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Oxidative stress indicators during the course of acute graft versus host disease

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ABSTRACT

Aim: This prospective study aimed to observe the changes in oxidative stress indicators, including total anti-oxidant status (TAS), total oxidant status (TOS), paraoxanase-1 (PON1), total thiol (TT), native thiol (NT), disulphide (DS) and nitric oxide (NO) levels from sequential blood samples obtained during a de-novo episode of acute graft versus host disease (aGvHD) and evaluate their association with disease severity and the risk of steroid resistant disease.

Material and Method: Sequential patients who underwent an allogeneic stem cell transplantation (ASCT) in our unit and subsequently developed a de-novo episode of aGvHD between January 2022 and May 2022 were included in case they gave informed consent. All patients were started high dose (2 mg/kg/day) methylprednisolone as institutional standard first-line treatment of aGvHD as soon as the clinical diagnosis is evident. All episodes were confirmed simultaneously with gastrointestinal (GI) endoscopy and/or skin biopsies. TAS, TOS, PON1, TT, NT, DS and NO were studied from blood samples collected on days 0, +3 and +7 of steroid treatment. Demographic characteristics, diagnoses, donor type, GvHD prophylaxis, stage and grade of aGvHD, performance status (PS), the presence of cytomegalovirus (CMV) reactivation and response to steroid therapy were also noted.

Results: A total of 15 cases was included. The median age was 49 (23-77). Males constituted 60.0% (n=9). The most frequent diagnosis and donor type were acute leukemia (53.3%, n=8) and matched related donor (46.7%, n=7), respectively. High grade aGvHD with Glucksberg grading and International Bone Marrow Transplant Registry severity index (IBMTR-SI) included 53,3% (n=8) and 86.7% (n=10) of cases, respectively. Non-responders (20.0%, n=3) significantly had advanced stage GI involvement, higher grade of aGvHD with Glucksberg grading and IBMTR-SI, and lower PS (p=0.005, p=0.04, p=0.006, and p=0.02, respectively). The changes in TAS, TOS, PON1, TT, NT, DS and NO levels on days 0, +3 and +7 of steroid treatment were not significant. Median PON1 levels on days 0, +3 and +7 of steroid treatment were significantly lower among non-responders ($p<0.01$, $p<0.02$, and $p=0.03$, respectively).

Conclusion: Steroid resistant aGvHD is an important cause of morbidity and mortality after ASCT. Advanced stage GI involvement and higher total grade of aGvHD is associated with steroid resistance. Lower PON1 levels may be employed as an early indicator of steroid resistance and thus may allow for the early start of more aggressive therapies. Cut-off values and possible confounders should be investigated in further studies.

Keywords: Oxidative stress, graft versus host disease, paraoxanase

INTRODUCTION

Acute graft versus host disease (aGvHD) is one of the leading causes of morbidity and mortality after allogeneic stem cell transplantation (ASCT), which is a curative treatment for various hematologic malignancies (1). The treatment for aGvHD should be promptly started after proper diagnosis, staging and grading. Staging and grading of aGvHD are made according to the severity and extent of organ involvement, which mainly include skin, gastrointestinal (GI) tract and liver. The two most commonly used grading systems include the Glucksberg grading (I to IV) and the International Bone Marrow Transplant Registry severity index (IBMTR-SI) (A to D) (2,3). The first-line treatment of aGvHD depends mainly on the use of high dose systemic glucocorticoids (4-6). However, grade I aGvHD, which includes only the limited involvement of skin, may be treated with topical steroids. The progression of grade I aGvHD to grade II can be prevented with systemic glucocorticoid treatment, whereas it has no effect on progression to grade III-IV disease (7). Methylprednisolone at doses of 2 mg/kg/day is generally the standard choice of therapy. Lower dose treatment (i.e.; 1 mg/kg/day) may also be effective in selected cases (8). Systemic steroids are associated with complete response rates of 25 to 40 percent and more than half of the patients relapse after initial response (4). Steroid resistant aGvHD (SR-aGvHD) is defined as progression of aGvHD by day +5 or a lack of response by day +7 of glucocorticoid treatment (9). The prognosis of SR-aGvHD is still dismal despite promising second-

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line treatments including extracorporeal plasmapheresis (ECP), ruxolitinib, etanercept and many others (9-11).

