Journals of Gerontology: MEDICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci. 2015 February;70(2):225–231
doi:10.1093/gerona/glu082

Advance Access publication June 10, 2014

Platelet–Monocyte Aggregate Formation and Mortality Risk in Older Patients With Severe Sepsis and Septic Shock

Matthew T. Rondina,[1,2,*] McKenzie Carlisle,[2,*] Tamra Fraughton,[2,3] Samuel M. Brown,[4,5] Russell R. Miller III,[5] Estelle S. Harris,[4] Andrew S. Weyrich,[2,4] Guy A. Zimmerman,[4] Mark A. Supiano,[6] and Colin K. Grissom[4,5]

[1]Division of General Internal Medicine,
[2]Program in Molecular Medicine,
[3]Department of Psychology, and
[4]Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Utah, Salt Lake City.
[5]Division of Pulmonary and Critical Care Medicine, Intermountain Medical Center, Murray, Utah.
[6]Division of Geriatric Medicine and Salt Lake City VA GRECC, University of Utah.

*These two authors contributed equally to the work and are co-first authors.

Address correspondence to Matthew T. Rondina, MD, Division of General Internal Medicine and Program in Molecular Medicine, University of Utah, 50 North Medical Drive, Salt Lake City, Utah 84132. Email: matthew.rondina@hsc.utah.edu

Background. Aging-related changes in platelet and monocyte interactions may contribute to adverse outcomes in sepsis but remain relatively unexamined. We hypothesized that differential platelet–monocyte aggregate (PMA) formation in older septic patients alters inflammatory responses and mortality.

Methods. We prospectively studied 113 septic adults admitted to the intensive care unit with severe sepsis or septic shock. Patients were dichotomized a priori into one of two groups: older (age ≥ 65 years, $n = 28$) and younger (age < 65 years, $n = 85$). PMA levels were measured in whole blood via flow cytometry within 24 hours of admission. Plasma levels of IL-6 and IL-8, proinflammatory cytokines produced by monocytes upon PMA formation, were determined by commercial assays. Patients were followed for the primary outcome of 28-day, all-cause mortality.

Results. Elevated PMA levels were associated with an increased risk of mortality in older septic patients (hazard ratio for mortality 5.64, 95% confidence interval 0.64–49.61). This association remained after adjusting for potential confounding variables in multivariate regression. Receiver operating curve analyses demonstrated that PMA levels greater than or equal to 8.43% best predicted 28-day mortality in older septic patients (area under the receiver operating curve 0.82). Plasma IL-6 and IL-8 levels were also significantly higher in older nonsurvivors. In younger patients, neither PMA levels nor plasma monokines were significantly associated with mortality.

Conclusions. Increased PMA formation, and associated proinflammatory monokine synthesis, predicts mortality in older septic patients. Although larger studies are needed, our findings suggest that heightened PMA formation in older septic patients may contribute to injurious inflammatory responses and an increased risk of mortality.

Key Words: Platelet—Platelet–monocyte aggregates—Inflammation—Sepsis—Mortality.

Received February 10, 2014; Accepted May 7, 2014

Decision Editor: Stephen Kritchevsky, PhD

BACTERIA, bacterial toxins, and host agonists in the septic milieu activate platelets, leukocytes, and other circulating hemostatic and immune cells (1). Activation of these cells leads to injurious thrombo-inflammatory responses, including upregulation of inflammatory monokines, the release of platelet proteins (eg, platelet factor 4, P-selectin), and enhanced platelet–monocyte aggregation (PMA). These responses contribute to the pathophysiology of septic syndromes and the risk of organ failure, lung injury, thrombosis, and death (2,3).

PMA formation is a sensitive marker of in vivo platelet activation (4,5) and is mediated via P-selectin translocated to the surface of the activated platelet (2). Upon PMA formation, proinflammatory monokine synthesis is upregulated (6), leading to imbalanced and inappropriate systemic inflammatory responses. These pathways have particular relevance in septic syndromes, where circulating agonists that activate platelets may result in enhanced PMA formation and inflammation, contributing to adverse outcomes.

The incidence of sepsis increases with age, with more than 60% of sepsis patients in the United States aged 65 years and older (7). Older septic patients also account for more than half of all days spent in the ICU (8,9) and are at higher risk of mortality from sepsis (7,10). Although the presence of comorbid conditions may contribute to the increased incidence and severity of sepsis in older patients, aging is also associated with alterations in platelet–monocyte interactions and associate immune functions (11–13). In older patients, platelets are hyperresponsive (14–16) and upon activation may more readily aggregate with or bind to platelets, leukocytes, and endothelial cells. This binding results in amplified inflammatory and thrombotic responses in sepsis, potentially contributing to an excess risk of organ failure, disability, and death (7).

