Building a model of sepsis: data integration unravels pathogenic mechanisms in severe *P. aeruginosa* infections.

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Abstract

Understanding how human hosts and bacterial pathogens interact with each other is essential to explaining the differences in severity and outcomes of systemic infectious diseases (sepsis). Such pathogens, like *Pseudomonas aeruginosa* (PA), remain a major public health concern. In this work we present a data integration workflow to build a model of interaction between *PA* and its human host. The pathogenic mechanisms of *PA* infection are described through accurate mapping of

protein-protein, and metabolite-protein direct interactions, between *PA* and human host, as well as a description of pathways activated in organs affected by severe *PA* infections.

In the first step, a literature review process was carried out based on previous experiences with virus-host interaction models. The collected Data was organized to build a conceptual model on multiple levels, providing a view of the relevant mechanisms involved in severe bacterial infections. All information about *PA* infection was categorized into three main groups: a) conceptual information about pathogenesis; b) molecular interactions between bacterium and humans; and c) gene expression signatures and dysregulated pathways in severe infections. A dataset of 89 *PA* – human interactions, involving 152 proteins/molecules (109 human proteins, 3 human molecules, 34 *PA* proteins, and 5 *PA* molecules) was reviewed and annotated, providing a new perspective on the *PA* -host interaction to improve understanding of the host biological responses. Such data were complemented by transcriptomic data on *PA* -infected lung samples, highlighting overexpression of proinflammatory pathways as well as modulation of the activities of *PA* on the host response in the lung.

Importance

The intricate interplay between host and microbial pathogens prompts us to use innovative data sources, combining omics data, clinical patient data, and pathogen features, through exploratory computational methods. A data curation process allowed us to put together a detailed description of the pathogenic mechanisms of *PA* in the human host, connecting basic knowledge about clinical aspects, interactions between bacterial and host proteins, and pathways dysregulated during *PA* infection. The results of our work allowed us to start putting together a model of *PA* infection, defining specific steps in *PA* infections and providing a significant step forward in understanding the deep interaction between *PA* and the human host in severe infections. This approach could be an effective way to predict specific clinical phenotypes in severe bacterial infections.

Keywords: *P. aeruginosa;* host-pathogen interaction; bacterial infection; disease model; sepsis.

Background

Computational modelling, omics data analysis, and clinical research proved to bridge the gap between conceptual models and clinical practice in infectious diseases [1, 2]. Molecular diagrams, structuring key pathophysiological mechanisms for specific diseases, and conceptual domains' identification could give a new understanding of biomedical knowledge[1, 3, 4]. A network-based

exploratory approach to molecular interactions helped us shed light on complex processes between host proteome and pathogen molecules, useful for severe human infections. In fact, during the COVID-19 pandemic, the search for novel strategies to fight this infection led to the new challenge of rapidly finding molecular interactions between emerging and completely unknown viruses and the human host [5]. The virus-host interactome has been valuable, showing models of interaction between SARS-CoV-2 and human host cells, explaining clinical manifestations[6-9] and enabling a timely drug repurposing process for COVID-19 treatment [10, 11]. In that context, the graphical representation of the molecular mechanisms of infection (or molecular map of disease) provided meaning to apparently unrelated interactions, becoming a dynamic model for omics data integration in COVID-19 investigation [12, 13].

Among all severe infectious diseases, sepsis due to several microbial pathogens, often with multidrug-resistant profile, remains one of the main causes of death among intensive care unit (ICU) patients and a warning for public health [14].

Recently, to provide early prediction about the estimated outcome in sepsis patients, a real-time deep-learning model was applied to sepsis patients, considering baseline acuity, comorbidities, seasonal effects, and secular trends over time [15], showing the strategic significance of computational modelling to improve the clinical outcomes in sepsis patients.

Currently, it is evident that microbial infection strongly depends on the host condition and the spatial interactions between the microbe and the host, as well as the other microorganisms [16, 17], but these aspects are still unexplored.

Among all possible bacteria causing severe infection, *Pseudomonas aeruginosa* (PA) is one of the most common pathogens for nosocomial infections, and, along with *Acinetobacter baumannii* and *Enterobacterales* resistant to carbapenem and third generation cephalosporins, it was listed among critical priority pathogens for WHO, while the European Centre for Disease Prevention and Control (ECDC) included it in the surveillance of antimicrobial resistance [18, 19]. It is considered as an opportunistic human pathogen, widely reported in Cystic Fibrosis (CF) patients, with high morbidity and mortality. *PA* is resistant to multiple drugs, makes many virulence factors, and produces the biofilm that lets it infect and colonize its host [20]. In recent years, *PA* has also represented an interesting model for computational exploration: network-assisted computational experiments allowed the identification of novel genes for virulence and antibiotic resistance, confirmed through experimental validation, simulating the event of cross-resistance against multiple drugs due to the same genes [21].

In this work, we aim to provide a data integration workflow to build an exhaustive model of interaction between *PA* and the human host. Data were collected through a wide review of literature data, a data curation process, and a meta-analysis of gene expression. We report the pathogenic mechanisms of *PA* infection, a list of direct interactions between the human host and *PA* through protein-protein or metabolite-protein interactions, as well as a description of pathways activated in organs affected by severe *PA* infections.

Materials and methods

Conceptual domains

First, we identified the conceptual domains that organize the information obtained from the literature, providing a hierarchical model of host-pathogen interaction, following a previous experience on COVID-19[2]. Three interaction levels within the host's system was identified: cell, tissue, and organ. For each level, we further identified conceptual domains, describing the interactions with the pathogen (Fig. 1). More details of this model was reported in S-Text 1.

Scoping review step

Every curator performed an independent literature review, following compliance with international reference guidelines using the scoping review method[22]. For each domain, the scoping review results were processed to identify features of *PA* interactions with the host and the direct or indirect effects that they cause within the host itself.

