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# Diagnostic Accuracy of Computer-aided Assessment of Intranodal Vascularity in Distinguishing Different Causes of Cervical Lymphadenopathy.

## Abstract

Ultrasound is useful in assessing cervical lymphadenopathy. Advancement of computer science technology allows accurate and reliable assessment of medical images. This study aims to evaluate the diagnostic accuracy of computer-aided assessment of intranodal vascularity index (VI) in differentiating the various common causes of cervical lymphadenopathy. Power Doppler sonograms of 347 patients with palpable cervical lymph nodes were reviewed (155 metastases, 23 lymphoma, 44 tuberculous lymphadenitis, 125 reactive). Ultrasound images of cervical nodes were evaluated, and intranodal VI was quantified using a customized computer program. The diagnostic accuracy of using intranodal VI in distinguishing different disease groups was evaluated and compared. Metastatic and lymphomatous lymph nodes tend to be more vascular than tuberculous and reactive lymph nodes. Intranodal VI demonstrated the highest diagnostic accuracy in distinguishing metastatic and tuberculous nodes with a sensitivity of 80%, specificity of 73%, positive predictive value of 91%, negative predictive value of 51% and overall accuracy of 68% when a cut-off VI of 22% was used. Computer-aided assessment provides an objective and quantitative way to evaluate intranodal vascularity. Intranodal VI is a useful parameter in distinguishing certain causes of cervical lymphadenopathy, and is particularly useful in differentiating metastatic and tuberculous lymph nodes. However, it has limited value in distinguishing lymphomatous nodes from metastatic and reactive nodes.

**Keywords:** Lymph nodes; Ultrasonography; Blood vessels; Image processing; Computer assisted; Power Doppler; Vascularity Index

## Introduction

Patients with head and neck cancers, lymphoma and tuberculosis often present with palpable neck nodes. Accurate diagnosis of the causes of cervical lymphadenopathy is important because the treatment of these diseases is different. In head and neck cancer patients, accurate assessment of metastatic cervical lymph nodes is particularly crucial because it helps to evaluate patient prognosis and aids treatment planning. In patients with proven head and neck primary squamous tumour, the presence of a metastatic cervical lymph node in one side of the neck reduces the 5-year survival rate to 50% and the presence of bilateral metastatic lymph nodes in the neck reduces the survival rate to 25% (Som 1992).

Ultrasound-guided fine-needle aspiration and cytology (FNAC) is a common diagnostic method for patients with cervical lymphadenopathy. The reported diagnostic accuracy of FNAC of cervical lymphadenopathy ranges between 82.2% and 88.7% (Hafez and Tahoun 2011; Rajbhandari et al. 2013). In identifying cervical tuberculous lymph nodes, FNAC has a diagnostic accuracy of 72.3% to 85.4% (Kim et al. 2013; Khan et al. 2015). However, 13.8% of patients undergoing FNAC required repeated aspirations and the reasons for repeat were predominantly due to inadequate aspirates (52.4%) and non-diagnostic descriptive reports (43.7%) (Goyal et al. 2014).

