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Artigo Original

Textural Analysis of Ultrasound Images of Reactive and Neoplastic Cervical Lymph Nodes: an exploratory study

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ABSTRACT:

Objective: To evaluate textural parameters of ultrasound images of reactive and neoplastic cervical lymph nodes, investigating differences between the groups and the potential of texture analysis to characterize these differences. **Materials and Methods:** We analyzed 36 B-mode (2D) cervical ultrasound images, corresponding to 16 reactive and 20 neoplastic lymph nodes, from examinations performed between 2010 and 2024 at a diagnostic imaging clinic in Parnaíba, Piauí, Brazil. Reactive lymph nodes were classified based on clinical course and response to treatment, while neoplastic lymph nodes were confirmed histopathologically. The images were analyzed using MaZda, with parameters based on the gray-level co-occurrence matrix (GLCM), considering different variables, directions, and distances between pixels. The Mann–Whitney test was used, with a significance level of 5%. **Results:** Four variables were statistically significant: Sum of Squares (SumOfSqs) in the direction S(1,-1), Entropy Difference (DifEntrp) S(2,0), Sum of Means (SumAverg) S(4,0), and Sum of Entropy (SumEntrp) S(0,4). Greater dispersion of SumOfSqs, DifEntrp, and SumEntrp was observed in reactive lymph nodes, while SumAverg was elevated in neoplastic lymph nodes. **Conclusion:** Textural analysis identified significant differences between the groups, suggesting its potential as a complementary quantitative approach to conventional ultrasound evaluation. Future studies may validate these findings and expand their clinical application.

Statement of Clinical Relevance: Texture analysis in ultrasound images has shown potential for differentiating reactive lymph nodes from lymph nodes of neoplastic origin. Variables such as SumOfSqs, DifEntrp, SumAverg, and SumEntrp showed statistical significance, suggesting that

certain texture descriptors may reflect differences in tissue organization and heterogeneity between reactive and neoplastic lymph nodes.

Keywords: Image Analysis; Lymph Node; Neoplasm; Ultrasound 11.

1. Introduction

Lymph nodes are integral structures of the lymphatic system and play a fundamental role in the immune response, filtering lymph and identifying infectious agents and abnormal cells. In the clinical evaluation of cervical lymph nodes, distinguishing between reactive changes and those of neoplastic origin is particularly important, as these conditions have different diagnostic, prognostic, and therapeutic implications [1].

Reactive lymph nodes are generally associated with inflammatory or infectious processes and may exhibit changes in volume, echogenicity, and vascularization resulting from the immune response. On the other hand, lymph nodes of neoplastic origin may be related to the presence of primary tumors or to metastatic involvement from neoplasms originating in other regions. Thus, the correct characterization of these changes poses a significant challenge in clinical practice, especially given that ultrasonographic features may overlap between benign and malignant conditions [2,3].

Ultrasound is widely used in the evaluation of cervical lymph nodes because it is an accessible, noninvasive method capable of providing detailed information on morphological and structural aspects, including size, shape, echogenicity, contours, hilum, and vascularization pattern. These parameters can aid in distinguishing between reactive and neoplastic lymph nodes. However, evaluation based exclusively on visual and morphological analysis has limitations, particularly when findings are nonspecific or overlap between different pathological conditions [4,5].

In this context, texture analysis has been investigated as a quantitative approach complementary to conventional medical image evaluation. This technique allows for the mathematical characterization of gray-scale distribution and tissue heterogeneity, enabling

the identification of patterns that may not be easily discernible by visual inspection. In ultrasound images of cervical lymph nodes, previous studies have demonstrated that textural parameters may differ between lymph nodes with and without neoplastic involvement, indicating potential to aid in the characterization of these changes [6,7].

Textural analysis can be performed using various mathematical methods, notably the Gray-Level Cooccurrence Matrix (GLCM), originally described by Haralick et al., which allows for the quantification of characteristics related to the spatial distribution of pixel intensity levels [10]. The MaZda software is a tool used to extract various textural descriptors from medical images, enabling the evaluation of parameters related to intensity, uniformity, entropy, contrast, and the spatial organization of tissues. Thus, its application makes it possible to transform visual image characteristics into quantitative measures suitable for statistical analysis [6–10].

Despite promising results, the literature shows significant heterogeneity regarding the selection of textural descriptors, image processing methods, definition of regions of interest, acquisition parameters, and statistical criteria employed. Furthermore, most studies focus on specific oncological contexts, particularly the identification of metastatic lymph nodes, using different sets of features and analytical strategies. This methodological variability hinders direct comparison of results and limits the generalizability of findings to different populations and clinical conditions [6,7].

In this regard, the use of a standardized approach to image acquisition and quantitative analysis—combined with the definition of a region of interest and the evaluation of parameters derived from GLCM-can contribute to a more objective characterization of the internal organization of lymph nodes. Textural analysis therefore has the potential to complement the information obtained by conventional ultrasound, particularly in situations where conventional ultrasound findings are insufficient to establish a reliable distinction between reactive and neoplastic changes. [4–7].

