

LYMPHATIC CAPILLARIES OF THE PERIOSTEUM: DO THEY EXIST?

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ABSTRACT

Normal periosteum from 12 humans was examined for the presence of lymphatic capillaries using immunohistochemistry (light microscopy) and transmission electron microscopy. Both techniques failed to demonstrate lymphatic capillaries suggesting that periosteum is devoid of these structures.

Lymphatic drainage from bone and periosteum is still an unresolved issue. Whereas most workers acknowledge that compact bone does not contain lymphatic vessels (1-5), some maintain that "osseous lymph" drains via lymphatics of the periosteum (2).

Large lymphatics have no characteristic morphologic feature that clearly distinguishes them from blood vessels of similar size. These lymphatics, therefore, can be recognized with some degree of certainty only by injection techniques, a method not readily clinically applicable. On the other hand, lymphatic capillaries lack a basal lamina and in this respect differ from blood capillaries (6). Accordingly, using light and transmission electron microscopy we examined human periosteum to detect small endothelial-lined vascular channels without basal lamina (i.e., lymphatic capillaries).

Whereas light microscopy (LM) has the advantage that relatively large areas can be examined, its disadvantages are that endothelium and basal lamina cannot

ordinarily be visualized in the same section, and nearby or adjacent sections (mirror sections) have to be compared. By contrast, transmission electron microscopy (TEM) allows endothelium and basal lamina each with its characteristic ultrastructure (6) to be detected in the same section. The chief disadvantage of TEM is that only comparatively small amounts of tissue can be examined at one time. Used together, however, LM and TEM should provide a reasonable assessment of whether periosteum normally contains lymphatic capillaries.

MATERIALS AND METHODS

Thirteen periosteal biopsies from 12 patients operated upon in the orthopedic department at Herlev Hospital were examined. Demographic findings including the site and indication for operation are summarized in *Tables 1 and 2*. Grossly, the specimens appeared as normal periosteum and were excised remote from the operated fractured or osteotomy site as was technically feasible. Each specimen measured at least 1x1cm and was removed with an osteotome in such a way that both periosteum and the underlying compact bone were represented. Only the edge of the specimen was traumatized by use of surgical forceps. Each specimen was fixed promptly after removal. After complete fixation the periosteum was gently freed from the underlying bone with a razor blade. For LM, the speci-

Table 1
Periosteal Biopsies for Light Microscopy

No.	Sex	Age	Biopsy Site	Operation	Distance from Primary Lesion
1	F	65	Proximal tibia	Transposition of tibial tuberosity	2cm
2	F	13	Proximal tibia	Removal of a single cartilag. exostosis	4cm
3	M	73	Lateral femur condyle	Osteosynthesis of lat. tibial condyle	Normal bone
4	M	73	Iliac crest	Osteosynthesis of lat. tibial condyle	Normal bone
5	M	76	Proximal femur	Osteosynthesis of femoral neck	15cm
6	M	13	Lateral femur condyle	Distal femoral epiphysiodesis	5cm

Biopsies No. 3 and 4 are from the same patient.

Table 2
Periosteal Biopsies for Transmission Electron Microscopy

No.	Sex	Age	Biopsy Site	Operation	Distance from Primary Lesion
1	F	61	Iliac crest	Osteosynthesis of lateral tibial condyle	Normal bone
2	F	74	Femoral neck	Moore prosthesis	3cm
3	F	28	Lateral tibial condyle	Window in cortex	Normal bone
4	M	23	Proximal tibia	Osteotomy	Normal bone
5	F	73	Proximal half of hallux	Keller's operation for hallux valgus	1cm
6	M	84	Proximal femur	Petrochanteric osteosynthesis	5cm
7	M	13	Distal femur	Osteotomy	Normal bone

mens were fixed overnight at room temperature in 10% buffered formalin, dehydrated and embedded in paraffin. Adjacent sections (mirror sections) were cut and matched pairs were stained by the immunoperoxidase method for factor VIII

(7) (factor VIII antibody was obtained from DAKO, Copenhagen) and for laminin (8) (laminin antibody was obtained from Dr. R. Albrechtsen, Copenhagen). Supplementary sections were stained with hematoxylin-eosin and reticulin for overall

orientation. For TEM, the specimens were fixed for two hours in Karnovsky's fixative (9) postfixed in 1% OsO₄, dehydrated and epoxy embedded using standard techniques. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a JEOL 100B electron microscopy (JEOL Inc., Tokyo).

