Lung Histology Predicts Outcome of Bronchiolitis Obliterans Syndrome after Hematopoietic Stem Cell Transplantation

Andreas Holbro [1], Thomas Lehmann [1], Sabine Girsberger [1], Martin Stern [1],
Franco Gambazzi [2], Didier Lardinois [2], Dominik Heim [1], Jakob R. Passweg [1],
André Tichelli [1], Lukas Bubendorf [3], Spasenija Savic [3], Katrin Hostettler [4],
Peter Grendelmeier [4], Joerg P. Halter [1,*], Michael Tamm [4]

[1] Department of Hematology, University Hospital Basel, Basel, Switzerland
[2] Division of Thoracic Surgery, University Hospital Basel, Basel, Switzerland
[3] Institute for Pathology, University Hospital Basel, Basel, Switzerland
[4] Department of Pneumology, University Hospital Basel, Basel, Switzerland

ABSTRACT

Bronchiolitis obliterans (BO) is a severe complication after allogeneic hematopoietic stem cell transplantation with an unfavorable prognosis. Lung biopsy remains the gold standard for diagnosis. In this retrospective single-center study, we describe 33 patients who underwent biopsy for suspected BO. Ten patients had constrictive BO (CBO); 9 had lymphocytic bronchiolitis (LB), characterized by lymphocytic infiltration of the bronchioles. Six additional patients (4, CBO; 2, LB) had concomitant infection; 8 had other pathological diagnoses. Seven patients with CBO and 3 with LB met the National Institutes of Health consensus BO syndrome definition criteria. An additional 7 patients with histologically confirmed CBO did not meet the consensus definition, 4 of them because of concomitant airway infection. At diagnosis, there were no significant differences between the CBO and LB groups in clinical presentation; pulmonary function tests (median forced expiratory volume in one second [FEV1] at baseline, 90.4% and 99% predicted, at time of video-assisted thoracoscopic surgery, 55.1% and 60.8% for CBO and LB groups, respectively); and chest scans. Treatment was similar in both groups but outcome was different depending on histological findings. FEV1 significantly improved in LB patients compared with CBO patients. Survivals at 1 and 3 years were 77% ± 12% and 60% ± 14% for patients with CBO and 91% ± 9% for patients with LB (P = .028). Lung biopsy in patients with suspected BO enables better characterization of the pattern of BO syndrome. In contrast to CBO, LB is associated with a good long-term prognosis.

© 2013 American Society for Blood and Marrow Transplantation.

INTRODUCTION

Despite careful patient selection for hematopoietic stem cell transplantation (HSCT) and improvements in supportive care, transplantation-related mortality, mainly due to infections and graft-versus-host disease (GVHD), remains a major issue [1-4]. Pulmonary complications are common and are associated with significant morbidity and mortality [4]. Most acute posttransplantation complications are related to infections, although noninfectious pulmonary toxicity contributes to late complications and are becoming increasingly recognized [5]. Lung injury can be infectious or noninfectious, acute or chronic, and can manifest with either a restrictive or an obstructive lung function pattern [6]. Late pulmonary complications are most often associated with chronic GVHD [7,8].

Bronchiolitis obliterans syndrome (BOS) is a complication with an increasing incidence of up to 15% in recent years. The reason for this increase is not completely understood. It might be partially explained by more successful treatment of other posttransplantation complications, thereby exposing more patients at risk for bronchiolitis obliterans (BO) and because of an increased use of unrelated donors [9-11]. In addition, differences in the incidence of BOS are in part due to the diagnostic methods. Risk factors for the development of BOS include pretransplantation lung function, type and intensity of the conditioning regimen, use of peripheral blood stem cells, immunoglobulin levels, and respiratory infections [12-14]. Moreover, the occurrence of GVHD at other sites is associated with BOS [15].

The pathogenesis of BO is not completely understood. Specific immune reactions by donor T cells, in addition to the recipient's innate immune system, are thought to play roles in the development of BO. According to the National Institutes of Health (NIH) consensus statement criteria, BO is considered diagnostic for pulmonary GVHD [16]. BO can be clinically diagnosed when all of the following criteria are met: (1) forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) ratio $<.7$ and FEV1 $< 75\%$ of predicted value; (2) evidence of air trapping or small airway thickening or bronchiectasis on high-resolution chest computed tomography (HR-CT), a residual volume $>120\%$, or a pathological confirmation of constrictive bronchiolitis; and (3) absence of respiratory tract infection. BO-organizing pneumonia that is not due to infection, but may represent a manifestation of either acute or chronic GVHD, is considered a common feature [16].

Nevertheless, the diagnosis of pulmonary chronic GVHD remains challenging, and the gold standard remains biopsy-proven BO [18,19]. However, it is not known if the histology results of patients with BOS are associated with the severity of the disease and its outcome. Once diagnosed, intensive,

long-term immunosuppression is generally recommended for proven BO [17]. However, the prognosis is unfavorable, mainly because of late definite diagnosis and resistance to therapy [18,19]. Progressive deterioration of lung function and death are common, either due to infections and/or respiratory failure.

Early testing with prognostic impact is, therefore, urgently needed. Our hypothesis was that histology may define disease severity and predict the outcomes for patients with BOS. We therefore reviewed all lung biopsies that were routinely performed in our patients with clinical suspicion of BO. We analyzed histological patterns, correlations with clinical characteristics, and the potential impact of biopsy results for predicting patient outcomes and guiding patient management.

MATERIALS AND METHODS

Patients and Study Design

This is a single-center cohort study including all consecutive patients who underwent lung biopsy for suspected BO after allogeneic HSCT from January 1989 to December 2010. During this period, 917 patients underwent allogeneic HSCT at our center.

