Salivary cytokines expression during hematopoietic cell transplantation: is there association with oral toxicity?

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The aim of this study is to determine the expression of salivary pro-inflammatory cytokines during the hematopoietic cell transplantation (HCT), and to verify whether there is an association between these cytokines with oral toxicity

Methods: We collected stimulated saliva from autologous and allogeneic HCT patients (n=72) at baseline (T0, before the HCT conditioning), during the neutropenia (T1), and after the marrow engraftment (T2). Salivary levels of IL-6, IL1 β , and TNF α were quantified by ELISA assay. Data about conditioning regimen, time duration of neutropenia, oral mucositis, xerostomia, and body weight loss was collected.

	Total (n=72)
Sex – n (%)	
Male	38 (52.8)
Female	34 (47.2)
Age – mean ($\pm$SD)	46.0 $\pm$ 17.2
Primary disease – n (%)	
Hodgkin lymphoma	4 (5.6)
Non-Hodgkin lymphoma	16 (22.2)
ALL	5 (6.9)
CLL	1 (1.4)
AML	7 (9.7)
CML	4 (5.6)
Multiple myeloma	12 (16.7)
Myelofibrosis	3 (4.2)
Myelodysplastic syndrome	6 (8.3)
Others	14 (19.4)

Characteristics of the patients.

	Total (n=72)
Type of transplantation	
Autologous	35 (48.6)
Allogeneic	23 (31.9)
Haploidentical	14 (19.4)
Conditioning – n (%)	
Bu/Flu (tiotepa, ATG or TMI)	19 (26.4)
Bu/Cy	4 (5.6)
Bu/Mel	1 (1.4)
Mel/Flu/TBI	8 (11.1)
Mel	12 (16.7)
Cy/Eto/Mel	2 (2.8)
BEAM/R-BEAM	15 (20.8)
Cy/Flu/TBI	6 (8.3)
Cy/methylprednisolone/ATG	3 (4.2)
Flu/TBI	2 (2.8)
GVHD prophylaxis	
Tacrolimus/MTX with or without ATG	17 (23.6)
Tacrolimus/MMF/CY	10 (13.9)
MMF/CSA with or without ATG	5 (6.9)
MTX/CSA	5 (6.9)
Time of neutropenia – mean ($\pm$SD)	11.6 $\pm$ 6.0
Body weight loss (%) – mean ($\pm$SD)	4.4 $\pm$ 3.0

Characteristics of the transplantation.

	Total (n=72)
Oral mucositis	
Time duration (in days) – mean $\pm$ SD	23.4 $\pm$ 4.8
Maximum degree – n (%)	
0	9 (12.5)
1	31 (43.0)
2	24 (33.0)
3	7 (9.7)
4	1 (1.3)
Xerostomia	
Frequency	25 (34.7)
Time duration (in days) – median (range)	0 (0-21)

Characteristics of the oral toxicity.

Results: The levels of salivary IL-6, IL-1 β , and TNF- α increased significantly at T1 compared to T0, with significant differences for IL-6 (p=0.039) and TNF- α (p<0.001). In an adjusted regression model analyzing the three cytokines, only high levels of IL-1 β were significantly associated with a long duration of oral mucositis (OR=0.44, p=0.017) and xerostomia (OR=0.49, p=0.038). The highest levels of IL-1 β at T1 were found in autologous HCT (OR=0.35, p=0.002), mainly after melphalan conditioning (OR=2.00, p=0.040). In addition, a significant association was found between body weight loss during the transplantation and high levels of salivary TNF- α at T0 (OR=2.35, p=0.010).

Box-plot for salivary IL-6 at T0, T1, and T2. Whiskers – Maximum value. X – mean value; central trace – median value. P value for Friedman test followed by Wilcoxon test with Bonferroni correction.

Box-plot for salivary IL-1 β at T0, T1, and T2. Whiskers – Maximum value. X – mean value; central trace – median value. Friedman test – p=0.929.

Box-plot for salivary TNF- α at T0, T1, and T2. Whiskers – Maximum value. X – mean value; central trace – median value. P value for Friedman test followed by Wilcoxon test with Bonferroni correction.

Logistic regression analysis with oral mucositis and xerostomia as dependent variable and cytokines (at T1)* as independent variables.

Duration of oral mucositis	OR	CI 95%	P value
First model			
IL-1 β	0.46	0.23-0.92	0.029
IL-6	0.98	0.50-1.96	0.976
TNF- α	0.82	0.41-1.62	0.568
Second model			
IL-1 β	0.44	0.22-0.86	0.017

Xerostomia	OR	CI 95%	P value
First model			
IL-1 β	0.48	0.23-0.97	0.041
IL-6	0.69	0.34-1.39	0.303
TNF- α	1.51	0.74-3.07	0.254
Second model			
IL-1 β	0.49	0.25-0.96	0.038

At T0 and T2, there was no significant association of oral mucositis and xerostomia with the cytokines

Body weight loss (%)	OR	CI 95%	P value
First model			
IL-1 β	1.14	0.56-2.34	0.710
IL-6	0.60	0.30-1.21	0.154
TNF- α	2.69	1.36-5.32	0.004
Second model			
TNF- α	2.35	1.22-4.51	0.010

Logistic regression analysis with body weight loss (%) as dependent variable and cytokines (at T0)* as independent variables.

Conclusion: There is an increase in salivary pro-inflammatory cytokines at neutropenia. Among the analyzed cytokines, only salivary IL-1 β exhibited a discrete association with oral mucositis and xerostomia. Salivary TNF- α at baseline was a predictive factor for body weight loss, suggesting that the saliva could signalize the systemic metabolic alterations caused by TNF- α during the HCT

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