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Review Article

Krebs von den Lungen 6 (KL6-): A Potential Predictive Biomarker for Bronchiolitis Obliterans in Pediatric and Young Adult Bone Marrow Transplant Recipients

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Abstract

Background

Bronchiolitis obliterans syndrome (BOS) is a serious complication of allogeneic bone marrow transplant (BMT) that is difficult to diagnose. There is a need for a biomarker to detect BOS in transplant recipients. Krebs von den Lungen-6 (KL-6) is a high molecular weight glycoprotein that is expressed on the epithelial surface of type II pneumocytes and bronchiolar epithelial cells in the lungs. We hypothesized that serum KL-6 levels would be elevated in subjects with known BOS.

Methods

This was a multi-center case control study that utilized convenience sampling. Serum samples were obtained from 20 subjects that underwent allogeneic BMT and 20 healthy controls between the ages of 6 months and 30 years. Of the 20 subjects that underwent BMT, 6 met the criteria for BOS. KL-6 levels were determined using a commercially available sandwich-type enzyme linked immunosorbent assay kit.

Results

Mean serum KL-6 levels (± standard deviation) for BMT subjects with BOS, BMT subjects without BOS and healthy controls were 641.5 (±517.1), 251.5 (±60.6) and 260.8 (±55.3) U/ml respectively. There was an overall difference in KL-6 levels between the three groups ($p<0.001$). Post hoc tests showed that KL-6 levels in BMT patients with BOS were significantly higher than those in both BMT patients without BOS ($p=0.001$) and healthy controls ($p=0.001$). There was no statistically significant difference in KL-6 levels between BMT subjects without BOS and healthy controls ($p=.99$).

Conclusions

KL-6 levels were significantly elevated in BMT subjects with BOS as compared those without BOS and healthy controls. KL-6 could serve as a biomarker to detect BOS in BMT recipients

Keywords: Bronchiolitis Obliterans Syndrome; Stem Cell Transplant; Rare Lung Diseases; Biomarkers; Pediatrics

Abbreviations

BMT: Bone Marrow Transplant;

BOS: Bronchiolitis Obliterans Syndrome;

cGVHD: Chronic Graft Versus Host Disease;

ELISA: Enzyme Linked Immunosorbent Assay;

FEV_1: Forced Expiratory Volume in first Second of Expiration;

FEV_1/FVC: Forced Expiratory Volume in First Second of Expiration/Forced Vital Capacity;

PFT: Pulmonary Function Testing;

KL-6: Krebs von den Lungen 6;

RV: Residual Volume;

RV/TLC: Residual Volume/Total Lung Capacity;

ELISA: Enzyme Linked Immunosorbent Assay

Introduction

Bronchiolitis obliterans syndrome (BOS) remains a rare and serious complication of allogeneic bone marrow transplant that affects approximately 6% of bone marrow transplant (BMT) recipients and up to 14% of patients with co-existing chronic graft-versus-host disease GVHD [1,2]. The 5 year survival rate is approximately 13% [2]. Bronchiolitis obliterans is a process that is characterized by a constrictive/obstructive bronchiolitis that begins with an inflammatory stage in which lymphocytic and neutrophilic infiltrates dominate the small airways [3]. This infiltration of the airways leads to the proliferation of fibroblasts and the accumulation of collagen that result in the irreversible obliteration (via fibrosis) of the airway lumen [3]. Clinically, the onset of BOS is insidious, non-specific, and challenging to diagnose. Symptoms of BOS include chronic cough, dyspnea, wheezing, or fatigue. However, early in the disease process, patients may be asymptomatic but given the morbidity and high mortality associated with BOS, early intervention may prove beneficial.

