



## Clinical significance of peripheral blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> myeloid cells in infants and children with infectious diseases and increased CRP

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### Abstract

**Background:** At early ages, recurrent or persistent infections are associated with increased serum C-reactive protein (CRP). Inflammatory mediators release inhibitory cells named myeloid-derived suppressor cell (MDSC) into circulating and tumor tissues. In the present study, we assayed the percentage and count of whole blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs or myeloid cells at early ages with infectious diseases and increased CRP.

**Methods:** In this study, the clinical significance of CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs or myeloid cells was evaluated in whole blood samples from 40 patients with infectious disease and 20 healthy controls by flow cytometry analysis. Subsequently, the Pearson correlation between the percentage and absolute count of MDSCs with clinical parameters were obtained by SPSS analysis. A p value of < 0.05 was considered statistically significant.

**Results:** We found a significantly higher level of MDSCs in infants and children with infectious diseases and increased CRP as compared to healthy controls (P=0.003). However, the results of analysis showed no correlation between MDSC percentage and count with grouped age and sex in patient groups.

**Conclusion:** Our findings showed a significant correlation between the high level of serum CRP and peripheral blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs at early ages. This study could be a roadmap for future studies to use increased CRP as a potential prognostic biomarker to target MDSCs in children with recurrent or persistent infections.

**Keywords:** CRP, MDSC, Infectious disease

**Conflicts of Interest:** None declared

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### Introduction

Myeloid-derived suppressor cells (MDSCs) are hetero-

geneous cells of immature myeloid cells recruited from

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#### ↑What is “already known” in this topic:

Previous studies indicated that MDSCs are abundant in tumor microenvironment because tumor microenvironment secretes inflammatory mediators such as IL-6 and C5a and lead to recruiting MDSC to tumor tissues. CRP is one of the inflammatory mediators that increases inflammations and infections. Resistance to treatment due to infectious diseases is frequent in infants and children and leads to chronic inflammation and incomplete eradication of infection. To date, the association between increased CRP and changes in peripheral blood MDSC in early ages has not been investigated.

#### →What this article adds:

Our results represent a positive correlation between the high level of serum CRP and peripheral blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs in infants and children with infectious diseases. CRP as an inflammatory mediator may stimulate myelopoiesis and affect releasing MDSCs from the bone marrow.

bone marrow toward inflammatory tumor microenvironments (1). These cells are phenotypically divided into at least 2 subsets: (1) granulocytic-MDSC (G-MDSC) with phenotypes; CD11b<sup>+</sup>/CD15<sup>+</sup>/CD33<sup>int</sup>/CD14<sup>-</sup>; and (2) monocytic-MDSCs (M-MDSC) with phenotypes; CD11b<sup>+</sup>/CD14<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-low</sup> (2, 3). Total MDSCs, including G-MDSCs (CD14<sup>-</sup>) and M-MDSCs (CD14<sup>+</sup>), are identified by CD11b<sup>+</sup>, CD33<sup>+</sup>, and HLA-DR<sup>-</sup> phenotypes (4, 5). One of the major problems in chronic inflammation and cancer that affects protective immune responses is emergency host responses to myelopoiesis and releasing MDSCs from the bone marrow (6). These cells have weak phagocytosis and can inhibit protective immune responses through secreting tumor growth factor-β (TGF-β), prostaglandins, and interleukin (IL)-10 (7).

The prolonged presence of the MDSCs can promote the inflammations in tumor microenvironment via reactive oxygen species production, activation of inducible nitric oxide synthase, and nitric oxide production. These oxygen metabolites are toxic for T cell proliferation and lead to inhibition of T-cell proliferation, poor antigen presentation, and promotion of anti-inflammatory cytokines (8, 9).

Furthermore, they have a major role in tumorigenesis and can lead to angiogenesis, tumor cell invasion, and metastasis (10). Releasing MDSCs from the bone marrow into lymph nodes and other tissues is mediated by different proinflammatory mediators produced by normal or malignant cells. These mediators, including prostaglandin E2, IL-6, vascular endothelial growth factor (VEGF), and complement fragment C5a, are not specific for tumor; in fact, MDSCs can accumulate in tissues of patients with chronic inflammatory diseases (11).

C-reactive protein (CRP) is a classical acute phase protein synthesized by hepatocytes in response to inflammation, trauma, and tissue damage. This molecule possesses a cyclic pentameric structure with calcium-dependent ligand binding and is a member of the pentraxin family. CRP is increased in blood circulation in response to infections and lead to acute and chronic inflammation and tissue damage. Assessment of CRP concentration is a nonspecific but sensitive marker for determining acute and/or chronic inflammatory conditions due to infections, some of the autoimmune diseases, and chronic obstructive pulmonary disease. In addition to increase of the CRP concentration in bacterial infection, CRP can also be used as a diagnostic factor in bacterial and viral infections (12, 13).

