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# Potential Diagnostic Value of Abnormal Pyroptosis Genes Expression in Myelodysplastic Syndromes (MDS): A Primary Observational Cohort Study

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**ABSTRACT**

**Background:** Myelodysplastic syndromes (MDS) are determined by ineffective hematopoiesis and bone marrow cytological dysplasia with somatic gene mutations and chromosomal abnormalities. Accumulating evidence has revealed the pivotal role of NLRP3 inflammasome activation and pyroptotic cell death in the pathogenesis of MDS. Although MDS can be diagnosed with a variety of morphologic and cytogenetic tests, most of these tests have limitations or problems in practice.

**Materials and Methods:** In the present study, we evaluated the expression of genes that form the inflammasome (*NLRP3, ASC, and CASP1*) in bone marrow specimens of MDS patients and compared the results with those of other leukemias to evaluate their diagnostic value for MDS.

Primary samples of this observational cohort study were collected from aspiration samples of patients with myelodysplastic syndromes (27 cases) and patients with non-myelodysplastic syndrome hematological cancers (45 cases). After RNA extraction and c.DNA synthesis, candidate transcripts and housekeeping transcripts were measured by real-time PCR method (SYBER Green assay). Using Kruskal-Wallis the relative gene expressions were compared and differences with *p* value less than 0.05 were considered as significant. Discrimination capability, cut-off, and area under curve (AUC) of all markers were analyzed with recessive operation curve (ROC) analysis.

**Results:** We found that Caspase-1 and ASC genes expressed at more levels in MDS specimens compared to non-MDS hematological malignancies. A relative average expression of 10.22 with *a p-value* of 0.001 and 1.86 with $p$=0.019 was detected for Caspase-1 and ASC, respectively. ROC curve analysis shows an AUC of 0.739 with $p$=0.0001 for Caspase-1 and an AUC of 0.665 with $p$=0.0139 for ASC to MDS discrimination.

**Conclusion:** Our results show that Caspase-1 and ASC gene expression levels can be used as potential biomarkers for MDS diagnosis. Prospective studies with large sample numbers are suggested.

**Keywords:** Myelodysplastic Syndromes; Inflammasome; Pyroptosis; *NLRP3*; *ASC*; *Casapase1*

## INTRODUCTION

Myelodysplastic Syndromes (MDS) are a group of clonal hematopoietic disorders that typically have features indicative of bone marrow failure such as inefficient hematopoiesis, morphological dysplasia and peripheral blood cytopenia[1]. The clinical phenotype is non-specific and can result in a variety conditions. The incidence of MDS and its mimics increases with age, making accurate diagnosis of the disease difficult. While MDS and associated disorders appear to have well-defined diagnostic boundaries, they are more difficult and vague to specify in practice[1]. In addition to the considerable heterogeneity of the disease, the diagnosis of myelodysplastic syndromes can be challenging due to the subjective assessments of cytological abnormalities, along with bone marrow aspirates with variable technical quality [2]. In addition, various hematological neoplasms such as chronic myelomonocytic leukemia (CMML), and benign conditions can show dysplastic abnormalities[3,4]. Apart from cytogenetic abnormalities, there are no specific and precise diagnostic biomarkers for MDS, and available methods have limitations and their pitfalls[5].

Recently, immunological disorders, particularly abnormalities of the innate immune system, have been shown to be important factors in the pathogenesis of MDS, distinguishing it from other hematological malignancies and similar benign diseases[6,7]. One of the mechanisms leading to critical progression and clonal expansion of hematopoietic stem cells in MDS is pyroptosis[8]. Activation of pattern recognition receptors (PRPs) by damage-associated molecular patterns (DAMPs) induces an inflammatory caspase-1-based cell death called pyroptosis. Pyroptotic cell death begins when an inflammasome complex forms by activating the cytosolic sensor of DAMPs, NLR family pyrin domain-containing 3 (NLRP3), and then leads to stimulation of an apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC). ASC then binds to pro-caspase-1, leading to its activation and cleavage to produce activated caspase-1[9,10]. Several cellular processes are involved in caspase-1 activation, including nuclear condensation, conversion of interleukin 1β (IL-1β), and IL-18 to their active forms, and pore formation which lead to the influx of cations and swelling of the cell[11]. Several studies have shown that MDS patients with genetic mutations can produce excessive reactive oxygen species (ROS), resulting in pyroptosis and activation of inflammasomes. There are also significant correlations between the number of genetic mutations and ROS, ASC, and IL-1β levels[6,12].

