

Sonographic Assessment of Peripheral Lymph Nodes In Early-Stage Mycosis Fungoides: Diagnostic Value And Prognostic Implications

Banu İsmail Mendi¹, Hatice Şanlı², Bökebatur Ahmet Raşit Mendi³, Ahmet Taha Aydemir⁴, İncilay Kalay Yıldızhan², Evren Üstüner⁵, Bengü Nisa Akay²

1 Department of Dermatology, Niğde Ömer Halisdemir University Faculty of Medicine, Niğde, Turkey

2 Department of Dermatology, Ankara University Faculty of Medicine, Ankara, Turkey

3 Department of Radiology, Ankara Yıldırım Beyazıt University Faculty of Medicine, Ankara, Turkey

4 Department of Dermatology, Yozgat City Hospital, Yozgat, Turkey

5 Department of Radiology, Ankara University Faculty of Medicine, Ankara, Turkey

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Corresponding Author: Banu İsmail Mendi, Department of Dermatology, Niğde Ömer Halisdemir University Faculty of Medicine. ORCID: 0000-0003-4890-1484. E-mail: banuismail92@gmail.com

ABSTRACT Introduction: Imaging studies are infrequently performed in patients with early-stage mycosis fungoides (MF), resulting in limited research on lymph nodes within this group.

Objectives: To determine which patient groups are more likely to have sonographically pathological lymph nodes and to assess the relationship between sonographic lymph node features and progression to advanced stage.

Methods: Characteristics of early-stage MF patients with lymphadenopathy were recorded retrospectively. Logistic regression analyses were performed to evaluate associations between patient characteristics and sonographic findings as well as to assess the relationship between sonographic features and progression to advanced disease stages.

Results: In the study, 70 patients were examined, revealing lymphadenopathy at a single site in 18.6% and multiple sites in 81.4%. The average long axis length was 23.4 mm, and the average short axis length was 8.4 mm. Diffuse cortical thickening was observed in 14.4%, asymmetric thickening in 3.8%, fatty hilum loss in 4.8%, heterogeneity in 1%, contour irregularity in 0.5%, and non-hilar,

disorganized blood flow in 0.5% of the lymph nodes. Biopsies were performed on 13 lymph nodes post-ultrasound, with 195 nodes monitored subsequently. Of these, 42 nodes did not regress, while 153 regressed. A total of 30 lymph nodes were biopsied, identifying 28 as N1, one as N2, and one as N3. Younger age and elevated beta-2 microglobulin levels correlated with pathological features and persistent lymphadenopathy.

Conclusions: Considering the potential morbidities linked to lymph node biopsy, evaluating ultrasound features might be essential to patient selection. An earlier biopsy could be justified in younger patients with increased beta-2 microglobulin levels.

Introduction

Mycosis fungoides (MF) is the most prevalent type of cutaneous T-cell lymphoma, with most patients presenting at an early stage. Progression to advanced stage occurs slowly, and approximately 16% of patients progress to later stages, while the majority remain in early-stage disease for extended periods [1]. In the TNMB staging system, early stage is characterized by patchy or plaque skin lesions, minimal or no blood involvement, or dermatopathic or low-grade lymph node involvement. The International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer (ISCL/EORTC) guidelines define peripheral lymph nodes as clinically abnormal if they measure 1.5 cm or greater in diameter or if any palpable node is firm, irregular, clustered, or fixed during a physical examination, regardless of size. Excisional biopsy is recommended as the preferred method for evaluating abnormal lymph nodes [2]. However, excisional lymph node biopsy is an invasive procedure that may lead to complications such as infection, bleeding, nerve palsy, and anesthesia-related issues. Locating the lymph node during the procedure can be challenging and often requires marking to ensure accurate removal [3]. Additionally, the guidelines highlight that reactive or dermatopathic lymph nodes are frequently observed in patients with MF, Sézary syndrome, or benign inflammatory dermatoses, and the possibility of multiple lymph node enlargements exists [2].