Donor T-cells play a pivot role in the pathogenesis of aGvHD. After the presentation of recipient antigens to donor T-cells during the ASCT process, donor T-cell activation and consequent development of an immune response against recipient's tissues take place. During this response an increased expression of pattern recognition receptors on antigen-presenting cells, a massive inflammatory cytokine secretion [mainly, tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6] and release of free radicals and oxidative stress products are observed (12, 13). There has been continuing efforts to define various diagnostic and prognostic markers for aGvHD, which yielded inconclusive results (14).

This prospective study aimed to observe the changes in oxidative stress indicators, including total anti-oxidant status (TAS), total oxidant status (TOS), paraoxanase-1 (PON1), total thiol (TT), native thiol (NT), disulphide (DS) and nitric oxide (NO) levels from sequential blood samples obtained during a de-novo episode of aGvHD and evaluate their association with disease severity and the risk of SR-aGvHD.

MATERIAL AND METHOD

The study was carried out with the permission of the Medicana Hospital Clinical Research Ethics Committee (Date: 24.11.2021, Decision No: BŞH-2022/39). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Sequential patients who underwent an ASCT in our unit and subsequently developed a de-novo episode of aGvHD between January 2022 and May 2022 were included in case they gave informed consent. aGvHD is classified into three subgroups according to the time of presentation and presenting features: 1) classic aGvHD-clinical features of aGvHD within 100 days of ASCT; 2) persistent, recurrent, late onset aGvHD-clinical features of aGvHD occurring beyond 100 days after ASCT; 3) overlap syndrome-clinical features of both aGvHD and chronic aGvHD at any time after ASCT (15,16). aGVHD was staged and graded according to Glucksberg and IBMTR-SI criteria. All patients were started high dose (2 mg/kg/day) methylprednisolone as institutional standard first-line treatment of aGvHD as soon as the clinical diagnosis is evident. All episodes were confirmed simultaneously with gastrointestinal (GI) endoscopy and/or skin biopsies. Steroid response was evaluated on days +5 and +7 of steroid therapy. We hypothesized that a possible earlier change on day +3 detected before the clinical judgement of steroid resistance may help to predict the response obtained on day 5. We also decided that another sample obtained on day +7 may help us to assess a correlation with the final clinical judgement for steroid resistant disease. In order to predict and to demonstrate a possible correlation with the steroid response, TAS, TOS, PON1, TT, NT, DS and NO were studied from blood samples collected on days 0, +3 and +7 of steroid treatment.

Demographic characteristics, diagnoses, donor type, GvHD prophylaxis, stage and grade of aGvHD, performance status (PS), the presence of concomitant cytomegalovirus (CMV) reactivation and response to steroid therapy were also noted. The study was approved by the local ethics committee of our hospital and all procedures was conducted in accordance with the ethical standards specified in the Declaration of Helsinki.

The analyses of TAS (mmol Trolox Eq/L), TOS (μmol H2O2 Eq/L), PON1 (U/L), TT (μmol/L), NT (μmol/L), NO (μmol/L) were performed with an autoanalyzer (Mindray BS 300) using commercial colorimetric assay kits (Rel Assay Diagnostics®, Turkey) from venous blood samples according to manufacturer's instructions as previously described (17). The concentration of DS, which indicates the amount of reduced thiols, was calculated as half of the difference between TT and NT.

