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Multi-omic and computational approaches for biomarker discovery and clinical translation in pediatric sepsis

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Sepsis remains one of the most complex and lethal syndromes in pediatric critical care, driven by dysregulated and heterogeneous host responses to infection. Despite decades of biomarker research, few biomarkers have been translated into routine clinical use for diagnosis or prognostication. This is largely because single marker approaches cannot capture the systemic complexity of sepsis pathobiology with high sensitivity and specificity. This review explores how the convergence of multi-omic technologies and computational biology is transforming biomarker discovery, from isolated molecular signals into integrated, systems-level understanding of disease, with an emphasis on pediatric sepsis. Recent omic studies reveal dysregulation across immune, endothelial, metabolic, and microbial networks in sepsis. Advances in bioinformatics and artificial intelligence now enable characterization of complex biological patterns that link molecular profiles into interpretable clinical phenotypes and outcomes. Multi-omic integration represents a paradigm shift in pediatric sepsis research, uniting biomarker discovery with clinical application through biologically coherent, computationally derived signatures. As these approaches mature, they promise to transform pediatric sepsis care from empiric treatment to precision medicine guided by the molecular pathways that define each patient's pathobiology.

KEYWORDS

bioinformatics, biomarker, critical illness, ICU, multi-omics integration, omics, pediatrics, sepsis

Introduction

Sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016), remains a leading cause of morbidity and mortality worldwide (Martin et al., 2009; Fleischmann et al., 2016; Fleischmann-Struzek et al., 2020). Despite decades of investigation, progress in developing effective targeted molecular therapeutics for sepsis has been limited (Marshall, 2014). A major barrier in sepsis research is the enormous biological and clinical heterogeneity among patients. Differences arise not only from the underlying pathogen, source of infection, age, and comorbidities, but also from the trajectory of the host immune response, which can range from hyperinflammation to immunosuppression (Yarnell and Fralick, 2024; Dellinger and

Bartock, 2025). This heterogeneity complicates the discovery, validation, and clinical translation of biomarkers, with many candidate markers showing promise in single cohorts for prognostication, or to serve as therapeutic targets, yet fail to replicate across diverse populations.

A biomarker is broadly defined as a measurable indicator of normal biological processes, pathogenic states, or responses to therapeutic interventions. To date, only a handful of biomarkers have consistently reached clinical application in sepsis, and even these have important limitations. Procalcitonin (PCT) is the most widely validated biomarker of bacterial infection, with trials demonstrating its utility in guiding antibiotic discontinuation (Bouadma et al., 2010; Shehabi et al., 2014; de Jong et al., 2016). C-reactive protein (CRP) is widely available as an acute-phase reactant and marker of inflammation, but lacks specificity (Jiang et al., 2023). Lactate has been firmly established as a prognostic biomarker, reflecting tissue hypoperfusion and correlating strongly with mortality risk (Rocha et al., 2013; Abdelaziz et al., 2024). Cytokines such as interleukin-6 and tumor necrosis factor- α provide mechanistic insight into host immune responses, but their short half-lives and variability have limited clinical implementation (Damas et al., 1992; Bouadma et al., 2010). Collectively, these markers provide incremental value but do not fully capture the systemic complexity of sepsis pathobiology.

Recognition of these limitations has fueled a shift from single-analyte biomarkers toward high-dimensional approaches that attempt to more accurately capture patient pathobiology. Advances in transcriptomics, proteomics, metabolomics, epigenomics, and metagenomics now allow simultaneous profiling of thousands of molecules, offering unprecedented resolution into the host–pathogen interface (Van Nynatten et al., 2025b; Van Nynatten et al., 2022; Tong et al., 2025; Leonard et al., 2024). Accordingly, this multi-omic profiling, in conjunction with rapid advances in computational analysis of large biologic data, is enabling the characterization of disease mechanisms that were previously unattainable and incomprehensible. In this review, we discuss emerging strategies for biomarker identification and validation, with an emphasis on critically-ill pediatric patients with sepsis; highlight examples of novel high-throughput omics technologies and computational analyses in pediatric sepsis biomarker discovery; and consider future clinical applications of biomarkers for identifying dysregulated molecular pathways in patient pathobiology.

Conceptual framework for biomarker development in sepsis

Classes of biomarkers

Traditionally, biomarkers were hypothesized to facilitate clinical diagnosis and disease monitoring. However, with advances in omics technologies and computational biology, biomarker classifications can now be further subdivided to better capture their diverse roles in translational research and precision medicine.

Predictive biomarkers identify those patients that are most likely to respond to a given therapy or intervention (Jørgensen, 2021). In the context of sepsis and other critical care syndromes, these

biomarkers help determine which patients would most benefit from enrollment in clinical trials based on their pathobiology. This is known as ‘predictive enrichment’. Prognostic biomarkers provide information about the outcomes that patients are likely to experience, such as mortality (Stanski and Wong, 2020; Leonard et al., 2024). This is known as ‘prognostic enrichment’, and such biomarkers are crucial for risk stratification, patient triage, and informing anticipated outcomes. Their value lies in identifying high-risk subgroups who require closer monitoring or intensified therapy. Mechanistic biomarkers directly link underlying biological pathways to clinical outcomes (Robinson et al., 2013), thus identifying pathways that may be targeted by new interventions. Dynamic biomarkers recognize that disease trajectories are not static. Temporal measurements can capture evolving host responses and therapeutic impacts in real time. These trajectories may better predict outcomes, identify windows of opportunity for intervention, and explain heterogeneity that single, cross-sectional measurements cannot.

Finally, multi-omic strategies integrate data not just from one biomarker type but from multiple platforms (genomics, transcriptomics, proteomics, metabolomics, etc). These integrated biomarkers reflect systems-level pathobiology and offer a more holistic framework for patient stratification (Maslove et al., 2022). As machine learning and network analysis approaches mature, such multi-dimensional signatures are poised to reshape biomarker validation and clinical application.

Characterizing molecular pathways

Traditional biomarker discovery in sepsis has often focused on individual markers measured in isolation. While measuring individual markers is pragmatic, they capture only a limited aspect of the host response to infection (Van Nynatten et al., 2025a). In contrast, multi-omic platforms (transcriptomic, proteomic, metabolomic, and epigenomic) provide a comprehensive, organism-wide, assessment of biology, enabling interrogation of entire cellular pathways and interacting networks rather than single molecules. This shift in framework highlights that the most informative biomarkers may not be individual analytes, but rather constellations of analytes that are aligned with or identify particularly dysregulated biological pathways. Pathway dysregulation captures the dysfunction of molecular networks, offering mechanistic insight into how disease processes unfold, not just merely that they exist. Collectively, multi-omic data demonstrates that clinical heterogeneity in pediatric sepsis arises from distinct patterns of pathway dysregulation rather than variations in individual analytes.

Examples of altered cellular pathways identified through multi-omic integration include immune activation and immunosuppression, endothelial dysfunction and coagulation, metabolic reprogramming and mitochondrial dysfunction, neuroendocrine and stress responses, cell death and repair, epigenetic and transcriptomic remodeling, and microbiome-host interactions. For example, whole-blood transcriptomics consistently identifies subclasses of pediatric sepsis patients characterized by suppressed adaptive immunity and glucocorticoid-receptor signaling pathways, alongside pro-inflammatory activation, with higher-risk phenotypes associated with worse outcomes (Yang

et al., 2023; Wong et al., 2014). These signatures have led to the development of multi-protein risk panels as described by the PERSEVERE study (and PERSEVERE-II/XP), linking immune activation and immunoparalysis to prognosis (Wong et al., 2009; Wong, 2013; Wong et al., 2012; Randolph, 2017; Wong, 2019; Wong et al., 2017a). Similarly, proteomic studies identify panels of inflammatory proteins in patient plasma (including markers such as IL-8, MCP-1, HSP-70, hyaluronan, M-CSF and IL-6) that associate with clinical variables and illness severity (Leonard et al., 2024). Plasma profiling of endothelial pathway proteins demonstrates an angiopoietin-2/angiopoietin-1 imbalance early in sepsis, correlating with organ dysfunction and severity, underscoring significant endothelial activation and microvascular injury (Melendez et al., 2019; Whitney et al., 2020; Richter et al., 2020). Metabolomics profiling in pediatric septic shock highlights metabolic reprogramming and mitochondrial stress (amino acid and energy-pathway shifts, elevated acylcarnitines) pathway dysregulation, consistent with an energy-failure phenotype (Mickiewicz et al., 2013). Complement and innate immune pathways are perturbed, with increased activation fragments and complement components associated with illness severity, suggesting overactive complement signaling in pediatric sepsis (Hazelzet et al., 1998; Li et al., 2021). In parallel, damage-associated signals and nucleosomal/DAMP readouts have been linked to illness severity, aligning with heightened cell death and danger signaling (Alcamo et al., 2019). Finally, epigenetic and transcriptomic remodeling, including steroid-associated repression of adaptive immunity programs, helps explain persistent immune dysfunction often observed in sepsis (Wong et al., 2014). Neonatal and younger pediatric methylome studies echo this theme, suggesting lasting immune reprogramming after sepsis (Wong et al., 2014; Lorente-Pozo et al., 2021). Collectively, these multi-omic data emphasize that pediatric sepsis is not characterized by a single unifying mechanism but by convergent dysregulation across immune, endothelial, metabolic, and stress-response networks, offering multiple potential targets for biomarker development and therapeutic enrichment strategies.