A multicenter study evaluating lymph nodes in early-stage MF demonstrated that many enlarged lymph nodes were missed by clinical examination alone, emphasizing the importance of lymph node imaging in early-stage patients [4]. The accurate identification and characterization of lymph nodes by imaging has important therapeutic and prognostic significance in patients with newly diagnosed malignancies [5]. Ultrasonography (US), due to its ease of use and availability, is highly effective in assessing cervical, axillary, and inguinal lymph nodes. Normal lymph nodes exhibit sonographic characteristics as somewhat flattened hypoechoic structures with varied quantities of hilar fat [6]. They may exhibit hilar vascularity but are often hypovascular [7]. The

sonographic features of the lymph nodes are altered by malignant infiltration, resulting in enlarged nodes that are typically rounded and exhibit peripheral or mixed vascularity [8]. Utilizing these criteria, US has demonstrated an accuracy of 89%–94% in distinguishing malignant from benign lymph nodes [9]. The aim of our study was to assess the utility of peripheral lymph node ultrasound in patients with early-stage MF. This evaluation was intended to objectively assess the subjective lymph node assessment through physical examination and to ensure the accurate and appropriate selection of patients for biopsy.

Material and Methods

Data from patients aged 18 years and older who presented to the Skin Lymphoma Outpatient Clinic of the Department of Dermatology at Ankara University between January 2006 and August 2024, were diagnosed with early-stage MF according to the ISCL criteria, and had sonographic lymph node enlargement (longest diameter ≥ 1.5 cm) were analyzed retrospectively [10]. Approval from the Ankara University Faculty of Medicine Institutional Review Board was obtained. Patients receiving systemic treatment for MF and/or those with secondary malignancy and/or systemic inflammatory disease were excluded from the study. Demographic, clinical, laboratory, dermatopathological, and lymph node sonographic data were recorded at the time of diagnosis, along with follow-up sonographic data from lymph nodes scheduled for monitoring. Given the subjective nature of clinical examination, all patients diagnosed with early-stage MF in our clinic are routinely screened for peripheral lymphadenopathy with US at the time of diagnosis. Ultrasound examinations of the cervical, axillary, and inguinal regions were performed using 7.5 MHz PLT-704AT linear transducers with the Toshiba Aplio 80-SSA system (Japan) between 2006 and 2018. From 2018 onward, 8–10 MHz GE 9L-D linear probes were used with the GE LOGIQ S8 XDclear system (USA). Lymph nodes identified as sonographically pathological and recommended for biopsy exhibit the following characteristics: i) absence of the fatty hilum, ii)

asymmetric or diffuse cortical thickening, iii) cortical heterogeneity, iv) irregular contours, v) non-hilar, cortical disorganized blood flow on color Doppler examination, and vi) short-axis diameter of ≥ 1.5 cm [11]. A demonstrative pathological lymph node ultrasound image is provided in Figure 1. Lymph nodes with a long axis measurement of ≥ 1.5 cm yet not fulfilling these criteria are monitored sonographically at intervals of three to six months. Biopsies are conducted on nodes that fail to regress (decrease in size). Excisional biopsies were obtained from lymph nodes meeting biopsy criteria. Lymph node pathology was evaluated in accordance with the NCI-VA classification system.

Statistical Analysis

Statistical analyses were performed using XLSTAT software, version 2025.1.2 (Addinsoft, Paris, France). Continuous variables are presented as mean \pm standard deviation or median (range), and categorical variables are presented as frequencies and percentages. Multivariate logistic regression analyses were used to evaluate the associations between pathological lymph node findings on US and patient characteristics as well as the relationship between non-regressive lymph nodes and patient characteristics. In these analyses, the presence of any pathological finding on US and non-regressive lymph node were defined as the dependent variable. Demographic, clinical, laboratory, and dermatopathological variables were included in the models as independent variables. In our analyses, independent variables with a prevalence below 10% were removed from the model to increase the statistical power of the multivariate logistic regression model and the reliability of the coefficient estimates. Including low-prevalence variables can negatively impact a model's predictive accuracy by leading to bias in regression coefficients, increased variability, and widening confidence intervals [12,13]. This approach is a frequently employed strategy, particularly in studies with limited sample sizes, to prevent model overfitting and obtain more robust results [14].

Due to the limited number of patients, a direct multivariate logistic regression model was used to reduce the risk of overfitting and obtain more robust results. Direct modeling was preferred over stepwise variable selection methods due to the potential for bias in coefficient estimates and elevated type I error rates, particularly with small sample sizes [15,16]. This approach ensures that the model is constructed on a theoretical basis and that all relevant variables are evaluated simultaneously. Furthermore, a similar multivariate logistic regression analysis was conducted for progression to advanced stages (IIB and beyond). In this analysis, the relevant dependent variable and the aforementioned demographic, clinical, and dermatopathological characteristics were included as independent variables in the model.

