

RESEARCH

Open Access



# TNF $\alpha$ induces Caspase-3 activity in hematopoietic progenitor cells CD34+, CD33+, and CD41 + of myelodysplastic syndromes

Anggraini Iriani<sup>1\*</sup> , Andhika Rachman<sup>2</sup> , Rahayuningsih D. Setiabudy<sup>3</sup>, Siti B. Kresno<sup>3</sup>, Aru W. Sudoyo<sup>2</sup>, Mansyur Arief<sup>4</sup>, Alida R. Harahap<sup>3</sup> and Marsya Kaila Fatina<sup>5</sup>

## Abstract

**Background** Cytopenia is the primary feature of Myelodysplastic Syndrome, even in the presence of hypercellular bone marrow. TNF $\alpha$  is recognized as both a proinflammatory, and proapoptotic cytokine with a well established role in promoting apoptosis in MDS. Therefore, TNF $\alpha$  has the potential to be a valuable biomarker for predicting the progression of cytopenia in MDS. This study aims to establish the role of TNF $\alpha$  exposure in triggering apoptosis through caspase-3 activity in CD34+, CD33+, and CD41 + cells in MDS.

**Methods** This study is an in vitro comparative experimental research. Bone marrow mononuclear cells were isolated as the source of hematopoietic progenitor cells. Subsequently, CD34+, CD33+, and CD41 + cells were exposed to rhTNF $\alpha$ , and the caspase-3 activity was measured using flowcytometry.

**Results** In MDS CD33 + and CD41 + caspase-3 activity of rhTNF $\alpha$  exposed cells was significantly higher than without exposed cells. The opposite result was found in CD34 + cells, where the caspase-3 activity without rhTNF $\alpha$  exposed cells was significantly higher than rhTNF $\alpha$  exposed cells.

**Conclusion** rhTNF $\alpha$  exposure led to an elevation in caspase-3 activity in MDS progenitor cells, especially in those that had differentiated into myeloid cell CD33 + and megakaryocyte cell CD41+, as opposed to the early progenitor cells CD34+.

**Keywords** Myelodysplastic syndrome, TNF $\alpha$ , caspase-3, CD34+, CD33+, CD41+

\*Correspondence:

Anggraini Iriani  
anggraini.iriiani@yarsi.ac.id

<sup>1</sup> Department of Clinical Pathology, Faculty of Medicine, Yarsi University - Yarsi Hospital, Jl. Letjen Suprpto Kav 13, Cempaka Putih, Jakarta 10510, Indonesia

<sup>2</sup> Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

<sup>3</sup> Department of Clinical Pathology, University of Indonesia, Jakarta, Indonesia

<sup>4</sup> Department of Clinical Pathology, Hasanuddin University, Makassar, Indonesia

<sup>5</sup> Faculty of Medicine, Andalas University, Padang, Indonesia



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

## Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous clonal stem cell disorder characterized by abnormalities in the differentiation and proliferation of hematopoietic precursors which leads to peripheral blood cytopenia, bone marrow hypercellularity, ineffective hematopoiesis, and dysplastic changes in myeloid, erythroid, and megakaryocyte lineages [1–4]. Cytopenia is the prominent feature of MDS amid the hypercellularity in the bone marrow [5, 6]. Some experts postulate that ineffective hematopoiesis in MDS may be caused by the increase in hematopoietic cell apoptosis, coupled with heightened intra-medullary proliferation or self-renewal capacity [7]. The bone marrow's microenvironment is a highly intricate structure that plays a crucial role in regulating the proliferation, differentiation, and self-renewal processes of progenitor cells by providing essential factors such as nutrients, growth factors, and cytokines. However, in MDS, the bone marrow microenvironment undergoes changes that involve interactions between various cell types and between cells and cytokines, which contribute to the pathogenesis of the MDS [8, 9].

There are several hypotheses explaining the heightened occurrence of apoptosis. Firstly, increased apoptosis serves as a mechanism through which hematopoiesis cells dispose of undesired cells. When bone marrow progenitor cells come across chronic peripheral cytopenia, the body's feedback mechanism triggers the production of cytokines to facilitate the formation of a new cell. Secondly, the elevation in apoptosis can be attributed to clonal abnormalities in MDS, implying that apoptosis is not solely an outcome but is intricately linked to the underlying cause or mechanism of abnormal biology in MDS clones [10, 11].

The TNF $\alpha$  gene is located on chromosome 6p21.3 and spans a length of 3 kb, encompassing four exons. The final exon is responsible for encoding approximately 80% of the secreted TNF $\alpha$ . TNF $\alpha$  itself is a type 2 transmembrane protein, composed of 212 amino acids and form a homotrimer structure. Various cell types, including macrophages, monocytes, T cells, B cells, NK cells, and Kupffer cells, produce TNF $\alpha$ . Its production is induced in response to cytokine signals, such as GM-CSF, IL-1, IL-2, IFN $\gamma$ , and immune complexes [12].

Apoptotic activity is assumed to be the primary mechanism underlying ineffective hematopoiesis, leading to cytopenia in the peripheral blood of MDS patients [8, 13]. In these patients, there is a known elevation in the level of TNF $\alpha$ , a cytokine that triggers apoptosis via the extrinsic pathway [14]. TNF $\alpha$  interacts with the TNF receptor initiating the activation of the caspase cascade. Caspase is an intracellular proteolytic enzyme responsible for triggering apoptosis [15]. Specifically, activation of

caspase-3 triggers a cell to initiate apoptosis. The activity of this enzyme is used as a marker for apoptotic signaling in cells. However, the exact location within MDS progenitor cells where this TNF $\alpha$ -induced apoptotic process occurs remains unknown, whether it takes place in the early progenitor cells (CD34 +) or in the differentiated myeloid progenitor cells (CD33 +) and megakaryocyte progenitor cells (CD41+) [10, 11].