Pulmonary function testing (PFT) can serve as a useful tool in establishing the diagnosis of BOS and specific NIH criteria have been defined for BMT recipients which include a sustained decrease in Forced Expiratory Volume in first second of expiration (FEV_1) of <75% predicted, Forced expiratory volume in first second of expiration/Forced vital capacity (FEV_1/FVC) <0.7 (ratio), and increased residual volume (RV) >120%. Other criteria include CT scan of the lungs demonstrating air trapping or bronchiectasis, one other manifestation of cGVHD and the absence of infection [1]. These criteria are specific to adult populations that can accurately perform PFT and can cooperate with inspiratory/expiratory CT scans. Among pediatric patients, especially under the age of 7 years, PFT can be cumbersome and inaccurate because of the child's inability to follow specific instructions and/or lack of cooperation with testing and the same holds true inspiratory/expiratory CT scans. For some pediatric patients the only diagnostic method is invasive lung biopsy. To date, specific criteria for the diagnosis of BOS in the pediatric population have not been established.

Transbronchial biopsy is utilized to diagnose BOS but the diagnostic sensitivity is only between 20% to 50% [4]. When positive, open lung biopsy provides a histological diagnosis but since this disease state is often non-contiguous within an individual bronchiole, the disease is easily misdiagnosed by biopsy alone [3]. In addition, lung biopsies are associated with significant morbidity, especially in patients that are immunocompromised and have co-morbid conditions. Complication rates of lung biopsy approach 13% to 19% [4,5] and include pneumothorax, persistent air leak, mechanical ventilation and death [5-7]. Consequently, there is a need for a non-invasive diagnostic biomarker in both pediatric and adult BMT recipients in order to detect BOS earlier and more accurately.

Krebs von den Lungen 6 (KL-6) is a high molecular weight glycoprotein that is classified as MUC1 mucin and is expressed primarily on the epithelial surface of type II pneumocyte cells in the lungs [8]. It has also been shown to be present on the surface of bronchiolar epithelial cells [8, 9]. In subjects who do not have lung disease, a small amount of KL-6 is present in serum and it is presumed that during homeostasis a small amount of the glycoprotein crosses through the lung/blood interface into the circulation [3]. However with injury in the bronchioles, KL-6 levels have been shown to increase due to inflammation and the proliferation of fibroblasts [3, 10]. Likewise, it has been shown that KL-6 is an active chemotactic factor for fibroblasts and increasing concentrations of KL-6 resulted in increased migration of lung fibroblasts [11,12]. As such, KL-6 likely plays a role in the early pathogenesis of BOS [11].

The objective of this study was to establish whether KL-6 levels are elevated in the serum of patients with known brochiolitis obliterans syndrome (BOS) that have undergone allogeneic bone marrow transplant and to establish whether any differ-

ences exist in KL-6 levels between healthy controls and those that have undergone BMT without evidence of BOS. We hypothesized that serum KL-6 levels would be elevated in bone marrow transplant recipients with known BOS when compared to healthy controls and to BMT recipients without BOS.

Methods

This was a case control study that utilized convenience sampling. It was a multi-center study. Institutional Board Review approval for the study was obtained from Children's Mercy Hospital (IRB approval CMH0811-175E) as well as from the other participating institution, University of Kansas Cancer Center (IRB approval HSC11929), prior to the enrollment of any subjects. Informed consent and assent (when appropriate) was obtained from all participants in the study prior to collecting samples or medical history. Forty subjects were recruited: 6 with known BOS who underwent BMT, 14 without BOS that underwent BMT, and 20 healthy control subjects with no systemic illness that would affect lung function. Inclusion criteria include the following: 1) 6 months to 30 years of age; 2) allogenic bone marrow transplant recipients with known bronchiolitis obliterans that was confirmed with pulmonary function testing (PFT) using NIH criteria and/or with the histological diagnosis obtained from lung biopsy with clinical symptoms; 2) allogeneic BMT recipients with no known lung disease or clinical symptoms of BOS; 3) healthy control subjects with no known lung disease or any other chronic medical condition that could affect lung function. Exclusion criteria include the following**:** 1) subjects over the age of 30 years or less than 6 months of age; 2) subjects with any other lung disease or complication such as asthma, infection (bacterial, fungal, viral); 3) subjects that had undergone more than one allogeneic bone marrow transplant; 4) history of tobacco smoking. The diagnosis of BOS was based upon clincial symptoms, changes in pulmonary functions tests and/or histologic diagnosis from lung biopsy. Because some of the patients were too young to perform PFT, strict adult NIH critieria could not be applied to all of our study subjects.