In light of this gap, the present study aimed to conduct an exploratory evaluation of textural parameters extracted from ultrasound images of reactive and neoplastic cervical lymph nodes, using MaZda software and GLCM-based descriptors. By comparing the two groups, we seek to identify differences in specific texture characteristics that may contribute to understanding the structural changes present in the lymph nodes and provide initial evidence for the development of quantitative approaches complementary to conventional ultrasound evaluation.

2. Materials & Methods

2.1 Study Design, Setting, and Ethical Approval

A retrospective exploratory comparative study was conducted using previously acquired cervical ultrasound images retrieved from the digital exam archive of a private diagnostic imaging clinic located in Parnaíba, Piauí, Brazil. Case selection and retrieval of corresponding clinical and histopathological information covered the period from 2010 to 2024.

The study was submitted to and approved by the Research Ethics Committee of the State University of Piauí (UESPI), under approval number 6,741,814.

2.2 Sample and Case Selection Criteria

The sample consisted of 36 cervical lymph nodes, 20 of which were classified as neoplastic and 16 as reactive. Selection was performed using convenience sampling, based on the availability of high-quality ultrasound images and corresponding clinical and/or histopathological information.

The following were included: lymph nodes located strictly in the cervical region; clear ultrasound images obtained in 2D B-mode, without significant artifacts and with adequate delineation of the lesion; histopathological confirmation of malignancy for cases included in the neoplastic group; and documented clinical follow-up demonstrating regression or complete resolution for cases included in the reactive group.

The following were excluded: non-cervical lymph nodes; ultrasound images with significant motion or acoustic artifacts or with inadequate delineation of the lesion; and cases suspected of neoplasia that did not have corresponding histopathological records at the partner laboratory.

2.3 Classification of Neoplastic and Reactive Lymph Nodes

The neoplastic group consisted of 20 lymph nodes with histopathologically confirmed malignant involvement. The cases included metastatic lesions, among them metastases from oral squamous cell carcinoma and carcinomas originating from other anatomical sites, as well as primary lymphoid malignancies, particularly Hodgkin's and non-Hodgkin's lymphomas. Selection was not restricted to a single histological subtype but was based on confirmation of malignant involvement of the parenchyma and consequent disruption of the tissue architecture.

Biopsies or surgical resections of neoplastic lymph nodes were performed in hospital and surgical departments in the Parnaíba region, Piauí, Brazil. The specimens and their respective histopathological reports were processed and issued by the Aldelab pathology laboratory, located in Parnaíba, Piauí, Brazil, with diagnostic evaluation conducted by specialist pathologists.

Reactive lymph nodes comprised 16 cases associated with regional inflammatory or infectious conditions. Classification as reactive was established based on the clinical history

, benign ultrasonographic features, and documented clinical follow-up, with regression or complete resolution following specific drug treatment for the identified etiology. These lymph nodes were not subjected to biopsy or surgical resection, as they showed a favorable clinical course after treatment.

The ultrasound images were acquired prior to surgical procedures. In neoplastic cases, archived images were selected showing lymph nodes with ultrasound features consistent with neoplastic involvement, belonging to the cervical chains that were later associated with cases with histopathological confirmation of malignancy. A histopathological example of a neoplastic lymph node and an illustrative image of reactive lymph node architecture are shown in Figure 1.

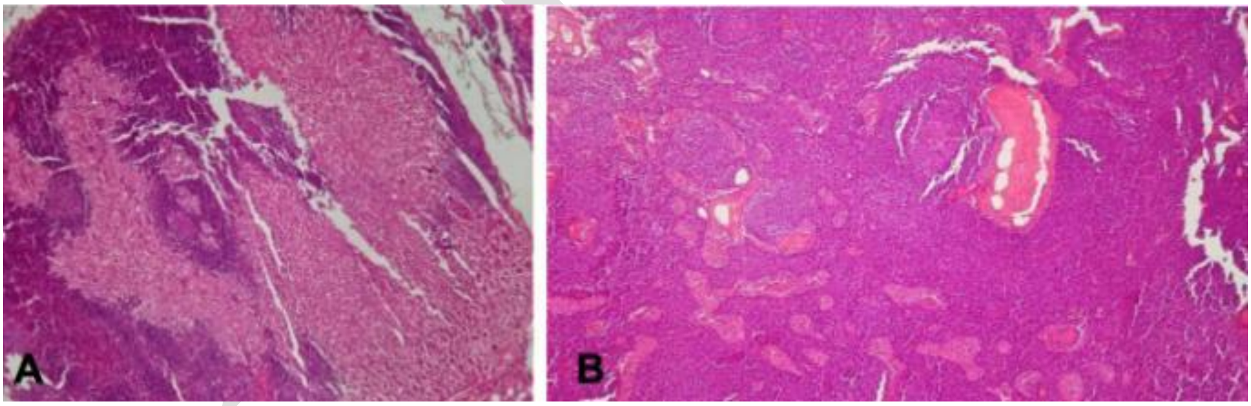

Figure 1. Representative histological sections (original magnification ×400). (A) Histopathologically confirmed neoplastic lymph node from the study cohort. (B) Illustrative archival section showing preserved/reactive lymph node architecture, included for educational and morphological comparison only and not obtained from a biopsied reactive lymph node in the study cohort.