The purpose of this study was to evaluate the expression of pyroptosis genes *(NLRP3, ASC, CASP1)* in bone marrow aspirate samples from patients suspected of having MDS and comparing the results to other hematologic malignancies to investigate whether these genes are potential diagnostic biomarkers.

## MATERIALS AND METHODS

### Primary samples

During one year from 2020 to 2021, primary samples of this observational cohort study were collected from Seyed Al-Shohada University Hospital in Isfahan, comprising bone marrow (BM) aspiration samples of patients with myelodysplastic syndromes (Table 1), patients with non-myelodysplastic syndrome hematological cancers, including 11 patients with acute lymphocytic leukemia (ALL), 14 patients with acute myeloid leukemia (AML), 7 patients with chronic lymphocytic leukemia (CLL), 5 patients with chronic myeloid leukemia (CML), 6 patients with multiple myeloma (MM), and 2 patients with hairy cell leukemia (HCL). Myelodysplastic syndrome diagnosis was done by morphological assessment, according to the 2016 revision of the WHO Classification of Myelodysplastic Syndromes[13]. Other clinical pathological findings were obtained to support the diagnosis (Table 1).

### Sample preparation and RNA extraction

Samples received from the center and its mononuclear cells were extracted and immediately stored at -70 ° C. After confirming the diagnosis, total RNA was extracted from samples through RNA extraction kit (Rojet, RNjia phenol free pb kit, Iran) based on manufacturer's instruction. RNA integrity was assessed by gel electrophoresis and its concentration at 260 nm was evaluated. Purity was assessed by calculating of the ratio for absorbance at 260 nm versus 280 nm (A260 nm/A280 nm) by using a Nano Drop.

**cDNA synthesis and quantitative RT-PCR**

After RNA extraction, cDNA was synthesized by using a cDNA synthesis kit (Pars tous, Mashhad, Iran), based on producer instruction. Briefly: mixing of 10 µl RT Mix, 2 µl Mix enzyme and 8 µl sample RNA and using following program: preincubation for 10 minutes at 25 °C followed by 60 minutes at 47 °C and 5 minutes at 85 °C. Finally, reaction was continued for 10 minutes at 4 °C and the synthesized cDNA was kept at -70 °C.

For real-time PCR method, SYBER Green master mix kit (Pars tous, Mashhad, Iran) and AB Step 1 real time PCR device (Applied Biosystems, USA) were used. The total reaction volume was 25 µl, including 12.5 µl Master mix, 1.25 µl Forward primer (Table 2), 1.25 µl Reverse primer (Table 2), 2.5 µl Template cDNA and 7.5 µl desalted water. To evaluate the reproducibility, real-time experiments were performed in duplicate. The temperature changes of the amplification reaction were followed as: starting step 15 minutes at 95 °C on time and then, 40 cycles of 20 seconds at 95 °C, 30 seconds at 60 °C, 30 seconds at 72 °C and as the final step, melting curve was drawn. PCR product sample sizes were evaluated by 2% agarose gel electrophoresis.

**Statistical analysis**

Data analysis was performed using SPSS 26. Kolmogorov-Smirnov and Shapiro tests were carried out to determine data distribution normality. Using Kruskal-Wallis test non-parametric data were compared and differences with p value less than 0.05 were considered as significant. Discrimination capability, cut-off, and area under curve (AUC) of all markers were analyzed with recessive operation curve (ROC) analysis.

**Table 1:** Basic characteristics of patients with myelodysplastic syndromes (MDS)

| **MDS patients(n=27)** | |
|---|---|
| MDS-SLD*; 5<br>MDS-MLD; 8<br>MDS-RS; 2<br>MDS-EB-1; 2<br>MDS-EB-2; 3<br>MDS-U; 7 | |
| Age range (y) | 70 ± 11 |
| Sex | Males (67%) – female (33%) |
| White blood cells/µl | 3962 (900 - 8500) ± 2399 |
| Red blood cell count/µl | 3687 (2240 - 5100) ± 1006 |
| Absolute neutrophil count/µl | 2648 (234 - 2800) ± 2134 |
| Platelets count/µl | 88664 (25000 - 275000) ±88143 |
| Hemoglobin (g/dl) | 8.72 (5.0 -12.8) ± 1.9 |