Lung biopsy to diagnose or exclude BO was considered by the treating physician in patients with respiratory symptoms, declining lung function test results, or both after exclusion of infections and other differential diagnoses by HR-CT scan and bronchoalveolar lavage (BAL). Pulmonary function tests (PFT) were usually performed for each patient using body plethysmography (Jaeger, Hoechberg, Germany) before transplantation (baseline); at 3 months, 6 months, and 1, 2 and 5 years after transplantation; and every 5 years thereafter. Additional PFT were performed in patients with pulmonary symptoms that could not be explained by active infections. Total lung capacity, FVC, FEV1, FEV1:FVC, and the CO diffusion capacity corrected for the hemoglobin level were determined. Results are reported as percentage of predicted normal values [20].

BO was considered in the differential diagnosis if a significant and persistent decline (>20% compared to pretransplantation baseline levels) in FEV1 occurred. However, if the treating physician suspected BOS based on symptoms and clinical findings, video-assisted thoracoscopic surgery (VATS) was also considered for patients with less pronounced changes in FEV1. Before surgery, routine work-up included a HR-CT and BAL. HR-CT scan results were analyzed by an experienced radiologist for intra- and extrapulmonary pathologies, including pulmonary infiltrates, bronchial wall thickening, increased bronchial diameter compared with the accompanying bronchial artery (bronchiectasis), and air trapping.

All patients underwent BAL to exclude infection as a cause of pulmonary symptoms and lung function decline. Cytopathological analysis of lavage fluid included total and differential cell counts and direct staining for bacterial and fungal organisms. In recent years, we added additional analyses, including immunofluorescence staining for pneumocystis, cytomegalovirus, and respiratory syncytial virus (RSV). Microbiological analysis included viral, bacterial, and fungal cultures. Since February 2009, analytic sensitivity was increased by analyzing BAL samples with an established multiplex PCR assay (RespiFinder, Maastricht, The Netherlands). RespiFinder can detect 14 RNA viruses, 1 DNA virus, and 4 bacteria: adenovirus, *Bordetella pertussis*, *Chlamydophila pneumoniae*, Coronavirus 229E/NL63/OC43, Human metapneumovirus, influenza A and B, influenza A/H5N1, *Legionella pneumophila*, *Mycoplasma pneumoniae*, parainfluenza (PIV) type 1 to 4, RSV A and B, and rhinovirus.

Proven infection was not an exclusion criterion for lung biopsy if patients showed a persistent decline in PFT. Lung biopsies were performed by VATS, except for the first 2 patients who had open lung biopsies. Biopsies were usually taken from 2 lobes, as well as from the areas most involved on HR-CT scans. Lung wedge resection was inflated and fixed in 4% buffered formalin. After paraffin embedding, specimens were cut into 4-µm sections and routinely stained with hematoxylin and eosin, Elastica van Gieson, alcian blue-periodic acid Schiff, and Grocott's methenamine silver stain. The specimens were reviewed in a blinded manner by a lung pathologist who was not involved in the initial diagnosis (L.B. or S.S.). If there were histological findings suggestive of a viral infection, such as nuclear enlargement, clearing, smudging, or multinucleation, immunohistochemical analyses for cytomegalovirus, herpes simplex virus, or adenovirus were also performed.

Two distinct histological patterns of bronchiolitis were identified: constrictive bronchiolitis obliterans (CBO) (Figure 1) and lymphocytic bronchiolitis (LB) (Figure 2) [21-23]. CBO was characterized by concentric or eccentric depositions of fibrous tissue within the submucosa of the bronchioles, with or without minimal chronic inflammation. In contrast, LB was characterized by a prominent lymphocytic infiltration of the bronchiolar wall with variable epithelial inflammation and damage. A third group comprised patients with other specific pathological diagnoses.
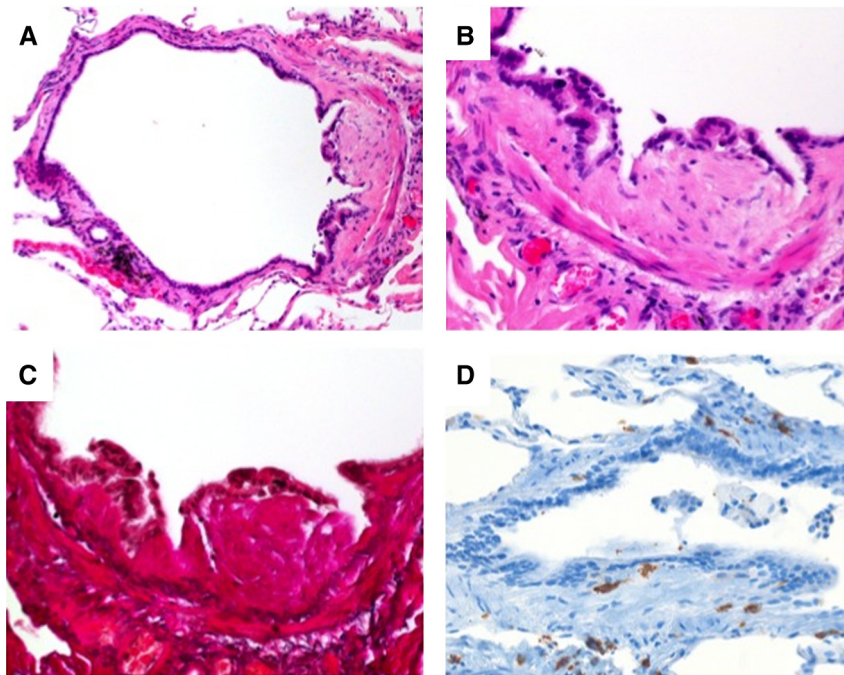
Figure 1. Constrictive bronchiolitis obliterans. (A) and (B) Show excentric subepithelial deposition of fibrous tissue within the submucosa of a bronchiole without lymphocytic inflammation (hematoxylin and eosin, magnification x200 and 400x, respectively). In the connective tissue stain, the subepithelial fibrosis and the separation of the epithelium from the elastic lamina is highlighted in (C). (Elastica van Gieson, magnification x400). (D) Demonstrates CD8 immunohistochemistry shows only scattered cytotoxic T-lymphocytes (anti-CD8 immunohistochemical reaction, magnification x400) (Axio Imager A1 microscope; Axio Vision 4.8. Software; Carl Zeiss, Jena, Germany).

