

Early lower expression of PD-L1 on M1 monocyte is associated with prevalent septic shock in older adult sepsis: an observational cohort study

Qian Gao

Capital Medical University

Beibei Liu

Capital Medical University

Li Yang

Capital Medical University

Shubin Guo

Capital Medical University

Tiecheng Yang

tiechengyang3953@163.com

Capital Medical University

Article

Keywords: sepsis, sepsis shock, monocyte subsets, PD-L1, aging

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11180912/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

To evaluate the early expression of programmed cell death receptor ligand-1 (PD-L1) on different subtype of monocyte and investigate their association with the severity of sepsis in the older adult population. Eighty older adult sepsis patients and 40 age and sex matched healthy controls were enrolled. The flow cytometry method was used to measure the monocyte subsets and PD-L1 expression on subsets of monocyte. PD-L1 expression on M1(CD14++CD16-) monocyte was lower in septic shock compared to patients without shock [1.19% (0.69%, 2.42%) vs. 1.96% (0.65%, 4.10%), $p = 0.012$]. PD-L1 expression on M1 monocytes was independently associated with septic shock in the multivariable logistic regression analysis (OR = 0.542, 95% CI: 0.324, 0.907, $p = 0.020$). The area under the curve (AUC) of PD-L1 expression on M1 monocytes for discriminating septic shock was 0.689 ($p = 0.006$). The AUC for the combined analysis of PD-L1 expression and the sequential organ failure assessment score was 0.826 ($p < 0.001$). In this exploratory cohort of older adults with sepsis, lower PD-L1 expression on M1 monocytes during the first 24 hours was associated with the presence of septic shock. This finding requires independent and longitudinal validation. Its prognostic value and pathophysiological significance remain undetermined.

Introduction

Sepsis represents a life-threatening infectious condition accompanied by varying degrees of organ dysfunction, arising from a complex interplay between the host's pro-inflammatory and anti-inflammatory responses¹. Monocytes isolated from sepsis patients display a reduced ability to secrete pro-inflammatory mediators, including interleukin (IL)-1 α , IL-6, and tumour necrosis factor (TNF)- α . In contrast, the production of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist remained unimpaired or even elevated, was a key hallmark of sepsis-associated immunosuppression^{2,3}. Human peripheral blood monocytes constitute a heterogeneous cell population, which can be categorized into three distinct subtypes based on the expression patterns of CD14 (a lipopolysaccharide receptor) and CD16 (the Fc γ III receptor) on their cell membranes^{4,5}. According to this classification system, monocytes are divided into: "classical monocytes" with high CD14 expression and no CD16 expression (CD14++CD16-); "intermediate monocytes" that co-express both receptors (CD14++CD16+); and "nonclassical monocytes" characterized by predominant CD16 expression (CD14+CD16++)⁴. Previous studies have indicated that the immunosuppressive phase of sepsis is typified by increased anti-inflammatory cytokine synthesis and decreased expression of human leukocyte antigen-DR (HLA-DR)⁶. Additionally, programmed cell death receptor-1 (PD-1) and its ligand PD-L1 co-inhibitory receptor molecules exert crucial regulatory effects on sepsis-induced immunosuppression⁷.

The global aging population is expanding at an alarming rate, and the immune system of older adult individuals exhibits distinct functional characteristics compared to that of young adults. Notably, most prior investigations into sepsis and monocyte function failed to stratify patients by age, resulting in a paucity of knowledge regarding monocyte behavior in older adult sepsis patients. In line with

recommendations that novel biomarkers should be utilized in conjunction with conventional indicators (4), this study compared PD-L1 expression levels on the surface of the three major monocyte subtypes to gain deeper insights into this cell population in older adult sepsis patients.

Results

Patient clinical baseline characteristics

A total of 80 patients were enrolled in this study following the screening of 146 Chinese patients aged ≥ 65 years with sepsis between October 2021 and October 2022 (Figure 1). Based on disease severity, these 80 patients were further categorized into two subgroups: 27 cases with septic shock and 53 cases with uncomplicated sepsis (without shock). Forty sex-, age-, and race-matched healthy volunteers were recruited from a routine health check-up center for inclusion in the present study. Detailed demographic and clinical characteristics of the enrolled patients were summarized in Table 1. Elevated SOFA scores in patients with septic shock reflected a more severe disease phenotype. Moreover, the 28-day mortality rate was notably higher in the septic shock subgroup compared with the non-shock sepsis subgroup. Although concentrations of conventional clinical inflammatory biomarkers (i.e., PCT and CRP) were numerically elevated in the septic shock cohort relative to the non-shock cohort, these differences did not reach statistical significance in our study. Furthermore, no statistically significant intergroup differences were observed in terms of age, sex distribution, peripheral blood leukocyte count, or monocyte count.

PD-L1 expression on different monocyte subtypes between groups

Peripheral blood monocyte subsets were categorized as M1 (classical monocytes, CD14⁺⁺CD16⁻), M2 (intermediate monocytes, CD14⁺⁺CD16⁺), and M3 (nonclassical monocytes, CD14⁺CD16⁺⁺) monocyte based on the surface expression of CD14 and CD16 molecules. We found that the percentage of M1 monocyte was higher in septic shock group and in 28-day death group comparing to septic group and 28-day survival group, respectively [68.8 % (50.7 %, 77.8 %) vs. 40.8 % (19.7 %, 70.7 %), $p = 0.011$; 70.9% (49.4%, 76.9%) vs 54.3% (22.2%, 74.7%), $P=0.038$] (Figure 2A). The proportion of M3 monocytes was significantly higher in sepsis patients than in healthy controls [3.2% (1.6%, 5.8%) vs. 1.8% (0.8%, 4.5%); $p = 0.034$] (Figure 2C). There were no disparity among the other groups on the three subsets monocyte nevertheless.