This study aims to demonstrate the impact of TNF $\alpha$  exposure on inducing apoptotic activity in hematopoietic progenitor cells, specifically CD34+, CD33+, and CD41 + cells in MDS. We aim to show that this exposure leads to an increase in caspase-3 activity particularly in cells that have undergone differentiation into myeloid, and megakaryocyte lineages.

## Materials and methods

This study employed a comparative in vitro experimental design involving the isolation of bone marrow mononuclear cells (BMMC) from 15 MDS bone marrow aspirates. Diagnosis of MDS was established based on clinical examination, complete hematological examination, peripheral blood and bone marrow morphology analysis [15], as well as cytogenetic testing according to the International System for Human Cytogenomic Nomenclature (ISCN) recommendations to align with the 2016 WHO diagnostic criteria [2]. The samples used consisted of CD34+, CD33+, and CD41 + cells that were isolated from the BMMC obtained from MDS patients. As a control group, we isolated BMMC from 8 lymphoma bone marrow aspirates, because it was not feasible to obtain bone marrow aspirates from healthy individuals, and lymphoma typically exhibits abnormalities in non-myeloid lineages, Table 1.

### BMMC isolation

Isolation of BMMC was performed using density gradient centrifugation with Ficoll-Paque medium (GE Healthcare™, Sweden). A cell density of 300,000 BMMC in 300  $\mu$ L of StemMACS HSC media (Miltenyi Biotec™, USA) was used in each exposure. Cells were prepared in separate tubes, with some exposed to 48ng/100mL recombinant human TNF $\alpha$  / rhTNF $\alpha$  (R&D systems™, USA) followed by incubation at 37°C with 5% CO<sub>2</sub> for 18 h. The remaining cells received the same treatment without exposure to TNF $\alpha$ ,

### Caspase 3 activity measurement

The BMMC from both the MDS and control groups underwent identical treatments, involving exposure to TNF $\alpha$  and no exposure. Each group was examined for caspase-3 activity, both with and without exposure to the TNF $\alpha$ . Then, the delta ( $\Delta$ ) caspase-3 activity was

**Table 1** Characteristic of MDS bone marrow aspirate

Bone marrow Aspirate	Bone Marrow Morphology	IPSS-R (score)
MDS 1	MDS-SLD	High (5)
MDS 2	MDS-SLD	Intermediate (4,5)
MDS 3	MDS-SLD	Very high (7)
MDS 4	MDS-SLD	NA
MDS 5	MDS-MLD	High (4,5)
MDS 6	MDS-MLD	Low (2,5)
MDS 7	MDS-MLD	Intermediate (4,5)
MDS 8	MDS-MLD	Very low (1)
MDS 9	MDS-MLD	NA
MDS 10	MDS-MLD	Intermediate (3,5)
MDS 11	MDS-MLD	NA
MDS 12	MDS-EB-1	Very high (7,5)
MDS 13	MDS-EB-1	Intermediate (4)
MDS 14	MDS-EB-1	Intermediate (4,5)
MDS 15	MDS-EB-1	High (6)

MDS Myelodysplastic syndrome, MDS-SLD Single lineage dysplasia, MDS-MLD Multilineage dysplasia, MDS-EB-1 Excess blast 1, NA Not applicable, IPSS-R international prognostic scoring system revised

**Table 2** Caspase-3 examination panel

Tube <sup>a</sup>	Treatment	Fluorochrome antibody
1	Without exposure	Negative control
2	Cisplatin 100 µg/(10 µL)	Positive control
3	Without exposure	CD34 APC, CD40 PE, CD154 FITC
4	Without exposure	CD34 APC, Caspase-3 FITC
5	rhTNFα 48 ng	CD34 APC, Caspase-3 FITC
6	Without exposure	CD33 PE, Caspase-3 FITC
7	rhTNFα 48 ng/well	CD34 APC, CD33 PE, Caspase-3 FITC
8	Without exposure	CD41 PE, Caspase-3 FITC
9	rhTNFα 48 ng/well	CD34 APC, CD41a PE, Caspase-3 FITC
10	Without exposure	CD73 PE, Caspase-3 FITC
11	rhTNFα 48 ng/well	CD34 APC, CD73 PE, Caspase-3 FITC

<sup>a</sup> Each tube contains BMMC with density of 300,000 cells/300µL media

measured, representing the difference between caspase-3 activity in BMMC exposed to rhTNFα and those without exposure. The BMMC were then labeled with specific fluorochrome antibodies; (CD34+ with APC, CD33+ with PE, and CD41+ with PE); and a fluorochrome FITC label was applied to assess caspase-3 activity (BD™, USA). The fluorescence intensity was quantified using the BD FACS-Calibur flowcytometry, Table 2.

The conceptual framework of this study is the BMMC from MDS patients, consist of CD34+, CD33+, and CD41+ in microenvironment of bone marrow there are conditions that support proapoptotic. This study tries to look at these in vivo condition in vitro. The apoptosis

process which is thought to be mainly induced by TNFα was tested by exposing rhTNFα to CD34+, CD33+, and CD41+, to find out in which progenitor lineage the apoptosis process occurs more dominantly by examining caspase-3 activity, Fig. 1.