Because this was a retrospective study based on archived images, it was not possible to ensure individual topographic correspondence, lymph node by lymph node, between each ultrasound image analyzed and the surgical specimen subsequently obtained. This limitation was taken into account in the interpretation of the results.

2.4 Acquisition and Processing of Ultrasound Images

All B-mode ultrasound images were acquired by a single experienced sonographer using a GE Healthcare LOGIQ E9 system equipped with a 7.5–12 MHz high-frequency linear transducer. Standardized acquisition parameters were employed, including optimized gain, a depth generally between 2 and 4 cm, and positioning of the focal zone in the region of the lymph node being evaluated, with the aim of minimizing operator-related variability.

Brightness and contrast parameters were adjusted for adequate visualization of the structures, using a noise reduction filter and standardized grayscale. The images were subsequently imported into MaZda software, version 4.60, in BMP format, for textural analysis.

2.5 Textural Analysis and Definition of the Region of Interest

Textural analysis was performed using MaZda software, version 4.60, with parameters derived from the Gray-Level Co-occurrence Matrix (GLCM). Different directions and distances between pixels were evaluated, allowing for the quantitative characterization of the spatial distribution of gray levels in the analyzed regions.

For each lymph node, a circular region of interest (ROI) with a diameter of 44 pixels was manually defined. The ROI was standardized and positioned in the region corresponding to the epicenter of the lesion or the nodal parenchyma. The definition and manual positioning of the circular ROI are illustrated in Figure 2. In neoplastic lymph nodes, especially those with an absence or loss of the fatty hilum, the ROI was centered on the region of altered parenchyma. In reactive lymph nodes, the ROI was positioned at the epicenter of the nodal parenchyma/cortex, avoiding the inclusion of extralymphatic structures and adjacent large vessels.

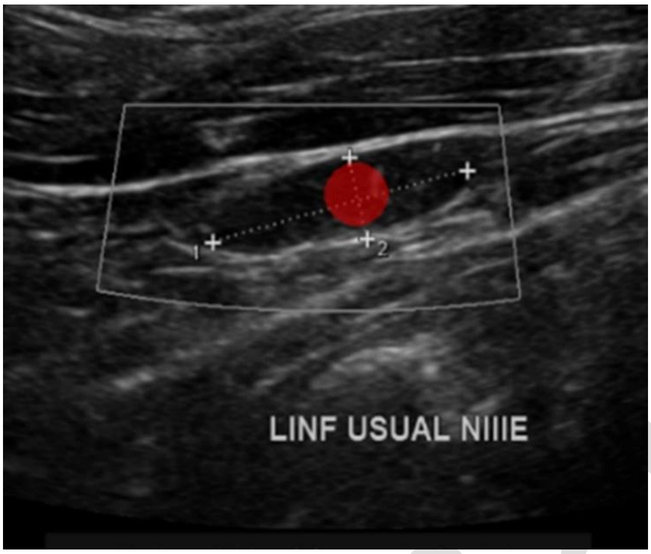

Figure 2. Sagittal ultrasound image showing the manual segmentation of a circular region of interest (ROI), with a diameter of 44 pixels, positioned in the center of the lesion.

Manual delineation of the ROIs was performed by a single evaluator who had been previously trained and calibrated to use the MaZda software and the analysis protocol. No formal assessment of intra-observer or inter-observer reproducibility was conducted for ROI segmentation, which constitutes a methodological limitation of the study.

The following textural descriptors derived from GLCM were analyzed: contrast (Contrast), inverse difference moment (InvDfMom), second-order angular moment (AngScMom), correlation (Correlation), sum of squares (SumOfSqs), entropy (Entropy), mean of the sum (SumAverg), sum of squares variance (SumVarnc), sum of squares entropy (SumEntrp), difference variance (DifVarnc), and difference entropy (DifEntrp). The report generated by the MaZda software, containing the extracted texture parameters, is shown in Figure 3.