In this study, we examined age-related associations among PMA formation, proinflammatory gene synthesis, and mortality in older and younger patients with severe sepsis and septic shock.

Methods

Patients

The Institutional Review Board approved this study, and all patients provided informed consent. Adults aged 18 years and older admitted to one of three ICUs with a principal diagnosis of severe sepsis or septic shock (17) were eligible for study participation. Patients were assigned a priori to one of two groups: young (age < 65 years, $n = 85$) and older (age ≥ 65 years, $n = 28$). Although there remains no precise age cutoff for older age and aging is a dynamic process with substantial variation among individuals, this assignment was chosen based on similar studies in the field (10). Patients who had received platelet transfusions at any point during their ICU course were excluded. Whole blood was collected within 24 hours of ICU admission.

All septic patients were followed prospectively. Acute physiology and chronic health evaluation II (APACHE II) scores were calculated upon study entry. The use of antiplatelet agents was carefully recorded. All clinical laboratory tests were measured through a reference laboratory according to established protocols and with appropriate quality controls. Survival status at 28 days following ICU admission, our primary endpoint, was captured prospectively by structured telephone follow-up. Deaths that occurred following hospital discharge, but before the 28-day endpoint, were further confirmed by review of medical and death records.

Flow Cytometry

Following informed consent, whole blood was carefully drawn into 8.6 mL sterile acid-citrate-dextrose (1.4 mL acid-citrate-dextrose/8.6 mL blood) Vacutainer tubes (Becton Dickinson), inverted to ensure adequate mixing, and transported at room temperature to the laboratory within 30 minutes. The first 3 mL of blood was discarded, and samples with gross hemolysis or clotting were not used. Flow cytometry was performed using a FACScan Analyzer (BD Biosciences) with CellQuest software for analysis (Becton Dickinson) within 24 hours of fixation. The flow cytometer was calibrated daily and cleaned carefully before each sample acquisition. All antibodies were obtained from BD Biosciences.

For detection of PMA formation, freshly drawn whole blood (800 µL) was diluted in 2.4 mL HEPES Tyrode's buffer. FACS buffer (5 mL, BD Biosciences) was added to the blood for an additional 10 minutes at room temperature in the presence of CD41–phycoerythrin (a marker for human platelets) and CD14–fluorescein isothiocyanate (a marker for human monocytes), as described (5,18). The sample was fixed by adding FACS buffer (800 µL, BD Biosciences). Monocytes were selected by gating more than or equal to 1,500 $CD41^+$ events on a two-parameter dot plot displaying side scatter vs CD14–fluorescein isothiocyanate (Supplementary Figure 1). Single platelets were excluded using a combination of forward scatter and side scatter and positive anti-CD14 fluorescence. Cells that stained double positive for both CD41 and CD14 were counted to quantify the percentage of monocytes with more than or equal to 1 adherent platelet.

Monokine Analysis

IL-6, IL-8, TNF-α, and IL-1β, produced upon monocyte activation and associated platelet-monocyte interactions (6,19) (and R.C. Campbell, unpublished data), were measured on plasma from a subset of randomly selected patients ($n = 25$ older and $n = 25$ younger) where plasma was available, using the Luminex Profiling System (20). Plasma was obtained from the same whole blood sample used for quantification of PMA levels. Utilizing this assay, the normal reference values for IL-6, IL-8, and TNF-α are 1.3 (±0.4), 3.7 (±0.4), 0.1 (±0.1) pg/mL, respectively.

Statistical Analyses

Continuous variables were first assessed for normality graphically and with tests of skew and kurtosis. Comparisons between groups were made using parametric two-tailed t tests or the nonparametric Mann–Whitney U test for continuous variables and the Pearson chi square test or Fisher's exact test for categorical variables. Logistic regression analyses evaluated the association between PMA levels (using PMA as a continuous variable) and 28-day mortality while controlling for the confounding variables of APACHE II, shock, platelet count, and age. Interaction analyses were used to further examine associations between PMA levels, age cohort (eg, <65 or ≥65), and 28-day mortality, using standard methods described previously (21). Briefly, PMA levels were dichotomized as