At early ages, recurrent or persistent infections are associated with an increase in CRP and previous studies investigated various aspects of the causes (14-16). Given that CRP is an inflammatory marker, the inflammatory mediators play a major role in the recruitment of MDSCs from bone marrow into the blood circulation and other tissues. However, the role of CRP as an inflammatory indicator in releasing MDSCs has not been identified. In this study, we preliminarily detected whole blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs or myeloid cells in infants, children, and adolescents with infectious diseases and increased CRP.

## Methods

### Reagents

CRP-Latex Immunoturbidimetric assay was prepared from Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA. PerCP anti-human HLA-DR Antibody was purchased from BioLegend Inc., 9727 Pacific Heights Blvd, San Diego, CA 92121. FITC anti-human CD11b antibody, R-PE anti-human CD33 antibody, and lysing solution were purchased from IQ Products BV Rozenburglaan 13a 9727 DL Groningen, the Netherlands.

### Study groups

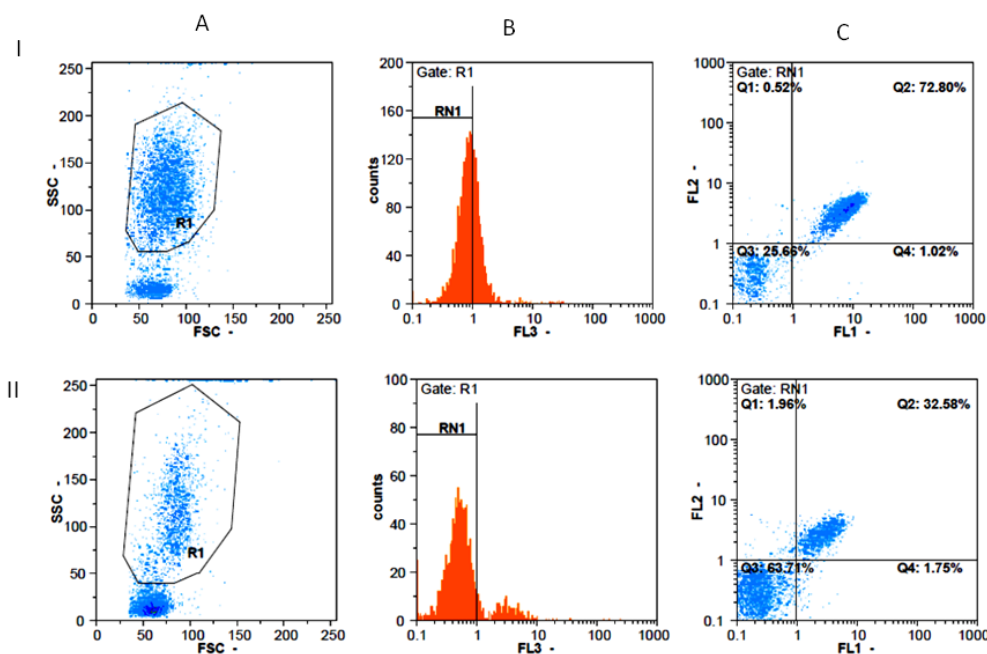
A total of 40 patients with infectious disease and increased CRP (<1 mg/mL) were included in this study. This study was conducted on infants, children, and adolescents (17). Serum samples of these patients were assessed for CRP in 2018 in Ali Asghar medical center, a major university-based referral pediatric hospital in Tehran, Iran. CRP was assessed by CRP-Latex Immunoturbidimetric assay according to the kit's instructions. In this study, 20 healthy controls (HCs) were included (12 males and 8 females with < 1 mg/dL of CRP). The age of healthy controls was almost similar to that of the patient group, with the mean age of 3.5 ± 5.5 years (age range of 3 days-12 years). This study was approved by the research ethics committee of Iran University of Medical Sciences.

