Multi-omics reveal neutrophil heterogeneity in sepsis (Review)

ZHI-QIANG LIN[1], DENG CHEN[1], PEI-DONG ZHANG[1], JIA-LIU LUO[1], SHUN-YAO CHEN[1], SHUAI-PENG GU[1], YU-JIE CHEN[1], YOU-XIE SHEN[1], TING-XUAN TANG[2], TE-DING CHANG[1], LI-MING DONG[1], CONG ZHANG[1] and ZHAO-HUI TANG[1]

[1]Department of Trauma Surgery, Emergency Surgery and Surgical Critical, Tongji Trauma Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China; [2]Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, P.R. China

Received May 6, 2025; Accepted September 12, 2025

DOI: 10.3892/ijmm.2025.5663

Abstract. Sepsis is a life-threatening disease characterized by a dysregulated immune response, and neutrophils serve an important role in pathogen clearance, multiple organ failure and immune regulation. With the discovery of multiple phenotypical and functional variants of neutrophils in sepsis, the heterogeneity of neutrophils is crucial, as it impacts the effectiveness of the immune response and the overall outcome of sepsis. Various genome, transcriptome, proteome and metabolome properties may contribute to this heterogeneity. Multi-omics approaches unveil complex details of neutrophil behavior in the context of sepsis, highlighting how neutrophil phenotypes are differentially recruited and activated in response to various stimuli. The present review aimed to provide an overview of the differences in neutrophil phenotypes and functions during sepsis, focusing on neutrophil heterogeneity identified via multi-omics methods. Comprehensive understanding of multi-omics data regarding neutrophil heterogeneity enhances the diagnostic accuracy of sepsis and provides a scientific basis for individualized treatment strategies, potentially improving patient outcomes by targeting specific neutrophil functions and states.

Correspondence to: Dr Cong Zhang, Department of Trauma Surgery, Emergency Surgery and Surgical Critical, Tongji Trauma Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jie Fang Da Dao, Wuhan, Hubei 430030, P.R. China
E-mail: tjcongzhang@hust.edu.cn

Abbreviations: scRNA-seq, single-cell RNA sequencing; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species; NET, neutrophil extracellular trap; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; eQTL, expression quantitative trait loci; IPA, ingenuity pathway analysis; MPO, myeloperoxidase; COVID-19, coronavirus disease 2019; GMP, granulocyte-monocyte progenitor; SRS1, sepsis response signature group 1; CEBPB, CCAAT/enhancer binding protein β; LDN, low-density neutrophil; AI, artificial intelligence

Key words: multi-omics, heterogeneity, neutrophil, sepsis

Contents

1. Introduction

Sepsis is defined as life-threatening organ dysfunction resulting from a dysregulated host response to infection (1). Each year, an estimated 48.9 million people worldwide develop sepsis, leading to approximately 11 million deaths and accounting for nearly one-fifth of all global mortality (2,3). Sepsis is a challenging health problem worldwide with lingering sequelae (4-6). Sepsis is considered a pathway to death initiated by the host immune defense system failing to restore homeostasis in response to an invading pathogen (7-12). Understanding the potential molecular and cellular features involved in complex immune pathological mechanisms is key for sepsis management. Neutrophils, as the most abundant type of white blood cell, are essential frontline responders to infection (13). In different stages of sepsis, neutrophils exhibit different transcriptomic profiles and biological functions (14), providing phenotypical diversity. Neutrophil subpopulations have been classified with different subsets causing various pathophysiological changes in the complex environment of sepsis, such as immune dysregulation, coagulation dysfunction and organ damage (15).

Neutrophil heterogeneity in sepsis has been extensively investigated (16-21). In disagreement with the previous consensus of neutrophil homogeneity, studies have indicated that neutrophils may remain in the circulation long enough to interpret environmental signals and execute specific molecular programs, providing a rationale for neutrophil diversity *in vivo*

and promoting neutrophil heterogeneity research (16-21). Researchers have identified different subsets of neutrophils via surface proteins, such as CD molecules (22-25). However, there are no uniform standards for different neutrophil subpopulations distinguished by phenotypes (26), resulting in confusing neutrophil nomenclature and disagreements regarding neutrophil function. It remains unclear whether neutrophil subsets present in sepsis result from bone marrow mobilization or are specific subsets formed under the influence of sepsis. Moreover, whether the neutrophil response to sepsis represents a transient and reversible activation, or profound gene reprogramming with long-lasting consequences for host immunity remains unknown. Ng *et al* (21) proposed that the integration of protein and transcript composition, functional properties, tissue distribution, and genomic organization provides a more definitive framework for classifying immune cells, thereby offering a more precise approach to defining true neutrophil heterogeneity (21). To define these features, multi-omics analyses provide a more comprehensive understanding of cellular heterogeneity.

Omics analyses evaluate the complete set of molecular data, comprising genome, transcriptome, proteome and metabolome (27). Single omics studies, predominantly single-cell RNA sequencing (scRNA-seq), have revealed notable heterogeneity of neutrophils (28-30). High-throughput analysis based on scRNA-seq enables broad screening of neutrophil heterogeneity, allowing precise identification of neutrophil subsets combined with more practical methods, such as flow cytometry (28). As a combination of single omics approaches, multi-omics unveils the information of a single regulatory layer and the association between different layers (31,32). Sepsis is heterogeneous and has a complicated immunological mechanism, resulting in modulation of neutrophil diversity (7,33). Multi-omics analyses can help to elucidate underlying differences of neutrophils in sepsis that have not been identified by previous classification methods.

The present review summarizes the phenotypical and functional heterogeneity of neutrophils, as well as advances in the discovery of neutrophil subsets in sepsis using single omics approaches and the complex biological mechanisms of neutrophils in sepsis using multi-omics analysis. The holistic perspective of multi-omics clarifies the dynamic interplay between neutrophils and the host immune response, and provides a foundation for the identification of novel biomarkers and therapeutic targets.

2. Neutrophil heterogeneity in sepsis

In sepsis, exposure to local or systemic extrinsic factors may modify neutrophil properties, resulting in the diversity of neutrophils (21). Neutrophil phenotypes and functions reflect key features of neutrophil heterogeneity (Fig. 1).

Phenotypical diversity of neutrophils in sepsis. Morphologically, the stages of neutrophil development include promyelocytes, myelocytes, metamyelocytes, band cells and segmented neutrophils, among which the segmented neutrophil is considered the mature form (34). Because of their characteristic multilobed nuclei at the mature stage, neutrophils are often referred to as polymorphonuclear leukocytes (PMNs). $CD11b^+$ $CD66b^+$ $CD15^+$ $CD14^-$ are commonly used phenotypical markers for human neutrophils (22). In homeostasis, as neutrophils mature, neutrophil surface markers changed to facilitate altered function. Immature neutrophils express more C-X-C chemokine receptor type 4 (CXCR4) than mature neutrophils, which may promote its retention in bone marrow (23). CD16b, CD35 and CD10 appear after neutrophil maturation, whereas CD49d and CD64 disappear (24).

During sepsis, neutrophils present with phenotypical alterations. Seree-Aphinan *et al* (25) found that a decrease in CXCR2 surface levels is associated with sepsis due to internalization of the CXCR2 receptor induced by circulating chemokines (35,36). Other studies also support decreased expression of the chemokine receptor CXCR2 on the surface of neutrophils in patients with septic shock (37,38). In addition, CD11b is decreased, but CXCR1 expression does not change significantly (37,38). Demaret *et al* demonstrated that $CD10^{dim}$ $CD16^{dim}$ neutrophils have an increased frequency in patients with septic shock (39). Neutrophils exhibit increased CD11b expression and decreased CD62L expression following stimulation with IL-8 or N-formyl-methionyl-leucyl-pheny lalanine (39). Similarly, suppressor cells mobilized during acute inflammation are characterized by normal expression of CD16, low expression of CD62L and high expression of CD11b and CD11c (40). Geng *et al* identified a third immune regulatory neutrophil in different inflammation conditions (41). This hybrid neutrophil subset extravasates at sites of inflammation or infection and expresses dendritic cell markers CD11c, major histocompatibility complex class II and costimulatory molecules. Ode *et al* (42), using a mouse model of sepsis, found that the frequency and number of Intercellular adhesion molecule-1 positive neutrophils are increased. CD64, a high-affinity immunoglobulin Fcγ receptor I, mediates phagocytosis of bacteria. Under homeostatic conditions, the expression of CD64 on neutrophils is comparatively low (42). By contrast, during infection, proinflammatory cytokines induce a 10-fold increase in CD64 expression (43,44). In addition, CD64 expression on neutrophils is specific for bacterial infection (45,46). Triggering receptor expressed on myeloid cells-1, a member of the immunoglobulin superfamily, is upregulated when PMNs are exposed to bacteria (47). Demaret *et al* (48) revealed that the expression of CD177 mRNA and protein is increased in circulating neutrophils in sepsis.

When differentiating solely by markers, however, it remains unclear whether certain neutrophil subsets are true lineages or simply represent differentiation and maturation states induced by the tissue environment.

Functional heterogeneity of neutrophils in sepsis. As first-line immune cells, neutrophils are required to adapt to diverse environments and respond promptly. To address this challenge, neutrophils have multiple capabilities. Typical functions of neutrophils encompass granule generation and degranulation, secretion of antimicrobial proteins, production of reactive oxygen species (ROS), phagocytosis, and formation of neutrophil extracellular traps (NETs) (49). NETs are DNA scaffolds containing granule-derived proteins, such as enzymatically active proteases and anti-microbial peptides, formed to immobilize invading microorganisms or in response to sterile

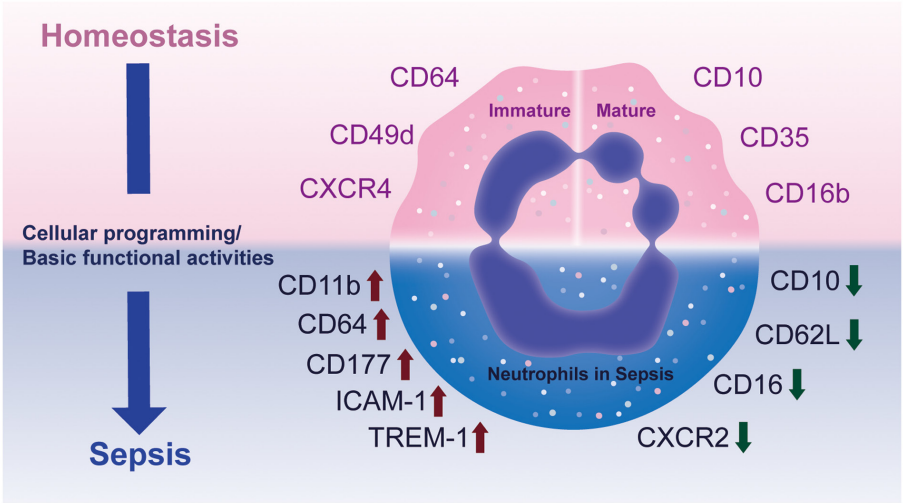

Figure 1. Phenotypical heterogeneity of neutrophils in homeostasis and sepsis. Under homeostatic conditions, immature neutrophils are characterized by expression of CD64, CD49d and CXCR4, whereas mature neutrophils typically express CD10, CD16b and CD35. During sepsis, both immature and mature neutrophils are exposed to inflammatory cues that reprogram cellular activities and functional states, leading to marked phenotypical shifts. Specifically, surface molecules such as CD11b, CD64, CD177, ICAM-1 and TREM-1 are upregulated, while CD10, CD62L, CD16 and CXCR2 are downregulated. These alterations reflect changes in neutrophil activation, adhesion and migration, however, the precise functional consequences and regulatory mechanisms underlying these transitions remain incompletely understood. CXCR, C-X-C chemokine receptor; ICAM-1, intercellular adhesion molecule-1; TREM-1, triggering receptor expressed on myeloid cells-1.

stimuli (50). Table I summarizes the functional diversity of neutrophils in sepsis (39,40,51-65).