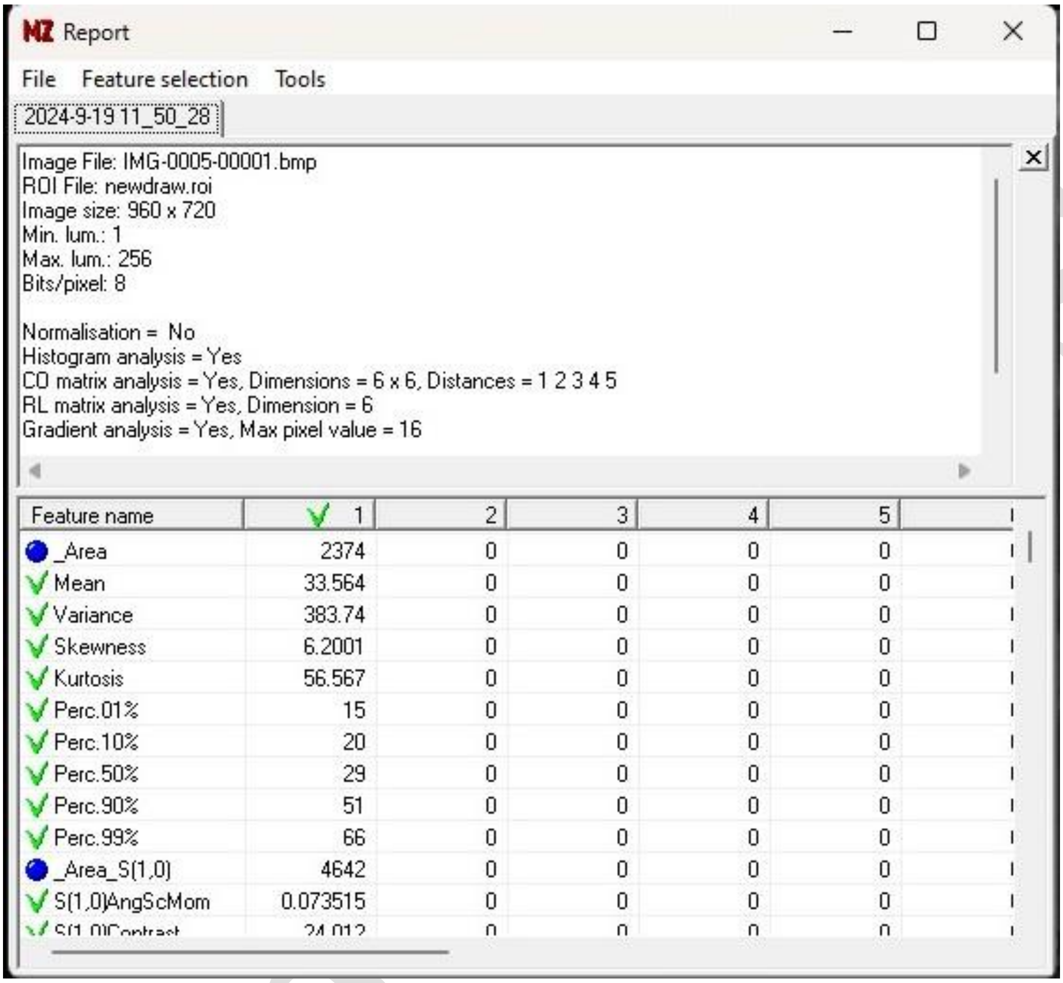

Figure 3. MaZda software report showing texture parameters extracted from cervical lymph node ultrasound images.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS software, version 26.0. Given the retrospective, exploratory, and pilot nature of the study, as well as the small sample size (n = 36), univariate analyses were conducted to compare the textural parameters between the reactive and neoplastic groups. The comparison was conducted using the Mann–Whitney U test, with a significance level of 5% ($p < 0.05$)..

No analyses of the ROC curve, area under the curve (AUC), sensitivity, or specificity were performed, since the study's objective was exploratory, aimed at identifying candidate textural descriptors that showed differences between the groups, rather than determining diagnostic accuracy or establishing clinical cutoff points. Furthermore, the small sample size could result in estimates of diagnostic performance with high uncertainty. Thus, the results were interpreted as preliminary evidence of textural differences and were not used to establish definitive diagnostic capability.

3. Results

A total of 36 ultrasound images of cervical lymph nodes were analyzed, with 20 belonging to the neoplastic group and 16 to the reactive group. The neoplastic lymph nodes had histopathological confirmation of malignancy, while the reactive lymph nodes were classified based on clinical course, ultrasonographic characteristics, and regression or disappearance following treatment. The ultrasonographic images were obtained prior to surgical procedures and selected retrospectively.

Analysis of the textural parameters derived from the gray-level co-occurrence matrix (GLCM) allowed for a comparison of the behavior of the descriptors between the two groups. The distribution of the mean ranks of the analyzed parameters is presented in Figure 4, providing an overview of the differences observed between neoplastic and reactive lymph nodes.

