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Original Articles

Malignant Lymphoma of True Histiocytic Origin: Histiocytic Sarcoma

A Morphological, Ultrastructural, Immunological, Cytochemical and Clinical Study of 10 Cases

P. van der Valk[1], J. te Velde[1], J. Jansen[2], D.J. Ruiter[1], P.J. Spaander[1], C.J. Cornelisse[1], and C.J.L.M. Meijer[1]

Department of Pathology[1] and Internal Medicine[2] of the University Medical Centre Leiden, The Netherlands

Summary. Ten tumors of true histiocytic origin (Histiocytic Sarcoma) are presented. The tumor cells were identified as histiocytes by immunological, cytochemical and ultrastructural criteria (cytoplasmic lysozyme activity, presence of C3 and Fcγ receptor, strong acid phosphatase and alpha-naphtyl acetate esterase activity, presence of lysosomes, absence of cell junctions and evidence of phagocytosis). The tumors identified in this way showed the following histological characteristics: diffuse proliferation of large tumor cells with ample cytoplasm, containing granular or occasionally diffuse diastase resistent PAS positive material, erythrophagocytosis, and haemosiderin pigment. The large or enormous nuclei were irregular, with occasional deep indentations, sharply defined nuclear membrane, coarse chromatin and conspicuous nucleoli. Despite the uniformity of these criteria differences in presence of $alpha_1$-antitrypsin, $alpha_1$-antichymotrypsin and 5 Nucleotidase activity and the number of lysosomes in the cytoplasm were found. The findings are suggestive of a spectrum of cytological changes in these Histiocytic Sarcomas.

The clinical picture ranged from monolocalization in a lymphoid organ to that of a diffuse Malignant Histiocytosis. The relationship between good response to therapy and complete remission and the absence of $alpha_1$-antitrypsin and a high number of lysosomes is discussed.

Key words: Histiocytic sarcoma – Histology – Marker studies – Morphometry – Clinical behavior

Introduction

In recent years it has become clear that the majority of the so-called "histiocytic" non-Hodgkin lymphomas in the Rappaport classification are in fact of lymphocytic origin. True histiocytic lymphomas are considered to be very rare. The

Offprint requests to: P. v. d. Valk, Pathologisch Anatomisch Laboratorium, University Medical Centre, P.O. Box 9603, 2300 RC Leiden, The Netherlands

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only acknowledged histiocytic tumor was Malignant Histiocytosis (Abele and Griffin 1972; Byrne and Rappaport 1973; Isaacson and Wright 1978; Lombardi et al. 1978; Meister et al. 1980a; Rausch et al. 1979), first described as Histiocytic Medullary Reticulosis by Scott and Robb-Smith (1939), a rapidly disseminating disease, affecting many extranodal sites.

However, Isaacson et al. (1979), in a study of 66 gastrointestinal non-Hodgkin lymphomas, recently found a remarkably high percentage (50%) of true histiocytic tumors among his cases. Thirty-three percent of the 66 non-Hodgkin lymphomas fulfilled the clinical and histological criteria of Malignant Histiocytosis (Byrne and Rappaport 1973); an additional 17% could not be classified as Malignant Histiocytosis, but were considered to be of histiocytic origin. Thus, apart from the clinical and pathological entity Malignant Histiocytosis, different tumors of histiocytic origin seem to exist.

In this study we present the histological and clinical data of 10 tumors of histiocytic origin, proven by combined histochemical, immunological and ultrastructural analysis. The results of the histological and marker studies and the stage of the disease are related to the clinical course and response to therapy. They indicate that true histiocytic lymphoma (histiocytic sarcoma) forms a spectrum of tumors, not only pathologically, but also clinically. The frequency of bone and skin lesions and the possible implications for chemotherapy underline the need to differentiate this type of tumor from the non-Hodgkin lymphomas.