Omics-driven discovery pipelines

The adoption of multi-omic technologies (the integrated profiling of genes, transcripts, proteins, metabolites and microbes) has ushered in a new era of biomarker discovery in critically-ill pediatric patients with sepsis, facilitating systems-level characterization of host responses. Each omic methodology provides unique information related to sepsis pathobiology: from inherited susceptibility to dynamic molecular responses and metabolic consequences of disease. Crucially, each modality also has clinical implications that influence its utility in biomarker development.

Genomics interrogates our inherited DNA sequence variation (single-nucleotide polymorphisms, copy number alterations, gene variants) that modulate the immune responses, susceptibility to infection and clinical outcomes that occur in sepsis. Assays range from SNP-genotyping arrays (high-throughput and lower cost but limited to known variants) to whole-exome sequencing (WES) and whole-genome sequencing (WGS) (comprehensive coverage but higher cost, greater computational burden and greater challenges in interpretation) (Wong, 2012; Atreya and Wong, 2019; Hurtado

and Sanchez-Pinto, 2025; Liu et al., 2021). Clinically, genomics is attractive because DNA is stable and sample collection is relatively simple and efficient. However, the static nature of the genome nucleotide sequence means it does not reflect the evolving host response during sepsis. Yet, in pediatric sepsis, multiple gene-association studies have identified polymorphisms in genes such as TLR4, LBP, BPI, HSP70 and IL6 that stratify risk of sepsis development and disease severity (Jabandziev et al., 2014). Rare deleterious variants in genes such as LTBP4 have been associated with severe pediatric sepsis phenotypes (PedSep-D) (Qin et al., 2025). NOD2/CARD15 gene SNPs contribute to sepsis susceptibility and clinical outcomes (Tekin et al., 2012), with significantly higher frequency of the R702W, G908R, and Leu1007fsinsC genotypes prevalent among children with sepsis admitted to pediatric intensive care units. Notably, the Leu1007fsinsC variant is also significantly associated with mortality. Thus, genomics lays the foundation for understanding predisposition to disease and may enable risk enrichment, but by itself lacks dynamic information about the current state of cellular dysregulation in sepsis.

Epigenomics investigates the modifiable chemical and structural modifications of chromatin, such as DNA methylation and histone modifications, that regulate gene expression without altering the original DNA sequence. Typical assays include bisulfite conversion followed by sequencing or methylation microarrays, ChIP-seq for histone marks or transcription-factor binding, and ATAC-seq for open chromatin. From a technical standpoint, epigenomics captures changes in DNA regulation in response to environment or infection during sepsis. However, many assays require large cell numbers or high-quality input material, are cell-type or tissue-specific (making PBMCs potentially less reflective of relevant organ compartments), and interpretation of epigenetic changes in children (with age-dependent changes) remains challenging. Clinically, epigenomic biomarkers have the potential to characterize dynamic genomic changes in sepsis, but turnaround time, cost and lack of standardized pediatric reference ranges hamper translation (Papareddy et al., 2025). Emerging pediatric studies suggest epigenetic reprogramming of immune and metabolic gene networks that occur in sepsis. For example, DNA methylation patterns in preterm infants with early-onset *versus* late-onset sepsis, reveal significant epigenetic reprogramming in leukocytes between septic and control groups, with differential methylation patterns that may contribute to sepsis-induced immunosuppression (Lorente-Pozo et al., 2021). Similarly, temporal genome-wide DNA methylation profiling in newborns identified 333 genomic regions with dynamic methylation changes that regulate transcription factors mediating shifts in neutrophil-to-lymphocyte ratios (NLR) (Martino et al., 2024). Healthy newborns with lower NLRs at birth were more likely to subsequently develop sepsis, and genetic variants were associated with baseline NLR levels, suggesting both epigenetic and genetic factors influence early immune development and sepsis susceptibility (Martino et al., 2024).

Transcriptomics quantifies gene-expression (RNA) to reflect active cellular responses during infection or injury. Transcriptomics measures which genes are actively transcribed into RNA, indicating which instructions the cell is currently following (Wang et al., 2023). Common platforms include microarrays, bulk RNA-seq (RNA profiling across multiple cells

or tissues) and single-cell RNA-seq (RNA profiling for individual cells). Bulk RNA-seq is relatively accessible but does not provide information about cellular heterogeneity and depends on robust RNA quality. scRNA-seq offers granular information about transcription at the single-cell level, but is expensive, lower throughput, and computationally intensive. Clinically, transcriptomics offers a window into the dynamic host response, enabling discovery of early biomarkers and endotypes, yet challenges remain in terms of sample timing, leukocyte composition effects, RNA stability and assay turnaround constraints (Wang et al., 2023).

In pediatric septic shock, transcriptome profiling has characterized distinct endotypes (A, B and C) that demonstrate differential immune suppression and glucocorticoid signaling, with endotype A also demonstrating higher illness severity and mortality (Wong, 2012; Wong et al., 2009; Wong et al., 2011). Recently, subclasses associated with higher organ failure and mortality based on distinct transcriptome patterns have been reported (Yang et al., 2023). Whole blood RNA sequencing has been able to identify two distinct pediatric septic shock subclasses with major biological and clinical differences. Subclass 1 is characterized by upregulation of innate immunity pathways, downregulation of adaptive immunity pathways including B and T cell activity, lower percentages of CD4 T cells and B cells, less diverse T cell receptor repertoires, elevated plasma inflammatory cytokines and endothelial injury biomarkers (Yang et al., 2023). Despite having similar illness severity at initial presentation Subclass 1 patients had significantly worse clinical outcomes including higher organ dysfunction scores, greater need for cardiovascular support, longer ICU stays, and fewer hospital-free days compared to Subclass 2 (Yang et al., 2023).

Proteomics measures proteins, the functional effectors of subcellular response during infection, using platforms such as mass spectrometry and high-throughput affinity-based multiplex assays (Olink PEA, SOMAscan aptamer arrays, Luminex bead arrays). Mass spectrometry allows unbiased discovery of thousands of proteins, not depending on pre-selected targets. However, it has practical limitations struggling to detect low-abundance proteins, require larger sample volumes, is lower throughput and relatively expensive for large studies. Multiplex affinity assays require less volume and are more clinically feasible, but depend on antibody/aptamer specificity, provide only relative quantification, and cover only pre-specified analytes. Clinically, proteins are the effectors carrying out biological responses during infection, making them biologically interpretable and clinically relatable. However, biomarker translation is impeded by patient heterogeneity, pre-analytical variability (sample handling, freeze-thaw), inter-laboratory variability and batch effects (Fan and Zeng, 2025).

Several proteomic studies have investigated protein markers in critically ill pediatric patients with sepsis. An aptamer-based platform measuring 1,305 proteins in serum samples from 40 children with sepsis compared to 30 post-cardiopulmonary bypass surgery controls, identified 111 proteins significantly differentially expressed between groups, with 55 previously reported in sepsis literature and 27 representing novel associations (Shubin et al., 2020). Using weighted gene correlation network analysis, they identified 76 proteins highly

correlated with clinical sepsis traits (Shubin et al., 2020). In a targeted mass spectrometry study, 40 pediatric sepsis patients compared to 24 healthy controls, identified 44 differentially expressed proteins. From this discovery set, six proteins were selected for further validation: lactoferrin, serum amyloid-A1, complement factor B, leucine-rich alpha-2 glycoprotein (LRG1), soluble interleukin-2 alpha chain receptor (sCD25), and soluble haptoglobin-hemoglobin receptor (Pilar-Orive et al., 2022). SAA-1, sCD25, and LRG1 were able to identify sepsis patients with high sensitivity and specificity (AUC > 0.9). In treatment response studies, serum proteomes compared before and after 7 days of continuous renal replacement therapy (CRRT) identified biomarkers reflecting the clinical benefits of continuous renal replacement therapy (CRRT) in pediatric sepsis. Among 145 differentially expressed proteins, lysozyme C (LYZ) and leucine-rich α 2-glycoprotein (LRG1) were significantly elevated after CRRT and correlated with organ recovery, suggesting they may serve as novel indicators of therapeutic response to CRRT in septic children (Cui et al., 2023). Collectively, proteomics connects what is happening inside cells and what's observed clinically (Fraser et al., 2020a; Fraser et al., 2020b; Patel et al., 2023; Iosef et al., 2023).