We identified 532 articles, after removing duplicates and non-English documents, using the suitable string for PubMed (Supplementary Text 1). Furthermore, 27 additional articles were added in initial list, considering also characterization of the host response in *PA* infection, both in mouse models and human patients, through “omics” data analysis.

Through these steps, 150 articles were selected, and enriched the conceptual domains of the overall three interaction levels: (1) “cellular interaction level”; (2) “tissue interaction level”; and (3) “organ interaction level”. Full-text articles were evaluated by overall curators to define at best the possible conceptual domains [23]. The working group authors evaluated every full-text article to define at best the possible subdomains. Every unique article was listed in Table-S1 with a reference ID (SR).

Molecular interaction database and human host - PA interactome

The curators have reported protein-protein (PPI) and molecule–protein interactions (MPI) between *PA* and human. All information about the curated interactions and details, such as type of interaction, Uniprot ID, reference, and subdomains of the model, were reported in the curated dataset (Table-S2). The network-based interaction model was built through the exploration of *PA*-host interactions data, based on the SARS-CoV-2-human host experience [6, 7], using data from the human PPI database, obtained by R packages PSICQUIC and biomaRt [24, 25]. A large PPI interaction database was assembled, including 13,334 nodes and 73,584 interactions, comprising *PA*-human host interactions. The mechanisms of infection were estimated by applying the RWR algorithm [26]. Every protein of the *PA* proteome was used as seed in only one run, imposing the limit of 200 closest host proteins to every *PA* protein. Graphical representations of networks were performed by GEPHI 0.9.2 [27]. To allow the gathering of results for network analysis, the R package enrichR was used to carry out gene set enrichment analysis[28], testing on Reactome 2022, KEGG 2021 and WikiPathways 2023 human pathways databases[29-31].

Meta-analysis of the whole transcriptome

A meta-analysis of gene expression was carried out in mouse lung samples to explore DEGs in lungs during *PA* infection, comparing them with corresponding lung samples in healthy controls, reporting in two bioprojects: 12 bulk RNAseq samples in *PA*-infected lung tissues (PRJNA975462; GEO: GSE233206, SRA Study SRP439193)[32] and 6 bulk gene expression data of acute and chronic *PA* pulmonary infection in mouse lung (PRJNA793679; GEO: GSE192890, SRA Study SRP353174)[33]. The SRA data was downloaded by Prefetch and converted into FASTQ files using the fastq-dump tool of the SRA Toolkit software v2.11.0 [34, 35]. Reads were aligned using HISAT2 to the mm10 mouse reference genome[36], while to identify differentially expressed (DE) genes DESeq2 was used[37] (R version 3.4.3, DESeq2 v.1.42.1). For each dataset, the gene counts were analyzed in DESeq2 to define changes in gene expression during *PA* infection, imposing as thresholds Log2FC >|1| and Benjamin-Hochberg False Discovery Rate<5% (BH-FDR). Since the two datasets have been made in different laboratories, a meta-analysis was carried out through metaRNASeq R packages, which allowed to perform a meta-analysis from two independent RNA-seq experiments and calculate the combination of the two p-values by Fisher methods[38]. As a pre-processing step, 21,010 genes, shared between two datasets, were analysed, obtaining a new combined BH adjusted p-value and average Log2FC, considering the batch effect due to two

different experiments. Gene counts with Log2FC >|1| and BH FDR<5% were considered as DEGs for the conditions.

Gene enrichment on DE genes in PA infection and healthy conditions

To give biological meaning to the gathered data in every step, gene enrichment analysis was carried out on each gene\proteins list using Reactome 2022, KEEG 2021, and WikiPathways 2023[29-31]. In this step, the enrichR R package was used to carry out gene set enrichment analysis, considering these statistical results: p-value (Fisher exact test), q-value (adjusted p-value for FDR)[28].

Results

Data integration for model of interaction between PA –human

The curation data process summarised all *PA* data in three main categories: specific conceptual information about the different aspects of *PA* pathogenesis; molecular interactions, proved by experimental models in vitro and in vivo, referred to *PA* and human; pathways identified in differentially expressed genes reported in severe *PA* infections. In Fig. 1, the structure of *PA* infection model was reported. At the cell level, specific aspects of *PA* -host interaction were considered: adhesion/colonization of *PA* into the host (PA-Ad); invasive mechanisms of *PA* and consequent innate immune response of the host (PA-In); role of *PA* exotoxins in infection (PA-Ex); Metabolomic mechanisms of *PA* (PA-Met). At the upper level, all information about *PA* infection was contextualized in three tissues, which are directly involved in *PA* infection, identified as conceptual domains: Endothelial tissue in severe *PA* infection (Endothelial Tissue - EnT); lower airway and alveolar epithelial tissue in the lung, also considering the genetic condition of CF (Airway Epithelial Tissue - AET); and other epithelial tissues, such as desquamated bronchial epithelium and urinary epithelium (Other Epithelial Tissues- ETs). Finally, the review activity was focused on two body districts directly hit by PA, defined as conceptual domains for the “organ” level: injuries due to *PA* infection in the lung, considering also CF, and damage reported to systemic *PA* infection in the bloodstream. The main pathogenic mechanisms in *PA* infection for each domain were reported in Table 1, while the results of conceptual summarization are extensively reported in Supplementary Text. Such information allowed us to define specific mechanisms and experimental models of *PA* infections.