Patients, Material and Methods

Ten patients, 5 males and 5 females, varying in age from 22 to 84 years, were studied. The clinical details are given in Table 1. Staging was performed according to the Ann Arbor scheme (Carbone et al. 1971).

Tissue Processing. Each tissue specimen was cut in five parts. One part was fixed in buffered formalin and embedded in paraplast for routine histological investigation. One part was fixed in a fixative according to Burkhardt for methylmethacrylate embedding (Te Velde et al. 1977). One part was fixed in cacodylate buffered glutaraldehyde for electron microscopy. One part was fixed in a sublimate formaldehyde mixture (Bosman et al. 1977) for immunohistochemistry and the fifth part was snap-frozen in liquid nitrogen and stored at $-70°$ C. For one patient (K) no material for electron microscopy was available.

Histology. Four µm thick paraplast sections were cut and stained with Haematoxilin-Eosin, Giemsa, PAS, Prussian blue and silver impregnation.

Methyl-Methacrylate Embedding. The technique of methyl-methacrylate embedding, developed by Burkhardt and described by Te Velde (Te Velde et al. 1977), was used. Two µm thick sections were cut and stained with Gallamine Giemsa, PAS, Gomori's reticulin stain and a combined stain according to Turnbull (for iron) and Trevan (for pyroninophilia).

Morphometry. Photographs of 7–10 characteristic fields of the tumor in Giemsa stained, methyl-methacrylate embedded sections, 1–2 µm thick, were made with a Zeiss photomicroscope (on a Kodak Pan F film), using a green filter (magnification 800×). The nuclear contour of all the photographed cells was traced on a graphic tablet interfaced to a PDP 11/10 minicomputer. From the digital contours of each nucleus the computer program calculated the area, perimeter and nuclear contour index (NCI), a size independent shape parameter for nuclear indentation, defined as the perimeter/area $^1/_2$ (Van der Loo et al. 1980; Meijer et al. 1980). The minimal value for

Table 1. Clinical data from 10 patients with histiocytic sarcoma

Pat.	Age/ sex	Presenting symptoms	Localization	Stage[a]	Initial therapy	Result	Relapse	Therapy relapse	Survival (months after diagnosis)
K.-H.	67/F	Left cervical mass	Left cervical lymph node	I	R.T.	C.R.	–	–	17+ free of disease
v. Z-R.	28/F	Left axillar mass	Left axillar lymph node	I	R.T.	C.R.	–	–	6+ free of disease
K.	57/M	Swollen tonsil	Tonsil	I	R.T.	C.R.	–	–	6+ free of disease
W.	28/M	Left submandibular mass	Left submandilar lymph node	I	Left cervical dissection	C.R.	±2 yrs later; Right cervical lymph node	Polychemotherapy II Result: C.R.	42+ free of disease
B.	77/M	Left inguinal mass Lymphoedema	Left inguinal and Left parailiac lymph nodes	II	Polychemotherapy I R.T.	P.R.	Right submandibular lymph node; Skin; Lung	Polychemotherapy III	11
P.H.	63/F	Retrosternal pain Coughing	Mediastinal lymph nodes	II	Polychemotherapy I	P.R.	Slow progression Involvement of lung, bone (11th thoracal vertebra	–	17+ tumor persisting
T.	84/F	Abdominal pain	Left inguinal lymph node Right and left axillary lymph nodes	III	Polychemotherapy II	P.R.	–	–	3+ tumor persisting
H.	22/F	Back pain	5th, 6th thoracic vertebrae Pectoral skin	IV	R.T.	C.R.	4 months later: 1st, 4th lumbar vertebrae	Polychemotherapy II Result: C.R.	25+ free of disease
d.J.	24/M	Fever; Low back pain Weight loss	Supraclavicular, axillary, pectoral, paraaortic lymph nodes; 1st lumbar vertebra; os ilium	IV	Polychemotherapy I	P.R.	–	–	11+ tumor persisting
v.O.	67/M	General weakness; fever; weight loss; hepatosplenomegaly; icterus	Submandibular, Hilar, inguinal lymph nodes; liver; spleen bone marrow; ileum	IV	Polychemotherapy I	P.R.	–	–	6; died of septicaemia

