The Role of Contrast-Enhanced Gray-Scale Ultrasonography in the Differential Diagnosis of Superficial Lymph Nodes

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Abstract: Lymph node micrometastases are common, but too often in clinical practice lack the tools for their accurate prebiopsy detection. The gray-scale contrast-enhanced ultrasonography technique permits high-resolution imaging of both the arterial and parenchymal phase and allows visualization of diffuse and partial alterations of nodal perfusion even in lymph nodes with a maximum diameter smaller than 1 cm.

The gray-scale contrast-enhanced ultrasonography can supply further useful information in case where doubt has arisen with conventional techniques. The results obtained show that it affords highly accurate differentiation between benign and metastatic lymph nodes.

Key Words: color Doppler sonography, contrast media, lymph nodes, oncological imaging, software, sonography

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The exclusion or identification of lymph node metastases is of primary importance in oncological staging because it directly affects not only prognosis, but also treatment decisions; equally important is the rapid definition of the nature of newly developed lymph node enlargement also in patients without a history of neoplastic diseases.

Gray-scale sonography and power Doppler sonography have been commonly used in the assessment of superficial lymph nodes (neck, axilla, and groin). The role of gray-scale sonography is well established,[1–4] and color Doppler, power Doppler, and pulsed Doppler sonography can supply additional information about the distribution of intranodal vessels, blood flow velocity, and vascular resistance. In particular, the absence of the echogenic hilus, round (longitudinal diameter–to–transverse diameter ratio of <2), and peripheral capsular vascularization are the signs regarded in the literature as being the characteristics of metastatic lymph nodes.[5–12]

These signs are connected to a global alteration of lymph node morphological characteristics or vascularization, which occurs in massive neoplastic invasion. On the contrary, small partial lesions are only incidentally detected because of the insufficient contrast resolution of current methods.

The use of microbubble contrast agent determined an important evolution of lymph node ultrasound examination.

The observation that microbubbles may be used as ultrasonography (US) contrast agent is not recent, as the first description dates back to 1968, when contrast phenomena were observed in the aorta during cardiac catheterization after injection of saline solution.[13]

The technology universally adopted is that of encapsulated bubbles of gas that are smaller than red blood cells and therefore capable of circulating freely in the body; these are the so-called blood pool agents.

First-generation high mechanical index contrast agents, based on air-filled microbubbles, were initially used on lymph nodes to enhance intranodal Doppler signals. This technique corresponds to the emission of a wideband signal when the microbubbles are destroyed by a high transmit power insonation and is limited by the transiency of the signal. Several studies have reported that this technique improved the diagnostic accuracy in the evaluation of cervical lymph nodes.[14,15]

These methods are unable to overcome the intrinsic limits of color and power Doppler, which can only provide information on the macrovascularization of lymph nodes; the detection of color signals on the entire lymph node surface, as happens with the modern high-sensitivity units even without contrast, is in part due to the fact that the small intranodal vessels are depicted by Doppler as having a larger lumen than in reality and therefore appear to occupy larger areas than they actually do.

However, this application should be considered left behind by introduction of second-generation contrast agents, which, being detected by gray-scale harmonic imaging techniques, allow real-time analysis of all vascular phases, including the parenchymal phase, and therefore provide extremely detailed information on nodal perfusion. The gray-scale contrast-enhanced US (CEUS) technique permits high-resolution imaging of both the arterial and parenchymal phases and allows visualization of diffuse and partial alterations of nodal perfusion even in lymph nodes with a maximum diameter smaller than 1 cm.

Second-generation contrast agents are composed by a sulfur hexafluoride–filled microbubbles and are encapsulated by a flexible phospholipid shell. Compared with contrast agents,air-filled microbubbles have a high and prolonged stability in the peripheral blood due to the low solubility of the gas and to stability of the phospholipids shell; the uniformity

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of microbubble diameter improves the backscattering and harmonic behavior at low acoustic power insonation.