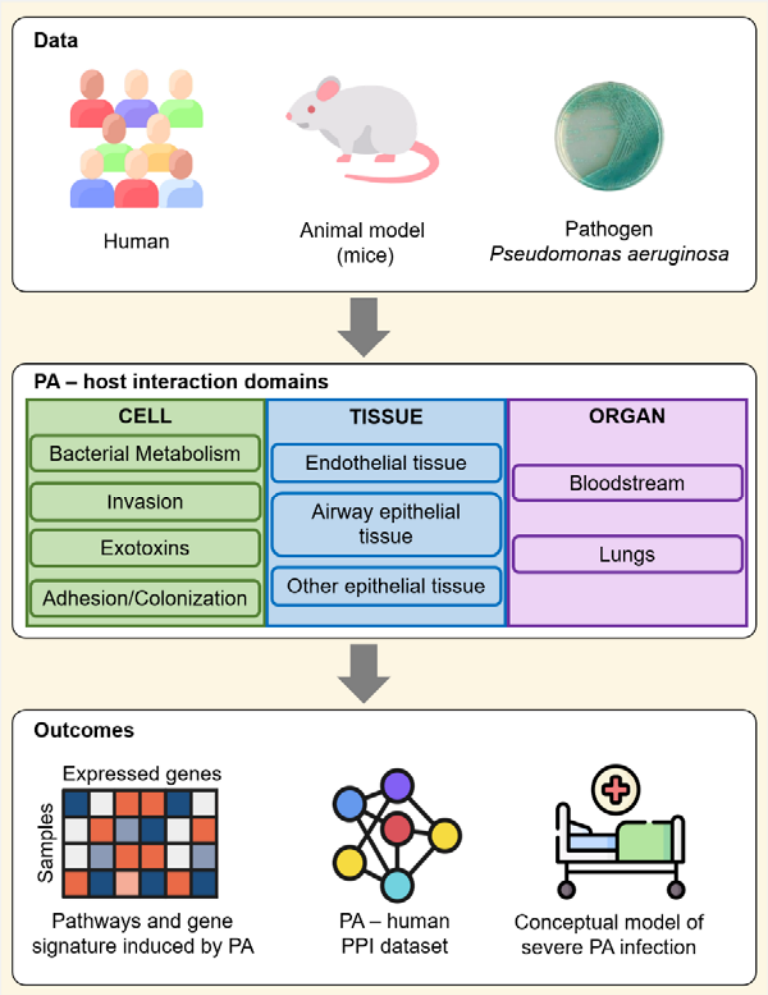


Figure 1: Structure of the conceptual model. For each level, we identified further conceptual domains, describing the interactions with PA.

Table 1: Main conceptual results summarized for each level and domains: Cell (A), Tissue (B) and Organ (C).

A) Cell Interaction Level

1) <u>PA Adhesion/Colonization (PA-Ad)</u>
- The flagellum and pilA contribute to PA adherence in the upper respiratory tract through interaction with IRF-1 (SR10; SR73; SR83) and NEU1 modulates the binding between the flagellum and MUC1 (SR10, SR73)
- The receptors for the pilus-specific interactions are asialo-GM1 and asialo-GM2, or sialic acid containing glycosphingolipids, as well as lactosylceramide and glycoproteins (SR39; SR47; SR79), inducing the expression of MMP7 (SR70)
- The psl is involved in biofilm formation, and it is required for adhesion to human cells and facilitates flagellin-mediated NF-κB activation (SR31).
- The estA, oprD, oprG, oprQ, PA3923 and Paf receptors bind alpha subunit 4 and 5 of LAMA1 and FN1 (SR33, SR56)
- Gb3 is bound by lecA, along with GPI-anchored CD59 (SR14)
- In PA uptake, CD18 receptor in human monocytes and murine neutrophils are strongly requested, as well as N-glycans facilitate integrin-mediated uptake, acting as cell surface ligands (SR2).
2) <u>PA Invasion (PA-In)</u>
- PA survives within macrophages due to mgtC and oprF (SR22)
- flagellin induces the release of EGFR and TGF-α ligand and phosphorylation of MUC1, allowing association with TLR5

(SR30)
- IMPa promotes the collective rolling adhesion of leukocytes, through CD43, CD44, CD55, and PSGL-1 ; interaction with selectins (SR25)
- The pumA inhibits NF-kB and, at the same time interacts with TIRAP, MyD88 and UBAP1, preventing cytokine and TLR receptor signaling (SR34)
- Human SP-A interacts with LPS, limiting TNF-α release (SR43)
- LL-37 inhibits LPS-induced IL-8 production (SR53) and induces mucA mutagenesis, leading to mucoid conversion (SR52)
- lasB degrades on human PBMCs the proteins elastin, collagen and laminin, IgG, C3 and α1-AT, as well as interferon-gamma and IL-2 (SR85);
- In mouse, LPS induces MUC5AC overproduction (SR68)
- Activation of CD95/CD95 ligand system after PA infection: triggers apoptosis; stimulates NF-kB, JNKs, GADD153 and PLA2; interferes with the functions of growth factor receptors, inducing defensins and/or cytokines secretion (SR69)
- In human immune cells, PTEN, complexed to CFTR, promotes intracellular killing of PAs (SR49)

3) Exotoxins (PA-Ex)
- exoS ribosylates Ras, inducing the phosphorylation of MAP kinase, ERK1/2 and PKB/Akt kinase, as well as blocking the activation of Ras, Rho family GTPases, ERM proteins and vimentin (SR82; SR87); inhibits anti-apoptotic pathway(s) controlled by ERK1/2 and p38, and causing aggregation/activation of FADDs within the host cell, inducing the host cell to secrete pro-apoptotic signals (SR46; SR54)
- exoT contains GAP activity towards Rho, Rac and CDC42, and targets CRK/CRKL, causing rearrangement of the actin cytoskeleton (SR116; SR8)
- In mouse, exoT is complexed with Crk and Cbl-b, which leads to the rapid degradation of ExoT by E3 ubiquitination (SR8; SR55)
- exoU requires the chaperone DNAJC5 to reach and act on plasma membrane lipids and promote toxic activity in human cells (SR7)
- exoA catalyzes the ADP-ribosylation of the eukaryotic eEF-2, affecting the protein synthesis of the host cells (SR9), it could be neutralized by HNP-1 (SR16)
- popD and popB interact with lipid bilayers and form pores in cell membrane (SR60)

