Study of proinflammatory and anti-inflammatory states in myelodysplastic syndrome patients

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Received 4 March 2019
Accepted 5 May 2019
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:733–740

Background
The myelodysplastic syndrome (MDS) are a group of clonal bone marrow neoplasms characterized by ineffective hematopoiesis, manifested by morphologic dysplasia in hematopoietic cells and by peripheral cytopenia(s) although previous studies have shown cytogenetic and molecular abnormalities, the underlying defect in the molecular pathway for inflammation milieu, extensive apoptosis, and dysplasia observed in the disease is yet to be studied.
Aim of the work
The aim of this study was to show the proinflammatory [tumor necrosis factor-α (TNF-α)/anti-inflammatory [interleukin-10 (IL-10)] balance in different subclassifications of MDS.
Patients and methods
From September 2017 through September 2018, serum levels were measured in 49 patients for TNF-α, IL-10 in patients diagnosed as having MDS. Also, these inflammatory cytokines had been measured in 46 apparently healthy participants as matched controls for the study. The diagnosis of MDS was confirmed by a hematopathologist after review of bone marrow aspiration and/or peripheral blood samples. Conventional cytogenetic studies were performed on bone marrow aspirate material using standard G-banding techniques. These patients were then subclassified according to the revised 2016 WHO classification for MDS.
Results
There is a statically significant difference between MDS patients and control group according to the results of serum level of TNF-α and IL-10. They were higher in MDS patients. Also, there was a statically significant difference between the subclassified groups of MDS patients according the results of serum level of TNF-α and IL-10. TNF-α was higher in MDS with multilineage dysplasia and MDS unclassifiable than the others. Also, IL-10 was higher in MDS with excess blasts 1 and MDS with excess blasts 2 than the others.
Conclusion
TNF-α and IL-10 are increased in MDS patients indicating an inflammatory disturbance. TNF-α and IL-10 serum level are inversely related to each other in the different subclasses of MDS.

Keywords:
cytokines, inflammation, interleukin-10, myelodysplastic syndrome, tumor necrosis factor-α

Egypt J Intern Med 31:733–740

1110-7782

Introduction

The myelodysplastic syndrome (MDS) are a group of clonal bone marrow (BM) neoplasms characterized by ineffective hematopoiesis, manifested by morphologic dysplasia in hematopoietic cells and by peripheral cytopenia(s) [1].

The revised classification introduces refinements in morphological interpretation and cytopenia assessment and addresses the influence of rapidly accumulating genetic information in MDS diagnosis and classification [1].

Cytopenia is a 'sine qua non' for any MDS diagnosis and in previous classifications, MDS nomenclature included references to 'cytopenia' or to specific types of cytopenia (e.g. 'refractory anemia') [2].

However, the WHO classification relies mainly on the degree of dysplasia and blast percentages for disease classification and specific cytopenias have only minor impact on MDS classification. Moreover, the lineage (s) manifesting significant morphologic dysplasia frequently do not correlate with the specific cytopenia(s) in individual MDS cases [3]. For these reasons, the terminology for adult MDS has changed to remove terms such as 'refractory anemia' and 'refractory cytopenia' and replaces them with 'myelodysplastic syndrome' followed by appropriate modifiers: single

versus multilineage dysplasia, ring sideroblasts, excess blasts, or the del (5q) cytogenetic abnormality [4].

Patients suffering from MDS clinically present with symptoms caused by cytopenia, that is, fatigue due to anemia, infections due to neutropenia, and bleeding due to a low platelet count. Many are dependent on blood transfusions at the time of diagnosis. Before diagnosis of MDS, other causes of dysplastic hematopoiesis need to be excluded as dysplastic hematopoiesis may also be seen in transitory reactive conditions [5].

Morphology (BM blast count, number of hematopoietic lineages affected), and cytogenetic analysis revealing risk defining cytogenetic aberrations allow classification into low-risk, intermediate (I or II) and high-risk disease with estimated survival differences between more than 5 years (low risk) and less than 1 year (high risk) according to the 'International Prognostic Scoring Systems' and the 'WHO adapted Prognostic Scoring System' [6].

Although most of the symptom burden in MDS stems from marrow failure and its associated cytopenias, it is not uncommon for patients with MDS to present with autoimmune and inflammatory conditions [7].