TECHNIQUE AND CLINICAL APPLICATION

Gray-Scale Sonography

Baseline ultrasonographic evaluation of lymph nodes should be performed using a 7.5-MHz central frequency linear transducers.

During B-mode scanning, the focus should always be placed at the level of the structures being studied to obtain the highest resolution.

Lymph node shape is a useful criterion for the differential diagnosis of lymphadenopathies and is generally evaluated on the basis of the longitudinal-transverse axis ratio (L/T ratio); lymph nodes with an L/T ratio higher than 2 have an oval or fusiform shape indicating benignity, whereas lymph nodes with an L/T ratio lower than 2 tend to be rounded and are more often malignant. This criterion, deemed useful in clinical practice, has various exceptions; in particular, a round shape can be found in normal parotid and submandibular lymph nodes and is quite common in inflammatory forms in early childhood.

Several authors have considered the size suggesting different cut-off sizes (maximum axial diameter, 5, 8, and 10 mm) to allow differentiation of benign and malignant lymph nodes; it has also been stressed that the lymph nodes of the upper cervical regions tend to be slightly larger than those located in lower cervical regions.[16]

The size criterion, however, is of relative value given that inflammatory lymph nodes can be as large as neoplastic lymph nodes, and neoplastic localizations are not a rare finding in small lymph nodes.

Normal and reactive lymph nodes have a large, hyperechogenic structure, defined as hilus, which is located in the central part of the lymph node and which continues with the surrounding fat (Fig. 1). This pattern was first reported in the mid-1980s; later studies demonstrated, also based on radiological-histological comparisons, that this appearance is the result of numerous interfaces located at the level of the lymph node medulla (blood vessels, lymphatic sinuses, fat).[17–21]

It should be noted, however, that the hilus may also appear complete in the early stages of neoplastic invasion, before infiltration of the medulla; on the contrary, the hilus may be absent in smaller lymph nodes less than 5 mm, in which the medullary interfaces cannot be visualized because of inadequate resolution.

Many studies on this parameter report that the echogenic hilus is absent in malignant lymph nodes, with a percentage between 72% and 95%.[1,4,16,22]

There may also be a reduction, interruption, or dislocation of the hilus as a sign of metastasis or lymphomatous infiltration.

The lymph node cortex has a hypoechogenic, homogeneous structure with smooth borders, and it evenly surrounds the hilus. The most frequent alterations to the normal cortical structure are calcifications and areas of necrosis. The presence of prevalently peripheral hyperechoic spots, indicating microcalcifications, has been found in about 50% to 69% of lymph nodes with metastasis from papillary carcinoma of the thyroid[4]; less frequently, microcalcifications may also be present in lymph node metastases from medullary carcinoma of the thyroid, whereas grosser calcifications may be found in tuberculous adenopathies, in other granulomatous inflammations, and after radiotherapy and chemotherapy.

Intranodal necrosis is characterized by anechoic areas that are frequent in metastases from squamous cell carcinoma and papillary carcinoma of the thyroid and in tuberculosis.

With regard to global alterations of the cortical structure, it should be recalled that lymphomas have been reported to have a strongly hypoechogenic, "pseudocystic" appearance with posterior wall shadowing; this pattern, however, is less evident with the more recent transducers, which often depict fine intranodal reticulation or a microdular appearance.

Color and Power Doppler

Upon evaluation by color or power Doppler, normal lymph nodes demonstrate vascularization at the level of the hilus, where the principal vessels flow, whereas they never demonstrate peripheral vessels at the level of the capsular contour (Fig. 2).

