or without SIRS, nonseptic	Sepsis, sepsis/ARDS	103	Blood
GSE40012	Discovery	SIRS	Sepsis from community acquired pneumonia	31	Blood
GSE74224	Discovery	Post-surgical infection-negative systemic inflammation	Sepsis patients from ICU	105	Blood
GSE66099	Validation	SIRS	Sepsis, Septic Shock	229	Blood

Table 2: The RBO results from comparing ranked DEG and pathways from GSEA or ssGSEA.

	DEG	GSEA	ssGSEA	FC GSEA	FC ssGSEA
GSE32707 vs GSE40012	7.53E-08	4.88E-02	5.45E-07	648 142.48	6.24
GSE32707 vs GSE74224	5.73E-05	1.05E-04	1.99E-04	0.83	2.47
GSE40012 vs GSE74224	7.94E-08	5.43E-02	8.89E-02	683 966.30	1 119 232.86

3 RESULTS AND DISCUSSION

We identified 5 datasets containing blood samples from adult patients with sepsis or severe infection. Three of these datasets were selected for biomarker discovery, and two were withheld for validation. Table 1 describes the experimental design of each dataset. Cases were considered to be sepsis or severe infection, and controls were post-surgical patients, patients with trauma, and or systemic inflammatory response syndrome (SIRS).

Complete ranked lists of DEGs were compared between the three discovery cohorts. The RBO for each comparison is reported in Table 2. Median RBO across the three comparisons was 7.94×10^{-08}. Pathways identified using GSEA were then ranked by and compared using the RBO metric. The RBO for the GSEA analysis is also reported in Table 2. Median RBO across the three GSEA comparisons was 4.88×10^{-02}. To illustrate the improvement in RBO similarity, we calculated the fold change improvement in RBO between DEG and GSEA identified pathways. As shown in Table 2, the improvement was large, with two comparisons having a fold change improvement of over 6×10^5.

While it is unsurprising that ranked lists of pathways are more reproducible than ranked genes, canonical methods like GSEA consider pathways at a dataset level rather than an individual level. Hence, we should consider whether sample level pathway analysis is more reproducible than DEGs using the same analysis techniques that would typically be used to identify transcriptomic biomarkers.

ssGSEA uses gene expression data to identify a pathway score for each sample in a data matrix. The median RBO across our comparisons for 'differentially expressed' pathways (DEPs) is 1.99×10^{-04}, with each comparison shown in Table 2. Similar to the GSEA pathways, all comparisons had considerable improvement compared to the DEG ranking.

Despite this improvement in reproducibility, the RBO was low across both ranked pathways and ranked genes, reflective of biological complexity. It is also unclear how these improvements in reproducibility will affect diagnostic performance at the clinical level. To study this further, we used the meta-analysis model proposed by Sweeney et al. (Sweeney et al., 2015; Sweeney and Khatri, 2017) to compare the results between the gene and pathway signatures.

A critical step of this method is filtering biomarkers based on summary effects size, significance, and heterogeneity (Cochran's Q) (Sweeney et al., 2015; Sweeney and Khatri, 2017). While filters based on the summary effects yielded a similar number of biomarkers, filtering based on heterogeneity removed far more genes than pathways. The heterogeneity step reduced the gene biomarker list to 48 positively and 6 negatively associated genes, conversely, the heterogeneity filter reduced the pathway biomarker list to 55 positive and 50 negative pathways.

The meta analysis results of top 10 associated genes, by magnitude of effect size, is shown in Figure 1a while the corresponding top pathways are shown in Figure 1b. The distributions of the summary estimates

Table 3: The genes selected by the greedy forward search in a meta-analysis framework for the diagnosis of sepsis.

Gene	FC	p-value	BH p-value	previously associated with sepsis
ADORA2A	1.033	1.63E-06	5.21E-04	Yes (Busse et al., 2016)
ARSD	0.863	9.56E-10	1.64E-06	Yes (Guillen-Guio et al., 2020)
LY6G6D	0.646	3.12E-06	7.78E-04	No
SIGLEC9	0.982	6.14E-12	5.70E-08	Yes (von Gunten et al., 2009)
ZBTB7B	0.648	2.87E-06	7.41E-04	Yes (Bhatty et al., 2012)
TTC17	0.651	2.59E-06	6.96E-04	No
GADD45G	0.643	3.47E-06	8.15E-04	Yes (Aare et al., 2012)
PPP1R9B	0.644	3.32E-06	8.02E-04	No
PARP10	0.634	4.72E-06	9.94E-04	Yes (Wasyluk and Zwolak, 2021)
MPZL2	-0.862	9.69E-10	1.64E-06	Yes (Ji et al., 2014)
DLG5	-0.710	3.36E-07	1.59E-04	Yes (Li et al., 2017)
EPHB4	-0.647	3.14E-06	7.78E-04	Yes (Coulthard et al., 2012)