Metabolomics and lipidomics capture small molecules that reflect the downstream biochemical consequences of gene and protein action and thus mirror real-time metabolic state (such as mitochondrial function, lipid metabolism, energy utilization). Assays include nuclear magnetic resonance (NMR) spectroscopy (broad but low sensitivity) and gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) (higher sensitivity and broader coverage). Targeted metabolomics quantifies a predefined set of known metabolites with high accuracy and sensitivity, making it ideal for hypothesis-driven or validation studies. In contrast, untargeted metabolomics broadly profiles all detectable metabolites to discover novel biomarkers or pathways, providing a comprehensive but less quantitative view of metabolic changes. Metabolomics is positioned to identify immediate physiologic perturbations, making it highly relevant for acute illness (Fraser et al., 2020c). However, interpretation is challenging because of dependence on sample timing, pre-analytical variables (nutrition, medications, sample handling), high inter-individual variability (especially in children of varying ages and developmental stages), and difficulty in tracing metabolites to specific tissues or cell types (Mickiewicz et al., 2013; Wildman et al., 2023).

In pediatric sepsis, metabolomic profiling has provided insight into the role of mitochondrial dysfunction in children with sepsis. Specifically, energy metabolism appears to be substantially altered in early stages of sepsis. A prospective observational study using metabolomic profiling in 161 children with sepsis found that mitochondrial dysfunction in peripheral blood mononuclear cells (PBMCs) is closely linked to an immunoparalysis phenotype and heightened systemic inflammation (Weiss et al., 2020). Children with sepsis demonstrated significantly lower mitochondrial respiration compared to controls. Those with low mitochondrial respiration had diminished *ex vivo* lipopolysaccharide (LPS)-stimulated tumor necrosis factor- α (TNF- α) production and reduced monocyte human leukocyte antigen-DR (mHLA-DR) expression, both markers of immune suppression. Furthermore, the subset of patients with immune paralysis or low mitochondrial respiration exhibited the highest circulating levels of inflammatory

cytokines, including interleukin (IL)-8, IL-10, and monocyte chemoattractant protein-1 (MCP-1), suggesting a paradoxical coexistence of immune suppression and exaggerated inflammation (Weiss et al., 2020).

Moreover, NMR-based metabolomics in critically-ill pediatric patients was able to identify key metabolites (2-hydroxybutyrate, lactate, glucose, ketone bodies) that distinguished patients with sepsis from controls with excellent accuracy (Mickiewicz et al., 2013). Metabolomics significantly outperformed traditional clinical predictors like procalcitonin and PRISM scores for mortality prediction (Mickiewicz et al., 2013). Indeed, NMR-based metabolomic profiling also seems effective in distinguishing infection type and severity. In 113 critically-ill children, metabolites provided excellent ability to identify bacterial and viral infection from post-cardiac surgery control subjects (Grauslys et al., 2020). Key differentiating metabolites included 3-hydroxybutyrate, lactate, phenylalanine, urea, and valine (increased in infection) and 2-hydroxyisobutyrate, isoleucine, and pyruvate (decreased in infection). The study also showed modest discrimination between sepsis with organ dysfunction *versus* infection without organ dysfunction, with five metabolites (histidine, glutamine, creatinine, creatine phosphate, alanine) showing lower levels in those with multiple organ dysfunction, suggesting distinct metabolic phenotypes that could aid in diagnosis and management of pediatric sepsis (Grauslys et al., 2020).

Similarly, wide-ranging alterations in lipid profiles occur in pediatric sepsis (Bermudes et al., 2018). Sphingolipids are crucial for the regulation of the endothelial barrier and the maintenance of vascular integrity. This regulation mainly occurs through the metabolism of sphingomyelin to ceramide by sphingomyelinases, the subsequent conversion of ceramide to sphingosine by ceramidases, and finally the generation of sphingosine-1-phosphate (S1P) from sphingosine *via* the sphingosine kinase (SPHK) 1 and 2 pathways (Fu et al., 2018). In a study of 48 children with sepsis, untargeted plasma lipidomics identified 1,257 differential lipids compared to controls, with widespread decreases in lipid abundance but marked increases in fatty acids (especially arachidonic acid) that correlated with higher pSOFA scores and worse outcomes (Awuti et al., 2025). Lower lysophosphatidylcholine (LPC) and higher fatty acid levels were associated with greater disease severity and poor prognosis, suggesting lipid disturbances reflect immune dysregulation and could serve as biomarkers for pediatric sepsis monitoring and risk stratification (Awuti et al., 2025). Moreover, integrative metabolomics/cytokine analyses in children with high-risk sepsis phenotypes (PHES vs. PMODS) demonstrated unique metabolic, immune and endothelial signatures that aligned with outcome trajectories (Hurtado and Sanchez-Pinto, 2025). Thus, metabolomics contributes a real-time functional view of host disruption, complementing upstream omic analyses.

The microbiome represents the collection of all microorganisms (bacteria, viruses, fungi, and other microbes) and their genetic material that live in a particular environment, modulating host immune function and sepsis susceptibility. The composition and functional profile of the microbiome can serve as a biomarker by reflecting host physiological and pathological states, offering insight into disease susceptibility, progression, and response to therapy

(Marchesi and Ravel, 2015). Metagenomic profiling enables characterization of these microbial ecosystems through sequencing-based approaches. Two principal techniques are used: 16S ribosomal RNA gene sequencing, which amplifies conserved bacterial regions for genus-level identification, and shotgun metagenomic sequencing, which sequences all DNA in a sample, permitting higher-resolution identification of bacteria, fungi, and viruses, as well as gene-function prediction. While 16S sequencing is cost-effective and analytically straightforward, it provides limited taxonomic depth and lacks information on microbial function. Shotgun metagenomics offers comprehensive resolution and functional annotation but demands greater sequencing depth, computational resources, and bioinformatic expertise. Both approaches are sensitive to contamination, require careful normalization for sequencing depth, and remain challenged by the absence of standardized analytical pipelines across laboratories (Quince et al., 2017; Knight et al., 2018).

From a clinical perspective, microbiome analysis reveals how microbial imbalances (dysbiosis) can impact systemic inflammation, immune development, and infection risk. In infants and children, gut microbiota changes rapidly, making it sensitive to disruptions from antibiotics, diet, or illness, which can affect immune function and sepsis susceptibility (Zhang et al., 2022). Microbiome profiling in preterm infants with necrotizing enterocolitis suggest that microbiome instability and lack of diverse, Bifidobacterium-rich communities, rather than a specific pathogen, characterize the preterm gut prior to NEC development (Stewart et al., 2016). A prospective study of 71 preterm infants with late-onset sepsis (LOS) and 164 controls found that intestinal dysbiosis preceding LOS was characterized by dominance of Bacilli (particularly coagulase-negative Staphylococci), decelerated development of microbial diversity, and a lack of anaerobic bacteria such as Clostridia (Graspeuntner et al., 2019). Using *in silico* metabolic modeling, LOS were found to have accumulation of bacterial fermentation products ethanol and formic acid prior to disease onset, which may contribute to intestinal barrier damage and bacterial translocation leading to sepsis (Graspeuntner et al., 2019). Moreover, because the microbiome and immune system interact closely, microbiome-derived biomarkers could improve diagnosis and treatment of pediatric sepsis. For example, metagenome profiling demonstrates children with sepsis have been shown to display reduced diversity and beneficial Bifidobacterium species, a lower Firmicutes/Bacteroidetes ratio, higher inflammatory markers, and worse gastrointestinal and organ function, supporting the role of gut dysbiosis in sepsis severity (Sankar et al., 2024).

Collectively, omics-based discovery pipelines advance biomarker development by enabling a mechanistic, multi-dimensional understanding of pediatric sepsis pathobiology. Genomics define baseline susceptibility, epigenomics capture regulatory adaptation, transcriptomics reveal active expression programs, proteomics quantify molecular effectors, and metabolomics reflect downstream functional consequences. Integrating these complementary layers strengthens the robustness of biomarker candidates, promotes pathway-centric rather than single-analyte discovery. An overview of key multi-omic pathways implicated in pediatric sepsis is summarized in Figure 1.