Ultrasound is a useful imaging tool and is more sensitive than neck palpation in the assessment of cervical lymphadenopathy (Haberal et al. 2004; Giacomini et al. 2013). On ultrasound examination of cervical lymph nodes, grey scale ultrasound assesses the nodal morphology whilst colour and power Doppler ultrasound evaluate intranodal vascularity. It has been reported that colour and power Doppler ultrasound is useful in assessing lymph node vascularity and helpful in the differential diagnosis of cervical lymphadenopathy (Ahuja and Ying 2003; Giacomini et al. 2013; Park and Kim 2014). Normal and reactive lymph nodes predominantly appear apparently avascular or show hilar vascularity (Ying and Ahuja 2003). Peripheral vascularity with or without hilar vascularity is common in metastatic and lymphomatous nodes, whereas displaced vascularity with apparently avascular areas is frequently found in tuberculous nodes (Ahuja and Ying 2003; Giacomini et al. 2013; Park and Kim 2014). Moreover, tumour angiogenesis and the related proliferation of intranodal vasculature cause metastatic and lymphomatous lymph nodes to have higher vascularity than benign and tuberculous nodes (Wu et al. 1998a). Therefore, the abundance of intranodal vascularity, which can be expressed as vascularity index (VI), could be a useful imaging parameter to distinguish different causes of cervical lymphadenopathy. This intranodal vascular abundance can be evaluated both qualitatively and quantitatively. In qualitative assessment, the intranodal vascularity is graded in a Likert scale according to the abundance of vascularity and vessels demonstrated within the lymph node (Wu et al. 2000; Ying et al. 2000). In quantitative assessment, the vascularity of lymph node is quantified using customized image post-processing program and is expressed as a numerical value (Wu et al. 1998b; Wu et al. 2000). A recent study showed that quantitative assessment of intranodal vascularity is more reliable and accurate than qualitative assessment method in differentiating reactive and metastatic lymph nodes (Lam et al. In press). Despite the above reports, overall, literature has limited information about the diagnostic accuracy of quantitative assessment of intranodal VI in distinguishing between the various causes of cervical lymphadenopathy. Therefore, this study was undertaken to evaluate the diagnostic accuracy of intranodal VI in distinguishing metastatic, lymphomatous, tuberculous and reactive lymph nodes, and to determine the optimum cut-off value of intranodal VI in the differential diagnosis. The clinical significance of the study is to provide an additional parameter which is objective and reliable for differential diagnosis of various causes of cervical lymphadenopathy.

## Materials and Methods

The study was approved by the Human Subject Ethics Subcommittee of the Department of Health Technology and Informatics, the Hong Kong Polytechnic University. We retrospectively reviewed power Doppler sonograms of 347 patients with palpable cervical lymph nodes. Patients were selected consecutively from our patient database, and patients without confirmed cytology/pathology results of the lymph nodes were excluded. All the data of the study was obtained from the routine clinical ultrasound examinations, and the ultrasound examinations were performed using a standard of care clinical protocol. Among the 347 patients, 155 patients had known head and neck cancer or primary tumour in other body regions and with proven metastatic cervical nodes (54 nasopharyngeal carcinoma, 30 oral cavity carcinomas, 25 papillary carcinoma of the thyroid, 23 pharyngeal and laryngeal carcinomas, 14 lung carcinoma, 6 breast carcinoma, 1 testis carcinoma, 1 colon carcinoma, 1 gastric carcinoma), 23 patients had lymphomatous nodes (20 patients were non-Hodgkin's lymphoma and 3 patients with Hodgkin's disease), 44 patients had tuberculous lymph nodes and 125 patients had reactive nodes (none of these patients had any known carcinoma; patients had clinical follow-ups in the out-patient department and remained well).

The neck ultrasound examination of the 347 patients was performed by the same operator using the same ultrasound scanning protocol, whereas the image analysis of intranodal VI was conducted by another operator. The operator was blinded to the FNAC result of the lymph nodes at the time of the image analysis. All ultrasound examinations were performed on a Philips IU22 ultrasound unit using a 5-12MHz linear transducer (Bothell, WA, USA). In each patient, the cervical nodes were assessed with grey scale and power Doppler ultrasound, and the lymph node that demonstrated the most abundant vascularity was included in the study. Settings of the power Doppler ultrasound were standardized for detecting blood vessels with low blood flow velocity: high sensitivity, low wall filter, pulsed repetition frequency (PRF) = 700Hz, and medium persistence. The colour gain was standardized and increased at the beginning to show colour noise (low amplitude, intermittent and scattered colour signals) and then decreased until the noise disappeared (Ying et al. 2000).