A UK cohort study identified neutrophil dysfunction in sepsis, including a notable and sustained reduction in NETosis (active NET release accompanied by cell death), along with defective neutrophil migration and delayed apoptosis (51). In addition, Demaret *et al* (39) found increased neutrophil production and decreased apoptosis in the bone marrow in sepsis. The oxidative burst and phagocytic function of neutrophils in patients with septic shock are increased, but their chemotactic function is strongly inhibited (39,52). Several studies have shown that the migration ability of neutrophils in sepsis patients is reduced (53-56). The underlying mechanism may be that lipopolysaccharide (LPS) and cytokines in sepsis activate G protein-coupled receptor kinase on circulating neutrophils, thereby inducing neutrophils to become desensitized to chemoattractant (57). Martins *et al* (58) also demonstrated that ROS production is upregulated in neutrophils during sepsis. Notably, neutrophil oxidative burst is decreased in the late stages of sepsis (39). These opposing findings suggested that septic shock may involve a transition from immune activation to immune suppression, during which neutrophils may exhibit immunosuppressive features that compromise the host antimicrobial defense (40,59). For example, Pillay *et al* (40) identified a neutrophil subpopulation that mediates the suppression of T cell proliferation by releasing neutrophils via local hydrogen peroxide.

The recruitment of bone marrow neutrophils increases and their apoptosis decreases during sepsis. Functional characteristics of neutrophils in patients with septic shock included enhanced respiratory burst and phagocytosis, as well as inhibited chemotaxis. High production of ROS and proinflammatory cytokines by neutrophils at sites far from the initial infection may be part of the pathophysiology of sepsis (56,60,61). Proinflammatory factors, including tumor necrosis factor-α (TNF-α), IL-1β, chemokines, leukotrienes, adhesion molecules, ROS, and nitric oxide, serve an important role in amplifying inflammation, recruiting immune cells and inducing tissue injury during sepsis (62). Decreased motility associated with acute neutrophil activation is hypothesized to play a role in the development of multiorgan failure following sepsis (63).

The functions of mature and immature neutrophils in sepsis are heterogenous. Drifte *et al* reported that immature circulating neutrophils of patients with septic shock support innate immune defenses to a lesser extent than mature neutrophils (64). Moreover, immature neutrophils possess a longer lifespan and a stronger capacity to resist spontaneous apoptosis (64). Compared with mature granulocytes, the phagocytosis and migration abilities of immature neutrophils are lower (64,65). The high TNF-α/IL-10 ratio in immature neutrophils suggests these cells adopt a proinflammatory profile, characterized by predominant production of inflammatory cytokines (64). Aged neutrophils also have different characteristics. Using a mouse model, Uhl *et al* (66) reported that the number of aged neutrophils returning to the bone marrow is decreased during an acute inflammatory response to endotoxemia, owing to rapid migration to sites of inflammation. Upon reaching inflamed tissues, aged neutrophils exhibit higher phagocytic activity than subsequently recruited non-aged neutrophils (66).

The abundant phenotypical and functional heterogeneity of neutrophils has been extensively studied, but the underlying mechanisms of diversity remain unclear (21,67). Neutrophils exhibit plasticity, allowing them to respond and adapt to various stimuli. Such context-dependent and reversible changes may resemble stable subsets, thereby confounding the interpretation of true heterogeneity. Because the methodologies used to study neutrophil heterogeneity need improvement and investigations of neutrophil heterogeneity are in an exploratory

Table I. Heterogeneity of neutrophil functions in sepsis.

Functional aspect	Observations in sepsis	(Refs.)
NET formation (NETosis)	Decreased NETosis observed in septic patients; associated antimicrobial defense	(51)
Apoptosis regulation	Increased neutrophil production and decreased apoptosis in bone marrow; delayed apoptosis in circulating neutrophils	(39,51,64)
Phagocytosis	Enhanced phagocytic function in septic shock; immature neutrophils show decreased phagocytosis	(39,52,64-66)
Oxidative burst/ROS production	Upregulated ROS generation early in sepsis; decreased oxidative burst in late sepsis; excessive ROS may drive tissue injury	(39,40,56,58-61)
Chemotaxis/migration	Strongly inhibited chemotaxis; defective migration in septic patients; mediated by GRK activation via LPS and cytokines	(39,52-57,64,65)
Proinflammatory cytokine release	High production of TNF-α, IL-1β, chemokines, leukotrienes, adhesion molecules, ROS and nitric oxide; immature neutrophils show high TNF-α/IL-10 ratio	(62,64)
Immunosuppressive features	Neutrophil subpopulation suppresses T cell proliferation via hydrogen peroxide release	(40)
Mature vs. immature neutrophils	Immature neutrophils exhibit prolonged lifespan, decreased phagocytosis/ migration and proinflammatory profile; mature neutrophils exhibit stronger innate immune functions	(64,65)
Aged neutrophils	Decreased return to bone marrow; preferential migration to inflammation sites; enhanced phagocytic activity compared with non-aged neutrophils	(66)

NET, neutrophil extracellular trap; ROS, reactive oxygen species; GRK, G protein-coupled receptor kinase; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.

stage, additional studies are needed to understand whether neutrophil heterogeneity is the result of cell programming or the basic functional activity of cells.

3. Neutrophil heterogeneity in sepsis by omics

The intricate nature of sepsis and its impact on neutrophil function has prompted researchers to investigate the molecular underpinnings of neutrophil heterogeneity using omics technologies (68-76). Each omics layer sheds light on neutrophil behavior in sepsis, providing insight into their roles in both host defense and immune dysregulation. Table II provides a brief summary of omics techniques (68-76).

Genome. Genomic studies have revealed polymorphisms and mutations within genes associated with neutrophil functions that may predispose individuals to sepsis or influence disease severity (77-80). Andiappan *et al* (77) investigated the expression quantitative trait loci (eQTL) of neutrophils and identified 21,210 eQTLs on 832 unique genes. The aforementioned study used Ingenuity Pathway Analysis to reveal an enrichment of neutrophil eQTLs in inflammatory disease, consistent with the established role of neutrophils in host defense against pathogens (77). Utilizing a genome-wide association study, Wang *et al* (78) investigated NET biomarkers to explore the causal association between NET and sepsis. Myeloperoxidase (MPO)-DNA complex is a biomarker of NET (79). With every standard deviation increase in the levels of the MPO-DNA complex, there is an ~18% increase in the risk of sepsis, a 51% increase in the risk of 28-day death from sepsis, ~38% rise in the risk of requiring intensive care due to sepsis and ~125% higher risk of 28-day death from sepsis requiring intensive care (80). Elevated NET levels may elevate the risk of sepsis onset, progression and mortality (80).

Beyond the genome, the epigenome provides an additional layer of regulation that shapes neutrophil heterogeneity. Epigenetic mechanisms such as histone modification, DNA methylation and chromatin accessibility dynamically modulate transcriptional programs, enabling neutrophils to adopt diverse functional states in response to microenvironmental cues. Using chromatin immunoprecipitation sequencing of histone H3K4me3, Piatek *et al* (80) characterized the epigenetic regulation of human neutrophil plasticity and heterogeneity following stimulation with LPS, TNF-α or IL-10. The aforementioned study revealed that changes in H3K4me3-marked transcriptional start sites are associated with diverse functional programs, including neutrophil activation, cytokine production, apoptosis, histone remodeling and NF-κB signaling pathways (80). IL-10 induces a distinct subset of apoptotic yet transcriptionally active neutrophils, which display a non-canonical NF-κB driven cytokine profile while simultaneously suppressing the canonical NF-κB pathway (80). These findings highlight that epigenomic profiling uncovers previously unrecognized heterogeneity in neutrophil functional states, and that H3K4me3-associated DNA binding sites may serve as potential therapeutic targets for immunomodulation.

Transcriptome. Building on the genomic foundation, transcriptomic studies have advanced understanding of neutrophil heterogeneity (81-88).

Table II. Omics levels, research scope and technology.

Omics level	Definition	Research scope	Technology/platform	Explanation	(Refs.)
Genomics	Comprehensive analysis of the genome, including DNA sequence, structural variants and mutations	WGS; WES	Short-read seq (Illumina, Inc.); long-read seq (PacBio, ONT)	WGS covers the entire genome to detect variants; WES focuses on protein-coding regions; long-read seq resolves structural variants and repetitive elements	(68-70)
Epigenomics	Study of genome-wide epigenetic modifications regulating gene expression without altering DNA sequence	DNA methylation profiling; chromatin accessibility mapping; protein-DNA interaction profiling	Bisulfite seq; ATAC-seq; ChIP-seq	DNA methylation profiling reveals CpG methylation; ATAC-seq measures chromatin accessibility; ChIP-seq identifies histone modifications or TF binding	(71)
Transcriptomics	Profiling of RNA expression levels to capture gene activity	Bulk RNA-seq; scRNA-seq; spatial transcriptomics	Short-read sequencing (Illumina, Inc.); *in situ* capture platforms (10x Genomics, Slide-seq)	Bulk RNA-seq quantifies average transcript abundance; scRNA-seq resolves cell-to-cell heterogeneity; spatial transcriptomics retains tissue architecture while measuring gene expression	(72)
Proteomics	Global profiling of protein abundance, modification and interactions	Protein identification and quantification	MS-based proteomics (LC-MS/MS, DIA, TMT); antibody-based proteomics (RPPA, CyTOF)	MS-based methods enable high-throughput protein identification and quantification; antibody-based methods provide sensitive detection of specific proteins	(73,74)
Metabolomics	Systematic identification and quantification of metabolites reflecting cell metabolic state	Targeted and untargeted metabolomics	LC-MS, GC-MS, NMR platforms	Targeted metabolomics measures predefined metabolites with high sensitivity; untargeted metabolomics broadly surveys metabolites to capture global metabolic changes	(75,76)

WGS, whole-genome sequencing; WES, whole-exome sequencing; ONT, Oxford Nanopore Technologies plc; PacBio, Pacific Biosciences; ATAC, assay for transposase-accessible chromatin; seq, sequencing; ChIP, chromatin immunoprecipitation; sc, single-cell; LC-MS/MS, liquid chromatography tandem mass spectrometry; DIA, data-independent acquisition; TMT, tandem mass tags; RPPA, reverse phase protein array; CyTOF, cytometry by time of flight; GC, gas chromatography; NMR, nuclear magnetic resonance.