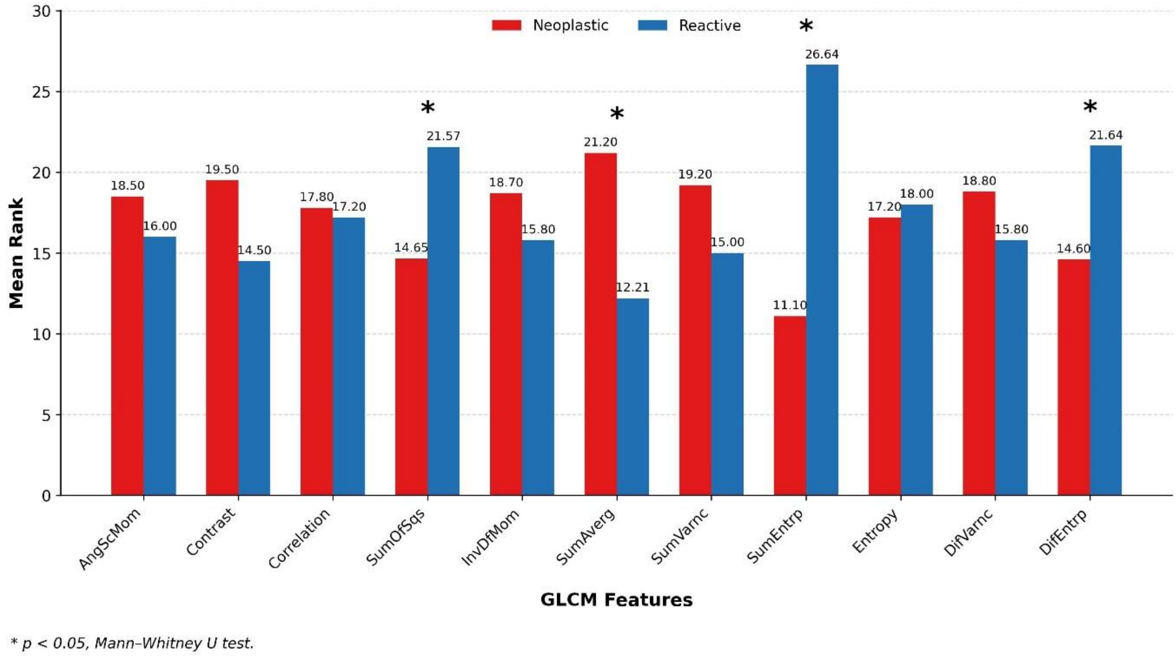

Figure 4. Mean ranks of GLCM texture variables extracted using MaZda in neoplastic (n = 20) and reactive (n = 16) cervical lymph nodes. Asterisks indicate statistically significant differences between groups (p < 0.05, Mann–Whitney U test).

Most of the descriptors evaluated showed no statistically significant difference between the groups (p > 0.05), including Angular Second Moment (AngScMom), Contrast, Correlation, Inverse Difference Moment (InvDfMom), Sum of Variance (SumVarnc), Entropy, and Difference of Variance (DifVarnc). This result demonstrates that differences between the groups were not consistently observed across all texture parameters.

On the other hand, four parameters derived from GLCM showed statistically significant differences between reactive and neoplastic lymph nodes (p < 0.05): Sum of Squares (SumOfSqs) in the S(1,-1) direction, Difference of Entropy (DifEntrp) in S(2,0), Sum of Means (SumAverg) in S(4,0), and Sum of Entropy (SumEntrp) in S(0,4). These four descriptors are highlighted in Figure 4 and presented individually in

