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ONCOLOGY

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Color-coded duplex sonography of the cervical lymph nodes: improved differential diagnostic assessment after administration of the signal enhancer SH U 508A (Levovist)®

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Abstract The vascularity of cervical lymph nodes can be documented by means of color-coded duplex sonography and malignant and benign lymph nodes distinguished on the basis of typical patterns of vascularity. However, not all intranodal vessels can be visualized by color-coded duplex sonography, and minute vessels are detectable only after the administration of a signal enhancer. This also makes it possible to assess the morphology of cervical lymph nodes that are inaccessible on plain sonography. In the present study we examined acute and chronic inflammatory and metastatic lymph nodes as well as malignant lymphomas to determine the extent to which a specific pattern of vascularity can be detected with color-coded duplex sonography after the injection of Levovist® as a signal enhancer. In addition, digital image processing was used to quantify the vascularity detected in relation to the cross-sectional area of the lymph nodes as seen at sonography and to determine whether there are any differences in lymph node types as regards an increase in the detection of vascularity. After injection of the marker a typical pattern of vascularity could be assigned to all lymph nodes examined and differences shown in quantifying vascularity: This increase was greatest in the acutely inflamed lymph nodes (36.0 ± 5.0%) and smallest in lymph nodes with chronic inflammation (2.3 ± 1.3%). These findings show that cervical lymph nodes of varying origin differ by virtue of their pattern of vascularity, with increased vascularity detectable after administration of a signal enhancer.

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Key words Color-coded duplex sonography · Cervical lymph node pathology · Lymph node vascularity · Signal enhancer

Introduction

Previous studies have shown that ultrasound can be used to detect abnormal cervical lymph nodes [6, 9]. Despite the high sensitivity of the method differentiation between benign and malignant lymph nodes can be difficult. Extirpation of a lymph node may be necessary in doubtful cases, with the risks of surgery and subsequent morbidity accepted as possible consequences. To avoid unnecessary interventions, criteria for malignancy in B-mode sonography include such findings as inhomogeneity, rupture of a node's capsule, and infiltration of adjacent structures. In addition, the vascularity of a lymph node in sonography can also be used as a criterion for malignancy [14]. The pathophysiological background for color-coded duplex sonography is the correlation of growth of a malignancy and angioneogenesis [3]. Owing to the limitations of available technology, however, very small vessels cannot be detected by routine duplex sonography, a situation that has produced some controversy about the correlation of typical patterns of vascularity with cervical lymphadenopathy due to various causes [2, 14].

If a signal enhancer is used during sonography even small vessels previously inaccessible to conventional color-coded duplex sonography can be detected. One such enhancer is Levovist® and is available for use in head and neck sonography. Levovist® is a 99.9% suspension of galactose microparticles which is stabilized with 0.1% palmitic acid and minute (< 3 μm) adherent bubbles of air as active constituents which amplify the color-coded duplex signal by up to 25 dB [8].

The present study examined the extent to which the administration of Levovist® improves color-coded duplex sonographic detection of vascularity in cervical lymph nodes of various causes (acute and chronic inflammation, malignant lymphomas, and metastatic tumors) and

demonstrates disease-specific patterns of intranodal vascularity. In addition, a procedure was developed with the help of digital image processing for quantifying the vascularity detected by color-duplex sonography in the cross-sections of lymph nodes. This procedure was then used to determine differences in lymph node enlargement due to various causes after the administration of Levovist®.

Materials and methods

All duplex-sonographic examinations were conducted in a nonselected population of patients with enlarged cervical lymph nodes of varying origin. The diagnosis of acute lymphonodulitis (n = 10) was made on the basis of clinical parameters. The diagnosis of a cervical lymph node metastasis from squamous cell carcinoma (n = 30), chronic inflammation (n = 10), and cervical lymphomas (n = 5) was verified by histology after completion of color-coded duplex sonography.

Ultrasound examinations

All examinations were performed with a high-resolution B scanner (Sonoline Elegra Advanced, Siemens, Erlangen, Germany) with a variable 5–9 MHz transducer. As previously described for lymph node sonography, the sensitivity for low blood flow velocities in color-coded duplex sonography was adjusted individually from case to case by changing the pulse repetition frequency so that as many intranodal vessels as possible could be detected [7]. Levovist® as signal enhancer was then prepared according to the manufacturer's instructions and injected intravenously 2 min later. Each patient was given an injection of 11 ml Levovist® at a concentration of 300 mg/ml. The first half of the total volume was given as a bolus; the second half was injected slowly as soon as a distinct increase in signals was detected. The examinations were recorded continuously by digital videography and evaluated later.