Bulk transcriptomic profiling in patients with sepsis has identified 37 differentially expressed genes, with functional enrichment and protein-protein interaction analyses highlighting immune and inflammatory signaling pathways, including the PI3K/AKT axis, as key regulators of neutrophil specialization (81). Validation in whole-blood neutrophil samples from patients with sepsis patients further confirmed these transcriptional alterations (81). These findings demonstrate sepsis-associated transcriptional alterations, but the limited resolution of bulk transcriptomics obscures cellular heterogeneity and prevents the identification of distinct neutrophil subsets. scRNA-seq provides a deeper resolution. Xie *et al* (28) identified eight transcriptional neutrophil clusters in mice, with G0-G4 representing bone marrow developmental stages and G5a-c representing three transcriptionally distinct mature neutrophil subsets

in peripheral blood. Bacterial infection accelerates the transition from immature to mature states without altering overall heterogeneity but induces stage-specific transcriptional reprogramming: Early progenitors upregulate genes regulating immune effector functions and ROS metabolism, while mature subsets exhibit increased cytokine production (28). Infection also reshapes transcription factor networks, with defense-associated factors such as interferon regulatory factor 7 activated in immature cells and metabolic regulators such as forkhead box protein P1 (Foxp1) and CCCTC-binding factor (Ctcf) downregulated in mature subsets, suggesting a redistribution of cell resources toward host defense (28). However, a murine model raises questions about their direct applicability to human sepsis, where immune and inflammatory contexts may differ (28). In human sepsis, Xu *et al* (82) identified novel neutrophil subtypes enriched in late differentiation stages, characterized by upregulation of alkaline phosphatase, liver/bone/kidney (ALPL), CD177 molecule (CD177), S100 calcium-binding protein A8 (*S100A8*), S100A9), and syntaxin binding protein 2 (STXBP2), providing potential biomarkers for therapy. Hong *et al* (83) further categorized neutrophils into four subsets (Neu1-Neu4) by single-cell transcriptomic profiling, with Neu1 expansion associated with septic shock and higher Sequential Organ Failure Assessment (SOFA) scores. A Neu1-specific gene module, including NFKBIA (NFKB inhibitor alpha, an inhibitor of NF-κB signaling), CXCL8 (C-X-C motif chemokine ligand 8, encoding interleukin-8), G0S2 (G0/G1 switch gene 2, a regulator of cell cycle and apoptosis), and FTH1 (ferritin heavy chain 1, involved in iron storage and oxidative stress response), demonstrates strong predictive value for shock. Consistently, Neu1 expresses cell surface markers such as CD123, CD38 and CD69, which have been linked to poor prognosis (84-87). However, whether these subsets represent stable cell states or transient activation phenotypes remains unresolved.

Beyond proinflammatory subtypes, immunosuppressive phenotypes have also been reported: Qi *et al* (88) identified a PD-L1^{high} neutrophil subset induced via the p38α pathway, involving mitogen- and stress-activated kinase 1 (MSK1) and MAPK-activated protein kinase 2 (MK2),, capable of suppressing T cell activation and promoting apoptosis or trans-differentiation (88).

Collectively, transcriptomics reveals that neutrophil heterogeneity in sepsis arises from dynamic transcriptional reprogramming, encompassing both proinflammatory and immunosuppressive subsets with distinct clinical relevance.

Proteome. Transcriptomic profiling has advanced understanding of neutrophil gene expression patterns in sepsis, but does not directly link transcriptional changes to protein function. Proteomics fills this gap by quantifying protein abundance and activity, thereby capturing more immediate functional adaptations of neutrophils.

Tak *et al* (89) identified CD62L^{dim} neutrophils as a distinct subset that typically resides outside circulation but is recruited during acute inflammation. Their proteomic signature, enriched in proteins associated with adhesion, activation and immune regulation, underscores functional specialization beyond conventional morphological classification (89). However, whether these proteomic differences translate into stable functional phenotypes or reflect transient activation states remains uncertain, especially since CD62L shedding can occur in multiple inflammatory contexts (89). This raises the question of whether CD62L^{dim} neutrophils represent a subset or activation-driven state detectable by proteomics but less evident in physiological conditions. In parallel, hypoxia, a hallmark of sepsis pathophysiology, triggers notable proteome remodeling (90). Watts *et al* demonstrated that hypoxic neutrophils upregulate inflammatory receptors, including formyl peptide receptor (FPR) and granulocyte-macrophage colony-stimulating factor receptor beta chain (GM-CSF receptor β), enhance lysosomal protein scavenging and sustain biosynthesis of granule and cytoskeletal proteins through *de novo* synthesis (91). These findings highlight the metabolic adaptability of neutrophils under stress (91). The reliance on murine models and controlled hypoxic exposure limits direct extrapolation to human sepsis, where hypoxia is heterogeneous, dynamic and often accompanied by additional insults such as acidosis or oxidative stress (90,91). Furthermore, whether such proteomic adaptations enhance host defense or contribute to maladaptive inflammation remains unresolved.

Taken together, proteomic profiling demonstrates that neutrophils are not passive effectors but metabolically flexible cells capable of remodeling the proteome in response to inflammatory and metabolic stressors. Nevertheless, studies are largely descriptive and rely on either *ex vivo* stimulation or animal models, which may not capture the temporal and spatial complexity of sepsis in patients (89-91).

Metabolome. Proteomics reveals alterations in signaling molecules, surface receptors and effector proteins that directly regulate neutrophil behavior but does not capture the intricate biochemical pathways that govern these changes in cell function and energy metabolism. Metabolic reprogramming refers to the dynamic reshaping of cell metabolic pathways in response to environmental and functional demands (92). In neutrophils, metabolomics has revealed that this process extends beyond glycolysis to include oxidative phosphorylation and the pentose phosphate pathway, enabling distinct effector functions such as chemotaxis, ROS generation and NET formation (93). This metabolic adaptability constitutes a fundamental basis of neutrophil heterogeneity, particularly in sepsis.

In a recent study, Li *et al* (94) observed significant alterations in neutrophil metabolism as severe corona virus disease 2019 (COVID-19) progresses, particularly in amino acid, redox and central carbon metabolism. Metabolic changes in neutrophils are associated with decreased activity of the glycolytic enzyme GAPDH (94). When GAPDH is inhibited, glycolysis is suppressed, leading to an increase in pentose phosphate pathway activity, but this also diminishes the neutrophil respiratory burst (94). Furthermore, inhibiting GAPDH triggers the formation of NETs, which depends on the activity of neutrophil elastase (94). This inhibition results in an increase in neutrophil pH and blocking this pH rise prevents both cell death and NET formation (94). These findings indicated that neutrophils in severe COVID-19 exhibit a heterogeneous and dysfunctional metabolic profile, which contributes to their impaired function.

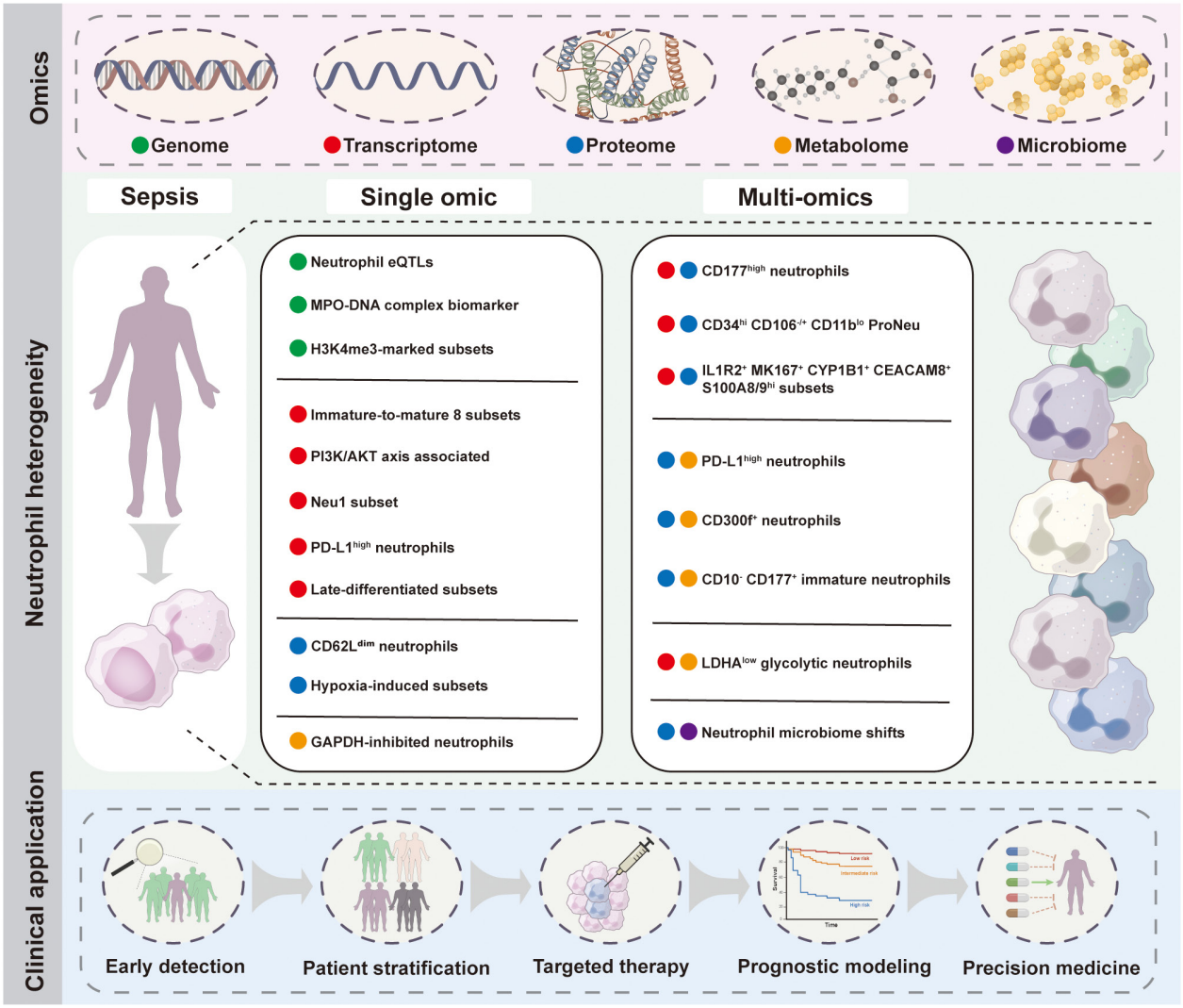

Figure 2. Multi-omics approaches reveal neutrophil heterogeneity and clinical implications in sepsis. Omics layers include genome, transcriptome, proteome, metabolome and microbiome. Neutrophil subsets and characteristics are identified through single-omics studies and multi-omics integration in sepsis. Potential clinical applications of neutrophil heterogeneity profiling include early detection, patient stratification, targeted therapy, prognostic modeling and precision medicine. eQTL, expression quantitative trait loci; MPO, myeloperoxidase; H3K4me3, histone H3 lysine 4 trimethylation; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; PD-L1, programmed death-ligand 1; MKI67, marker of proliferation Ki-67; CYP1B1, cytochrome P450 family 1 subfamily B member 1; CEACAM8, carcinoembryonic antigen-related cell adhesion molecule 8; S100A8/9, S100 calcium-binding protein A8/9; LDHA, lactate dehydrogenase A; Neu1, neutrophil subtype 1.

4. Multi-omics to explore neutrophil heterogeneity in sepsis

Each layer of omics offers insight into genomic variants, transcriptional changes, protein modification or metabolic shift. However, they often present a limited view of the complex interplay between these biological layers. For example, genomics identifies genetic predispositions that shape neutrophil responses, but does not capture the dynamic processes that occur during gene expression and protein translation. Similarly, transcriptomics reveals altered gene expression profiles associated with sepsis but may overlook the functional implications of changes at the protein level. Transitioning from single to multi-omics allows for a more holistic perspective by integrating data across different layers of biological information. Multi-omics approaches not only facilitate the identification of distinct neutrophil subsets and their functional states in real time but also enhance understanding of how metabolic changes influence neutrophil activity during sepsis (Fig. 2).

