

Histological features of the nasal mucosa in hematopoietic stem cell transplantation

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ABSTRACT

Background: Immunosuppression is the leading cause of recurrent sinus infections after hematopoietic stem cell transplant (HSCT), with increased incidence of sinusitis in patients with chronic graft versus host disease (GVHD). Histological descriptions of the oral mucosa, lung ciliary epithelium, and intestinal mucosa related to HSCT have been described. However, few have described the nasal mucosa. We, therefore, sought to elucidate the histological and ultrastructural features of the nasal mucosa in patients after HSCT to better understand the pathophysiology of the immune response.

Methods: Uncinate processes from 24 HSCT patients and 12 immunocompetent patients were subjected to histological analyses via light and transmission electron microscopy (TEM).

Results: TEM revealed aberrant cilia structure, altered mitochondria quantity, microvilli, and cytoplasm vacuolization. All HSCT patients with rhinosinusitis had significant loss or absence of cilia ($p = 0.018$). Apoptotic bodies were increased and Goblet cells decreased in nasal epithelium from patients with chronic GVHD ($p = 0.04$).

Conclusion: This tissue destruction likely enhances pathogen penetration resulting in recurrent infection.

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It is currently thought that immunosuppression is the sole cause of the increased incidence of recurrent or chronic sinusitis in patients undergoing hematopoietic stem cell transplant (HSCT). However, prolonged antibiotic administration, in addition to immunosuppressant agents, steroids, radiotherapy, chemotherapy, diabetes, prolonged hospitalization, and graft versus host disease (GVHD) have also been described as etiologic factors underlying sinusitis.^{1–11} The estimated risk of bacterial sinusitis in transplanted patients is ~37%, compared with 23% in immunocompetent patients.^{2,3} Furthermore, increased incidence of sinusitis in patients with chronic GVHD (GVHDc) has been reported, suggesting endoscopic surgery as a reasonable treatment option in this cohort.^{1–3,7–11}

GVHD is a potential complication of HSCT and is similar to an autoimmune disease, clinically resembling vascular disease. These processes may have debilitating consequences on patient quality of life because of substantive organ damage requiring prolonged immunosuppression.^{9–20} Previous studies have shown abnormalities in the oral and intestinal mucosa, characterized by thickening of the mucosa and the lichenoid aspect, in addition to decreased lymphocytic regulators (CD4⁺) and increased cytotoxic lymphocytes (CD8⁺).^{12–20} Although the diagnosis of GVHD has been standardized, the precise pathophysiology of the disease remains unknown and, furthermore, abnormalities in the nasal mucosa are poorly described.^{9–20}

Previously, Cordonier (1996) described ciliary alterations in the nasal mucosa in HSCT patients.¹⁵ However, this study did not describe abnormalities of the cilia in patients with HSCT compared with GVHD and rhinosinusitis. Anatomic, ciliary, and mucoviscosity abnormalities have been shown to trigger or exacerbate rhinosinusitis in immunocompetent patients.^{21–23} In transplanted

patients, apart from immunosuppression, there are possible histological and mucociliary abnormalities that may contribute to the increased incidence or recurrence of rhinosinusitis.¹⁵ Therefore, in the current study, we sought to describe histological and ultrastructural abnormalities of the nasal mucosa between in patients undergoing HSCT with and without GVHDc.

METHODS

This prospective, exploratory study evaluated patients undergoing HSCT in the Departments of Hematology and Otorhinolaryngology. The study was approved by the Ethics in Research Committee at University of Campinas. Twenty-four immunosuppressed patients and 21 immunocompetent patients were included in the current study. The immunosuppressed patients were then further stratified into two subgroups: HSCT patients ($n = 5$) and HSCT patients with GVHDc ($n = 19$). HSCT patients were included regardless of donor type or prophylaxis against GVHD and regardless of the incidence of rhinosinusitis. Exclusion criteria included serious risk of bleeding from thrombocytopenia and patients infected by cytomegalovirus or herpesvirus. The hematologic and immunologic status of all subjects was evaluated *via* standard neutrophil, lymphocyte, and platelet counts.

Endoscopic mucosal biopsies were performed on the uncinate process of the medial meatus on all the patients under anesthesia. Histological evaluation was performed *via* light and transmission electron microscopy (TEM). The specimens were stained with hematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome. For TEM, samples were cut into fine fragments ~0.3 mm in diameter and placed on fine permeable cardboard over a wax disk with the epithelium upward. The specimens were then immersed in a fixing solution of 3% glutaraldehyde and maintained at 4°C for 3 hours. Specimens were then washed and stored until analysis.