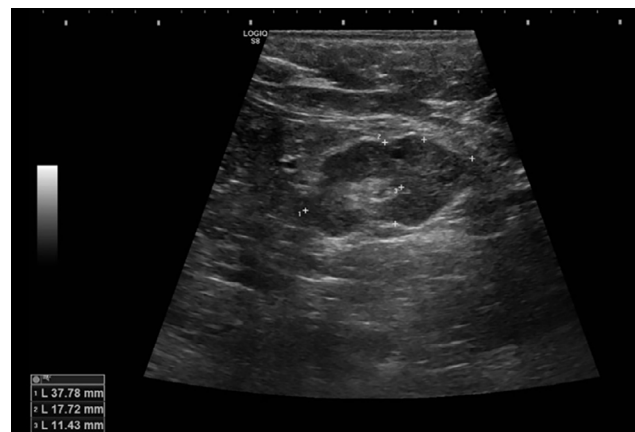


Figure 1. In this 51-year-old male patient, the left axillary lymph node demonstrates abnormally increased size (37.8 \times 17.7 mm) and cortical thickness (11.4 mm).

Analysis results are presented with odds ratios (OR) and 95% confidence intervals (CI). The significance level for all statistical tests was set at $P < 0.05$.

Results

The study included seventy patients, with 36 (51.4%) being female and 34 (48.6%) male. The average age was 50.7 years. The mean follow-up duration was 99.2 months. Among the patients, 36 (51.4%) were classified as T1 and 34 (48.6%) as T2. Thirty-one patients presented with only patch lesions, while 39 had both patch and plaque lesions. Dermatopathological analysis revealed that 44 (62.0%) were of the classical type and 15 (21.1%) were of the folliculotropic type (Table 1). Thirteen patients (18.6%) progressed to advanced stage: ten to stage IIB, one to stage IIIA, one to stage IIIB, and one to stage IVA.

Among the 70 patients, 13 (18.6%) exhibited lymph node enlargement confined to a single region, while the remaining 57 (81.4%) showed enlargement across multiple regions. Of the enlarged lymph nodes, 89 (42.8%) were in the axillary region, 83 (39.9%) were in the inguinal region, and 36 (17.3%) were in the cervical region. The average long axis length was 23.4 mm, and the average short axis length was 8.4 mm. US reports indicated diffuse cortical thickening in 30 lymph nodes, asymmetric cortical thickening in eight lymph nodes, loss of fatty hilum in 10 lymph nodes, heterogeneity in two lymph nodes, contour irregularity in one lymph node, and disorganized blood flow in one lymph node (Table 2).

Among the 208 lymph node regions, 13 were biopsied without follow-up due to the presence of multiple pathological features observed sonographically, while 195 were monitored over time. Of the lymph nodes under observation, 42 showed no decrease in size, whereas 153 did. The average duration for regression during follow-up was 9.1

Table 1. Demographic, clinical, laboratory, histopathological, and immunohistochemical features of the patients.

Characteristics	Level	Values	
		n	%
Age at Diagnosis (years)	Mean	50.3	-
	Median	51.5	-
Sex	Male	34	48.6
	Female	36	51.4
Follow-up Duration (Months)	Mean	99.2	-
	Median	53.5	-
T Stage	T1	36	51.4
	T2	34	48.6
B Stage	B0	70	100
Morphology	Erythematous	47	67.1
	Hyperpigmented	9	12.9
	Hypopigmented	2	2.9
	Folliculotropism	6	8.6
	Poikiloderma	1	1.4
	PPD-like	3	4.3
	Ichthyosiform	2	2.9
Patch/Plaque	Patch	31	44.3
	Patch+Plaque	39	55.7
LDH	Normal	62	88.6
	High	8	11.4
Beta-2 Microglobulin	Normal	53	75.7
	High	17	24.3
Eosinophilia	Normal	67	95.7
	High	3	4.3
Dermatopathology	Classic	40	57.1
	Folliculotropism	15	21.1
	LCT	2	2.8
	PPD-like	3	4.2
	CD4 (+) CD8 (-)	57	81.4
	CD4 (-) CD8 (+)	11	15.7
	CD4 (+) CD8 (+)	1	1.4
	CD30 (+)	1	1.4
Lymph Node Enlargement	One Region	13	18.6
	Multiple Regions	57	81.4
Progression to Advanced Stage	No	57	81.4
	Yes	13	18.6