### Flowcytometry analysis

Measurement of active caspase-3 was conducted using flow cytometry, which involves passing cells (events) through a detector. A number of cells were examined and analyzed on a scatter diagram. The x-axis represented the intensity of FITC against caspase-3 in the cells, while the y-axis described the fluorescence intensity against cell markers (CD34, CD33, CD41). Cells displaying a fluorescent pattern were identified and compared with the entire cell population, after which the percentage was calculated.

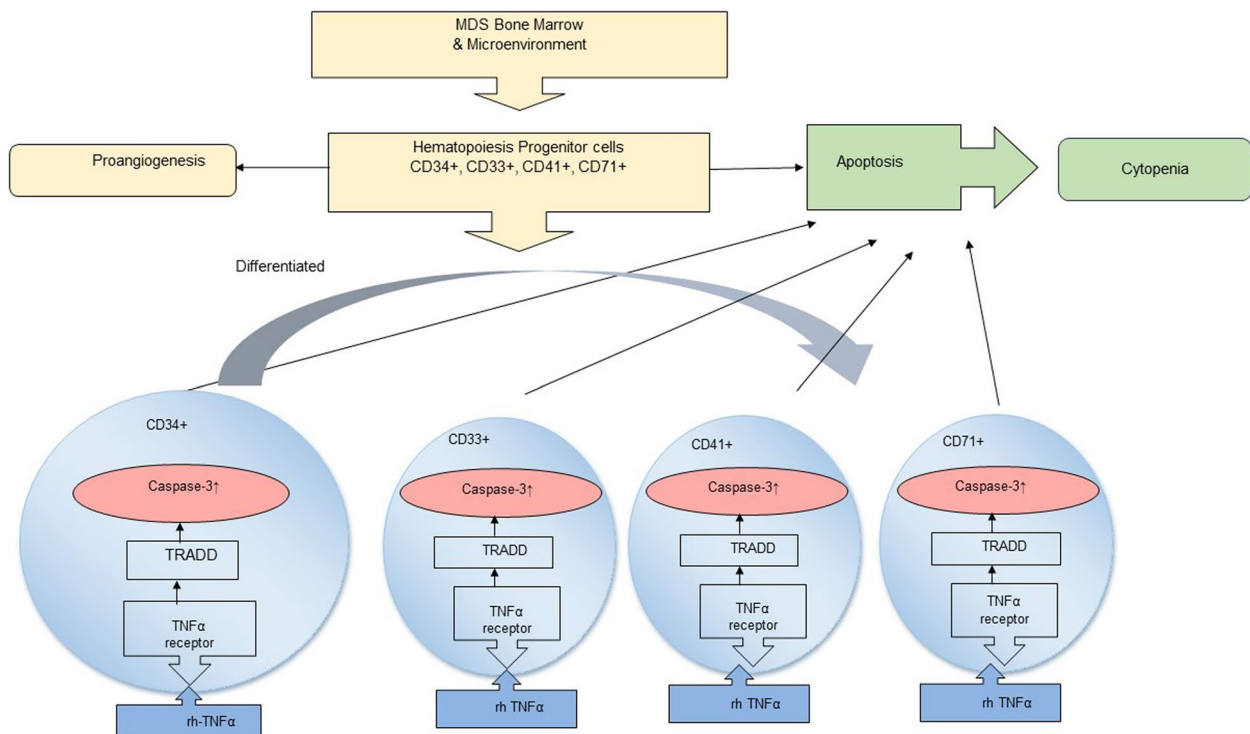
### Statistical analysis

To analyze the data for differences between paired groups (those without exposure and those exposed to rhTNFα) a paired t-test was employed, whereas, for unpaired groups (comparing the MDS group to the control group) an independent t-test was used.

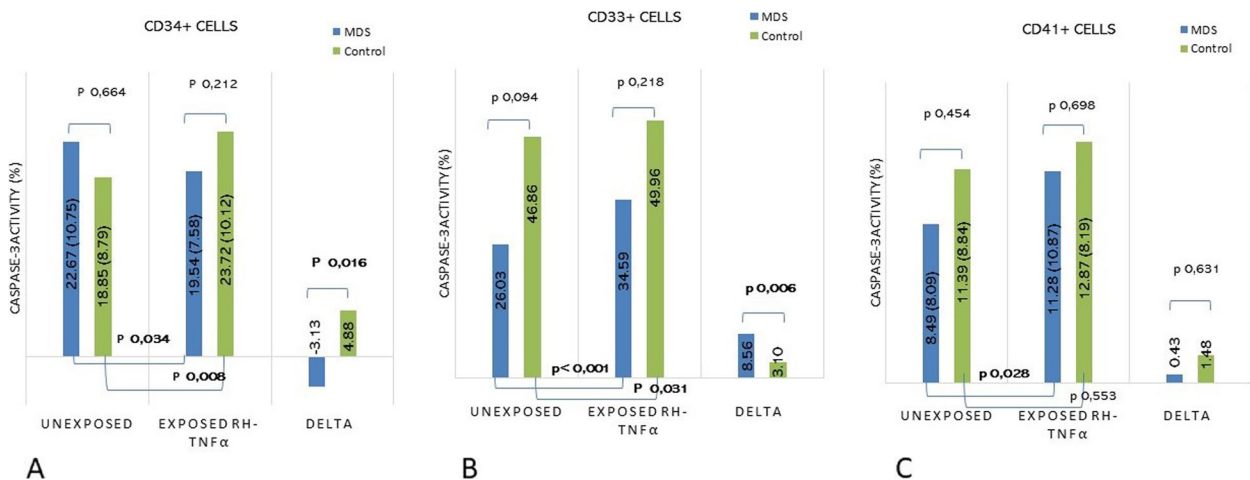
The comparison of Δ caspase-3 activity between the CD34+, CD33+, and CD41+ cell groups in MDS and the corresponding control cell groups was conducted. Both groups under conditions without exposure and after exposure to rhTNFα. A one-way ANOVA test was performed for more than two unpaired groups. If the results of the One-Way ANOVA test indicated a *p*-value of <0.001. Therefore, it can be concluded that there were at least two distinct variations in the caspase-3 activity mean among the progenitor cell groups, which were significantly different in both the MDS and control groups.