above the mean (high) or below the mean (low). An interaction term was then created between the age cohort (older or younger) and the dichotomized PMA levels and analyzed with regard to mortality status. Receiver operating characteristic (ROC) analyses were conducted to examine the performance of PMA levels in discriminating between septic survivors and nonsurvivors in the two cohorts and to determine the optimal cutoff value for PMA levels to be used in remaining analyses with mortality outcomes. In these ROC analyses, we plotted 28-day mortality as the classification variable and PMA levels as the prognostic variable. The cutoff prognostic value of PMA levels was selected for a likelihood ratio between sensitivity and 1-specificity. Using this cutoff as a dichotomous variable (0 = below cutoff and 1 = greater than or equal to cutoff), Cox regression analyses were performed to examine the contribution of PMA levels on 28-day mortality. Analyses were performed using STATA program (version 11.0, StataCorp, College Station, TX) and SPSS (version 18.0, IBM, Armonk, NY). Significance was predetermined as two-tailed $p \leq .05$.

Results

We enrolled 113 septic patients during the period from August 1, 2008 to July 31, 2010. There were no significant differences between older and younger patients in APACHE II score, gender, comorbidities, or platelet counts (Table 1). Although a higher proportion of older patients had septic shock on admission, this difference did not achieve statistical significance (53.6% vs 35.3%, $p = .1$). Consistent with their increased incidence of shock, 28-day mortality was also higher numerically in older patients (25.0% vs 16.5%, $p = .4$). Ventilator-free days at 28 days were lower in older patients (Table 1).

Univariate regression analyses demonstrated that PMA levels in older septic patients significantly and positively correlated with 28-day mortality (Supplementary Table 1). Other usual predictors of mortality and potential confounding variables were also tested in stepwise, univariate regression models of mortality. Admission APACHE II score trended toward an association with mortality in older patients but did not reach statistical significance. When controlling for these covariates in a multivariate regression model of mortality, PMA levels

Table 1. Characteristics of the Study Cohorts

	Septic Patients (n = 113)		
	Age ≥ 65 (n = 28)	Age < 65 (n = 85)	p Value
Male, n (%)	15 (53.6)	30 (35.3)	.119
Age (y)	75.5 (8.1)	43.4 (13.8)	—
APACHE II	18.6 (4.9)	17.9 (7.2)	.736
Median (IQR) ICU LOS (d)	5 (3, 8)	9 (5, 22)	.282
Severe sepsis, n (%)	13 (46.4)	55 (64.7)	.119
Septic shock, n (%)	15 (53.6)	30 (35.3)	.119
RBC transfusion*, n (%)	1 (3.6)	6 (7.1)	.256
Ventilator-free days	3.7 (0.72)	8.5 (1.3)	.027
28-d mortality, n (%)	7 (25.0)	14 (16.5)	.401
Source of sepsis†			
Pulmonary, n (%)	2 (7.1)	44 (51.8)	<.05
Bacteremia, n (%)	12 (42.9)	23 (27.1)	.093
Genitourinary, n (%)	3 (10.7)	8 (9.4)	.545
Intraabdominal/gastrointestinal, n (%)	2 (7.1)	10 (11.8)	.529
Skin/soft tissue, n (%)	1 (3.6)	3 (3.5)	.686
Unknown, n (%)	12 (42.9)	17 (20.0)	.018
Comorbid conditions/medications/labs			
Diabetes, n (%)	8 (28.6)	16 (18.8)	.294
CHF, n (%)	3 (10.7)	2 (2.4)	.096
Active cancer, n (%)	4 (14.3)	5 (5.9)	.222
CVD, n (%)	6 (21.4)	8 (9.4)	.107
Aspirin‡, n (%)	5 (17.9)	2 (2.4)	.062
Platelet count, k/μL	179 (107)	180 (105)	.944
WBC, k/μL	14.9 (5.8)	11.7 (5.9)	.014
Hemoglobin, mg/dL	10.4 (1.5)	10.7 (2.1)	.603
Fibrinogen, mg/dL	570 (214.5)	571.7 (207.1)	.985
Serum creatinine, mg/dL	1.5 (0.8)	1.4 (1.5)	.322
Glucose, mg/dL	116.4 (40.6)	120 (38)	.921

Notes: All values represent the mean ± *SD* unless otherwise specified. APACHE = acute physiology and chronic health evaluation; CHF = congestive heart failure; hs-CRP = high-sensitivity C-reactive protein; CVD = cardiovascular disease including stroke, transient ischemic attack, and myocardial infarction; IQR = interquartile range; LOS = length of stay; NA = not applicable; NR = not recorded; Septic shock = hypotension requiring vasopressor support upon admission.

*Transfusion of ≥1 unit of packed red blood cells at any point during their ICU course.