Smaller lymph nodes, with maximum transverse diameter smaller than 5 mm, may apparently be avascular; this

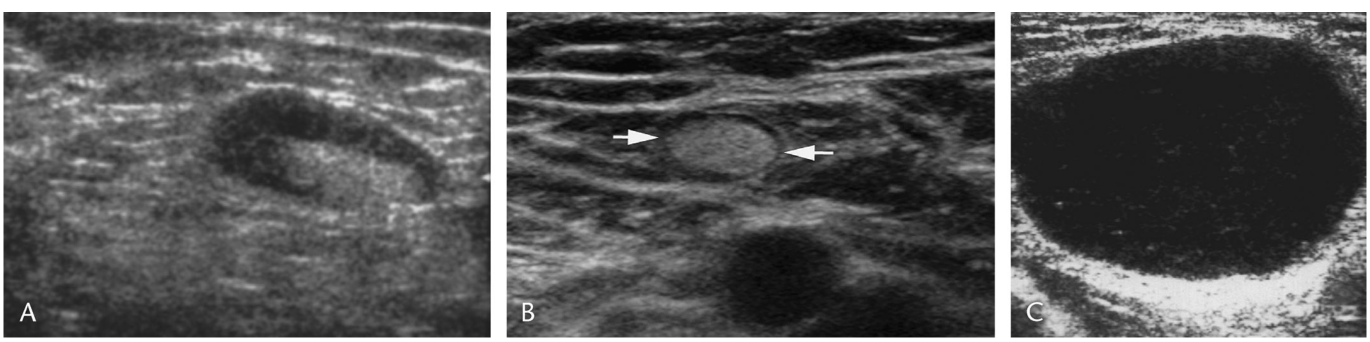

FIGURE 1. Conventional US. A, Normal cervical lymph node (arrows) with elongated shape, echogenic hilus, and regular outlines. B, Lymph node with large echogenic hilus from marked adipose metaplasia; the central hyperechogenicity occupies a large part of the lymph node, and the cortex is reduced to a thin peripheral hypoechogenic layer. C, Enlarged lymph node with an L/T axis ratio lower than 2 and absence of echogenic hilus (metastasis from squamous cell carcinoma).

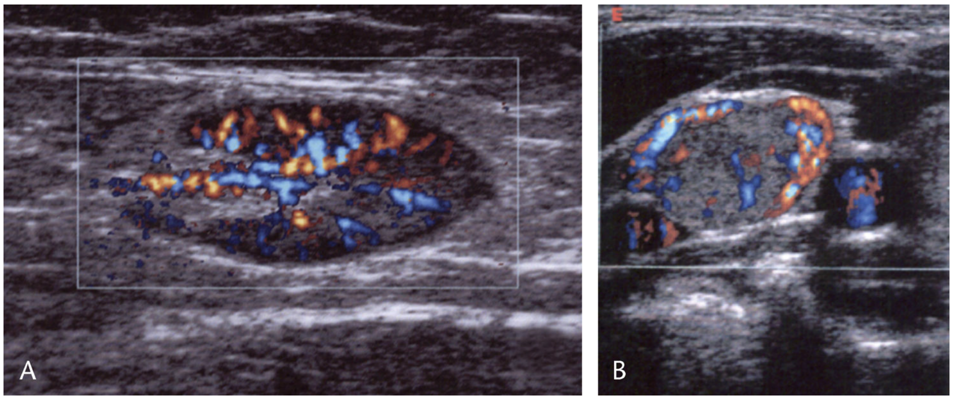

FIGURE 2. Color Doppler. A, Normal vascularization showing main artery at the hilus and arterioles directed toward the cortex. B, Metastatic lymph node with mainly peripheral vascularization and persistence of a few cut-off hilar branches.

pattern is more frequent in cervical than in axillary and inguinal lymph nodes, especially at the level of the posterior neck triangle.

Furthermore, the avascular pattern is more common in elderly patients. Among the cervical lymph nodes, the greater vascularization is seen at the submandibular level, probably as a result of inflammatory events of the upper airways, which even when asymptomatic can activate regional lymph nodes.

Metastatic lymph nodes tend to have peripheral vascularization along the capsular contour with various vascular poles or mixed, peripheral and hilar, vascularization.

This pattern of peripheral vascularization may be explained by the fact that, in metastases, the tumor cells reach the lymph node via the afferent lymphatic vessels that pierce the convex profile of the capsule and from here colonize the marginal sinus and the outer parts of the lymph node and then invade the inner part reaching the medulla and hilus.