### Results

The mean of Δ activity caspase-3 in CD34+ between MDS and control groups showed that there was a significant difference with *p*-value 0.016. The paired t-test to analyze caspase-3 activity in CD34+ MDS without exposure and those exposed to rhTNFα showed a significant difference with *p*-value 0.034, as well as in control with *p*-value 0.008. In the MDS group the mean of caspase-3 activity in CD34+ without exposure (22.67%) was higher than in CD34+ exposed to rhTNFα (19.53%), with a negative Δ activity caspase-3. In control group the mean caspase-3 activity in CD34+ exposed to rhTNFα (23.72%) was higher than without exposure (18.84%), Fig. 2. This shows that CD34+ has weird behavior because even without exposure to rhTNFα the caspase-3 activity tends to be higher.



**Fig. 1** The idea is that exposure of rhTNFα to the BMBC of MDS, CD34+, CD33+, and CD41 + would induce apoptosis through extrinsic pathway via TRADD and activate cascade caspase to increase caspase-3 activity

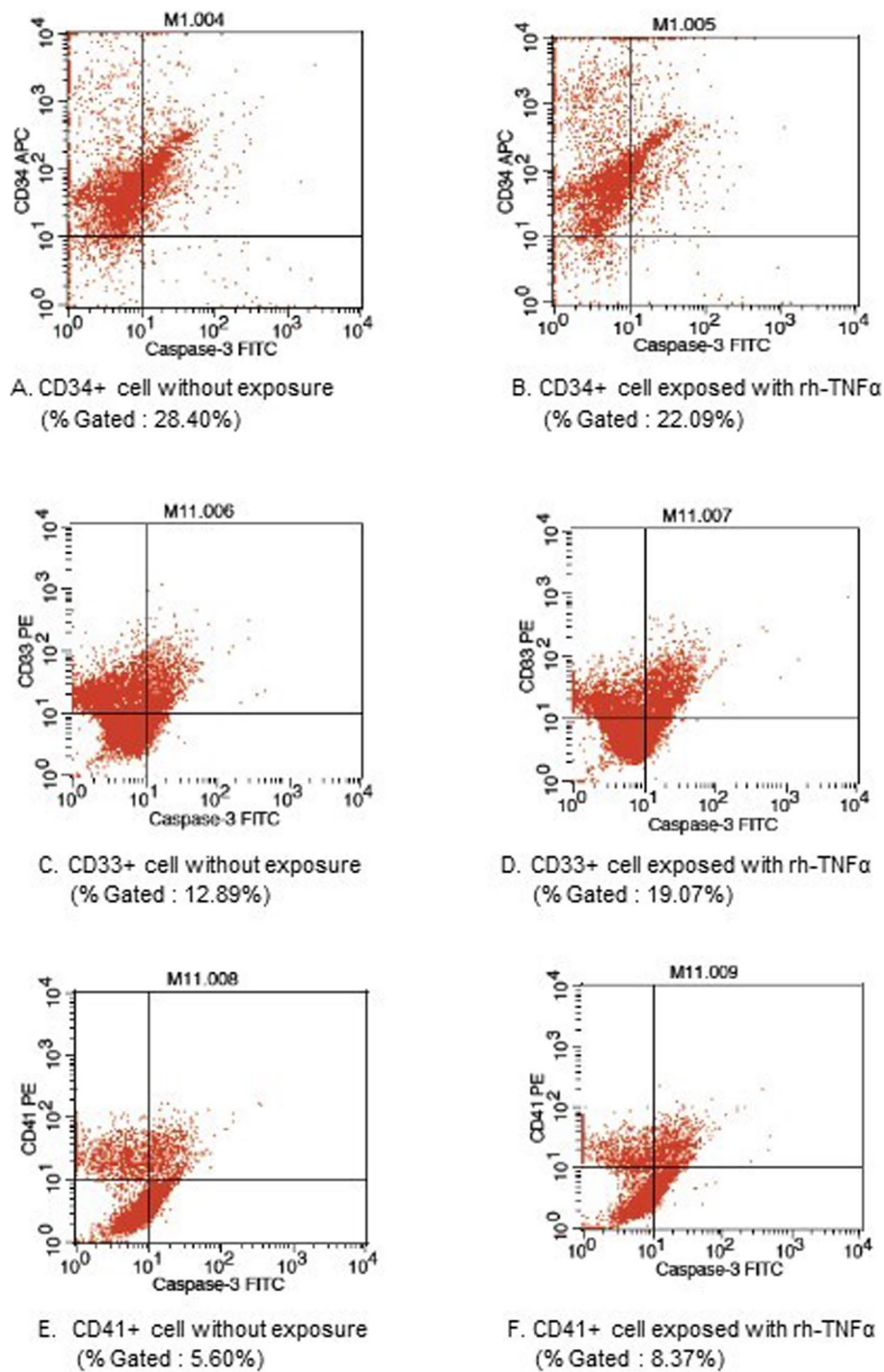


**Fig. 2** The caspase-3 activity in MDS and control BMBC unexposed and exposed to rhTNFα. **A** Caspase activity in CD34 + cells, **B** Caspase-3 activity in CD33+, **C** Caspase activity in CD41 + cells. Data presented in mean (SD). Blue box: MDS group; green box: control group. Left side: the group of cells unexposed to rhTNFα; middle side: the group of cells exposed to rhTNFα; right side: delta value. Delta formula: caspase-3 activity after exposure rhTNFα subtracted by caspase-3 activity before exposure