KL-6 levels were determined using a commercially available sandwich-type enzyme linked immunosorbent assay (ELISA) kit as per the manufacturer's instructions (Sanko Junyaku Co., Ltd., Tokyo, Japan). A Five milliliter blood sample was obtained by venipuncture during a scheduled clinic visit. Samples were then centrifuged at 2150 Relative Centrifugal force for 10 minutes and the serum was extracted and stored at -70 degrees Celsius in cryovial tubes until samples were processed as per the manufacturer's protocol. The plate reader used to read the ELISA samples was the PerkinElmer Victor2 1420 Multilabel counter (MTX Lab Systems, Vienna, VA). All subjects were de-identified and given study numbers. The technician performing the ELISA testing was blinded to the study status of the subjects.

Comparison analyses of KL-6 levels between subjects post BMT with known BOS, subjects post BMT with no known lung disease and healthy age-matched controls were performed and validity scores were applied. One-way ANOVA with Tukey's post-hoc comparisons was used to compare serum KL-6 levels of BMT subjects with known BOS to those of BMT subjects without BOS and healthy controls. Receiver operator characteristics curve and Spearman's correlation co-efficient were performed. All statistical analyses were generated using SPSS version 18 statistical software (IBM, Armonk, New York). A p-value <0.05 was considered statistically significant.

Results

The mean age of healthy subjects was 17 years, BMT recipients without BOS were 16 years and BMT recipients with BOS were 17 years. Thirty-three percent of subjects were male and sixty seven percent were female. Of the 6 subjects that underwent BMT with the diagnosis of BOS, 4 had an underlying malignant condition and 2 had myelodysplastic syndrome that required BMT. Among BMT recipients that did not have the diagnosis of BOS (n=14), 64% (9/14) also had an underlying malignant condition. All but one patient with BOS had chronic GVHD. Of those subjects with BOS, all but 1 (83%) received a matched unrelated donor transplant. Table 1 depicts specific subject demographics and characteristics for BMT recipients with the diagnosis of BOS and those without BOS. Table 2 describes the subject's criteria for the BOS diagnosis.

Mean serum KL-6 levels ($\pm$ standard deviation) for BMT subjects with BOS, BMT subjects without BOS and healthy controls were 641.5 ($\pm$517.1), 251.5 ($\pm$60.6) and 260.8 ($\pm$55.3) U/ml respectively. All samples were analyzed in duplicate. Overall there was a significant difference between the groups in terms of KL-6 levels ($p<0.001$). KL-6 levels in BMT subjects with BOS were significantly higher than those of BMT patients without BOS ($p = 0.001$). BMT subjects with BOS also had significantly higher KL-6 levels than the healthy controls ($p=0.001$) (see Figure 1). There was no statistical difference between BMT subjects without BOS and healthy controls ($p=.99$). Among bone marrow transplant recipients, KL-6 levels were negatively correlated with predicted FEV_1 from pulmonary function testing using Spearman's correlation (rho=-0.555, $p=0.026$) (see Figure 2). One subject with BMT and BOS had a KL-6 level much larger than all of the other KL-6 levels (1602U/ml). The above analyses were repeated after excluding this value and no significant changes were found. The BMT with BOS group still had significantly higher KL-6 values as compared to both the BMT with no BOS group and the healthy controls (both $p =0.001$). The correlation between KL-6 and FEV_1 was still negative (rho=-0.545, $p=0.036$).