R.T. = radiotherapy (tumor dose); C.R. = complete remission; P.R. = partical remission

[a] At time of diagnosis

Polychemotherapy I: cyclophosphamide, adriamycin, vincristine, prednisone
Polychemotherapy II: cyclophosphamide, vincristine, prednisone
Polychemotherapy III: carmustine, vinblastine bleomycin

Table 2. Morphological, histological, immunological and cytochemical data from 10 patients with

Pat.	Tissue for diagnosis	Lys	C3	Fcγ	Ac.P	ANAE	$Alpha_1$ AT	$Alpha_1$ ACT
K.-H.	Lymph node	+	±	±	++	++	−	±
v.Z-R.	Lymph node	+	±	+	++	++	locally ±	±
K.	Tonsil	+	+	±	++	++	locally ±	locally ±
W.	Lymph node	++	±	±	+	+	locally ±	−
B.	Lymph node	±	±	±	++	++	±/+	−
P.-H.	Lymph node	±	+	+	+/++	+/++	+	+/++
T.	Lymph node	±	±	±	+/++	+/++	+	+
H.	Subcutis	+/++	±	±	++	++	locally ±	n.d.
d.J.	Lymph node	+	+	+	++	++	+	+++
v.O.	Lymph node	+	+	+	++	++	+	±
Reactive histiocytes from lymph nodes ($n=2$)		++	+	+	++	++	−/+	−/+

− = absent; ± = weakly present; + = present; ++ = strongly present

Lys = lysozyme; Ac.P = Acid phosphatase; ANAE = alpha-naphtyl acetate esterase; $Alpha_1$ AT = $alpha_1$-antitrypsin; $Alpha_1$ ACT = $alpha_1$-antichymotrypsin; 5 NT = 5-Nucleotidase; ATP = adenosinetriphosphatase; n.d. = not done

this index is found for a perfect circle and is 3.545. From each tumor 110–120 cells were measured. Morphometry was not done in cases H and K, since no methylmethacrylate sections were available.

Immunohistochemistry. Alpha, mu and gamma heavy chains, kappa and lambda light chains, lysozyme, $alpha_1$-antitrypsin and $alpha_1$-antichymotrypsin were demonstrated by the PAP technique (Taylor and Mason 1974). The rabbit antisera against IgA, IgG, IgM and kappa and lambda light chains were purchased from Dakopatts (Denmark). The specificity of these antisera was confirmed by immunoelectrophoresis, and by cytoplasmatic immunofluorescence and immunoperoxidase staining on monoclonal bone marrow preparations (Hijmans et al. 1969). The rabbit antiserum against lysozyme was also purchased from Dakopatts, and tested on tissues in which lysozyme is known to be present, such as cartilage, bronchial submucosal glands, and Kupfer cells (Mason and Taylor 1975). The rabbit anti-$alpha_1$-antitrypsin, prepared by Dr. H. Kramps, Division of Pulmonary diseases, Department of Internal Medicine of the Leiden University Medical Center, was absorbed with serum from a patient with a total $alpha_1$-antitrypsin deficiency. The antiserum raised in rabbits against $alpha_1$-antichymotrypsin was purchased from Behringwerke (Amsterdam, The Netherlands). The goat-anti-rabbit IgG was prepared in our laboratory, and the PAP-complex prepared in rabbits was obtained from Dakopatts. Control studies were done according to Sternberger (1977) and included replacement of the first layer of antibodies by non-immune rabbit serum or by the specific antiserum absorbed by the respective antigens.