†Patients may have had ≥1 foci of infection; thus total numbers may exceed the number of patients in each cohort.

‡Defined as taking aspirin at any dose as an outpatient, prior to ICU admission.

remained significantly and positively correlated with mortality in older septic patients (Supplementary Table 1). There were no significant differences in the distribution of diabetes, cardiovascular disease, or clopidogrel use or in admission serum creatinine levels between older septic survivors vs nonsurvivors (data not shown). In younger septic patients, PMA levels were not significantly correlated with mortality in either univariate or multivariate regression models. Rather, admission APACHE II score and the platelet count significantly correlated with mortality in younger patients.

PMA levels were approximately twofold higher in older nonsurvivors compared with older survivors (Figure 1A). In contrast, PMA levels did not differ between younger nonsurvivors and survivors (Figure 1A). Interaction analyses confirmed a significant interaction between higher PMA levels and 28-day mortality in older, but not younger, patients (Figure 1B). Specifically, as age and PMA increased (from younger age to older age and from lower PMA levels to higher PMA levels), 28-day mortality also increased.

ROC analyses demonstrated that PMA levels had excellent performance for predicting 28-day mortality in older septic patients (Figure 2A). In these older patients, a PMA level greater than or equal to 8.43% provided the best performance for discriminating between survivors and nonsurvivors. At this cutoff, the area under the ROC curve was 0.82 with a sensitivity of 83%, specificity of 68%, positive likelihood ratio of 2.62, and negative likelihood ratio of 0.24. In contrast, PMA levels did not discriminate survivors from nonsurvivors in younger septic patients (Figure 2B).

Using the threshold value of 8.43% identified in ROC analyses, PMA levels were dichotomized in older septic patients into two categories: low (PMA level <8.43%) and high (≥8.43%). These cutoffs were then used in Cox proportional hazard regression. Older septic patients with higher PMA levels had almost a sixfold higher risk of mortality (hazard ratio 5.64, 95% confidence interval 0.64–49.61; Figure 2C). PMA levels were not predictive of mortality in younger septic patients (hazard ratio 0.89, 95% CI 0.30–2.68; Figure 2D).

Plasma levels of IL-6 and IL-8, proinflammatory monokines synthesized by monocytes upon platelet-monocyte binding (22), were higher in older nonsurvivors compared with survivors (Figure 3). The most significant differences were observed with IL-8, which has previously been reported as a predictor of 28-day mortality (23). In contrast, in younger patients, IL-6 and IL-8 levels did not differ significantly between survivors and nonsurvivors. Plasma levels of TNF-α trended similarly but did not reach statistical significance (data not shown).

Discussion

Aging has been associated with changes in platelet reactivity and associated thrombo-inflammatory responses (11,24–26). In sepsis, aging is also a significant risk factor for mortality (27). Although many of these age-related changes remain incompletely characterized, interplay between agonists or pathogens in the septic milieu and hyperreactive platelets and monocytes may contribute to impaired immunity and adverse clinical outcomes in older septic patients (12,13).

Among these dysregulated cellular responses, platelet–monocyte interactions have particular physiologic relevance in sepsis. PMAs form in response to activating signals commonly present in the septic milieu and are a sensitive marker of in vivo platelet activation (2,4,5,18,28). PMA formation leads to increased proinflammatory monokines, amplifying ongoing thrombo-inflammatory responses (6,29). Although increased PMA levels are associated with adverse outcomes in cardiovascular disease (30) and stroke (31), interactions among PMA formation, age, and mortality in septic syndromes have not been previously examined.

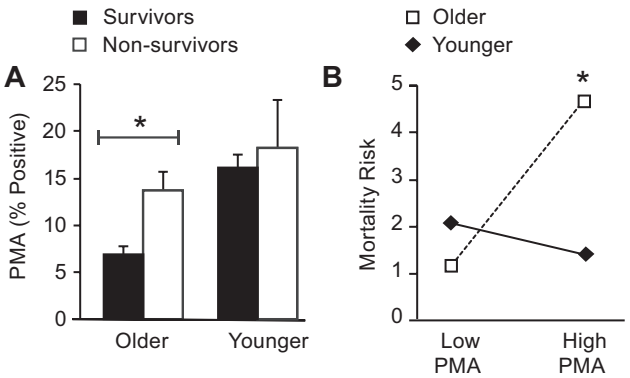

Figure 1. Increased levels of platelet–monocyte aggregates (PMAs) in older patients correlate with a greater risk of mortality. (**A**) Levels of PMA in whole blood were significantly higher in older, nonsurviving, septic patients ($n = 7$), compared with older, surviving, septic patients ($n = 21$, $p \leq .05$). PMA levels did not differ between younger septic nonsurvivors ($n = 71$) and survivors ($n = 14$, $p =$ NS). Values represent the mean ± SEM. (**B**) Interaction analyses confirmed that higher PMA levels in older septic patients were significantly associated with a greater risk of mortality (*$p \leq .05$). In younger septic patients, there was no significant interaction between PMA levels and mortality.