4) PA Metabolisms (PA-Met)
- Azurin exerts potent cytotoxicity towards host cells, inhibiting cell proliferation by increasing the secretion of aldolase A (SR127)
- 3OC12-HSL inhibits the proliferation of T-lymphocytes, modulates cytokine synthesis and induces the activation of MAPK-p38 (SR64)
- The autoinducer PAI-1 activates the cellular cyclooxygenase 2 gene in human fibroblasts and ECs (SR48)
- In mouse, PNC induces rapid neutrophil death due to increased release of myeloperoxidase and lysozyme and activation of Asm and ROS generation in mitochondria. In human keratinocytes, PNC alters mitochondrial function by causing a decrease in NADPH and ATP levels (SR65; SR87); it induces ceramide formation, causing cell death, and negatively regulates the induction of the proinflammatory cytokine IL-8 (SR24)
- pvrA gene is upregulated during infection and co-ordinately regulates the genes involved in PC and long chain fatty acid catabolism. PC is also one of the major nutrient sources for PA during lung infection (SR103)

B) Tissue Interaction Level

1) Endothelial tissue (EnT)
- APOE3 exhibits antibacterial activity against PA, reducing NF-κB activation in human and mouse monocytes (SR5)
- In mouse, exoS and exoT affect the Lim kinase-cofilin pathway, promoting actin filament severing (SR62)
- In PA-induced acute pneumonia in mouse, cofilin was activated and exoS and exoT inactivated RHO, RAC, and CDC42 GTPases (SR62)
- PA alters endothelium producing metalloproteinases or serine-proteinases that are also virulence factors, such as lasB/pseudolysin which can severely affect the adherence, as well as having a cytotoxic effect (SR57)

2) Airway epithelial tissue (AET)
- PA binds the pseudostratified epithelium via pilY1 and activates the PI3K/PIP3/Akt pathway, promising apical membrane remodelling in the basolateral membrane (SR21)
- PA flagella induces TLR5 activation in macrophages while IL-6, IL-8 and CXCR1 activation cause respiratory burst in neutrophils migrated in AET (SR74; SR66)
- In lesions, the interaction between glycolipid molecules and pilA can cause disruption of tight, adherent epithelial junctions in

AET cells, allowing bacterial invasion (SR37; SR57); it activates IRF-1 in AET cells, rapidly inducing a cytotoxic effect (SR63; SR84)
- exoA interacts with epithelial ADAM10, stimulating transepithelial leukocyte migration and altering protein permeability and epithelial regeneration and integrity (SR15)
- PNC alters ciliary function in airway cells and tracheal mucus velocity in vivo (SR86)
- PA produces PA-IL and PA-IIL which facilitate airway infection, contributing to adhesion by binding to cilia (SR20)
- lasB induces ECs detachment and death through proteolysis of FN1 and degradation of vWf and inter-endothelial junctional proteins (SR56)
- PA stimulates IL-6 production in 16HBE airway ECs, induces CXCL8, TACE expression, and modulates TNF-α reactivity in airway cells (SR77)
- In CF, Vav3 drives the ectopic b1 integrin/FN1 complex, predisposing AET to PA adhesion (SR29)
- In CF, CTFR is involved in the mechanism of PA uptake: CFTR-PA interaction leads to nuclear translocation of NF-kB, promoting inflammation (SR59)
- In macrophages of healthy subjects, CFTR works with TLR4 as a receptor for phagocytosis of PA. The genetic loss of CTFR in CF patients allows the disruption of AET (SR58)

3) Other epithelial tissues (ETs)
- HSPGs are upregulated on the apical cell surface, resulting in increased binding to PAs and subsequent tissue damage (SR44)
- pilA mediate binding and entry onto the apical surface via N-glycans (SR44)
- Flagellar cap protein allows to entry through HSPGs on the basal cell surface and binding to N-glycans on the apical surface of polarised epithelium (SR44)
- T3SS and LPS influence epithelial barrier function (SR61)
- exoS binds FXYD3 and leads to inhibition of Na,K-ATPase, causing increased tight junctions permeability towards ionic and non-ionic solutes (SR40)
- T3SS, along with exoS, exoU, exoT and exoY, are associated with PA keratitis (SR84) and enable PA invasiveness through disruption of tight junctions in eye (SR84)
- In the urinary tract, PA secretes specific virulence factors involved in upregulation of iron acquisition pathways via PA iron-binding Fur regulator (SR50)

C) Organ Interaction Level

1) Lung
- The CF pulmonary tissue is characterized by activation of different proinflammatory signaling cascades, along with chronic bacterial infection in the lower airways (SR74; SR76)
- PA LPS is specifically recognized and bound by CFTR, allowing the PA uptake and activation of NF-kB (SR60)
- CFTR, together TLR4, works as a receptor for phagocytosis of PA, and the activation of TLR5 by flagellin is required to trigger proinflammatory cytokine production (SR58; SR74)
- TRPV4 gene enhances host innate immune defenses and protects the lung from injury after PA pneumonia (SR11)
- In CF innate immunity, PA elastase induces cleavage of monoclonal and polyclonal IgG, resulting in inhibition of PA phagocytosis (SR75)
- PA induces NET formation due to LL-37, and the release of proinflammatory cytokines together with CLEC5A (SR99)
- In patients with COPD, laminin deposition is increased in the airways because of abundance of receptors of PA (SR6)
- MIF reduces lung inflammation after PA infection in mouse (SR39)
- In PA-infected alveolar macrophages, cytokine signaling and interleukin signaling are upregulated, regulating the actin cytoskeleton and the JAK/STAT and MAPK signaling pathways (SR90)
- In chronic PA-infection, neutrophils and interstitial macrophages cells increase, while that of alveolar macrophages cells decrease significantly (SR105)
- The PA infection in mouse lung inhibits the super pathway of quinolone, alkylquinolone biosynthesis and 2-heptyl-3-hydroxy-4(1H)-quinolone biosynthesis in gut microorganisms (SR98)

