Lymphocytopenia in Children With Lymphatic Malformation

Richard M. Tempero, MD, PhD; Mark Hannibal, MD, PhD; Laura S. Finn, MD; Scott C. Manning, MD; Michael L. Cunningham, MD, PhD; Jonathan A. Perkins, DO

Objective: To determine whether an immunologic abnormality exists in patients with lymphatic malformation (LM).

Design: Retrospective case series.

Setting: Tertiary care pediatric hospital.

Patients: Twenty-one consecutive patients (11 male and 10 female) undergoing LM treatment.

Interventions: Clinical data (ie, age, clinical LM stage, radiographic appearance, and histologic findings) were correlated with complete blood cell count and detailed lymphocyte differential. Complete blood cell counts and lymphocyte subsets were measured in 21 and 18 patients, respectively.

Results: The average age at the time of testing was 67 months (range, 1-231 months). The patients were categorized according to LM stage, including 4 (19%) with stage 1, 4 (19%) with stage 2, 4 (19%) with stage 3, 7 (33%) with stage 4, and 2 (10%) with stage 5 disease.

Radiographic LM appearance was macrocystic in 6 patients (29%), mixed macrocystic and microcystic in 8 (38%), and microcystic in 7 (33%). Complete blood cell count data demonstrated lymphocytopenia in 6 patients (29%). The results of the lymphocyte subset tests showed concomitant T-, B-, and natural killer (NK)–cell deficiency in 6 (33%) of 18 patients. All 6 patients with T-cell lymphocytopenia had normal neutrophil and platelet counts. Spearman rank and χ² analyses showed that LM stage 4 or 5 and microcystic LM were significantly associated with lymphocytopenia (P=.002 and P=.008, respectively). Histologic analysis did not demonstrate increased lymphocytes in any LM specimens.

Conclusion: We found T, B, and NK lymphocytopenia in patients with large bilateral or microcystic LM. Although the relationship between lymphocytopenia and infection was not addressed in this study, the recognition of lymphocytopenia in patients with LM may have important clinical and prognostic implications.


Author Affiliations:
Departments of Otolaryngology–Head and Neck Surgery (Drs Tempero, Manning, and Perkins) and Pediatrics (Drs Hannibal and Cunningham) and Division of Genetics and Developmental Medicine (Dr Hannibal), University of Washington, and Department of Pathology (Dr Finn), Division of Pediatric Otolaryngology (Drs Manning and Perkins), and Children’s Craniofacial Center (Drs Cunningham and Perkins), Children’s Hospital and Regional Medical Center, Seattle.

Lymphatic Malformations (LMs) are uncommon congenital vascular anomalies that occur throughout the body but most often involve the head and neck (90% of patients). Clinical features can be diverse but primarily relate to the regional presence of the LM. In certain patients, the form and function of adjacent anatomic structures is significantly disturbed. It has long been recognized that the pathophysiology of LM and the clinical course of patients with LM varies with the type of LM. Macrocytic LMs are effectively treated with several methods. Surgical resection and/or intraluminal sclerotic agents such as OK-432 (Picinabil) are indicated for LM size reduction. Larger LMs with microcystic characteristics are often difficult to eradicate, and full extirpation is rare. Extensive clinical observation has been used to derive prognostic factors and staging systems, based on LM structure, size, and location, that can predict clinical outcomes.

One remarkable clinical observation is that infections localized to LM occur in 30% to 70% of patients with LMs. Clinical presentation is diverse, ranging from skin cellulitis overlying localized LM inflammation to recurrent, occasionally severe, viral and bacterial infections of the respiratory system. In some series, recurrent LM inflammation is associated with development of localized tissue hypertrophy, complicating LM management. An isolated case of persistent T-cell deficiency with LM and recurrent infection has been reported. The underlying pathol-
Mixed and suprhyoid disease. A detailed lymphocyte differential count that included the absolute number and percentage of T cells (T4 [CD3+ and CD4+] and T8 cells [CD3+ and CD8+]), B cells (CD19+), and natural killer (NK) cells (CD16+ and CD56+) was obtained (Epics XL flow cytometer; Coulter Beckman, Fullerton, Calif). Cell surface markers were used to specifically identify T4, T8, B, and NK cells. All normal values for lymphocyte counts and hematopoietic tests were adjusted for age. To be considered lymphocytopenic, a patient’s lymphocyte counts had to be less than the lower limit of the reference range for their age (ie, T4 cell absolute number for ages 5–10 years, 300 cells/µL). For purposes of analysis, lymphocytopenia was defined as an absolute lymphocyte count of less than 1500 cells/µL. In the detailed lymphocyte differential count, lymphocytopenia was defined as follows: T4 cells less than 300 cells/µL, T8 cells less than 300 cells/µL, B cells less than 200 cells/µL, and NK cells less than 150 cells/µL.