Autoimmune and inflammatory conditions can appear before, during, or after the diagnosis of MDS. Observational studies suggest that chronic immune stimulation can promote the development of myeloid malignancies [8].

MDS is frequently associated with immune dysregulation. Coexistence with autoimmune diseases, impaired cellular immunity, and abnormal secretion of cytokines have been reported [9].

The polymorphisms of tumor growth factor-β1, interferon-γ, tumor necrosis factor- α (TNF-α), and interkleukin-10 (IL-10) may account for the propensity to immune-mediated killing of hematopoietic stem cells and/or ineffective hematopoiesis characteristic of aplastic anemia and MDS [10].

Low-risk MDS is dominated by proinflammatory cells, while immunosuppressive cells (regulatory T cells and myeloid-derived suppressor cells) [11,12] are more important in high-risk disease where they possibly provide immune evasion for MDS blasts and aid in transformation to AML. The bone marrow environment is important for normal maturation of bone marrow progenitor cells, and signaling through direct cell contact, as well as soluble mediators, are both necessary for correct maturation of progenitor cells [13,14]. Thus, investigation of systemic (i.e. serum or plasma levels) levels of soluble mediators may reveal information regarding the pathophysiology in MDS, for example immunoregulation, as well as regulation of hematopoiesis [15].

IL-10, a multifunctional cytokine with both immunosuppressive and antiangiogenic properties, is involved in the pathogenesis of many cancers. Increased serum levels of IL-10 are found in patients with some cancers [16].

Patients and methods

Patients

From September 2017 through September 2018, serum levels were measured in 49 patients for TNF-α, IL-10 in patients diagnosed as having MDS. Also, these inflammatory cytokines had been measured in 46 apparently healthy participants as matched controls for the study.

The diagnosis of MDS was confirmed by a hematopathologist after review of bone marrow aspiration and/or peripheral blood samples. Conventional cytogenetic studies were performed on BM aspirate material using standard G-banding techniques. These patients were then subclassified according to the revised 2016 WHO classification for MDS [1].

The diagnosis of MDS was made by history taking, physical examination, investigations including complete blood count (hemoglobin level, white blood cell count, absolute neutrophil count, platelet count), blood film (reticulocytes and percentage of blasts), lactate dehydrogenase (LDH), erythrocyte sedimentation rate (ESR), serum ferritin level, BM aspiration (to assess cellularity, dysplastic changes, and blast cells percentage), trephine bone marrow biopsy with the reticulin stain to assess the grade of fibrosis and cytogenetic analysis by fluorescence in situ hybridization and karyotyping cases and were then classified.

Exclusion criteria

Other causes of peripheral blood and BM cytopenia and dysplasia such as vitamin B12 and folic acid deficiency, infections, hematological malignancies, and drug intake were excluded.

Healthy volunteers who had no fever for at least 1 week before testing, had taken no medications, were not pregnant, and who were free from known chronic or acute disease were served as normal controls. Some of these controls were used in previous studies.

Methods

The patients were subjected to full clinical assessment including full history taking and complete clinical examinations, supplemented by targeted laboratory assessment including complete blood count with blood film for detecting hemoglobin and hematocrit level, white blood cell and platelet counts, percentage of blasts using Sysmex XT-1800i automated hematology analyzer (Sysmex, Kobe, Japan), reticulocyte count by brilliant cresyl blue stain, and LDH levels were done using Cobas c501 AutoAnalyzer (Roche, stutgart, Germany), serum ferritin level using Cobas e601 Auto analyzer (Roche), ESR, BM aspiration (to assess cellularity, dysplastic changes, and blast cells percentage), trephine BM biopsy with the reticulin stain to assess the grade of fibrosis and cytogenetic analysis using karyotyping and fluorescence in situ hybridization techniques.