Assessment of vascularity patterns

All lymph nodes were examined for the distribution of intranodal vessels before injection of Levovist® and at the time point of maximum signal increase. To the extent that vessels were detectable by color-coded duplex sonography, a distinction was made between a *radial pattern of vascularity*, a pattern in which vessels run radially from the hilum to the lymph node periphery, a *hilar pattern*, a pattern in which vessels were limited to the hilum, a *heterogeneous pattern* in which vessels were distributed individually and diffusely over the lymph node stroma, and a *complete pattern of vascularity* in which the entire stroma was virtually filled with vessels.

Quantification of lymph node vascularity

All studies were videographed examinations and each sonogram was stored digitally in the red-green-blue (RGB) format (Fig. 1). Each color-coded pixel in the image represented an intranodal vessel and was defined by a triple (r, g, b) [5]. A gray value (lymph node stroma) was seen when all three color canals were equal, whereby the threshold of the gray value as an elliptical area around the gray value diagonals could be varied. All values outside this ellipse were then interpreted as color values and marked. After morphological filtering to eliminate noise processes while maintaining shape [4], associated regions were obtained to represent the vascularized areas of the lymph node and were calculated as a percentage of the total sonographic cross-sectional area of the lymph node stroma.

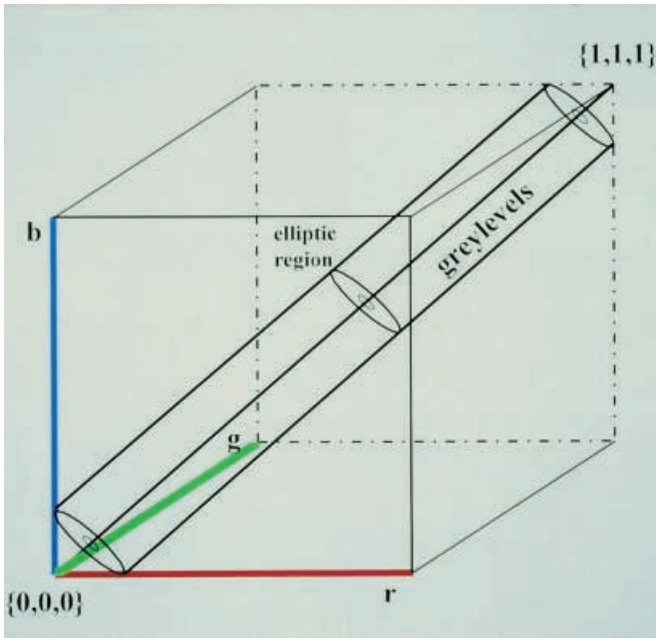


Fig. 1 The red-green-blue (RGB) color space. The three spatial axes (r, g, b) represent the three basic colors, while the color values perceived as gray values are defined by an ellipsoid surrounding the main diagonals of the color cube

The mean and standard deviation of the vascularity of the individual groups before and after administration of Levovist® were calculated using the SPSSPC+, version 4.0.1 (SPSS, Chicago, USA). Differences between the various groups were regarded as significant at $P \leq 0.01$ using the Mann-Whitney U test.

Results

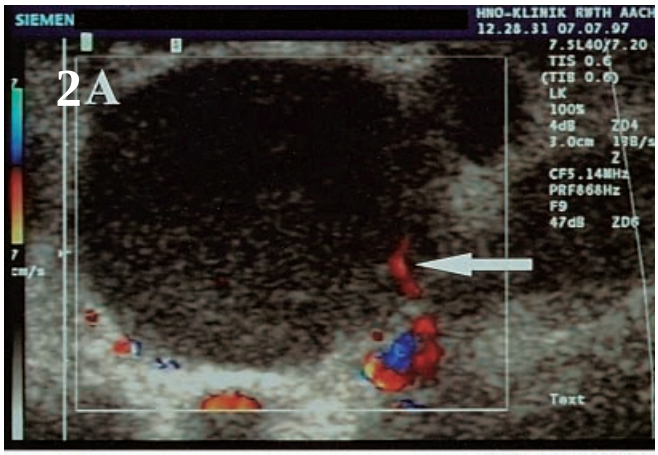
In all cases examined, duplex sonography detected substantially more vessels after the administration of Levovist®. An increase in the color-coded duplex signal was seen within 1 min after the injection of signal enhancer.