Abbreviations: LDH: lactate dehydrogenase, PPD-like: pigmented purpura dermatosis-like mycosis fungoides, LCT: large cell transformation

months. Biopsies were performed on 17 of the lymph nodes that did not regress. No pathological feature was observed in the initial and follow-up ultrasonographic evaluations of the 25 lymph nodes that did not regress and were not biopsied.

Table 2. Sonographic features of the lymph nodes.

Characteristics	Level	Values	
		N	%
Long axis dimension (mm)	Mean	23.4	-
	Median	22.0	
Short axis dimension (mm)	Mean	8.4	-
	Median	8.0	
Short axis	1.5 cm>	204	98.0
	1.5 cm≤	4	2.0
Diffuse cortical thickening	Yes	30	14.4
	No	178	85.6
Asymmetric cortical thickening	Yes	8	3.8
	No	200	96.2
Loss of fatty hilum	Yes	10	4.8
	No	198	95.2
Heterogeneity	Yes	2	1.0
	No	206	99.0
Contour irregularity	Yes	1	0.5
	No	207	99.5
Non-hilar disorganized blood flow	Yes	1	0.5
	No	207	99.5

The reasons for not performing biopsies on these lymph nodes included the patient's refusal to undergo biopsy and procedural morbidity. The mean follow-up period for lymph nodes that did not regress and were not biopsied was 24.2 months. Of the 30 lymph nodes biopsied, 28 were classified as N1, one as N2, and one as N3 (Figure 2).

Analyzing the relationship between demographic, clinical, laboratory, and dermatopathological characteristics of the patients and any pathological finding on US, age at diagnosis and beta-2 microglobulin levels were found to be significant. Pathological findings on US decreased with increasing age, while the likelihood of pathological findings increased with elevated beta-2 microglobulin levels ($P<0.05$) (Table 3).

Multivariate regression analysis demonstrated that age at diagnosis and beta-2 microglobulin levels were significantly associated with non-regressing lymph nodes ($P<0.05$). An inverse correlation was observed between age at diagnosis and the non-regressive lymph nodes, whereas elevated beta-2 microglobulin levels were positively correlated with lack of regression (Table 4).

Upon evaluating disease and lymph node characteristics alongside progression to advanced stages, neither the presence of lymph nodes with pathological features, non-regressing lymph nodes, nor the number of areas with lymph node enlargement demonstrated statistical significance (Table 5). Sex emerged as the sole significant variable, with males exhibiting a significantly elevated risk of disease progression.