*SLD=single lineage dysplasia; MLD=multi lineage dysplasia; RS= ring sidroblast; U=unclassifiable

**Table 2:** Sequences of primers used for direction Real time PCR and PCR product size lengths

| | | |
|---|---|---|
| **NLRP3. Forward** | **5′-CATGAGTGCTGCTTCGACAT-3′** | **121 bp** |
| NLRP3. Reverse | 5′-GCTTCAGTCCCACACACAGA-3′ | |
| ASC. Forward | 5′-GGCTGCTGGATGCTCTGTA-3′ | 114 bp |
| ASC. Reverse | 5′-AGGCTGGTGTGAAACTGAAGA-3′ | |
| Caspase-1. Forward | 5′-TTATTGGAAGACTCATTGAAC-3′ | 160 bp |

## RESULTS

### The expression level alterations of *NLRP3*, *ASC* and *CASP1*

Real-time PCR outputs for the expression of *NLRP3, ASC and CASP1* genes against *GAPDH* reference gene were collected. Data were analyzed by Livak and Pfaffl and T-test statistical methods and the RFCs (Relative Fold Change) of all the three genes were calculated.

The expression level of the *NLRP3* gene was lower than that of the control group but insignificant (Relative average expression = 0.68, p=0.071, Figure 1A). The expression level of *ASC* gene, was higher than control group (but not so high) and significant (Relative average expression = 1.86, p=0.019, Figure 1B). The expression level of *CASP1* gene in patients with MDS was significantly higher compared to the control group (Relative average expression = 10.22, p=0.001, Figure 1C).

### The potential of *ASC, and Caspase1* as discriminating biomarkers

To determine whether the expression of the *ASC* and *CASP1* genes could be reliable biomarkers in the diagnosis of MDS, ROC-curve analysis was applied and the area under curve (AUC) determined (Figure 2A and 2B). Due to insignificant difference in expression of *NLRP3* between two groups, ROC-curve analysis was not applied for this transcript.

The area under the curve (AUC) was 0.739 (p=0.0001) and 0.665 (p=0.0139) for *CASP1* and *ASC* respectively. ROC outputs characterized the optimal relative expression cut-off point 1.61 for *CASP1* with 85.19% sensitivity and 63.83% specificity (57.5% PPV,88.2% NPV) and for *ASC* gene the cut-off point value was 1.68 with 70.37% sensitivity and 70.21% specificity (57.6% PPV,80.5% NPV) (Table3).