Table 4: The pathways selected by the greedy forward search in a meta-analysis framework for the diagnosis of sepsis.

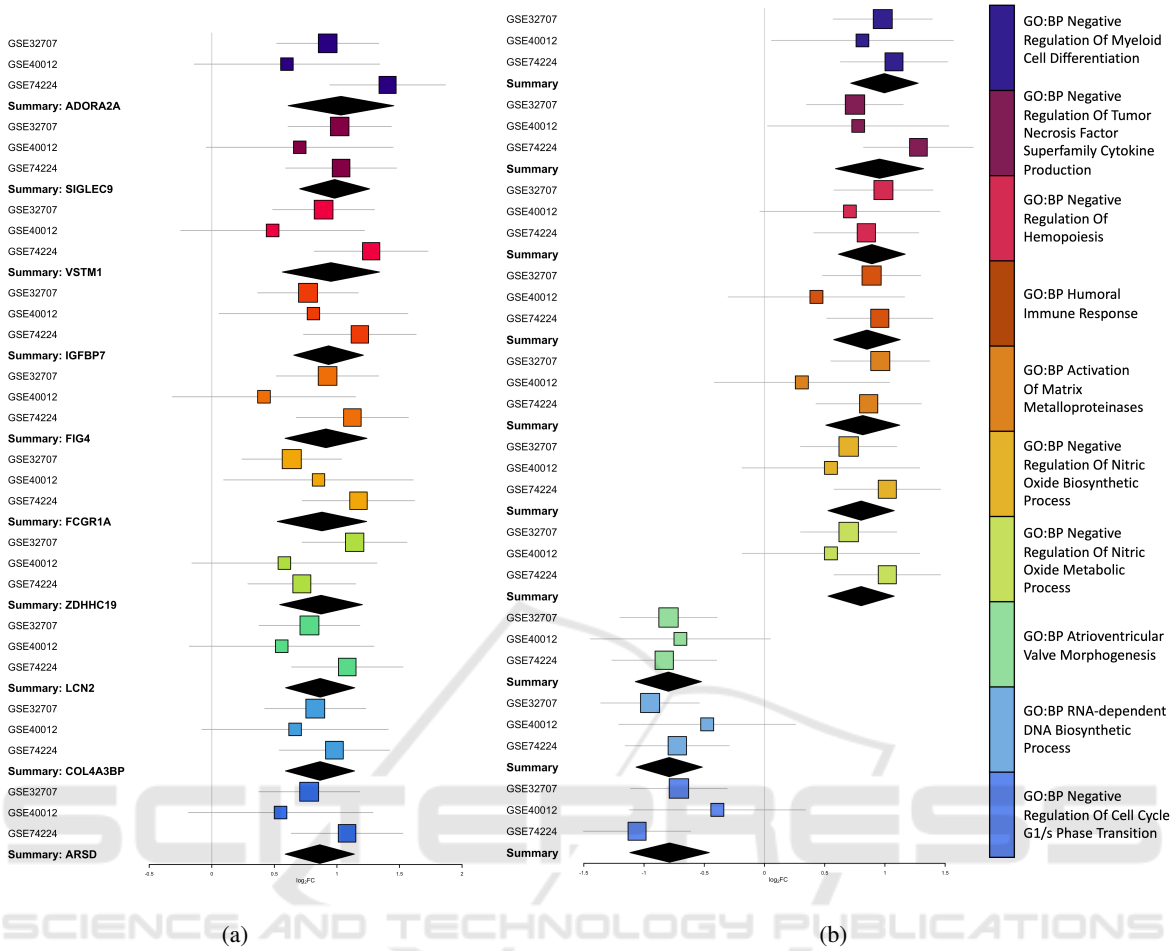
Name	ID	FC	p-value	BH p-value	gene count
Negative regulation of myeloid cell differentiation	GO:0045638	0.996	3.23E-12	2.82E-08	59
Cell killing	GO:0001906	0.723	2.14E-07	6.67E-05	27
Negative regulation of tumor necrosis factor superfamily cytokine production	GO:1903556	0.955	3.13E-07	8.64E-05	29
Regulation of fatty acid metabolic process	GO:0019217	-0.698	8.28E-06	7.72E-04	62
APEX1-Independent resolution of AP sites via the single nucleotide replacment pathway	R-HSA-5649702	-0.695	1.26E-05	9.95E-04	7
Polyamine biosyntethic process	GO:0006596	-0.624	6.31E-06	6.47E-04	9
Signalling by PTK6	R-HSA-8848021	-0.650	2.72E-06	3.41E-04	61
Regulation of cell cycle G1/S phase transition	GO:1902806	-0.781	2.45E-08	2.33E-05	110