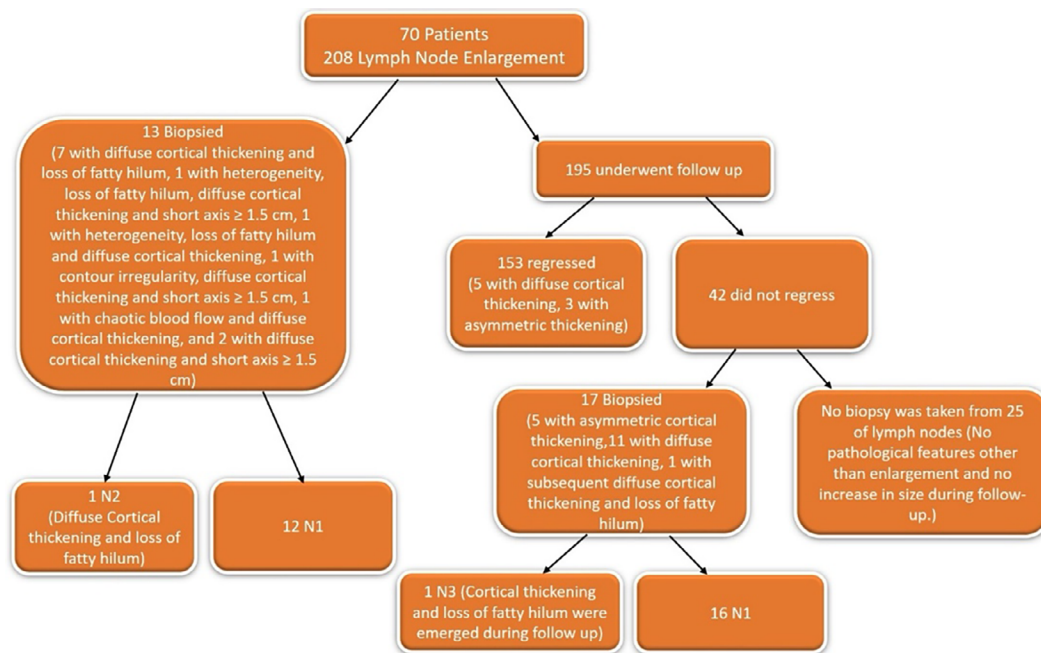


Figure 2. Illustration of procedures performed on lymph nodes.

Table 3. The relationship between patient characteristics and lymph nodes that had any pathological feature (diffuse cortical thickening/asymmetric cortical thickening/fatty hilus loss/heterogeneity in the cortex/contour irregularity/disorganized blood supply on Doppler/short axis ≥ 1.5 cm) using a logistic regression model.

Variable	p-Value	Odds Ratio	95% Confidence Interval	
			Lower	Upper
Age at Diagnosis	0.013*	0.940	0.896	0.987
Sex	Female	-	-	-
	Male	0.186	2.232	0.679
T stage at diagnosis	T1	-	-	-
	T2	0.088	2.944	0.853
Patch/plaque	Patch	-	-	-
	Patch+Plaque	0.614	0.708	0.185
LDH	Normal	-	-	-
	High	0.197	4.129	0.478
Beta-2 microglobulin	Normal	-	-	-
	High	0.035*	4.929	1.121
Classic type in dermatopathology	No	-	-	-
	Yes	0.386	2.445	0.323
Folliculotropism in dermatopathology	No	-	-	-
	Yes	0.145	5.243	0.565
CD8 (+) in immunohistochemical analysis of skin biopsy	No	-	-	-
	Yes	0.605	1.789	0.197

Abbreviations: LDH: lactate dehydrogenase, *statistical significance

Discussion

To our knowledge, this is the first study to evaluate the sonographic features of lymph nodes in early-stage MF patients. Lymphadenopathy in these patients is often underestimated

due to the reliance on clinical examination alone, which is subjective and may overlook many enlarged lymph nodes, as imaging is infrequently utilized [4]. Our study comprised 70 patients in early-stage with lymph node enlargement detected by US, yielding several noteworthy observations.

Table 4. The relationship between patient characteristics and non-regressing lymph nodes during follow-up using a logistic regression model.

Variable		p-Value	Odds Ratio	95% Confidence Interval	
				Lower	Upper
Age at diagnosis		0.041*	0.960	0.919	1.002
Sex	Female	-	-	-	-
	Male	0.541	1.413	0.467	4.276
T stage at diagnosis	T1	-	-	-	-
	T2	0.624	1.316	0.439	3.943
Patch/Plaque	Patch	-	-	-	-
	Patch+Plaque	0.923	0.942	0.280	3.163
LDH	Normal	-	-	-	-
	High	0.954	0.946	0.140	6.385
Beta-2 microglobulin	Normal	-	-	-	-
	High	0.038*	4.226	1.081	16.520
Classic type in dermatopathology	No	-	-	-	-
	Yes	0.785	0.785	0.138	4.474
Folliculotropism in dermatopathology	No	-	-	-	-
	Yes	0.802	1.278	0.188	8.680
CD8 (+) in immunohistochemical analysis of skin biopsy	No	-	-	-	-
	Yes	0.278	0.345	0.050	2.357

Abbreviations: LDH: lactate dehydrogenase, *statistical significance.

Table 5. The association between progression to advanced stage and the characteristics of patients and lymph nodes using a logistic regression model.