2) Bloodstream and systemic infection
- In septic shock, TREM-1 amplifies the inflammatory response and its deletion protected mice during septic shock by modulating inflammatory responses, even against PA (SR4)
- During systemic infection, QS genes in PA are down-regulated, while expression of pqsH increases (SR92)
- In sepsis of thermally injured mice, PA affects the blood metabolome, involving abundance of thymidine, thymine, uridine and uracil (SR96)
- Hxu system is an important signal transduction pathway that contributes to adaptive PA pathogenesis in BSI. In PA-induced murine sepsis, the deletion of hxuIRA genes reduce the BSI infection effects, whereas their overexpression increases the BSI effects (SR100)
- The immune cells after stimulation with PA shows that myeloid cells response more than CD4+ and CD8+ T cells (SR104)

Molecular interactions between human cells proteome and PA molecules during infection

We manually curated the molecular interactions between *PA* and human proteins during different steps of the infection. We found many direct interactions between human and *PA* molecules from 150 articles, mostly protein-protein and molecule–protein interactions (PPI and MPI).

Table-S2 shows the details of the curated interactions, such as type, Uniprot ID, reference, and subdomains of the model.

We identified 152 proteins/molecules: 109 human proteins, 3 human molecules, 34 *PA* proteins, and 5 *PA* molecules. There were 189 PA–human interactions and only 7 human-human interactions. Every interaction reported here was enclosed in domains of cell interaction levels: Adhesion process (PA-Ad), Invasion and injury of tissue (PA-Inv), Exotoxin production (PA-Ex) and bacterial metabolism (PA-meta) (S-Fig. 1). Gene enrichment analysis was applied on all the proteins in the data set. In Table-S3 in the Supplementary Text, the top ten most enriched pathways were reported for three databases: KEGG 2021, Wikipathway 2023, and Reactome 2022. It is worth noting that the Wikipathway showed enrichment of the “Pathogenic Escherichia Coli Infection WP2272" pathway, while KEGG showed "Pertussis" (FDR<0.0001%). In Reactome, the top three pathways with FDR<0.0001%, such as Signalling By Interleukins R-HSA-449147 (Fig. 2). Some PA proteins were shown to play a main role in modulating these pathways, such as exoS and exoT, which inhibit interleukin proteins or degrade Occludin (OCLN) that regulates cell death[39]. Notably, among the proteins in Signalling By Interleukins R-HSA-449147, fibronectin (FN1), which is involved in cell adhesion, blood coagulation, and innate immunity.