Histologic review was conducted to explore the hypothesis that lymphocytes were selectively depleted from the peripheral blood and sequestered in the LM. The reviewer (L.S.F.) was blinded to LM stage, radiographic description, and associated hematologic data. All hematoxylin–eosin–stained slides from each case were scanned (original magnification ×40), and the slide with the most inflammation was selected; sections that included lymph nodes or thymus were excluded. Each selected slide was reviewed at ×200 magnification to locate and quantify LM inflammation. The number of these fields with inflammation and the number per slide were determined, yielding the percentage of surface area per slide with inflammation. Inflammation in the interstitium or lymphatic lumens was recorded in a semiquantitative manner as follows: scattered lymphocytes indicated rare to few cells; aggregate lymphocytes, clusters of cells (>30 cells).

Descriptive statistics were used to determine the prevalence of LM type (eg, macrocystic or microcystic), LM stage, histologic characteristics, and hematologic abnormality in our study sample. The relationships between LM stage and the various hematologic measures were assessed using Spearman rank correlation analysis. Spearman correlation is a nonparametric, rank-based method that is appropriate for use with continuous and ordinal scale variables. For LM type, which is a nominal (categorical) variable, the nonparametric Kruskal–Wallis test was used to compare lymphocyte counts among the 3 diagnosis categories. Comparison of histologic measurements on LM specimens between subjects with and without lymphocytopenia was conducted with the Wilcoxon rank sum test. This study was approved by the Institutional Review Board of the Children’s Hospital and Regional Medical Center.

Figure 1. Significant negative correlation exists between absolute lymphocyte number and lymphatic malformation (LM) stage (A) and microcystic radiographic characteristics (B) in 21 patients. The LM stages are described in the “Methods” section.

Methods

As a part of the routine medical care, hematologic data (ie, complete blood cell count) were collected from 21 consecutive patients with LM not undergoing active treatment for more than 7 days, from January 1, 1999, through December 31, 2004, at Children’s Hospital and Regional Medical Center, Seattle, Wash. In patients with a history of unexplained recurrent LM inflammation or swelling, a lymphocyte differential count was obtained. Inclusion criteria were an established or new diagnosis of LM (categorial) variable, the nonparametric Kruskal–Wallis test. This study was conducted with the Wilcoxon rank sum test. This study was approved by the Institutional Review Board of the Children’s Hospital and Regional Medical Center.

Results

We included 11 male and 10 female patients aged 1 month to 19 years (mean age, 67 months [SD, 56 months]) at the time of testing. Nineteen patients had a history of LM inflammation. Medical therapy consisted of intermittent oral antibiotics and oral corticosteroids in 18 patients and antibiotics alone on 1 occasion in the remaining 3. Invasive LM treatment consisted of surgery in 14 patients, sclerotherapy in 1, and combined surgery and sclerotherapy in 1. These procedures were performed more than 6 months before testing. The remaining 5 patients had not had invasive therapy at the time of the study. Results of LM staging indicated that 4 patients had stage 1 disease; 4, stage 2; 4, stage 3; 7, stage 4; and 2, stage 5. Radiographic imaging showed that 7 (33%) of 21 pa-

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tients had microcystic LM characteristics; 6 (28%), macrocystic; and 8 (38%), mixed.

Complete blood cell count data were obtained in all 21 patients and demonstrated absolute lymphocytopenia in 6 (29%). The mean age of patients with lymphocytopenia at the time of testing was 90.3 months (SD, 44.5 months). A significant negative correlation existed between absolute lymphocyte number and LM stage \( (P = .002; \text{Spearman rank} \gamma = -0.64) \) (Figure 1A). A significant association was also shown between absolute lymphocyte number and microcystic LM characteristics \( (P = .007) \) (Figure 1B).

The results of lymphocyte subset analysis, obtained in 18 patients with recurrent LM inflammation, showed a significant lymphocytopenia in T4 (CD4\(^+\)), T8 (CD8\(^+\)), B (CD19\(^+\)), and NK (CD16\(^+\)) cells that correlated negatively with LM stage (Spearman rank \( \gamma \), \(-0.80 [P = .001], -0.83 [P < .001], -0.55 [P = .02], \) and \(-0.56 [P = .02] \), respectively) (Figure 2A-C). However, a nonsignificant correlation existed between LM stage compared with a normal peripheral hematocrit reading and absolute neutrophil and platelet counts (Spearman rank \( \gamma \), \(-0.15 [P = .31], 0.27 [P = .24], \) and \(-0.06 [P = .81] \), respectively).