Cytokine level measurements

Serum levels of IL-10 was measured using a commercially available enzyme-linked immunoadsorbent assay ELISA kit (Diaclone Research, Besancon, France) and TNF-α level was assessed by ELISA kits that were purchased from R&D Systems (Minneapolis, Minnesota, USA) according to the manufacturer's instructions with detection limit of up to 3 and 6.23 pg/ml for IL-10 and TNF-α, respectively. Briefly, 50 µl of standards and samples were added in duplicate to the precoated strip well plates; then, 50 µl of biotinylated antibody was added to each as a conjugated antibody for 2 h and then washed three times. A measure of 100 µl of streptavidin horseradish peroxidase was also added to each well for 30 min, followed by 100 µl of TMB substrate solution for 30 min. Finally, 100 µl of stop solution was added to the well and the absorbance of the plate was detected on a plate reader at 450 nm. All recordings were done at room temperature. Samples were measured in duplicate, and all values were expressed as the mean of the two determinations. A standard curve was created using the known concentrations of the respective recombinant cytokine. The concentration of each was then determined using its own standard curve.

All cytokine measurements were performed on frozen samples over a period of a few weeks with kits that were purchased simultaneously.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with Statistical Package of Social Sciences, version 22 and Epi Info 2000 programs (SPSS Inc., Chicago, Illinois, USA), where the following statistics were applied.

Types of statistics

Descriptive statistics, for example number, percentage, mean, and SD.

Arithmetic mean was used as a measure of central tendency. SD was used as a measure of dispersion and percentage.

(1) χ^2-test was used to measure the association between qualitative variables.
(2) Fisher's exact test was used for 2×2 qualitative variables when more than 25% of the cells have an expected count of less than 5.
(3) Mann–Whitney test was used to compare mean and SD of two sets of quantitative data when this data is not normally distributed.
(4) Spearman's correlation was used to study the correlation between two variables when this data is not normally distributed.
(5) The*P* value is considered statistically significant when it is less than 0.05.

Results

Table 1 represents the demographic data of the studied groups.

The demographic data of the participants were in MDS patients' age of 46.2±16.3 years ranging from 16 to 85 years. There were 18 (36.7%) men and 31 (63.3%) women. There was no statically significant difference with the control group.

Table 2 shows the comparison between cases and controls according to the serum level of TNF-α and IL-10.

From this table, there is a statically significant difference between MDS patients and control group

Table 1 Demographic data of the studied groups

	Patients (*n*=49)	Control (*n*=46)	Test	*P* value
Age (years)				
Mean±SD	46.2±16.3	49.4±8.3	1.3[a]	0.18
Range	16–85	2–65		
Sex [*n* (%)]			0.005[b]	0.98
Male	18 (36.7)	17 (37.0)		
Female	31 (63.3)	29 (63.0)		

[a]Mann–Whitney *U*-test. [b]χ^2-test.

Table 2 Comparison between cases and controls according to the serum level of tumor necrosis factor-α and interleukin-10

	Patients (*n*=49)	Control (*n*=46)	*U*[a]	*P* value
TNF-α (pg/ml)				
Mean±SD	36.012±16.9	8.2±4.3	7.9	0.001
Median	33.4	7.1		
Range	11–67.0	4–19		
IL-10 (pg/ml)			8.3	0.001
Mean±SD	76.9±56.8	1.9±0.87		
Median	50.0	2.0		
Range	15–171	0.2–5.0		

IL-10, interleukin-10; TNF-α, tumor necrosis factor.
[a]Mann–Whitney *U*-test.

according to the results of serum level of TNF-α and IL-10. They were higher in MDS patients.

Table 3 shows the comparison of other laboratory investigations in MDS cases and controls.

According to other investigations there were statically significant differences in hemoglobin level, platelets count, serum LDH, ESR, and serum ferritin levels between MDS patients and controls, being higher in the later three and lower in the former two.

Table 4 represents the demographic data in different subclassified MDS cases.

Table 3 Comparison of other laboratory investigations in myelodysplastic syndrome cases and controls

	Patients (*n*=49)	Control (*n*=46)	*U*[a]	*P* value
Hb			7.3	0.001
Mean±SD	8.1±2.1	12.9±2.1		
Median	8.0	13.8		
Range	4.0–13.0	9.0–16.0		
Total leukocytes			1.5	0.117
Mean±SD	6.8±7.01	5.8±1.7		
Median	3.6	5.6		
Range	0.3–34.0	3.2–11.1		
Platelets			4.7	0.001
Mean±SD	123.6±126.9	204.4±42.7		
Median	87	198		
Range	10–550	129–381		
ESR			8.061	0.001
Mean±SD	88.3±33.7	11.7±6.6		
Median	90	10		
Range	9–150	2–32		
LDH			8.3	0.001
Mean±SD	477.1±230.6	144.6±11		
Median	460	147		
Range	152–1200	120–162		
Ferritin			8.1	0.001
Mean±SD	754.9±486.2	77.6±41.3		
Median	640	85		
Range	7–3000	4.5–155		

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; LDH, lactate dehydrogenase. [a]Mann–Whitney *U*-test.