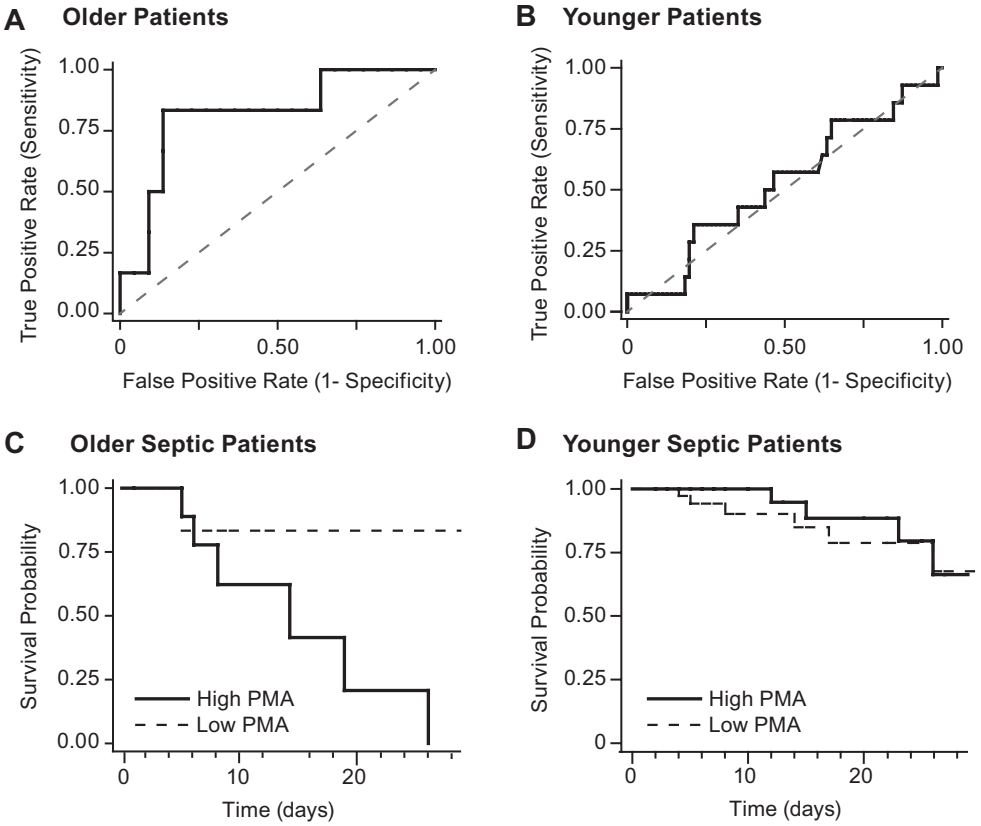

Figure 2. Receiver operating characteristic (ROC) analyses demonstrated that platelet–monocyte aggregate (PMA) levels in older septic patients were a sensitive marker for subsequent mortality. (**A**) In older septic patients, a PMA level ≥ 8.43% provided the best performance for discriminating between survivors and nonsurvivors. At this cut-off, the area under the ROC curve was 0.82 with a sensitivity of 83%, specificity of 68%, positive likelihood ratio of (LR+) 2.62, and negative likelihood ratio of (LR–) 0.24. (**B**) PMA levels were neither a sensitive nor specific marker of mortality in younger patients (area under the ROC curve 0.52, sensitivity 35% LR+ 1.15, specificity 69%, LR– 0.93). (**C**) In older septic patients, elevated PMA levels (PMA ≥ 8.43%) were associated with almost a sixfold higher risk of mortality (hazard ratio 5.64, 95% confidence interval 0.64–49.61). (**D**) In contrast, PMA levels were not associated with mortality in younger septic patients (hazard ratio 0.89, 95% confidence interval 0.30–2.68).