PD-L1 expression on M1 monocyte was higher in older adult with sepsis than control group, but this tendency was not significant [1.79% (0.59%, 3.28%) vs. 1.61% (0.65%, 2.67%), $p = 0.072$]. PD-L1 expression on M1 monocyte was lower in septic shock group compared to septic group [1.19% (0.69%, 2.42%) vs. 1.96% (0.65%, 4.10%), $p = 0.012$]. PD-L1 expression on M1 monocyte was lower in septic shock group compared to septic group [1.19% (0.69%, 2.42%) vs. 1.96% (0.65%, 4.10%), $p = 0.012$]. PD-L1 expression on M1 monocyte was upregulated in 28-day death group than 28-day survival group, but still not significant [1.84% (0.39%, 4.01%) vs. 1.52% (0.45%, 2.39%), $p = 0.068$]. The PD-L1 expression on M2, M3 monocyte exhibited no significant differences. Detailed data were presented in Figure 3.

The association between PD-L1 expression on M1 monocyte and septic shock in patients with older adult population

In the univariate analyses, we have shown that the proportion of M1 monocyte, PD-L1 expression on M1 monocyte exhibited significant difference between the septic shock group and that without shock group. In the following multivariable logistic regression analysis, PD-L1 expression on M1 monocyte ($\beta = -0.612$, OR = 0.542, 95%CI: 0.324, 0.907, $p = 0.020$) and SOFA score ($\beta = 1.366$, OR = 3.919, 95%CI: 1.893, 8.114, $p = 0.000$) still exhibited significant effect in predicating septic shock. While, we had not found any significant effect for predicating septic shock with the percentage of M1 monocyte (Table 2).

The ROC curve analysis demonstrated that the AUC value for PD-L1 expression on M1 monocytes in predicting septic shock was 0.689 ($p = 0.006$), whereas the AUC value corresponding to SOFA score was 0.781 ($p < 0.001$). Furthermore, the AUC derived from the combination of PD-L1 expression percentage on M1 monocytes and the SOFA score reached 0.826 ($p < 0.001$), a value superior to that of SOFA score by the Delong test ($P=0.022$). Detailed data pertaining to these ROC curve analyses are depicted in Figure 4.

Discussion

Sepsis triggered complex crosstalk between host pro-inflammatory and anti-inflammatory responses¹. The acute inflammatory cytokine storm accounted for early-phase mortality in sepsis, whereas persistent immunosuppression represented a major driver of long-term death⁸. Impaired pro-inflammatory cytokine release capacity of monocytes from patients with sepsis in response to endotoxin (lipopolysaccharide, LPS), other Toll-like receptor (TLR) agonists, and various other bacterial compounds constituted a key hallmark of the immune dysfunction associated with sepsis^{2,3}. Sepsis triggered premature immunosenescence in young patients, subsequently causing immune disorders encompassing exaggerated inflammatory responses and persistent immunosuppression. For older adult patients with sepsis, the synergistic interplay of sepsis and aging drove more substantial immunosenescence-associated alterations, which translated to severe immune dysfunction and unfavorable clinical outcomes. These prematurely aged immune cells can further cause excessive innate immune responses and defective adaptive immune functions, which ultimately resulted in overwhelming inflammation and the shift to immunosuppressive state in sepsis. Notably, the terms “premature aging” and “senescence” represented related yet distinct cellular states. “Premature aging” referred to immune cells displaying aging or dysfunction before reaching their physiological lifespan, a state that can be induced by infection or inflammation. Conversely, “senescence” described an irreversible arrest of cellular proliferation resulting from intrinsic aging processes. In older adult patients with sepsis, the combined effects of both sepsis-induced premature aging and pre-existing cellular senescence may cause more significant immunosenescence-associated alterations, resulting in a poorer prognosis⁹. M1 monocytes represented the dominant subset in healthy individuals¹⁰. The majority of evidence indicated that these cells exhibit a pro-inflammatory phenotype, attributable to their robust capacity to secrete pro-

inflammatory cytokines in response to microbial challenge¹¹. Studies had shown that the proportion of M1 monocyte exhibited a decreased tendency in the older adult population¹². Transcriptomic analysis further suggested that M1 monocytes exhibit age-associated decline in proliferative capacity¹³. Immunosenescence was associated with aging which was characterized with immune dysfunction and gradual deterioration of the immune system¹⁴. Senescent cells were capable of secreting copious amounts of proinflammatory cytokines, which initiated inflammatory responses and remodelled the tissue microenvironment¹⁵. Accumulating evidence indicated that senescent cells persist and accumulate without efficient immune surveillance and clearance during the aging process, thereby exacerbating inflammatory states and contributing to the development of multiple age-related disorders.^{16,17}.