RESULTS

Periosteum from different sites varied in thickness, but otherwise the microscopic structure was similar. Close to the bone the collagen was dense with few vessels. More remote from the bone the tissue texture was looser with a greater number of vessels. No histologic evidence of inflammation or trauma was detected. Irrespective of the method of examination, only blood capillaries were depicted (Figs. 1-3). In none of the specimens were lymphatic capillaries seen.

DISCUSSION

Lymphatics in the periosteum are rarely mentioned (2,10,11,18), although such vessels have been demonstrated in human alveolar bone (3). Based on anatomical and histological studies, tissue

fluid of bone has been thought to "percolate" through the Haversian canals and gain access to lymphatic vessels within the periosteum (10), or simply drain via the Haversian system independent of the lymphatic network (12). Although lymphangiomas and lymphangiomatosis of bone are a distinct entity (13), these malformations or hamartomas probably originate from medullary bone and not from the periosteum (14). Malignant bone tumors, on the other hand, occasionally metastasize to regional lymph nodes rather than the more common hematogenous route of spread (15). Whereas this phenomenon suggests a lymphatic drainage pathway for bone, it seems unlikely that once bone tumors have disseminated (e.g., to the regional nodes) the tumor is still confined locally to cortical bone. In other words, at this stage the tumor has probably invaded adjacent tissues with known lymphatic vessels. Thus, the existence of benign and nodal spread of malignant bone tumors does not definitely establish the existence of a periosteal lymphatic drainage system.

In contrast to large lymphatic trunks which have no discernible morphological marker to distinguish them from blood vascular counterparts, lymphatic capillaries lack a basal lamina characteristic of blood capillaries. This basal lamina is

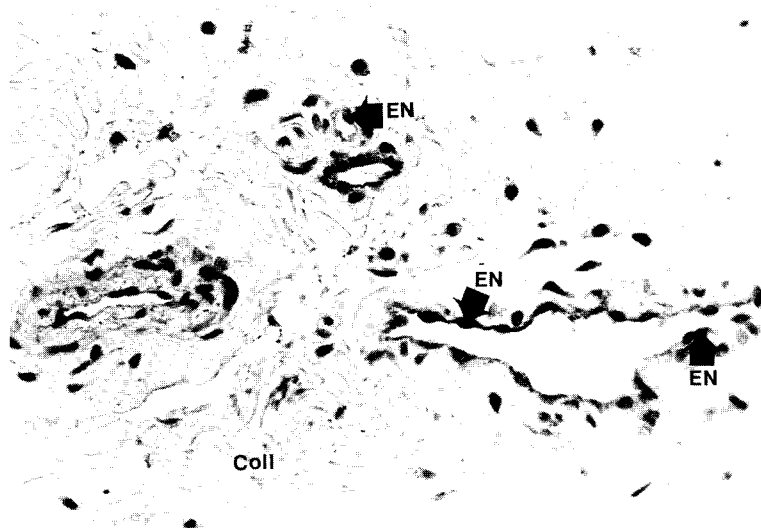


Fig. 1. Light microscopic immunohistochemistry of normal human periosteum. Staining for factor VIII identifies vascular endothelial cells (EN) (660x). Coll-collagen.



Fig. 2. Mirror section from the same specimen as in Fig. 1. Positive staining for laminin identifies basal lamina (BL) (660x). Coll-collagen.

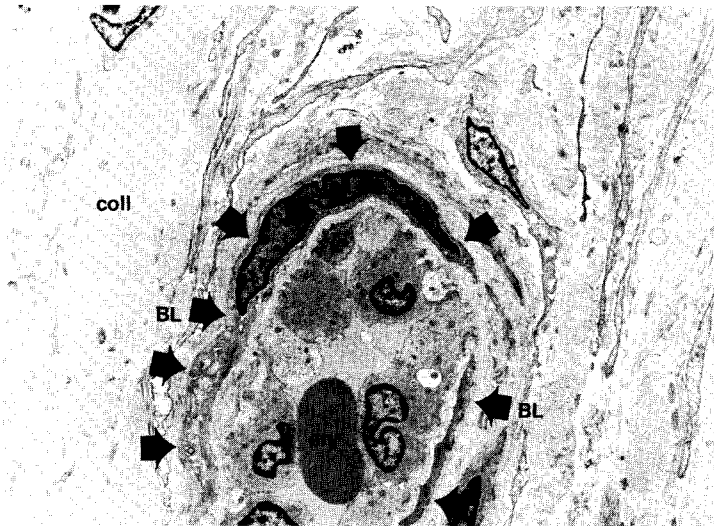


Fig. 3. Transmission electron microscopy of normal human periosteum. Both endothelium and basal lamina (BL) can be identified on the same section. No vessels lacking the basal lamina were detected (800x). Coll-collagen; erythrocyte.