Our study suggests that increased PMA formation in older, but not younger, septic patients correlates with 28-day mortality. Although confidence intervals were wide, reflecting the smaller sample size and greater variance common in clinical studies, increased PMA levels in older septic patients were associated with almost a sixfold higher risk of mortality. Moreover, in older patients, increased PMA levels more strongly correlated with mortality than other traditional risk factors, such as APACHE II or shock. Plasma levels of the proinflammatory monokines IL-6 and IL-8 were also significantly higher in older nonsurvivors. PMA formation in older adults may lead to enhanced monokine synthesis in older adults in vitro (M.T. Rondina, unpublished observations). IL-6 and IL-8 have correlated with adverse outcomes in sepsis (23,32,33) and in older adults (34). Thus, taken together, our findings support the supposition that PMA formation and subsequent downstream monokine synthesis in older adults may contribute to dysregulated inflammatory syndromes and adverse clinical outcomes.

The strengths of this study include its prospective design, the large number of patients enrolled for a study of this nature, and the comprehensive assessment of in vivo PMA formation and plasma monokine levels. To our knowledge, this is the only prospective clinical study to examine interactions between PMA levels, age, and clinical outcomes in septic syndromes. Exclusion of patients who received platelet transfusions eliminates a potential source of confounding. There are several limitations to the study. Although the overall sample size (n = 113 patients) was large for studies of this nature, the number of older nonsurvivors was relatively small, leading to wider confidence intervals. In this aspect, larger studies are needed to confirm our findings. Nevertheless, despite this sample size of older nonsurvivors, the effect size was large (approximately sixfold higher risk of mortality) and remained after controlling for confounding variables. Our study population also included a small number of patients prescribed aspirin prior to admission (n = 7/113, 6.2%). Nevertheless, we do not feel that this aspirin use was a significant limitation. PMA formation is not dependent on the cyclooxygenase pathway and is unaffected by aspirin therapy (35). In addition, when we excluded these seven patients from our analyses, correlations between high PMA levels and mortality in older septic patients persisted, although the effect was attenuated due to the smaller sample size (odds ratio 2.91, 95% confidence interval 0.32–26.3).

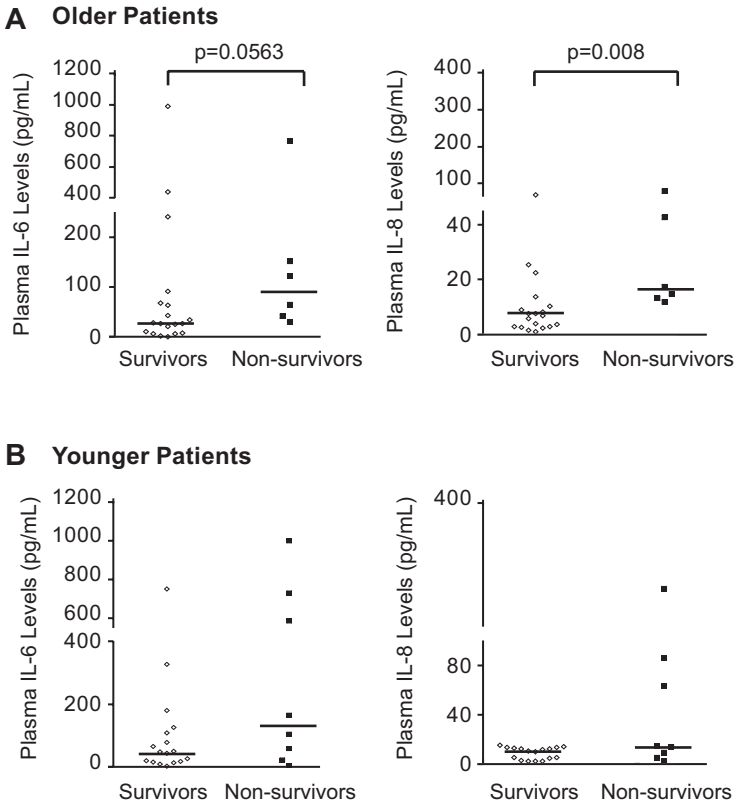

Figure 3. Proinflammatory plasma monokines were higher in older septic nonsurvivors compared with survivors. Plasma levels of IL-6 and IL-8, which are commonly synthesized upon platelet–monocyte aggregate (PMA) formation, were determined from the same blood sample used for PMA measurements by flow cytometry. (**A**) In older septic patients, levels of IL-6 and IL-8 were higher in nonsurvivors compared with survivors. (**B**) In comparison, in younger septic patients, levels of IL-6 and IL-8 were not significantly different between survivors and nonsurvivors. The solid bar shown represents the median value (**$p \leq .05$).