histiocytic sarcoma

5 NT	ATP	Lysosomes	Mean nuclear area (μm^2) ± S.D.	Percentage of nuclei > 80 µm	Mean NCI ± S.D.	Percentage of nuclei with NCI > 5.5	Erythrophagocytosis	Mitotic figures[a]	Necrosis
+	+	3	42.24 ± 22.87	4.1	4.67 ± 0.67	13.9	+	2–4	+
+	+	2	45.25 ± 16.73	4.3	4.61 ± 0.69	10.3	++	0–1	–
+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	0–1	+
+	+	2/3	39.60 ± 21.67	5.6	4.67 ± 0.69	9.7	+	2–3	+
–	±	1	57.55 ± 32.24	13.6	4.75 ± 0.77	14.4	+	3–5	–
+	+	n.d.	47.06 ± 17.69	4.6	4.56 ± 0.75	11.0	+	1–3	–
–	±	1	90.88 ± 47.45	52.9	4.55 ± 0.65	11.6	+	2–4	–
+	+	4	n.d.	n.d.	n.d	n.d	+	3–4	–
–	–	1	57.87 ± 27.18	28.5	4.92 ± 0.74	17.1	+	3–4	+
–	+	1	37.73 ± 23.58	4.1	4.74 ± 0.77	19.5	+++	0–2	+
–/+	–/+	n.d	34.34 ± 11.71	0	4.09 ± 0.36	0	–	–	–

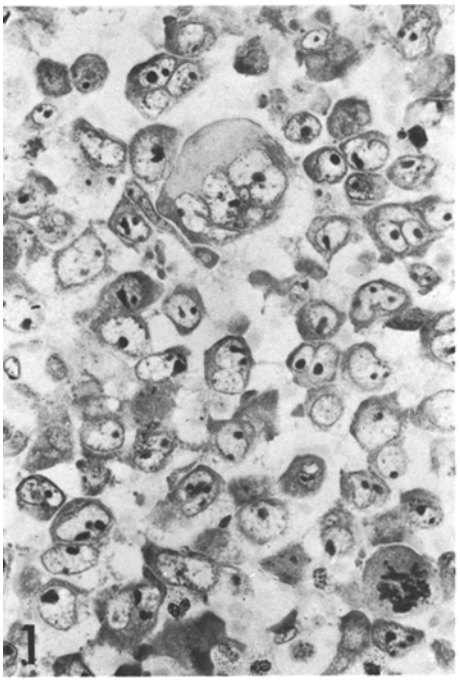
0 = absent; 1 = scarce; 2 = several; 3 = many; 4 = abundant

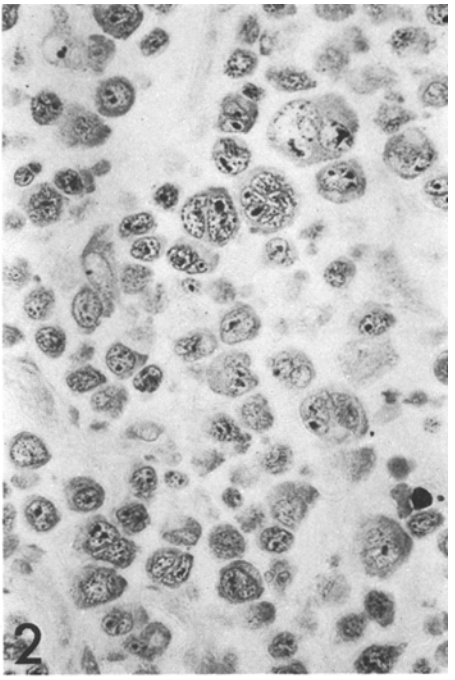
[a] per high powerfield magnification 500 ×

