CytokineFinder: a method for identifying and ranking cytokines using transcriptomics

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INTRODUCTION

- The COVID-19 pandemic has generated a vast amount of cytokine data as a result of **cytokine storms**, a topic of significant scientific interest within the last few years.
- Cytokines are signaling molecules between cells and are crucial players in allergies, asthma, and other immune system disorders.
- Understanding these communication pathways offers valuable insights for clinicians.
- The number of studies effectively utilizing this data remains limited.
- A comparative analysis of existing analytical methods is necessary to identify reliability due to the sheer volume of methods.

HYPOTHESIS

By systematic benchmarking of gene expression data sets with various methods, we will be able to identify an effective approach to detect key cytokines.

METHODS

Authors	Journal	Disease	Sample Size	GEO	Target Cytokine
Sandborn *et al,* 2014	Gastroenterology	UC	183	GSE92415	*TNF*
Defois *et al,* 2023	Cell Commun Signal	OA	5	GSE215039	*IL-1B*
Defois *et al,* 2023	-	OA	5	-	*IL-6*
Defois *et al,* 2023	-	OA	5	-	*TNF*
Houssiau *et al,* 2021	Ann Rheum Dis	SLE	90	GSE185047	*IFNA2*

Table 1. Ongoing list of study data currently analyzed with successful identification of target cytokines following a selection criteria where total study size is at least 5, within the last 10 years, and cover a variety of human diseases.

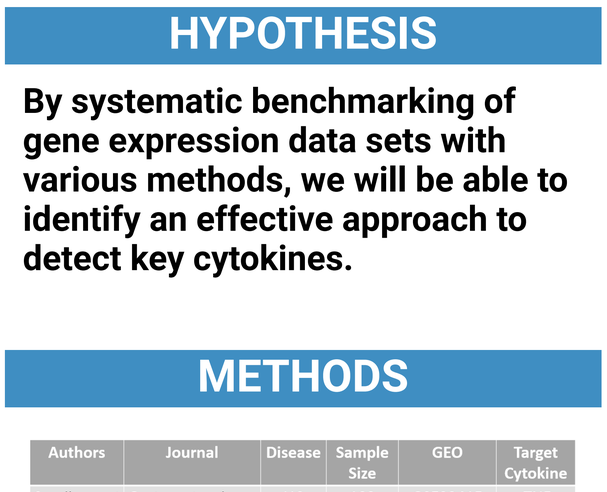

Figure 1. General method overview. Given a public dataset with cytokine data, can we identify the target cytokine based on current available databases? Intentions are to create a framework for benchmarking. Differential expression analysis was applied and benchmarking of several downstream methods were considered. We took a list of databases and subset DE genes based on ligand-receptor relationships.

BENCHMARKING EVALUATIONS

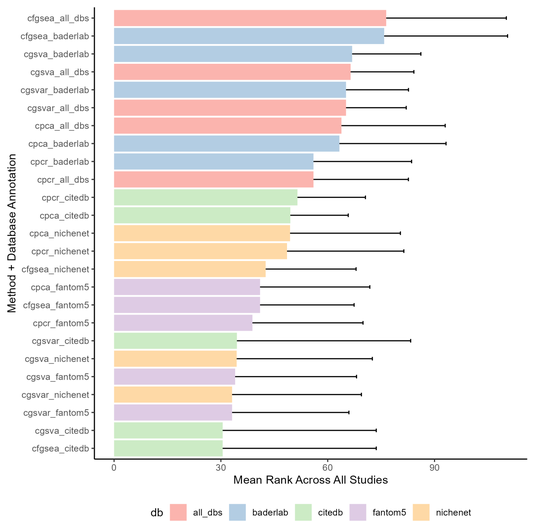

Figure 2. Overall mean performance ranking of cytokine identification based on the method and database selected. All rankings are based on converted mean percentiles thus, the higher the rank, the better a method + database annotation combination performed. Cytokine detection of *TNF, IL-1B, IL-6,* and *IFNA2* is highly varied.

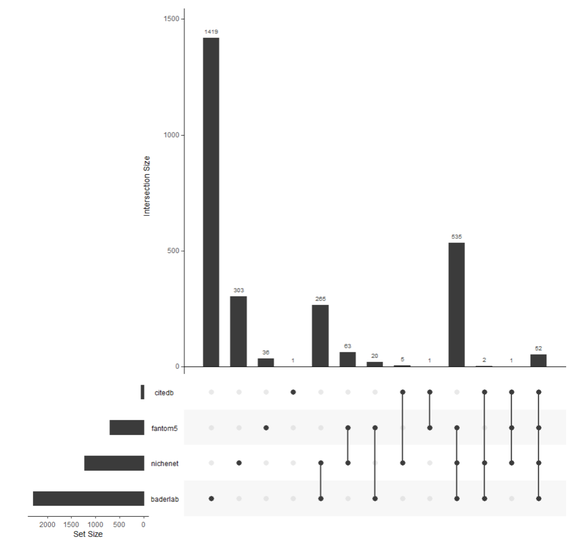

Figure 3. An upset plot representing the total number of ligand-receptor relationships relevant to cytokines in each database and comparing overlap of features with other independent databases.

- In this study, methods that used Baderman Lab ligand-receptor annotations to detect the cytokine of interest were ranked higher than other databases.
- We found NicheNet and Fantom5 databases consistently ranked lower in performance which is consistent to annotation quantity
- There was high variability in rankings for the *cFGSEA* method which could mean methods may be sensitive to annotation quality.
- Our results shown signifies the need for development of higher quality databases.