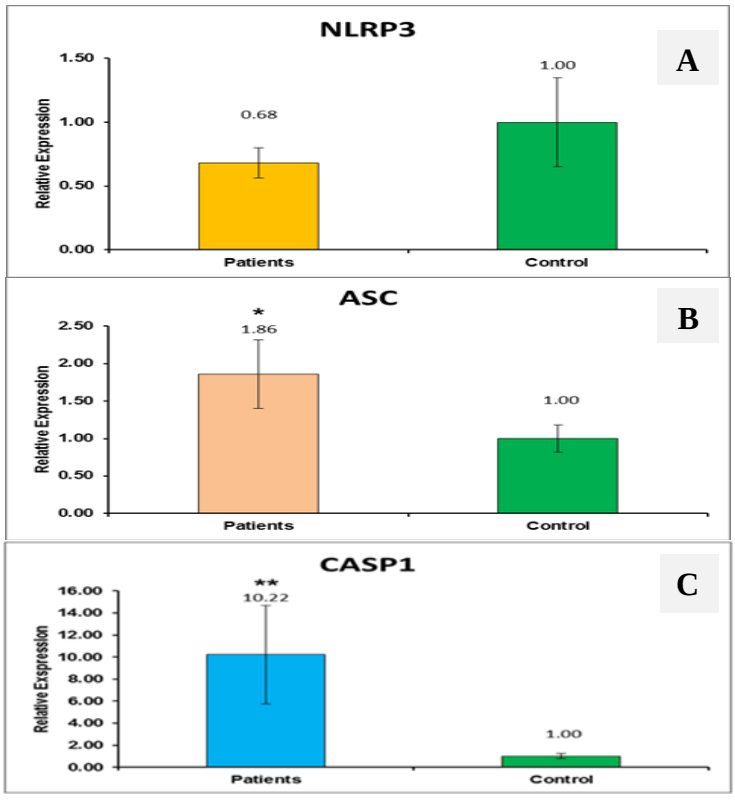


Figure 1. The relative expression of genes related to pyroptosis cell death (*ASC*, *NLRP3*, and *CASP1*) in patients with MDS compared to the control group. The gene expression level of *NLRP3* **(A),** *ASC* **(B)** and *CASP1* (C) in the bone marrow (BM) aspiration samples of 27 MDS patients (N = 27) and the control group (N = 45) including 11 patients with acute lymphocytic leukemia (ALL), 14 patients with acute myeloid leukemia (AML), 7 patients with chronic lymphocytic leukemia (CLL), 5 patients with chronic myeloid leukemia (CML), 6 patients with multiple myeloma (MM), and 2 patients with hairy cell leukemia (HCL). Asterisks represent for statistical significance (* means p<0.05 and ** means p<0.001).

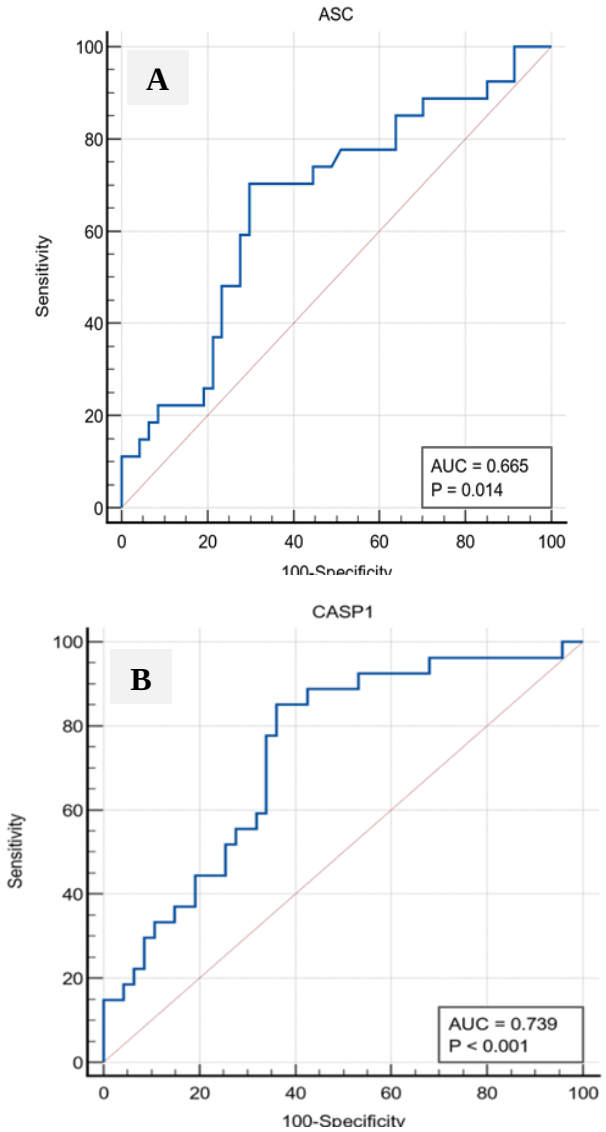


Figure 2. ROC curves of sample sets analyzed for relative expression levels of *ASC* **(A)** and *CASP1* **(B)**. Area under curve, cut-off value, sensitivity and specificity for all studied genes are shown.

**Table (3):** Cut-off point analysis

| Biomarker | Cut-off point (RFC) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| Caspase-1 | >1.61 | 85.19 | 63.83 | 57.5 | 88.2 |
| ASC | >1.68 | 70.37 | 70.21 | 57.6 | 80.5 |

Control vs MDS. AUC: Area under the curve; STD. Error: Standard Error; C.I.: Confidence Interval.PPV: Positive Predictive Value; NPV: Negative Predictive Value; RFC: Relative fold change