Transcriptome and proteome. Integration of transcriptomic and proteomic data has advanced understanding of neutrophil heterogeneity and its functional consequences during sepsis. By linking gene expression with protein abundance and post-translational modification, these approaches not only delineate the molecular programs of neutrophils but also capture how these programs are dynamically reconfigured under septic conditions.

The source of neutrophil heterogeneity remains debated. On one hand, studies have suggested that circulating neutrophils follow a predefined differentiation trajectory derived from hematopoietic progenitor cells (95,96). On the other hand, *in vitro* experiments have shown that stimulation of mature neutrophils with inflammation-associated molecules can also reshape their transcriptome, indicating that microenvironmental cues serve a crucial role in driving functional diversity (88,97). Thus, both developmental origin and peripheral reprogramming may contribute to heterogeneity.

Kaiser *et al* (98) used transcriptomics and proteomics to reveal mechanisms associated with functional reprogramming of peripheral neutrophils during acute infection. Transcriptome analysis revealed increased expression of classical neutrophil markers such as CXCL8, SOD2, S100A8/A9, CSF3 receptor and myeloid cell nuclear differentiation antigen (MNDA) (98). Activation markers and antimicrobial genes are upregulated, including cystatin F (CST7), S100 calcium binding protein A12 (S100A12), interleukin 1 receptor type 2 (IL1R2) and annexin A1 (ANXA1) (98). The aforementioned study used mass spectrometry to assess whether post-infection transcriptomic alterations are reflected in the proteome of human neutrophils (98). Corresponding upregulation at both transcriptional and protein levels included interferon-induced transmembrane protein 3 and alkaline phosphatase (98). In addition, patients with acute bacterial infections show enrichment of $CD177^{high}$ neutrophils (98). Both transcription and protein expression of CD177 are positively associated with disease severity (98). These results suggest that the transcriptome response of neutrophils is effectively translated into the proteome during bacterial inflammation. However, the aforementioned study did not resolve how transcriptomic shifts intersect with upstream regulatory mechanisms such as chromatin accessibility and transcription factor activity (95), leaving the drivers of neutrophil reprogramming incompletely understood. Moreover, although CD177 expression is associated with disease severity, its biological role remains unclear, especially since up to 10% of individuals lack CD177 without apparent immune defects (99-101). This raises the possibility that CD177 serves as a context-dependent marker rather than a direct effector. Finally, the cohort size and baseline heterogeneity may limit generalizability, and larger longitudinal studies are required to clarify the stability and functional relevance of $CD177^{+}$ neutrophils in bacterial infection (99-101).

In parallel, Kwok *et al* (102) identified a programmed neutrophil lineage within granulocyte-monocyte progenitors (GMPs) and analyzed the heterogeneous neutrophil subsets at different stages of this lineage in sepsis. During the differentiation of GMPs into preNeus, the aforementioned study described two phenotypically distinct types of neutrophil progenitors, termed proNeus (102). These include the $CD34^{hi}$ $CD106^{-}$ $CD11b^{lo}$ proNeu1 and the $CD34^{lo}$ $CD106^{+}$ $CD11b^{hi}$ proNeu2 subset (102). Proteomics shows progressive upregulation of CD11b and downregulation of CD34 expression during the differentiation of GMP into preNeus (102). Other differentially expressed cell surface markers include CD81, CD49a, CD106 and CD63, which may serve as positive or exclusive markers (102). Transcriptomics confirms the downregulated expression of lineage-associated genes and upregulated expression of granule protein genes in the GMP differentiation trajectory during sepsis, suggesting that sepsis promotes ProNeu1 differentiation (102). ProNeu1 and proNeu2 are both early neutrophil progenitor cell populations, but their functions are not identical (102). ProNeu1 exhibits a stronger capacity for proliferation than proNeu2 (102). During the early stages of sepsis inflammation, proNeu1 expands specifically and extensively, which decreases monocyte differentiation (102). However, proNeu2 remains largely unchanged during infection (102).

A recent multi-omics study by Kwok *et al* demonstrated that septic neutrophils acquire immunosuppressive properties and are enriched in patients with the sepsis response signature group 1 (SRS1) (103). Single-cell transcriptomics and cell surface protein profiling reveal the expansion of immature and functionally distinct neutrophil subsets, including $CEACAM8^{+}$ degranulating cells, $S100A8/9^{hi}$ cells, $IL1R2^{+}$, peptidyl arginine deiminase type 4^{+}, MPO^{+} and proliferative $MK167^{+}$ $CYP1B1^{+}$ neutrophils, many of which are specific to sepsis rather than sterile inflammation (103). Coculture assays further demonstrate that these septic subsets inhibit $CD4^{+}$ T cell activation (103). Epigenomic profiling of hematopoietic stem and progenitor cells revealed CCAAT/enhancer-binding protein α (CEBPA)- and β (CEBPB)-driven regulatory programs consistent with the activation of both steady-state and emergency granulopoiesis, linking neutrophil reprogramming to systemic alteration in hematopoiesis (103). Importantly, within the SRS1 subtype, neutrophil subsets such as $IL1R2^{+}$ and $MK167^{+}$ $CYP1B1^{+}$ cells are significantly enriched and exhibit STAT3- and CEBPB-dependent gene expression programs, indicating that neutrophil heterogeneity is not only a reflection of sepsis pathology but also a driver of immunosuppressive disease endotypes (103). However, the single-cell cohorts analyzed may not represent the spectrum of sepsis, and causal involvement of transcription factors such as CEBPB and STAT3 requires direct experimental validation.

Transcriptome and metabolome. Integration of transcriptomic and metabolomic approaches provides insights into how metabolic reprogramming shapes neutrophil heterogeneity during sepsis.

Neutrophils are reliant on glycolysis to fuel key antimicrobial functions, including chemotaxis, phagocytosis, oxidative burst and NET formation (104-106), yet their metabolic flexibility is altered in the septic milieu. Pan *et al* (107) demonstrated that sepsis-tolerant neutrophils exhibit reduced glycolytic activity associated with downregulation of LDHA via the PI3K/Akt/hypoxia-inducible factor (HIF)-1α pathway, leading to impaired chemotactic and phagocytic capacity. Metabolomic profiling further revealed that lactate levels are diminished in septic neutrophils compared with non-septic infected controls, highlighting a distinct metabolic state of glycolytic suppression (107).

Lactate, long regarded as a metabolic byproduct, is a immunomodulatory metabolite capable of exerting feedback control over immune cell metabolism (108,109). While evidence in monocytes and macrophages has demonstrated lactate-driven immunosuppression (110,111), its role in neutrophil biology remains less well understood. Pan *et al* (107) suggested that reduced lactate production in sepsis may represent a unique metabolic signature of neutrophil dysfunction, warranting further exploration of lactate-mediated feedback in neutrophil plasticity. At the molecular level, transcriptomic changes in key glycolytic enzymes, including LDHA, pyruvate dehydrogenase kinase 1, glucose transporter 1 (GLUT1) and pyruvate kinase M2 (PKM2) converge with metabolomic alterations, reinforcing the key role of glycolysis in neutrophil effector responses (107). Stabilization of HIF-1α restores LDHA expression and glycolytic activity, thereby rescuing neutrophil chemotaxis and phagocytosis, underscoring the PI3K/Akt/HIF-1α axis as a central regulator of neutrophil function in sepsis (107).

Collectively, combined transcriptomic and metabolomic analyses uncover a metabolically defined neutrophil subpopulation in sepsis characterized by glycolytic suppression and functional impairment (107). This multi-omics perspective not only advances understanding of neutrophil heterogeneity but also highlights metabolic checkpoints such as glycolysis and HIF-1α signaling as potential therapeutic targets to restore neutrophil immunity in sepsis (107).

Proteome and metabolome. Exploring the association between protein expression profiles and metabolic signatures reveals key regulatory hubs and pathways that influence neutrophil activation, survival and effector functions.

Using a proteomic approach, Parthasarathy *et al* (112) examined the differential expression of neutrophil subsets in patients with sepsis. The expression of CD10, CD16 and CD86 is downregulated, while human leukocyte antigen- DR isotype (HLA-DR), CD11b, CD80, CD184, CD63 and CD66b are upregulated in sepsis (39,112,113). The aforementioned study identified a specific mature neutrophil subset with high expression of CD274 (PD-L1) and CD300f. Previous studies have shown that blocking of PD-L1 improves survival in patients with sepsis (114-116), and CD300f deletion stimulates neutrophil recruitment to the site of infection and decreases septic death in mice (117). According to the expression of CD177, immature neutrophil subsets are divided into two subgroups: $CD10^{-}$ $CD177^{+}$ and $CD10^{-}$ $CD177^{-}$ (113). CD177 rapidly mobilizes specific particles to the cell surface following cell activation (112). Differential expression of CD184 (CXCR4) and HLA-DR in $CD10^{-}$ $CD177^{+}$ immature neutrophil subsets is observed in patients with sepsis (118). Aged neutrophils expressing CD184 exhibit a higher migratory activity and phagocytic capacity than the subsequently recruited non-aged neutrophils (65). HLA-DR expression was increased in $CD10^{-}$ $CD177^{+}$ septic neutrophils (112). The expression and role of HLA-DR on neutrophils remains unclear (119,120). Soluble factors pentraxin 3 (PTX3), angiopoietin-2 (Ang-2), endothelial cell-specific molecule-1 (Endocan), growth arrest-specific 6 (Gas6) and the inflammatory marker procalcitonin are upregulated in sepsis (112). These factors are elevated in patients with sepsis and contribute to vascular leak and endothelial dysfunction (121,122). Correlation analysis shows that CD10 is inversely correlated with these factors (112). Notably, immature neutrophils store and release PTX3 during inflammation, and this factor predicts disease severity and mortality in sepsis (123-126). Immature neutrophils may be a driver of vascular inflammation or leak in sepsis.

While these observations provide insights into the functional diversity of neutrophil subsets and their potential links to soluble mediators and vascular pathology, limitations remain. Distinguishing sepsis from other infections or organ failure remains clinically challenging. Integrated multi-omics approaches have revealed associations between neutrophil phenotypes, soluble mediators and metabolic alterations. However, these findings are largely observational and derived from limited patient cohorts (112). Therefore, further mechanistic studies are needed to validate whether specific neutrophil subsets and their products directly drive vascular leakage and immune dysregulation in sepsis.

Proteome and microbiome. The combined investigation of proteome and microbiome has revealed the functional dynamics of neutrophils in the context of sepsis.

Wang *et al* (127) isolated neutrophils from patients with sepsis after surgery and characterized intracellular bacterial communities, also termed as the neutrophil-specific microbiome. The aforementioned study showed that the proportion of actinobacteria decreased, while the levels of proteobacteria increased (127). Compared with healthy controls, the abundance of *Escherichia/Shigella*, *Klebsiella* and *Bradyrhizobium* is higher in patients with sepsis (127). The neutrophil-specific microbiome of patients with sepsis exhibits heterogeneity (127). Dysregulation of the circulating microbiota may increase the risk of postoperative infectious events (128). In addition, quantitative proteomic analysis of neutrophils derived from patients with demonstrates proteins involved in bactericidal activities of neutrophils are downregulated, especially in patients with septic shock (127). Significant downregulation of some immunomodulatory-associated proteins is also observed in sepsis patients, including integrin α-M, IgA Fc receptor and lactotransferrin (127). MMP9 is significantly downregulated in patients with septic shock, indicating that the migratory activity of neutrophils is impaired (128). Proteomic analysis reveals a decrease in neutrophil function in sepsis (127).