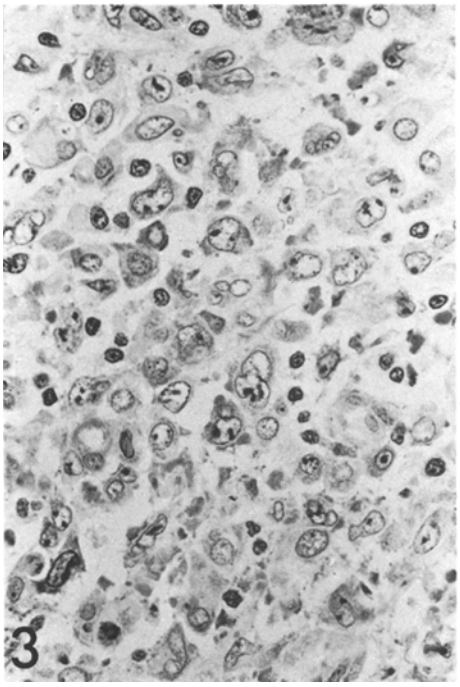
Immunofluorescence. The indirect immunofluorescence technique was done on 4 µm thick cryostat sections, generally using the same antisera as described above. The fluorescein conjugated horse-anti-rabbit IgG (HaRIgG/FITC) was obtained from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands). However, IgG was demonstrated by a direct immunofluorescence technique, using an FITC tagged antibody raised in rabbits against human IgG (Dakopatts, Denmark). Controls were performed as described by Sternberger (1977).

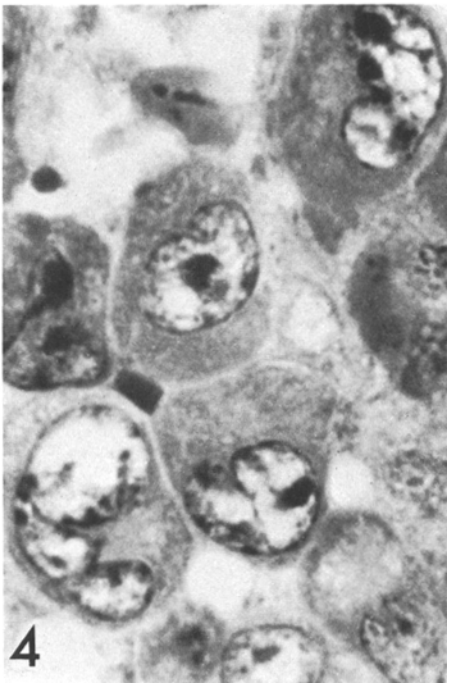
Enzyme Histochemistry. All enzyme stainings were performed on 8 µm thick cryostat sections. Alpha-naphtyl acetate esterase was demonstrated by the method of Gomori, as modified by Barka and Anderson (1965) using alpha-naphtyl acetate as a substrate with 30 min of incubation; alpha-naphtol-ASD-chloroacetate esterase activity was assayed with the method of Leder and Stutte (1975) with a 20 min incubation. Acid and alkaline phosphatase activity were demonstrated, at pH 5.0 and 9.6 respectively, according to Gomori (1952, 1946), using β sodium glycerophosphate as a substrate with a 2 h incubation. 5-Nucleotidase and Adenosine triphosphatase staining was performed according to Wachstein and Meisel (1957) with incubation times of 2 and $1^1/_2$ h, respectively. Peroxidase staining was performed according to Graham and Karnovsky (1966); the incubation time with diamino-benzidine was 1 h.

Rosette Assay. Tissue localization of cells bearing receptors for the Fc fragment of IgG (Fcγ) or the activated third component of complement (C3) were demonstrated as described earlier









Figs. 1–4. Histiocytic Sarcoma (Giemsa stained, methyl-methacrylate embedded sections). **Fig. 1.** Lymph node (patient T). Note the enormous, multinucleated cell. Some tumor cells show features of immunoblasts. **Fig. 2.** Lymph node (patient B). Note the hyperlobulation of the nuclei. **Fig. 3.** Lymph node (patient v.O). Note the striking nuclear polymorphism. **Fig. 4.** Lymph node (patient d.J). Note the nuclear indentations, condensed chromatin along the sharply defined nuclear membrane and the prominent nucleoli. **Figs. 1–3.** $\times 450$. **Fig. 4.** $\times 1{,}600$

by us (Meijer and Lindeman 1975; Meijer et al. 1977) using sheep erythrocytes coated with rabbit IgG antibody (EAIgG) or sheep erythrocytes coated with rabbit IgM antibody and mouse complement. As controls served sheep erythrocytes either uncoated or coated with rabbit IgM antibody.