Table 4 Demographic data in different subclassified myelodysplastic syndrome cases

	MDS with multilineage dysplasia (MDS-MLD) (*n*=22)	MDS with single linage dysplasia (MDS-SLD) (*n*=9)	MDS with excess blasts 1 (MDS-EB1) (*n*=10)	MDS with excess blasts 2 (MDS-EB2) (*n*=5)	MDS unclassifiable (MDS-U) (*n*=3)	Test	*P* value
Age (years)						4.2*	0.378
Mean ±SD	54.4±16.01	61.6±10.9	53.2±22.4	66.0±6.5	47.7±19.9		
Range	30–84	47–82	16–85	55–70	26–65		
Sex [*n* (%)]						3.4**	0.491
Male	7 (31.8)	4 (44.4)	4 (40.0)	3 (60.0)	0 (0.0)		
Female	15 (68.2)	5 (55.6)	6 (60.0)	2 (40.0)	3 (100.0)		

MDS, myelodysplastic syndrome; MDS-EB1, MDS with excess blasts 1; MDS-EB2, MDS with excess blasts 2; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single-lineage dysplasia; MDS-U, MDS unclassifiable. [a]Kruskal–Wallis test. [b]χ^2-test.

Table 5 Serum levels of tumor necrosis factor-α and interleukin-10 in different subclassifications of myelodysplastic syndrome

	MDS with multilineage dysplasia (MDS-MLD) (n=22)	MDS with single lineage dysplasia (MDS-SLD) (n=9)	MDS with excess blasts 1 (MDS-EB1) (n=10)	MDS with excess blasts 2 (MDS-EB2) (n=5)	MDS unclassifiable (MDS-U) (n=3)	K^a	P value	Post-hoc P value
TNF-α (pg/ml)								P_1=0.001P_2=0.001P_3=0.001P_4=0.723P_5=0.026P_6=1.00P_7=0.001P_8=0.030P_9=0.001P_{10}=0.001
Mean ±SD	51.3±8.3	24.2±6.22	15.4±3.7	23.8±3.7	48.4±0.65	38.9	0.001	
Median	54.0	22.0	15.3	24.0	48.8			
Range	33.0–67.0	18–35	11–22	19–29	47.7–49			
IL-10						38.1	0.001	P_1=0.001P_2=0.001P_3=0.001P_4=0.7981P_5=0.001P_6=0.001P_7=0.025P_8=0.815P_9=0.001P_{10}=0.001
Mean ±SD	34.8±14.2	59.6±11.3	156±7.5	163.8±9.5	29±7.5			
Median	35.0	60.0	156	169	28			
Range	15–60	39-80	147–170	148–171	22–37			

IL-10, interleukin-10; MDS, myelodysplastic syndrome; MDS-EB1, MDS with excess blasts 1; MDS-EB2, MDS with excess blasts 2; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single-lineage dysplasia; MDS-U, MDS unclassifiable; TNF-α, tumor necrosis factor. P_1=MDS-MLD versus MDS-SLD. P_2=MDS-MLD versus MDS-EB1. P_3=MDS-MLD versus MDS-EB2. P_4=MDS-MLD versus MDS-U. P_5=MDS-SLD versus MDS-EB1. P_6=MDS-SLD versus MDS-EB2. P_7=MDS-SLD versus MDS-U. P_8=MDS-EB1 versus MDS-EB2. P_9=MDS-EB1 versus MDS-U. P_{10}=MDS-EB2 versus MDS-U. [a]Kruskal–Wallis test.