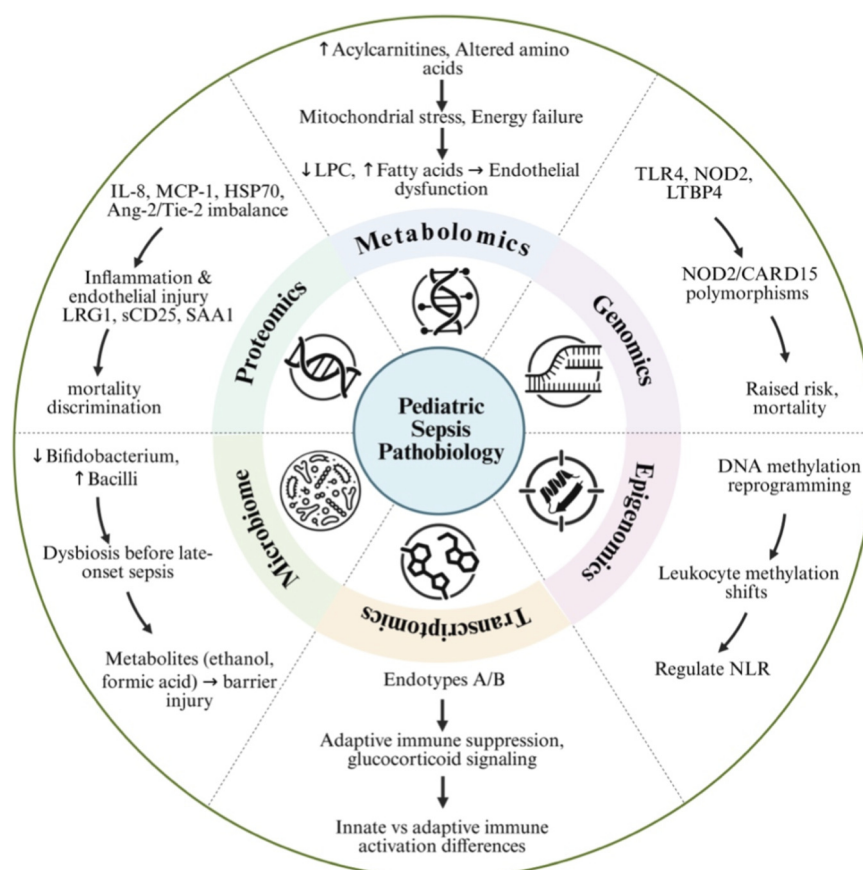


FIGURE 1

Convergent molecular pathways dysregulated in pediatric sepsis identified through multi-omic profiling. Schematic summarizing key molecular pathways implicated in pediatric sepsis across multiple omic layers. At the core is the host response to infection, surrounded by interacting genomic, epigenomic, transcriptomic, proteomic, metabolomic, and microbiome signatures. Genomic studies identify polymorphisms in innate immune genes (e.g., NOD2/CARD15, TLR4, LTBP4) associated with sepsis susceptibility and mortality risk. Epigenomic analyses demonstrate dynamic leukocyte DNA methylation changes that reprogram immune gene expression. Whole-blood transcriptomics has identified reproducible pediatric septic shock endotypes characterized by adaptive immune suppression and altered glucocorticoid signaling. Proteomic profiling highlights inflammatory and endothelial injury markers (e.g., IL-8, MCP-1, HSP70, Ang-2, Tie-2, LRG1, sCD25) associated with illness severity and outcomes. Metabolomic studies reveal mitochondrial stress, elevated acylcarnitines, altered amino acid profiles, and lipid perturbations linked to endothelial dysfunction and energy failure. Microbiome analyses demonstrate early dysbiosis, including reduced *Bifidobacterium* and increased *Bacilli*, preceding late-onset sepsis and contributing to barrier dysfunction. Collectively, these interconnected omic layers illustrate pediatric sepsis as a systems-level disorder driven by coordinated pathway dysregulation rather than isolated biomarkers.

Validation pathways: from discovery to clinical utility

As omics technologies increasingly identify pathway-focused biomarkers, there is a need for robust analytical validation to ensure assay reproducibility and standardization, biological validation to confirm findings across independent cohorts, and clinical validation to establish meaningful associations with patient outcomes and therapeutic interventions (Jiang et al., 2025).

Analytical validation

By and large, biomarker identification in critically ill pediatric patients with sepsis has relied on high-throughput proteomic assays. Such assays include Olink proximity extension assays (PEA), SOMAscan aptamer technology, mass spectrometry (MS), and Luminex multiplex assays. Olink's proximity extension assays use

pairs of oligonucleotide-labeled antibodies that generate amplifiable DNA barcodes upon dual binding to a target protein, enabling highly specific detection with low sample volume (Wang et al., 2024). SOMAscan aptamer technology employs chemically modified single-stranded DNA aptamers with high binding specificity to thousands of proteins, allowing wide proteome coverage (Joshi and Mayr, 2018). Mass spectrometry works by ionizing molecules, separating the resulting ions based on their mass-to-charge ratio, and detecting them to identify and quantify compounds (Glish and Vachet, 2003). Luminex multiplex bead-based immunoassays use fluorescently barcoded microspheres coupled to capture antibodies, permitting simultaneous quantification of specific subsets of proteins, though the total number of proteins measurable is substantially lower than in Olink or SOMAscan (Khalifian et al., 2015).

Antibody-based platforms such as Olink PEA and Luminex offer high sensitivity and multiplexing but are vulnerable to variability in

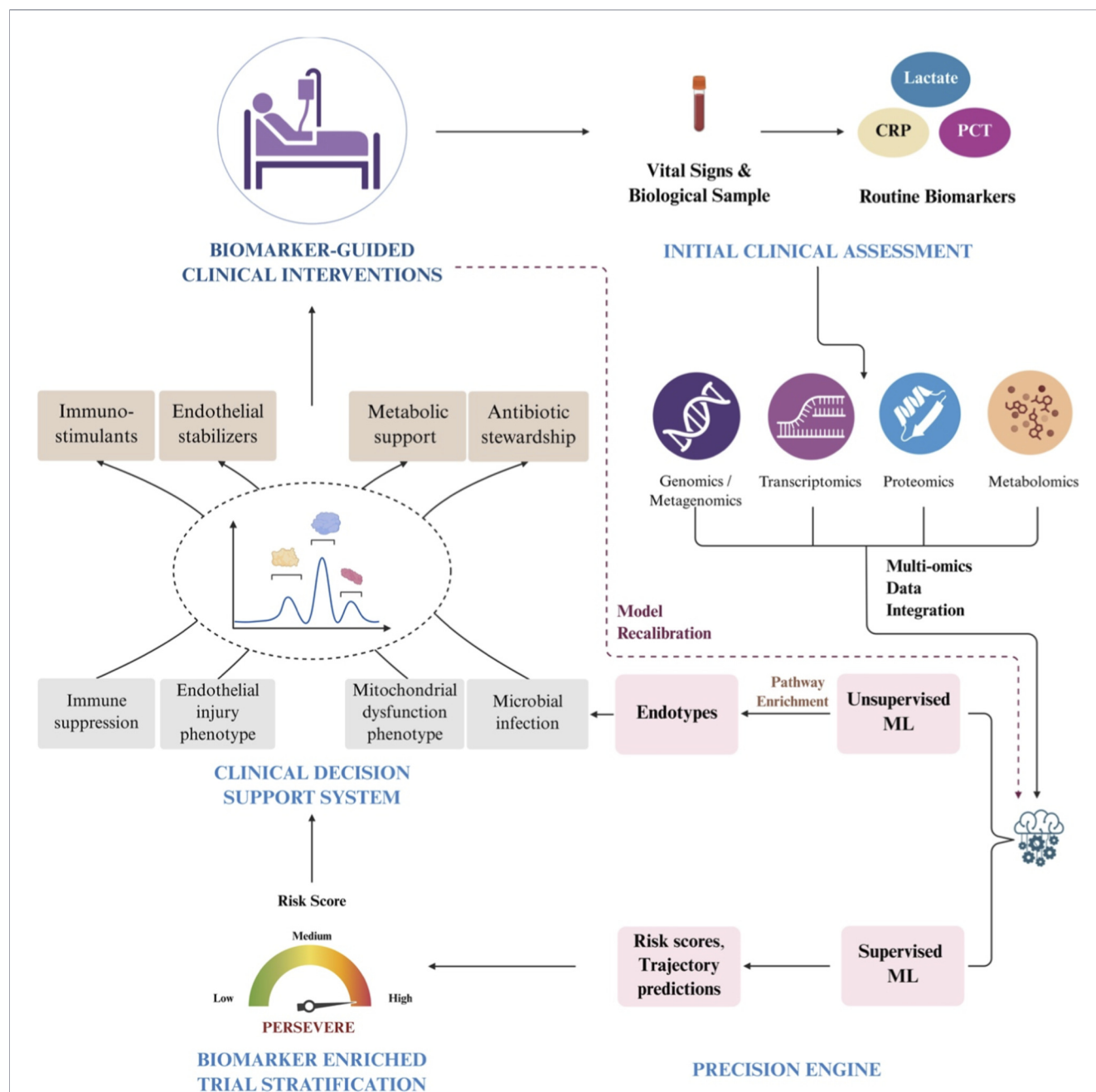


FIGURE 2

Precision medicine workflow integrating biomarkers, machine learning, and clinical outcomes. Translating biomarker discovery into clinical practice requires an integrative precision-medicine framework in which routine clinical data, multi-omic profiling, and machine-learning models are unified to guide care. The workflow begins with initial bedside evaluation, including vital signs and routine biomarkers (e.g., lactate, CRP, PCT). In parallel, research-grade multi-omic data (genomics/metagenomics, epigenomics, transcriptomics, proteomics, and metabolomics) are generated and integrated computationally. Unsupervised machine-learning approaches identify biologically coherent patient endotypes through pathway enrichment, while supervised models generate risk scores and outcome trajectories. Outputs from this precision engine inform clinical decision-support systems, enabling risk-guided monitoring, biomarker-informed antibiotic stewardship, and alignment of therapies with treatable biological traits (immune suppression, endothelial dysfunction, metabolic failure). Validated biomarker signatures and risk models further support biomarker-enriched clinical trial stratification. Clinical outcomes and biomarker responses are iteratively fed back to recalibrate and refine predictive models, supporting continuous learning and improved clinical performance.

antibody affinity, batch effects, and cross-reactivity, necessitating robust calibration across laboratories. Aptamer-based assays like SOMAscan expand proteome coverage, but similar to Olink, typically provide relative or normalized values rather than absolute quantification. This presents challenges in identifying

universal biomarker thresholds or validating cutoffs required for integration into clinical practice (Wang et al., 2025). Mass spectrometry remains the gold standard for specificity, yet it is resource-intensive and prone to greater inter-laboratory variability. Therefore, robust assay calibration is crucial for translating

candidate biomarkers into clinical use and ensuring analytical validation.