The PD-1/PD-L1 signalling pathway played a critical role in autoimmune diseases, infectious diseases, tumour immunity, and drug resistance mechanisms¹⁸. Report shown that senescence and aging were associated with upregulation of PD-L1¹⁹. The senescence-associated secretory phenotype, characterized by the secretion of a large amount of proinflammatory cytokines, chemokines induced the PD-L1 upregulation by several mechanism such as NF- κ B and p38 MAPK pathways^{2,20,21}. The upregulating PD-L1 may create an overall immunosuppressive environment that inhibited immune-mediated clearance of damaged cell²², therefore may lead to insufficient clearance of pathogenic microorganisms and progress to sepsis. While it was widely recognized that excessive early inflammatory responses in sepsis were essential for the development of multiple organ dysfunction and septic shock although both proinflammatory and anti-inflammatory responses were concurrently present in sepsis^{23,24}. One speculative interpretation of our findings is that, in older adults with sepsis, lower early PD-L1 expression on M1 monocytes may reflect an insufficient compensatory anti-inflammatory response; however, our observational data cannot establish the direction of this association, and reverse causation—for example, shock-related immunosuppression down-regulating PD-L1—cannot be excluded. This was consistent with our findings indicated that early PD-L1 expression on M1 monocyte was markedly decreased in older adult patients with septic shock. In the multivariable analysis, lower PD-L1 expression on M1 monocytes was independently associated with septic shock; this is a statistical association and does not demonstrate a biologically protective effect. These findings further suggested that it were predominantly characterized by excessive inflammatory response at the early phase in sepsis with older adult, and such early hyperinflammation remained a key driver for the development of septic shock in older adult. However, accumulating evidence indicates that persistent inflammation, as well as short-term inflammatory insults during sepsis, may drive accelerated immune cell aging, which in turn could aggravate the inflammatory cytokine storm and facilitate progression toward an immunosuppressive state²⁵. This can explain why older adult sepsis patients who had decreased expression of PD-L1 on M1 monocytes were prone to have adverse outcomes, such as septic shock in our study. However, this finding may be inconsistent with previous studies regarding immune suppression in older adult patients with sepsis, yet it may reflect the genuine early-phase inflammatory response in older adult population. Our findings reflected an early "snapshot" of the immune response

although it was not be interpreted as representing the entire trajectory of PD-L1 expression. Aging status secreted a large array of inflammatory cytokines that triggered inflammation response and altered tissue microenvironment¹⁵. Study showed that the anti-PD-L1 therapy markedly decreased the level of pro and anti-inflammatory cytokines such as IL-6 and IL-10 in sepsis mice and the survival rate of sepsis model mice was significantly improved by anti-PD-L1 antibody treatment¹⁸. However, those conclusions were limited to animal model studies. Because clinical trials had not given the direct benefit result of PD-1 or PD-L1 blockade in human^{26,27}. Under physiological immune conditions, the PD-L1 signaling pathway serves a crucial anti-inflammatory function^{28,29}. During pathogen invasion, robust inflammatory responses were rapidly activated to eradicate invading microorganisms. Meanwhile, the host initiated a protective negative feedback mechanism to restrict excessive inflammation, which would otherwise damage endogenous normal cell³⁰. This process involved the upregulation of anti-inflammatory signaling, thereby maintaining immune homeostasis and preventing inflammatory imbalance. We hypothesize that age-related cellular senescence may blunt the compensatory upregulation of PD-L1-mediated inhibitory signalling after infection, thereby permitting exaggerated early inflammation. However, because our study did not include sepsis patients younger than 65 years, we cannot establish that this association is specific to older adults. Maybe early intervention with PD-L1-related agents not anti-PD-L1 may help attenuate septic shock induced by excessive inflammatory responses in older adult with sepsis. But our study was not designed to evaluate therapeutic interventions, whether modulation of the PD-1/PD-L1 pathway influences outcomes in older adults with sepsis can only be addressed in dedicated mechanistic studies and adequately powered clinical trials.

Several limitations of this study should be emphasized. First, PD-L1 expression was measured at a single early time point; we cannot determine whether lower PD-L1 expression preceded, accompanied, or followed the physiological changes leading to septic shock, and reverse causation cannot be excluded. Second, with only 27 septic shock events and stepwise variable selection, the regression models remain vulnerable to overfitting, and the reported AUCs are likely optimistic despite bootstrap internal validation. Third, PD-L1 expression was not associated with 28-day mortality, and no prognostic inference can be drawn from our data. Finally, although all analyses were performed in a two-single cohort without external validation.

The current study focused on the association between early-phase PD-L1 expression on monocytes and disease severity. Notably, additional data regarding the dynamic alterations in monocyte PD-L1 expression over time needed to better mirror the real-world clinical course of sepsis in this the older adult group. Moreover, the absence of data from non-older adult sepsis patients younger than 65 years make the result of this study cannot truly reveal the immune status of older adults with sepsis. So, prioritizing investigations of dynamic biomarkers with an expanded cohort of sepsis patients under 65 years old serving as controls will be the focus of our future work.

Methods

Study subjects and healthy controls

The participants recruited for this study presented to the emergency departments (EDs) of two distinct hospitals, which reported annual ED visit volumes of roughly 250,000 and 200,000 cases, respectively. Eligible subjects were all aged 65 years or older and diagnosed with sepsis in accordance with the 2016 International Sepsis-3 Diagnostic Criteria ³¹. For the sake of clinical standardization, organ dysfunction was defined as an elevation of the Sequential Organ Failure Assessment (SOFA) score by no less than 2 points. Clinically, septic shock was identified by the necessity of vasopressor administration to sustain a mean arterial pressure of ≥ 65 mmHg, accompanied by a plasma lactate concentration exceeding 2 mmol/L (>18 mg/dL), in the absence of hypovolemia. The exclusion criteria included the following: mortality within 48 hours after sepsis onset and septic shock presentation at admission; sepsis-related manifestations emerging more than 72 hours prior to hospital admission; presence of congenital or acquired immunodeficiency disorders; chronic administration of corticosteroids or other immunosuppressive agents; comorbidities of human immunodeficiency virus (HIV) infection or malignant neoplasms; positive findings on coronavirus disease 2019 (COVID-19) antigen and/or nucleic acid detection assays; and refusal of study participation.

The healthy control group was recruited from the physical examination centre of the two participating hospitals. Individuals with coronary heart disease, diabetes mellitus, hypertension, other severe systemic diseases affecting the heart, brain, lungs, liver or kidneys, or those who declined study participation were excluded from the control group.