Lymphomas are frequently characterized by hypertrophic vessels with enlarged lumen arising from the hilus and by peripheral vessels, which, however, are less frequent than in metastases. These neoplasms originate within the lymph node, of which they invade the central portion, whereas the periphery may remain intact for a long time.

The vascularization pattern in lymphomas may be poorly characteristic, and, especially in low-grade lymphomas, it partly overlaps the appearance of inflammatory lymph nodes. It must be noted that in these cases, B-mode US can be of great help and is often more significant than Doppler analysis, as it shows grossly enlarged lymph nodes, with globose morphology and altered echogenic or absent hilus.

However, the most significant finding for the differentiation of benign and malignant lymph nodes is the detection of peripheral vascularization, an aspect that should always lead to further diagnostic investigation. Vascularization extending to the whole lymph node, but harmonic and with no peripheral vessels, indicates benignity. Finally, avascular but morphologically altered lymph nodes may be metastases with extensive necrotic areas and obliteration of the newly formed vessels. The complete absence of visible vessels has been reported also in tubercular lymphadenopathies as a result of necrosis or fibrosis.[16]

The evaluation of the Resistive and Pulsatility Indexes by means of pulsed Doppler has a limited clinical role because it considerably increases examination time and because the results reported in the literature are somewhat controversial.[23]

Gray-Scale CEUS Sonography

In our experience, CEUS examinations were performed on the lymph nodes being considered by means of an apparatus (MyLab Ultrasound System, Esaote, Genova, Italy; Esaote that was specifically designed for sonographic examinations with a contrast agent. A contrast agent composed of sulfur hexafluoride–filled microbubbles (SonoVue, Bracco International, B.V., Amsterdam, Netherlands) was used.

A 7.5-MHz dedicated linear transducer was used in conjunction with new continuous-mode, contrast-enhanced harmonic imaging technology. During transmission, low values of mechanical index (ie, 0.05–0.2) and of acoustic pressure allow the microbubbles to oscillate at maximum intensity without being destroyed. The acoustic pressure was set at 45 kPa in

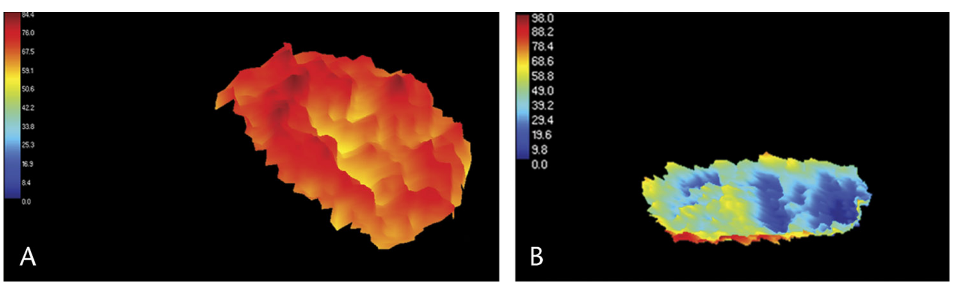

FIGURE 3. Chromatic maps. A, Reactive lymph node demonstrates a homogeneous vascularization. B, Metastatic lymph node with inhomogeneous vascularization and hypoperfused areas (blue areas).

each patient, and the mechanical index was selected automatically by the sonography scanner in relation to beam-focus depth. During reception, the signal emitted by the microbubbles is received in a selective manner, thereby eliminating all signals that are not useful. This technique, developed by Esaote and Bracco in conjunction, is referred to as contrast-tuned imaging technology. This contrast-specific technique uses the transmission of the specific resonance frequency of sulfur hexafluoride–filled microbubbles and the selective registration of harmonic frequencies. This allows a significant reduction of the insonation power with a reduction of the nonlinear harmonic behavior of the stationary tissues.