between the most significant genes and pathways is similar (Kolmogorov-Smirnov p=0.1641), suggesting that biomarker signatures should be competitive between genes and pathways.

We identified a reduced biomarker signature in both gene expression and pathway matrices using a greedy forward search algorithm, where biomarkers were selected based on maximizing the AUC between cases and controls in the discovery datasets. The final meta-score gene signature for sepsis genes can be found in Table 3 and the meta-score pathway signature in Table 4.

AUROC for the discovery datasets were promising, using either the gene meta-score or pathway meta-score. The AUROCs using the gene meta-score are 0.815, 0.925, and 0.952 for GSE32707, GSE40012, and GSE74224 respectively. The AUROCs using the pathway meta-score are 0.833, 0.884, and 0.925 in GSE32707, GSE40012, and GSE74224 respectively. AUROCs were not significantly different between the gene signature and the pathway signature in the discovery datasets.

The AUROC for the validation datasets is shown in Figure 2. Considerable improvement is seen in the pathway signature in both datasets. Of note, both signatures perform poorly in GSE66099, which is an amalgamation of 6 unique sepsis datasets and includes patients with septic shock. In particular, the gene based signature performs only slightly better than random guessing while the pathway based signature exhibits poor performance with an AUROC of 0.667. Despite the improvement in a pathway based signature, more work is needed to increase reproducibility and replicability in validation datasets before such a signature would be considered for use in a clinic. However, the improvement of AUROC in the



(a) (b)

Figure 1: The top 10 associated genes and pathways from from the filtered meta analysis results.

unique validation datasets does suggest that a pathway based signature is clinically useful in the development of molecular biomarker signatures.

While pathways are more reproducible compared to genes in this work, much of previous work in molecular diagnostics has focused on a minimal set of biomarkers to be measured in order to minimize diagnostic costs (Sweeney et al., 2015). However, due to the falling costs of whole genome RNA sequencing, we are approaching a time when sequencing the entire transcriptome will be as cost effective, if not cheaper, then targeted technologies. Such approaches open up the possibility of using these pathway based signatures without additional costs.

One of common critiques of the use of AI in diagnosis is a lack of clinical interpretability, with clinicians feeling uncomfortable with the 'black box' approach to diagnosis. While pathway signatures cannot serve to 'lift the hood' under complicated AI algorithms, they do allow researchers, clinicians, and patients to better understand the biological inputs underpinning the diagnostic prediction models (Wang et al., 2020). As the pathways here are generated from the ranked DEGs, the meta-score pathway signature both preserves more information that just DEGs while also increasing interpretability of biomarkers.

Sepsis is characterized by dysregulation in the host immune system and inflammatory response, which then causes severe oxidative stress.(Macdonald et al., 2003) Excessive free radicals or inadequate defenses can cause lipid peroxidation, impact cell and mitochondrial membrane stability and lead to cell death and tissue damage. (Macdonald et al., 2003; Fanucchi, 2014) Accordingly, we would expect to see pathways involved in immune response, heat and oxidative stress, and apoptosis. Nuclear factor κB (NFκB) is a ubiquitous transcription factor that is thought to regulate innate immunity and be involved in inflammation, cancer, and nervous system function. (Macdonald et al., 2003; Salminen et al., 2008; Albensi, 2019) By over-regulating downstream proteins, this transcription factor may contribute to the