Subject	Diagnosis	Age	sex	Source	Related	Match	GVHD grade	KL6 Level	Status
	BOS								
1	**MDS**	**17**	**F**	**BM**	**N**	**10/10**	**4**	**324**	**alive**
2	**ALL**	**17**	**M**	**CORD**	**N**	**6/6**	**0**	**667**	**dead**
3	**AML**	**5**	**M**	**BM**	**N**	**9/10**	**3**	**1601**	**dead**
4	**MDS**	**23**	**F**	**PB**	**N**	**9/10**	**4**	**205**	**alive**
5	**ALL**	**26**	**F**	**PB**	**N**	**10/10**	**4**	**743**	**alive**
6	**AML**	**27**	**F**	**PB**	**Y**	**10/10**	**4**	**288**	**alive**
	NO BOS								
7	**Omen syndrome**	**3**	**M**	**BM**	**N**	**10/10**	**0**	**222**	**alive**
8	**ALL**	**10**	**F**	**BM**	**Y**	**10/10**	**0**	**290**	**alive**
9	**Hurler's disease**	**3**	**M**	**CORD**	**N**	**6/6**	**0**	**178**	**alive**
10	**ALL**	**16**	**F**	**PB**	**Y**	**10/10**	**0**	**251**	**alive**
11	**Beta Thalassemia major**	**15**	**M**	**BM**	**Y**	**10/10**	**0**	**196**	**alive**
12	**AML**	**17**	**F**	**BM**	**Y**	**10/10**	**0**	**217**	**alive**
13	**JMML**	**12**	**M**	**PB**	**N**	**10/10**	**0**	**310**	**alive**
14	**Aplastic anemia**	**15**	**F**	**BM**	**Y**	**10/10**	**1**	**210**	**alive**
15	**ALL/AML**	**4**	**F**	**BM**	**Y**	**10/10**	**0**	**166**	**alive**
16	**AML**	**25**	**F**	**PB**	**N**	**9/10**	**0**	**222**	**alive**
17	**AML**	**22**	**M**	**PB**	**N**	**8/8**	**2**	**288**	**alive**
18	**AML**	**30**	**M**	**PB**	**N**	**8/8**	**0**	**282**	**alive**
19	**ALL**	**29**	**M**	**CORD**	**N**	**6/6**	**0**	**384**	**alive**
20	**Aplastic anemia**	**25**	**F**	**BM**	**N**	**10/10**	**0**	**304**	**alive**

Table 1. Subject Characteristics. ALL- Acute Lymphoblastic Leukemia; AML - Acute Myelogenous Leukemia; JMML - Juvenile Myelomonocytic Leukemia; MDS - Myelodysplastic syndrome; BM bone marrow; PB peripheral blood; N No; Y Yes

Subject	Diagnosis	Age	NIH Criteria	Biopsy Proven	KL6 Level
1	**MDS**	**17**	**Yes**	**Yes**	**324**
2	**ALL**	**17**	**No**	**No**	**667**
3	**AML**	**5**	**Yes**	**Yes**	**1601**
4	**MDS**	**23**	**Yes**	**No**	**205**
5	**ALL**	**26**	**Yes**	**Yes**	**743**
6	**AML**	**27**	**Yes**	**No**	**288**

Table 2. Diagnostic Criteria for BOS Among Subjects. ALL acute lymphoblastic leukemia; AML acute myelogenous leukemia; MDS myelodysplastic syndrome

The Receiver Operating Characteristic Curve (ROC) was calculated in order to attempt to establish a serum KL-6 level that could best predict the presence of BOS in BMT patients (see figure 3). The area under the curve is 0.810 and the p-value is 0.032. Using a value of 288.5 or greater, the KL-6 test provides a sensitivity of 83% (CI .36-.99) and a specificity of 71% (CI .42-.92) with a positive predictive value of 56% (CI .21-.86) and negative predictive value of 91% (CI .59-.99) (see Figure 3).