All patients gave their informed consent for the examination including intravenous administration of the contrast agent. A 4.8-mL bolus of contrast agent was injected into a peripheral vein and was followed by an injection of 10 mL of physiological saline solution. Immediately after the injections, the lymph nodes were scanned in contrast-tuned imaging technology mode with a frame rate of 15 frames per second.

The transducer was placed directly on the patient's skin without interposition of any pad and was kept in a fixed position to highlight all the phases of enhancement of the lymph node being examined.

The beam focus was placed at the level of the lymph node being examined or immediately below it, and beam gain was set, in all cases, at the minimum level.

The apparatus in question affords the recording and filing of the images in digital format, and all the dynamic phases of the examinations performed during 25 seconds were saved using this system.

Quantitative Analysis

The digital recordings were sent from the sonography scanner to a PC and were then processed by means of experimental software (Qontraxt, Amid and R&D, Bracco, Milan, Italy).

This software is able to analyze the signal intensity of each single pixel of each frame and thereby to generate chromatic maps that allow immediate evaluation of the perfusion

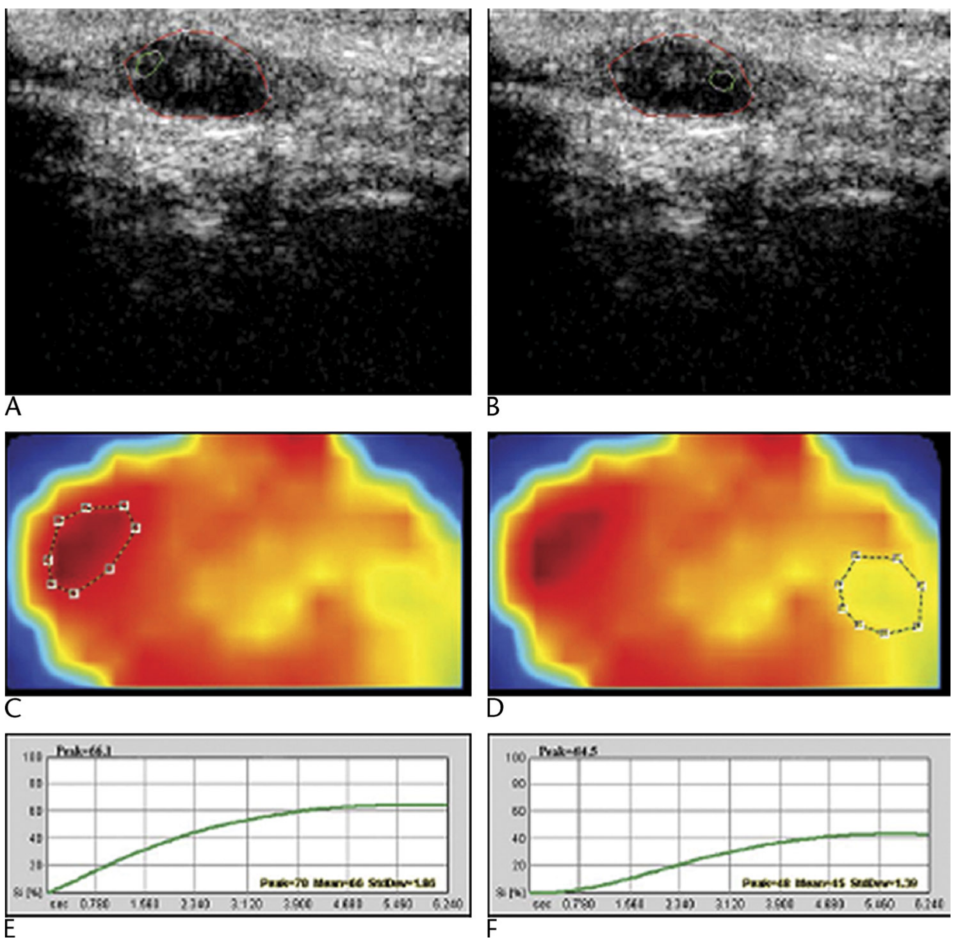

FIGURE 4. Inguinal reactive lymph node (AJR). A and B, Reference scans delimit areas (circle) corresponding to lymph node under examination to be processed by software for evaluation of maximum (A) and minimum (B) signal intensity areas. C and D, After analyzing all frames of recording as expression of maximum (C) or minimum (D) signal intensity in each pixel, software (Qontraxt; Amid) creates chromatic maps composed of scale of primary colors varying from red (maximum signal intensity) to blue (minimum signal intensity). E and F, Software automatically supplies signal intensity–time curves and numeric values of peak, mean, and SD (StdDev) signal intensities for areas of maximum (E) and minimum (F) signal intensity. Time in seconds is shown on *x* axis, and *y* axis shows signal intensity as percentage, with 100% being maximum intensity.