Using light microscopy, the following dependent variables were analyzed: (1) composition and density of infiltrate inflammatory; (2) intraepithelial lymphocytes and eosinophils; (3) glandular density; (4) apoptotic bodies; and (5) lamina propria thickness, fibrosis, and edema. Using an ocular micrometer, maximal thickness of the lamina propria was measured in 10 high-power fields (trichrome), selected randomly. All samples were analyzed by two pathologists. The parameters analyzed *via* TEM were (1) cilia quantity

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Table 1 Optic microscopy results on nasal mucosa of hematopoietic stem cell transplant (HSCT) patients and immunocompetent controls

Optic Microscopy	HSCT	Controls	p Value
Lymphocytic infiltrate inflammatory	6 (33%)	3 (30%)	0.91
Infiltrate inflammatory density (low/moderate)	13 (72%)	7 (70%)	0.04
Intraepithelial lymphocytes (HPF)	12	4.4	0.62
Intraepithelial eosinophils (HPF)	4.2	0.9	0.31
Glandular density (HPF)	11	7.7	0.97
Fibrosis (mild/severe)	7 (41%)	1 (10%)	0.39
Edema (mild/severe)	5 (29%)	1 (10%)	0.36
Apoptotic bodies (HPF)	6.6	0.75	0.89
Lamina propria (mm)	0.09	0.07	0.94

HPF = 10 high power fields (400×).

Table 2 Electron microscopy results on nasal mucosa of hematopoietic stem cell transplant (HSCT) patients and immunocompetent controls

Electron Microscopy	HSCT	Controls	p Value
Decrease or absence of cilia	17 (77%)	0	0.001
Abnormal cilia structure	6 (50%)	3 (33%)	0.042
Cilia orientation	Normal	Normal	1
Squamous metaplasia	4 (18%)	0	0.29
Microvilli	15 (68%)	4 (44%)	0.25
Goblet cells (decreased or absence)	11 (50%)	2 (22%)	0.08
Intracellular mitochondria (decrease or absence)	12 (54%)	0	0.017
Cytoplasm vacuolization	9 (40%)	0	0.032

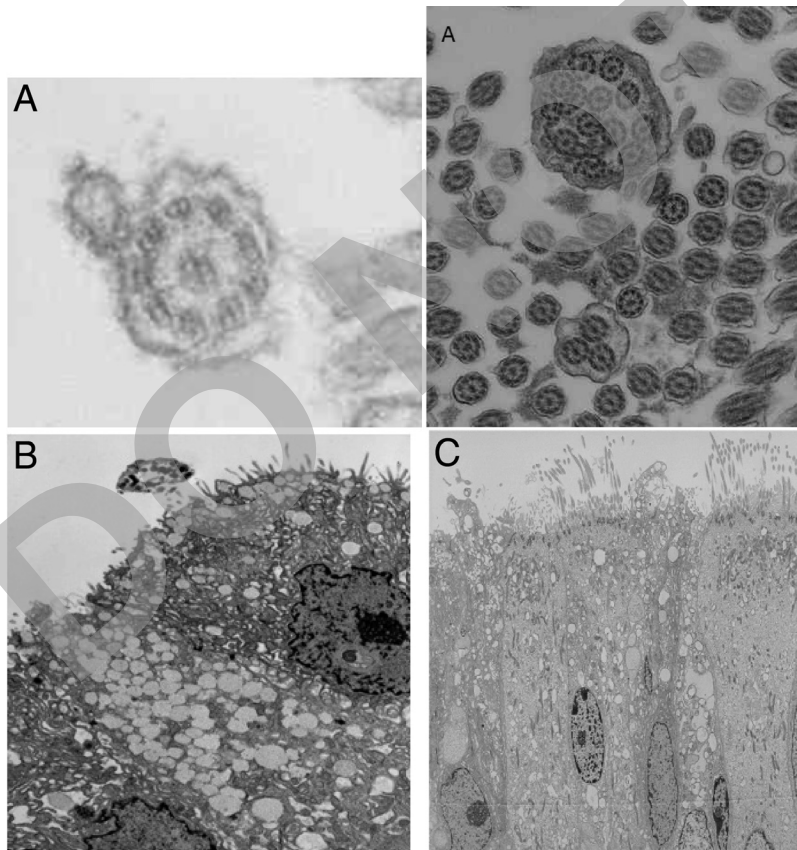


Figure 1. (A, A) Transmission electron microscopy (16,700–6000×): structural abnormalities in the epithelium of the hematopoietic stem cell transplant (HSCT) patients. (B) Nasal epithelium without cilia in the HSCT patients. (C) Cytoplasm vacuolization of the ciliated cell in the HSCT patient.