### Flow cytometry

Flow cytometry assessment was performed in whole blood samples on day 0 within 2 hours after the blood draw. One milliliter (ML) of whole peripheral blood specimens was collected from patients and healthy controls (18, 19). The samples containing anticoagulants (Ethylenediaminetetraacetic acid (EDTA)) were stained with conjugated antibodies to fluorescent materials using the whole blood lysis method and analyzed on the flow cytometer (Partec, Germany) using Flomax software. The samples from control children were also stained and analyzed simultaneously with the patients' samples. MDSCs were detected by expression of CD11b and CD33 on HLA-DR negative population. Briefly, whole blood samples were incubated with anti-CD11b, CD33, and HLA-DR mAb; lysing solution was then added and the samples were washed and resuspended in phosphate buffer. At the analysis steps, granulocytes and monocytes were selected in a gate by drawing a region around the leukocyte population on side scatter (SSC) and the forward scatter (FCS) dot plots (Fig. 1 A and B). Subsequently, HLA-DR negative population was gated and selected and CD11b and CD33 positive cells were analyzed. Surface markers were evaluated by considering the relative number of positive cells.

### Statistical analysis

Prism™ version 6.0 software (GraphPad Inc., CA, USA) was used for diagram and curve presentation and data were analyzed using SPSS version 20. The data were normally distributed by one-sample Kolmogorov-Smirnov analysis; thus, we used parametric tests (independent sample t test). Receiver operating characteristic



**Fig. 1.** Detection of MDSCs by flow cytometry analysis. MDSC were detected by gating a: granulocytes/monocytes (R1), b: gating HLA-DR negative (RN1), and c: finally, MDSC were detected by either CD11b or CD33 expression. I: represents a patient sample and II: a control sample.

(ROC) curves and the area under the curve (AUC) were used to discriminate the patients and controls for percentage and count of whole blood MDSCs. Pearson's  $\chi^2$  and Pearson's R tests were used to analyze the significance of the correlation between MDSC percentage and count with clinical parameters. A p value of  $< 0.05$  was considered statistically significant.

## Results

### Characterization of the study group

Overall, the mean  $\pm$  SD age of the study population was  $4 \pm 0.46$  years (ranged 3 days–18 years) and the mean  $\pm$  SD age of patients was  $4 \pm 0.47$  years (ranged 3 days–18 years). Patients group included 21 neonates and infants (1 day–2 years), 17 children (2–12 years), and 2 adolescents (12–18 years). Mean  $\pm$  SD CRP concentration (mg/dL) of case group was  $68 \pm 3.87$  (ranged 2.5–126 mg/dL) and all healthy controls  $< 1$  mg/dL (Table 1). The patients were diagnosed with different bacterial infections and most of them had a positive blood culture for bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Acinetobacter baumannii*. Two patients died due to acute and severe infections.

### Flow cytometric analysis of MDSC population

We found a higher percentage of CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs in whole blood of the patients with increased CRP compared to the healthy control group ( $p=0.003$ ) (Fig. 1 C and Fig. 2 A). The MDSC percentages of the 2 patients who died due to severe infection were 72.81% and 70.5% and those of other patients were between 22.69% to 89.43%. In addition, a borderline significant difference was found when we compared the absolute count of the MDSCs between the patients and healthy controls ( $p=0.057$ , Fig. 1 C and Fig. 2 B). Further analysis based on ROC curve analysis showed an AUC of 0.71 for MDSC percentage and 0.65 for the total MDSC count (Fig. 2 C and D) (20). These data indicated a difference and discrimination for whole blood MDSC between patients with infectious diseases and increased CRP and healthy controls.

### Association of whole blood MDSCs with clinical parameters

The mean  $\pm$  SD of age ( $4 \pm 0.47$  years), CRP ( $68 \pm 3.87$  mg/dL), MDSC percentage ( $63 \pm 1.5$ ) and MDSC count ( $1396 \pm 767$ ) were selected as a cutoff value to classify each of these parameters into patient group. The results of

**Table 1.** Characterization of studied groups

Group	Number	Age/year		CRP mg/dL		MDSC percentage		MDSC absolute count	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Healthy control	20	3.5	0.46	68	3.87	48.98	1.98	1012	715
Infectious patients	40	4	0.47	0.15	0.046	63.2	1.5	1396	767