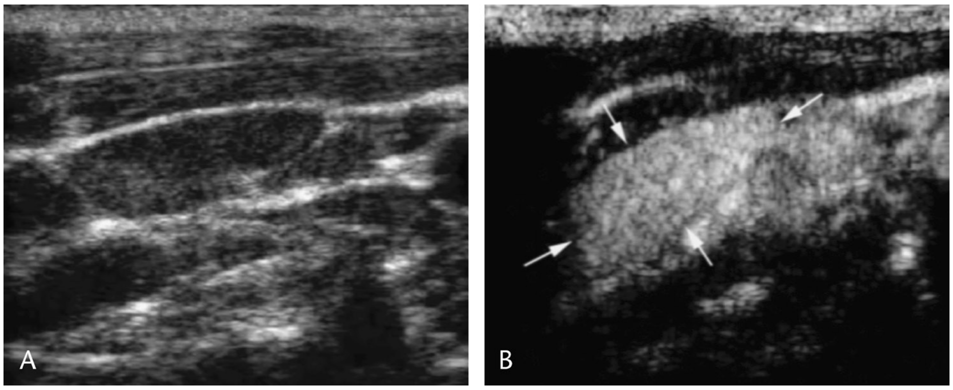

FIGURE 5. Reactive neck node. A, Baseline sonogram before injection of the contrast agent. The node is oval and has regular margins and a homogeneous hypoechoic structure. B, Contrast-enhanced sonogram in the parenchymal phase showing intense and widespread enhancement of lymph node echogenicity.

properties of the entire organ under examination or of regions of interest (ROIs), irrespective of shape or dimensions, as selected by the operator (Fig. 3). On the basis of this analysis, the software enables numeric values to be obtained for each point in the region under examination, during a temporal sequency selected by the operator, as the final result. These values are correlated to the quantity of contrast medium that reached the sector in question. In practice, a virtual image is obtained of the lymph node composed of a scale of primary colors varying from red (maximum signal intensity) to blue (minimum signal intensity). The level of enhancement is not uniform over the entire lymph node volume: generally, the normal hilar region or areas of inflammation tend to give high signal intensity, whereas signal intensity corresponding to fatty infiltration or areas of necrosis is lower. For these reasons, the position and dimensions of the ROIs should be chosen on the basis of the chromatic maps obtained.

By moving a cursor over the image, it is possible to explore the various color zones and obtain, at every point therein, the numeric value of signal intensity expressed as a percentage (maximum intensity = 100%).

Contemporaneously, the system generates a signal intensity–time curve in relation to the selected point or region (Fig. 4). All procedures are fully automated; human intervention is limited to selection of the organ or the ROI and specification of the initial and final frames of the perfusion interval.

The software incorporates a graphic interface that is intuitive to use, with the objective of providing a product that can be used by personnel without specialized training.