Fig. 2 A, B Color-coded duplex sonography of a metastatic lymph ▶ node. While only a peripheral vessel *(large arrow)* is detectable before the injection of Levovist® as signal enhancer (**A**), small vessels *(small arrows)* can also be detected throughout the entire stroma after its injection (**B**), resulting in a heterogeneous pattern of vascularity

Fig. 3 A, B In chronic inflammatory lymph nodes, vessels can be detected only in the hilar region *(arrow)* before (**A**) and after (**B**) administration of the signal enhancer

Fig. 4 A In acute inflammatory lymph nodes, prominent vessels running from the hilum *(arrow)* to the periphery can be detected even before signal enhancement. **B** After signal enhancement, the entire lymph node stroma is saturated with vessels

Fig. 5 A In the malignant lymphoma shown, vessels originating from the hilum *(star)* can be detected before administration of the signal enhancer. **B** After administration of Levovist®, vessels with an irregular course can also be seen in the peripheral region *(arrows)*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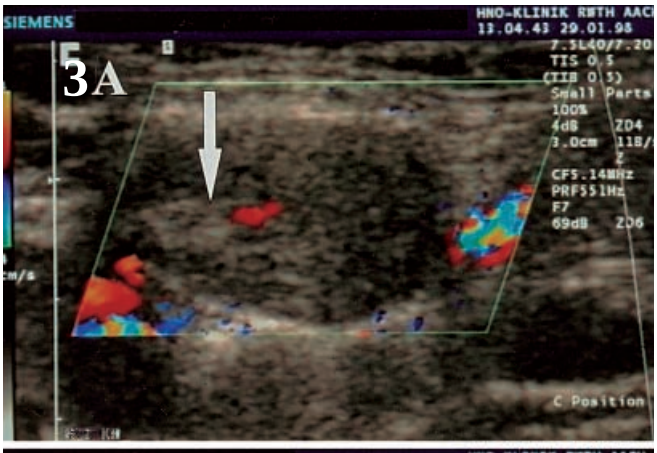
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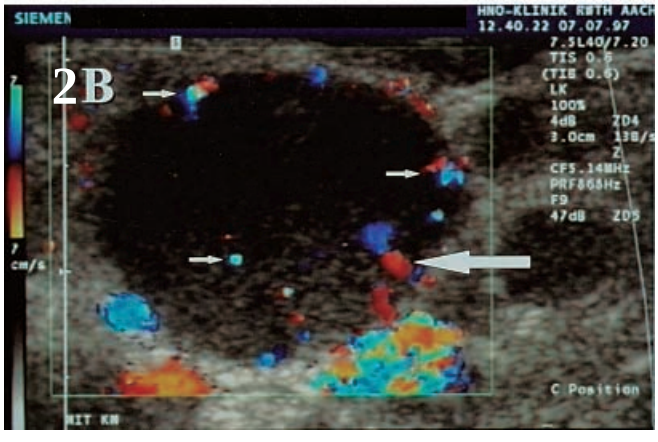
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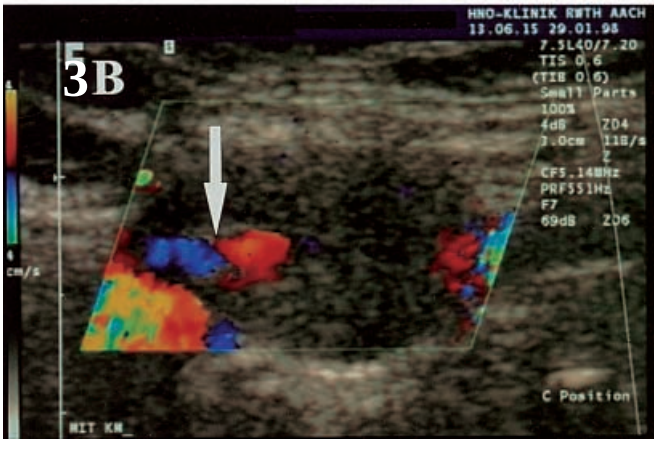