The mean of  $\Delta$  activity caspase-3 in CD33+ between MDS and control groups showed that there was a significant difference with  $p$ -value 0.006. The paired t test to analyze caspase activity in CD33+ without exposure and exposed to rhTNFα showed a significant difference in

both groups, MDS with  $p$ -value < 0.001 and control with  $p$ -value 0.031. The paired t-test in CD41+ MDS without exposure and exposed to rhTNFα showed a significant difference with  $p$ -value 0.028. One of the results of caspase-3 activity shows in Fig. 3.





**Fig. 3** Caspase-3 Activity on MDS BMCC by Flowcytometry Analysis. A result of caspase-3 activity is indicated by gating in the upper right quadrant (UR). **A** CD34+ cell without exposure, **B** CD34+ cell exposed to rh-TNF $\alpha$ , **C** CD33+ cell without exposure, **D** CD33+ cell exposed to rh-TNF $\alpha$ , **E** CD41+ cell without exposure, **F** CD41+ cell exposed to rh-TNF $\alpha$

Due to the statistical significance in the one-way ANOVA test among the different cell group variants, we proceeded to conduct Tamhane's post hoc test to

identify which groups exhibit differences. The disparity in mean  $\Delta$  caspase activity is negative (-), indicating that CD34+ caspase activity is lower than caspase-3 activity

in its comparator progenitor cells, namely CD33+ and CD41+ cells. A significant difference in mean  $\Delta$  caspase-3 activity was observed within the MDS group, specifically between CD34+ and CD33+ cells, as well as between CD34+ cells and CD41+ cells, with  $p$ -values of < 0.001 and 0.013, respectively.

## Discussion

### Exposure to rhTNF $\alpha$ induced apoptosis in MDS hematopoietic progenitor cells

The purpose of rhTNF $\alpha$  exposure is to induce these pro-apoptotic cytokines and observe the apoptotic response, specifically caspase-3 activity in CD34+, CD33+, and CD41+ MDS BMNC. Apoptosis may occur through either the intrinsic or extrinsic pathway. The TNF $\alpha$  apoptosis mechanism model operates through the extrinsic pathway.

The TNF $\alpha$  signaling pathway mechanism for initiating the apoptotic process begins with the binding of TNF $\alpha$  to TNFR-1 and TNFR-2 receptors. TNFR-1 mediates the cell death program by initially binding directly to the adapter protein TNF receptor-associated death domain (TRADD), then indirectly connecting to the FAS-associated protein with a death domain (FADD). This sequential process ultimately leads to the activation of the caspase cascade, culminating in the execution phase facilitated by caspase-3 [16–18].

Exposure of rhTNF $\alpha$  to hematopoietic progenitor cells is expected to result in the binding of rhTNF $\alpha$  to its receptor, TNFR1. This binding is anticipated to initiate cell death signals through the death-inducing signaling complex (DISC) and activate TRADD. Subsequently, caspase activation will be triggered, ultimately leading to the activation of caspase-3, which serves as the executor of apoptosis in hematopoietic progenitor cells in MDS, as shown in Fig. 1.

The role of TNF $\alpha$  in triggering apoptosis is supported by a research report that discovered a positive correlation between elevated serum TNF $\alpha$  levels and anemia in MDS patients. Furthermore, an increase in TNF $\alpha$  levels in the bone marrow was linked to heightened apoptosis during the early stages of MDS [19]. Several studies have also documented increased activity of caspase-1 and caspase-3 in hematopoiesis cells of MDS patients, with caspase-3 activity being ten times higher than that of caspase-1. Notably, the heightened caspase-3 activity is positively associated with increased TNF $\alpha$  levels [20, 21].

However, it is possible for caspase-3 activation to occur through interactions aside from rhTNF $\alpha$  exposure, although such instances were negligible in this study. We determined this by calculating the delta value ( $\Delta$ ) for Caspase-3 using the formula: the caspase-3 activity data after rhTNF $\alpha$  exposure subtracted by data before exposure

(as the baseline). The positive and statistically significant delta values indicate that rhTNF $\alpha$  exposure can indeed increase caspase-3 activity.

The involvement of TNF $\alpha$  as an apoptosis trigger in MDS was confirmed by the elevated levels of TNF $\alpha$  in the serum of MDS patients. This increase in TNF $\alpha$  showed a positive correlation with clinical anemia, and an elevated rate of apoptosis in the early stages of MDS [19].

In MDS, there is an increase in the activity of both caspase-1 and caspase-3, with caspase-3 activity being ten times higher than caspase-1 [7, 13, 14]. This increased caspase-3 activity is associated with a high level of TNF $\alpha$  [20, 21]. Iriani et al. [22] discovered that the high levels of TNF $\alpha$  in MDS are initiated by an increase in the expression of TNF $\alpha$  mRNA, triggered by sCD40L stimulation.