GRAY-SCALE CEUS APPEARANCE

Gray-scale CEUS improves the diagnostic accuracy in the differential diagnosis between benign and metastatic superficial lymph nodes by assessment of characteristic enhancement patterns. Compared with cytological and/or histological diagnosis, this technique displayed a high sensitivity, specificity, and accuracy (92%, 93%, and 92.8%, respectively) in distinguishing metastatic from reactive lymph nodes.[24]

Normal and reactive lymph nodes give rise to diffused intense and homogeneous contrast enhancement due to intense vascularization with a rich cortical capillary circulation[25] (Figs. 5 and 6).

Lymph node metastases are generally less vascularized than healthy nodal parenchyma; therefore, they show up as well-localized areas of perfusion deficits after microbubble

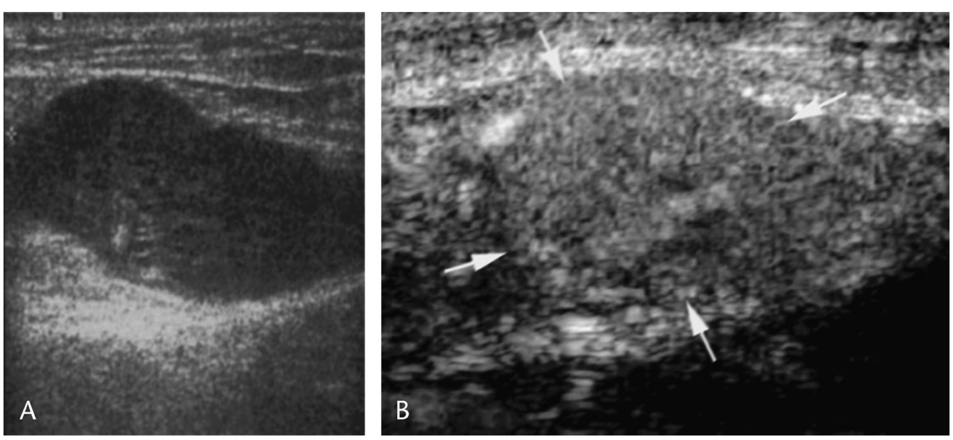

FIGURE 6. Reactive axillary lymph node. A, Baseline sonogram showing a round lymph node (arrows) without an echogenic hilus. B, Contrast-enhanced sonogram showing intense and homogeneous enhancement of echogenicity (arrows).

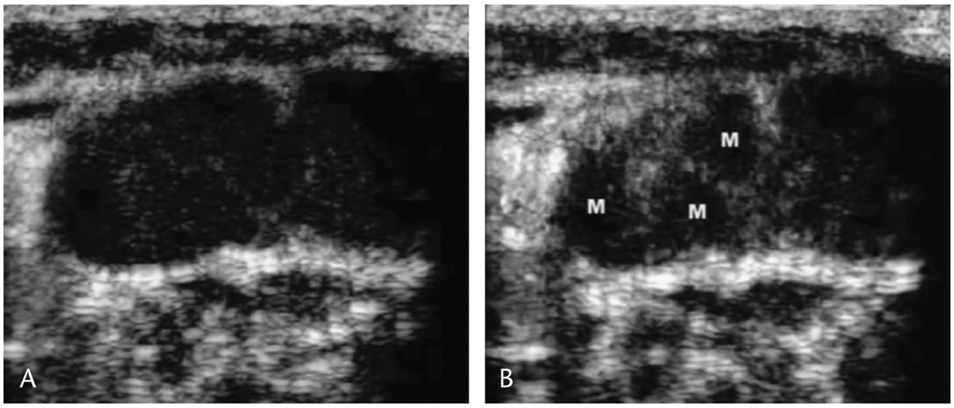

FIGURE 7. Cervical lymph node with metastasis. A, Baseline sonogram before injection of the contrast agent showing an oval lymph node and a homogeneous structure. B, Contrast-enhanced sonogram in the parenchymal phase shows 3 evident central perfusion defects (M).

injection (Figs. 7 and 8). Furthermore, scant or absent perfusion due to large confluent areas of necrosis and neoplastic infiltration involving the entire lymph node are frequent in metastatic lymph nodes.[5,26–28] This aspect, which is considered, at computed tomography and magnetic resonance imaging as well, as a specific sign of metastasis,[29] may sometimes be the result of an inflammatory process, such as in tubercular nodes and lymphadenitis.[30]

The vascularization pattern in lymphomas, on the contrary, has a more variable appearance, which is partially similar to the pattern of reactive and metastatic lymph nodes. It may be poorly characteristic, and, especially in low-grade lymphomas, it partly overlaps the appearance of inflammatory lymph nodes.[24,31] For this reason, results are difficult to distinguish with this technique.