**DISCUSSION**

MDS diagnosis is one of the main challenges in clinical hematology. It is largely based on the morphological examination of blood and bone marrow samples. It has been elucidated that the recognition of dysplastic changes varies significantly between observers, even among experienced hematopathologists[4]. Cytogenetic analysis plays a very important role in diagnosis, however, only half of patients have MDS-type karyotype abnormalities[14]. Furthermore, Cytogenetic analysis is a time-consuming process, especially for leukemia cell with a low proliferation index such as MDS leukemia cells. Therefore, this technique does not provide a quick and comprehensive diagnosis. Compared to cytogenetic analysis, fluorescence in situ hybridization (FISH) is a more sensitive and faster technique, but it brings no additional benefit to the karyotype[15]. Some studies have shown promising results for flowcytometry[16,17]. However, its limitations should be addressed and incorporated; Demand for the use of multiple antibody combinations and concerns of high cost, technical complexity and need for experienced analysts and finally there are reactive situations that mimic MDS-like immunophenotype abnormalities. Flow cytometry is recommended by the WHO guideline as an ancillary but not confirmatory test[18]. With the advancement of high-throughput technologies such as next generation sequencing (NGS), our knowledge of the molecular pathology of myeloid malignancies has grown remarkably [19]. In fact, most MDS patients (90%) have at least one somatic mutation, but, these mutations are also detected in a group of closely related disorders such as clonal cytopenia of undetermined significance (CCUS) and idiopathic cytopenia of undetermined significance (ICUS)[20]. Currently, NGS testing play only a limited role in the definitive diagnosis of MDS. Recently, increased attention has been paid to abnormal blood count parameters derived from new automated counters as they offer a simple, rapid, and inexpensive approach to screening for MDS[21,22]. Although, new tools are now being developed to improve MDS diagnosis, the distinction remains difficult[5]. Overall, identifying simple, rapid, and comprehensive diagnostic biomarkers is the focus of MDS studies. The results of recent studies [23, 24] and their attention to the role of immune system mechanisms, including activation of inflammasome, in the pathogenesis of MDS prompted us to search for immune-related biomarkers for better and faster diagnosis of this disease.

Although the association between inflammation and MDS has been known for a long time, understanding of the specific role of inflammation and mechanisms of the innate immune system in MDS pathogenesis, particularly pyroptosis, has only recently been recognized. Overall, pyroptosis as a pathologic cellular mechanism can lead to critical progression and clonal expansion of hematopoietic stem cells in MDS patients by activating the NLRP3-inflammasome cascade[25]. Reactive oxygen species (ROS) production, activation of redox-sensitive NLRP3 inflammasome and β-catenin are triggered by the alarmin S100A9, resulting in pyroptotic cell death and proliferation of MDS clones. According to recent findings, the pro-inflammatory environment is associated with PRR and DAMPs signaling, changes in the BM microenvironment, genetic mutations, activated inflammasomes, and the proliferation of hematopoietic stem and progenitor cells[(26-28)]. Clarifying the role of pyroptosis in the pathogenesis of MDS may lead to the identification of new diagnostic and therapeutic strategies that hold promise for the treatment of this disease[9]. The NLRP3-inflammasome complex also plays a central role in normal hematopoiesis and proliferation of CD34+ hematopoietic stem cells[29,30].

We hypothesized that high expression of *NLRP3*, *ASC*, and *CASP1* might correlate with MDS development and measurement of these genes may provide valuable diagnostic biomarkers for MDS patients compared to other hematological malignancies. The data collected after our experiments showed a significant increase of *CASP1* expression in the MDS group compared to the control group (p=0.001). However, the significance of the difference in *ASC* expression between case and control groups was less than what we observed for *caspase-1* (p=0.019), and there was also no significant variation in *NLRP3* gene expression between two groups (p=0.07). Our group also compared the diagnostic value of abnormal

expression of these genes involved in MDS pathogenesis. Based on the ROC curves, *caspase-1* has shown promising results as a more valuable diagnostic biomarker for MDS discrimination (AUC=0.739).