Beyond elucidating the molecular heterogeneity of neutrophils, multi-omics suggests potential biomarkers and therapeutic targets with translational relevance. Table III summarizes representative neutrophil-associated biomarkers identified from multi-omics, along with their potential diagnostic or prognostic value in sepsis (28,77,78,80-83,88,89,91, 94,98,102,103,107,112,115,117,127).

5. Challenges and limitations

Despite the advances in understanding neutrophil heterogeneity in sepsis through multi-omics, key challenges hinder the full potential of these approaches.

Patient heterogeneity is a key obstacle to characterizing neutrophil heterogeneity in sepsis through multi-omics approaches. Variations in demographic and physiological backgrounds (age, sex, genetic factors) shape neutrophil development and lifespan, potentially masking sepsis-specific changes (129-132). Underlying comorbid conditions may introduce baseline alterations in neutrophil function, thereby contributing to heterogeneity and complicating the attribution of omics signatures solely to sepsis (133). In addition, pathogen type, primary infection site and therapeutic intervention may induce distinct neutrophil activation programs, while clinical trajectories range from hyperinflammation to immunosuppression, generating variable molecular patterns (33,134-136). These sources of variability make datasets harder to compare, decrease reproducibility and blur the true sepsis-associated neutrophil signatures. To overcome this challenge, future studies should incorporate strategies such as stratified analyses, matched cohort designs and standardized metadata collection to distinguish patient-level variability from the intrinsic biological diversity of neutrophils in sepsis.

Sample preparation is a key step in multi-omics studies, but introduces inherent biases, particularly during cell isolation and processing. For example, methods based on density

Table III. Clinical translation and biomarker potential of neutrophil signatures identified by multi-omics in sepsis.

Biomarker/subtype	Omics source	Clinical relevance	(Refs.)
Neutrophil eQTL	Genomics	Identification of 21,210 neutrophil eQTLs (832 genes) underscores the role of neutrophils in host defense and inflammation	(77)
MPO-DNA complex	Genomics	Elevated MPO-DNA associated with increased risk of sepsis (18%), 28-day mortality (51%) and ICU requirement (38%) and mortality (125%)	(78)
H3K4me3-marked subsets	Epigenome	IL-10 induces transcriptionally active neutrophils, contributing to inflammation resolution; H3K4me3 positioning suggests potential immunotherapeutic targets	(80)
Transcriptional neutrophil clusters	Transcriptome (scRNA-seq)	Infection accelerates immature-to-mature transition; developmental stage-dependent neutrophil responses to infection	(28)
DEGs	Transcriptome (bulk RNA-seq)	DEGs enriched in immune/inflammatory signaling and PI3K/AKT pathway; AKT1 validated as a potential therapeutic target in sepsis	(81)
Neutrophil subtypes enriched at late differentiation stage	Transcriptome (scRNA-seq)	A total of two novel subsets enriched in human sepsis; five hub genes validated in mice; candidate biomarkers and therapeutic targets (ALPL, CD177, S100A8/A9, STXBP2)	(82)
Neu1	Transcriptome (scRNA-seq)	Neu1 associated with septic shock and SOFA score; Neu1_C module predictive for shock (AUC=0.81); expresses poor prognosis markers (CD123, CD38, CD69)	(83)
PD-L1^{high} neutrophils	Transcriptome (scRNA-seq)	Immunosuppressive subset in sepsis (p38α/MSK1/MK2 dependent); directly inhibits T cell response; associated with immune suppression and poor host defense; potential therapeutic target via PD-L1 blockade	(88)
CD62L^{dim} neutrophils	Proteome	Distinct subset recruited during acute inflammation; proteomic profile enriched in adhesion/activation pathways; potential biomarker of systemic inflammation	(89)
Hypoxia-induced neutrophil proteomic remodeling	Proteome	Inflammatory receptor upregulation (FPR, GM-CSFRβ) and lysosomal protein scavenging sustain neutrophil metabolism under stress; metabolic pathways as potential therapeutic targets in hypoxic inflamed tissue	(91)
GAPDH-inhibited neutrophils in severe COVID-19	Metabolome	Dysregulated amino acid, redox and carbon metabolism; suppressed glycolysis with PPP shift; GAPDH inhibition triggers pathogenic NET formation; links neutrophil metabolic dysfunction to tissue damage and highlights GAPDH as a potential therapeutic target	(94)
CD177^{high} neutrophils	Transcriptome and proteome	Expanded during acute bacterial infection; TLR4/NF-κB driven transcriptional plasticity enhances antibacterial programs; CD177 upregulation is associated with inflammation severity and may serve as a marker of acute infectious states	(98)
CD34^{hi} CD106^{-} CD11b^{lo} ProNeu1	Transcriptome and proteome	Early committed progenitor; expands in early sepsis with high proliferative capacity, suppressing monocyte output; potential biomarker of emergency granulopoiesis	(102)
CD34^{lo} CD106^{+} CD11b^{hi} ProNeu2	Transcriptome and proteome	Intermediate progenitor; stable during sepsis; reference subset for maturation trajectory	(102)
IL1R2^{+}, MK167^{+} CYP1B1^{+}, CEACAM8^{+}, S100A8/9^{hi}	Transcriptome (scRNA-seq) and proteome	Sepsis-specific; inhibit CD4^{+} T cells; enriched in SRS1 endotype; associated with STAT3/CEBPB-driven immunosuppression	(103)
LDHA^{low} glycolytic neutrophils	Transcriptome and metabolome	Exhibit suppressed glycolysis with impaired chemotaxis and phagocytosis; dysfunction mediated by PI3K-Akt-HIF-1α-LDHA axis; reversible by HIF-1α activation, suggesting therapeutic potential for restoring immune competence in sepsis	(107)
CD10^{-} CD177^{+} immature neutrophils	Proteome and metabolome	Associated with SOFA score and endothelial dysfunction (PTX3, Ang-2, endocan); PTX3 release associated with severity and mortality; associated with metabolic dysregulation and identified as a feature for patient stratification	(112)

Table III. Continued.

Biomarker/subtype	Omics source	Clinical relevance	(Refs.)
PD-L1^{high} neutrophils	Proteome and metabolome	PD-L1 blockade improves survival in murine CLP sepsis model	(115)
CD300f^{+} neutrophils	Proteome and metabolome	Disruption or deletion of CD300f decreases septic mortality in murine CLP model of sepsis	(117)
Neutrophil microbiome shifts	Proteome and microbiome	Associated with impaired bactericidal activity and migration; downregulation of ITGAM, LTF and MMP9; associated with septic shock	(127)

eQTL, expression quantitative trait loci; MPO, myeloperoxidase; DEG, differentially expressed gene; seq, sequencing; sc, single-cell; SOFA, Sequential Organ Failure Assessment; PD-L1, programmed death ligand 1; CD62L, CD62 ligand; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α; ChIP, chromatin immunoprecipitation; H3K4me3, histone H3 lysine 4 trimethylation; PI3K, phosphoinositide-3-kinase; STXBP2, syntaxin-binding protein 2; MK2, MAPK-activated protein kinase 2; FPR, formyl peptide receptor; GM-CSFR, granulocyte-macrophage colony-stimulating factor receptor; PPP, pentose phosphate pathway; TLR4, toll-like receptor 4; C/EBP, CCAAT/enhancer-binding protein; LDHA, lactate dehydrogenase A; HIF-1α, hypoxia-inducible factor 1 α; PTX3, pentraxin 3; Ang-2, angiopoietin-2; endocan, endothelial cell-specific molecule-1; CLP, cecal ligation and puncture; ITGAM, integrin α M; LTF, lactotransferrin.

gradient centrifugation may inadvertently exclude low-density neutrophils (LDNs), which are typically found in the peripheral blood mononuclear cell fraction (137). By contrast, commonly used methods obtain samples from peripheral blood or tissue, followed by red blood cell lysis or enzymatic digestion (138). The cells are centrifuged, resuspended, washed and filtered to ensure high-quality viable cells. Neutrophils are purified by either positive or negative selection using magnetic beads or fluorescence-activated cell sorting and subjected to scRNA-seq for downstream analysis (28). Other improvements include initial selection of total neutrophils using magnetic beads followed by density gradient separation of LDNs (139,140). In addition, density gradient-based approaches to neutrophil isolation may result in preparations containing small numbers of contaminating leukocytes, primarily eosinophils, a potential source of bias despite the small contribution of these leukocytes to the overall gene expression profile (141). These technical variations can lead to discrepancies between *in vitro* or *ex vivo* findings and the actual *in vivo* behavior of neutrophils, particularly within the complex microenvironment of sepsis.

The temporal dynamics of neutrophil changes during sepsis are not well understood. The heterogeneity of neutrophils in sepsis results from a complex interaction between intrinsic factors and disease-associated changes over time. Sepsis is a dynamic condition, marked by rapid shifts in immune response, from excessive inflammation in the early stage to immunosuppression in the later stages (33). However, most current multi-omics studies focus on single time-point analyses, providing a static snapshot of neutrophil phenotypes, which may overlook the ongoing changes in neutrophils during the immune transition in sepsis (83,112). Therefore, longitudinal multi-omics studies are necessary to capture the immune switching of neutrophils over time and improve understanding of this dynamic process.

Each omics layer provides a unique resolution and focus for capturing biological heterogeneity, yet these differences also impose analytical limitations. The integration of multi-omics data from high-throughput platforms inherently faces challenges due to their diverse characteristics (142). Picard *et al* (143) highlighted that the heterogeneity of data complicates the integration process. For example, transcriptomic data is often subjected to RNA-seq normalization, while proteomic data typically rely on mass spectrometry-specific scaling methods, leading to differing data ranges and distribution patterns. These disparities necessitate alignment procedures before integration. Data quality also poses a concern. Issues such as noise, missing values and batch effects notably impact the outcomes of the analysis (144). In addition, there are kinetic differences between RNA and protein expression. For example, Hoogendijk *et al* (145) reported that nearly 30% of the transcriptome -proteome pairs showed inconsistent dynamics during neutrophil differentiation. This discrepancy may contribute to the inconsistency between the transcriptome data from scRNA-seq and the protein-based surface marker data from mass cytometry. These mismatches complicate the classification of cell populations and the annotation of functional roles.

6. Conclusion

Sepsis is a life-threatening syndrome characterized by a systemic inflammatory response to infection, leading to multiorgan dysfunction and potential mortality. The pathophysiology of sepsis involves complex interactions between pathogens, the immune system and various host factors. Advancements in research have demonstrated the key role of immune cells in sepsis, particularly neutrophils, which are frontline responders in the immune system. The functional and phenotypical heterogeneity of these immune cells during sepsis can influence outcomes.

In terms of phenotypical diversity, neutrophils in sepsis exhibit various surface markers and morphologies that reflect their activation state, origin and roles in the inflammatory response. Functionally, neutrophils exhibit heterogeneity in their ability to degranulate, phagocytose, release ROS, form NETs, migrate, undergo apoptosis and mediate immunosuppression. Understanding these functional differences is key for

developing targeted therapeutic strategies aimed at modulating neutrophil activity during sepsis.