DISEASE-SPECIFIC CYTOKINE TARGET ACTIVITY

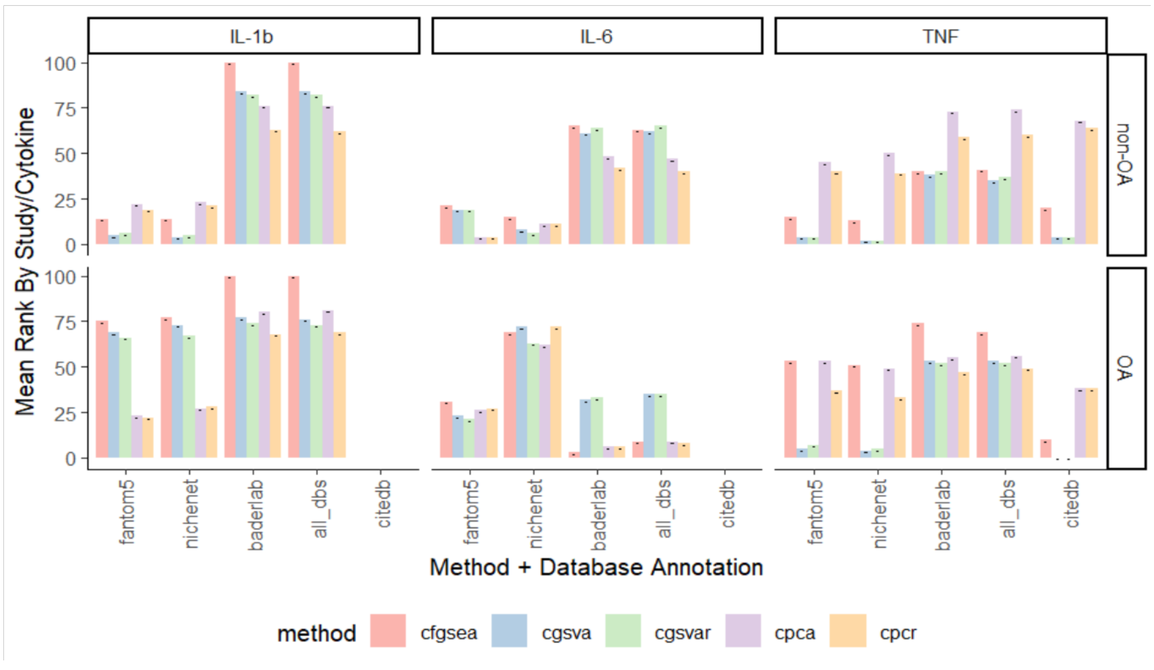

Figure 4. An evaluation of **GSE215039** comparing cytokine detection and ranking between chondrocyte states (osteoarthritis vs non-osteoarthritis) across the series of methods and databases. Differentially expressed receptors were subset along with the cytokine of interest (as some cytokine names have their own receptors) and a mean rank was computed for each method. Each cytokine was represented by n=5.

A NOVEL LANDSCAPE OF CYTOKINE ACTIVITY

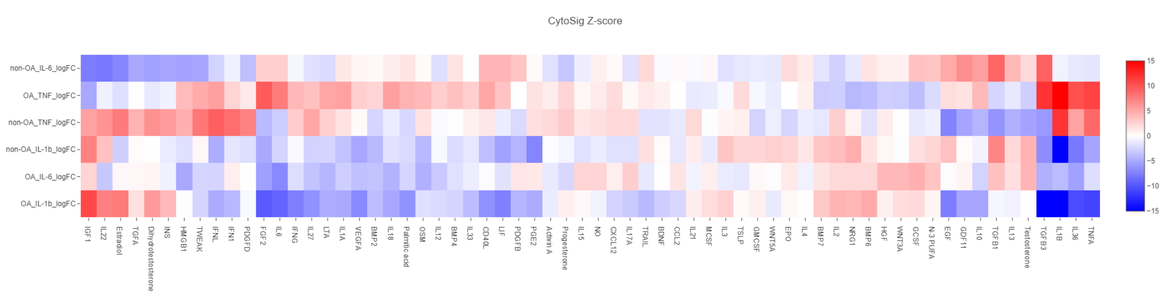

Figure 5. Z-Score distribution of **GSE2150039** for CytoSig, a novel method for detecting cytokine activity signatures. The higher the absolute Z-score value, the more likely a cytokine is active. We observe variable detection in cytokine activity with high prediction for *TNF* and *IL-1B.*

FUTURE DIRECTIONS

- More data sets to analyze
  - Include other conditions relevant to immune response (ie. **sepsis)**
- Assess performance of the novel method, CytoSig which predicts cytokine activity independently of a database
- Include different types of disease and anti-cytokine treatment combinations (N = 10), assess gene expression and cytokine protein paired data (N = 10), and identify temporal cytokine expression via cytokine stimulation
- As cytokines are a way for immune cells to communicate, it is important to consider how cytokine activity may differ between immune cells
- Single cell RNAseq (scRNAseq) may be an avenue to explore cell-specific cytokine activity
- Identify gene hubs and model cytokine networks to characterize immune response, interpret potential novel pathways

CONCLUSION

- **Several of the selected methods are sensitive to database annotation quality**
- **Benchmarking methodologies is important to consider as more methodologies become available**
- **Cytokine detection provides utility for a range of diseases**

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