Althoug it is plausible that increases in PMA only reflect illness severity and do not contribute to proinflammatory responses, we feel this is unlikely. APACHE II scores, the presence of shock, red blood cell transfusions, and platelet counts did not differ significantly between older and younger patients, suggesting that our findings were not solely due to differences in illness severity, transfusions, or circulating platelets. Moreover, in multivariate regression analyses controlling for potential confounders, PMA levels remained a predictor of mortality in older, but not younger, septic patients.

In prior studies, total and differential WBC counts positively associated with age, contributing to differential cytokine synthesis (36). Similarly, total WBC counts in our septic patients (n = 113) also correlated with age (β-coefficient 0.89, p = .002). We did not record differential WBC counts nor did we perform characterization of monocyte subtypes or intracellular cytokine staining. These, and other, future studies are needed to further dissect mechanisms regulating these responses in older adults.

In conclusion, PMA levels in older septic patients upon ICU admission correlate strongly with mortality risk and may be a novel marker of mortality risk in older septic patients. As these differences were not seen in younger patients, we hypothesize that aging-related changes in PMA formation and proinflammatory monokine synthesis may contribute to the higher risk of adverse outcomes in older septic patients. These data provide new evidence that aging may alter normal platelet and monocyte responses, contributing to injurious thrombotic and inflammatory syndromes.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

Funding

This work was supported by the National Institutes of Health and National Institute on Aging (HL091754, HL066277, AG040631, R37HL044525, K23HL092161, K23GM094465.

Acknowledgments

We thank Dr. Timothy LaPine, and Dr. Harry Hill at the ARUP Institute for Clinical and Experimental Pathology at the University of Utah for expertise with the multiplex detection assays. We also thank Ms. Diana Lim for figure preparation and Ms. Alexandra Greer for editorial assistance.

References

1. Rondina MT, Schwertz H, Harris ES, et al. The septic milieu triggers expression of spliced tissue factor mRNA in human platelets. *J Thromb Haemost*. 2011;9:748–758. doi:10.1111/j.1538-7836.2011.04208.x
2. Vieira-de-Abreu A, Rondina MT, Weyrich AS, Zimmerman GA. Inflammation. In: Michelson AD ed. *Platelets*. 3rd ed. New York: Elsevier; 2012:733–766.