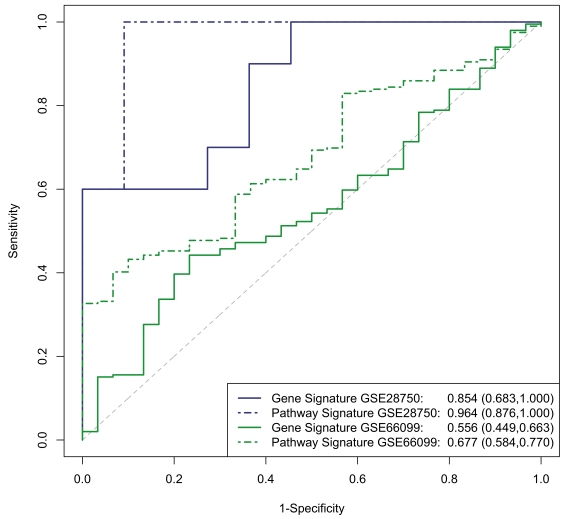


Figure 2: The gene and pathway meta-score ROC curves in the validation datasets.

immune dysregulation in sepsis. Several of the pathways included in our signature are thought to directly or indirectly regulate or be regulated by NFκB, including: Negative regulation of myeloid cell differentiation (Achyut et al., 2017), Cell killing (Fan et al., 2008), Negative regulation of tumor necrosis factor superfamily cytokine production (Hayden and Ghosh, 2014), Regulation of fatty acid metabolism (Kracht et al., 2020), Polyamine biosynthetic process (Facchini et al., 2005), Regulation of cell cycle G1/S phase transition (Ledoux and Perkins, 2014). The tight interconnection of these pathways supports the biological relevance of our signature, and suggests the relationship between these processes as a target for future research in sepsis.

4 CONCLUSION

This work, while promising, is not without limitation. Future work should consider whether the increases seen in reproducibility of biomarkers is true across many diseases and compare across both microarray and RNA sequencing. As well, we sought only to validate these findings in datasets collected on the same tissue. Additional work should consider whether pathway signatures are more reproducible across different tissue types compared to gene expression signatures. Moreover, comparing the performance of pathway based approaches in different diagnostic models should be considered.

We demonstrate that pathway signatures are more replicable than gene signatures and that pathway signatures can be easily amended to existing signature identification models to improve validation accuracy in new datasets. Future work emphasizing methods for pathway-based signatures should occur as RNA sequencing costs fall and the possibility of cost-effective pathway signatures becomes reality.

REFERENCES

Aare, S., Radell, P., Eriksson, L. I., Chen, Y.-W., Hoffman, E. P., and Larsson, L. (2012). Role of sepsis in the development of limb muscle weakness in a porcine intensive care unit model. *Physiological genomics*, 44(18):865–877.

Achyut, B. R., Angara, K., Jain, M., Borin, T. F., Rashid, M. H., Iskander, A. S. M., Ara, R., Kolhe, R., Howard, S., Venugopal, N., Rodriguez, P. C., Bradford, J. W., and Arbab, A. S. (2017). Canonical nfκb signaling in myeloid cells is required for the glioblastoma growth. *Scientific Reports*, 7(1):13754.

Albensi, B. C. (2019). What is nuclear factor kappa b (nf-κb) doing in and to the mitochondrion? *Frontiers in Cell and Developmental Biology*, 7:154.

Alpern, D., Gardeux, V., Russeil, J., Mangeat, B., Meireles-Filho, A. C. A., Breysse, R., Hacker, D., and Deplancke, B. (2019). BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. *Genome Biology*, 20(1):71.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., and Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nature Genetics*, 25(1):25–29.

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M., Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H., Zhang, N., Robertson, C. L., Serova, N., Davis, S., and Soboleva, A. (2012). NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research*, 41(D1):D991–D995.

Bhatty, M., Fan, R., Muir, W. M., Pruett, S. B., and Nanduri, B. (2012). Transcriptomic analysis of peritoneal cells in a mouse model of sepsis: confirmatory and novel results in early and late sepsis. *BMC genomics*, 13(1):1–13.

Busse, H., Bitzinger, D., Höcherl, K., Seyfried, T., Gruber, M., Graf, B. M., and Zausig, Y. A. (2016). Adenosine a2a and a2b receptor substantially attenuate ischemia/reperfusion injury in septic rat hearts. *Cardiovascular drugs and therapy*, 30(6):551–558.