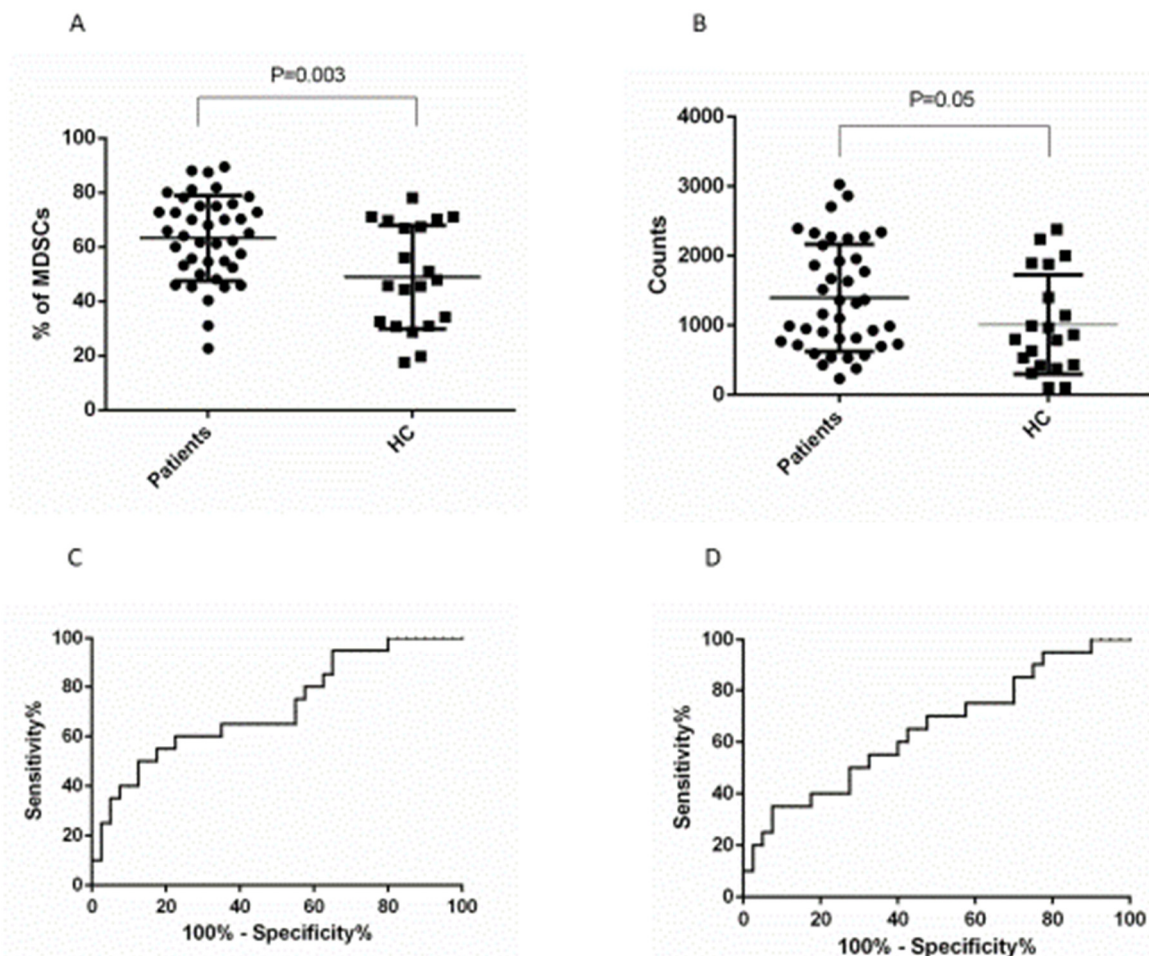


Fig. 2. MDSC expression in 40 patients and 20 healthy controls; whole blood percentage of MDSCs (A), absolute counts (B). Receiver operated characteristic (ROC) curve analyses presented for MDSC percentage (C); Area under the curve (AUC) = 0.71, 95% CI:0.57–0.85 and MDSC absolute count (D); AUC = 0.65, 95% CI:0.5–0.8. Statistical analysis of A and B curves were done by independent samples t test. HC: healthy controls.

the analysis showed no correlation between MDSC percentage and MDSC count with grouped age, grouped CRP and sex (Tables 2 and 3).

### Discussion

In this study, total MDSCs or myeloid cells with phenotypes- CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> in peripheral blood of

Table 2. Association between whole blood MDSCs (percentage) and grouped clinical parameters of infectious patients based on cutoff point described in the text (P value; Pearson  $\chi$ )

Characterization of clinical parameters	Level	P value	Percentage			
			<63%		>63%	
			No	Percent	No	Percent
Year of Age	<4	0.836	13	32.5	15	37.5
	>4		6	15	6	15
Sex	Male	0.564	11	28.9	14	36.8
	Female		7	18.4	6	15.8
CRP	<68 (mg/dL)	0.342	11	27.5	9	22.5
	>68 (mg/dL)		8	20	12	30

Table 3. Association between whole blood MDSCs (count) and grouped clinical parameters of infectious patients based on cutoff point described in the text (P value; Pearson  $\chi$ )