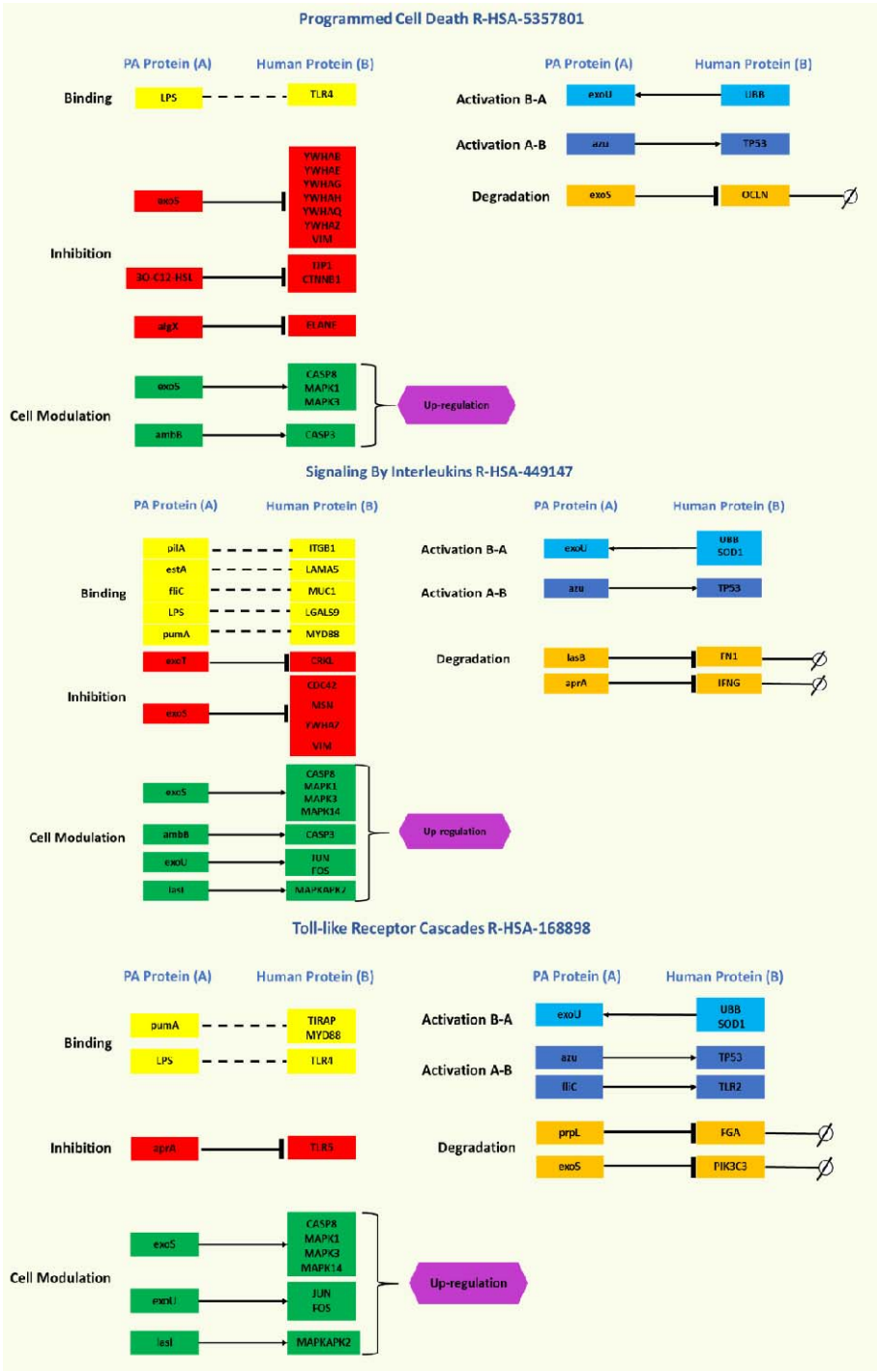


Figure 2: PA-human interaction diagrams for the most significant pathways, from GSEA on Reactome.

A network of interactions between *PA* and host proteins was applied (Fig. 3). Such network showed the overall cell response to the infection, highlighting new possible pathogenic mechanisms. Some peculiar pathways were shown as involved: Complement Cascade Pathway (Reactome R-HSA-166658; 18/55; FDR<0.0001%) would be affected by outer membrane proteins oprH, oprQ, and the elastase lasB, contrasting cell damage by binding complement proteins like C3. Another aspect was the interaction of oprH, oprQ, and lasB with blood clotting proteins (VWF, SERPINF2, PLAUR, PLAT, and PLG). GSEA on the Wikipathway indicated the interaction with coagulation cascades (Complement and Coagulation Cascade WP558; 20/58; FDR<0.0001%), indicating their potential involvement in thrombosis. The key role of exoS in *PA* infection was also supported: our network-based model suggested a combined action of exoS, exoY, and exoT, which could trigger cell toxicity after binding to cytoplasmic proteins 14-3-3 (i.e. YWHAB).

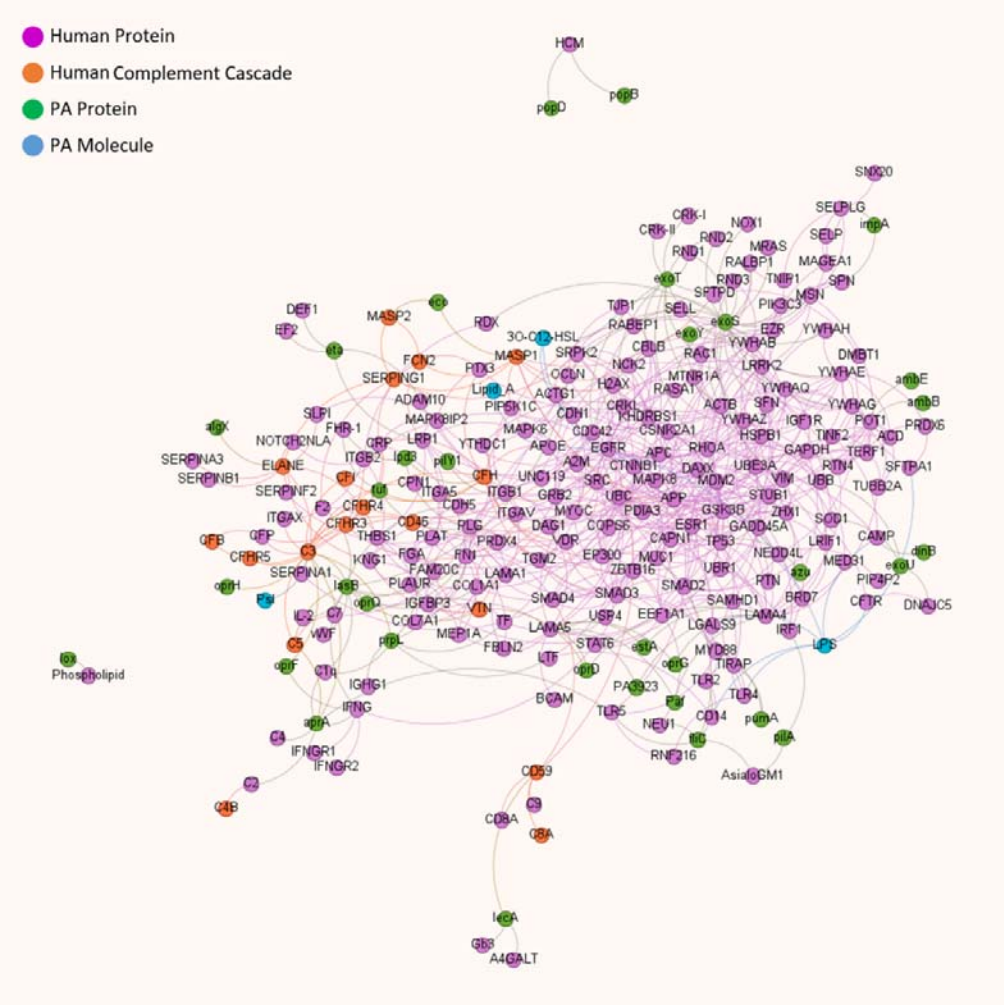
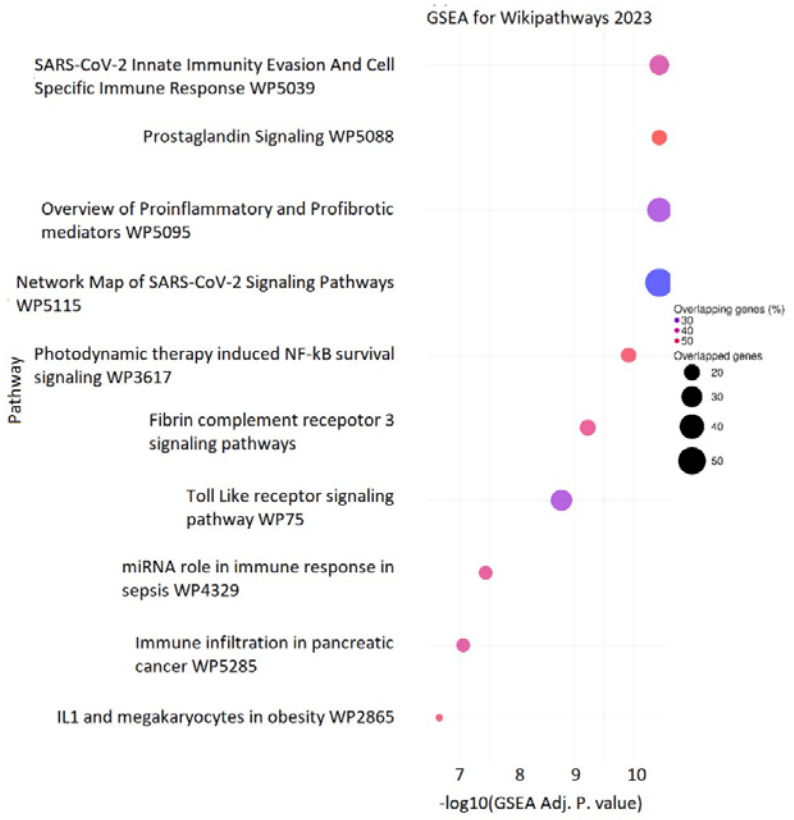