The primary objective of the study was to determine a significant difference in serum levels of TAS, TOS, PON1, TT, NT, DS and NO between steroid responder and non-responders on days 0, +3 and +7 of steroid treatment. The secondary objectives included the observation of longitudinal changes in serum levels of TAS, TOS, PON1, TT, NT, DS and NO on days 0, +3 and +7 of steroid treatment and to define differences in disease and treatment related characteristics between steroid responder and non-responders.

Median, minimum and maximum values were calculated for non-normally distributed continuous variables. Categorical variables were presented as frequencies and percentages. Comparisons between groups were made by Chi-square or Fisher test for categorical variables and Mann-Whitney U test and Kruskal-Wallis test for continuous variables, respectively. Wilcoxon and Friedman's tests were used for the comparison of repeated measures.

Binary logistic regression was used to assess the independent effects of selected parameters on SR-aGvHD. Factors associated with a statistical significance ($p<0.5$) in the univariate analysis were entered via stepwise exclusion into the model. Hosmer Lemeshow goodness of fit statitstics were used to assess a model fit. Multi-collinearity was excluded. Cohort size limited the number of factors in each model to those with suggested association in univariate analysis. Statistical software package IBM SPSS Statistics for Windows version 25.0

(IBM Corp. released 2017. Armonk, NY, USA) was used in all statistical analyses and a 5% type 1 error (two-sided) was considered statistically significant.

RESULTS

A total of 15 patients was included. The median age was 49 (23-77). Males constituted 60.0% (n=9). The most frequent diagnosis and donor type were acute leukemia (53.3%, n=8) and matched related donor (46.7%, n=7), respectively. Most patients received post-transplant cyclophosphamide (PT-Cy)+calcineurin inhibitor (CNI)/sirolimus+mycophenolate mofetil (MMF) for GvHD prophylaxis (n=8, 53.3%). High grade aGvHD with Glucksberg scale (III or IV) and IBMTR-SI (C or D) included 53.3% (n=8) and 66.7% (n=10) of cases, respectively. General characteristics of patients are given in **Table 1**.

Table 1. General characteristics of patients

Age, median (minimum-maximum)	49 (23-77)
Gender, n (%)	
Male	9 (60.0)
Female	6 (40.0)
Diagnosis, n (%)	
Acute myeloblastic leukemia	6 (40.0)
Acute lymphoblastic leukemia	2 (13.3)
Myelodysplastic syndrome	2 (13.3)
Non-Hodgkin lymphoma	2 (13.3)
Aplastic anemia	1 (6.7)
Chronic myelomonocytic leukemia	1 (6.7)
Primary myelofibrosis	1 (6.7)
Donor type, n (%)	
Matched related	7 (46.7)
Matched unrelated	2 (13.3)
Single antigen mismatched unrelated	3 (20.0)
Haploidentical	3 (20.0)
GvHD prophylaxis, n (%)	
CNI+Mtx	3 (20.0)
CNI/sirolimus+MMF	4 (26.7)
PT-Cy+CNI/sirolimus+MMF	8 (53.3)
Glucksberg grade of acute GvHD, n (%)	
I	7 (46.7)
II	1 (6.7)
III	2 (13.3)
IV	5 (33.3)
IBMTR-SI of acute GvHD, n (%)	
A	3 (20.0)
B	7 (46.7)
C	2 (13.3)
D	3 (20.0)
Concommitant CMV reactivation, n (%)	6 (40.0)
Subtype of acute GvHD, n (%)	
Classic	11 (73.3)
Late onset	4 (26.7)

GvHD: graft versus host disease; CNI: calcineurin inhibitor; Mtx: methotrexate; MMF: mycophenolate mofetil; PT-Cy: post-transplant cyclophosphamide; IBMTR: International Bone Marrow Transplant Registry severity index; CMV: cytomegalovirus

Non-responders (20.0%, n=3) significantly had advanced stage GI involvement, higher grade of aGvHD with Glucksberg grading and IBMTR-SI, and lower PS (p=0.005, p=0.04, p=0.006, and p=0.02, respectively) (**Table 2**).