Importantly, emerging pathway-based approaches (in which biomarkers cluster into coherent signatures of immune suppression, endothelial activation, or coagulopathy) (Van Nynatten et al., 2026) may reduce reliance on precise cutoffs or age-adjusted reference values (Fraser et al., 2025). By identifying the underlying dysregulated biology, these signatures enable stratification by mechanism rather than by arbitrary thresholds, potentially simplifying translation across diverse pediatric populations (Wong et al., 2017a; Ishaque et al., 2023). Figure 2 illustrates an integrative precision-medicine workflow for pediatric sepsis, progressing from initial clinical assessment and routine biomarkers to the clinical application of machine-learning-based models. Clinical outcomes and biomarker responses are subsequently fed back to recalibrate and refine the model.

Biological and clinical validation

In addition to ensuring robust inter-assay reproducibility, biomarkers must also be validated across different cohorts to determine whether dysregulated pathways persist across independent populations, care settings, and demographic groups. In pediatric patients, this has been accomplished most rigorously in the PERSEVERE (pediatric sepsis biomarker risk model) program, in which multi-marker panels prognostic of mortality in pediatric septic shock were derived, then subsequently validated in multiple independent cohorts, establishing generalizability beyond the training set (Wong et al., 2012). Twelve candidate serum proteins, previously identified from genome-wide association studies (Wong et al., 2009; Shanley et al., 2007), combined with clinical variables, were used as input into classification and regression tree analysis to generate a decision tree for predicting 28-day all-cause mortality in a cohort of 135 children with sepsis, which was then validated in a separate independent test cohort of children with sepsis. Subsequent studies have repeatedly tested this panel in distinct phenotypes such as septic shock and thrombocytopenia-associated organ failure (TAMOF) (Wong, 2019), endothelial dysfunction (Atreya et al., 2022), or with different outcomes such as kidney injury (Stanski et al., 2020), where it continues to reliably predict mortality.

Similarly, transcriptomic profiling to generate endotypes has provided further evidence of biological validation, as findings have been reproducible and confirmed that biomarkers identify true pathobiology rather than individual cohort idiosyncrasies. Pediatric septic shock endotypes (A and B), distinguished by differences in 100 genes reflecting adaptive immunity and glucocorticoid receptor signaling, were validated across multiple cohorts, with endotype A associated with worse clinical outcomes including mortality and complicated disease course (Wong et al., 2018; Wong et al., 2017b). More recently, derivation-validation studies integrating additional markers of endothelial injury (soluble thrombomodulin and vascular cell adhesion molecule-1) with clinical data (such as PaO₂/FiO₂ ratio) have produced externally tested risk models for pediatric septic shock that identify children at high risk for acute respiratory dysfunction (Williams et al., 2025). Recent pediatric analyses have also replicated subphenotypes using independent, prospectively enrolled cohorts (Atreya et al., 2024).

Among 1,071 children with septic shock requiring vasoactive support on day 1, two phenotypes were identified: Phenotype 1 (19.5%) and Phenotype 2 (80.5%). Phenotype 1 was associated with approximately fourfold higher adjusted odds of a complicated course compared with Phenotype 2, and was characterized by elevated Angiopoietin-2/Tie-2 ratio, Angiopoietin-2, soluble thrombomodulin, IL-8, and ICAM-1, along with lower levels of Tie-2 and Angiopoietin-1 (Atreya et al., 2024). Collectively, these findings demonstrate that biological and clinical validation of pediatric sepsis biomarkers is both achievable and feasible, as shown by replication across diverse, independent cohorts.

Emerging themes and novel directions: machine learning and bioinformatics

Machine learning

Machine learning (ML) brings novel computational capability to characterize complex pathobiology of multifactorial diseases such as sepsis (Sahu et al., 2026). Machine learning is able to recognize intricate and often nuanced patterns in large biological data sets, that are beyond the capacity of traditional statistical methods. This promotes identification of novel clinical and biological insights. Conceptually, machine learning approaches can be categorized as supervised, unsupervised, or reinforcement learning, each offering distinct advantages for omic driven biomarker discovery. In biomarker discovery, supervised learning uses labeled datasets (where outcomes such as disease presence, severity, or survival are known) to train models that identify molecular patterns predictive of those outcomes. For example, supervised algorithms can learn which proteins or transcripts best distinguish septic from non-septic patients. In contrast, unsupervised learning analyzes unlabeled data to uncover natural groupings or patterns within complex molecular datasets, revealing hidden biological subgroups or endotypes that may share underlying pathophysiology. Reinforcement learning, though less commonly applied, involves algorithms that are learnt through trial and error by receiving feedback on their performance, enabling adaptive decision-making over time. In the context of biomarker discovery, reinforcement learning can be used to iteratively optimize feature selection, study design, or therapeutic targeting based on performance feedback.

Supervised learning algorithms, such as random forests, support vector machines, and gradient-boosting models, use labeled datasets to predict predefined outcomes, such as mortality or disease severity. Through iterative training, these models describe nonlinear relationships between molecular features and clinical parameters. In pediatric sepsis, a supervised ML pipeline combining differential gene-expression analysis with feature selection was able to generate a 10-gene signature that reliably predicted sepsis mortality (AUC 0.89) (Abbas and El-Manzalawy, 2020). This approach exemplifies how ML can transform large-scale transcriptomic data into biologically coherent and clinically actionable prognostic panels, beyond prognostic ability of individual markers. Similarly, supervised ML analysis with a random forest approach to distinguish children with sepsis from non-infectious SIRS responses incorporated four clinical variables (duration of

PICU stay before onset, presence of a central line, core temperature, and number of previous SIRS/sepsis episodes) and four laboratory parameters (IL-6, platelet count, procalcitonin, and CRP). This integrated algorithm achieved a robust diagnostic performance (AUC 0.78) in identifying sepsis (Lamping et al., 2018).

Unsupervised methods such as hierarchical clustering, principal component analysis (PCA), and related dimensionality-reduction algorithms (t-SNE, UMAP) also identify occult molecular patterns within pediatric sepsis cohorts. As previously discussed, unsupervised hierarchical clustering of genome-wide expression data identified reproducible pediatric septic shock endotypes distinguished by repression of adaptive-immune and glucocorticoid-responsive pathways and by markedly different mortality risks (Wong et al., 2009). More recently, whole-blood RNA-sequencing again identified two sepsis subclasses with divergent innate-versus adaptive-immune transcriptional programs, endothelial activation patterns, and clinical outcomes (Yang et al., 2023). In the context of omics, unsupervised ML facilitates endotype discovery by grouping patients with shared molecular characteristics, independent of cohort clinical parameters. This approach can reveal unrecognized biological subgroups that differ in immune, endothelial, or metabolic profiles, providing a framework for mechanistic insight. Similarly, unsupervised k-means clustering analysis applied to a dataset of 404 children with sepsis-associated organ failure admitted to the intensive care unit identified four distinct clinical phenotypes, each associated with different patterns of organ dysfunction and mortality risk. PedSep-A included younger, previously healthy children, predominantly with isolated respiratory failure and the lowest mortality rate (2%). PedSep-B was characterized by patients with neurological impairment, frequent need for intubation, and an intermediate mortality rate (12%). PedSep-C included children with cardiovascular dysfunction, lymphopenia, and elevated ferritin levels, also associated with an intermediate mortality rate (10%). Finally, PedSep-D represented the most severe phenotype, marked by multiple organ failures and the highest mortality rate (34%) (Qin et al., 2022).