Figure 2. Lymphocytic bronchiolitis. (A) and (B) Show lymphocytic inflammation of the bronchiolar epithelium (hematoxylin and eosin, magnification x200 and x400, respectively). (C) Shows in the connective tissue stain, the epithelium lies just above the elastic lamina 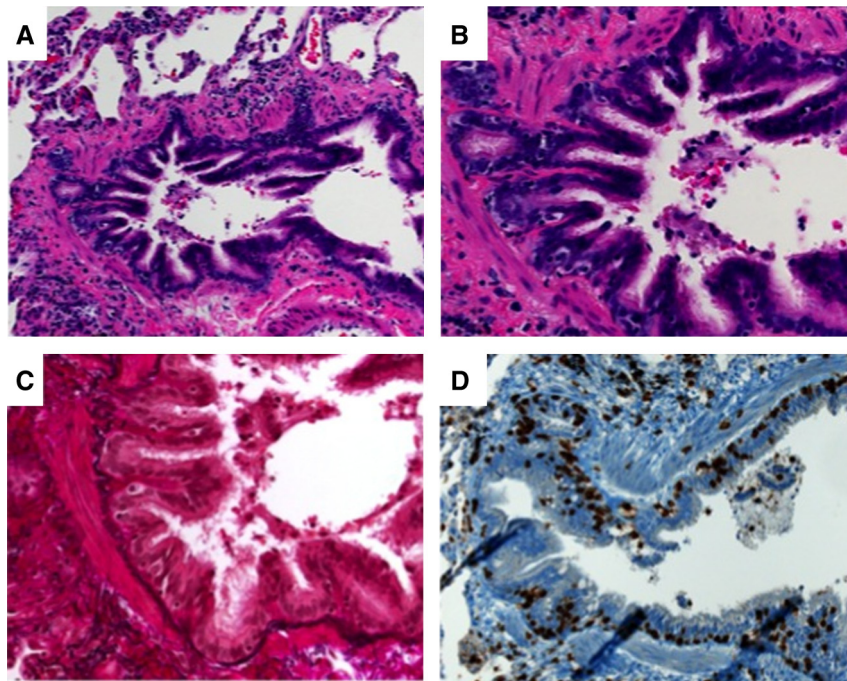without subepithelial fibrosis (Elastica van Gieson, magnification x400). (D) Shows the intraepithelial inflammation consists of CD8 positive cytotoxic lymphocytes (anti-CD8 immunohistochemical reaction, magnification x400) (Carl Zeiss).

After the diagnosis of lung pathology, regular follow-ups and PFT were routinely performed.

Therapies and Outcomes

After obtaining biopsy results, all patients with biopsy-proven bronchiolitis received new or increased immunosuppressive therapy, including a calcineurin inhibitor (mainly tacrolimus, except for patients with severe renal insufficiency, for whom sirolimus was used, or intolerance, for whom cyclosporine was used), mycophenolic acid, and prednisone. Initiation of prednisone was delayed until 7 to 10 days after surgery to allow wound healing. Initial dose was usually 2 mg/kg for 14 days, followed by 1 mg/kg for another 2 weeks with subsequent tapering of 10 mg every 2 weeks down to 20 mg daily. Afterwards, steroids were tapered more slowly to 15 mg, 10 mg, 7.5 mg, and 5 mg every 4 weeks, depending on stabilization or improvement of PFT. Mycophenolic acid was added, based on in vitro data showing a potent antiproliferative effect on lung fibroblasts [24]. As soon as the systemic steroid dose was ≤10 mg, treatment with inhalation steroids twice daily was started. Azithromycin 500 mg 3 times weekly was added in 2006 after its anti-inflammatory action became evident [25,26]. Type of therapy and outcome, based on improvement in PFT results, and survival were analyzed.

Data on diagnosis, conditioning regimen, graft source, GVHD prophylaxis, smoking history, allergies, and GVHD were collected retrospectively by chart review. Follow-ups were current as of July 31, 2011.

Approval from the local institutional review board was obtained for this study.

Statistical Analysis

Patient survival was determined using the Kaplan-Meier method. Mann-Whitney U test was used to compare variables among the CBO and LB groups. For multiple comparisons, Kruskal-Wallis test was used. For comparisons of FEV1 between CBO, LB, and CBO with infection (CBOi), analysis of variance was used. For multiple comparisons, a P value of < .01 was considered to be statistically significant. For other analyses, a 2-sided P value of < .05 was considered statistically significant. Analyses were performed using IBM SPSS version 19 (IBM, Armonk, NY).