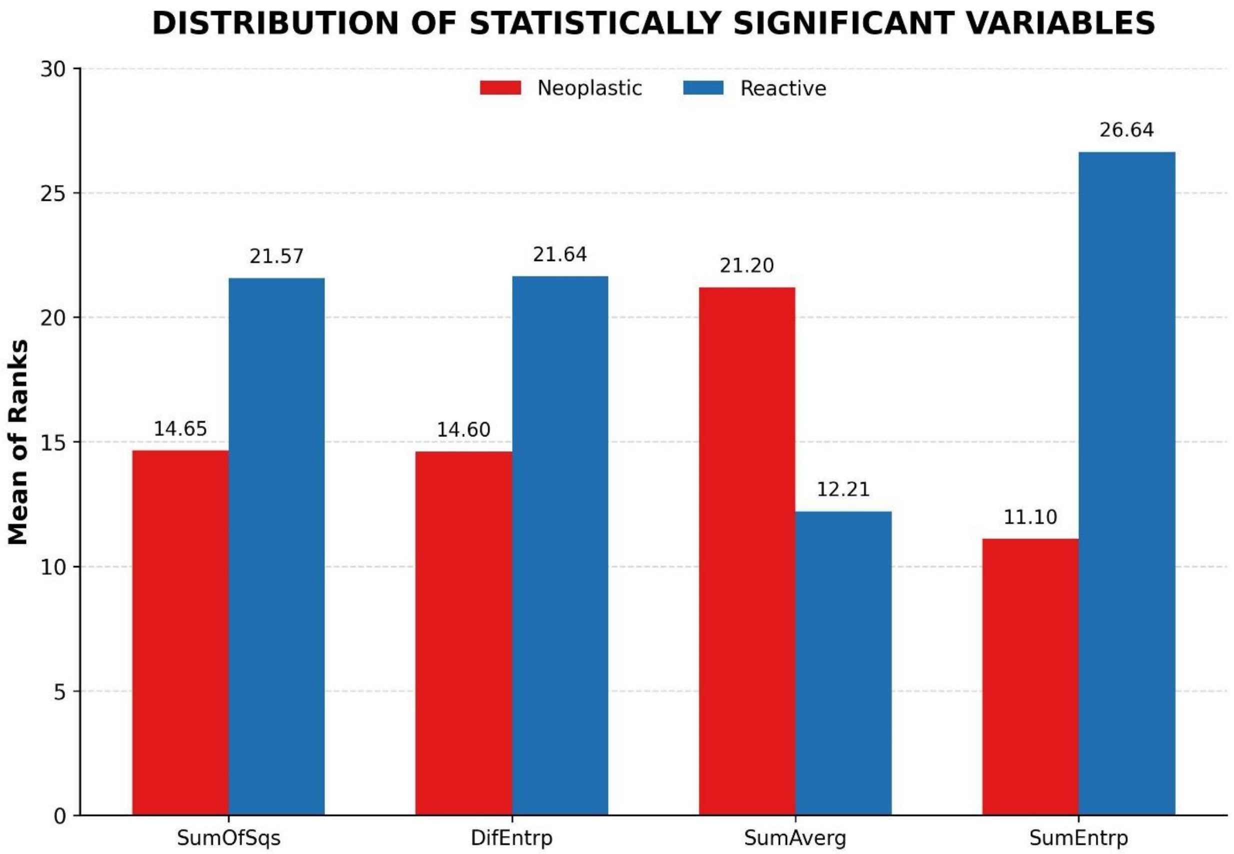

Figure 5. Distribution of the statistically significant GLCM texture variables between neoplastic and reactive cervical lymph nodes. SumOfSqs, DifEntrp, SumAverg, and SumEntrp showed statistically significant differences between groups ($p < 0.05$, Mann–Whitney U test).

Among the significant parameters, SumOfSqs, DifEntrp, and SumEntrp showed higher mean ranks in the reactive group. For SumOfSqs, the mean rank was 21.57 in the reactive group and 14.65 in the neoplastic group. For DifEntrp, the values were 21.64 and 14.60, respectively. As for SumEntrp, the reactive group had a mean rank of 26.64, while the neoplastic group had 11.10.

Conversely, the SumAverg parameter showed a higher mean rank in the neoplastic group (21.20) than in the reactive group (12.21). Thus, the four significant descriptors exhibited distinct distribution patterns between the groups, as shown in Figure 5.

Figure 6 shows the distribution of the mean values of the textural parameters and illustrates the overlap between the neoplastic and reactive groups. This overlap demonstrates that some of the observed values fall within a common range between the groups, reinforcing the fact that the differences identified in certain descriptors do not represent a complete separation of the distributions.

