"Circulating Concentrations of HMGB1 in Patients with Myelodysplastic Syndrome: Exploratory Study"

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INTRODUCTION

Myelodysplastic syndrome (MDS) is associated with a chronic innate immune signaling and related inflammatory pathways.

HMGB1 is an incompletely studied intra-nuclear molecule that may play a role in the pathophysiology of MDS. HMGB1 is involved in nucleosome stability and is a critical member of the transcriptional regulatory network which regulates hematopoietic stem cell. HMGB1 can interact with a number of TLRs (TLR2, TLR4 and TLR9) , and activate the NF-kB, resulting in production and release of IL-1β, IL-8, IL-6, and TNF-α, resulting in inflammation.

It has been observed *in vitro* that, in CD34⁺ cells derived from patients with MDS, inhibiting HMGB1 promotes MDS-cell death, and alter innate immune responses via suppression of NF-kB pathways. However, it is unknown if plasma concentrations of HMGB1 are increased in patients with MDS compared with those with aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and healthy people.

OBJECTIVE(S)

The main objective of the study was described the plasma concentrations of HMGB1 in patients with MDS, AA, PNH and healthy ageing people.

METHOD(S)

We included patients older than 18 years with the diagnosis of MDS according to the WHO 2016 criteria. As controls, we included patients with diagnosis of AA, PNH, and healthy volunteers adjusted to the cases by gender and age (±5 years).

We excluded those with medical conditions known or suspected to increase HMGB1, including epilepsy, acute or chronic infection, rheumatic diseases, congestive heart failure, acute myocardial infarction, stage-5 chronic renal failure, acute liver disease, and active cancer.

After signing informed consent, peripheral blood was obtained, centrifuged to separate plasma, and samples stored at -80ºC until analysis. Concentrations of plasma cytokines were analyzed in duplicates using multiplex, while HMGB1 was detected using Shino-Test ELISA kits.

RESULT(S)

Between June 2018 and September 2019, 66 samples of patients with MDS, 27 of AA, 17 of PNH and 65 healthy controls matched by gender and age (±5 years) were collected.

Patients with MDS (n = 66) had a median age of 67.87 years (IQR: 61-76 years), 59.09% women (n = 39), and, according to the WHO 2016 classification 92.42% remained within MDS-DU/DM/SA and 5q (n=61) while 7.57% were MDS-EB (n=5).

Through bivariate analysis, we found statistically significant differences in the concentrations of HMGB1, IL-1β, IL-8, and TNF-α (p=0.0001, p=0.0002, p=0.001, and p=0.0001, respectively) between the different groups. In the post-hoc analysis using Scheffe it was observed that HMGB1 is different with each of the groups (Table 1 and Figure 1), IL-1β and IL-8 showed no difference in the post-hoc analysis.

Table 1. Concentrations of HMGB1 in Bone Marrow Failure

Median (IQR)	MDS n=66	AA n=27	PNH n=17	Healthy n=65	p
HMGB1 ng/ml	4.93 (2.30-8.05)	2.57 (1.72-3.69)	1.66 (0.93-2.54)	1.92 (0.87-2.48)	**0.0001**
IL-1ß pg/ml	0.36 (0.27-0.51)	0.23 (0.19-0.34)	0.21 (0.15-0.29)	0.19 (0.16-0.36)	**0.0002**
IL-8 pg/ml	8.26 (3.70-13.98)	4.31 (2.19-9.31)	2.28 (1.44-6.84)	4.41 (2.21-8.16)	**0.0010**
IL-6 pg/ml	1.60 (0.98-3.04)	1.38 (1.15-1.99)	1.59 (0.71-4.65)	1.85 (1.11-3.96)	0.4008
FNT-alpha	3.94 (3.25-5.42)	3.52 (2.36-4.91)	2.86 (2.11-4.77)	6.53 (4.50-9.62)	**0.0001**

*Kruskal-Wallis, p<0.05
HMGB1 (MDS vs AA, p=0.002), (MDS vs PNH, p=0.001), (MDS vs Healthy, p=0.001)

We observed higher plasma concentrations of TNF-α among the healthy control group *vs.* the rest of the bone marrow failures (p=0.003 MDS, p=0.002 AA, p=0.009 PNH), but we observed no differences between the bone marrow failure groups (according to Scheffe). A positive correlation was found between HMGB1 and IL-1β (r=0.43, p=0.0002); and HMGB1 and IL-8 (r=0.57, p=0.0001).