RESULTS
Patient Characteristics

During the last nearly 30 years, surgical lung resections have been regularly performed at our center in hematologic patients in neutropenia or posttransplantation. In earlier years, the main reason for lung resections was fungal infection [27]. Since 2003, the majority of surgical lung resections in patients after allogeneic HSCT (ie, in 45 of 59 patients) have been for diagnostic reasons, early or late after transplantation. Diagnostic questions depended on the time posttransplantation and clinical characteristics. Twenty-seven biopsies were performed to diagnose or exclude BO, which, during this time period, comprised 90% of all patients in whom BO was suspected based on the criteria described. Three patients did not proceed to VATS because of either patient deferral (n = 2) or lung reserve was considered too poor (n = 1). Biopsies in 6 more patients were performed for suspicion of BO between 1989 and 2002, representing about 30% of all patients who would have qualified for this procedure during that time period. Exact reasons for not undergoing lung biopsy could not be ascertained in all cases, but included both medical reasons (comorbidities, lung function was considered too poor to allow lung surgery with acceptable risk) and patient refusal.

Hence, 33 patients for whom a lung biopsy was performed because of an initial suspicion of BO were identified. Ten (30%) had histologically proven CBO; 4 (12%) had CBOi (one with PIV, 3 with RSV); 9 (27%) had LB; and 2 (6%) had LB with concomitant infection (PIV and RSV, one each). In one patient, both CBO and LB lesions were identified. Assuming that the constrictive lesions would be primarily determining for the further course of the disease, this patient was included in our CBO group for further analysis. In the remaining 8 patients (24%), other histological diagnoses were made, including mucus plugging (n = 4), organizing pneumonia (n = 2), veno-occlusive disease of the lung, and fibrosis (n = 1 each). Patient and disease characteristics are shown in Table 1.

Table 1
Patient, Disease, and Transplant Characteristics

Characteristic	CBO	CBO + Infection	LB	LB + Infection	Others	Total
No. of patients	10	4	9	2	8	33
Males (%)	6 (60%)	2 (50%)	5 (56%)	1 (50%)	6 (75%)	20 (61%)
Diagnosis						
PMF	1	0	0	0	0	1
ALL	2	0	4	0	2	8
AML	2	3	3	0	3	11
NHL/CLL	2	0	1	2	2	7
CML	2	1	0	0	1	4
RCC	1	0	0	0	0	1
MDS	0	0	1	0	0	1
Age at diagnosis, median (range), yr	47 (10-59)	45 (15-49)	45 (16-55)	54 (53-55)	45 (18-58)	45 (10-59)
No. of Tx. before allo-HSCT, median (range)	2.5 (0-4)	2.5 (1-3)	4 (1-6)	4 (2-6)	2 (1-8)	3 (0-8)
Patients with previous HSCT						
Auto-HSCT	1	0	1	1	1	4
Syngenic-HSCT	1	0	0	0	0	1
Allo-HSCT	0	0	1	0	0	1
Patients with RT before allo-HSCT (%)	2 (20%)	1 (25%)	4 (44%)	0	1 (13%)	8 (24%)
Remission at allo-HSCT[*]						
Early	4	3	6	1	4	18
Intermediate	2	0	0	0	1	3
Advanced	2	1	2	1	0	6
NHL chemosensitive	1	0	0	0	2	3
NHL chemoinsensitive	1	0	1	0	1	3
Age at allo-HSCT, median (range), yr	49 (15-59)	45 (15-49)	46 (17-55)	55.5 (56-57)	46 (19-59)	46 (15-59)
Donor						
RD	8	3	6	1	6	24
UD	2	1	3	1	2	9
HLA-match						
HLA-identical	9	4	8	2	7	30
HLA-mismatch	1	0	1	0	1	3
CMV (donor/recipient)						
−/−	5	2	1	1	4	13
−/+	2	1	3	0	2	8
+/−	1	0	1	0	1	3
+/+	2	1	5	1	1	9
Sex match (donor/recipient)						
F/M	2	1	3	0	3	9
M/M	4	1	2	1	3	11
M/F	1	1	3	1	1	7
F/F	3	1	1	0	1	6
Stem cell source						
BM	1	3	2	0	1	7
PBSC	9	1	7	2	7	26
Conditioning regimen						
Intensive	6	4	8	0	7	25
RIC	4	0	1	2	1	8
TBI-based conditioning						
200cGy	4	0	1	2	1	8
>/= 1000cGy	2	3	5	0	3	13
No	4	1	3	0	4	12
Busulfan-containing conditioning						
Yes	3	1	3	0	3	10
No	7	3	6	2	5	23
GVHD-Prophylaxis						
CYA	2	1	1	0	2	6
CYA + MTX	4	3	7	0	5	19
CYA + MMF	4	0	1	2	1	8

CBO indicates constrictive bronchiolitis obliterans; LB, lymphocytic bronchiolitis; PMF, primary myelofibrosis; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; NHL/CLL, non-Hodgkin lymphoma/chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; RCC, renal cell carcinoma; MDS, myelodysplastic syndrome; Tx., therapies; allo-HSCT, allogeneic hematopoietic stem cell transplantation; auto-HSCT, autologous hematopoietic stem cell transplantation; RT, radiotherapy; RD, related donor; UD, unrelated donor; HLA, human leukocyte antigen; CMV, cytomegalovirus; F, female; M, male; BM, bone marrow; PBSC, peripheral blood stem cells; RIC, reduced-intensity conditioning; TBI, total body irradiation; Gy, Gray; GVHD, graft-versus-host disease; CYA, cyclosporine; MTX, methotrexate; MMF, mycophenolate.
Data are presented as n, unless otherwise specified.
[*] According to Center for International Blood and Marrow Transplant Research.

Transplantation Characteristics

Table 1 shows the transplantation characteristics based on the pathological findings. Median age at transplantation was 46 years (range, 15 to 59 years). Most patients had a matched related or a 10/10 HLA-matched unrelated donor. Peripheral blood stem cells were the most frequently used stem cell source. Twenty-five patients (76%) followed intensive mye-loablative conditioning regimens, including busulfan for 10 patients (30%). For the majority, GVHD prophylaxis included cyclosporine and methotrexate (n = 19; 58%).