On power Doppler ultrasound examination of each lymph node, multiple sonograms at different scan planes were obtained and the sonogram that showed the most abundant intranodal vascularity was selected for the measurement of intranodal VI. In the assessment of intranodal VI, the degree of vascularity was evaluated using the software program Matlab® (version 7.3.0.267 R2006b; The MathWorks, Natick, MA, USA) and a customized algorithm for colour signal quantification of Doppler images (Ying et al. 2009; Lam et al. In press). Power Doppler ultrasound images retrieved from the ultrasound unit were converted into tagged image file format (TIFF). The ultrasound images were then processed on a computer workstation with the Matlab and Microsoft Paint (version 5.1; Microsoft Corporation, Redmond, WA, USA) installed. Using Microsoft Paint, the boundaries of the lymph node (i.e. region of interest, ROI) were manually outlined on the ultrasound image. The image with the ROI outlined was then analyzed with Matlab. Using the customized algorithm, the ROI was initially extracted from the ultrasound image and the total number of pixels of the ROI was evaluated by the algorithm. Subsequently, the colour pixels coded by power Doppler ultrasound were extracted from the ROI, and the number of colour pixels was counted (Figures 1 and 2). The VI of the lymph node was then calculated by the following equation (Lam et al. In press):

$$\text{VI (\%)} = \frac{\text{Number of colour pixels within the ROI}}{\text{Total number of pixels within the ROI}} \times 100$$

Our previous study has demonstrated that this image analysis technique has a high inter-rater (0.83-0.96) and intra-rater (0.97-0.99) reliability (Lam et al. In press).

The VI of the metastatic, lymphomatous, tuberculous and reactive lymph nodes was expressed as mean ± 1 standard deviation (SD). Kruskal-Wallis Test was used to calculate the significance of difference in the VI among different groups of lymph nodes. Dunn's multiple comparisons test was used as the post-hoc test to calculate the significance of difference among the study groups in pairs. When a significant difference was found between two types of lymph nodes, receiver-operating characteristic (ROC) curve in differentiating these lymph nodes was plotted. The performance of using VI to distinguish different pathological lymph nodes was evaluated and compared using the area under the curve (AUC). Kruskal-Wallis Test and the post-hoc test were performed, and the AUC of the ROC curves was calculated using Statistical Product and Service Solutions (SPSS) version 20 (IBM, Armonk, New York, USA). The ROC curves were used to determine the optimum cut-off of VI in distinguishing different pathological lymph nodes. The point of the curve nearest to the top left-hand corner corresponded to the cut-off that had high sensitivity and specificity in the differential diagnosis. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of VI in the differential diagnosis were also evaluated. A p value $< 0.05$ is considered to be significant, and a 95% confidence interval is used.

## Results

The intranodal VI of the 347 lymph nodes was evaluated (155 metastatic nodes, 23 lymphomatous nodes, 44 tuberculous nodes, 125 reactive nodes), and the VI of cervical nodes in different disease groups is shown in Table 1. Results showed that metastatic nodes (38 ± 20%) had significantly higher VI than reactive nodes (27 ± 15%) ($p < 0.05$). Lymphomatous nodes (33 ± 20%) were more vascular than reactive nodes but the difference was not statistically significant ($p > 0.05$). Moreover, results illustrated that tuberculous nodes (17 ± 13%) had significantly lower VI than metastatic, lymphomatous and reactive nodes ($p < 0.05$) (Figure 3).

ROC curves were plotted when there was significant difference in the mean VI between the disease groups. Therefore, ROC curves to determine the performance of intranodal VI in differentiating metastases and reactive, metastases and tuberculosis, lymphoma and tuberculosis, as well as reactive and tuberculosis were obtained (Table 2).

In distinguishing metastatic and reactive lymph nodes, the AUC of the ROC curve was 0.66 and the optimum cut-off of VI was 24% with sensitivity, specificity, PPV, NPV and overall diagnostic accuracy of 72%, 52%, 65%, 60% and 63% respectively.

In distinguishing lymphomatous and tuberculous lymph nodes, the AUC of the ROC curve was 0.76 and the optimum cut-off of VI was 18% with sensitivity, specificity, PPV, NPV and overall diagnostic accuracy of 83%, 61%, 53%, 87% and 69% respectively.