Table 2. Characteristics of patients according to response to steroid therapy

	Non-responder	Responder	P
Gender, n (%)			0.11
Male	3 (100.0)	6 (50.0)	
Female	-	6 (50.0)	
Age, median (min-max)	59 (38-64)	48 (23-77)	0.72
Donor type, n (%			0.73
Haploidentical	1 (33.3)	2 (16.7)	
Single antigen mismatched unrelated	1 (33.3)	2 (16.7)	
Matched related	1 (33.3)	6 (50.)	
Matched unrelated	-	2 (16.7)	
GvHD prophylaxis, n (%)			0.63
CNI+Mtx	1 (33.3)	3 (25.0)	
CNI/sirolimus+MMF	-	3 (25.0)	
PT-Cy+CNI/ sirolimus+MMF	2 (66.7)	6 (50.0)	
Stage of skin involvement, n (%)			0,44
None	-	1 (8.3)	
1	-	5 (41.7)	
2	1 (33.3)	4 (33.3)	
3	1 (33.3)	1 (8.3)	
4	1 (33.3)	1 (8.3)	
Stage of gastrointestinal involvement, n (%)			**0.005**
None	-	7 (58.3)	
1	-	2 (16.7)	
2	-	3 (25.0)	
3	2 (66.7)	-	
4	1 (33.3)	-	
Stage of liver involvement, n (%)			0.11
None	2 (66.7)	11 (91.7)	
1	-	1 (8.3)	
3	1 (33.3)	-	
Performance status, n (%)			**0.02**
<2	-	9 (75.0)	
≥2	3 (100.0)	3 (25.0)	
Glucksberg grade of aGvHD, n (%)			**0.04**
I or II	-	8 (66.7)	
III or IV	3 (100.0)	4 (33.3)	
IBMTR-SI of aGvHD, n (%)			**0.006**
A or B	-	10 (83.3)	
C or D	3 (100.0)	2 (16.7)	
Concommitant CMV reactivation, n (%)	2 (66.7)	4 (33.3)	0.29
Subtype of aGvHD, n (%)			0.24
Classic	3 (100.0)	8 (66.7)	
Late onset	-	4 (33.3)	

aGvHD: acute graft versus host disease; CNI: calcineurin inhibitor; Mtx: methotrexate; MMF: mycophenolate mofetil; PT-Cy: post-transplant cyclophosphamide; IBMTR-SI: International Bone Marrow Transplant Registry severity index; CMV: cytomegalovirus

Median PON1 levels on days 0, +3 and +7; and median NT levels on day +7 of steroid treatment were significantly lower among non-responders (p<0.01, p<0.02, p=0.03, and p=0.03, respectively) (**Table 3**). Median TAS, TOS, PON1, TT, NT, DS and NO levels on days 0, +3 and +7 of steroid treatment were similar between patients having IBMTR-SI low (A or B) and high (C or D) grade aGvHD (**Table 3**). However, there was a tendency for lower NT levels on days 0 and +7 among patients with high IBMTR-SI (C or D) of aGvHD (p=0.07, and p=0.06, respectively) (**Table 3**). The longitudinal changes in TAS, TOS, PON1, TT, NT, DS and NO levels on days 0, +3 and +7 of steroid treatment were not significant (p=0.53, p=0.31, p=0.93, p=1.0, p=0,76, p=0.18, and p=0.91, respectively).

The distribution of age, gender, diagnosis, performance status and steroid response were similar between different donor types (**Table 4**). PT-Cy based GvHD prophylaxis was more frequently used for haploidentical and unrelated donors (p=0.02). aGvHD of Glucksberg grades III to IV were more frequent in ASCTs from haploidentical and single antigen mismatched unrelated donors (p=0.009) (**Table 4**).

A binary logistic regression model including PON-1 levels on day 0 of steroid treatment and aGvHD of Glucksberg grades I/II versus III/IV revealed no significant associations of these parameters on SR-aGvHD (p=0.99 and p=0.99, respectively).