Variable		p-Value	Odds Ratio	95% Confidence Interval	
				Lower	Upper
Age at Diagnosis		0.728	1.013	0.943	1.087
Sex	Female	-	-	-	-
	Male	0.049*	16.070	1.010	25.564
T stage at diagnosis	T1	-	-	-	-
	T2	0.462	2.409	0.231	25.127
Patch/plaque	Patch	-	-	-	-
	Patch+Plaque	0.487	2.590	0.177	37.949
Number of areas with lymph node enlargement		0.056	0.542	0,290	1.015
Lymph node with any pathological features	No	-	-	-	-
	Yes	0.374	0.307	0.023	4.139
Non-regressing lymph node	No	-	-	-	-
	Yes	0.072	7.505	0.833	67.583
Classic type in dermatopathology	No	-	-	-	-
	Yes	0.143	0.051	0.001	2.733
Folliculotropism in dermatopathology	No	-	-	-	-
	Yes	0.265	6.343	0.246	16.323
CD8 (+) in immunohistochemical analysis of skin biopsy	No	-	-	-	-
	Yes	0.250	0.145	0.005	3.896

*statistical significance.

For example, although 18.6% of patients exhibited lymph node enlargement at a single site, the overwhelming majority (81.4%) demonstrated enlargement in multiple sites. Hodak et al.'s study, which examined the necessity of lymph node imaging in early-stage MF, also reported a 60% (18/30) rate of lymph node involvement in multiple regions among patients who underwent imaging [4]. The higher rate in our study compared to Hodak et al.'s may be attributed to our clinic's practice of performing peripheral lymph node ultrasound on every MF patient at diagnosis, regardless of stage. Hodak et al.'s study noted differences in imaging outcomes based on lesion type.

The 2007 revision of the EORTC guidelines state that a lymph node should be considered abnormal when the longest transverse diameter is 1.5 cm or greater [2]. However, in various sections of the same guidelines and in subsequent guidelines and studies, only those with a longest diameter of ≥ 1.5 cm are deemed abnormal, without specifying "transverse" [4,17,18]. This ambiguity affects the staging of many patients and may lead to unnecessary interventional procedures. In our study, four patients presented with lymph nodes exhibiting a short-axis diameter of ≥ 1.5 cm. These nodes also demonstrated additional sonographic abnormalities: one patient exhibited pathological findings such as diffuse cortical thickening, loss of fatty hilum, and cortical heterogeneity, another showed contour irregularity and diffuse cortical thickening, and two had diffuse cortical thickening. Biopsies were performed on all identified lymph nodes.

Pathological lymph nodes have several sonographic characteristics on ultrasonography that differentiate them from normal or reactive nodes. A principal finding is the absence or displacement of the central echogenic hilum, frequently linked to altered nodal architecture [11]. These nodes usually appear as hypoechoic and are more likely to have a round shape rather than an oval one [8]. Cortical thickening, whether focal, asymmetric, eccentric, or diffuse, serves as another significant sign [19]. Margins may appear irregular or unclear, potentially indicating capsular involvement or peri-nodal reaction. Doppler ultrasound provides further diagnostic insights as pathological nodes may exhibit non-hilar, peripheral, mixed, or disorganized vascularity, unlike the characteristic regular hilar flow observed in normal nodes [20]. These ultrasonographic characteristics, although not pathognomonic, suggest potential underlying pathology and can direct subsequent diagnostic assessment [11]. In the retrospective evaluation conducted in light of the aforementioned findings, the ultrasonography reports of our sample demonstrated a short axis diameter of ≥ 1.5 cm in four patients, diffuse cortical thickening in 30 patients, asymmetric cortical thickening in eight patients, fatty hilum loss in 10 patients, cortical heterogeneity in two patients, contour irregularity in one patient, and non-hilar disorganized blood

flow in Doppler examination in one patient. Taking into consideration these described findings when performing a biopsy on the lymph nodes may be a more accurate approach to avoiding causing morbidity.

The etiology of lymphadenopathy is broad, including malignancies, infections, autoimmune diseases, medications, and iatrogenic causes [21,22]. Furthermore, since lymphadenopathy can frequently occur in multiple areas in diseases such as MF [2] and because biopsy is associated with morbidity, obtaining the recommended excisional biopsy in all these patients may be challenging. Excisional biopsy can result in bleeding, nerve palsy, and infection [3]. Therefore, especially if sonographic pathological features are absent, only patients with lymph node enlargement in the long axis can be monitored sonographically. On lymph node imaging, size reduction on follow-up has been extensively documented in the literature [23-27]. In our study, 195 lymph nodes were monitored sonographically, and, in accordance with existing literature, 153 (78.5%) showed regression in size.