Data collection

Venous blood specimens were obtained within 24 hours of meeting the diagnostic criteria for sepsis. The baseline clinical characteristics of patients, encompassing age, gender, and results of laboratory tests, were systematically documented following the onset of sepsis. The SOFA score was calculated at the time of sepsis diagnosis. The presentation of septic shock was recorded after the first 72 hours after sepsis diagnosis. 28-day clinical outcome (survival or mortality) was ascertained during the scheduled follow-up period. For healthy control subjects, blood samples were procured at the time of their attendance at the physical examination centre. This study was performed in adherence to the principles of the Declaration of Helsinki and was approved by the institutional review boards of the two participating hospitals (ethics approval number: 2021-686, sjtkyll-ix-2020(8)). Written informed consent was provided by all study participants prior to their enrolments.

Flow cytometry

Whole blood analyses were conducted within 2 hours of collecting peripheral blood into ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes, using human-specific monoclonal antibodies targeting CD45 (Clone HI30), CD14 (Clone M5E2), CD16 (Clone 3G8), and CD274 (Clone MIH1), alongside isotype-matched control antibodies. All antibodies were pre-titered and optimized based on the conjugated fluorophore. Cells were stained on ice in the dark for 30 minutes and

subsequently washed twice. A minimum of 10,000 monocyte events were acquired using a BD FACS Aria II flow cytometer. Monocyte gating was performed based on forward scatter, side scatter, and positive expression of CD14 and CD16, with gating thresholds calibrated against isotype-matched control staining. Single-cell gating was achieved by plotting forward scatter area (FSC-A) versus forward scatter height (FSC-H). Results were expressed as percentages and mean fluorescence intensities(MFI). MFI was measured but not selected as a final factor due to its limited prognostic value. Data analysis was carried out using FlowJo software (Version 10.0.8; Tree Star, Ashland, OR, USA).

Statistical analysis

Normally distributed data are expressed as mean $\pm$ standard deviation, and non-normally distributed data as median (interquartile range). Baseline characteristics of the patient cohort and healthy controls were compared using independent-samples t-tests, Mann-Whitney U tests, and chi-square tests, as deemed appropriate. Among group comparisons of monocyte subset proportions and PD-L1 expression levels on distinct monocyte subsets were performed using the Mann-Whitney U test. Binary logistic regression analysis was employed to identify variables independently associated with severity in sepsis patients. Univariate analysis were included in the initial multivariable logistic regression model. A enter method stepwise elimination procedure was applied. The area under curve (AUC) of the receiver operating characteristic (ROC) curve was calculated to assess the predictive efficacy of relevant parameters for sepsis shock in this sepsis population. De-Long was used to test the significant of AUC. All statistical tests were two-tailed, with statistical significance established at a P value < 0.05. All data analyses were conducted using SPSS software (Version 27.0).

Abbreviations

PD-L1 programmed cell death receptor ligand-1

AUC area under the curve

ROC receiver operating characteristic

IL interleukin

TNF tumor necrosis factor

PD-1 programmed cell death receptor-1

ED emergency department

SOFA sequential organ failure assessment

HIV human immunodeficiency virus

COVID-19 coronavirus disease 2019

EDTA ethylenediaminetetraacetic acid

PCT procalcitonin

CRP C reactive protein

ESR erythrocyte sedimentation rate

Declarations

Data availability

The data that support the findings of this study are available from ResMan

(<http://www.medresman.org.cn/login.aspx>) but restrictions apply to the

availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of ResMan.

Acknowledgement: We are grateful to Melissa Crawford, PhD, of Liwen Bianji (Edanz) (www.liwenbianji.cn/), for her professional English language editing of the draft version of this manuscript. This work was supported by Research Open Project of Beijing Key Laboratory of Cardiopulmonary Cerebral Resuscitation (2020XFN-KFKT-01)

Author contributions

QG and TCY designed the study. BBL acquired the data. QG and LY performed the analysis and interpretation of data. QG and BBL wrote the manuscript. TCY revised the manuscript.

Conflict of Interest

All authors declare no financial or non-financial competing interests.

References

1. Hotchkiss, R. S., Monneret, G. & Payen, D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* **13**, 862-874, doi:10.1038/nri3552 (2013).
2. Cavaillon, J. M. & Adib-Conquy, M. Bench-to-bedside review: endotoxin tolerance as a model of leukocyte reprogramming in sepsis. *Crit Care* **10**, 233, doi:10.1186/cc5055 (2006).
3. Biswas, S. K. & Lopez-Collazo, E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol* **30**, 475-487, doi:10.1016/j.it.2009.07.009 (2009).
4. Ziegler-Heitbrock, L. et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74-80, doi:10.1182/blood-2010-02-258558 (2010).