Given the enormous amount of biologic data that omics technologies provide, techniques including feature selection and dimensionality-reduction (least-absolute-shrinkage-and-selection-operator (LASSO), Boruta, or recursive feature elimination) have become indispensable for model parsimony and interpretability. Essentially, a parsimonious model uses the fewest possible parameters while still adequately explaining the data. Feature reduction simplifies large biomarker datasets by removing redundant, noisy, or weakly informative molecular signals while retaining those most strongly associated with underlying biology or clinical outcomes. For example, in critically-ill pediatric patients with sepsis a Random Forest classifier with Boruta feature-reduction was used to identify inflammatory plasma biomarkers that most strongly distinguish children with sepsis from healthy controls (Leonard et al., 2024). Out of 58 measured cytokines, Boruta retained a small subset (IL-8, MCP-1, HSP70, hyaluronan, M-CSF, and IL-6) that provided near-perfect discrimination between groups (Leonard et al., 2024). This approach demonstrates how feature-selection can distill large omic outputs into concise, biologically meaningful panels to improve interpretability and predictive performance of omic data.

Natural language processing (NLP) refers to the use of computational techniques to extract structured information from unstructured text, including published manuscripts and omic databases. In the context of omics research, NLP is a novel tool to bridge the gap between molecular data and biological meaning. Omics studies often generate lists of hundreds or thousands of genes, proteins, or metabolites that differ between patient groups. NLP tools can automatically search and interpret the scientific literature and databases (PubMed, Gene Ontology, Human Protein Atlas, UniProt) to determine what is already known about those molecules, including their biological functions, pathways, tissue or cell-type expression, and disease associations (Oikonomou et al., 2024). This expedites hypothesis-generation for future mechanistic studies to elucidate pathobiology, while also efficiently identifying patterns and relationships that otherwise could take months or years to uncover.

Finally, the next frontier in machine learning omic interpretation is the integration of explainable artificial intelligence (XAI) and causal inference to characterize dysregulated pathways. Explainable AI has begun to address one of the main challenges in applying machine learning to sepsis biology titled the “black box” problem. XAI methods, such as SHapley Additive exPlanations (SHAP) or Local Interpretable Model-Agnostic Explanations (LIME), make complex models more transparent by showing how individual biomarkers or features influence predictions. In pediatric sepsis, recent studies have applied SHAP to interpret survival and diagnostic models, identifying which clinical and biomarker variables most strongly contribute to risk prediction (Huang et al., 2025). These approaches enhance interpretability and trust by linking computational outputs to biological reasoning, helping researchers understand why a model predicts severe disease or poor outcome.

Causal inference builds upon these explainable AI frameworks by moving from correlation to causation. Using graphical models such as Bayesian networks and algorithms like PC or LiNGAM, causal inference identifies directional relationships between molecular features and clinical outcomes, distinguishing upstream drivers from downstream effects. Through *do-calculus* and counterfactual reasoning, it estimates how hypothetical interventions (such as modulation of specific biomarkers) would alter disease trajectories (Dibaieinia et al., 2025). Integrating causal discovery with omics-based AI therefore transforms descriptive associations into mechanistic insight, enabling identification of biologically actionable targets for precision sepsis therapeutics (Lecca, 2021). Collectively, the integration of omics, machine learning, bioinformatics, and explainable modeling represents a critical step toward unifying exploratory omic studies with clinical translation.

Bioinformatics and multi-omic data integration approaches

Each omic platform (genomics, transcriptomics, proteomics, metabolomics) captures only a fraction of sepsis biology. Genomic and epigenomic signals reveal predisposition and regulation; transcriptomics reflects real-time immune activation; proteomics identifies circulating effectors; and metabolomics describe the physiological consequences of these processes. Integrating these datasets provides a multidimensional view of

host response. This systems-level integration enables the identification of robust biomarker panels and mechanistic pathways that single biomarker classifications often overlook (Hasin et al., 2017; Krassowski et al., 2020).

Early and late integration represent two complementary strategies for combining omic data depending on the research question and data structure (Sibilio et al., 2025). In early integration, multiple omic datasets, such as transcriptomic and proteomic measures, are consolidated into one high-dimensional dataset and analyzed concomitantly. This approach lets algorithms detect shared patterns of variation across different biological molecules, uncovering cross-omic interactions. Early integration is particularly advantageous when biospecimens are collected from the same patients and can capture simultaneous biological events, providing insight into multi-scale mechanisms (for example, how transcriptional activation drives downstream protein and metabolic changes) (Hasin et al., 2017; Argelaguet et al., 2018).

In contrast, late integration treats each omic output (proteins, metabolites, etc) independently and merges their outputs only after separate analyses are completed. This strategy allows investigators to compare and validate findings across omics platforms, asking whether transcriptomic and proteomic results implicate the same pathways even if derived from different cohorts or time points. Late integration is especially useful in translational research where datasets vary in quality or completeness, offering a robust means to cross-validate biomarker candidates across omics modalities. In short, early integration emphasizes biological synergy, while late integration emphasizes reproducibility and interpretability.

Network and multi-block approaches integrate multi-omic data by examining how molecular layers interact rather than analyzing or merging them in isolation. These methods identify groups of genes, proteins, and metabolites that change together across patients (so-called molecular modules) that likely represent coordinated biological pathways. Tools such as Multi-Omics Factor Analysis (MOFA+), DIABLO (mixOmics), iCluster, and multi-block partial least squares (MB-PLS) extract shared latent factors across omic datasets, capturing the dominant axes of biological variability. By uncovering these cross-layer relationships, network and multi-block frameworks provide a systems-level view of disease biology and highlight pathway-level mechanisms underlying biomarker signatures (Singh et al., 2019; Shen et al., 2009; Huang et al., 2017). In sepsis, this can reveal, for example, that an inflammatory transcriptomic module co-varies with endothelial proteins and metabolic intermediates, pointing to a coupled immune-endothelial-metabolic network driving organ dysfunction. Similarly, Weighted Gene Co-Expression Network Analysis (WGCNA) can be extended to integrate multiple omics by constructing correlation networks that link co-expressed transcripts with their downstream protein or metabolite products (Langfelder and Horvath, 2008). These methods reduce complexity while retaining biological meaning, enabling identification of mechanistically coherent biomarker sets rather than isolated molecules. For pathway discovery, they show how different biological processes communicate (Karczewski and Snyder, 2018).

Pathway-based integration translates multi-omic associations into biological and clinical meaning. After identifying significant genes, proteins, or metabolites within each omic layer, these molecules are mapped onto curated knowledgebases such as Reactome, STRING, or Cytoscape to visualize their convergence within shared pathways

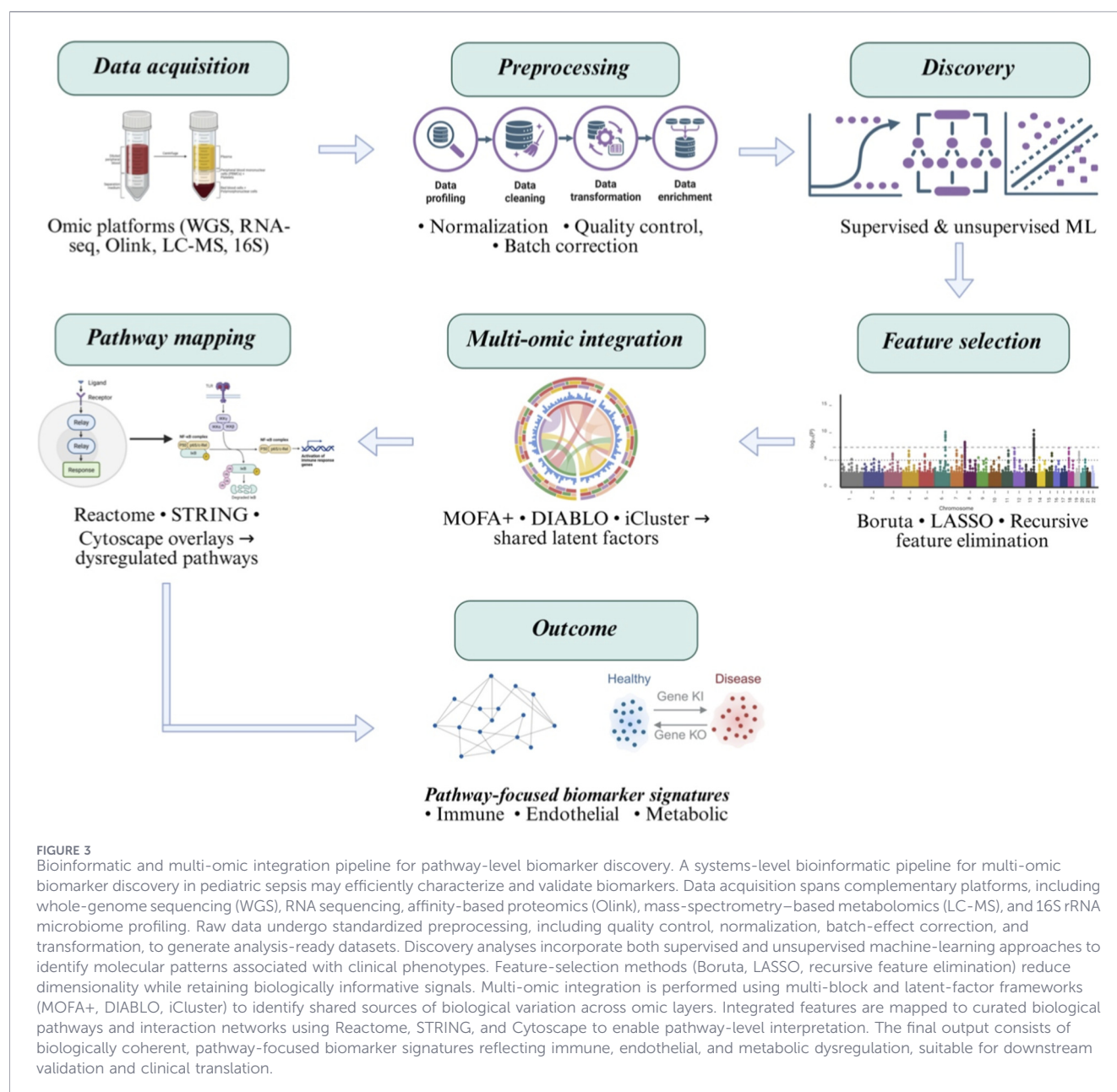
(Fabregat et al., 2018; Szklarczyk et al., 2019; Shannon et al., 2003). This approach situates omic outputs (such as proteins) in the context of known biological networks. For example, this may reveal coordinated perturbations in the complement cascade, coagulation system, or mitochondrial metabolism amongst the analytes measured. The strength of this method lies in its interpretability: rather than isolated lists of biomarkers, it enables construction of mechanistic narratives such as “innate immune activation” and “endothelial dysfunction” occur in parallel. By highlighting where molecular layers intersect, pathway overlays help distinguish causal drivers from downstream by-products and guide prioritization of candidates for validation. Using such methodology, pediatric sepsis has been characterized by coordinated proteomic and pathway-level perturbations rather than robust single-protein biomarkers. Early sepsis showed significant enrichment of inflammatory and interleukin signaling pathways, while later stages were marked by suppression of transcriptional pathways (Stranges et al., 2025; Stranges et al., 2026). Network analysis identified distinct inflammatory and brain-associated protein clusters bridged by TNF and IL-1 β , implicating neuroimmune crosstalk in sepsis-associated encephalopathy (Stranges et al., 2025). In essence, pathway-based integration transforms large-scale omic data into biologically coherent and actionable insights. Figure 3 provides a detailed workflow for multi-omics integration with bioinformatics to develop biomarkers.