This table shows the demographic data of the different subclassified groups of MDS: they were 22 MDS with multilineage dysplasia (MDS-MLD), nine MDS with single-lineage dysplasia (MDS-SLD), 10 MDS with excess blasts 1 (MDS-EB1), five MDS with excess blasts 2 (MDS-EB2), and three MDS unclassifiable (MDS-U) and there was no statically significant difference between them according to the age or sex.

Table 5 shows the serum level of TNF-α and IL-10 in different subclassifications of MDS.

From this table, there is a statically significant difference between the subclassified groups of MDS patients according the results of serum level of TNF-α and IL-10. TNF-α was higher in MDS with multilinage dysplasia and MDS unclassifiable than the others. And IL-10 was higher in MDS with excess blasts 1 (MDS-EB1) and MDS with excess blasts 2 (MDS-EB2) than the others.

Table 6 shows the other laboratory investigations in different subclassifications of MDS cases.

According to other investigations the hemoglobin level, platelets count, serum LDH, and serum ferritin level had no statically significant differences between the subclassified groups of MDS patient.

Table 7 represents the correlation between serum TNF-α and IL-10 with other investigations in MDS patients.

There were no positive correlations of serum level of TNF-α IL-10 and any of age, hemoglobin level, leukocytes, platelets, ferritin, ESR, and LDH. But there was an inversely positive correlation between serum level of TNF-α and IL-10 and MDS patients.

Discussion

The cytokine network is important in orchestrating immune responses, and previous studies suggest that this network is dysregulated in MDS. However, the cytokine network interacts with several other and biologically different immune mediators, for example, soluble adhesion molecules and the protease system [17].

MDS represent a heterogeneous group of clonal myelopoietic stem-cell disorders, characterized by persistent peripheral cytopenia with morphological and functional abnormalities of hematopoietic cells, often contrasted by BM hypercellularity, with an

Table 6 Other laboratory investigations in different subclassifications of myelodysplastic syndrome cases

	MDS with multilineage dysplasia (MDS-MLD) (*n*=22)	MDS with single linage dysplasia (MDS-SLD) (*n* =9)	MDS with excess blasts 1 (MDS-EB1) (*n* =10)	MDS with excess blasts 2 (MDS-EB2) (*n* =5)	MDS unclassifiable (MDS-U) (*n* =3)	*K*[a]	Post-hoc *P* value
Hb						4.8	0.306
Mean±SD	7.6±1.8	9.8±2.7	8.2±1.8	7.3±1.1	8.5±3.1		
Median	7.8	9.0	8.5	7.7	7.8		
Range	4.3–12.0	6.4–13.5	5–11.9	5.7–8.5	5.8–12.0		
Total leukocytes						9.3	0.052
Mean±SD	4.9±5.1	12.2±8.9	6.7±7.5	6.9±8.4	4.9±2.6		
Median	3.2	10.5	4.5	2.5	3.8		
Range	0.9–22.0	4.6–34.0	1.3–26	0.3–20.0	3–8		
Platelets						3.8	0.427
Mean±SD	105.7±96.2	190.2±168.5	138.1±158.3	73.4±77.6	91.3±137.4		
Median	89.0	220	83.5	54	14.000		
Range	10–414	24–550	18–540	11–208	10.0–250		
Ferritin						7.1	0.127
Mean±SD	810.7±621.3	543.3±186	913.5±450.1	748.6±134.9	462±193.8		
Median	655	573	886.5	794	410		
Range	7–3000	156–790	440–1693	582–900	300–677		

Hb, hemoglobin; MDS, myelodysplastic syndrome; MDS-EB1, MDS with excess blasts 1; MDS-EB2, MDS with excess blasts 2; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single-lineage dysplasia; MDS-U, MDS unclassifiable. [a]Kruskal–Wallis test.

increased risk of transformation into acute myeloid leukemia [18].

The striking feature of this study is the significantly increased level of inflammatory cytokines, TNF-α and IL-10, in MDS patients (Table 2). The elevated TNF-α is in accordance with several studies which reached the same finding with no effect of age-dependent variation like that of Feng *et al.* [19] and Kim *et al.* [20] who could not detect any variation in the systemic levels for any of the cytokines studied and the age.