5. Lund, H., Boysen, P., Akesson, C. P., Lewandowska-Sabat, A. M. & Storset, A. K. Transient Migration of Large Numbers of CD14(++) CD16(+) Monocytes to the Draining Lymph Node after Onset of Inflammation. *Front Immunol* **7**, 322, doi:10.3389/fimmu.2016.00322 (2016).
6. Landelle, C. et al. Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock. *Intensive Care Med* **36**, 1859-1866, doi:10.1007/s00134-010-1962-x (2010).
7. Boomer, J. S. et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* **306**, 2594-2605, doi:10.1001/jama.2011.1829 (2011).
8. Wang, N., Lu, Y., Zheng, J. & Liu, X. Of mice and men: Laboratory murine models for recapitulating the immunosuppression of human sepsis. *Front Immunol* **13**, 956448, doi:10.3389/fimmu.2022.956448 (2022).
9. He, W. et al. Single-cell landscape of immunological responses in elderly patients with sepsis. *Immun Ageing* **21**, 40, doi:10.1186/s12979-024-00446-z (2024).
10. Calderon, T. M. et al. Dopamine Increases CD14(+)CD16(+) Monocyte Transmigration across the Blood Brain Barrier: Implications for Substance Abuse and HIV Neuropathogenesis. *J Neuroimmune Pharmacol* **12**, 353-370, doi:10.1007/s11481-017-9726-9 (2017).
11. Cros, J. et al. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* **33**, 375-386, doi:10.1016/j.immuni.2010.08.012 (2010).
12. Seidler, S., Zimmermann, H. W., Bartneck, M., Trautwein, C. & Tacke, F. Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. *BMC Immunol* **11**, 30, doi:10.1186/1471-2172-11-30 (2010).
13. Wong, K. L. et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. *Blood* **118**, e16-31, doi:10.1182/blood-2010-12-326355 (2011).
14. Ayoub, M., Abou Jaoude, C., Ayoub, M., Hamade, A. & Rima, M. The Immune System and Cellular Senescence: A Complex Interplay in Aging and Disease. *Immunology*, doi:10.1111/imm.70036 (2025).
15. Tchkonina, T., Zhu, Y., van Deursen, J., Campisi, J. & Kirkland, J. L. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. *J Clin Invest* **123**, 966-972, doi:10.1172/JCI64098 (2013).
16. Childs, B. G. et al. Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov* **16**, 718-735, doi:10.1038/nrd.2017.116 (2017).
17. van Deursen, J. M. Senolytic therapies for healthy longevity. *Science* **364**, 636-637, doi:10.1126/science.aaw1299 (2019).
18. Yang, L., Gao, Q., Li, Q. & Guo, S. PD-L1 Blockade Improves Survival in Sepsis by Reversing Monocyte Dysfunction and Immune Disorder. *Inflammation* **47**, 114-128, doi:10.1007/s10753-023-01897-0 (2024).

19. Onorati, A. et al. Upregulation of PD-L1 in Senescence and Aging. *Mol Cell Biol* **42**, e0017122, doi:10.1128/mcb.00171-22 (2022).
20. Chien, Y. et al. Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. *Genes Dev* **25**, 2125-2136, doi:10.1101/gad.17276711 (2011).
21. Freund, A., Patil, C. K. & Campisi, J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* **30**, 1536-1548, doi:10.1038/emboj.2011.69 (2011).
22. De Cecco, M. et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. *Nature* **566**, 73-78, doi:10.1038/s41586-018-0784-9 (2019).
23. Schorr, C. A. & Dellinger, R. P. The Surviving Sepsis Campaign: past, present and future. *Trends Mol Med* **20**, 192-194, doi:10.1016/j.molmed.2014.02.001 (2014).
24. Torres, L. K., Pickkers, P. & van der Poll, T. Sepsis-Induced Immunosuppression. *Annu Rev Physiol* **84**, 157-181, doi:10.1146/annurev-physiol-061121-040214 (2022).
25. Wang, Y., Zhang, H. & Miao, C. Unraveling immunosenescence in sepsis: from cellular mechanisms to therapeutics. *Cell Death Dis* **16**, 393, doi:10.1038/s41419-025-07714-w (2025).
26. Hotchkiss, R. S. et al. Immune Checkpoint Inhibition in Sepsis: A Phase 1b Randomized, Placebo-Controlled, Single Ascending Dose Study of Antiprogrammed Cell Death-Ligand 1 Antibody (BMS-936559). *Crit Care Med* **47**, 632-642, doi:10.1097/CCM.0000000000003685 (2019).
27. Hotchkiss, R. S. et al. Immune checkpoint inhibition in sepsis: a Phase 1b randomized study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of nivolumab. *Intensive Care Med* **45**, 1360-1371, doi:10.1007/s00134-019-05704-z (2019).
28. Budimir, N., Thomas, G. D., Dolina, J. S. & Salek-Ardakani, S. Reversing T-cell Exhaustion in Cancer: Lessons Learned from PD-1/PD-L1 Immune Checkpoint Blockade. *Cancer Immunol Res* **10**, 146-153, doi:10.1158/2326-6066.CIR-21-0515 (2022).
29. Yi, M. et al. Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol Cancer* **21**, 28, doi:10.1186/s12943-021-01489-2 (2022).
30. Cecconi, M., Evans, L., Levy, M. & Rhodes, A. Sepsis and septic shock. *Lancet* **392**, 75-87, doi:10.1016/S0140-6736(18)30696-2 (2018).
31. Singer, M. et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **315**, 801-810, doi:10.1001/jama.2016.0287 (2016).

Tables

Table 1 Baseline characteristics of all the enrollments

	Sepsis with older adult		P value	Controls
	Septic shock	Septic		
	(n=27)	(n=53)		(n=40)
Age (yrs)	80.00±8.38	81.10±8.59	0.085	76±5.75
Male, n (%)	17(60.7)	26(50)	0.512	24(40)
WBC ($\times 10^9/L$)	11.86(6.92,16.36)	11.61(7.24,17.62)	0.526	/
M ($\times 10^9/L$)	0.43(0.23,0.61)	0.46(0.33,0.76)	0.410	/
MP (%)	4.60(2.03,6.78)	4.50(2.20,6.15)	0.438	/
PCT (ng/ml)	18.73±41.44	13.50±24.00	0.076	/
CRP (mg/L)	141.25±90.52	120.59±77.48	0.094	/
ESR (mm/h)	52.00±32.79	53.38±32.88	0.135	/
SOFA score	12.17±1.49	6.33±2.46	0.001	/
28-day mortality	9(33.3)	3(5.66)	0.003	