The study of neutrophil heterogeneity in sepsis through multi-omics approaches has provided insights into the complex and adaptive nature of these immune cells. Multi-omics studies have revealed that the differentially expressed genes, proteins and metabolites underlying neutrophil heterogeneity are not independent events but reflect interconnected layers of regulation. Transcriptomic analyses have identified altered expression of transcription factors and signaling molecules that reprogram neutrophil activation, survival and differentiation, while proteomic profiling has identified changes in effector functions, including degranulation, phagocytosis and cytokine release (81,88). Metabolomic data have further indicated shifts in glycolysis, oxidative phosphorylation and amino acid metabolism that provide energetic and biosynthetic support for these functions. In addition, epigenomic profiling has elucidated associations between chromatin accessibility and histone modifications with NF-κB signaling, apoptosis and cytokine regulation, thereby uncovering previously unrecognized neutrophil subsets (80,107). Multi-omics integration has identified transcription factor-driven programs, such as CEBPA/CEBPB- and STAT3-dependent networks, which connect emergency granulopoiesis with the emergence of sepsis-specific neutrophil subsets (103). Collectively, these interconnected regulatory networks provide a mechanistic basis by which neutrophils acquire distinct functional states, thereby contributing to the phenotypical and functional heterogeneity observed in sepsis.

Acknowledgements

Not applicable.

Funding

The present study was supported by Hubei Province health and family planning scientific research project (grant no. WJ2023M015).

Availability of data and materials

Not applicable.

Authors' contributions

ZT designed the study. DC, PZ, JL, SC, SG, YC, YS, TT, LD and TC performed the literature review. ZL wrote the manuscript. CZ edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, *et al*: The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 315: 801-810, 2016.
2. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S, *et al*: Global, regional, and national sepsis incidence and mortality, 1990-2017: Analysis for the Global Burden of Disease Study. Lancet 395: 200-211, 2020.
3. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, Angus DC and Reinhart K; International Forum of Acute Care Trialists: Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. Am J Respir Crit Care Med 193: 259-272, 2016.
4. Prescott HC and Angus DC: Enhancing recovery from sepsis: A review. JAMA 319: 62-75, 2018.
5. Prescott HC, Langa KM and Iwashyna TJ: Readmission diagnoses after hospitalization for severe sepsis and other acute medical conditions. JAMA 313: 1055-1057, 2015.
6. Shankar-Hari M, Saha R, Wilson J, Prescott HC, Harrison D, Rowan K, Rubenfeld GD and Adhikari NKJ: Rate and risk factors for rehospitalisation in sepsis survivors: Systematic review and meta-analysis. Intensive Care Med 46: 619-636, 2020.
7. van der Poll T, van de Veerdonk FL, Scicluna BP and Netea MG: The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol 17: 407-420, 2017.
8. Chen S, Zhang C, Luo J, Lin Z, Chang T, Dong L, Chen D and Tang ZH: Macrophage activation syndrome in Sepsis: from pathogenesis to clinical management. Inflamm Res 73: 2179-2197, 2024.
9. Chang TD, Chen D, Luo JL, Wang YM, Zhang C, Chen SY, Lin ZQ, Zhang PD, Tang TX, Li H, *et al*: The different paradigms of NK cell death in patients with severe trauma. Cell Death Dis 15: 606, 2024.
10. Chen S, Zhang C, Chen D, Dong L, Chang T and Tang ZH: Advances in attractive therapeutic approach for macrophage activation syndrome in COVID-19. Front Immunol 14: 1200289, 2023.
11. Chen D, Zhang C, Luo J, Deng H, Yang J, Chen S, Zhang P, Dong L, Chang T and Tang ZH: Activated autophagy of innate immune cells during the early stages of major trauma. Front Immunol 13: 1090358, 2023.
12. Yang J, Chang T, Tang L, Deng H, Chen D, Luo J, Wu H, Tang T, Zhang C, Li Z, *et al*: Increased expression of Tim-3 is associated with depletion of NKT Cells In SARS-CoV-2 infection. Front Immunol 13: 796682, 2022.
13. Fine N, Tasevski N, McCulloch CA, Tenenbaum HC and Glogauer M: The Neutrophil: Constant defender and first responder. Front Immunol 11: 571085, 2020.
14. Kangelaris KN, Clemens R, Fang X, Jauregui A, Liu T, Vessel K, Deiss T, Sinha P, Leligdowicz A, Liu KD, *et al*: A neutrophil subset defined by intracellular olfactomedin 4 is associated with mortality in sepsis. Am J Physiol Lung Cell Mol Physiol 320: L892-L902, 2021.
15. Shen XF, Cao K, Jiang JP, Guan WX and Du JF: Neutrophil dysregulation during sepsis: an overview and update. J Cell Mol Med 21: 1687-1697, 2017.
16. Silvestre-Roig C, Hidalgo A and Soehnlein O: Neutrophil heterogeneity: Implications for homeostasis and pathogenesis. Blood 127: 2173-2181, 2016.
17. Naranbhai V, Fairfax BP, Makino S, Humburg P, Wong D, Ng E, Hill AV and Knight JC: Genomic modulators of gene expression in human neutrophils. Nat Commun 6: 7545, 2015.
18. Coit P, Yalavarthi S, Ognenovski M, Zhao W, Hasni S, Wren JD, Kaplan MJ and Sawalha AH: Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. J Autoimmun 58: 59-66, 2015.
19. Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K and Koenderman L: In vivo labeling with 2H2O reveals a human neutrophil lifespan of 5.4 days. Blood 116: 625-627, 2010.

20. Ericson JA, Duffau P, Yasuda K, Ortiz-Lopez A, Rothamel K, Rifkin IR and Monach PA; ImmGen Consortium: Gene expression during the generation and activation of mouse neutrophils: Implication of novel functional and regulatory pathways. PLoS One 9: e108553, 2014.
21. Ng LG, Ostuni R and Hidalgo A: Heterogeneity of neutrophils. Nat Rev Immunol 19: 255-265, 2019.
22. Grecian R, Whyte MKB and Walmsley SR: The role of neutrophils in cancer. Br Med Bull 128: 5-14, 2018.
23. Day RB and Link DC: Regulation of neutrophil trafficking from the bone marrow. Cell Mol Life Sci 69: 1415-1423, 2012.
24. Elghetany MT: Surface antigen changes during normal neutrophilic development: A critical review. Blood Cells Mol Dis 28: 260-274, 2002.
25. Seree-Aphinan C, Vichitkunakorn P, Navakanitworakul R and Khwannimit B: Distinguishing sepsis from infection by neutrophil dysfunction: A promising role of CXCR2 surface level. Front Immunol 11: 608696, 2020.
26. Silvestre-Roig C, Fridlender ZG, Glogauer M and Scapini P: Neutrophil diversity in health and disease. Trends Immunol 40: 565-583, 2019.
27. Wang X, Fan D, Yang Y, Gimple RC and Zhou S: Integrative multi-omics approaches to explore immune cell functions: Challenges and opportunities. iScience 26: 106359, 2023.
28. Xie X, Shi Q, Wu P, Zhang X, Kambara H, Su J, Yu H, Park SY, Guo R, Ren Q, *et al*: Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. Nat Immunol 21: 1119-1133, 2020.
29. Shaath H, Vishnubalaji R, Elkord E and Alajez NM: Single-cell transcriptome analysis highlights a role for neutrophils and inflammatory macrophages in the pathogenesis of severe COVID-19. Cells 9: 2374, 2020.
30. Deerhake ME, Reyes EY, Xu-Vanpala S and Shinohara ML: Single-Cell transcriptional heterogeneity of neutrophils during acute pulmonary cryptococcus neoformans infection. Front Immunol 12: 670574, 2021.
31. Civelek M and Lusis AJ: Systems genetics approaches to understand complex traits. Nat Rev Genet 15: 34-48, 2014.
32. Johnson Chavarria EC: A primer of human genetics. Yale J Biol Med 89: 603, 2016.
33. van der Poll T, Shankar-Hari M and Wiersinga WJ: The immunology of sepsis. Immunity 54: 2450-2464, 2021.
34. Borregaard N: Neutrophils, from marrow to microbes. Immunity 33: 657-670, 2010.
35. Stadtmann A and Zarbock A: CXCR2: From bench to bedside. Front Immunol 3: 263, 2012.
36. Phillipson M and Kubes P: The neutrophil in vascular inflammation. Nat Med 17: 1381-1390, 2011.
37. Chishti AD, Shenton BK, Kirby JA and Baudouin SV: Neutrophil chemotaxis and receptor expression in clinical septic shock. Intensive Care Med 30: 605-611, 2004.
38. Rios-Santos F, Alves-Filho JC, Souto FO, Spiller F, Freitas A, Lotufo CM, Soares MB, Dos Santos RR, Teixeira MM and Cunha FQ: Down-regulation of CXCR2 on neutrophils in severe sepsis is mediated by inducible nitric oxide synthase-derived nitric oxide. Am J Respir Crit Care Med 175: 490-497, 2007.
39. Demaret J, Venet F, Friggeri A, Cazalis MA, Plassais J, Jallades L, Malcus C, Poitevin-Later F, Textoris J, Lepape A and Monneret G: Marked alterations of neutrophil functions during sepsis-induced immunosuppression. J Leukoc Biol 98: 1081-1090, 2015.
40. Pillay J, Kamp VM, van Hoffen E, Visser T, Tak T, Lammers JW, Ulfman LH, Leenen LP, Pickkers P and Koenderman L: A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. J Clin Invest 122: 327-336, 2012.
41. Geng S, Matsushima H, Okamoto T, Yao Y, Lu R, Page K, Blumenthal RM, Ward NL, Miyazaki T and Takashima A: Emergence, origin, and function of neutrophil-dendritic cell hybrids in experimentally induced inflammatory lesions in mice. Blood 121: 1690-1700, 2013.
42. Ode Y, Aziz M and Wang P: CIRP increases ICAM-1(+) phenotype of neutrophils exhibiting elevated iNOS and NETs in sepsis. J Leukoc Biol 103: 693-707, 2018.
43. Hoffmann JJ: Neutrophil CD64: A diagnostic marker for infection and sepsis. Clin Chem Lab Med 47: 903-916, 2009.
44. Hoffmann JJ: Neutrophil CD64 as a sepsis biomarker. Biochem Med (Zagreb) 21: 282-290, 2011.
45. Cid J, Aguinaco R, Sánchez R, García-Pardo G and Llorente A: Neutrophil CD64 expression as marker of bacterial infection: A systematic review and meta-analysis. J Infect 60: 313-319, 2010.
46. Li S, Huang X, Chen Z, Zhong H, Peng Q, Deng Y, Qin X and Zhao J: Neutrophil CD64 expression as a biomarker in the early diagnosis of bacterial infection: A meta-analysis. Int J Infect Dis 17: e12-e23, 2013.
47. Bouchon A, Facchetti F, Weigand MA and Colonna M: TREM-1 amplifies inflammation and is a crucial mediator of septic shock. Nature 410: 1103-1107, 2001.
48. Demaret J, Venet F, Plassais J, Cazalis MA, Vallin H, Friggeri A, Lepape A, Rimmelé T, Textoris J and Monneret G: Identification of CD177 as the most dysregulated parameter in a microarray study of purified neutrophils from septic shock patients. Immunol Lett 178: 122-130, 2016.
49. Amulic B, Cazalet C, Hayes GL, Metzler KD and Zychlinsky A: Neutrophil function: From mechanisms to disease. Annu Rev Immunol 30: 459-489, 2012.
50. Boeltz S, Amini P, Anders HJ, Andrade F, Bilyy R, Chatfield S, Cichon I, Clancy DM, Desai J, Dumych T, *et al*: To NET or not to NET: Current opinions and state of the science regarding the formation of neutrophil extracellular traps. Cell Death Differ 26: 395-408, 2019.
51. Patel JM, Sapey E, Parekh D, Scott A, Dosanjh D, Gao F and Thickett DR: Sepsis Induces a Dysregulated Neutrophil Phenotype That Is Associated with Increased Mortality. Mediators Inflamm 2018: 4065362, 2018.
52. Martins PS, Kallas EG, Neto MC, Dalboni MA, Blecher S and Salomão R: Upregulation of reactive oxygen species generation and phagocytosis, and increased apoptosis in human neutrophils during severe sepsis and septic shock. Shock 20: 208-212, 2003.
53. Alves-Filho JC, Spiller F and Cunha FQ: Neutrophil paralysis in sepsis. Shock 34 (Suppl 1): S15-S21, 2010.
54. Reddy RC and Standiford TJ: Effects of sepsis on neutrophil chemotaxis. Curr Opin Hematol 17: 18-24, 2010.
55. Tavares-Murta BM, Zaparoli M, Ferreira RB, Silva-Vergara ML, Oliveira CH, Murta EF, Ferreira SH and Cunha FQ: Failure of neutrophil chemotactic function in septic patients. Crit Care Med 30: 1056-1061, 2002.
56. Brown KA, Brain SD, Pearson JD, Edgeworth JD, Lewis SM and Treacher DF: Neutrophils in development of multiple organ failure in sepsis. Lancet 368: 157-169, 2006.
57. Arraes SM, Freitas MS, da Silva SV, de Paula Neto HA, Alves-Filho JC, Auxiliadora Martins M, Basile-Filho A, Tavares-Murta BM, Barja-Fidalgo C and Cunha FQ: Impaired neutrophil chemotaxis in sepsis associates with GRK expression and inhibition of actin assembly and tyrosine phosphorylation. Blood 108: 2906-2913, 2006.
58. Martins PS, Brunialti MK, Martos LS, Machado FR, Assunçao MS, Blecher S and Salomao R: Expression of cell surface receptors and oxidative metabolism modulation in the clinical continuum of sepsis. Crit Care 12: R25, 2008.
59. Kalyan S and Kabelitz D: When neutrophils meet T cells: Beginnings of a tumultuous relationship with underappreciated potential. Eur J Immunol 44: 627-633, 2014.
60. Kovach MA and Standiford TJ: The function of neutrophils in sepsis. Curr Opin Infect Dis 25: 321-327, 2012.
61. Chen D, Tang TX, Deng H, Yang XP and Tang ZH: Interleukin-7 biology and its effects on immune cells: Mediator of generation, differentiation, survival, and homeostasis. Front Immunol 12: 747324, 2021.
62. Bone RC, Grodzin CJ and Balk RA: Sepsis: A new hypothesis for pathogenesis of the disease process. Chest 112: 235-243, 1997.
63. Alves-Filho JC, de Freitas A, Spiller F, Souto FO and Cunha FQ: The role of neutrophils in severe sepsis. Shock 30 (Suppl 1): S3-S9, 2008.
64. Drifte G, Dunn-Siegrist I, Tissières P and Pugin J: Innate immune functions of immature neutrophils in patients with sepsis and severe systemic inflammatory response syndrome. Crit Care Med 41: 820-832, 2013.
65. Taneja R, Sharma AP, Hallett MB, Findlay GP and Morris MR: Immature circulating neutrophils in sepsis have impaired phagocytosis and calcium signaling. Shock 30: 618-622, 2008.
66. Uhl B, Vadlau Y, Zuchtriegel G, Nekolla K, Sharaf K, Gaertner F, Massberg S, Krombach F and Reichel CA: Aged neutrophils contribute to the first line of defense in the acute inflammatory response. Blood 128: 2327-2337, 2016.
67. Yang P, Li Y, Xie Y and Liu Y: Different faces for different places: Heterogeneity of neutrophil phenotype and function. J Immunol Res 2019: 8016254, 2019.
68. van Dijk EL, Jaszczyszyn Y, Naquin D and Thermes C: The third revolution in sequencing technology. Trends Genet 34: 666-681, 2018.