MDS hematopoietic stem and progenitor cells (HSPCs) undergo activation of NLRP3 complexes and overexpression of inflammasome proteins, leading to caspase-1 activation and production of IL-1β, and IL-18, and pyroptotic cell death, regardless of disease genotype[7, 9]. Several studies have demonstrated the importance of overexpression of the *CASP1* and *ASC* genes in MDS progression. The exact molecular mechanisms of altered gene expression are not clear. However, epigenetic changes play an important role in the abnormal gene expression associated with MDS[31, 32]. An investigation by Grace A. Ward et al. have indicated that oxidized mitochondrial DNA (ox-mtDNA) is significantly increased in peripheral blood plasma of MDS patients, which is due to greater activity of the inflammasome complex. In addition, Ox-mtDNA may be a biomarker of pyroptosis in MDS associated with S100A8, S100A9 alarmins and circulating ASC specks. In their study, another experiment using immortalized bone marrow-derived mononuclear stem cells (BM-MNC) from murine models with common somatic MDS gene mutations found that caspase-1 activity was 2 to 4.5 times higher in this group than in wild-type cells from control mice[33]. Basiorka et al. have reported that ASC specks detected in peripheral blood plasma could serve as a biologically meaningful biomarker for MDS[12]. We obtained similar results for *caspase-1* and *ASC* as potential biomarkers for MDS diagnosis.

Another study by Basiorka et al. showed significant upregulation of inflammasome transcripts such as *caspase-1* and *NLRP3* in MDS BM-MNC samples compared to age-matched normal controls. However, mRNA levels of *caspase-3* as a canonical apoptotic caspase were the same as normal BM-MNC. This evidence could demonstrate the importance of pyroptosis versus apoptosis in MDS pathogenesis. Furthermore, in this study the levels of pro-IL-1β, activated IL-1β and ASC monomers increased in MDS BM-MNC compared to normal specimens[11]. In our study, the increased expression of the *CASP1* and *ASC* genes is consistent with Basiorka findings and can confirm their results. However, in the case of the *NLRP3* gene, there is a controversy that could be due to differences in the control groups. Our control group included patients with non-MDS hematological malignancies, however, the control group in the above study included normal subjects. This could indicate that *NLRP3* gene expression may elevated not only in MDS but also in other hematological malignancies.

Our group recently showed the significance of innate immune abnormalities as potential diagnostic biomarkers for MDS[34]. However, further experiments and clinical studies are needed to confirm these findings.

## CONCLUSION

In conclusion, our study shows that caspase-1 and ASC transcript levels have diagnostic potentials for MDS. In present study, the control arm included only 6 types of hematological malignancies, which had a limited number and an uneven distribution of cases. A more comprehensive study, including all leukemias with a sufficient number of patients, is needed to get promising results. Due to the lack of comparable results associated with MDS, we hope that these findings will be explored by other investigators. It is also recommended that these biomarkers be examined in peripheral blood samples from MDS patients.

## Consent to participate

Informed consents was given from all participated patients.

## CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

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## Ethical Approval Code:

IR.MUI.MED.REC.1399.076

**REFERENCES**

1. Tanaka TN, Bejar R. MDS overlap disorders and diagnostic boundaries. Blood. 2019;133(10):1086-95.
2. Bejar R. Myelodysplastic Syndromes Diagnosis: What Is the Role of Molecular Testing? Curr Hematol Malig Rep. 2015;10(3):282-91.
3. Carbuccia N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. Leukemia. 2009;23(11):2183-6.
4. Font P, Loscertales J, Benavente C, et al. Inter-observer variance with the diagnosis of myelodysplastic syndromes (MDS) following the 2008 WHO classification. Ann Hematol. 2013;92(1):19-24.
5. Mufti GJ, McLornan DP, van de Loosdrecht AA, et al. Diagnostic algorithm for lower-risk myelodysplastic syndromes. Leukemia. 2018;32(8):1679-96.
6. Sallman DA, List A. The central role of inflammatory signaling in the pathogenesis of myelodysplastic syndromes. Blood. 2019;133(10):1039-48.
7. Trowbridge JJ, Starczynowski DT. Innate immune pathways and inflammation in hematopoietic aging, clonal hematopoiesis, and MDS. J Exp Med. 2021;218(7): e20201544.
8. Lin Y, Gao QP, Ye BX. Research Progress on the Role of Pyroptosis in the Pathogenesis of Myelodysplastic Syndrome -Review. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2018;26(3):937-41.
9. Sallman DA, Cluzeau T, Basiorka AA, et al. Unraveling the Pathogenesis of MDS: The NLRP3 Inflammasome and Pyroptosis Drive the MDS Phenotype. Front Oncol. 2016;6:151.
10. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 2016;16(7):407-20.
11. Basiorka AA, McGraw KL, Eksioglu EA, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. Blood. 2016;128(25):2960-75.
12. Basiorka AA, McGraw KL, Abbas-Aghababazadeh F, et al. Assessment of ASC specks as a putative biomarker of pyroptosis in myelodysplastic syndromes: an observational cohort study. Lancet Haematol. 2018;5(9):e393-e402.
13. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
14. Haase D. Cytogenetic features in myelodysplastic syndromes. Ann Hematol. 2008;87(7):515-26.
15. Van De Loosdrecht AA, Alhan C, Béné MC, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica. 2009;94(8):1124-34.
16. Westers TM, Ireland R, Kern W, et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia. 2012;26(7):1730-41.
17. van de Loosdrecht AA, Westers TM. Cutting edge: flow cytometry in myelodysplastic syndromes. J Natl Compr Canc Netw. 2013;11(7):892-902.
18. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022;36(7):1703-19.
19. Sanz GF, Ibañez M, Such E. Do next-generation sequencing results drive diagnostic and therapeutic decisions in MDS? Blood Adv. 2019;3(21):3454-60.
20. Duncavage EJ, Schroeder MC, O'Laughlin M, et al. Genome Sequencing as an Alternative to Cytogenetic Analysis in Myeloid Cancers. N Engl J Med. 2021;384(10):924-35.
21. Pozdnyakova O, Niculescu RS, Kroll T, et al. Beyond the routine CBC: machine learning and statistical analyses identify research CBC parameter associations with myelodysplastic syndromes and specific underlying pathogenic variants. J Clin Pathol. 2023;76(9):624-631.
22. Ravalet N, Foucault A, Picou F, et al. Automated Early Detection of Myelodysplastic Syndrome within the General Population Using the Research Parameters of Beckman-Coulter DxH 800 Hematology Analyzer. Cancers (Basel). 2021;13(3):389.
23. Graf JR, Forster S, Bruehl FK, et al. Diagnostic and Prognostic Implications of Caspase-1 and PD-L1 Co-Expression Patterns in Myelodysplastic Syndromes. Cancers (Basel). 2021;13(22):5712.
24. Lyu C, Liu K, Jiang Y, et al. Integrated analysis on mRNA microarray and microRNA microarray to screen immune-related biomarkers and pathways in myelodysplastic syndrome. Hematology. 2021;26(1):417-31.
25. Basiorka A, McGraw K, Eksioglu E, et al. Activation of redox-sensitive inflammasomes underlies the biological phenotype of myelodysplastic syndromes. Leuk Res. 2015;39(1):S14.
26. Balandran JC, Lasry A, Aifantis I. The Role of Inflammation in the Initiation and Progression of Myeloid Neoplasms. Blood Cancer Discov. 2023; 4(4):254-266.
27. Hemmati S, Haque T, Gritsman K. Inflammatory Signaling Pathways in Preleukemic and Leukemic Stem Cells. Front Oncol. 2017;7:265.

28. Mitroulis I, Kalafati L, Bornhauser M, et al. Regulation of the Bone Marrow Niche by Inflammation. Front Immunol. 2020;11:1540.
29. Ratajczak MZ, Bujko K, Cymer M, et al. The Nlrp3 inflammasome as a "rising star" in studies of normal and malignant hematopoiesis. Leukemia. 2020;34(6):1512-23.
30. Dadkhah M, Sharifi M, Sharifi MJ, et al. Effects of glucose on the proliferation of human umbilical cord blood hematopoietic stem cells. Cell Tissue Bank. 2023;24(2):485-94.
31. Issa JP. Epigenetic changes in the myelodysplastic syndrome. Hematol Oncol Clin North Am. 2010;24(2):317-30.
32. Sharifi MJ, Zaker F, Nasiri N, et al. Epigenetic changes in FOXO3 and CHEK2 genes and their correlation with clinicopathological findings in myelodysplastic syndromes. Hematol Oncol Stem Cell Ther. 2020;13(4):214-9.
33. Ward GA, McGraw KL, Abbas-Aghababazadeh F, et al. Oxidized mitochondrial DNA released after inflammasome activation is a disease biomarker for myelodysplastic syndromes. Blood Adv. 2021;5(8):2216-28.
34. Khalilian P, Eskandari N, Sharifi MJ, et al. Toll-Like Receptor 4, 2, and Interleukin 1 Receptor Associated Kinase4: Possible Diagnostic Biomarkers in Myelodysplastic Syndrome Patients. Adv Biomed Res. 2024;13:17.