Figure 3. Network of human PPI and PA-human interactions, with the top 200 nearest proteins found by RWR. Nodes and edges have different colours to show different kinds of molecules.

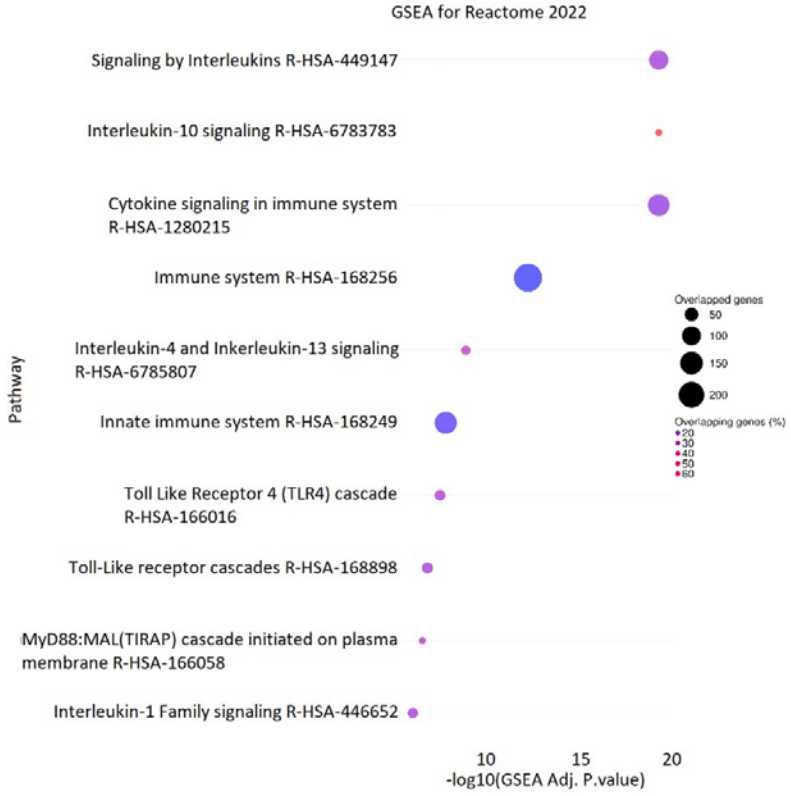
Meta-analysis of whole transcriptome in PA -infected lung tissues

We performed a meta-analysis of gene expression in mouse lung samples to identify DEGs in the lung during *PA* infection, compared to healthy controls, using two bulk RNAseq datasets: GSE233206 and GSE192890. To bring out a unique signal of infection due to *PA* in organs, the meta-analysis on over DEGs in both bioprojects allowed us to obtain a new list of DEGs. 1,560 genes were up-regulated in response to *PA* infection in the lung and 383 were down-regulated (Log2FC > 1; FDR BH<0.05, Table-S4).

In Wikipathways, the up-regulated DEGs in combined gene list showed an enrichment of inflammation pathways in infection, such as “Overview of Proinflammatory And Profibrotic Mediators WP5095” (39/129) (FDR<0.0001%). The GSEA on Reactome showed high consistency with the model proposed in the scoping review process. The most significant pathways (FDR<0.000001%) were Signalling By Interleukins R-HSA-449147 (103/453), Interleukin-10 Signalling R-HSA-6783783 (31/45), Cytokine Signalling In Immune System R−HSA−1280215 (145/702) (Fig. 4a/b). However, no host gene targets for *PA* proteomes, identified as belonging to proinflammatory pathways, were upregulated. These results suggest that this pathway is directly involved in initiating the innate response to *PA* infection, but also highlight the potential role of *PA* proteins in modulating and limiting this response, especially for interleukins



a)



b)

Figure 4. GSEA with Reactome 2022 (a) and Wikipathways 2023 (b), based on up-regulated DEGs in *PA* -infected samples, obtained from meta-analysis in two Bioprojects in mouse lung tissues.