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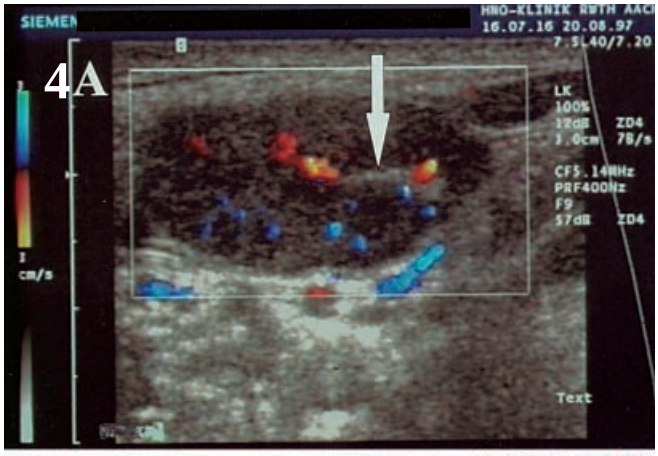
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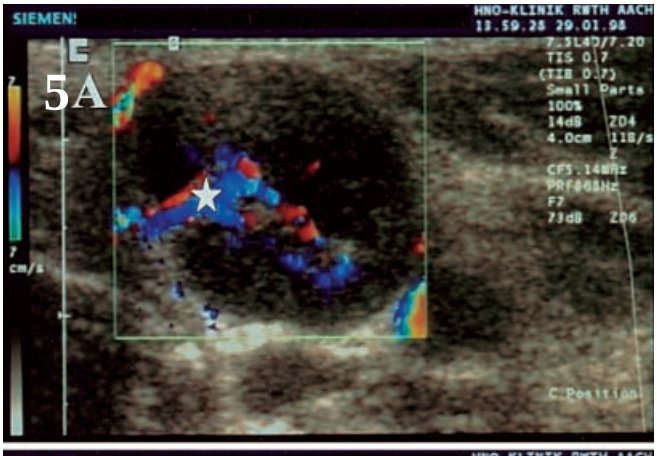
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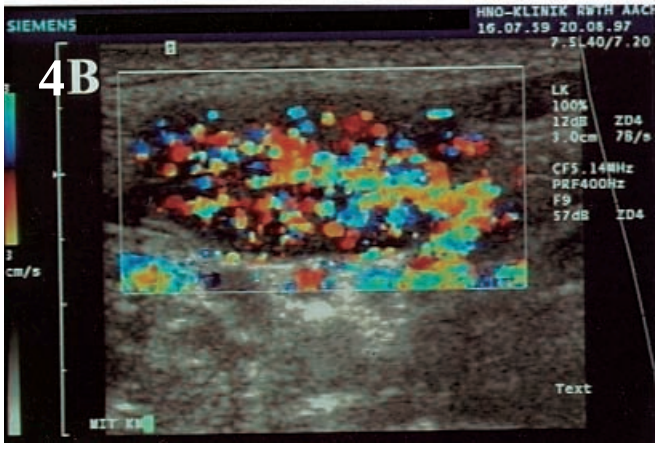
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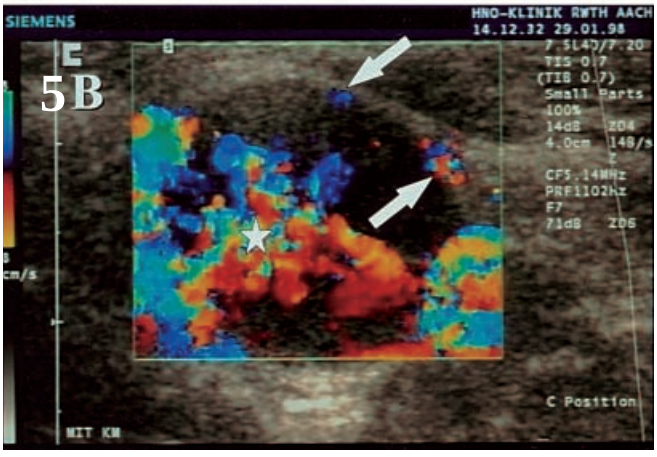
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No allergic or cardiovascular reactions occurred in the examinations performed, and only two patients reported a local feeling of warmth in the region of the injection site. Before the administration of Levovist® duplex sonography was unable to detect any vessels in metastatic lymph nodes, particularly in those smaller than 1 cm. In the other lymph node metastases the vessels were distributed diffusely in the stroma (Fig. 2 A). After the administration of the Levovist® a heterogeneous pattern with peripheral vessels running an irregular course were detected in all cases examined (Fig. 2 B). No vessels corresponding to normal lymph node anatomy and originating from the hilum were observed in the lymph node metastases examined. In the chronic inflammatory lymph nodes the vessels detectable at duplex sonography both before (Fig. 3 A) and after signal enhancement (Fig. 3 B) were limited to the hilum. The detection of vascularity increased substantially after the injection of Levovist®.