DISCUSSION

SR-aGvHD continues to be a major clinical problem following ASCT. The standard choice of effective second-line treatments also has not been established yet. Thus, the earlier identification of steroid resistant cases may allow for the earlier start of more aggressive first-line therapies, which may provide more favorable outcomes. Ongoing trials evaluating the role of individual biomarkers or their combinations in the diagnosis and prognosis of aGvHD yielded inconsistent results (14, 18).

The pathogenesis of GvHD involves four main phases: 1) conditioning regimen induced tissue injury; 2) activation of host antigen presenting cells; 3) activation of donor T-cells and resultant cytokine storm; 4) end-organ damage due to activated T cells, natural killer (NK) cells, macrophages and cytokines (19). The tissue damage during the early phases of ASCT, which leads to an increased activity of innate immune cells, including neutrophils, macrophages and monocytes, results in release of ROS. This increase in ROS due to neutrophil activity has been linked to an increased GvHD risk (20).

Oxidative stress modifies and regulates the functions of various immune cells (21). It creates inflammatory signals on macrophages via signal transducer/transcription activator 1 (STAT-1), mitogen-activated protein kinases (MAPK) and NF-κB mechanisms and modulate nicotinamide adenine dinucleotide phosphate

Table 3. Changes in studied parameters according to response to steroid therapy and IBMTR-SI

	Non-responders median (minimum-maximum)	Responders median (minimum-maximum)	P	IBMTR-SI A or B median (minimum-maximum)	IBMTR-SI C or D median (minimum-maximum)	P
TAS on day 0	0.91 (0.72-1.21)	0.94 (0.53-1.4)	0.89	0.94 (0.55-1.4)	0.91 (0.53-1.21)	0.81
TAS on day +3	1.41 (0.6-1.55)	1.07 (0.46-1.58)	0.48	1.07 (0.46-1.58)	1.07 (0.6-1.55)	1.0
TAS on day +7	1.57 (1.03-2.11)	0.92 (0.67-1.08)	0.08	0.92 (0.71-1.08)	0.985 (0.67-2.11)	0.51
TOS on day 0	7.59 (3.31-8.09)	3.55 (1.97-13.7)	0.39	3.55 (1.97-13.7)	5.57 (2.46-8.09)	0.71
TOS on day +3	3.97 (2.72-6.65)	2.9 (1.36-9.05)	0.31	3.2 (1.36-9.05)	2.72 (1.42-6.65)	0.74
TOS on day +7	5.23 (2.59-7.87)	2.69 (1.12-6.1)	0.35	2.69 (1.12-6.1)	2.915 (2.07-7.87)	0.51
PON1 on day 0	86 (52-97)	308 (106-420)	**<0.01**	297.5 (106-420)	97 (52-383)	0.39
PON1 on day +3	72 (57-106)	295 (104-458)	**0.02**	294 (104-403)	106 (57-458)	0.55
PON1 on day +7	58 (54-61)	349 (87-427)	**0.03**	332 (87-427)	205 (54-380)	0.34
TT on day 0	270 (247-316)	324 (225-386)	0.11	323.5 (225-386)	282 (247-362)	0.24
TT on day +3	292 (195-374)	312 (207-373)	0.82	305 (207-351)	312 (195-374)	0.55
TT on day +7	243 (157-329)	372 (230-441)	0.24	372 (230-420)	307.5 (157-441)	0.57
NT on day 0	200 (198-220)	239 (103-288)	0.19	248 (103-288)	200 (179-223)	0.07
NT on day +3	215 (183-231)	250 (132-312)	0.48	250 (132-296)	219 (183-312)	0.84
NT on day +7	124 (79-169)	270 (177-317)	**0.03**	270 (180-317)	173 (79-276)	0.06
DS on day 0	25.0 (24.5-58.0)	54.3 (21.5-80.5)	0.31	44.0 (21.5-80.5)	51.5 (24.5-69.5)	0.81
DS on day +3	30.5 (6.0-79.5)	30.5 (14.5-49.5)	1.0	29.5 (14.5-49.5)	30.5 (6.0-79.5)	0.64
DS on day +7	59.5 (39.0-80.0)	48.0 (9.0-92.5)	0.64	38.5 (9.0-92.5)	67.3 (39.0-82.5)	0.13
NO on day 0	13.57 (10.71-19.64)	18.57 (10.71-34.64)	0.28	19.29 (10.71-34.64)	13.93 (10.71-19.64)	0.13
NO on day +3	11.07 (10.36-28.21)	17.14 (11.43-25.36)	0.39	19.64 (11.43-25.36)	15.36 (10.36-28.22)	0.29
NO on day +7	24.64 (17.14-32.14)	16.43 (13.21-38.93)	0.35	16.43 (13.21-20.36)	24.64 (16.43-38.93)	0.16