Chen, J. J., Tsai, C.-A., Tzeng, S., and Chen, C.-H. (2007). Gene selection with multiple ordering criteria. *BMC Bioinformatics*, 8(1):74.

Cohen, J., Vincent, J.-L., Adhikari, N. K. J., Machado, F. R., Angus, D. C., Calandra, T., Jaton, K., Giulieri, S., Delaloye, J., Opal, S., Tracey, K., van der Poll, T., and

Pelfrene, E. (2015). Sepsis: a roadmap for future research. *The Lancet Infectious Diseases*, 15(5):581–614.

Coulthard, M. G., Morgan, M., Woodruff, T. M., Arumugam, T. V., Taylor, S. M., Carpenter, T. C., Lackmann, M., and Boyd, A. W. (2012). Eph/ephrin signaling in injury and inflammation. *The American journal of pathology*, 181(5):1493–1503.

Crow, M., Lim, N., Ballouz, S., Pavlidis, P., and Gillis, J. (2019). Predictability of human differential gene expression. *Proceedings of the National Academy of Sciences of the United States of America*, 116(13):6491–6500.

Dolinay, T., Kim, Y. S., Howrylak, J., Hunninghake, G. M., An, C. H., Fredenburgh, L., Massaro, A. F., Rogers, A., Gazourian, L., Nakahira, K., Haspel, J. A., Landazury, R., Eppanapally, S., Christie, J. D., Meyer, N. J., Ware, L. B., Christiani, D. C., Ryter, S. W., Baron, R. M., and Choi, A. M. K. (2012). Inflammasome-regulated Cytokines Are Critical Mediators of Acute Lung Injury. *American Journal of Respiratory and Critical Care Medicine*, 185(11):1225–1234.

Facchini, A., BorzÍ, R. M., Marcu, K. B., Stefanelli, C., Olivotto, E., Goldring, M. B., Facchini, A., and Flamigni, F. (2005). Polyamine depletion inhibits nf-κb binding to dna and interleukin-8 production in human chondrocytes stimulated by tumor necrosis factor-α. *Journal of Cellular Physiology*, 204(3):956–963.

Fan, Y., Dutta, J., Gupta, N., Fan, G., and Gélinas, C. (2008). Regulation of programmed cell death by nf-κb and its role in tumorigenesis and therapy. In *Programmed Cell Death in Cancer Progression and Therapy*, volume 615, pages 223–250. Springer Netherlands, Dordrecht. Series Title: Advances in Experimental Medicine and Biology.

Fanucchi, M. V. (2014). Development of antioxidant and xenobiotic metabolizing enzyme systems.

Ferrer, R., Martin-Loeches, I., Phillips, G., Osborn, T. M., Townsend, S., Dellinger, R. P., Artigas, A., Schorr, C., and Levy, M. M. (2014). Empiric Antibiotic Treatment Reduces Mortality in Severe Sepsis and Septic Shock From the First Hour: Results From a Guideline-Based Performance Improvement Program*. *Critical Care Medicine*, 42(8):1749–1755.

Guillen-Guio, B., Lorenzo-Salazar, J. M., Ma, S.-F., Hou, P.-C., Hernandez-Beeftink, T., Corrales, A., García-Laorden, M. I., Jou, J., Espinosa, E., Muriel, A., et al. (2020). Sepsis-associated acute respiratory distress syndrome in individuals of european ancestry: a genome-wide association study. *The Lancet Respiratory Medicine*, 8(3):258–266.

Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14:7.

Hayden, M. S. and Ghosh, S. (2014). Regulation of nf-κb by tnf family cytokines. *Seminars in Immunology*, 26(3):253–266.

Haynes, W. A., Vallania, F., Liu, C., Bongen, E., Tomczak, A., Andres-Terre, M., Lofgren, S., Tam, A., Deisseroth, C. A., Li, M. D., Sweeney, T. E., and Khatri, P. (2016). Empowering multi-cohort gene expression analysis to increase reproducibility. *Pacific Symposium on Biocomputing*.

Ji, S., Pan, Y., Lu, Q., Sun, Z., and Liu, Y. (2014). Screening of differentially expressed genes between multiple trauma patients with and without sepsis. *Genet Mol Res*, 13(1):1855–64.