Data are expressed as mean $\pm$ SD, median (Q1, Q3), number (%); WBC white blood

cell count; M blood monocyte count; MP blood monocyte percentage; PCT procalcitonin; CRP C reactive protein; ESR erythrocyte sedimentation rate; SOFA sequential organ failure assessment;

Table 2 Logistic Regression-Based Analysis of Independent Determinants of Septic Shock

variable	b	SE	Wald	P value	Odds ratio	95 % confidence interval for EXP(B)	
						Lower limit	Upper limit
M1 monocyte	0.018	0.020	0.844	0.358	1.019	0.979	1.060
PD-L1 expression on M1 monocyte	-0.612	0.263	5.429	0.020	0.542	0.324	0.907
SOFA	1.366	0.371	13.537	<0.001	3.919	1.893	8.114
constant	-12.097	3.461	12.216	<0.001	0.000		

PD-L1 programmed cell death receptor ligand-1; M1 CD14++CD16– monocyte; SOFA sequential organ failure assessment;

Figures

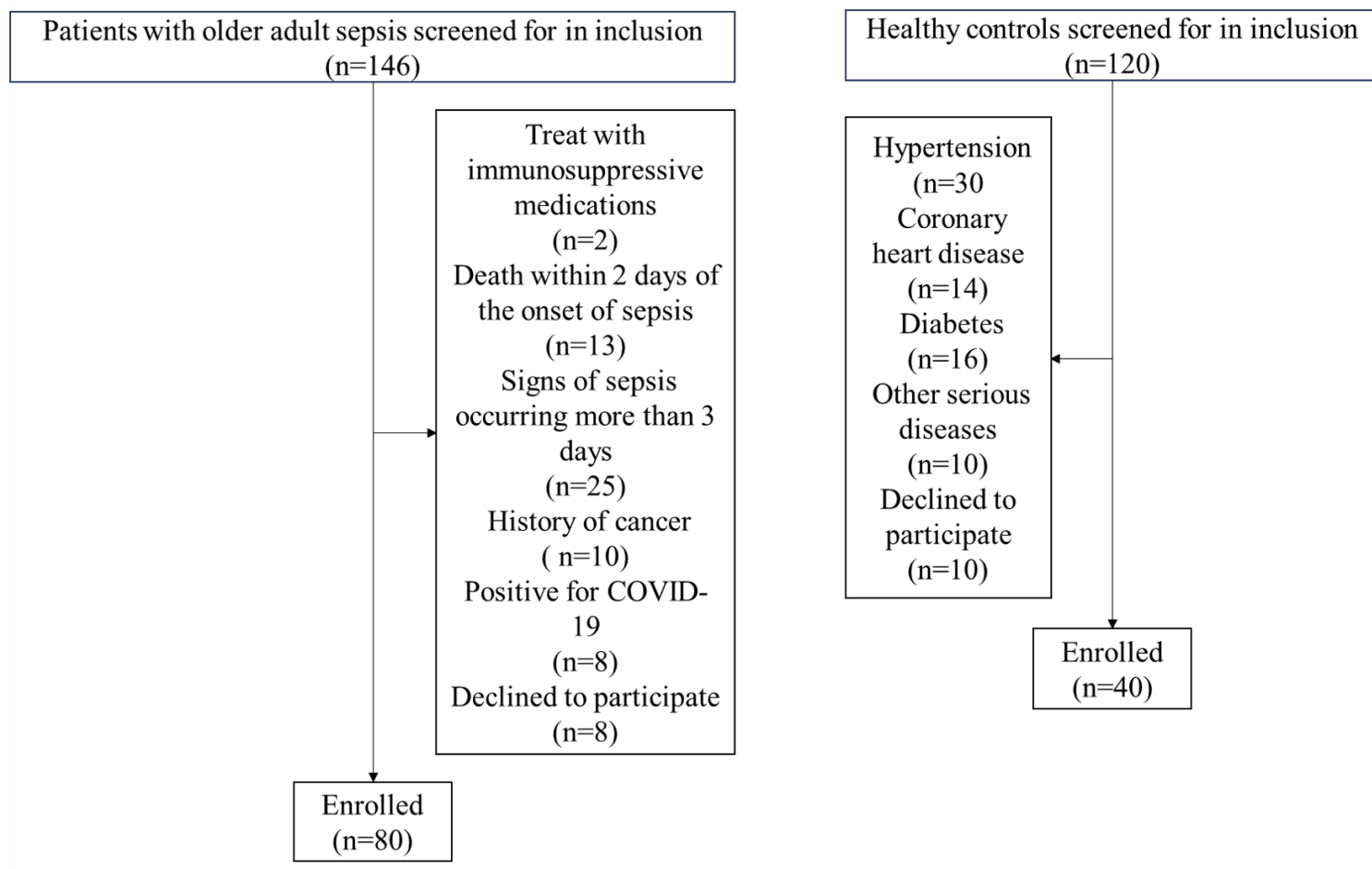


Figure 1

Flow diagram depicting the enrolment of patients and controls, with detailed reasons for exclusion provided.