IBMTR-SI: International Bone Marrow Transplant Registry severity index; TAS: total anti-oxidant status (mmol Trolox Eq/L); TOS: total oxidant status (μmol H2O2 Eq/L); PON1: paraoxanase-1 (U/L); TT: total thiol (μmol/L); NT: native thiol (μmol/L); DS: disulphides (μmol/L); NO: nitric oxide (μmol/L)

Table 4. Characteristics of patients according to donor type

	Haploidentical donor	Single antigen mismatched unrelated donor	Matched related donor	Matched unrelated donor	P
Age, median (minimum-maximum)	38 (26-70)	51 (31-59)	47 (23-77)	59 (49-68)	0.93
Gender, n (%)					0.70
Male	2 (66.7)	1 (33.3)	5 (71.4)	1 (50.0)	
Female	1 (33.3)	2 (66.7)	2 (28.6)	1 (50.0)	
Diagnosis, n (%)					0.63
Acute leukemia and myelodysplastic syndromes	5 (71.4)	1 (50.0)	2 (66.7)	3 (100.0)	
Other	2 (28.6)	1 (50.0)	1 (33.3)	-	
GvHD prophylaxis, n (%)					**0.02**
CNI+Mtx	-	-	4 (57.1)	-	
CNI/sirolimus+MMF	-	-	3 (42.9)	-	
PT-Cy+CNI/sirolimus+MMF	3 (100.0)	3 (100.0)	-	2 (100.0)	
Performance status, n (%)					0.32
<2	5 (71.4)	2 (100.0)	1 (33.3)	1 (33.3)	
≥2	2 (28.6)	-	2 (66.7)	2 (66.7)	
Glucksberg grade of aGvHD, n (%)					**0.009**
I or II	-	-	5 (71.4)	2 (100.0)	
III or IV	3 (100.0)	3 (100.0)	2 (28.6)	-	
IBMTR-SI of aGvHD, n (%)					0.16
A or B	1 (33.3)	1 (33.3)	6 (85.7)	2 (100.0)	
C or D	2 (66.7)	2 (66.7)	1 (14.3)	-	
Streoid response, n (%)					0.73
Non-responder	1 (33.3)	1 (33.3)	1 (14.3)	-	
Responder	2 (66.7)	2 (66.7)	6 (85.7)	2 (100.0)	

aGvHD: acute graft versus host disease; CNI: calcineurin inhibitor; Mtx: methotrexate; MMF: mycophenolate mofetil; PT-Cy: post-transplant cyclophosphamide; IBMTR-SI: International Bone Marrow Transplant Registry severity index

(NADPH) oxidase (NOX) to produce more ROS (22). ROS originating from NOX stimulates antigen presentation of dendritic cells to CD8+ T-cells (23). Toll-like receptor (TLR) mediated ROS provide maturation signals for CD4+ T-cells (24). Eventually, the disturbance of oxidative equilibrium within CD4+ T-cells may end up with hyper-inflammation and tissue necrosis (25).