According to the TNF-α level overall it was increased in MDS patients and this meets the findings in many studies like Kittang *et al.* [15] and also Molnar *et al.* [21] who had found that its expression was increased in MDS patients,

Like this present study in comparison between MDS subclass and the serum TNF-α level, it is increased in MDS-MLD than the classes with more blast and excess blast cells and this in agreement with Molnar *et al.* [21].

In this study, we failed to find any correlation between leukocyte count and degree of anemia in different subclasses of MDS in contrast with Parnes *et al.* [22] who had found that high-expressing TNF-308 and TNF-238 genotypes were independently associated with neutropenia and severity of anemia, respectively, in a large cohort of treatment-naive, de-novo MDS patients, and also Stiffer *et al.* [23] had reported that TNF-α was overexpressed in MDS patients, especially in those with refractory anemia and its expression correlated with BM cellularity and with the magnitude of anemia. Also, we failed to find a correlation between TNF-α level and platelet count but the higher its serum level the lower the platelets count in this study.

IL-10 is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies [24].

In this study, the serum level of IL-10 was significantly elevated in MDS patients than the control group and this in acceptance with many studies like Pardanani *et al.* [25] and Feng *et al.* [26].

Different WHO subclassification of MDS was found in variable levels of serum IL-10 as it was highest in MDS-EB2 and MDS-EB1 and this was inversely correlated with the serum level of TNF-α and we found no correlation with the leukocytic count degree of anemia or even platelet count and the serum level IL-10 in acceptance with Tsimberidou *et al.*[27].

Elevated serum ferritin, due to ineffective erythropoiesis and increased iron absorption from the gut, is often observed in nontransfused MDS patients, suggesting involvement of iron overload in its pathogenesis [28].

Table 7 Correlation between serum levels of tumor necrosis factor-α and interleukin-10 with other investigations in myelodysplastic syndrome patients

Variables	IL-10	TNF
Age (years)		
R	0.104	–0.091
P value	0.475	0.532
Hb (g/dl)		
R	0.117	–0.185
P value	0.422	0.202
Total leukocytes		
R	0.130	–0.196
P value	0.372	0.178
Absolute leukocytes		
R	0.127	–0.175
P value	0.402	0.246
Platelets		
R	0.003	–0.065
P value	0.985	0.657
ESR		
R	0.093	–0.056
P value	0.525	0.701
LDH		
R	0.259	–0.236
P value	0.073	0.102
Ferritin		
R	0.150	–0.021
P value	0.303	0.884
IL-10		
R	–	–0.763
P value	–	0.001
TNF-α		
R	–0.763	–
P value	0.001	–

ESR, erythrocyte sedimentation rate; Hb, hemoglobin; IL-10, interleukin-10; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor.

We found serum ferritin was significantly elevated in MDS patients than control and in many other studies like that of Kikuchi *et al.* [28], Chee *et al.* [29], Cermak *et al.* [30] and Park *et al.* [31].

Similarily it had been found by Matter *et al.* [32], that serum ferritin level ranges from 89 to 2700 ng/ml with a mean level of 933 ng/ml. There was a strong correlation between ferritin and blood units transferred to patients. This supported the study made by Valent *et al.* [33] in Australia, which had found that in low-risk MDS patients with transfusion-dependent anemia, one important clinical feature is iron overload which develops in most cases. Some of these patients develop massive iron overload over time with consecutive organopathy (hepatopathy, cardiomyopathy, and others) despite chelation therapy.

Regarding cytopenia, our study showed that pancytopenia was the main clinical feature, 26 (53%) of the patients were presented by it, 10 (20%) cases were presented with anemia and thrombocytopenia, six (12%) cases presented with anemia only, four (8%) cases were presented with anemia and neutropenia and a same percentage presented with thrombocytopenia only. Only one (2%) case had thrombocytopenia and neutropenia.

Similarily it had been found by Anwar *et al.* [34] that pancytopenia was observed in 80 (45%) patients and bicytopenia in 74 (42%) patients (anemia and thrombocytopenia). However, 23 of them only (13%) had cytopenia of one-cell lineage and also by Gupta *et al.* [35],India, said that pancytopenia was the main complaint, as 64% of patients presented with it, while thrombocytopenia alone was seen in only two cases.