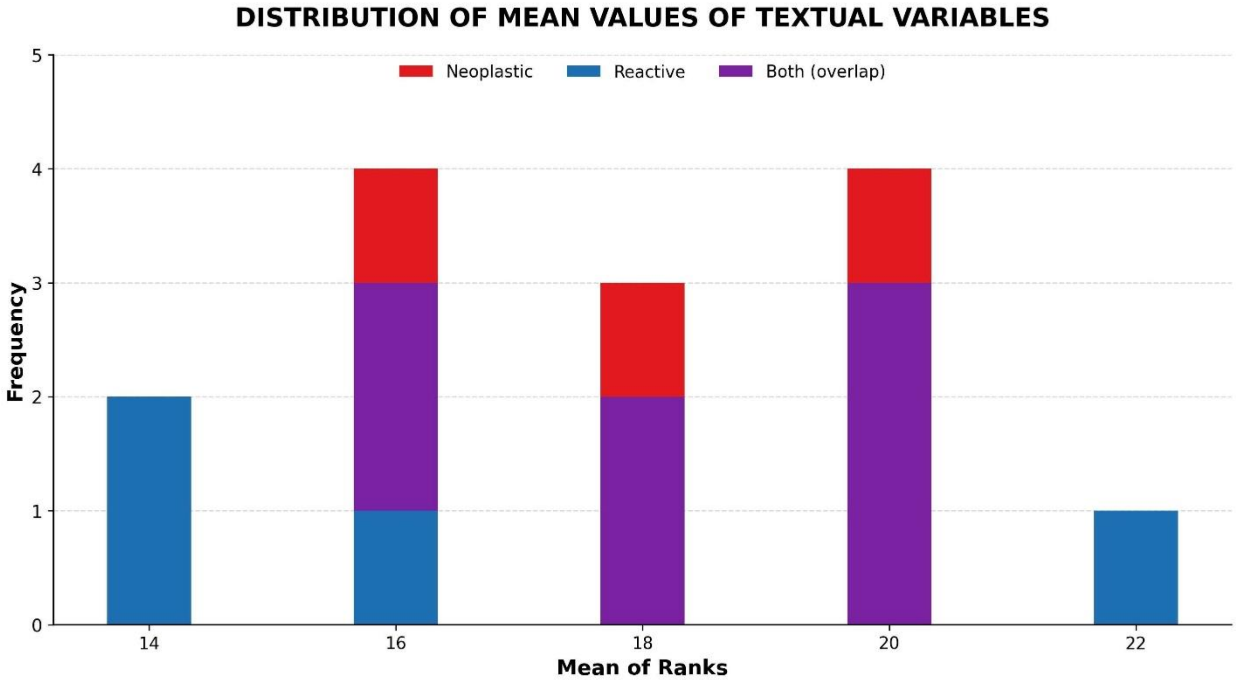

Figure 6. Distribution and overlap of mean-rank values for the analyzed texture variables in neoplastic and reactive cervical lymph nodes. “Both (overlap)” indicates overlap between the distributions of the neoplastic and reactive groups.

Taken together, the results demonstrate that, although most descriptors did not show statistically significant differences, four specific parameters — SumOfSqs, DifEntrp, SumAverg, and SumEntrp — exhibited differences between the studied groups. These findings suggest that certain quantitative characteristics related to the distribution and spatial organization of gray levels exhibit distinct behaviors between reactive and neoplastic lymph nodes.

However, the results should be interpreted in light of the exploratory and pilot nature of the study. No diagnostic performance analyses-such as ROC curves, area under the curve (AUC), sensitivity, and specificity-nor multivariate predictive models were performed. These analyses were not conducted due to the small sample size (n = 36), as their application to a small sample could produce unstable estimates and lead to overfitting.

Thus, the four identified parameters should not be interpreted as validated diagnostic markers, but rather as candidate textural descriptors that showed statistically significant differences between groups in the studied sample. The results constitute initial evidence that may guide further investigations with larger samples and specific study designs to validate these findings.

Thus, the results demonstrate the feasibility of extracting and quantitatively comparing texture descriptors in ultrasound images of cervical lymph nodes, with the identification of four parameters that exhibited differential behavior between the studied groups. Although these findings still require validation in future studies, they contribute to the investigation of quantitative approaches complementary to conventional ultrasound evaluation.

4. Discussion

Texture analysis has been used as a quantitative approach for characterizing tissues in different imaging modalities, allowing for the assessment of patterns related to the distribution of gray levels that may not be fully identified by conventional visual evaluation. In ultrasound images of cervical lymph nodes, previous studies have demonstrated differences in texture parameters between metastatic and non-metastatic or tumor-free lymph nodes, suggesting that this approach may provide additional information for the characterization of these structures [4,6,7].

Conventional ultrasound plays an important role in the evaluation of cervical lymph nodes, allowing for the analysis of morphological characteristics such as hilum, cortical thickness, shape, echogenicity, contours, and vascularization. These characteristics can aid in differentiating between reactive and neoplastic lymph nodes, as shown in Table 1 [1,5,11,12]. However, morphological changes may overlap across different conditions, making it important to investigate complementary quantitative methods.

Table 1. Comparison of ultrasound characteristics between benign and malignant lymph nodes.

Parameter	Benign lymph node	Malignant lymph node
Hilo	Central	Eccentric
Cortex	Tapered	Thickened
Morphology	Oval	Rounded
Echogenicity	Homogeneous	Heterogeneous
Contour	Regular, smooth, and well-defined	Irregular, spiky, and poorly defined.
Vascularization	Poor vascularization	Hypervascularized

In this context, analysis based on the gray-level co-occurrence matrix (GLCM) allows for the quantification of spatial relationships between pixel intensity levels, enabling the derivation of various texture descriptors [8–10]. The present study used this approach to compare reactive and neoplastic lymph nodes and identified four descriptors with statistically significant differences: SumOfSqs, DifEntrp, SumAverg, and SumEntrp. These results indicate that certain quantitative image patterns exhibited distinct behavior between the evaluated groups.

The findings of the present study may also be related to the results of previous studies. Kawashima et al. [4] demonstrated differences in textural parameters between metastatic and nonmetastatic cervical lymph nodes, while Abbasian Ardakani et al. [6] observed that ultrasound texture analysis could help differentiate between metastatic and tumor-free lymph nodes. Kim et al. [7], in turn, identified an association between ultrasound texture parameters and lymph node metastasis in patients with papillary thyroid carcinoma. Although the descriptors and the populations evaluated differ, these studies, together with the results obtained in this study, reinforce the possibility that quantitative information present in ultrasound images contributes to the characterization of lymph nodes.