In differentiating metastatic and tuberculous nodes, the AUC of the ROC curve was 0.82 and the optimum cut-off of VI was 22% with sensitivity, specificity, PPV, NPV and overall diagnostic accuracy of 80%, 73%, 91%, 51% and 68% respectively.

In differentiating reactive and tuberculous nodes, the AUC of the ROC curve was 0.69 and the optimum cut-off of VI was 17% with sensitivity, specificity, PPV, NPV and overall diagnostic accuracy of 71%, 59%, 83%, 42% and 68% respectively.

In the comparison of the AUC of different ROC curves, results showed that the AUC for metastases vs tuberculosis was significantly higher than the AUC for metastases vs reactive and AUC for reactive vs tuberculosis ($p < 0.05$). There was no significant difference in the AUC for other comparisons ($p > 0.05$).

## Discussion

Results of the study demonstrated that metastatic and lymphomatous lymph nodes tended to be more vascular than reactive and tuberculous lymph nodes, similar to a previous study in which malignant lymph nodes had a higher intranodal vascularity than benign nodes (Wu et al. 1998a). The higher intranodal vascularity of malignant lymph nodes is likely the result of angiogenesis leading to increased number of tumour vessels within the lymph node. In the present study, tuberculous lymph nodes showed a relative lower intranodal vascularity. This may be due to the high incidence of intranodal necrosis of tuberculous nodes in which intranodal lymphoid tissues become necrotic and the intranodal blood vessels are displaced by the necrosis and/or endarteritis associated with tuberculosis. This finding is consistent with previous studies that a vascular pattern with apparently avascular areas and displaced vascularity is common in tuberculous nodes (Ahuja et al. 2001; Park and Kim 2014).

This study illustrates that the AUC of ROC curve in the differentiation of metastatic and tuberculous nodes was the highest among different differential diagnoses, and was significantly higher than that in differentiating reactive and tuberculous lymph nodes as well as in distinguishing reactive and metastatic nodes. This finding suggested that intranodal VI is a useful parameter in distinguishing metastatic and tuberculous lymph nodes particularly in areas of the world where tuberculosis is highly prevalent. The better performance of using intranodal VI in the differentiation of metastatic and tuberculous lymph nodes when compared to others is because the significant difference in the VI of metastatic and tuberculous nodes (mean VI: 38% and 17% respectively) which allowed more accurate differentiation between these two pathological conditions.

The present study investigated the diagnostic accuracy of using intranodal VI and suggested the optimum cut-off of VI in distinguishing different causes of cervical lymphadenopathy. However, one must be aware that intranodal VI analysis should not be used as the sole method in the differential diagnosis of cervical lymphadenopathy. It is complementary and must be used in conjunction with grey scale and power Doppler ultrasound which assess the morphology and vascular distribution of lymph nodes respectively (Ahuja and Ying 2003; Giacomini et al. 2013). Grey scale ultrasound has a high sensitivity in differentiating metastatic and benign lymph nodes (96.8%) but the specificity is low (32%) (Baatenburg de Jong et al. 1989). Evaluation of intranodal VI may improve the specificity by increasing the true-negative rate because benign lymph nodes tend to have lower VI and they are readily identified in the VI image analysis. Although the lower VI of tuberculous nodes may be due to the high incidence of intranodal necrosis, it must be remembered that intranodal necrosis is also common in metastatic nodes from squamous cell carcinoma and papillary carcinoma of the thyroid (Ustun et al. 2002; Kessler et al. 2003; Giacomini et al. 2013). Therefore, in the assessment of necrotic lymph nodes with low VI, other ultrasound features such as nodal vascular distribution, matting and adjacent soft tissue edema should be used to identify tuberculous nodes whereas ultrasound features such as location, shape, punctate calcification and peripheral vascularity are used to identify metastatic lymph nodes (Ariji et al. 1998; Ahuja and Ying 2003; Rosario et al. 2005; Park and Kim 2014).