A substantial portion of MDS cells undergo intramedullary apoptosis, which can be challenging to detect morphologically, as it predominantly occurs at an early stage. Therefore, the assessment of apoptosis in MDS should involve early markers of apoptosis, such as caspase-3.

### Apoptosis in CD34+

In Fig. 2A, the  $\Delta$  caspase-3 value in the MDS group was negative, whereas it was positive, with a significant  $p$ -value. The  $\Delta$  value was used to gauge the extent of the impact of rhTNF $\alpha$  exposure. This suggests that, in the MDS group, apoptosis had occurred in CD34+ cells even without exposure to rhTNF $\alpha$ , in contrast to the control group. This implies that CD34+ MDS cells naturally tend to undergo apoptosis.

Reports indicate that CD34+ MDS cells tend to undergo apoptosis, with their susceptibility to apoptosis being most pronounced among CD34+ progenitor cells. [11, 23] This susceptibility is attributed to defects in the progenitor stem cells. Due to these clonal abnormalities, daughter cells would develop into defective cells that are prone to apoptosis. Another study found that MDS CD34+ cells displayed higher apoptotic activity than normal cells, with the apoptosis rate in MDS CD34+ cells being 2.08 times faster than their proliferation rate [24]. In MDS, despite the bone marrow continuing to produce cells, the cell cycle is shorter. It is also known that over two-third of MDS cells at various stages of differentiation in the bone marrow undergo apoptosis.

### Apoptosis in CD33+

In Fig. 2B, the  $\Delta$  caspase-3 value in the MDS group was higher than that in the control group, with a significant  $p$ -value. This suggests that the exposure of rhTNF $\alpha$  led to increased apoptosis in both groups, but the impact of increasing apoptosis in CD33+ MDS cell group was more significant.

Raza et al. [11] found that in MDS, exposure of TNF $\alpha$  would result in increased apoptosis in both CD33+ and CD34+ cells. In MDS, despite the excess apoptosis in the bone marrow, granulocyte in the peripheral circulation tend to be more resistant to apoptosis. This occurs because sensitive granulocytes undergo apoptosis earlier in the bone marrow, while apoptosis-resistant granulocytes continue to enter the circulation. Apoptosis in the myeloid lineage is also affected by *myeloid-derived suppressor cells* (MDSCs). In MDS, there is an increase in circulating MDSCs. MDSCs induce apoptosis in hematopoietic progenitor cells and trigger the secretion of suppressive cytokines, IL10 and TGF $\beta$ , which suppress the development of immature myeloid cells [25].

#### Apoptosis in CD41+

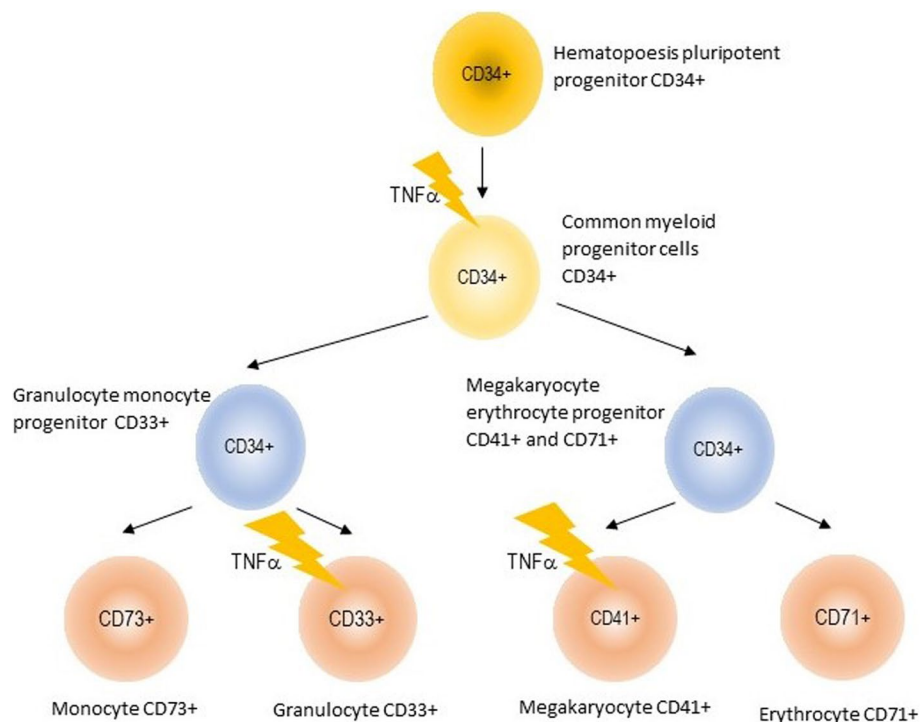
In Fig. 2C, caspase-3 activity in CD41+ in the MDS group exposed to rhTNF $\alpha$  was significantly higher compared to the group without exposure. This indicated that TNF $\alpha$  exposure to MDS megakaryocyte-platelet lineage cells leads to increased apoptosis. Cevic et al. [26], also observed that TNF $\alpha$  increased caspase-3 activity in platelets.

Apoptosis in megakaryocyte-platelets lineage of MDS can occur through the extrinsic pathway involving TNF $\alpha$ ,

but it predominantly takes place via the intrinsic pathway. This is supported by the finding of higher levels of Bax and Bak proteins [27], as well as the possibility of mitochondrial activation or the release of cytochrome C from the cytosol [28].