readily identified in LM by anti-laminin staining (8,16,17) and in TEM by its characteristic ultrastructure (6). Endothelium in both lymphatic and blood capillaries can be positively identified in LM by anti-factor VIII staining (7) and in TEM by its well-defined ultrastructure (6). Thus, by comparing mirror sections in LM and by

direct observation in TEM, one should be able to detect lymphatic capillaries with some degree of confidence. Our failure to demonstrate lymphatic capillaries in human periosteum suggest that either they are not there or, alternatively, they are so few and far apart that they cannot be visualized by methods which have proved

reliable in other investigations of this type (6,16,17).

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REFERENCES

1. Cooper, RR, JW Milgram, RA Robinson: Morphology of the osteon. *J. Bone and Joint Surgery* 48A (1966), 1239-1271.
2. Seliger, WG: Tissue fluid movement in compact bone. *Anat. Records* 166 (1970), 247-256.
3. Barker, JH: Lymphatic vessels in human alveolar bone. *Lymphology* 15 (1982), 1-13.
4. Kelly, PJ, LFA Peterson: Lymphatics of the bone. In *Blood Vessels and Lymphatics*, Abramson, DI (Ed.), Academic Press, New York (1962), 544.
5. Hudson, G, JM Yoffey: Ultrastructure of reticuloendothelial elements in guinea-pig bone marrow. *J. Anat.* 103 (1965), 515-525.
6. Leak, LV: Lymphatic vessels. In *Electron Microscopy in Human Medicine. Vol. 5. Cardiovascular System, Lymphoreticular and Hematopoietic System*, Johannessen, JV (Ed.), McGraw-Hill International Book Company, New York (1980), 164-212.
7. Sehested, M, K Hou-Jensen: Factor VIII related antigen as an endothelial cell marker in benign and malignant diseases. *Virchows Arch. (Pathol. Anat.)* 391 (1981), 217-225.
8. Albrechtsen, R, UM Wewer, LA Liotta: Basement membranes in human cancer. In *Pathology Annual*, Sommers, SS, PP Rosen, RE Fechner (Eds.), Part 2, 21 (1986), pp. 251-276.
9. Karnovsky, MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27 (1965), 137A.
10. Oshkaderov, B: Lymphatic vessels in long bones of man and domestic animals. *Khirurgia (Moskva)* 12 (1949), 49-54.
11. Ossof, RH, DE Bytell, MH Hast, et al: Lymphatics of the floor of the mouth and periosteum: Anatomical studies with possible clinical correlations. *Otolaryngol Head Neck Surg* 88 (1980), 652-657.
12. Anderson, DW: Studies of the lymphatic pathways of bone and bone marrow. *J Bone and Joint Surgery* 42A (1960), 716-717.
13. Hafner, E, WA Fuchs, K Kuffer: Lymphangiography in lymphangiomatosis of bone. *Lymphology* 5 (1972), 129-131.
14. Spjut, HJ, HD Dorfman, RE Fechner, et al: Tumors of bone and cartilage. In *Atlas of Tumor Pathology, Second Series*, Fascicle 5, Castle House Publications, London (1979), 329-330.
15. Makai, F, A Belan, P Malek: Lymphatic metastases of bone tumors. *Lymphology* 3 (1971), 109-116.
16. Barsky, SH, A Baker, GP Siegel, et al: Use of anti-basement membrane antibodies to distinguish blood vessel capillaries from lymphatic capillaries. *Am. J. Surg. Pathol.* 7 (1983), 667-677.
17. Hultberg, BM, S Daugaard, HF Johansen, et al: Malignant haemangiopericytomas and haemangioendotheliosarcomas: An immunohistological study. *Histopathology* 12 (1988), 405-414.
18. Deysine, M: Lymphatic space in cortical bone. *Anatomy and physiology. Abstracts 4th Congress of International Lymphological Society, Tucson, Arizona, March 6-10, 1973.*

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