Discussion

In this paper, we provide a data integration useful to build a model of *PA* infection. Such information enabled us to create a comprehensive and detailed database of interactions between *PA* and the human host through a useful approach to construct an organized repository of biological information supported by different levels. A model of infection aims to reflect the extreme complexity of severe infection caused by bacteria, reporting mechanisms of infection at specific biological levels and identifying the role of the pathogen to induce the response against the pathogen, even applying network-based explorative models [6, 7, 40]. *PA* represents a warning alert for public health because of multi-drug resistance (MDR) and ability to induce acute or chronic infection in many pulmonary disease patients[41], but meanwhile, it represents a microorganism model to study severe systemic infection. Although many pathogenic aspects of this bacterium remain unclear, basic knowledge about pathogenesis, virulence, host response, and therapeutic development in *PA* infection has already been unravelled. Its rapid adaptation to gain resistance to antibiotic treatments and the capability to produce biofilm, allowing it to survive in a hypoxic atmosphere, makes it extremely difficult to treat[41, 42]. In this context, the literature curation data process and application of the computational explorative approach can lead to gathering information about *PA* infection, providing a new tool to investigate the deep interaction between *PA* and host immune responses and cell signalling pathways. This experience allowed us to establish an annotated database of PPI/MPI between *PA* and the human host, highlighting the complexity of the relationship between the pathogen and its host and showcasing the many strategies employed by *PA* to establish infection. Firstly, the main role of exoS during *PA* infection was further confirmed: in fact, this computational experiment suggested a coordinated activity among exoS, exoY, and exoT[43]. In our workflow, exoS inhibits several proteins of interleukin pathways or degrades Occludin (OCLN), an integral membrane protein involved in cytokine-induced regulation of the tight junction permeability barrier, inducing cell death. The ADP RT activity of exoS allowed it to modulate host cell apoptosis, inducing PA -afflicted host cell apoptosis by targeting a variety of Ras proteins[44]. The Complement Cascade Pathway could be modulated by outer membrane proteins oprH, oprQ, and the elastase lasB, which would play a role in a cytotoxic effect, binding complement proteins (i.e. C3), as well as adhesion[45]. This result mirrors the mechanism of

activation of the complement system, in which C3 is the main actor against bacteria, through a link with oprF, a porin involved in ion transport (Na^+ and Cl^-), but also in anaerobic processes and biofilm[46, 47].

An interesting result is surely the interaction of oprH, oprQ, and lasB with coagulation proteins, suggesting their activity in the thrombotic process. Cleavage of a C-terminal- peptide FYT21 derived from thrombin, by *PA* lasB, inhibits activation of the transcription factors nuclear factor NFκ-B and activator protein 1 (AP-1). An interesting new point of view was led by the modulative effect of *PA* protein set on Signalling by Interleukins pathway, reporting a significant link with *PA* infection in the lung. The *PA* proteomics seems to play a modulating role on host response, where specific bacterial proteins aprA and lasB, as well as exoS, have an inhibiting effect on overall interleukin pathways [43, 48, 49]. However, although *PA* limited the interleukin response, the decrease of *PA* in CF patients is linked to proinflammatory cytokines decreasing[50]. Such an effect was confirmed in *PA* infection, where IL-1β production is negatively induced by *PA*-derived DnaK by cross-talk between J NK and PI3K/PDK1/FoxO1 pathways[51].

Our findings provide valuable insights into the complex interplay between *PA* and the human host and pave the way for building disease maps specifically for severe infection in *PA*. Surely, understanding molecular perturbations in severe outcomes of bacterial systemic infection can be improved by static diagrams of molecular interactions, scaffold for omics data integration from real cases, predictive computational models, and employing of text mining and AI-assisted analysis (i.e. LLM) to identify drug targets[13], as well as to develop a digital model for immune systems for sepsis and pneumonia infection[52]. Our study has some limitations. Although several significant *PA* -human interactions have been gathered and reviewed, there may be other interactions not described in our model. In the future, an enlarging of the PPI/MPI dataset should be upgraded, considering new cutting-edge papers about in vitro and in vivo experiments. The PPIs/MPIs were collected from in vitro *PA* -human host interaction, not considering tissues and organs involved in *PA* infection. Thus, although the pathogenic mechanisms described are based on experimental data, these would need to be confirmed in use cases of *PA* severe systemic infections.

Finally, the DE metanalysis was carried out in the mouse model and with a limited sample size. Although such data cannot be overlayered on the *PA* -human interaction diagram and it should be confirmed with clinical data., this result provided an overview of gene expression host signature in *PA* infection.

Conclusion

The results of our work allowed us to start filling up a model for *PA* infection, defining specific steps in *PA* infections, and providing a significant step forward in understanding the deep interaction between *PA* and the human host. Our study represents a significant step forward in understanding the deep interaction between *PA* and the human host in a systemic infection context, applying a data integration workflow. This allowed us to shed light on the molecular pathophysiology in invasive bacterial infection and how direct interactions among proteins of *PA* and host, as well as involvement of pathways, could be linked to clinical phenotypes in sepsis. This effort will provide a valuable resource for future clinical research of infectious diseases, influencing the development of more effective treatments and looking at applying precision medicine approaches to severe bacterial infections and improving personalized care and patient-tailored treatments in severe systemic infections.

Contributors

FM and CF designed the study. FM, CR and CF developed the search strategy with feedback from LL and BS. FM, CR, VD and MP screened and selected studies. CR, VD, MP and BR extracted the data and prepared the data for analysis. FM, LL, BS, LG, MGB, GC and CF verified the study data. FM and LL analysed the data. FM, CR, VD and MP wrote the first draft of the manuscript. FM, MGB, GC and CF, writing—review and editing. MGB and CF, supervision. FM, and CF, project administration; FM, LG, and CF, funding acquisition. All authors critically revised the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The datasets generated and analysed in this meta-analysis are published in the supplementary text and in supplementary tables. Additional information collected from studies and the bioinformatics pipelines is available from the corresponding author on reasonable request.

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