In contrast to the patterns seen in the nodes affected by chronic inflammation and metastatic tumor, a pronounced radial vascularity was observed in acute inflammatory lymph nodes even before signal enhancement (Fig. 4 A). The injection of Levovist® led to the detection of significantly more vessels in all cases. The color-coded duplex sonograms showed that almost the entire lymph node parenchyma was filled with vessels (Fig. 4 B). Before signal enhancement the malignant lymphomas examined were distinguished by a radial pattern that was less pronounced than the pattern seen in the acute inflammatory lymph nodes (Fig. 5 A). After signal enhancement vessels were also found distributed irregularly throughout the stroma (Fig. 5 B).

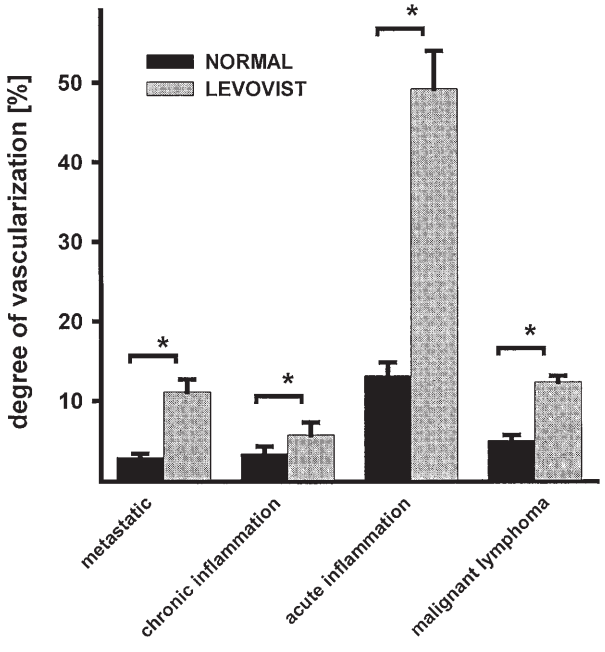

Fig. 6 Percentage of the sonographic lymph node cross-section accounted for by the vascularity detected before and after signal enhancement. *$P \leq 0.01$ by Student's *t* test

Cervical lymph nodes of different origin were characterized by a different degree of vascularity. The greatest degree of vascularity before and after administration of Levovist® was detected in the acute inflammatory lymph nodes (Fig. 6). In the malignant and chronic inflammatory lymph nodes the percentage of detected vessels before signal enhancement averaged 5% or less. After signal enhancement the percentage of vascularity detected increased to more than 10% in the malignant lymph nodes (metastases and malignant lymphomas) and increased only slightly (2%) in the chronic inflammatory lymph nodes.

Discussion

Sonography of the head and neck is a relatively quick and inexpensive imaging modality whose quality and sensitivity is often about the same as that of other modalities [11]. The technique is now characterized by a high sensitivity for the detection of cervical lymph nodes. Nevertheless, extirpation of a node is often required since pathology cannot be defined reliably enough in B mode studies. Consequently, color-coded duplex sonography is used to assess intranodal vessels, although the specificity of the ultrasound examination increases to only 77% [13]. The value of specific patterns of vascular distribution in the differential diagnosis of cervical lymph nodes of various origins is controversial [2, 10, 14]. One reason for this may be the previously inadequate detection of blood flow in minute vessels. To overcome these technical limitations signal enhancers are now available that permit the detection of even minor blood flow in the smallest lymph node vessels. The preparation of Levovist® used in our study is a very well tolerated signal enhancer which can increase the strength of the color-coded duplex signal by 25 dB. The only contraindications to the use of Levovist® are galactosemia and New York Heart Association stage IV heart failure [8].

The present study was conducted to determine whether administration of Levovist® as a signal enhancer permits better differentiation of cervical lymph nodes of different origin on the basis of their pattern of vascularity. In addition, an attempt was made to establish an objective, examiner-independent parameter for assessing vascularity. Parameters such as the perfusion and resistance index can vary greatly even within one entity [12] and are unsuitable for assessing the status of cervical lymph nodes. Consequently a new parameter was introduced that calculates the percentage of the lymph node cross-section accounted for by the vascularity detected by color-coded duplex sonography using digital image processing.