Symptoms at Diagnosis

Median time interval between HSCT and CBO diagnosis was 739 days (range, 136 to 3305 days) and, for interval between HSCT and LB diagnosis, the mean was 550 days (range, 111 to 2159 days) ($P = .253$). At diagnosis, the main symptoms were cough, mainly nonproductive (n = 5 in the CBO group, n = 6 in the LB group), and dyspnea (n = 9 in the CBO group, n = 8 in the LB group).

PFT Results

Median baseline FEV1 values before allogeneic HSCT were not different between patients who were later diagnosed with CBO (90.4%; range, 68% to 107%), CBOi (109%; range, 89% to 120%) or with LB without infection (99%; range, 56% to 114%). (Table 2, Figure 3) Furthermore, at the time of biopsy, there were no significant differences in the median FEV1 values between the 3 groups: CBO, 55.1% (range, 35.2% to 83.2%); CBOi, 41.6% (range, 34.1% to 53.2%); and LB, 60.8% (range, 44.1% to 85.9%). The median decline in FEV1 from baseline to the time when biopsy was performed was 43.8% for CBO, 65.4% for CBOi, and 23.6% for LB. These declines in PFT were statistically significant for all the 3 groups (CBO: $P = .001$; CBOi: $P = .04$; LB: $P = .002$).

Computed Tomography Findings Before VATS

Three patients had normal HR-CT: 2 in the CBO and 1 in the LB group. Increased bronchial wall thickening was the most frequent radiological finding in both groups: 8 of 10 in the CBO and 9 of 9 in the LB group. (Table 2) Sixteen (64%) patients had in- and expiratory HR-CT scans (2 of 4 CBOi patients, 1 of 4 CBOi patients, 5 of 9 LB patients, and 2 of 2 LBi patients). Mosaic patterns after expiration or infiltrates were seen in only a few patients. None of the radiological findings were pathognomonic for either CBO or LB. Because the histological diagnosis was known, the sensitivity of CT findings could be determined. Among patients with confirmed CBO, increased bronchial wall thickness was

found in 80%, increased bronchial diameter compared with the corresponding artery in 50%, and a mosaic pattern after expiration in 29%. The corresponding values in patients with LB were 100%, 78%, and 20%, respectively.

Table 3 summarizes the NIH-criteria according to the histological group for each patient.

Bronchoscopy and BAL Results

All patients underwent BAL procedures. Results are shown in Table 2. Differential cell counts were available for 28 patients (85%). Concomitant viral infection was identified in 6 patients (2 with PIV, 4 with RSV). Patients with LB, compared with patients with CBO, had significantly higher proportions of lymphocytes ($P = .009$). There were no other significant differences in the differential cell counts between these 2 groups.

VATS

VATS procedures were performed a median of 7 days after BAL. There were no in-hospital and 30-day postoperative mortalities for the entire group. Twenty-six patients (84%) did not experience any postoperative complications. Six patients had surgery-related minor complications: prolonged pain (n = 4) and prolonged air leak (n = 1). One patient experienced pain and hypesthesia after open lung surgery and another patient developed late empyema (69 days after VATS) that was possibly related to this procedure. For one intubated patient, the indication for VATS was persistent pneumothorax with suspected BO; the final diagnosis was veno-occlusive diseases of the lung. This patient remained intubated and underwent successful urgent lung transplantation 34 days after VATS.

Therapy

Most patients, all in the CBO group and 8 of 9 (89%) in the LB group, were treated with calcineurin inhibitors (cyclosporine or tacrolimus). One patient with LB was treated with

Table 2
Radiologic Findings, Bronchoalveolar Lavage, and Pulmonary Function Test Results

Results	CBO	CBO with Infection	LB
Symptoms			
None	1	0	0
Cough	0	0	1
Dyspnea	5	1	3
Cough and Dyspnea	4	3	5
Radiologic findings			
Infiltrates	1	2	2
Increased bronchial wall thickness	8	2	9
Thickness bronchial wall > arterial wall	5	2	7
Mosaic pattern	2	0	1
Bronchoalveolar lavage findings*			
Cell count, median (range), x10^6/L	239.55 (26-621.9)	69.9 (50.2-89.6)	103.35 (29-830.9)
Macrophages, median (range), %	87.5 (51-95)	86.5 (86-87)	60 (43-90)
PMN, median (range), %	6 (2-45)	9.5 (9-10)	11.5 (0.43)
Lymphocytes, median (range), %	4 (1-12)	4 (3-5)	14 (6-51)
Eosinophils, median (range), %	0 (0-1)	0	1 (0-10)
FEV1 (% predicted), pretransplantation			
Mean (range), %	90.8 (68-107)	103.0 (89-111)	90.4 (57-114)
Median, %	90.0	109.0	99.0
Interquartile ranges 25/50/75	83.5/90.0/100.5	89.0/109.0	75.0/99.0/105.0
FEV1 (% predicted), time of biopsy			
Mean (range), %	53.8 (35-83)	42.75 (34-53)	62.4 (44-86)
Median, %	55.0	42.0	61.0
Interquartile ranges 25/50/75	42.8/55.0/60.3	35.5/42.0/50.8	53.0/61.0/72.0
Time to biopsy since transplantation, median (range), days	739 (136-3305)	296 (125-1903)	550 (111-2159)