Karp, P. D., Billington, R., Caspi, R., Fulcher, C. A., Latendresse, M., Kothari, A., Keseler, I. M., Krummenacker, M., Midford, P. E., Ong, Q., Ong, W. K., Paley, S. M., and Subhraveti, P. (2019). The BioCyc collection of microbial genomes and metabolic pathways. *Briefings in Bioinformatics*, 20(4):1085–1093.

Khatri, P., Sirota, M., and Butte, A. J. (2012). Ten Years of Pathway Analysis: Current Approaches and Outstanding Challenges. *PLoS Computational Biology*, 8(2):e1002375.

Kim, K. S., Jekarl, D. W., Yoo, J., Lee, S., Kim, M., and Kim, Y. (2021). Immune gene expression networks in sepsis: A network biology approach. *PLOS ONE*, 16(3):e0247669.

Kracht, M., Müller-Ladner, U., and Schmitz, M. L. (2020). Mutual regulation of metabolic processes and proinflammatory nf-κb signaling. *Journal of Allergy and Clinical Immunology*, 146(4):694–705.

Ledoux, A. and Perkins, N. (2014). Nf-κb and the cell cycle. *Biochemical Society Transactions*, 42(1):76–81.

Li, Z., Zhang, Y., Liu, Y., Liu, Y., and Li, Y. (2017). Identification of key genes in gram-positive and gram-negative sepsis using stochastic perturbation. *Molecular medicine reports*, 16(3):3133–3146.

Lim, K., Li, Z., Choi, K. P., and Wong, L. (2015). A quantum leap in the reproducibility, precision, and sensitivity of gene expression profile analysis even when sample size is extremely small. *Journal of Bioinformatics and Computational Biology*, 13(04):1550018.

Macdonald, J., Galley, H., and Webster, N. (2003). Oxidative stress and gene expression in sepsis. *British Journal of Anaesthesia*, 90(2):221–232.

Martens, M., Ammar, A., Riutta, A., Waagmeester, A., Slenter, D., Hanspers, K., A. Miller, R., Digles, D., Lopes, E., Ehrhart, F., Dupuis, L. J., Winckers, L. A., Coort, S., Willighagen, E. L., Evelo, C. T., Pico, A. R., and Kutmon, M. (2021). WikiPathways: connecting communities. *Nucleic Acids Research*, 49(D1):D613–D621.

Mayday, M. Y., Khan, L. M., Chow, E. D., Zinter, M. S., and DeRisi, J. L. (2019). Miniaturization and optimization of 384-well compatible RNA sequencing library preparation. *PLOS ONE*, 14(1):e0206194.

McHugh, L., Seldon, T. A., Brandon, R. A., Kirk, J. T., Rapisarda, A., Sutherland, A. J., Presneill, J. J., Venter, D. J., Lipman, J., Thomas, M. R., Klein Klouwenberg, P. M. C., van Vught, L., Scicluna, B., Bonten, M., Cremer, O. L., Schultz, M. J., van der Poll, T., Yager, T. D., and Brandon, R. B. (2015). A Molecular Host Response Assay to Discriminate Between Sepsis and Infection-Negative Systemic Inflammation in Critically Ill Patients: Discovery and Val-

idation in Independent Cohorts. *PLOS Medicine*, 12(12):e1001916.

Mootha, V. K., Lindgren, C. M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., Houstis, N., Daly, M. J., Patterson, N., Mesirov, J. P., Golub, T. R., Tamayo, P., Spiegelman, B., Lander, E. S., Hirschhorn, J. N., Altshuler, D., and Groop, L. C. (2003). PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics*, 34(3):267–273.

Parnell, G. P., McLean, A. S., Booth, D. R., Armstrong, N. J., Nalos, M., Huang, S. J., Manak, J., Tang, W., Tam, O.-Y., Chan, S., and Tang, B. M. (2012). A distinct influenza infection signature in the blood transcriptome of patients with severe community-acquired pneumonia. *Critical Care*, 16(4):R157.

Pradines, J. R., Farutin, V., Cilfone, N. A., Ghavami, A., Kurtagic, E., Guess, J., Manning, A. M., and Capila, I. (2020). Enhancing reproducibility of gene expression analysis with known protein functional relationships: The concept of well-associated protein. *PLOS Computational Biology*, 16(2):e1007684.

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., and Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, 43(7):e47–e47.

Salminen, A., Huuskonen, J., Ojala, J., Kauppinen, A., Kaarniranta, K., and Suuronen, T. (2008). Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. *Ageing Research Reviews*, 7(2):83–105.