Electron Microscopy. Small pieces of tissue (± 1 mm^3) were fixed in 1.5% cacodylate buffered glutaraldehyde, postfixed in 2% cacodylate buffered osmiumtetroxyde, dehydrated in a graded series of alcohol solutions, and embedded in Epon. Ultrathin sections (± 600 Å) were cut on a LKB III microtome and stained with uranyl acetate and lead citrate. The sections were examined in a Siemens Elmiskop 1a or a Philips 201 C electron microscope.

Results

Histology. The main histological data are summarized in Table 2. All tumors, consisting of cells with intracytoplasmatic lysozyme, C3 and Fcγ receptors and acid phosphatase and alpha-naphtyl acetate esterase activity and, ultrastructurally, lysosomes in the cytoplasm essentially showed the same characteristic picture: a diffuse proliferation of large cells with abundant, basophilic cytoplasm and large nuclei. There was a considerable variation in nuclear size, with a few small and some gigantic nuclei; multinucleated cells could be seen in each slide. The nuclei were polymorphic, sometimes oval to kidney-shaped, sometimes with bizarre shapes, showing deep indentations (Figs. 1–4). The chromatin was coarse, and the nucleoli were prominent: their relative size varied for each case, occasional nuclei contained several nucleoli. Some chromatin was condensed at the nuclear membrane, giving the nuclei a "punched out" feature. The multinucleated cells did not resemble Reed-Sternberg cells, because they lacked rod-like nucleoli and a perinucleolar halo. In fact most of the nucleoli were attached to the nuclear membrane. In some tumors the cells closely resembled immunoblasts (Fig. 1).

In eight patients the diagnostic tissue specimen was a lymph node, in one a tonsil (K) and in one a subcutaneous lesion (H). In seven of the eight lymph nodes the proliferation replaced the normal nodal architecture completely (K-H, v. Z-R, P-H, T, d.J, v.O). The eight h node was only partially involved (W). Three tumors showed cohesive growth (W, H, v.O), in the remaining tumors the growth pattern was non-cohesive. In three lymph nodes the tumor had destroyed the capsule and invaded the perinodal tissue (P-H, d.J, v.O).

All tumors showed frequent mitotic figures and phagocytosis with intracellular débris was prominent. In methyl-methacrylate sections with their excellent morphological quality, erythrophagocytosis could be observed in almost all tumors (the exception was patient K's tumor); it was most pronounced in patient v.O (Fig. 5). The tumors of patients H., v.Z-R, K and W showed admixture of lymphoid cells and some granulocytes.

Silver staining revealed disruption of the preexisting fibrous skeleton and a focal reactive fibrosis was observed. In the PAS staining many tumor cells showed PAS positive material in their cytoplasm, coarsly granular, but also more diffusely distributed. Prussian blue staining showed haemosiderin to be present in all tumors in varying quantity.

Morphometry. Quantitative analysis of the nuclear size and the degree of indentation was done by means of morphometry (Table 2). The nuclear parameters of the cells in our histiocytic sarcomas were compared with those of the cells