3. Garraud O, Berthet J, Hamzeh-Cognasse H, Cognasse F. Pathogen sensing, subsequent signalling, and signalosome in human platelets. *Thromb Res*. 2011;127:283–286. doi:10.1016/j.thromres.2010.10.015
4. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation*. 2001;104:1533–1537.
5. Rondina MT, Grissom CK, Men S, et al. Whole blood flow cytometry measurements of in vivo platelet activation in critically-Ill patients are influenced by variability in blood sampling techniques. *Thromb Res*. 2012;129:729–735. doi:10.1016/j.thromres.2011.11.031
6. Weyrich AS, Elstad MR, McEver RP, et al. Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest*. 1996;97:1525–1534.
7. Girard TD, Opal SM, Ely EW. Insights into severe sepsis in older patients: from epidemiology to evidence-based management. *Clin Infect Dis*. 2005;40:719–727.
8. Martin GS, Mannino DM, Moss M. The effect of age on the development and outcome of adult sepsis. *Crit Care Med*. 2006;34:15–21.
9. De Gaudio AR, Rinaldi S, Chelazzi C, Borracci T. Pathophysiology of sepsis in the elderly: clinical impact and therapeutic considerations. *Curr Drug Targets*. 2009;10:60–70.
10. Baldwin MR, Narain WR, Wunsch H, et al. A prognostic model for 6-month mortality in elderly survivors of critical illness. *Chest*. 2013;143:910–919. doi:10.1378/chest.12-1668
11. Seidler S, Zimmermann HW, Bartneck M, Trautwein C, Tacke F. Age-dependent alterations of monocyte subsets and monocyte-related chemokine pathways in healthy adults. *BMC Immunol*. 2010;11:30. doi:10.1186/1471-2172-11-30
12. Plackett TP, Boehmer ED, Faunce DE, Kovacs EJ. Aging and innate immune cells. *J Leukoc Biol*. 2004;76:291–299.
13. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. *Nat Rev Immunol*. 2013;13:875–887. doi:10.1038/nri3547
14. Cesari M, Kritchevsky SB, Leeuwenburgh C, Pahor M. Oxidative damage and platelet activation as new predictors of mobility disability and mortality in elders. *Antioxid Redox Signal*. 2006;8:609–619.
15. Reilly IA, Doran JB, Smith B, FitzGerald GA. Increased thromboxane biosynthesis in a human preparation of platelet activation: biochemical and functional consequences of selective inhibition of thromboxane synthase. *Circulation*. 1986;73:1300–1309.
16. Mohebali D, Kaplan D, Carlisle M, Supiano MA, Rondina MT. Alterations in platelet function during aging: clinical correlations with thromboinflammatory disease in older adults. *J Am Geriatr Soc*. 2014;62:529–535. doi:10.1111/jgs.12700
17. Bone RC, Balk RA, Cerra FB, et al. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992;20:864–874.
18. Rondina MT, Brewster B, Grissom CK, et al. In Vivo Platelet Activation in Critically Ill Patients With Primary 2009 Influenza A (H1N1). *Chest*. 2012, 141:1490–1495. doi:10.1378/chest.11-2860
19. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res*. 2013;112:1506–1519. doi:10.1161/CIRCRESAHA.113.300512
20. Fulton RJ, McDade RL, Smith PL, Kienker LJ, Kettman JR Jr. Advanced multiplexed analysis with the FlowMetrix system. *Clin Chem*. 1997;43:1749–1756.
21. Aiken LS, West SG. *Multiple Regression: Testing and Interpreting Interactions*. London: Sage Publications; 1991.
22. Schmidt R, Bültmann A, Fischel S, et al. Extracellular matrix metalloproteinase inducer (CD147) is a novel receptor on platelets, activates platelets, and augments nuclear factor kappaB-dependent inflammation in monocytes. *Circ Res*. 2008;102:302–309.
23. Bozza FA, Salluh JI, Japiassu AM, et al. Cytokine profiles as markers of disease severity in sepsis: a multiplex analysis. *Crit Care*. 2007;11:R49.
24. Johnson M, Ramey E, Ramwell PW. Sex and age differences in human platelet aggregation. *Nature*. 1975;253:355–357.
25. Sie P, Montagut J, Blanc M, et al. Evaluation of some platelet parameters in a group of elderly people. *Thromb Haemost*. 1981;45:197–199.
26. Fagiolo U, Cossarizza A, Scala E, et al. Increased cytokine production in mononuclear cells of healthy elderly people. *Eur J Immunol*. 1993;23:2375–2378.
27. Dreiher J, Almog Y, Sprung CL, et al.; SEPSIS-ISR Group. Temporal trends in patient characteristics and survival of intensive care admissions with sepsis: a multicenter analysis. *Crit Care Med*. 2012;40:855–860. doi:10.1097/CCM.0b013e318236f7b8
28. Freedman JE, Loscalzo J. Platelet-monocyte aggregates: bridging thrombosis and inflammation. *Circulation*. 2002;105:2130–2132.
29. Weyrich AS, Denis MM, Kuhlmann-Eyre JR, et al. Dipyridamole selectively inhibits inflammatory gene expression in platelet-monocyte aggregates. *Circulation*. 2005;111:633–642.
30. Furman MI, Barnard MR, Krueger LA, et al. Circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction. *J Am Coll Cardiol*. 2001;38:1002–1006.
31. Lukasik M, Dworacki G, Kufel-Grabowska J, Watala C, Kozubski W. Upregulation of CD40 ligand and enhanced monocyte-platelet aggregate formation are associated with worse clinical outcome after ischaemic stroke. *Thromb Haemost*. 2012;107:346–355. doi:10.1160/TH11-05-0345
32. Andaluz-Ojeda D, Bobillo F, Iglesias V, et al. A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis. *Cytokine*. 2012;57:332–336. doi:10.1016/j.cyto.2011.12.002
33. Calfee CS, Ware LB, Glidden DV, et al.; National Heart, Blood, and Lung Institute Acute Respiratory Distress Syndrome Network. Use of risk reclassification with multiple biomarkers improves mortality prediction in acute lung injury. *Crit Care Med*. 2011;39:711–717. doi:10.1097/CCM.0b013e318207ec3c
34. Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatr Soc*. 2002;50:1268–1271.
35. Klinkhardt U, Bauersachs R, Adams J, Graff J, Lindhoff-Last E, Harder S. Clopidogrel but not aspirin reduces P-selectin expression and formation of platelet-leukocyte aggregates in patients with atherosclerotic vascular disease. *Clin Pharmacol Ther*. 2003;73:232–241.
36. Leng S, Xue QL, Huang Y, et al. Total and differential white blood cell counts and their associations with circulating interleukin-6 levels in community-dwelling older women. *J Gerontol A Biol Sci Med Sci*. 2005;60:195–199.