Conclusion

In MDS patients the inflammatory milieu in the bone marrow affects the pathogenesis and pathology of the disease, so the expression of different cytokines in the peripheral blood will be affected as well. We found striking high serum levels of TNF-α (proinflammatory) and IL-10 (anti-inflammatory) cytokines, which confirms the inflammatory process and its role and these cytokine-elevated levels are inversely correlated in different subclasses of MDS patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau M, *et al.* The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016; 127:2391–2405.

2 Verburgh E, Achten R, Louw VJ, Brusselmans C, Delforge M, Boogaerts M, *et al.* A new disease categorization of low-grade myelodysplastic syndromes based on the expression of cytopenia and dysplasia in one versus more than one lineage improves on the WHO classification. Leukemia 2007; 21:668–677.

3 Germing U, Strupp C, Giagounidis A, Haas R, Gattermann N, Starke C, *et al.* Evaluation of dysplasia through detailed cytomorphology in 3156 patients from the Düsseldorf Registry on myelodysplastic syndromes. Leuk Res 2012; 36:727–734.

4 Maassen A, Strupp C, Giagounidis A, Kuendgen A, Nachtkamp K, Hildebrandt B, *et al.* Validation and proposals for a refinement of the WHO 2008 classification of myelodysplastic syndromes without excess of blasts. Leuk Res 2013; 37:64–70.

5 Frietsch JJ, Dornaus S, Neumann T, Scholl S, Schmidt V, Kunert C, *et al.* Paraneoplastic inflammation in myelodysplastic syndrome or bone marrow failure: case series with focus on 5-azacytidine and literature review. Eur J Haematol 2014; 93:247–259.

6 Schanz J, Tüchler H, Solé F, Mallo M, Luño E, Cervera J, *et al.* New comprehensive cytogenetic scoring system for primary myelodysplastic

syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol 2012; 30:820–829.

7 Wolach O, Stone R. Autoimmunity and inflammation in myelodysplastic syndromes. Acta Haematol 2016; 136:108–117.

8 Wilson AB, Neogi T, Prout M, Jick S. Relative risk of myelodysplastic syndromes in patients with autoimmune disorders in the general practice research database. Cancer Epidemiol 2014; 38:544–549.

9 Braun T, Fenaux P. Myelodysplastic syndromes (MDS) and autoimmune disorders (AD): cause or consequence? Best Pract Res Clin Haematol 2013; 26:327–336.

10 Serio B, Selleri C, Maciejewski JP. Impact of immunogenetic polymorphisms in bone marrow failure syndromes. Mini Rev Med Chem 2011; 11:544–552.

11 Kordasti SY, Ingram W, Hayden J, Darling D, Barber L, Afzali B, *et al.* CD4 +CD25 high Foxp3+ regulatory T cells in myelodysplastic syndrome (MDS). Blood 2007; 110:847–850.

12 Kittang A, Kordasti S, Sand KE, Costantini B, Kramer AM, Perezabellan P, *et al.* Expansion of myeloid derived suppressor cells correlates with number of t regulatory cells and disease progression in myelodysplastic syndrome. Oncoimmunology 2016; 5:e1062208.

13 Verfaillie CM. Adhesion receptors as regulators of the hematopoietic process. Blood 1998; 92:2609–2612.

14 Kornblau SM, Cohen AC, Soper D, Huang YW, Cesano A. Age-related changes of healthy bone marrow cell signaling in response to growth factors provide insight into low risk mds. Cytom B Clin Cytom 2014; 86:383–396.

15 Kittang AO, Sand K, Brenner AK, Rye KP, Bruserud Ø. The systemic profile of soluble immune mediators in patients with myelodysplastic syndromes. Int J Mol Sci 2016; 17:1080–1094.

16 Kasamatsu T, Saitoh T, Minato Y, Shimizu H, Yokohama A, Tsukamoto N, *et al.* Polymorphisms of IL-10 affect the severity and prognosis of myelodysplastic syndrome. Eur J Haematol 2016; 96:245–251.

17 Hatfield KJ, Reikvam H, Bruserud O. The crosstalk between the matrix metalloprotease system and the chemokine network in acute myeloid leukemia. Curr Med Chem 2010; 17:4448–4461.