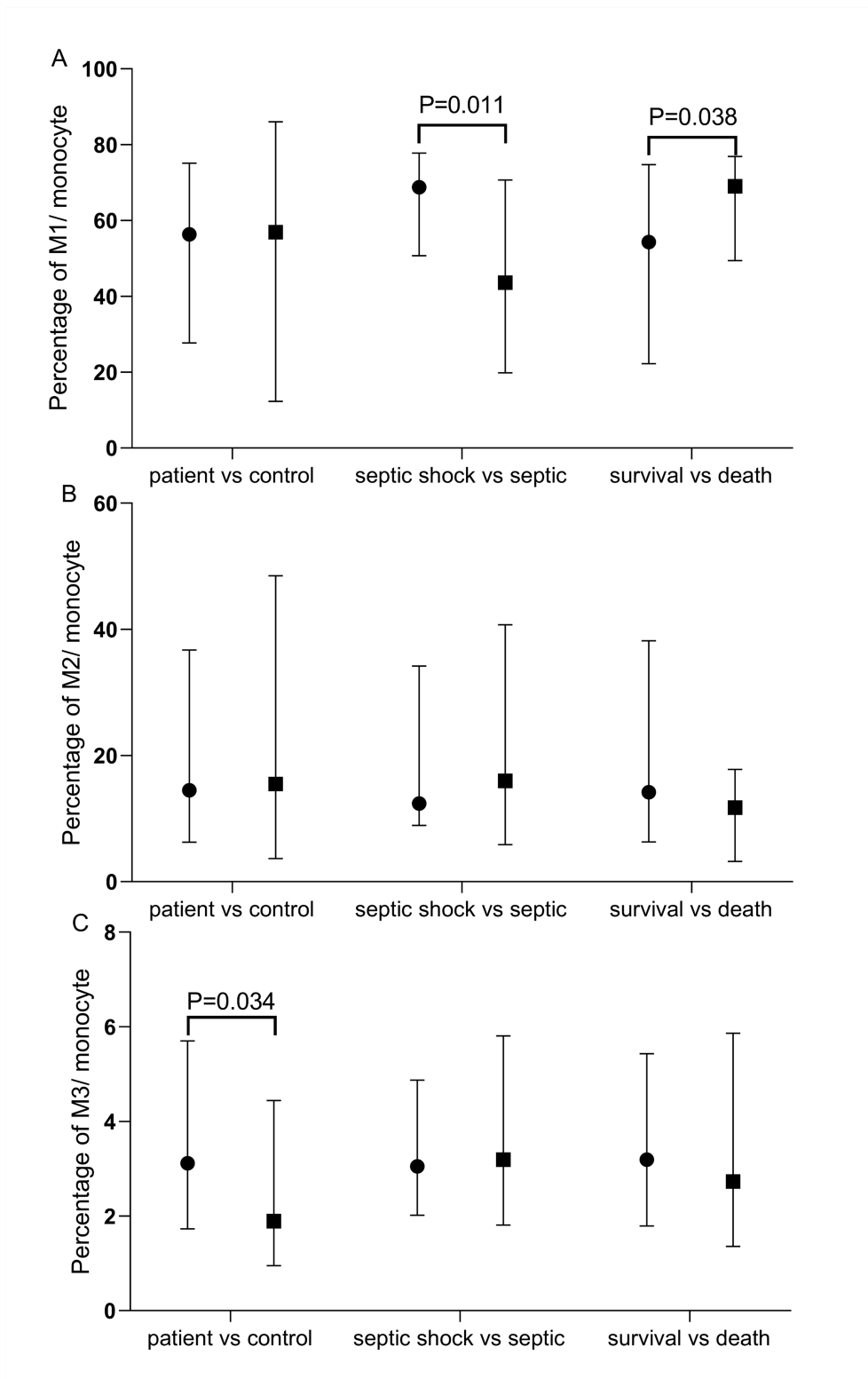


Figure 2

Peripheral blood monocyte subsets distribution in different comparison groups. Panels 2a, 2b, and 2c illustrate MO1 (CD14++CD16-), MO2 (CD14++CD16+), and MO3 (CD14+CD16++) monocytes, respectively, in the following comparison groups: patients vs. controls, septic shock vs. sepsis without shock, and 28-day survival and 28-day death groups.