Following the maximum release of platelets (1000–3000 platelets), senescent megakaryocytes, characterized by a thin nucleus and cytoplasm, immediately undergo apoptosis and are phagocytosed by macrophages [29]. The apoptosis in MDS megakaryocytes occurs due to ineffective platelet production caused by the premature apoptosis of mature megakaryocytes in the intramedullary. Cytogenetic abnormalities in MDS clones primarily led to defects in megakaryocytes, manifesting in morphological abnormalities, and disturbances in nucleus differentiation and maturation. Moreover, these abnormalities are influenced by the MDS bone marrow microenvironment.

To address the hypothesis regarding whether apoptosis in MDS progenitor cell occurs during the early stage or at a differentiation stage, a post hoc analysis was performed to examine the apoptotic activity of caspase-3 in CD34+ cells, representing early progenitors, and in CD33+ and CD41+ cells, representing differentiated progenitors. This study demonstrates that exposure to



**Fig. 4** Scheme of Apoptosis Mechanism on MDS Hematopoiesis Progenitor Cell by TNF $\alpha$ . Early hematopoiesis progenitor cell express CD34+ surface marker. Then it will differentiate into specific myeloid progenitor (CD33+), megakaryocyte-thrombocyte progenitor (CD41+). This image illustrates that TNF $\alpha$  induces apoptosis in MDS hematopoiesis progenitor cell, especially to cell that have differentiated into myeloid CD33+ and megakaryocyte-thrombocyte CD41+

rhTNF $\alpha$  significantly increases caspase-3 activity, especially in hematopoietic progenitor cells that differentiate into the myeloid lineage CD33+ and the megakaryocyte-thrombocyte lineage CD41+, as opposed to early progenitor lineage CD34+, Fig. 4.

These findings shed light on why cytopenia in MDS is more pronounced than the increase in blast cells. Similar results were reported by Xia et al. [30], who examined apoptotic activity in MDS CD34+ (progenitor cells) and MDS CD34- (mature cells), and found higher apoptotic activity in mature cells. However, their study did not distinguish the specific stage of differentiation among mature cells.

These results contrast with the previous hypothesis, which suggested that apoptosis in MDS mainly occurs in early progenitor cells CD34+.

The reason MDS develops slowly but progressively lies in the fact that CD34+ MDS cells are long-term repopulating stem cells (LT-HSC) with a high self-renewal capacity but a slow proliferation rate. The MDS cell clone could persist for up to 17 years without clinically significant changes in bone marrow or peripheral blood. However, after prolonged periods, the bone marrow exhibits an 8.15% presence of dysplastic progenitor cells. These abnormal progenitor cells would significantly reduce the number of mature cells in the peripheral blood, leading to cytopenia [31].

This explanation helps clarify the typical phenomenon in MDS, where there is hypercellularity in the bone marrow but cytopenia in the peripheral blood. It suggests that apoptosis occurs mainly in differentiated progenitor cells rather than in early progenitor cells. Unlike other malignant cells that can further develop, MDS clonal cells fail to mature, leading to an accumulation of cells with increased apoptotic abilities.

## Conclusion

We have demonstrated that rhTNF $\alpha$ , a proapoptotic cytokine, plays a significant role in initiating apoptotic activity through caspase-3 in hematopoietic cells of MDS. This effect is particularly pronounced in cells that have already differentiated into myeloid lineage CD33+ and the megakaryocyte-thrombocyte lineage CD41+, as opposed to early progenitor cells CD34+. The results of this study have effectively substantiated the hypothesis that apoptosis predominantly takes place in differentiated progenitor cells, specifically in the myeloid and platelet lineages, as opposed to early progenitor cells in MDS hematopoiesis. This revised hypothesis differs from the initial one, which suggested that apoptosis mainly occurs in CD34+ early progenitor cells in MDS.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12860-023-00495-0>.

**Additional file 1: Supplementary Table 1** . Characteristics of The Study Subjects. **Supplementary Table 2** . Caspase-3 Activity in CD34+, CD33+ and CD41+ Cells Without exposure and exposure to rhTNF $\alpha$ . **Supplementary Table 3** . One Way Anova test and Post Hoc test to D Caspase-3 in CD34+, CD33+ and CD41+ Cells.

## Acknowledgements

Not applicable.

## Authors' contributions

AI, RDS, SBK AWS conceptualization and design the research; AI, AR, RDS, ARH, MKF collected samples, data analysis and interpretation; AR, RDS, SBK, AWS, MA, MKF critical revision and review manuscript. The authors read and approved the final manuscript.

## Funding

Part of this research was founded by the Research Development of Medical Science and Technology, Ministry of Health Republic of Indonesia no LB.02.01/1.1/4676.

## Availability of data and materials

The datasets used and/or analyzed during the current study are available in our institutionalized database and the Copy Right of this study has been deposited in the repository of Yarsi University, or please contact the corresponding author upon reasonable request.

## Declarations

### Ethics approval and consent to participate

This study has received ethical approval from the Ethics Committee of the Faculty of Medicine University of Indonesia No.673/UN2.F1/ETIK. This study was conducted in accordance with the declaration of Helsinki. Moreover, informed consent forms were duly signed by all participants.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

Received: 22 September 2022 Accepted: 8 November 2023

Published online: 21 November 2023

## References

- Tefferi A, Vardiman JW. Mechanisms of disease myelodysplastic syndromes. *N Engl J Med*. 2009;361:1872–85.
- Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–405.
- Ma X. Epidemiology of myelodysplastic syndromes. *Am J Med*. 2012;125:52.
- Germing U, Neukirchen J, Haas R. The epidemiology of myelodysplastic syndromes. *Clin Leuk*. 2008;2:34–8.
- Valent P, Horny HP, Bennett JM, Fonatsch C, Germing U, Greenberg P, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. *Leuk Res*. 2007;31:727–36.
- Bain BJ. *Leukemia diagnosis*. Wiley Blackwell; 2010.