Quantitative analyses of vascularity of cervical lymph nodes have been previously reported (Wu et al. 1998a; Kagawa et al. 2011) which demonstrated that metastatic nodes had higher vascularity than benign nodes, which is similar to the result of the present study. However, previous studies focused on the differentiation of metastatic and benign lymph nodes, whereas the present study evaluates the diagnostic accuracy of intranodal VI in differentiating between various causes of cervical lymphadenopathy and suggests optimal cut-off of VI for these differential diagnoses. Therefore, the present study provides additional information on the use of VI in the assessment of cervical lymphadenopathy. The increased intranodal vascularity in metastatic and lymphomatous nodes due to tumour angiogenesis, the normal vascular architecture of reactive nodes gives moderate amount of intranodal vascularity, and the high incidence of intranodal necrosis in tuberculous nodes leads to low intranodal vascularity. These explain why VI could distinguish different causes of cervical lymphadenopathy.

The computed-aided assessment method used in the present study evaluated the VI of lymph nodes on two-dimensional (2-D) sonograms. It is presumed that the intranodal vascularity may be more accurately quantified in a three-dimensional matrix. Further study is suggested to combine this computer-aided assessment method with three-dimensional (3-D) ultrasound so that volumetric analysis of the VI of lymph nodes can be performed. Similar study has been performed for VI of laryngeal tumours using 3-D ultrasound which suggested VI is a useful parameter for predicting lymph node metastases (Zhou et al. 2009). However, quantitative evaluation of intranodal VI takes time for post-processing of ultrasound images and if an algorithm for automatic image processing can be developed it would substantially reduce the examination time and be more applicable in routine clinical practice. In the present study of evaluating 2-D sonograms, it took about 15-20 minutes to transfer the image from the ultrasound unit to the image analysis workstation, and to evaluate the image and quantify the VI of a lymph node.

Although power Doppler ultrasound is sensitive in detecting fine blood vessels, some vasculature within lymph nodes could be very small and may not demonstrate colour signals on the sonogram. Similar to the assessment of other nodal vascular features such as vascular distribution, standardized Doppler settings such as PRF and wall filter are essential to ensure high reproducibility. In routine clinical practice, standardized power Doppler settings for detection of small blood vessels are used for the assessment of cervical lymph nodes. However, the adjustment of colour gain could be subjective which may affect the reliability of intranodal vascularity assessment.

In conclusion, quantitative evaluation of intranodal VI is useful for the differential diagnosis of certain causes of cervical lymphadenopathy. It is particularly useful in distinguishing metastatic and tuberculous lymph nodes, and the optimum cut-off value for the differential diagnosis is 22%. However, it has limited value in distinguishing lymphomatous nodes from metastatic and reactive nodes.

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## Figure legends

Figure 1 A: Longitudinal power Doppler sonogram of a metastatic upper cervical lymph node. B: Longitudinal power Doppler sonogram of a lymphomatous submandibular lymph node. C: Longitudinal power Doppler sonogram of a reactive parotid lymph node. D: Transverse power Doppler sonogram of a tuberculous lymph node in supraclavicular fossa. The lymph node has intranodal necrosis (arrows) and shows displacement of vascularity (arrowheads).

Figure 2 The sequence of image analysis of the power Doppler sonograms of the lymph nodes shown in Figure 1 (A: metastasis; B: lymphoma; C: reactive; D: tuberculosis). In images A1, B1, C1 and D1, the region of interest ROI (i.e. the lymph node) was extracted by trimming the unwanted area from the four sides of the ROI (i.e. top, right, bottom and left sides). In images A2, B2, C2 and D2, the ROI was further extracted from the outlined area, and the total number of pixels within the ROI was counted by the computer algorithm. In images A3, B3, C3 and D3, the colour pixels coded by the power Doppler ultrasound were extracted by eliminating the grey scale pixels, and the number of colour pixels was counted by the computer algorithm. The calculated vascularity index of the metastatic (A), lymphomatous (B), reactive (C) and tuberculous (D) lymph node was 59.8%, 34.1%, 13.4% and 7.6% respectively.

Figure 3 Histogram shows the vascularity index of metastatic, lymphomatous, reactive and tuberculous lymph node.