CBO indicates constrictive bronchiolitis obliterans; LB, lymphocytic bronchiolitis; PMN, polymorpho-nuclear cells; FEV1, forced expiratory volume.
Data are presented as n unless otherwise indicated.
 * Cell differentiation was not available for 2 patients in the CBO and CBO with infection group and for one patient in the Others group.
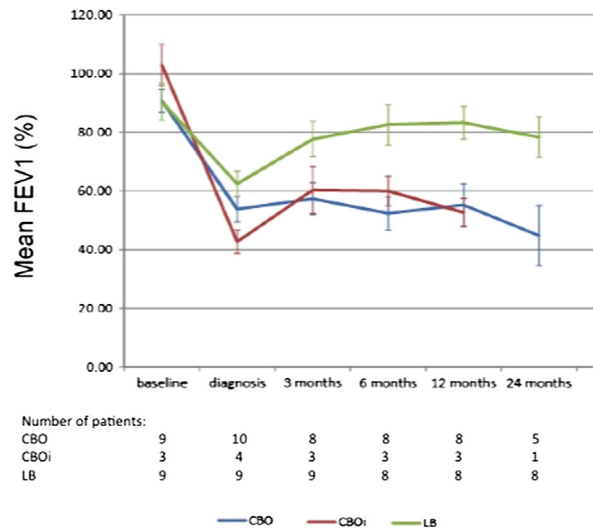
Figure 3. Mean forced expiratory volume in 1 second (FEV1) for the 3 groups with constrictive bronchiolitis obliterans (CBO), CBO with infection (CBOi), and lymphocytic bronchiolitis (LB) as percentage of predicted values. The time points shown are at baseline before hematopoietic stem cell transplantation, at the time of video-assisted thoracoscopic surgery (diagnosis), and after 3, 6, 12 and 24 month, respectively. Error bar indicates the standard error of the mean.

sirolimus rather than a calcineurin inhibitor. Further, 8 of 10 in the CBO and 6 of 9 in the LB group received mycophenolate mofetil as well as macrolides (5 of 10 in the CBO group; 3 of 9 in the LB group). All patients with CBO were treated with systemic steroids, as were 8 of 9 patients with LB; one of these was treated with <10 mg/day. Median duration of steroid treatment with a dosage of ≥10 mg/day was 32 weeks (range, 8 to 174 weeks) for the CBO and 13 weeks (range, 0 to 64 weeks) for the LB group. There was only a slight but not significant trend for less intensive steroid treatment in the LB group ($P = .09$).

Patients with infections (either RSV or PIV) were treated with intravenous immunoglobulins and, for RSV, ribavirin [28].

Follow-Up

Median follow-up time after obtaining a lung biopsy was 715 days (range, 39 to 1914) in the CBO group and 1224 days (range, 171 to 5666) in the LB group ($P = .221$). Median follow-up time after biopsy for surviving patients was 863 days (range, 130 to 1671) in the CBO and 1258 days (range, 453 to 5666) in the LB group ($P = .38$). One patient in the CBO group underwent lung transplantation; hence, follow-up was censored at the time of lung transplantation. This patient is alive 26 months after lung transplantation with an FEV1 of 106.9% of predicted value.

During follow-up after biopsies, there was no significant increment in the median FEV1 in the CBO and the CBOi group. In contrast, the LB group exhibited a statistically significant increase in the median FEV1 6 months after diagnosis ($P = .035$), maintaining this improvement at 12 months ($P = .017$). Median FEV1 between the 3 groups differed statistically upon 6 month after diagnosis. Neither the CBO nor the CBOi group regained their baseline values (Figure 3). In the LB group, FEV1 at 3 and 6 months after diagnosis were also significantly lower compared to baseline, but this difference was no longer detected after 12 and 24 months, when there was only a slight trend for a lower FEV1 compared to baseline. At last follow-up, there was a statistically significant difference in the median FEV1 values between the 2 groups: CBO, 44.4% (range, 16.8% to 86.7%) versus LB, 75.3% (range, 50.9% to 116%; $P = .014$).

Survival

For survival analysis, patients with infection present at the time of diagnosis were included in the corresponding groups, assuming that after resolution of the initial infection, survival would be mainly dependent on the histologic characteristics. Figure 4 shows the Kaplan-Meier survival curves

Table 3
Histology Versus NIH Criteria

Patient No.	Gender	NIH Criteria Fulfilled	Infection	FEV1 < 75% of Predicted	FEV1/FVC < 0.7	RV > 120%	HR-CT Criteria
CBO							
1	M	+	−	+	+	+	−
2	M	+	−	+	+	+	+
3	F	+	−	+	+	+	−
4	M	+	−	+	+	−	+
5	M	−	−	−	−	−	+
6	F	+	−	+	+	+	+
7	M	+	−	+	+	+	+
8	F	−	−	+	−	nd	−
9	M	−	−	+	+	−	−
10	F	+	−	+	+	+	+
LB							
1	M	+	−	+	+	−	+
2	M	+	−	+	+	+	+
3	M	−	−	+	−	−	+
4	M	+	−	+	+	+	+
5	F	−	−	+	−	+	+
6	F	−	−	+	−	−	−
7	F	−	−	−	−	−	+
8	M	−	−	−	−	−	+
9	F	−	−	+	−	+	+