Although advanced age is known to be a factor associated with poor prognosis in Caucasian MF patients, studies have reported that MF may be more aggressive at a younger age in different races [28]. The literature highlights that younger age can increase the risk of lymph node involvement in malignancies [29-31]. Specifically, a 2018 study on non-small cell lung cancers indicated that younger age was associated with lymph node involvement but was also correlated with a reduced risk of cancer-related mortality [32]. In our study, younger age was linked to both sonographic pathological findings and persistent lymphadenopathy. Furthermore, beta-2 microglobulin, identified in the literature as associated with poor prognosis [33], was also found to correlate with pathological features on sonography and absence of lymph node regression. In line with our findings and the existing literature, closely monitoring lymph nodes and considering early histopathological evaluation in young individuals and those with elevated beta-2 microglobulin levels are recommended.

The 2007 EORTC revision introduced the Nx category for lymph nodes that were not biopsied but demonstrated enlargement on clinical or radiological examination [2]. The literature presents conflicting evidence regarding the prognostic significance of the Nx classification. Nikolau et al. reported that Nx was associated with disease progression and survival in patients with both early and advanced stages of MF [34]. In contrast, Suzuki et al. found no significant association between Nx and survival in a comparable patient cohort [35]. Green et al. evaluated prognostic features of MF, documenting cutaneous manifestations including alopecia, ulceration, and erosions, systemic symptoms such as malaise and chills, and the TN stage, which integrates the number of clinically-detected enlarged lymph nodes with

T stage classification. Their findings indicated that TN stage correlated with survival outcomes [36]. A subsequent investigation by the same research group identified the number of clinically enlarged lymph nodes as an adverse prognostic factor for survival; however, the authors noted that this factor demonstrated less prognostic significance than did T stage in both publications [37]. In our investigation, the number of lymph nodes measuring ≥ 1.5 cm on sonography showed no association with disease progression. This discrepancy may be attributed to our exclusive focus on early-stage patients and the inclusion of dermatopathological features in our analysis. Additionally, previous studies have demonstrated that clinical lymph node enlargement may result from etiologies unrelated to MF [2] and that detection rates vary considerably based on a patient's body habitus. While multiple lymph nodes may be palpable in patients with lean body composition, detection becomes challenging in obese patients. It is unclear whether reported clinically enlarged lymph nodes include those at the 1.5 cm threshold or exclusively those with substantially greater dimensions. Sonographic lymph node assessment provides more objective data, facilitating more precise evaluation of the relationship with disease progression. Our study additionally examined the sonographic features of lymph nodes measuring ≥ 1.5 cm on ultrasound and their changes during follow-up. No comparable study regarding MF addressing these specific parameters exists in the current literature. Although lymph node characteristics and persistence during follow-up showed no association with progression, this finding may reflect the limitations of our sample size. Future investigations incorporating larger patient cohorts will provide further clarification of these relationships.

The research had several limitations. Firstly, the study was retrospective in nature. Secondly, the sample size was limited as the study was conducted at a single center. Lastly, biopsies could not be obtained from all patients whose lymph nodes did not regress.

Conclusion

Our study is the first to comprehensively evaluate sonographic features in early-stage MF patients with lymphadenopathy. Our findings demonstrate that sonography provides useful information for lymph node assessment, revealing pathological features in a subset of patients that may guide clinical decision-making. The significant associations between beta-2 microglobulin and sonographic pathological findings and between younger age and both sonographic pathological findings and non-regressive lymph nodes suggest that these parameters may serve as indicators for earlier biopsy consideration. While the existence of pathological lymph node features did not correlate with disease

progression in our study, this may reflect sample size limitations rather than a true absence of any association. The high regression rate (78.5%) observed in monitored lymph nodes suggests that lymph node enlargement in these patients may be due to reactive processes, supporting a conservative approach for nodes without pathological sonographic features, potentially minimizing unnecessary invasive procedures and associated morbidities. Future multicenter studies with larger sample sizes are required to validate these findings and establish standardized criteria for lymph node evaluation in MF.

Ethics Statement: The study was approved by the Ankara University Faculty of Medicine Institutional Review Board (Decision number: İ09-735-24) and was conducted according to the principles of the Declaration of Helsinki.

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