(absence, decreased, or normal), (2) cilia structure and orientation, (3) squamous metaplasia, (4) microvilli (presence or absence), (5) goblet cells (absence, decreased, increased, and normal), (6) intracellular mitochondria (presence or absence), and (7) cytoplasm vacuolization (presence or absence). TEM specimens were analyzed by a single rater.

At least 5 specimens were collected from the 24 transplanted patients. Two of these specimens were randomly selected for analyses. In the immunocompetent control group, 12 total specimens were analyzed *via* light microscopy and 9 specimens *via* TEM because of technical issues. Data were compared between the three groups with contingency statistic analysis (chi-square) *via* Wil-

Table 3 Optic microscopy of nasal mucosa of hematopoietic stem cell transplant (HSCT) with and without chronic graft versus host disease (GVHDc)

Optic Microscopy	HSCT	HSCT with GVHDc	p Value
Inflammatory infiltrate	80% Lymphocytic	77% Lymphocytic	0.11
Inflammatory Infiltrate density (low/mild)	5 (100%)	8 (61.5%)	0.70
Intraepithelial lymphocytes	12	7.2	0.146
Intraepithelial eosinophils	4.2	3.6	0.65
Glandular density	11	8.6	0.164
Fibrosis (moderate/severe)	1 (20%)	6 (50%)	0.36
Edema (moderate/severe)	1 (20%)	4 (30%)	0.68
Apoptotic bodies	2.3	6.6	0.04
Lamina propria (mm)	0.05	0.09	0.461

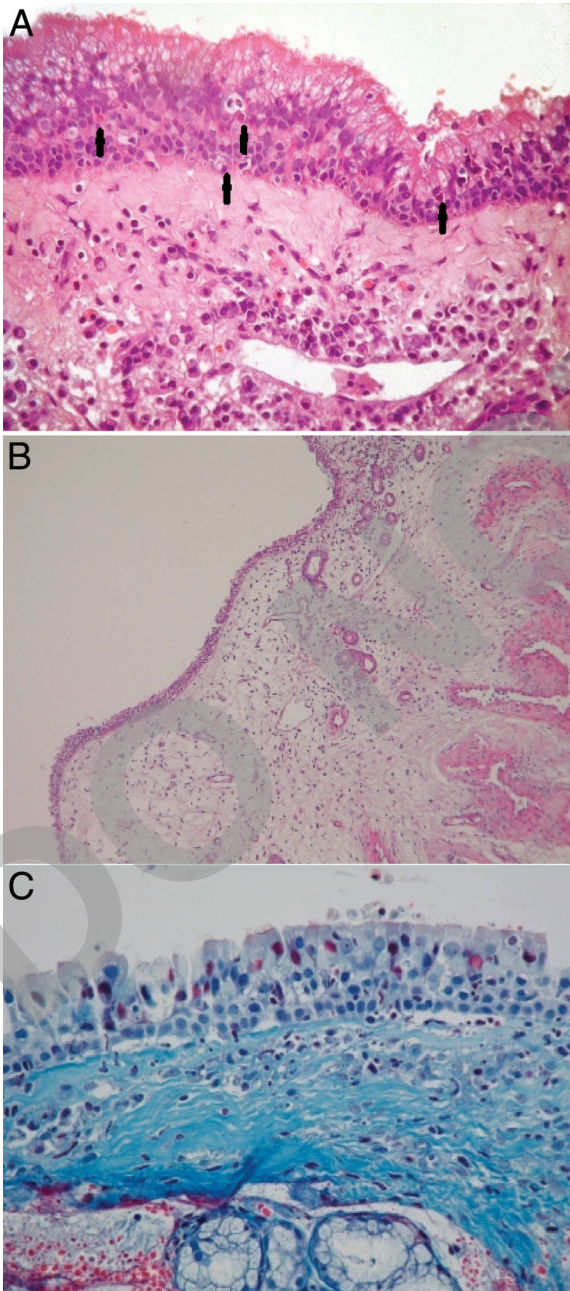


Figure 2. Optic microscopy: (A, arrow) intraepithelial apoptotic bodies, (B) epithelial edema, and (C) fibrosis on hematopoietic stem cell transplant (HSCT) with chronic graft versus host disease (GVHDc).