The role of oxidative stress in the regulation of T-cell activation, proliferation and differentiation has been emphasized in many preclinical studies (21, 26). Nuclear factor kappa B (NF-κB), which can be activated by cytokines, activators of protein kinase C, viruses and oxidative stress, is an important pathway in T-cell activation and results in transcription of IL-2, TNF-α, interferon-γ, and their receptors (13). Thus, parameters evaluating oxidative stress seem to be attractive candidates as biomarkers, when the pivotal role of T-cells in the development of aGvHD and the high oxidative stress load generated during the process of ASCT are considered.

Endogenous NO production has been reported to exert protective effects against GvHD (27). According to previous reports, the activation of inducible nitric oxide synthase may be responsible from the increased serum NO levels observed preceding the onset of clinical GvHD (28). However, we failed to observe neither a significant change in serum NO levels during the course of aGvHD, nor any difference when compared for steroid response and grade of aGvHD.

The unfavorable effects of increased oxidative stress may be augmented in case of insufficient anti-oxidant reserves, which is evaluated by TAS, TT and NT measurements. TAS, which is an indicator of anti-oxidant activity, did not change significantly during the course of aGvHD and when compared for steroid response and grade of aGvHD. Glutathione system, also called dynamic thiol-disulphide homeostasis, constitutes the main buffer mechanism against oxidative stress. This system involves molecules with labile sulfhydryl groups, which undergo repeated reversible redox reactions catalyzed by NADPH and include glutathione, homocysteine, cysteine, cysteinylglycine and γ- glutamylcysteine (19). Glutathione may inhibit GvHD reactions via suppression of Th-17 differentiation and stimulation of T-regs (21). Although we did not observe a significant association of TT and DS during the course of aGvHD, there was a tendency for lower NT levels in the presence of higher grade of aGvHD.

PON1 is an enzyme secreted from liver. It is found mainly in the form of a stable complex together with high-density lipoprotein (HDL) and apolipoprotein A1 (ApoA1) in the circulation (21). It exerts protective effects against lipid peroxidation and stress-induced ROS formation in the endoplasmic reticulum of human endothelial cells and eliminates homocysteine-thiolactone, which is a toxic metabolite associated with the development of autoimmune, cardiovascular, neurological and malignant diseases (29). Our findings show that there is an impaired PON1 activity in steroid non-responders. This may be either a result of the complex pathophysiological interactions observed during the course of aGvHD, or may demonstrate an individual susceptibility originating from the reported interindividual variations in the enzymatic activity of PON1 isoforms (30). Due to the study design, it is not possible to evaluate whether the observed decrease in PON1 levels is the cause or result of GvHD, however, it may serve as a biomarker in both circumstances. Further studies are needed to evaluate whether there exists a causal effect. PON1, which is regarded as a potential biomarker for cellular stress, may also serve as a biomarker for aGvHD (31).

Our study is the first to explore the changes in oxidative stress parameters during the course of aGvHD. The sample size is modest, however, it may be considered big enough to have a preliminary opinion whether there exists an association between the disease and studied parameters. This research has been designed as a pilot study, and it will take some more time to recruit more patients, when the relatively low incidence of this disease in the general population is considered. Despite its limitations the prospective design and proper definition of risk groups for aGvHD allowed for important observations.

CONCLUSION

SR-aGvHD is an important cause of morbidity and mortality after ASCT. Advanced stage GI involvement and higher total grade of aGvHD is associated with steroid resistance. Lower PON1 levels may be employed as an early indicator of steroid resistance and thus may allow for the early start of more aggressive therapies. Cut-off values and possible confounders should be investigated in further studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Medicana Hospital Clinical Research Ethics Committee (Date: 24.11.2021, Decision No: BŞH-2022/39).

Informed Consent: Verbal and written informed consents were obtained from all patients before enrollment.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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