69. Warr A, Robert C, Hume D, Archibald A, Deeb N and Watson M: Exome sequencing: Current and future perspectives. G3 (Bethesda) 5: 1543-1550, 2015.
70. Goldman AD and Landweber LF: What is a genome? PLoS Genet 12: e1006181, 2016.
71. Wang KC and Chang HY: Epigenomics: Technologies and applications. Circ Res 122: 1191-1199, 2018.
72. Wang Z, Gerstein M and Snyder M: RNA-Seq: A revolutionary tool for transcriptomics. Nat Rev Genet 10: 57-63, 2009.
73. Timp W and Timp G: Beyond mass spectrometry, the next step in proteomics. Sci Adv 6: eaax8978, 2020.
74. Fu Q, Vegesna M, Sundararaman N, Damoc E, Arrey TN, Pashkova A, Mengesha E, Debbas P, Joung S, Li D, *et al*: A proteomics pipeline for generating clinical grade biomarker candidates from data-independent acquisition mass spectrometry (DIA-MS) discovery. Angew Chem Int Ed Engl 63: e202409446, 2024.
75. Moco S, Vervoort J, Moco S, Bino RJ, De Vos RC and Bino R: Metabolomics technologies and metabolite identification. TrAC Trends in Analytical Chemistry. 2007;26: 855-866, 2007.
76. Bedair M and Sumner LW: Current and emerging mass-spectrometry technologies for metabolomics. TrAC Trends in Analytical Chemistry 27: 238-250, 2008.
77. Andiappan AK, Melchiotti R, Poh TY, Nah M, Puan KJ, Vigano E, Haase D, Yusof N, San Luis B, Lum J, *et al*: Genome-wide analysis of the genetic regulation of gene expression in human neutrophils. Nat Commun 6: 7971, 2015.
78. Wang J, Zhang Y, Cheng L, Geng Y, Lu J and Zhou J: Neutrophil extracellular trap increase the risk of sepsis: A two-sample, one-way Mendelian randomization study. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 35: 1045-1052, 2023 (In Chinese).
79. Zhang H, Wang Y, Qu M, Li W, Wu D, Cata JP and Miao C: Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis. Clin Transl Med 13: e1170, 2023.
80. Piatek P, Namiecinska M, Lewkowicz N, Kulińska-Michalska M, Jabłonowski Z, Matysiak M, Dulska J, Michlewska S, Wieczorek M and Lewkowicz P: Changes WIthin H3K4me3-marked histone reveal molecular background of neutrophil functional plasticity. Front Immunol 13: 906311, 2022.
81. Liu Z, Chen Y, Pan T, Liu J, Tian R, Sun S, Qu H and Chen E: Comprehensive analysis of common different gene expression signatures in the neutrophils of sepsis. Biomed Res Int 2021: 6655425, 2021.
82. Xu P, Tao Z and Zhang C: Integrated multi-omics and artificial intelligence to explore new neutrophils clusters and potential biomarkers in sepsis with experimental validation. Front Immunol 15: 1377817, 2024.
83. Hong Y, Chen L, Sun J, Xing L, Yang Y, Jin X, Cai H, Dong L, Zhou L and Zhang Z: Single-cell transcriptome profiling reveals heterogeneous neutrophils with prognostic values in sepsis. iScience 25: 105301, 2022.
84. Goswami DG, Garcia LF, Dodoo C, Dwivedi AK, Zhou Y, Pappas D and Walker WE: Evaluating the Timeliness and Specificity of CD69, CD64, and CD25 as Biomarkers of Sepsis in Mice. Shock 55: 507-518, 2021.
85. Zhou Y, Zhang Y, Johnson A, Venable A, Griswold J and Pappas D: Combined CD25, CD64, and CD69 biomarker panel for flow cytometry diagnosis of sepsis. Talanta 191: 216-221, 2019.
86. Meghraoui-Kheddar A, Chousterman BG, Guillou N, Barone SM, Granjeaud S, Vallet H, Corneau A, Guessous K, de Roquetaillade C, Boissonnas A, *et al*: Two new neutrophil subsets define a discriminating sepsis signature. Am J Respir Crit Care Med 205: 46-59, 2022.
87. Wang P, Wang J, Li YH, Wang L, Shang HC and Wang JX: Phenotypical changes of hematopoietic stem and progenitor cells in sepsis patients: Correlation with immune status? Front Pharmacol 11: 640203, 2020.
88. Qi X, Yu Y, Sun R, Huang J, Liu L, Yang Y, Rui T and Sun B: Identification and characterization of neutrophil heterogeneity in sepsis. Crit Care 25: 50, 2021.
89. Tak T, Wijten P, Heeres M, Pickkers P, Scholten A, Heck AJR, Vrisekoop N, Leenen LP, Borghans JAM, Tesselaar K and Koenderman L: Human CD62L(dim) neutrophils identified as a separate subset by proteome profiling and in vivo pulse-chase labeling. Blood 129: 3476-3485, 2017.
90. Mallat J, Rahman N, Hamed F, Hernandez G and Fischer MO: Pathophysiology, mechanisms, and managements of tissue hypoxia. Anaesth Crit Care Pain Med 41: 101087, 2022.
91. Watts ER, Howden AJ, Morrison T, Sadiku P, Hukelmann J, von Kriegsheim A, Ghesquiere B, Murphy F, Mirchandani AS, Humphries DC, *et al*: Hypoxia drives murine neutrophil protein scavenging to maintain central carbon metabolism. J Clin Invest 131: e134073, 2021.
92. Sun L, Yang X, Yuan Z and Wang H: Metabolic reprogramming in immune response and tissue inflammation. Arterioscler Thromb Vasc Biol 40: 1990-2001, 2020.
93. Kumar S and Dikshit M: Metabolic insight of neutrophils in health and disease. Front Immunol 10: 2099, 2019.
94. Li Y, Hook JS, Ding Q, Xiao X, Chung SS, Mettlen M, Xu L, Moreland JG and Agathocleous M: Neutrophil metabolomics in severe COVID-19 reveal GAPDH as a suppressor of neutrophil extracellular trap formation. Nat Commun 14: 2610, 2023.
95. Khoyratty TE, Ai Z, Ballesteros I, Eames HL, Mathie S, Martín-Salamanca S, Wang L, Hemmings A, Willemsen N, von Werz V, *et al*: Distinct transcription factor networks control neutrophil-driven inflammation. Nat Immunol 22: 1093-1106, 2021.
96. Grieshaber-Bouyer R, Radtke FA, Cunin P, Stifano G, Levescot A, Vijaykumar B, Nelson-Maney N, Blaustein RB, Monach PA and Nigrovic PA; ImmGen Consortium: The neutrotime transcriptional signature defines a single continuum of neutrophils across biological compartments. Nat Commun 12: 2856, 2021.
97. Tsukahara Y, Lian Z, Zhang X, Whitney C, Kluger Y, Tuck D, Yamaga S, Nakayama Y, Weissman SM and Newburger PE: Gene expression in human neutrophils during activation and priming by bacterial lipopolysaccharide. J Cell Biochem 89: 848-861, 2003.
98. Kaiser R, Gold C, Joppich M, Loew Q, Akhalkatsi A, Mueller TT, Offensperger F, Droste Zu Senden A, Popp O, di Fina L, *et al*: Peripheral priming induces plastic transcriptomic and proteomic responses in circulating neutrophils required for pathogen containment. Sci Adv 10: eadl1710, 2024.
99. Grieshaber-Bouyer R and Nigrovic PA: Neutrophil heterogeneity as therapeutic opportunity in immune-mediated disease. Front Immunol 10: 346, 2019.
100. Eulenberg-Gustavus C, Bähring S, Maass PG, Luft FC and Kettritz R: Gene silencing and a novel monoallelic expression pattern in distinct CD177 neutrophil subsets. J Exp Med 214: 2089-2101, 2017.
101. Wu Z, Liang R, Ohnesorg T, Cho V, Lam W, Abhayaratna WP, Gatenby PA, Perera C, Zhang Y, Whittle B, *et al*: Heterogeneity of human neutrophil CD177 expression results from CD177P1 pseudogene conversion. PLoS Genet 12: e1006067, 2016.
102. Kwok I, Becht E, Xia Y, Ng M, The YC, Tan L, Evrard M, Li JLY, Tran HTN, Tan Y, *et al*: Combinatorial single-cell analyses of granulocyte-monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. Immunity 53: 303-318.e5, 2020.
103. Kwok AJ, Allcock A, Ferreira RC, Cano-Gamez E, Smee M, Burnham KL, Zurke YX; Emergency Medicine Research Oxford (EMROx); McKechnie S, Mentzer AJ, *et al*: Neutrophils and emergency granulopoiesis drive immune suppression and an extreme response endotype during sepsis. Nat Immunol 24: 767-779, 2023.
104. Borregaard N and Herlin T: Energy metabolism of human neutrophils during phagocytosis. J Clin Invest 70: 550-557, 1982.
105. Bao Y, Ledderose C, Graf AF, Brix B, Birsak T, Lee A, Zhang J and Junger WG: mTOR and differential activation of mitochondria orchestrate neutrophil chemotaxis. J Cell Biol 210: 1153-1164, 2015.
106. Rodríguez-Espinosa O, Rojas-Espinosa O, Moreno-Altamirano MM, López-Villegas EO and Sánchez-García FJ: Metabolic requirements for neutrophil extracellular traps formation. Immunology 145: 213-224, 2015.
107. Pan T, Sun S, Chen Y, Tian R, Chen E, Tan R, Wang X, Liu Z, Liu J and Qu H: Immune effects of PI3K/Akt/HIF-1α-regulated glycolysis in polymorphonuclear neutrophils during sepsis. Crit Care 26: 29, 2022.
108. Ratter JM, Rooijackers HMM, Hooiveld GJ, Hijmans AGM, de Galan BE, Tack CJ and Stienstra R: In vitro and in vivo effects of lactate on metabolism and cytokine production of human primary PBMCs and monocytes. Front Immunol 9: 2564, 2018.
109. Dietl K, Renner K, Dettmer K, Timischl B, Eberhart K, Dorn C, Hellerbrand C, Kastenberger M, Kunz-Schughart LA, Oefner PJ, *et al*: Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. J Immunol 184: 1200-1209, 2010.