7. Steensma DP, List AF. Genetic testing in the myelodysplastic syndromes: molecular insights into hematologic diversity. *Mayo Clin Proc.* 2005;80:681–98.
8. Aleshin A, Greenberg PL. Molecular pathophysiology of the myelodysplastic syndromes insight for targeted therapy. *Blood.* 2018;2(20):2787–97.
9. Comont T, Treiner E, Vergez F. From immune dysregulations to therapeutic perspectives in myelodysplastic syndromes: a review. *Diagnostic.* 2021;11(11):1982.
10. Bennett JM, Kouides PA, Forman SJ. The myelodysplastic syndromes morphology, risk assessment, and clinical management. *Int J Hematol.* 2002;76(Suppl 2):228–38.
11. Raza A, Gezer S, Mundle S, Gao X, Alvi S, Borok R, et al. Apoptosis in bone marrow biopsy samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. *Blood.* 1995;86(1):268–76.
12. Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, et al. He role of TNF- $\alpha$  in autoimmune disease and current TNF- $\alpha$  inhibitors in therapeutics. *Int J Mol Sci.* 2021;22(5):2719.
13. D'Silva S, Rajadhyaksha SB, Singh M. Immune dysregulation in MDS: the role of cytokines and immune cells. In: Fuchs O, editor. *Recent development in myelodysplastic syndromes.* Intechopen. 2019. p. 1–17.
14. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. *Blood.* 2019;133(10):1039–48.
15. Bain BJ, Bates I, Laffan MA, Lewis SM. *Dacie and Lewis practical haematology.* Elsevier Churchill Livingstone; 2012.
16. Gu C, Zhang J, Chen Y, Lei J. A trigger model of apoptosis induced by tumor necrosis factor signaling. *BMC Syst Biol.* 2011;5(Suppl 1):S13.
17. Webster JD, Vucic D. The balance of TNF mediated pathways regulates inflammatory cell death signaling in healthy and diseased tissues. *Front Cell Dev Biol.* 2020;8:365.
18. Loo GV, Bertrand MJM. Death by TNF a road to inflammation. *Nat Rev Immunol.* 2023;23:289–303.
19. Kerbauy DB, Deeg HJ. Apoptosis and anti apoptotic mechanisms in the progression of MDS. *Exp Hematol.* 2007;35:1739.
20. Ali A, Mundle SD, Ragasa D, Reza S, Shetty V, Mativi BY. Sequential activation of caspase 1 and caspase 3 like proteases during apoptosis in myelodysplastic syndromes. *J Hematol Stem Cell Res.* 1999;8(4):343–56.
21. Mundle SD, Reza S, Ali A, Mativi BY, Shetty V, Venugopal P, et al. Correlation of tumor necrosis factor  $\alpha$  (tnf $\alpha$ ) with high caspase 3 like activity in myelodysplastic syndromes. *Cancer Lett.* 1999;140(1–2):201–7.
22. Iriani A, Setiabudy RD, Kresno SB, Sudoyo AW, Bardosono S, Rachman A, et al. Expression of mRNA TNF $\alpha$  and level of protein TNF $\alpha$  after exposure sCD40L in bone marrow mononuclear cells of myelodysplastic syndromes. *Stem Cell Investig.* 2021;8(6):1–9.
23. Bachegonda L, Gligich O, Mantzaris I, Schinke C, Wyville D, Carrillo T, et al. Signal transduction inhibitors in treatment of myelodysplastic syndromes. *J Hematol Oncol.* 2013;6:1–11.
24. Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. *Blood.* 2000;96:3932–8.
25. Fozza C, Crobu V, Isoni MA, Dore F. The immune landscape of myelodysplastic syndromes. *Crit Rev Oncol Hematol.* 2016;107:90–9.
26. Cevic O, Adiguzel Z, Baykal AT, Sener A. TNF $\alpha$  induced caspase 3 activation related inos gene expression in adp activated platelet. *Turk J Biol.* 2017;41(1):31–41.
27. Josefsson EC, Vainchenker W, James C. Regulation of platelet production and life span: role of Bcl-xL and potential implications for human platelet diseases. *Int J Mol Sci.* 2020;165:1–12.
28. Kile BT. The role of apoptosis in megakaryocytes and platelet. *Br J Haematol.* 2014;165:217–26.
29. Radley JM, Haller CJ. Fate of senescent megakaryocyte in bone marrow. *Br J Haematol.* 1983;53:227–87.
30. Xia B, Guo Q, Zhao D, Zhao HF, Han XP, Wang H, et al. Significance of cd34- and cd34+ cell apoptosis and proliferation in bone marrow of patient with mds and the impact on survival. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* 2012;20(6):1392–7.
31. Walenda T, Stiehl T, Braun H, Frobel J, Ho AD, Schroeder T, et al. Feedback signals in myelodysplastic syndromes increased self renewal of the malignant clone suppresses normal hematopoiesis. *PLoS Comput Biol.* 2014;2014(4):1–9.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Ready to submit your research? Choose BMC and benefit from:**

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

**At BMC, research is always in progress.**

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