NIH indicates National Institutes of Health; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; HR-CT, high-resolution computed tomography; CBO, constrictive bronchiolitis obliterans; LB, lymphocytic bronchiolitis; M, male; F, female; nd, not done.
Patients with infection were omitted because absence of infection is a prerequisite for the diagnosis of bronchiolitis obliterans according to the NIH.
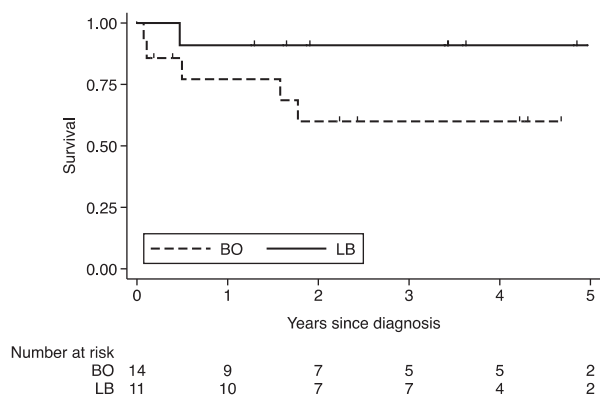
Figure 4. Kaplan-Meier survival curves for patients with constrictive bronchiolitis obliterans (BO, with and without infection, n = 14) and those with lymphocytic bronchiolitis (LB, with and without infection, n = 11); $P = .028$.

for patients with CBO and LB. Patient survivals at 1 and 3 years were 77% ($\pm$12%) and 60% ($\pm$14%), respectively, for patients with CBO and 91% ($\pm$9%) for patients with LB ($P = .028$).

During the observation period, 50% (7 of 14) of the patients with CBO died, whereas only 9% (1 of 11) of the patients with LB died. For patients with CBO, the causes of death were relapse and/or progression of an underlying disease (n = 3), sepsis (n = 1), respiratory failure (n = 2), and secondary malignancy (n = 1). Sepsis was the cause of death for the patient with LB. Three of the eight patients with histological diagnoses other than CBO and LB died during the follow-up period from respiratory insufficiency, secondary malignancy, or myocardial infarction.

DISCUSSION

In this retrospective, single-center study we included all patients who, after allogeneic HSCT, underwent lung biopsy for suspected BO. Although the practice for decision-making was not entirely consistent over the 2 decades and was been less rigorous in earlier years, a rather strict algorithm has been followed during the last 10 years, when most of the biopsies were performed. Even though this study was not designed to validate the current NIH consensus criteria for BOS, several important observations can be made.

First, in patients with similar clinical presentation, we found 2 distinct histological entities: classic CBO characterized by depositions of fibrous tissue, and LB characterized by lymphocytic infiltration of the bronchiolar wall without a significant fibrosis. Neither PFT results nor HR-CT findings at the time of VATS were predictive for the histologic findings. Although BAL showed a significantly higher proportion of lymphocytes in the LB group, this was not found to be useful for clinical practice, as there was a wide and overlapping distribution range.

Second, in our cohort, 7 patients with CBO and 2 with LB met the current NIH consensus BOS definition [16]. On the other hand, 7 patients, 50% of our patient cohort with histologically confirmed CBO, did not meet the consensus definition—4 of them because of concomitant airway infection. Furthermore, although this is a small patient number, signs of air trapping in HR-CT scans were only seen in about 30% of histologically proven CBO. Hence the current definition includes patients with different histological entities and may miss some patients with CBO. This may have an impact

on the interpretation of therapeutic interventional trial results if no histologic confirmation of BO is done. If these results are confirmed, the current NIH consensus BOS definition may need to be modified to increase sensitivity. In addition, the suspicion for evolving BO should remain high even in the presence of an infection. Finally, our results confirm that biopsy remains the gold standard for diagnosis of BO, as outlined in the NIH consensus documents. This statement is further supported by the fact that we found other distinct pathological diagnoses in 8 (24%) of our patients with strong suspicion of BOS. At least some of them didn't require intensified immunosuppressive therapy.

Third, if currently available surgical techniques are applied by skilled surgeons, VATS can be performed with a very low complication rate and perioperative risk. These results compare favorably with previous studies that reported lung biopsy is associated with significant morbidity and mortality, ranging from 13% to 47% [29,30]. Both skilled surgeons and progress made in minimally invasive surgical techniques and perioperative patient care might have contributed to this improvement.

Our study has several limitations. The most important is its retrospective nature and the small number of patients. However, because of the rarity of BO and the fact that only a few centers seek a histological diagnosis, our study is, to the best of our knowledge, the largest of its kind reported so far. The retrospective design and the lack of repeated biopsies during follow-up only allow generating some hypotheses. Hence it remains an open question if CBO and LB represent 2 different stages of the same disease process or if they are 2 different entities. It seems reasonable that an inflammatory process like LB precedes the fibrosis that is observed in CBO [31,32], as it has also been described in a case report [33] but the pathophysiology of CBO remains to be further elucidated. Because this was not a prospective study, histologic findings did not translate yet into different treatment strategies. Treatment was mainly guided by the further course of PFT. With similar immunosuppressive treatment, recovery of the lung function measured by FEV1 and survival were strikingly different between these 2 groups. Hence, if our hypothesis is true, our results suggest that intensive immunosuppression may be able to reverse the pathophysiological process if bronchiolitis is detected at an early stage when inflammatory changes predominate, and prevent the development of fibrosis, thus altering the otherwise poor prognosis of evolving BO. When constrictive changes are already established, intensive immunosuppression will not lead to improvements in airway narrowing, and may even contribute to further deterioration of lung function by increasing the susceptibility to infections or increasing the risk for relapse and/or the development of secondary malignancy. For these patients, new antiproliferative approaches need to be investigated.

In conclusion, lung biopsies for patients with suspected BO better characterize the BOS pattern following allogeneic HSCT. In contrast to CBO, LB, which may represent an inflammatory status preceding CBO, is associated with a good long-term prognosis. Further understanding of the pathophysiology of BOS is necessary to develop appropriate strategies for the early diagnosis and treatment of this severe post-transplantation complication.

ACKNOWLEDGMENTS

Hospital Basel for their excellent contributions to patient care and sample analysis.