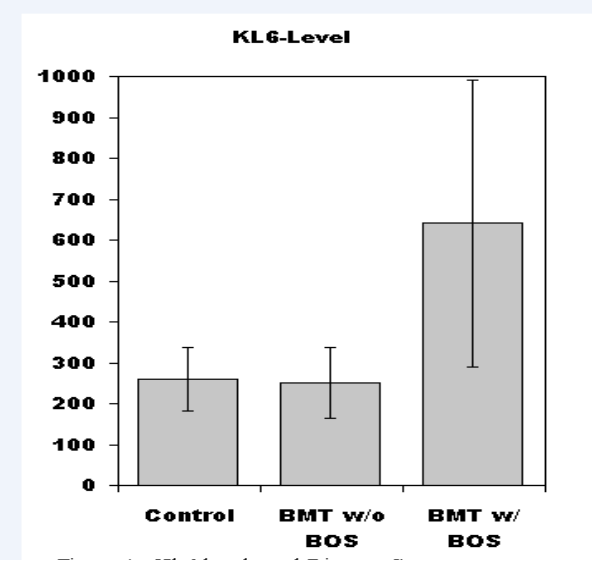

Figure 1. KL-6 levels in BMT subjects with BOS were higher than those of BMT patients without BOS (p = 0.001) and healthy controls (p=0.001). No statistical difference between BMT subjects without BOS and healthy controls (p=0.99).

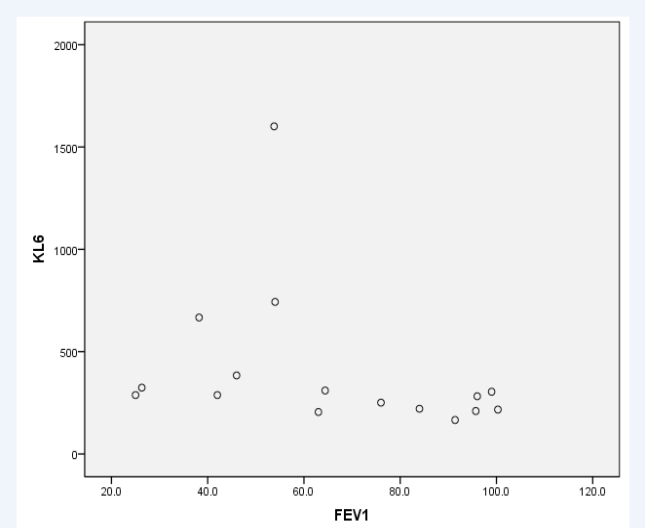

Figure 2. KL-6 levels were negatively correlated with predicted FEV1 from pulmonary function testing using Spearman's correlation (rho=-0.555, p=0.026).

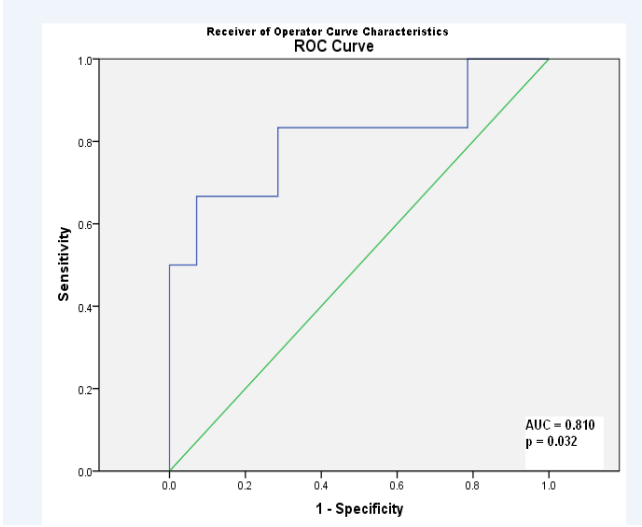

Figure 3. Using a value of 288.5 or greater, the KL-6 test provides a sensitivity of 83% (CI .36-.99) and a specificity of 71% (CI .42-.92) with a positive predictive value of 56% (CI .21-.86) and negative predictive value of 91% (CI .59-.99).