Automated quantification of sonographic signal intensity, which is related to contrast enhancement of lymph nodes tissue, by using signal intensity–time curves, also permits to obtain quantitative and objective data to differentiate areas of normal tissue from perfusion defects. It has be found that the values obtained in the evaluation of the area with the least increase of signal intensity present a statistically significant difference ($P < 0.001$) between benign and metastatic lymph nodes.[32]

The use of CEUS has been also documented, after subcutaneous injection of microbubble contrast agent, to identify sentinel lymph nodes, which are defined as the first lymph node to receive lymphatic drainage from a neoplasm and are at highest risk for regional metastasis. A tissue-specific US contrast agent that when injected intravenously is taken by the macrophages of the reticuloendothelial system (Sonazoid; Amersham Health, Oslo, Norway) was used after subcutaneous peritumoral administration to identify the draining lymphatic channels and the sentinel lymph nodes in a swine model with melanoma.[33] This examination was correlated with those found with the lymphoscintigraphy and with the surgical dissection after an injection of blue dye. The overall sensitivity of sentinel lymph node detection was 90% for lymphatic US and 81% for lymphoscintigraphy. This technique performed with SonoVue showed that the sensitivity and specificity were 95% and 63%, respectively.[34]

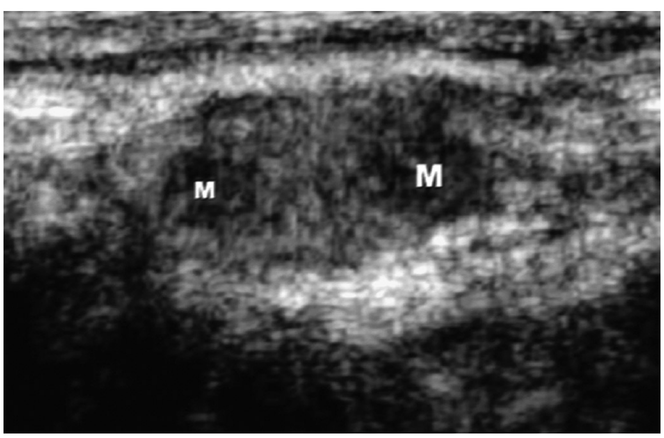

FIGURE 8. Metastatic lymph node. Parenchymal phase after injection of contrast medium shows an intense and dishomogeneous contrast enhancement due to the presence of 2 areas of perfusion defects.

CONCLUSIONS

High-resolution US and color or power Doppler are routinely used in the study of lymph nodes. The evaluation of morphology, echo structure, and vascularity of lymph nodes, together with the clinical and laboratory data, enables the selection of those cases to be examined by US-guided fine-needle aspiration. The results of US-guided fine-needle aspiration cytology obviously depend on the experience of the operator, and even in experienced hands, approximately 15% of samples may not lead to an accurate diagnosis because of difficulties of interpretation by the cytologist or of inadequate sampling material.

Gray-scale CEUS can supply further useful information in case where doubt has arisen with conventional techniques. The results obtained show that it affords highly accurate differentiation between benign and metastatic lymph nodes. Furthermore, the detection of defect areas in lymph nodes smaller than 1 cm, in addition to the diagnostic importance, can prove useful in indicating the most suitable area for taking a cytological sample by means of ultrasonographically guided fine-needle aspiration cytology, thereby affording the possibility of further improving the sensitivity of this already very-high-specificity method.

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