Table 4 Electron microscopy of nasal mucosa of hematopoietic stem cell transplant (HSCT) with and without chronic graft versus host disease (GVHDc)

Electron Microscopy	HSCT	HSCT with GVHDc	p Value
Decreased/absence of cilia	4 (80%)	13 (76%)	1
Cilia structure	3 (60%)	3 (33%)	0.08
Cilia orientation	Normal	Normal	1
Squamous metaplasia	2 (40%)	2 (11%)	0.21
Microvilli	5 (100%)	10 (59%)	0.13
Goblet cells	2 (40%)	9 (53%)	0.005
Intracellular mitochondria (decreased/absent)	4 (80%)	8 (47%)	0.48
Cytoplasm vacuolization	3 (60%)	6 (35%)	0.60

coxon (Version 2.7.0, R Foundation for Statistic Computing, Vienna, Austria).

RESULTS

The age of the transplant patients ranged from 19 to 64 years (mean, 39 years), and the posttransplant interval ranged from 15 days to 11 years. Differences in neutrophil, lymphocyte, and peripheral platelet counts were not significant between the transplant and control groups.

The results of the light microscopy analyses are presented in Table 1. In the immunosuppressed group, intraepithelial lymphocytes and eosinophils were increased by a factor of 2.7 and 4.7 compared with immunocompetent controls, respectively. These differences, however, did not achieve statistical significance. Furthermore, apoptotic bodies were increased by approximately eightfold and nasal mucosa edema and fibrosis were more severe in HSCT patients.

Summary data from the TEM analyses is presented in Table 2 and Fig. 1. Ultrastructural abnormalities were more diverse and frequent (50%) in HSCT patients including compound cilia, absent inner arm dynein, and triple central microtubules. The only structural abnormality found in the control group was compound cilia. Squamous metaplasia occurred only in the HSCT group. Microvilli were increased by ~3.8 times in transplanted mucosa. However, increased goblet cells were observed in the control specimens (~5.5 times).

The histological characteristics of the specimens from HSCT patients with and without GVHD are presented in Table 3 and Fig. 2. Apoptotic bodies were significantly higher in the nasal mucosa of GVHDc patients. In addition, fibrosis was ~3.5 times increased in the GVHDc specimens. The results of the TEM analyses from this cohort are shown in Table 4 and Fig. 3. The only significant finding was decreased goblet cells in the GVHDc specimens. Qualitatively, more microvilli and cilia absence were observed, but this variables were not statistically significant.

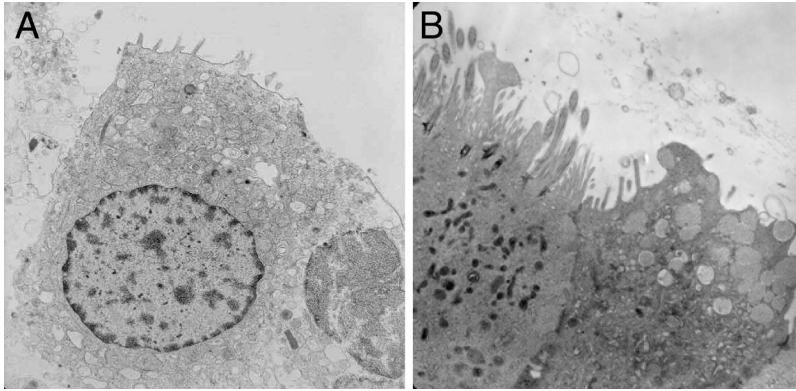


Figure 3. Transmission electron microscopy (6000 $\times$): (A and B) ciliated cell of a hematopoietic stem cell transplant (HSCT) patient with and without chronic graft versus host disease (GVHDc).

DISCUSSION

Recurrent bacterial rhinosinusitis is more frequent in immunosuppressed patients and treatment is challenging.^{2,3,6,7,15} Rhinosinusitis is even more frequent after HSCT patients with acute and GVHDc.^{1,2,3,7,8} The increased incidence of these infections is likely associated with the immunologic deficiencies related to hematologic disease, the transplant, and GVHD.^{1-8,15} Rieger and Cols (2006) showed an imbalance of CD4 and CD8 in intestinal mucosa of post-HSCT patients with GVHDc, suggesting GVHD manifests as tissue damage caused by decreased suppressor or regulator lymphocytes (CD4⁺) and a predominance of cytotoxic lymphocytes (CD8⁺).¹²