Discussion

Bronchiolitis obliterans is a diagnostic dilemma in both adult and pediatric populations. However, pediatric patients present a significant challenge, especially under the age of 7 years, because of their inability to perform PFT thus invasive lung biopsy alone remains the gold standard of diagnosis to date. Several biomarkers (sIL-2Rά, IL-6, TGF-β, sCD13, sBAFF, IL-8) have been identified to predict the development of both acute and chronic GVHD among BMT recipients [13,14]. However none of these markers are organ specific, especially for the lung. Our study suggests that KL-6 combined with clinical symptoms and PFT, if age appropriate, may serve as a useful, specific predictive biomarker to screen for BOS prior to lung biopsy or when PFT cannot be performed. There is a statistically significant relationship between elevated KL-6 levels and the diagnosis of BOS (p<.001). Using ROC analysis, we found that KL-6 is a specific biomarker for BOS in BMT recipients. Although our sample size was small, we were able to establish a diagnostic cut-off level for KL-6 (288 U/ml) that provided a sensitivity of 83% and negative predictive value of 91%. KL-6 levels do not appear to be increased in the BMT setting unless there is a diagnosis of BOS.

As diagnostic criteria evolve, KL-6 may potentially serve as an additional tool to detect BOS early in the disease process. For example, Chien et al. demonstrated that a decrease in FEV_1>5%/year and a FEV_1/FVC ratio of less than 0.8% identified airflow obstruction and this correlated with a 2.3 fold higher risk of mortality rates among BMT patients (p<0.001)

[15]. These subtle changes in FEV_1 annually likely represent the early stages of BOS [2]. In our study, 3 subjects (subjects 13, 17, 19), all of which had never been given the diagnosis BOS, were found to have decreased FEV_1 and increased RV from their baseline pre-transplantation testing in reviewing their medical records but they did not meet the strict NIH criteria for the diagnosis of BOS. Each of these subjects had a KL-6 level ≥288 U/ml. However, they remained clinically asymptomatic. These patients could potentially represent early asymptomatic disease state that is not detected with current criteria.

Recent data have also suggested that KL-6 is a useful serum marker for BOS in lung transplant patients [3, 10]. Walter et al. revealed that KL-6 serum levels in both pediatric and adult lung transplant recipients with known BOS were significantly elevated in lung transplant patients with BOS compared to lung transplant patients without BOS and to the healthy control subjects ($p<0.01$) [3]. Further work by Ohshimo et al. demonstrated a correlation between KL-6 levels and the decline in FEV_1. Their work also showed serum KL-6 levels to be a superior to neutrophilia in bronchoalveolar lavage fluid in detecting BOS [16]. Haberman et al. demonstrated in a prospective study that KL-6 levels that increased >200U/ml from baseline pre-transplantation levels in lung transplant recipients generates a sensitivity of 67% and a specificity of 95% for the diagnosis of BOS [10]. Our study also demonstrates similar differences among bone transplant recipients. A recent publication by Gassas et al. demonstrated a correlation between elevated KL-6 levels in pediatric patients in the asymptomatic phase of BOS after allogeneic stem cell transplant [17]. Our study lends further support to the usefulness of KL-6 as a marker for BOS in the stem cell transplant setting in both the pediatric and young adult population.

Increased KL-6 levels have been documented in other diseases including interstitial lung disease, sarcoidosis, radiation pneumonitis, pneumocystic pneumonia, Langerhan's cell histiocytosis in the lung, advanced measles and tuberculosis as well as other types of lung injury [18-24]. It has not routinely been associated with most bacterial, viral or fungal infections, asthma, chronic obstructive pulmonary disease [3]. Bone marrow transplant patients are screened routinely for viral infections and are evaluated for both bacterial and fungal infections in the face of any clinical symptoms. When KL-6 levels were obtained in our study, the subjects had no evidence of active viral, bacterial or fungal infection which suggests that the elevated KL-6 levels were due to BOS and not infection.

One of the limitations of this study is the small sample size of the study. This accounts for some of the width of confidence intervals as well as the large standard deviation for KL-6 levels among BMT recipients with BOS. However, the recent study conducted by Haberman et al. among lung transplant patients with BOS also revealed a wide standard deviation for KL-6 among lung transplant recipients with BOS and this study suggested that a fixed static KL-6 level is not as useful in identifying BOS as following trends in these levels after lung transplant and establishing a threshold of increase above baseline in order to better diagnose BOS [10]. Large prospective studies that follow KL-6 levels pre-BMT and then serially after BMT, might also reveal a similar finding and establish a useful threshold level of increase in the BMT setting as well. Until further studies are completed in the BMT setting, we have established a KL-6 value (288U/ml) among BMT recipients that correlated with the diagnosis of BOS. Establishing this static level among BMT recipients, especially when combined with newer proposed guidelines such as a decrease in FEV_1>5%/year, may assist in the early detection of BOS.