Clinical translation and predictive enrichment

The ultimate goal of biomarker discovery is clinical translation whereby molecular signatures can be used to guide therapy, improve trial design, and ultimately enhance patient outcomes. Translation requires three components: (i) analytical readiness of the assay, (ii) clinical evidence linking the biomarker to outcomes or treatment response, and (iii) regulatory and operational frameworks for bedside deployment. Plasma protein panels identified using the PERSEVERE cohort illustrate this trajectory in critically ill pediatric patients with sepsis. Initially derived from discovery cohorts, these biomarker panels have since been prospectively validated across multiple centers and tested for bedside feasibility, with turnaround times compatible with enrollment windows in septic shock trials (Wong et al., 2012; Wong et al., 2015; Basu et al., 2011). While PERSEVERE-based models are primarily prognostic, they provide an important framework for biological stratification that may support predictive enrichment strategies when combined with mechanistic biomarkers. These models exemplify how biomarker programs can advance along the translational pipeline.

Predictive enrichment

Beyond risk stratification, biomarkers offer an opportunity for predictive enrichment. Predictive enrichment is a strategy that selectively identifies patients most likely to benefit from therapy based on underlying biology for trial enrollment. This approach aims to limit the heterogeneity of treatment effects observed in larger populations, where individuals may respond differently to the same treatment due to biological differences (Khan et al., 2021). Such a concept is being validated in adult ARDS populations, where

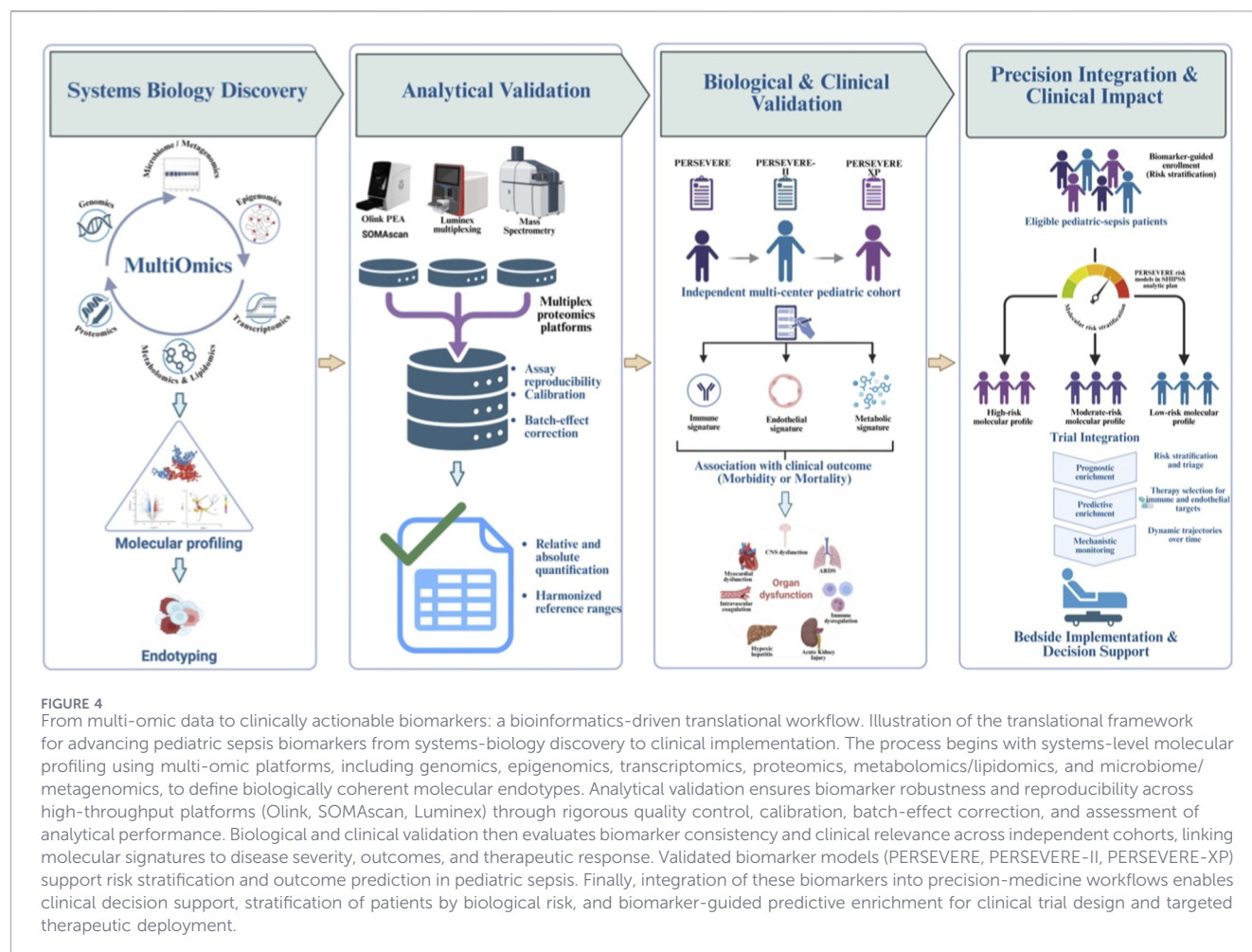


patients with hyperinflammatory *versus* hypoinflammatory ARDS demonstrated different responses to simvastatin in retrospective analyses of the HARP2 trial (Calfée et al., 2018). Although no pediatric studies have yet implemented this methodology, there is growing potential for the emergence of biomarker-based predictive enrichment trials. The ongoing SHIPSS trial (NCT03401398) of hydrocortisone in pediatric septic shock prospectively incorporates PERSEVERE risk models and transcriptomic endotypes into its analytic plan, providing an important precedent for how enrichment strategies may be operationalized, even if they do not yet determine enrollment (Basu et al., 2025).

Precision therapeutics

Several candidate biomarkers demonstrate how pathway-focused identification can also inform opportunities for novel sepsis

therapeutics within the context of predictive enrichment. Emerging therapies target pathways such as immunomodulation or endothelial dysregulation. Monocyte HLA-DR downregulation, an indicator of immune suppression, has been associated with poor outcomes and nosocomial infections in pediatric sepsis (Hall et al., 2011; Remy et al., 2018), suggesting that immunostimulating therapies may help restore immune function in those enriched for biomarkers of immunosuppression. This mirrors exploratory adult studies in which low mHLA-DR has guided immunostimulant therapy (Meisel et al., 2009; Döcke et al., 1997). Furthermore, integrating PERSEVERE biomarkers with markers of endothelial dysfunction (such as ICAM-1, Ang-2, and Tie-2) was shown to improve discrimination for death in pediatric septic shock compared to PERSEVERE biomarkers alone (Atreya et al., 2022). This supports the plausibility of using endothelial activation biomarkers to identify subgroups who may benefit from endothelial-targeted therapies.