Histologic review of available hematoxylin-eosin–stained LM specimens was conducted in 7 patients (4 with and 3 without lymphocytopenia) to assess the presence and location of lymphocytes. The number of fields (at \( \times 200 \) magnification) per slide averaged 81 (range, 63-96). The LM tissue from patients with lymphocytopenia had an average of 10% (range, 3%-24%) of fields with lymphocyte presence. In comparison, the LM tissue from patients with normal lymphocyte counts had an average of 31% (range, 22%-45%) of fields with lymphocyte presence. There was no significant difference between these 2 groups \( (P = .08) \). The LM lumen (Figure 3) did not contain significant numbers of scattered or aggregated lymphocytes in any specimen (scattered, \( P = .33 \); aggregated, \( P = .66 \)). Interstitial LM tissue had few scattered lymphocytes in all specimens, whereas 1 specimen, from a patient with a normal lymphocyte count, had clusters in cell aggregates (Figure 3B and Figure 4). There was a difference in scattered and aggregate lymphocyte presence in patients with normal lymphocyte counts compared with those with low lymphocyte counts (scattered, \( P = .03 \); aggregate, \( P = .02 \)).

**COMMENT**

This prospective case series describes a significant relationship between T-, B-, and NK-cell lymphocytopenia and patients with large bilateral microcystic LM. Indices of bone marrow function (platelet count, hematocrit, and neutrophil count) were low in several patients with stages 4 and 5 LM and lymphocytopenia; however, a positive or negative trend could not be established between these indices and LM stage. From these data, evidence for central immune suppression or bone marrow failure and pancytopenia could not be generated. Rather, these findings support a model of selective lymphocytopenia.

Lymphocytopenia can result from 3 abnormalities: decreased lymphocyte production, altered lymphocyte traffic, and lymphocyte loss. The etiology of lymphocytopenia in patients with LM is not clear; however, it is
strongly associated with advanced LM stage and structure. Extensive medical management, frequently required for large microcystic LM, could decrease lymphocyte production, as has been demonstrated in animal models. In our series, patients were not receiving active medical treatment at the time of testing, so drug-induced lymphocytopenia is less likely. The 2 patients (with stage 5 disease) in our series who required mediastinal dissection and partial thymectomy had lymphocytopenia. Partial thymectomy may affect normal thymic T-cell maturation, reducing T-cell production, as has been described after thymectomy for other conditions. Viruses and certain robust inflammatory responses can induce transient lymphocytopenia through lymphocyte destruction or loss. In 5 of 6 patients, lymphocytopenia was noted during periods of good health.

Could the LM have negative regulatory effects on peripheral immune response and lymphocyte trafficking? In a case report of lymphocytopenia and LM, a “reservoir” model for lymphocytopenia is proposed. In this model, peripheral T-cell lymphocytopenia is secondary to lymphocyte accumulation in the malformation. This model is similar to intestinal lymphangiectasia, where lymphocytes are lost in the gastrointestinal tract because of blocked intestinal lymphatics. Histopathologic review of available LM tissue showed limited numbers of mononuclear cells in and around the malformation. There was no increase in malformation inflammatory cell numbers in patients with lymphocytopenia. Our results do not support the hypothesis that LM sequesters lymphocytes.

The limitations of this study are the small number of patients enrolled, the small number of histologic specimens, the lack of serial immunologic evaluation, and the lack of other studies of immunologic function. The small number of patients and lack of multiple measures of immune function make it impossible to establish a causal relationship between T-cell lymphocytopenia and infection.

From a clinical standpoint, these results may have important clinical and prognostic implications. An associa-
tion appears to exist between individuals with large bilateral microcystic LM with recurrent infection and lymphocytopenia. Future prospective longitudinal immunologic data collection will help determine the etiology of lymphocytopenia with LM, whether this is independent of LM treatment, and how lymphocytopenia affects treatment outcomes. The recognition of lymphocytopenia in patients with LM should prompt the referral to clinical immunologists or infectious disease providers to complement care. With further investigation, it may be possible to establish lymphocytopenia as another variable, in addition to structure, size, and location, to use in the staging of LM.

Submitted for Publication: February 24, 2005; final revision received June 15, 2005; accepted August 24, 2005.

Correspondence: Jonathan A. Perkins, DO, Division of Otolaryngology–Head and Neck Surgery, Children’s Hospital and Regional Medical Center, 4800 Sand Point Way NE, MailStop G-0035, PO Box 5371, Seattle, WA 98105 (jonathan.perkins@seattlechildrens.org).

Financial Disclosure: None.

Previous Presentation: This study was presented at the 20th Annual Conference of the American Society of Pediatric Otolaryngology; May 30, 2005; Las Vegas, Nev.

Acknowledgment: We thank Kristy Seidel, BS, for statistical support and Kathleen J. Beaudry, MA for manuscript preparation.

REFERENCES