In the present study, SumOfSqs, DifEntrp, and SumEntrp had higher mean ranks in reactive lymph nodes, while SumAverg had a higher mean rank in neoplastic lymph nodes. These differences demonstrate that the descriptors did not exhibit uniform behavior across the groups, indicating that different mathematical characteristics of the spatial distribution of gray levels may respond differently depending on the condition of the lymph node. Although these parameters cannot be interpreted in isolation as diagnostic markers, their identification provides relevant candidates for future investigations [8–10,18].

The literature, however, reveals significant diversity in the methodologies used for texture analysis, including differences in the selected descriptors, image processing, definition of regions of interest, acquisition conditions, and statistical strategies [4,6–8,18]. This variability can influence the results obtained and hinders direct comparison between studies. Furthermore, research in other areas of medical imaging has demonstrated the applicability of texture analysis for characterizing changes in head and neck tissues, reinforcing the relevance of this approach in the dental and maxillofacial context [9,13–17,19,20].

The use of B-mode ultrasound images, combined with the standardized definition of the region of interest and the extraction of descriptors using MaZda, enabled a systematic quantitative analysis of the lymph nodes. However, the use of a single evaluation and the absence of a formal analysis of intra- and inter-observer reproducibility must be considered, since the definition of the region of interest and the processing procedures can influence texture parameters [8,15,18,19].

Furthermore, it is important to consider the relationship between texture analysis and conventional ultrasound findings. Although Table 1 presents characteristics frequently used in lymph node assessment, no statistical correlation analysis was performed in this study between these characteristics and the textural descriptors. Thus, the results demonstrate quantitative differences between the groups but do not allow for the establishment of a direct relationship between the four significant parameters and specific morphological characteristics of the lymph nodes [1,4–7,11,12].

Another aspect to consider is the retrospective nature of the study. The images were obtained prior to surgical procedures and subsequently retrieved from the examination archive, and were associated with cases classified as reactive or neoplastic. Although the neoplastic cases were confirmed histopathologically and the reactive cases were supported by clinical course and regression following treatment, the retrospective nature of the study limits the ability to absolutely guarantee topographical correspondence between the region analyzed in the image and the specimen subsequently removed. This limitation should be taken into account when interpreting the results.

Finally, the absence of analyses such as the ROC curve, area under the curve, sensitivity, and specificity does not allow us to determine, at this stage, the individual diagnostic performance of the identified descriptors. Previous studies using these approaches have demonstrated the possibility of evaluating the discriminatory potential of texture analysis [4,6,7]. In the present study, however, the small sample size and exploratory nature of the study justified prioritizing the comparison between groups. Thus, the four identified parameters should be understood as candidate descriptors, which may be investigated in larger samples through multivariate and diagnostic performance analyses.

Overall, the results demonstrated significant differences in SumOfSqs, DifEntrp, SumAverg, and SumEntrp between reactive and neoplastic lymph nodes, while most of the descriptors evaluated did not show significant differences. These findings reinforce the potential of ultrasonographic texture analysis as a quantitative approach complementary to the conventional evaluation of cervical lymph nodes, while also providing specific parameters that can be explored in future studies with larger case numbers and validation methodologies.

5. Conclusion

Texture analysis of cervical ultrasound images demonstrated differences in selected textural parameters between reactive and neoplastic lymph nodes. Four descriptors — SumOfSqs, DifEntrp, SumAverg, and SumEntrp — showed statistically significant differences between the groups, suggesting that variations in the distribution of gray levels and spatial organization may reflect differences in the tissue characteristics of reactive and neoplastic lymph nodes.

These findings highlight the potential of texture analysis of ultrasound images as a complementary quantitative approach in the evaluation and characterization of cervical lymph nodes. However, the results should be interpreted in light of the exploratory nature of this study. Future investigations, with larger samples and standardized methodologies, are needed to validate the identified texture descriptors and clarify their discriminatory potential and clinical applicability.

Authors' Contributions

ASBP: Study conception, methodology definition, research planning, investigation, data acquisition, manuscript writing, revision and editing, and supervision.

LBT: Data analysis, results interpretation, data curation, manuscript writing, revision and editing.

RESS: Data analysis, results interpretation, data curation, manuscript writing, revision and editing.

AGAJ: Data analysis, results interpretation, data curation, manuscript writing, revision and editing.

NLSA: Critical manuscript review, writing, revision and editing, and supervision.

CAMF: Critical manuscript review, writing, revision and editing, and supervision.

PCM: Scientific guidance of the study, supervision, critical manuscript review, and revision and editing.

MS: Scientific guidance of the study, supervision, critical manuscript review, and revision and editing.

VAMM: Scientific guidance of the study, supervision, critical manuscript review, and revision and editing.

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Conflict of interest

The authors declare that there are no conflicts of interest related to the preparation and publication of this manuscript.

Ethical approval

This study was conducted in accordance with all provisions of the guidelines and policies of the local committee for the supervision of research involving human subjects at the State University of Piauí (UESPI). The protocol for this study was analyzed and approved by the Research Ethics Committee of the State University of Piauí (UESPI), under opinion number 6741814.

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