REFERENCES

1. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med*. 2010;363: 2091-2101.
2. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106:2912-2919.
3. Afessa B. Pretransplant pulmonary evaluation of the blood and marrow transplant recipient. *Chest*. 2005;128:8-10.
4. Bhatia S, Francisco L, Carter A, et al. Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. *Blood*. 2007;110:3784-3792.
5. Tichelli A, Rovo A, Gratwohl A. Late pulmonary, cardiovascular, and renal complications after hematopoietic stem cell transplantation and recommended screening practices. *Hematology Am Soc Hematol Educ Program*. 2008;125-133.
6. Walter EC, Orozco-Levi M, Ramirez-Sarmiento A, et al. Lung function and long-term complications after allogeneic hematopoietic cell transplant. *Biol Blood Marrow Transplant*. 2010;16:53-61.
7. Sakaida E, Nakaseko C, Harima A, et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. *Blood*. 2003;102: 4236-4242.
8. Freudenberger TD, Madtes DK, Curtis JR, et al. Association between acute and chronic graft-versus-host disease and bronchiolitis obliterans organizing pneumonia in recipients of hematopoietic stem cell transplants. *Blood*. 2003;102:3822-3828.
9. Chien JW, Duncan S, Williams KM, Pavletic SZ. Bronchiolitis obliterans syndrome after allogeneic hematopoietic stem cell transplantation-an increasingly recognized manifestation of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2010;16:S106-S114.
10. Chien JW. Preventing and managing bronchiolitis obliterans syndrome after allogeneic hematopoietic cell transplantation. *Expert Rev Respir Med*. 2011;5:127-135.
11. Au BK, Au MA, Chien JW. Bronchiolitis obliterans syndrome epidemiology after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:1072-1078.
12. Santo Tomas LH, Loberiza FR Jr, Klein JP, et al. Risk factors for bronchiolitis obliterans in allogeneic hematopoietic stem-cell transplantation for leukemia. *Chest*. 2005;128:153-161.
13. Holland HK, Wingard JR, Beschorner WE, et al. Bronchiolitis obliterans in bone marrow transplantation and its relationship to chronic graft-v-host disease and low serum IgG. *Blood*. 1988;72:621-627.
14. Yoshihara S, Tateishi U, Ando T, et al. Lower incidence of Bronchiolitis obliterans in allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. *Bone Marrow Transplant*. 2005;35:1195-1200.
15. Duncker C, Dohr D, Harsdorf S, et al. Non-infectious lung complications are closely associated with chronic graft-versus-host disease: a single center study of incidence, risk factors and outcome. *Bone Marrow Transplant*. 2000;25:1263-1268.
16. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945-956.
17. Hildebrandt GC, Fazekas T, Lawitschka A, et al. Diagnosis and treatment of pulmonary chronic GVHD: report from the consensus conference on clinical practice in chronic GVHD. *Bone Marrow Transplant*. 2011.
18. Williams KM, Chien JW, Gladwin MT, Pavletic SZ. Bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. *JAMA*. 2009;302:306-314.
19. Dudek AZ, Mahaseth H, DeFor TE, Weisdorf DJ. Bronchiolitis obliterans in chronic graft-versus-host disease: analysis of risk factors and treatment outcomes. *Biol Blood Marrow Transplant*. 2003;9:657-666.
20. Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Resp J Suppl*. 1993; 16:5-40.
21. Yousem SA. The histological spectrum of pulmonary graft-versus-host disease in bone marrow transplant recipients. *Hum Pathol*. 1995;26: 668-675.
22. Yokoi T, Hirabayashi N, Ito M, et al. Broncho-bronchiolitis obliterans as a complication of bone marrow transplantation: a clinicopathological study of eight autopsy cases. Nagoya BMT Group. *Virchows Arch*. 1997; 431:275-282.
23. Beschorner WE, Saral R, Hutchins GM, et al. Lymphocytic bronchitis associated with graft-versus-host disease in recipients of bone-marrow transplants. *N Engl J Med*. 1978;299:1030-1036.
24. Azzola A, Havryk A, Chhajed P, et al. Everolimus and mycophenolate mofetil are potent inhibitors of fibroblast proliferation after lung transplantation. *Transplantation*. 2004;77:275-280.
25. Yates B, Murphy DM, Forrest IA, et al. Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med*. 2005;172:772-775.
26. Khalid M, Al Saghir A, Saleemi S, et al. Azithromycin in bronchiolitis obliterans complicating bone marrow transplantation: a preliminary study. *Eur Respir J*. 2005;25:490-493.
27. Nebiker CA, Lardinois D, Junker L, et al. Lung resection in hematologic patients with pulmonary invasive fungal disease. *Chest*. 2012;142: 988-995.
28. Khanna N, Widmer AF, Decker M, et al. Respiratory syncytial virus infection in patients with hematological diseases: single-center study and review of the literature. *Clin Infect Dis*. 2008;46:402-412.
29. Hayes-Jordan A, Benaim E, Richardson S, et al. Open lung biopsy in pediatric bone marrow transplant patients. *J Pediatr Surg*. 2002;37: 446-452.
30. White DA, Wong PW, Downey R. The utility of open lung biopsy in patients with hematologic malignancies. *Am J Respir Crit Care Med*. 2000;161:723-729.
31. Socie G, Blazar BR. Acute graft-versus-host disease: from the bench to the bedside. *Blood*. 2009;114:4327-4336.
32. Baird K, Pavletic SZ. Chronic graft versus host disease. *Curr Opin Hematol*. 2006;13:426-435.
33. Nakamoto-Matsubara R, Nishikii H, Yamada K, et al. Early pathologic findings of bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation: a proposal from a case. *Case Rep Hematol*. 2012; 2012:957612. http://dx.doi.org/10.1155/2012/957612.