110. Nolt B, Tu F, Wang X, Ha T, Winter R, Williams DL and Li C: Lactate and immunosuppression in sepsis. Shock 49: 120-125, 2018.
111. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, *et al*: Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature 513: 559-563, 2014.
112. Parthasarathy U, Kuang Y, Thakur G, Hogan JD, Wyche TP, Norton JE Jr, Killough JR, Sana TR, Beakes C, Shyong B, *et al*: Distinct subsets of neutrophils crosstalk with cytokines and metabolites in patients with sepsis. iScience 26: 105948, 2023.
113. Marini O, Costa S, Bevilacqua D, Calzetti F, Tamassia N, Spina C, De Sabata D, Tinazzi E, Lunardi C, Scupoli MT, *et al*: Mature CD10(+) and immature CD10(-) neutrophils present in G-CSF-treated donors display opposite effects on T cells. Blood 129: 1343-1356, 2017.
114. Wang JF, Li JB, Zhao YJ, Yi WJ, Bian JJ, Wan XJ, Zhu KM and Deng XM: Up-regulation of programmed cell death 1 ligand 1 on neutrophils may be involved in sepsis-induced immunosuppression: An animal study and a prospective case-control study. Anesthesiology 122: 852-863, 2015.
115. Zhang Y, Zhou Y, Lou J, Li J, Bo L, Zhu K, Wan X, Deng X and Cai Z: PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. Crit Care 14: R220, 2010.
116. Brahmamdam P, Inoue S, Unsinger J, Chang KC, McDunn JE and Hotchkiss RS: Delayed administration of anti-PD-1 antibody reverses immune dysfunction and improves survival during sepsis. J Leukoc Biol 88: 233-240, 2010.
117. Izawa K, Maehara A, Isobe M, Yasuda Y, Urai M, Hoshino Y, Ueno K, Matsukawa T, Takahashi M, Kaitani A, *et al*: Disrupting ceramide-CD300f interaction prevents septic peritonitis by stimulating neutrophil recruitment. Sci Rep 7: 4298, 2017.
118. Hu N, Mora-Jensen H, Theilgaard-Mönch K, Doornbos-van der Meer B, Huitema MG, Stegeman CA, Heeringa P, Kallenberg CG and Westra J: Differential expression of granulopoiesis related genes in neutrophil subsets distinguished by membrane expression of CD177. PLoS One 9: e99671, 2014.
119. Davis RE, Sharma S, Conceição J, Carneiro P, Novais F, Scott P, Sundar S, Bacellar O, Carvalho EM and Wilson ME: Phenotypic and functional characteristics of HLA-DR(+) neutrophils in Brazilians with cutaneous leishmaniasis. J Leukoc Biol 101: 739-749, 2017.
120. Chakravarti A, Rusu D, Flamand N, Borgeat P and Poubelle PE: Reprogramming of a subpopulation of human blood neutrophils by prolonged exposure to cytokines. Lab Invest 89: 1084-1099, 2009.
121. Vincent JL and Beumier M: Diagnostic and prognostic markers in sepsis. Expert Rev Anti Infect Ther 11: 265-275, 2013.
122. Parlato M and Cavaillon JM: Host response biomarkers in the diagnosis of sepsis: A general overview. Methods Mol Biol 1237: 149-211, 2015.
123. Daigo K and Hamakubo T: Host-protective effect of circulating pentraxin 3 (PTX3) and complex formation with neutrophil extracellular traps. Front Immunol 3: 378, 2012.
124. Jaillon S, Peri G, Delneste Y, Frémaux I, Doni A, Moalli F, Garlanda C, Romani L, Gascan H, Bellocchio S, *et al*: The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. J Exp Med 204: 793-804, 2007.
125. Caironi P, Masson S, Mauri T, Bottazzi B, Leone R, Magnoli M, Barlera S, Mamprin F, Fedele A, Mantovani A, *et al*: Pentraxin 3 in patients with severe sepsis or shock: The ALBIOS trial. Eur J Clin Invest 47: 73-83, 2017.
126. Lee YT, Gong M, Chau A, Wong WT, Bazoukis G, Wong SH, Lampropoulos K, Xia Y, Li G, Wong MCS, *et al*: Pentraxin-3 as a marker of sepsis severity and predictor of mortality outcomes: A systematic review and meta-analysis. J Infect 76: 1-10, 2018.
127. Wang C, Li Q, Tang C, Zhao X, He Q, Tang X and Ren J: Characterization of the blood and neutrophil-specific microbiomes and exploration of potential bacterial biomarkers for sepsis in surgical patients. Immun Inflamm Dis 9: 1343-1357, 2021.
128. Kalkoff M, Cursons RT, Sleigh JW and Jacobson GM: The use of real time rtPCR to quantify inflammatory mediator expression in leukocytes from patients with severe sepsis. Anaesth Intensive Care 32: 746-755, 2004.
129. Lu RJ, Taylor S, Contrepois K, Kim M, Bravo JI, Ellenberger M, Sampathkumar NK and Benayoun BA: Multi-omic profiling of primary mouse neutrophils predicts a pattern of sex-and age-related functional regulation. Nat Aging 1: 715-733, 2021.
130. Bongers SH, Chen N, van Grinsven E, van Staveren S, Hassani M, Spijkerman R, Hesselink L, Lo Tam Loi AT, van Aalst C and Leijte GP, *et al*: Kinetics of neutrophil subsets in acute, subacute, and chronic inflammation. Front Immunol 12: 674079, 2021.
131. Kingren MS, Starr ME and Saito H: Divergent sepsis pathophysiology in older adults. Antioxid Redox Signal 35: 1358-1375, 2021.
132. Arcaroli J, Fessler MB and Abraham E: Genetic polymorphisms and sepsis. Shock 24: 300-312, 2005.
133. Shurtz-Swirski R, Sela S, Herskovits AT, Shasha SM, Shapiro G, Nasser L and Kristal B: Involvement of peripheral polymorphonuclear leukocytes in oxidative stress and inflammation in type 2 diabetic patients. Diabetes Care 24: 104-110, 2001.
134. van der Poll T and Opal SM: Host-pathogen interactions in sepsis. Lancet Infect Dis 8: 32-43, 2008.
135. Ma Y, Zhao Y and Zhang X: Factors affecting neutrophil functions during sepsis: Human microbiome and epigenetics. J Leukoc Biol 116: 672-688, 2024.
136. Shukla P, Rao GM, Pandey G, Sharma S, Mittapelly N, Shegokar R and Mishra PR: Therapeutic interventions in sepsis: Current and anticipated pharmacological agents. Br J Pharmacol 171: 5011-5031, 2014.
137. Sun R, Huang J, Yang Y, Liu L, Shao Y, Li L and Sun B: Dysfunction of low-density neutrophils in peripheral circulation in patients with sepsis. Sci Rep 12: 685, 2022.
138. Dagur PK and McCoy JP Jr: Collection, storage, and preparation of human blood cells. Curr Protoc Cytom 73: 5.1.-5.1.16, 2015.
139. Hardisty GR, Llanwarne F, Minns D, Gillan JL, Davidson DJ, Gwyer Findlay E and Gray RD: High purity isolation of low density neutrophils casts doubt on their exceptionality in health and disease. Front Immunol 12: 625922, 2021.
140. Yennemadi AS, Keane J and Leisching G: The isolation and characterization of low-and normal-density neutrophils from whole blood. J Vis Exp 7 (216), 2025.
141. Thomas HB, Moots RJ, Edwards SW and Wright HL: Whose gene is it anyway? The effect of preparation purity on neutrophil transcriptome studies. PLoS One 10: e0138982, 2015.
142. Bersanelli M, Mosca E, Remondini D, Giampieri E, Sala C, Castellani G and Milanesi L: Methods for the integration of multi-omics data: mathematical aspects. BMC Bioinformatics 17 (Suppl 2): S15, 2016.
143. Picard M, Scott-Boyer MP, Bodein A, Périn O and Droit A: Integration strategies of multi-omics data for machine learning analysis. Comput Struct Biotechnol J 19: 3735-3746, 2021.
144. Flores JE, Claborne DM, Weller ZD, Webb-Robertson BM, Waters KM and Bramer LM: Missing data in multi-omics integration: Recent advances through artificial intelligence. Front Artif Intell 6: 1098308, 2023.
145. Hoogendijk AJ, Pourfarzad F, Aarts CEM, Tool ATJ, Hiemstra IH, Grassi L, Frontini M, Meijer AB, van den Biggelaar M and Kuijpers TW: Dynamic transcriptome-proteome correlation networks reveal human myeloid differentiation and neutrophil-specific programming. Cell Rep 29: 2505-2519.e4, 2019.