In all cases examined ($n = 55$) the percentage of vascularity detected by color-coded duplex sonography increased within the first minute after the injection of Levovist®. In particular, the frequent clinical problem of unequivocal differentiation of metastatic disease from chronic inflammatory change in lymph nodes is difficult to make with-

out the administration of a signal enhancer, since there may be no quantitative difference present regarding vascularity. When lymph nodes examined have a diameter of 1 cm or less, it is almost impossible to distinguish pathology in many cases because no intranodal vessels are demonstrable. However, vessels could be detected after the administration of our signal enhancer (Fig. 7). Metastatic lymph nodes were characterized by a heterogeneous pattern of vascularity after signal enhancement, with vessels observed not only in the hilar region ("normal anatomy") but also diffusely in the lymph node stroma and in the periphery [13]. In these cases small angioneogenetic vessels of the metastatic lymph nodes were seen only after administration of the signal enhancer. Additionally, tumor growth could displace regular vessels, with the result that the physiological lymph node structure appeared to be obliterated. In contrast, this structure is preserved in chronic inflammatory lymph nodes, and vascularity is thus confined to the hilum even after the administration of Levovist® [10, 15, 16]. The difference in lymph node vascularity caused by the neogenesis of vessels in metastatic lymph nodes is the reason for our being able to better detect abnormal vascularity. This improved differentiation of metastatic tumor from chronic inflammation in cervical lymph nodes resulting from the administration of Levovist® is of great importance in clinical practice particularly in the follow-up of patients with an epithelial malignancy originating in the upper aerodigestive tract and in the preoperative assessment of the N0 neck.

In acutely inflamed lymph nodes a pronounced radial pattern of vascularity can be detected before the administration of Levovist®. In borderline cases assessment based on the pattern of vascularity alone may lead to confusion with a malignant lymphoma. Quantification, however, reveals a much greater vascularity in acute inflammatory lymph nodes than in other lesions. After signal enhancement a specific pattern of vascularity filling almost the entire stroma with color-coded pixels is seen only in acute inflammatory lymph nodes (Fig. 7). The pathophysiological reason for this pronounced vascularity is an intranodal immune reaction, which causes widening of the vascular bed secondary to the release of cytokines [1]. This finding in color-coded duplex sonography can be used to support the diagnosis of acute lymphadenopathy made on the basis of clinical parameters.

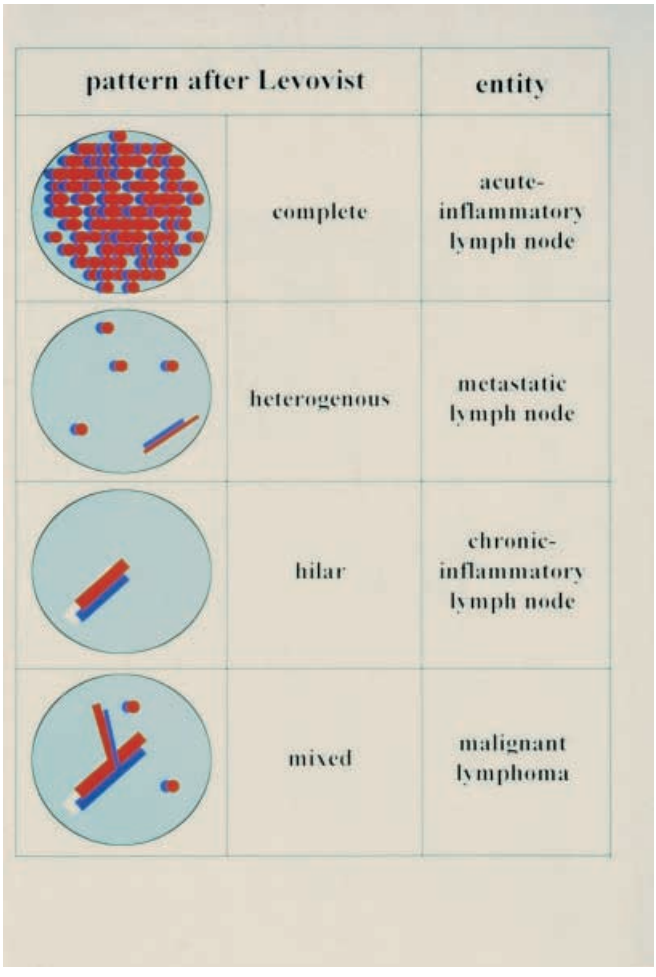

Fig. 7 Schematic representation of various patterns of vascularity after Levovist® injection in the groups of enlarged cervical lymph nodes due to acute and chronic inflammation, malignant lymphoma, and metastatic squamous cell carcinoma

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