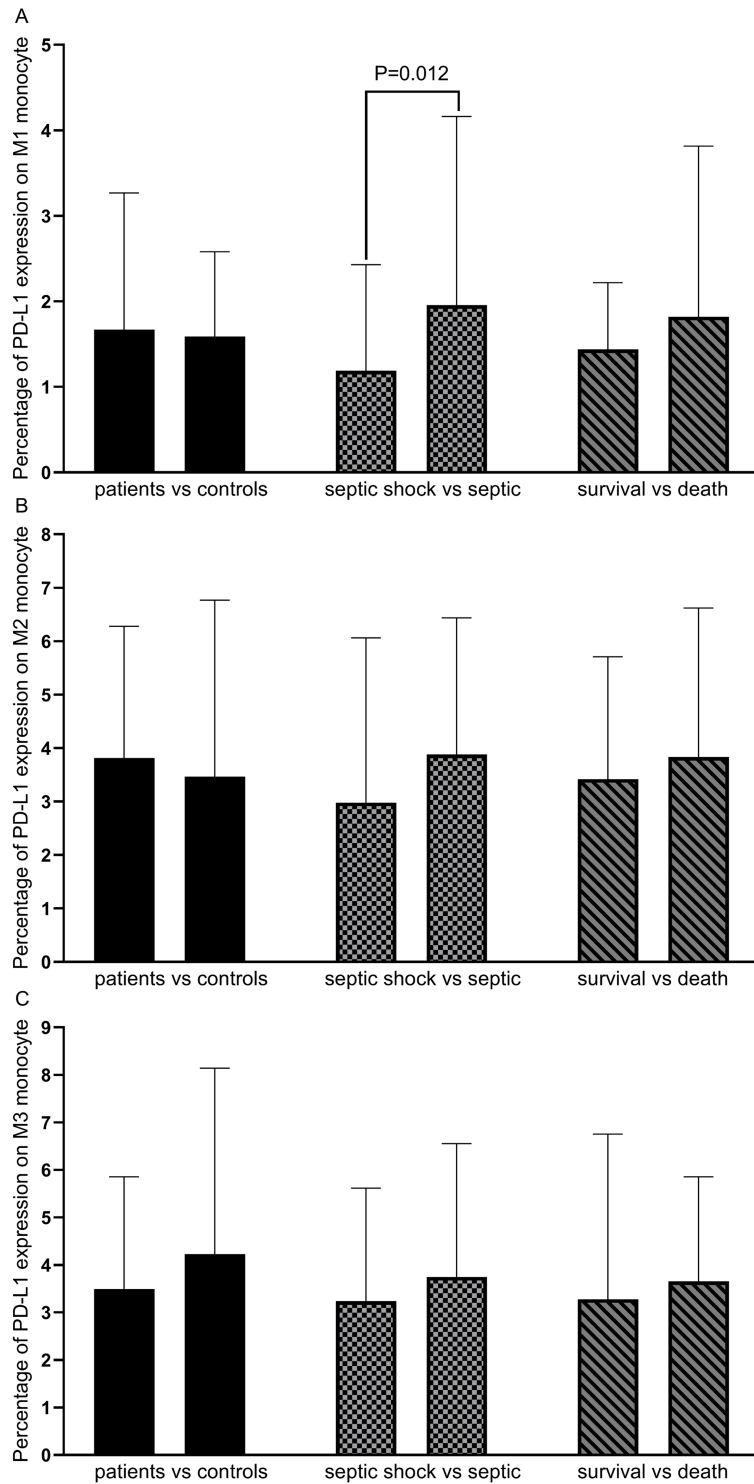


Figure 3

PD-L1 expression on M1 monocyte in different comparison groups: patients vs. controls, septic shock vs. sepsis without shock, and 28-day survival and 28-day death.

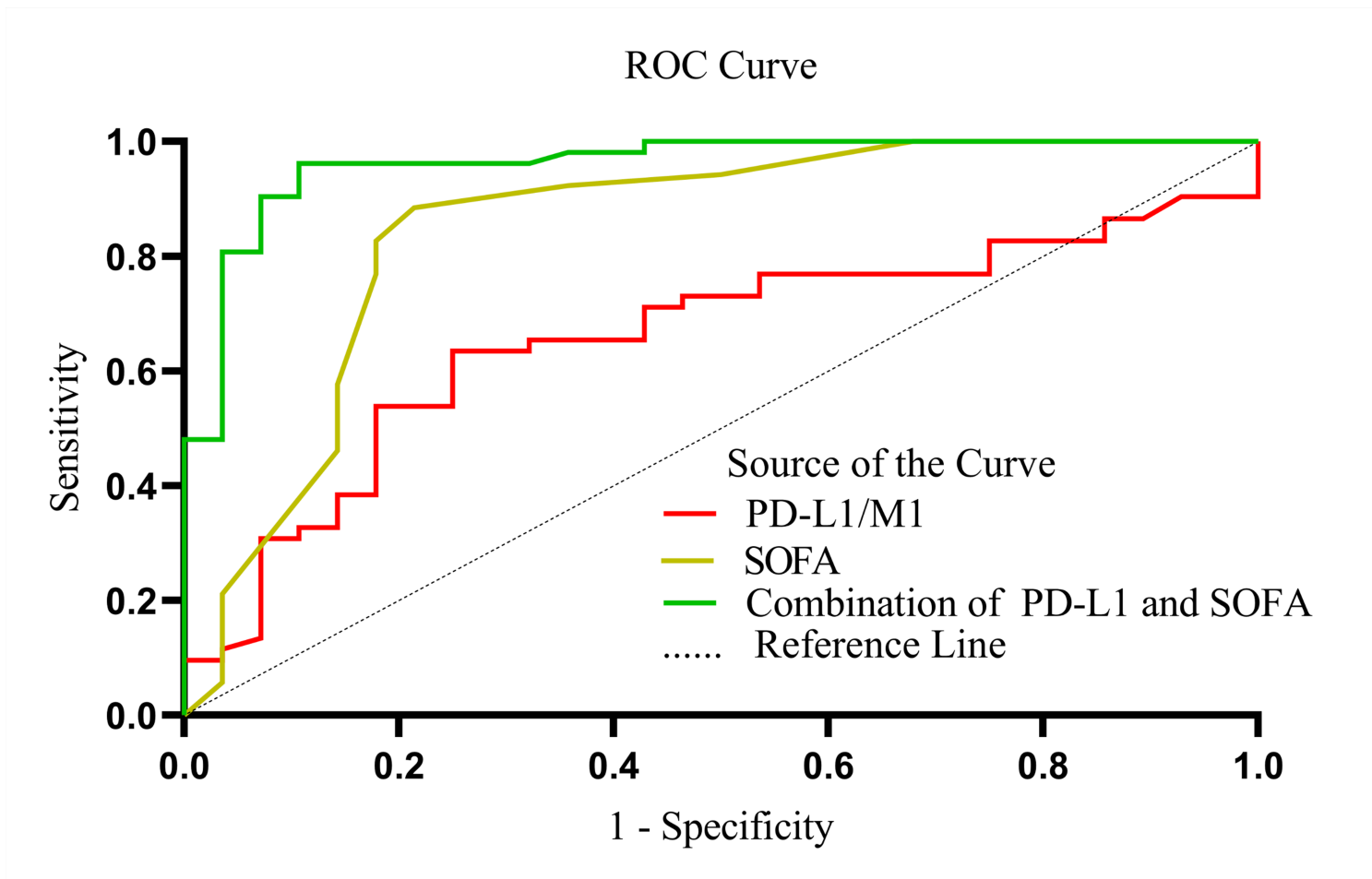


Figure 4

Receiver operating characteristic (ROC) curve for discriminating septic shock in all sepsis patients.