Characterization of clinical parameters	Level	P value	Count			
			<1396		>1396	
			No	Percent	No	Percent
Year of Age	<4	0.530	17	42.5	11	27.5
	>4		6	15	6	15
Sex	Male	0.899	14	36.8	11	28.9
	Female		7	18.4	6	15.8
CRP	<68 (mg/dL)	0.110	14	35	6	15
	>68 (mg/dL)		9	22.5	11	27.5

neonates, infants, children, and adolescents with increased CRP due to infectious diseases were assayed. Previous studies and this study defined the MDSCs with phenotypes- CD33<sup>+</sup>CD11b<sup>+</sup>HLA-DR<sup>-</sup> in whole blood (4, 21). These phenotypes are very common and have been used in various types of malignancies (22). In the present study, the percentage of MDSCs is also shown as the CD33<sup>+</sup>CD11b<sup>+</sup> (%) of gated HLA-DR<sup>-</sup> cells (21). Sade-Feldman et al detected the percentage of MDSC in fresh whole blood approximately in a range of 13%-70% in healthy participants (21). Consistent with the previous data, we assayed CD33<sup>+</sup>CD11b<sup>+</sup>HLA-DR<sup>-</sup> MDSCs in whole blood of the studied population (patients with infectious disease and healthy control) in a range of 17%-89%. Given that the MDSCs are derived from the myeloid lineage and morphologically (size and cytoplasmic granules) closer to granulocytes and monocytes (23), it may be better to search for these cells on granulocytes and monocytes population in peripheral blood instead of peripheral blood mononuclear cells.

CRP is a sensitive but nonspecific marker in the inflammatory condition. Serum CRP level is affected by physiological and pathological factors such as acute and chronic infections and the use of anti-inflammatory and infectious drugs. This marker leads to production and secretion of inflammatory mediators such as IL-6 and C5a. These mediators play a central role in recruiting MDSCs from the bone marrow into peripheral blood and other tissues (13). Previous studies indicated that these cells are abundant in the tumor microenvironment because tumor microenvironment containing immune cells secrete inflammatory cytokines and lead to recruiting MDSC to tumor tissues. In addition, tumor cells secrete inflammatory mediators such as IL-6, VEGF, and prostaglandin E2 recruiting MDSC toward tumor microenvironments. MDSCs cause tumor survival because they produce reactive oxygen species such as peroxynitrite and indolamine 2,3-dioxygenase and arginase I that inhibits proliferation and activation of tumor-specific T lymphocytes (11). Hence, releasing inflammatory mediators are dominant in recruiting MDSC from bone marrow and inflammation with stimulating MDSCs into peripheral blood may suppress protective immune responses via these cells. In this study, to investigate the increased CRP changes as an inflammatory mediator in peripheral blood MDSCs, we assayed peripheral blood MDSCs in patients with infectious disease and increased CRP in an age range of 3 days to 18 years. Given the inflammatory mediators in recruiting MDSC from bone marrow, our results indicated that increasing CRP can impact the blood MDSCs and significantly elevate MDSC percentage in patients with the infectious disease compared with healthy controls.

At early ages, there are recurrent or persistent infections that lead to high-level CRP that are associated with chronic inflammations (24). This disorder can be partly due to increased peripheral blood MDSCs that require further investigation in future studies. Recent reports indicated that a high level of serum inflammatory cytokines can make an immune-inhibitory microenvironment containing MDSCs and regulatory T cells in melanoma pa-

tients (25, 26). Resistance to treatment due to infectious diseases is frequent in infant and children that lead to chronic inflammation and incomplete eradication of infection. Chronic inflammation leads to disorder in protective immune responses that can be partly due to increased blood MDSCs; however, strong confirmation of these results will require conducting many studies in the future and it is likely to promote the protective immune system with targeting MDSCs in patients with recurrent infection at earlier ages.

### Conclusion

Taken together, our findings showed a positive correlation between the high level of serum CRP and peripheral blood CD11b<sup>+</sup>/CD33<sup>+</sup>/HLA-DR<sup>-</sup> MDSCs in infants and children, as the CRP increases, the percentage of blood MDSCs increases. CRP as an inflammatory mediator can stimulate myelopoiesis and affect releasing MDSCs from the bone marrow. This preliminary study provides grounds for further studies to investigate increased CRP changes on expression and releasing MDSCs from the bone marrow into peripheral blood in acute and chronic inflammation for therapeutic strategies to treat children with recurrent and persistent infectious diseases.

### Acknowledgments

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### Conflict of Interests

The authors declare that they have no competing interests.

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