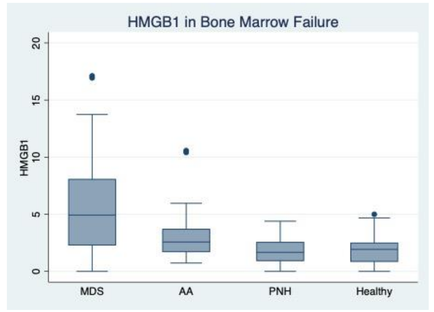

Figure 1. Concentration of HMGB1 in Bone Marrow Failure

Table 2 shows the results of HMGB1 concentrations in the MDS group according to the different characteristics, of which the following stand out: very low, and low risk patients had higher plasma concentrations of HMGB1 than the intermediate, high and very high risk groups (6.19ng/ml, IQR: 2.91-8.58ng/ml *vs.* 3.08ng/ml, IQR: 2.12-6.38ng/ml; p=0.046 respectively).

Table 2. Concentrations of HMGB1 in MDS

	HMGB1 Median (IQR)	p
Type of MDS OMS 2016		
*MDS-UD/MD/RS/-5q	5.33 (2.35-8.19)	0.073
*MDS-EB	3.04 (1.70-4.65)	
Risk IPSS-R		
*Very low/low	6.19 (2.91-8.58)	0.046
*Intermediate/High/Very high	3.08 (2.12-6.38)	
Risk for LRPSS		
*0-4	6.00 (2.61-8.51)	0.085
*≥5	3.00 (2.12-5.85)	
Cellularity bone marrow		
**Hypercellular	4.02 (2.07-8.35)	0.648
**Normocellular	5.39 (3.14-8.94)	
**Hypocellular	5.60 (2.79-7.32)	
Treatment		
*Yes	3.32 (2.12-6.38)	0.044
*No	5.80 (2.91-9.44)	
Reumatology Disease		
*Yes	4.93 (2.48-8.58)	0.851
*No	4.98 (2.30-7.81)	

*Mann-Whitney, p<0.05. **Kruskal-Wallis, p<0.05

When comparing the plasma concentrations of HMGB1 in patients with hypocellular MDS *versus* patients with AA, we found higher concentrations in MDS (n = 14, 5.60ng/ml, IQR: 2.79-7.32ng/ml *vs.* 2.57ng/ml, IQR: 1.72- 3.69ng/ml; p=0.006, respectively) (figure 2).

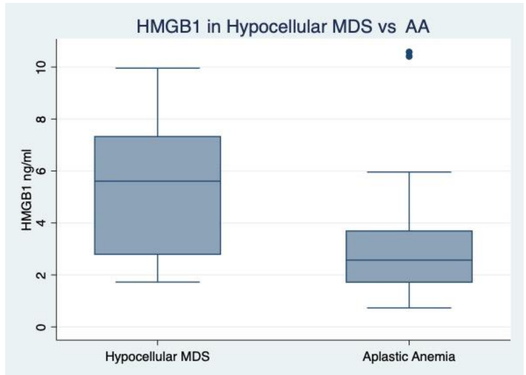

Figure 2. Concentration of HMGB1 in Hypocellular MDS vs AA

CONCLUSION(S)

These observations suggest that HMGB1 is increased in patients with MDS when compared to healthy people and other bone marrow failure syndromes. Circulating HMGB1 was higher in patients with low and very low risk MDS, suggesting that it is involved in the chronic inflammatory mechanism of the pathogenesis of MDS. The observation of higher concentrations of HMGB1 in the group of hypocellular SMD in comparison to AA suggests HMGB1 determination may be clinically useful as a differential diagnosis tool.

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CONTACT INFORMATION

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