A possible criticism of our study is that not all KL-6 levels were obtained at the time of initial diagnosis of BOS and in the case of 2 subjects (subject 2 and 3), they already had very advanced disease when the samples were collected. These two subjects' KL-6 levels were exceptionally elevated as compared to other subjects and both of these patients died of BOS related complications. Subject 4 had significant clinical findings that included hypoxia, fatigue, dyspnea and required home oxygen months prior to obtaining the KL-6 level. This subject had been undergoing active treatment at the time the KL-6 level was obtained and had clinically improved, was no longer requiring oxygen and the KL-6 level was within a normal range. While the timing of sample collection during the disease process may be viewed by some as a limitation, it also provided us the opportunity to evaluate KL-6 levels in the spectrum of disease progression and resolution and theKL-6 levels correlated with the clinical findings of these extreme phenotypes. Certainly further longitudinal studies would be helpful to further delineate the usefulness of KL-6 as a marker of disease severity.

In conclusion, KL-6 is a potential biomarker for diagnosing BOS and following therapeutic response among BMT recipients. In particular, this biomarker may be useful in pediatric patients that cannot perform PFT when combined with clinical symptoms and radiographic findings. For patients capable of performing PFT, correlation studies that analyze RV, Residual volume/Total lung capacity RV/TLC, and a decrease in FEV_1 5%/year from baseline values, could also be useful in establishing KL-6 as a biomarker for disease. Prospective multi-center studies will be needed in order to better define the parameters of this marker in the bone marrow transplant setting. As drug therapies become more sophisticated with targeted molecules and medicine demands more cost effective and less invasive diagnostic methods as well as therapeutic monitoring techniques, equally sophisticated non-invasive biomarkers will be needed to serve as predictive diagnostic tools and pharmacodynamic endpoints for therapeutic response and drug

monitoring.

Conclusions

1. Bronchiolitis obliterans syndrome is an important and increasingly recognizable complication after allogenic-SCT

2. Bronchiolitis obliterans syndrome results in progressive circumferential fibrosis and ultimate cicatrization of the small terminal airways, manifesting as new fixed airflow obstruction.

3. Patients lack respiratory symptoms during mild stages of bronchiolitis obliterans syndrome, resulting in rare detection in the earliest stages of disease. Hence, KL-6 could be helpful in early detection of the disease.

4. The diagnosis of bronchiolitis obliterans syndrome is often challenging to establish, especially among pediatric patients who cannot perform pulmonary function testing due to young age. KL-6 is a potential non-invasive biomarker that could be useful in the early detection of bronchiolitis obliterans syndrome among bone marrow transplant recipients.

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References

1. Chien JW. Preventing and managing bronchiolitis obliterans syndrome after allogeneic hematopoietic cell transplantation. Expert Rev Respir Med. 2011, 5(1): 127-135.

2. Williams KM, Chien JW, Gladwin MT, Pavletic SZ. Bronchiolitis obliterans after allogeneic hematopoietic stem cell transplantation. JAMA. 2009, 302(3): 306-314.

3. Walter JN, Fan LL, Bag R, Zhang H, Doan M et al. Serum KL-6 as a marker for bronchiolitis obliterans syndrome after lung transplantation. *Transplantation.* 2006, 82(5): 709-711.

4. 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(1 Suppl): S106-114.

5. Bensard DD, McIntyre RC, Jr., Waring BJ, Simon JS. Comparison of video thoracoscopic lung biopsy to open lung biopsy in the diagnosis of interstitial lung disease. Chest. 1993, 103(3): 765-770.

6. 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(3 Pt 1): 723-729.

7. Ayed AK, Al-Shawaf E. A survey of 150 video-assisted thoracoscopic procedures in Kuwait. Med Princ Pract. 2004, 13(3): 159-163.

8. Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. Am J Respir Crit Care Med. 1999, 159(2): 646-678.

9. Smith KJ, Fan LL. Insights into post-infectious bronchiolitis obliterans in children. Thorax. 2006, 61(6): 462-463.

10. Haberman B, Doan ML, Smith EO, Schecter MG, Mallory GB et al.. Serum KL-6 level and the development of bronchiolitis obliterans syndrome in lung transplant recipients. Pediatr Transplant. 2010, 14(7): 903-908.

11. Hirasawa Y, Kohno N, Yokoyama A, Inoue Y, Abe M et al. KL-6, a human MUC1 mucin, is chemotactic for human fibroblasts. Am J Respir Cell Mol Biol. 1997, 17(4): 501-507.

12. Ohshimo S, Yokoyama A, Hattori N, Ishikawa N, Hirasawa Y et al. KL-6, a human MUC1 mucin, promotes proliferation and survival of lung fibroblasts. Biochem Biophys Res Commun. 2005, 338(4): 1845-1852.

13. Fujii H, Cuvelier G, She K, Aslanian S, Shimizu H et al. Biomarkers in newly diagnosed pediatric-extensive chronic graft-versus-host disease: a report from the Children's Oncology Group. Blood. 2008, 111(6): 3276-3285.

14. Paczesny S, Krijanovski OI, Braun TM, Choi SW, Clouthier SG et al. A biomarker panel for acute graft-versus-host disease. Blood. 2009, 113(2): 273-278.

15. Chien JW, Martin PJ, Gooley TA, Flowers ME, Heckbert SR et al. Airflow obstruction after myeloablative allogeneic hematopoietic stem cell transplantation. Am J Respir Crit Care Med. 2003, 168(2): 208-214.

16. Ohshimo S, Bonella F, Grammann N, Starke K, A Cui et al Serum KL-6 as a novel disease marker in adolescent and adult Cystic Fibrosis. Sarcoidosis vasculitis and diffuse lung diseases. 2009, 26(1): 47-53.

17. Gassas A, Tal Schechter, Joerg Krueger, Hayley Craig-Barnes, Lillian Sung et al Serum Krebs Von Den Lungen-6 as a Biomarker for Early Detection of Bronchiolitis Obliterans

Syndrome in Children Undergoing Allogeneic Stem Cell Transplantation. Biology of Blood and Marrow transplantation. 2015, 21(8): 1524–1528.

18. Oyama T, Kohno N, Yokoyama A, Hirasawa Y, Hiwada K et al. Detection of interstitial pneumonitis in patients with rheumatoid arthritis by measuring circulating levels of KL-6, a human MUC1 mucin. Lung. 1997, 175(6): 379-385.

19. Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R et al. Comparative evaluation of serum markers in pulmonary sarcoidosis. Chest. 2010, 137(6): 1391-1397.

20. Goto K, Kodama T, Sekine I, Kakinuma R, Kubota K et al. Serum levels of KL-6 are useful biomarkers for severe radiation pneumonitis. Lung Cancer. 2001, 34(1): 141-148.

21. Hamada H, Kohno N, Yokoyama A, Hirasawa Y, Hiwada K et al. KL-6 as a serologic indicator of Pneumocystis carinii pneumonia in immunocompromised hosts. Intern Med. 1998, 37(3): 307-310.

22. Matsubayashi T, Miwa Y, Saito I, Matsubayashi R. KL-6: marker for pulmonary involvement in Langerhans cell histiocytosis in infants. J Pediatr Hematol Oncol. 2004, 26(9): 584-586.

23. Narita M, Nakayama M, Yamada S, Togashi T. Elevated KL-6 levels in fatal measles pneumonia. Eur J Pediatr. 2001, 160(7): 454-455.

24. Inoue Y, Nishimura K, Shiode M, Akutsu H, Hamada H et al. Evaluation of serum KL-6 levels in patients with pulmonary tuberculosis. Tuber Lung Dis. 1995, 76(3): 230-233.