Acquired or decreased ADAMTS13 activity has been associated with disease severity and mortality in sepsis (Nguyen et al., 2007; Lin et al., 2016), raising the possibility that plasma exchange could provide clinical benefit for those enriched for decreased ADAMTS13 activity. Additionally, RNA expression signatures distinguishing bacterial from viral infections may facilitate targeted administration of antibacterial or antiviral therapies (Herberg et al., 2016). Figure 4 provides a comprehensive overview of bioinformatics and multi-omics integration, outlining a step-by-step workflow. It begins with data acquisition from various omics technologies, then proceeds to preprocessing, followed by the application of supervised and unsupervised models, and feature selection. Finally, identifying shared latent factors and mapping them to biological pathways helps to pinpoint involved biomarkers.

Barriers to translation

Despite promising discovery and validation work, several barriers continue to limit the clinical adoption of pediatric sepsis biomarkers. Regulatory approval remains a major hurdle. Without regulatory clearance, biomarkers cannot be deployed as tools for clinical decision-making. Standardization is another significant challenge. Biomarker assays often lack harmonization across platforms and laboratories, with variability in calibration, reference ranges, and sample handling. This is especially

problematic in pediatrics, where developmental stage and age-specific physiology influence baseline biomarker values. Establishing reproducible, cross-site standards is essential before biomarkers can support multicenter trials or clinical practice. Finally, clinician uptake will likely depend on clear demonstration of utility. Intensivists are more likely to adopt biomarkers that provide incremental prognostic or therapeutic value beyond existing clinical scores, deliver rapid turnaround compatible with ICU decision windows, and are integrated seamlessly into electronic health records. Without transparent algorithms, multicenter reproducibility, and evidence that biomarker-informed strategies improve outcomes, clinician enthusiasm may remain limited.

Limitations and ethical considerations

While the identification and validation of biomarkers present opportunities for patient phenotyping, several limitations and ethical issues must be addressed to ensure equitable and responsible translation into clinical practice. One major limitation is the variability in the reproducibility of findings in omics. High-throughput omics studies are subject to numerous sources of technical and statistical variability, including batch effects, differences in platforms, data normalization, sample processing, data transformation, and the choice of computational pipelines. These factors can lead to

heterogeneous findings (Straube et al., 2015; Goh et al., 2022). Batch effects have been shown to both reduce statistical power and generate spurious associations if batches are correlated with biological or clinical outcomes (Yu et al., 2024; Krassowski et al., 2020). Without rigorous experimental design, adequate sample sizes, appropriate controls, reference standards, and transparent reporting of all analytical steps, there is a risk that candidate biomarkers will fail independent validation or clinical testing.

Bias in datasets is another key limitation. Many omics datasets are heavily skewed toward certain geographic regions (often high-income countries), socioeconomic strata, and ethnicities (primarily of European descent), resulting in underrepresentation of populations that may have distinct biological variations, environmental exposures, and disease trajectories (Soares et al., 2023). Such bias limits the generalizability of biomarkers and may exacerbate health disparities if biomarkers validated in one group are applied to another without recalibration (Alemu et al., 2025; Chang et al., 2024). Ethical implications also arise around patient stratification when using biomarkers to determine therapy allocation. While stratification can optimize benefits and reduce harm, it can also determine who receives treatment access, who may be excluded, and how decisions are made. There is potential for unfair or unequal allocation if stratification criteria are biased in terms of socioeconomic status, geographic location, or ethnicity (Brothers and Rothstein, 2015).

Future outlook

The future of biomarker discovery and validation in sepsis is decisively moving toward integration and clinical translation. Multi-omic platforms combined with artificial intelligence (AI) and clinical decision support tools offer unprecedented opportunities to synthesize high-dimensional biological signals into actionable insights at the bedside. Recent reviews demonstrate how AI-driven integration of genomics, transcriptomics, proteomics, and metabolomics can refine immune endotyping and predict outcomes, setting the stage for clinically meaningful decision-support algorithms (Gao et al., 2025; Papareddy et al., 2025).

A second critical frontier is the development of bedside-ready assays. While high-throughput proteomics and transcriptomics have illuminated key pathways, their clinical impact depends on translation into rapid, reliable, and regulatory-cleared diagnostics. Advances in multiplex immunoassays, microfluidics, and point-of-care molecular diagnostics are beginning to bridge this gap (He et al., 2024; Alevizou et al., 2025). Biomarkers most likely to reach clinical practice are those measurable within routine workflows, underscoring the need for pragmatic assay design (Bourika et al., 2025).

The potential of adaptive clinical trials guided by real-time biomarkers represents another transformative step. Adaptive enrichment and Bayesian platform trials allow dynamic stratification of patients according to evolving biomarker profiles, improving efficiency and therapeutic targeting (Talisa et al., 2018). Recent adult translational studies, such as omics-based subgrouping of septic shock patients to guide fluid resuscitation (Zhang et al., 2024) and biomarker-guided antibiotic stewardship trials (Gupta et al., 2025), provide proof-of-concept for embedding biomarkers into prospective trial frameworks.

Ultimately, the vision is for biomarker-driven sepsis care to serve as a bridge to true precision medicine. In this model, critically ill patients would be rapidly profiled using validated multi-omic panels, decision-support algorithms would guide therapy selection, and adaptive monitoring would adjust treatment according to evolving biology.

Conclusion

Sepsis exemplifies the complexity of critical illness, where diverse host responses and overlapping pathobiological pathways challenge traditional biomarker paradigms. Integrating multi-omic technologies with machine learning, bioinformatics and network biology enables a shift from single-analyte markers toward systems-level models that capture immune, endothelial, metabolic, and microbial dysregulation. Emerging pediatric studies demonstrate that such pathway-focused biomarkers can stratify risk, illuminate mechanism, and inform therapeutic targeting. As analytical rigor and validation frameworks mature, the convergence of omics, bioinformatics, and artificial intelligence offers a path toward clinically actionable, biologically grounded biomarkers. Ultimately, translating these insights into rapid diagnostics and adaptive trial designs will accelerate the realization of precision medicine in pediatric sepsis.

Author contributions

LVN: Writing – original draft, Data curation, Methodology, Investigation. VS: Data curation, Investigation, Methodology, Writing – original draft. DS: Methodology, Data curation, Investigation, Writing – original draft. DDF: Conceptualization, Writing – review and editing, Supervision, Funding acquisition, Resources, Project administration.

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Glossary

ARDS	acute respiratory distress syndrome	TLR	Toll-like receptor
ATAC-seq	assay for transposase-accessible chromatin using sequencing	TNF-α	tumor necrosis factor-alpha
AUC	area under the receiver operating characteristic curve	UMAP	uniform manifold approximation and projection
BPI	bactericidal/permeability-increasing protein	WES	whole-exome sequencing
CARD15	caspase recruitment domain-containing protein 15	WGCNA	weighted gene co-expression network analysis
ChIP-seq	chromatin immunoprecipitation sequencing	WGS	whole-genome sequencing
CRP	C-reactive protein	XAI	explainable artificial intelligence.
CRRT	continuous renal replacement therapy		
DAMPs	damage-associated molecular patterns		
DNA	deoxyribonucleic acid		
FDR	false discovery rate		
GC-MS	gas chromatography-mass spectrometry		
GO	Gene Ontology		
HLA-DR	human leukocyte antigen-DR		
ICU	intensive care unit		
IL	interleukin		
LC-MS	liquid chromatography-mass spectrometry		
LPS	lipopolysaccharide		
LPC	lysophosphatidylcholine		
LRG1	leucine-rich α 2-glycoprotein		
ML	machine learning		
MOFA	multi-omics factor analysis		
MS	mass spectrometry		
NLR	neutrophil-to-lymphocyte ratio		
NMR	nuclear magnetic resonance		
NLP	natural language processing		
PCA	principal component analysis		
PBMCs	peripheral blood mononuclear cells		
PCT	procalcitonin		
PEA	proximity extension assay		
PICU	pediatric intensive care unit		
PRISM	Pediatric Risk of Mortality score		
RNA-seq	ribonucleic acid sequencing		
scRNA-seq	single-cell RNA sequencing		
SHAP	SHapley Additive exPlanations		
SIRS	systemic inflammatory response syndrome		
SNP	single-nucleotide polymorphism		
SOMAScan	slow off-rate modified aptamer scan		
SPHK	sphingosine kinase		
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins		
TAMOF	thrombocytopenia-associated multiple organ failure		
TBI	traumatic brain injury		