Scicluna, B. P., Klein Klouwenberg, P. M. C., van Vught, L. A., Wiewel, M. A., Ong, D. S. Y., Zwinderman, A. H., Franitza, M., Toliat, M. R., Nürnberg, P., Hoogendijk, A. J., Horn, J., Cremer, O. L., Schultz, M. J., Bonten, M. J., and van der Poll, T. (2015). A Molecular Biomarker to Diagnose Community-acquired Pneumonia on Intensive Care Unit Admission. *American Journal of Respiratory and Critical Care Medicine*, 192(7):826–835.

Sholder, G., Lanz, T. A., Moccia, R., Quan, J., Aparicio-Prat, E., Stanton, R., and Xi, H. S. (2020). 3'Pool-seq: an optimized cost-efficient and scalable method of whole-transcriptome gene expression profiling. *BMC Genomics*, 21(1):64.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., and Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, 102(43):15545–15550.

Sutherland, A., Thomas, M., Brandon, R. A., Brandon, R. B., Lipman, J., Tang, B., McLean, A., Pascoe, R., Price, G., Nguyen, T., Stone, G., and Venter, D. (2011). Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. *Critical Care*, 15(3):R149.

Sweeney, T. E. and Khatri, P. (2017). Benchmarking Sepsis Gene Expression Diagnostics Using Public Data*:. *Critical Care Medicine*, 45(1):1–10.

Sweeney, T. E., Shidham, A., Wong, H. R., and Khatri, P. (2015). A comprehensive time-course–based multicohort analysis of sepsis and sterile inflammation reveals a robust diagnostic gene set. *Science Translational Medicine*, 7(287):287ra71–287ra71.

Tan, P. K. (2003). Evaluation of gene expression measurements from commercial microarray platforms. *Nucleic Acids Research*, 31(19):5676–5684.

Tweedie, S., Braschi, B., Gray, K., Jones, T. E. M., Seal, R., Yates, B., and Bruford, E. A. (2021). Genenames.org: the HGNC and VGNC resources in 2021. *Nucleic Acids Research*, 49(D1):D939–D946.

van Zanten, A. R. H. (2014). The Golden Hour of Antibiotic Administration in Severe Sepsis: Avoid a False Start Striving for Gold*. *Critical Care Medicine*, 42(8):1931–1932.

von Gunten, S., Jakob, S. M., Geering, B., Takala, J., and Simon, H.-U. (2009). Different patterns of siglec-9-mediated neutrophil death responses in septic shock. *Shock*, 32(4):386–392.

Wang, F., Kaushal, R., and Khullar, D. (2020). Should health care demand interpretable artificial intelligence or accept "black box" medicine?

Wasyluk, W. and Zwolak, A. (2021). Parp inhibitors: An innovative approach to the treatment of inflammation and metabolic disorders in sepsis. *Journal of Inflammation Research*, 14:1827.

Webber, W., Moffat, A., and Zobel, J. (2010). A similarity measure for indefinite rankings. *ACM Transactions on Information Systems*, 28(4):1–38.

Wu, G. and Haw, R. (2017). Functional Interaction Network Construction and Analysis for Disease Discovery. In Wu, C. H., Arighi, C. N., and Ross, K. E., editors, *Protein Bioinformatics*, volume 1558, pages 235–253. Springer New York, New York, NY. Series Title: Methods in Molecular Biology.

Zarringhalam, K., Enayetallah, A., Reddy, P., and Ziemek, D. (2014). Robust clinical outcome prediction based on Bayesian analysis of transcriptional profiles and prior causal networks. *Bioinformatics*, 30(12):i69–i77.

Zhang, M., Yao, C., Guo, Z., Zou, J., Zhang, L., Xiao, H., Wang, D., Yang, D., Gong, X., Zhu, J., Li, Y., and Li, X. (2008). Apparently low reproducibility of true differential expression discoveries in microarray studies. *Bioinformatics*, 24(18):2057–2063.

Zhang, M., Zhang, L., Zou, J., Yao, C., Xiao, H., Liu, Q., Wang, J., Wang, D., Wang, C., and Guo, Z. (2009). Evaluating reproducibility of differential expression discoveries in microarray studies by considering correlated molecular changes. *Bioinformatics*, 25(13):1662–1668.