Junghansa and Col (2002) reported no difference in the incidence of fungal sinusitis in myeloablative and nonmyeloablative transplants during the 1st postoperative year. The authors also reported less bacteria in the nonmyeloablative transplants because of the short-term neutropenia (10 days).²⁴ It is possible that structural abnormalities of the nasosinusal mucosa were present, but identifying a single etiologic factor responsible for these abnormalities is unlikely given that patients are subjected to full-body radiation, chemotherapy, and viral infections that are known to be deleterious to the respiratory mucosa. However, after recovery of the primary functions of the hematologic system (3 months), HSCT patients continued to present with bacterial rhinosinusitis, worsening with the development of GVHDc.^{3-8,24,25} Similar to our population, these patients presented with a normal hemogram, but were considered immunosuppressed due to the transplant period (<5 years), immunosuppressive medications (cyclosporine or corticoid), and GVHDc.^{9,10,19,20,24,25}

Cordonnier and Col (1996) described squamous metaplasia and defects in the axoneme of the central and peripheral microtubules. However, the authors did not include a control group.¹⁵ In contrast, our data suggest higher lymphocytic inflammatory infiltrate density in patients after HSCT and an increase in intraepithelial apoptotic bodies in HSCT patients with GVHDc. These data coincide with histological abnormalities, already described in other organs affected by GVHDc, suggesting increased cytotoxicity in the sinonasal epithelium.¹¹⁻¹⁹ The ultrastructural abnormalities appear to be more diverse and are more frequent in HSCT patients. Decreased or absent cilia and cytoplasm vacuolization and the decrease of mitochondria in the nasal epithelium of HSCT patients possibly reveal an abnormality in ciliated cell function and, consequently, in mucociliary clearance. Although this difference did not achieve statistical significance, it appears that post-HSCT patients with GVHDc have, apart from decreased goblet cells, an evolution of more severe edema and/or fibrosis similar to previous findings in esophageal, vaginal, and oral mucosa.^{11,12,15-17} This finding may explain the sensation of dry nose and thicker secretions in these patients. It is possible that the relatively small number of GVHD patients in the current study limited statistical power.

It is known that structural abnormalities and motility of the pseudostratified ciliated epithelium can cause inadequate mucociliary

transport and an evolution or exacerbation of rhinosinusitis in immunocompetent patients.^{21-23,26} Previous studies suggest that 5-9% of healthy controls present with ciliary structural abnormalities, as well as the presence of compound cilia and central microtubule abnormalities.²² These aberrant findings do not necessarily restrict ciliary motility in immunocompetent patients. The most deleterious abnormality in the ultrastructure would be in the outer arm dynein of peripheral microtubules. In the maxillary mucosa of immunocompetent patients with chronic rhinosinusitis and nasal polyps, ultrastructural abnormalities were observed, including atypical ciliated epithelium with decreased ciliated cells, increased goblet cells, flattening of basal cells, submucosal fibrosis, and even squamous metaplasia.²² Relevant to the current study, the immunosuppressed patients had ciliated cells, but with few or no cilia, despite these abnormalities not being specific or pathognomonic of either HSCT or GVHDc.

The structure of the nasal mucosa consists of an epithelial layer of ciliated, pseudostratified, columnar cells joined by tight junctions, interspersed with goblet cells. Beneath the epithelium lymphocytes, plasma cells, macrophages, dendritic cells, vascular arcades, and glands can be found. Ciliary motility and the structural integrity of the epithelium serve as mechanical factors limiting antigenic stimulation. As previously documented, these form a mechanical and innate immune barrier across the nasal mucosa to appropriately repel the constant load of exogenous stimulation and limit activation of an acquired immune response. Genetic and/or acquired defects of this complex process may, at least theoretically, lead to the development of the chronic inflammation seen in chronic rhinosinusitis.²⁶ Cumulatively, our data suggest that viral or bacterial infections do not seem to be the main source of tissue damage in patients with GVHD associated with HSCT. Instead, cytotoxic lymphocytes (CD8⁺) of the graft acting against the mucosa appear to be a relevant factor. Accordingly, tissue destruction likely facilitates bacterial and viral penetration, resulting in recurrence or exacerbation of the infection.

CONCLUSION

Patients undergoing HSCT presented with histological and ultrastructural abnormalities when compared with immunocompetent controls. However, ultrastructural differences were not different between HSCT patients with GVHDc and patients without GVHDc, with the exception of increased intraepithelial apoptotic bodies and decreased goblet cells.

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