18 Raza A, Mundle S, Shetty V, Alvi S, Chopra H, Span L, *et al.* A paradigm shift in myelodysplastic syndromes. Leukemia 1996; 10:1648–1652.

19 Feng X, Scheinberg P, Wu CO, Samsel L, Nunez O, Prince C, *et al.* Cytokine signature profiles in acquired aplastic anemia and myelodysplastic syndromes. Haematologica 2011; 96:602–606.

20 Kim HO, Kim HS, Youn JC, Shin EC, Park S. Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. J Transl Med 2011; 9:113.

21 Molnár L, Berki T, Hussain A, Németh P, Losonczy H. The role of TNF-alpha in myelodysplastic syndrome: immunoserologic and immunohistochemical studies. Orv Hetil 2000; 141:1807–1811.

22 Parnes A, Nikiforow S, Berliner N, Vanasse GJ. Single nucleotide polymorphisms in the human TNF gene are associated with anaemia and neutropenia in a cohort of patients with de novo myelodysplastic syndrome. Br J Haematol 2010; 150:700–701.

23 Stifter G, Heiss S, Gastl G, Tzankov A, Stauder R. Over-expression of tumor necrosis factor-alpha in bone marrow biopsies from patients with myelodysplastic syndromes: relationship to anemia and prognosis. Eur J Haematol 2005; 75:485–491.

24 Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, *et al.* Biology of interleukin-10. Cytokine Growth Factor Rev 2010; 21: 331–344.

25 Pardanani A, Finke C, Lasho TL, Al-Kali A, Begna KH, Hanson CA, *et al.* IPSS-independent prognostic value of plasma CXCL10,IL-7 and IL-6 levels in myelodysplastic syndromes. Leukemia 2012; 26:693–699.

26 Feng X, Scheinberg P, Wu CO, Samsel L, Nunez O, Prince C, *et al.* Circulating cytokine profiles of patients with acquired aplastic anemia and myelodysplastic syndrome. Blood 2008; 112:1038.

27 Tsimberidou AM, Estey E, Wen S, Pierce S, Kantarjian H, Albitar M, *et al.* The prognostic significance of cytokine levels in newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndromes. Cancer 2008; 113:1605–1613.

28 Kikuchi S, Kobune M, Iyama S, Sato T, Murase K, Kawano Y, *et al.* Prognostic significance of serum ferritin level at diagnosis in myelodysplastic syndrome. Int J Hematol 2012; 95:527–534.

29 Chee CE, Steensma DP, Wu W, Hanson CA, Tefferi A. Neither serum ferritin nor the number of red blood cell transfusions affect overall survival in refractory anemia with ringed sideroblasts. Am J Hematol 2008; 83:611–613.

30 Cermak J, Kacirkova P, Mikulenkova D, Michalova K. Impact of transfusion dependency on survival in patients with early myelodysplastic syndrome without excess of blasts. Leuk Res 2009; 33:1469–1474.

31 Park S, Sapena R, Kelaidi C, Vassilieff D, Bordessoule D, Stamatoullas A, *et al.* Ferritin level at diagnosis is not correlated with poorer survival in non RBC transfusion dependent lower risk de novo MDS. Leuk Res 2011; 35:1530–1533.

32 Mattar M, Mohamed S, El Husseiny N. Myelodysplastic syndrome: an Egyptian experience. J Blood Disord Transfus 2012; 3:121.

33 Valent P, Krieger O, Stauder R, Wimazal F, Nösslinger T, Sperr WR, *et al.* Iron overload in myelodysplastic syndromes (MDS) diagnosis, management, and response criteria: a proposal of the Austrian MDS platform. Eur J Clin Investig 2008; 38:143–149.

34 Anwar N, Arshad A, Nadeem M, Khurram S, Fatima N, Sharif S, *et al.* Clinicohematological and cytogeneticprofile of myelodysplastic syndromes in Pakistan-compare and contrast. Mol Cytogenet 2017; 10:17.

35 Gupta R, Rahman Kh, Rahman Kh, Kumari S, Yadav G, Nityanand S. Clinico-pathological spectrum and novel karyotypic findings in myelodysplastic syndrome: experience of Tertiary Care Center in India, Mediterr J Hematol Infect Dis 2017; 9:e2017048.