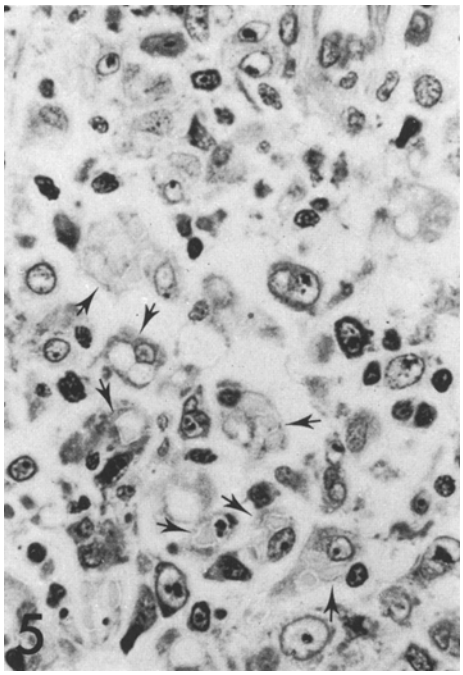


Fig. 5. Lymph node (patient v.O). Abundant erythrophagocytosis (*arrows*). Giemsa stained, methyl-methacrylate embedded section. ×550

Table 3. Morphometrical reference material from 5 non-Hodgkin lymphomas

		Mean nuclear area (μm^2) ±S.D.	Percentage of nuclei $>80\ \mu m^2$	Mean NCI ±S.D.	Percentage of nuclei with NCI > 5.5
Immunoblastic lymphoma (B)	1	37.08 ±15.25	0	4.18 ±0.52	1.7
Immunoblastic lymphoma (B)	2	28.91 ±17.23	0	4.17 ±0.51	0.8
Immunoblastic lymphoma (B)	3	35.64 ±15.73	1.7	4.15 ±0.47	3.3
Convoluted T-lymphoblastic lymphoma		31.07 ±8.36	0	4.33 ±0.54	4.3
Large cleaved F.C.C. tumor		34.05 ±17.29	0.9	4.10 ±0.48	3.4

in non-Hodgkin lymphomas, which may resemble histiocytic sarcoma histologically. Three B-immunoblastic sarcomas, one large cleaved follicle centre cell tumor and one convoluted T-lymphoblastic lymphoma were measured. The diagnosis in these cases was established using the same methods of investigation as described above. Additionally we measured the nuclei of a number of reactive

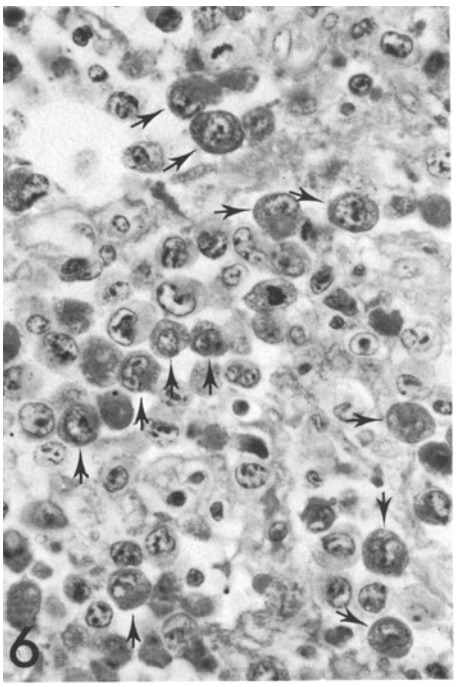
histiocytes as a reference, taking one lymph node with marked reactive sinus histiocytosis and one lymph node with the clinical and pathological entity sinus histiocytosis with massive lymphadenopathy (Rosai and Dorfman 1969). The mean values and their standard deviation of the nuclear area and of the NCI are shown in Tables 2 and 3. Furthermore, we counted the cells with an area exceeding 80 μm^2 and cells with an NCI larger than 5.5. These findings are also shown in Tables 2 and 3. In particular, cells with an NCI exceeding 5.5 were far more numerous in histiocytic sarcoma. All histiocytic sarcomas had higher mean values than the five lymphomas and the reactive histiocytes; for area and for the NCI ($P<0.05$). In most cases the histiocytic sarcomas had a greater standard deviation, illustrating their great variation in size.

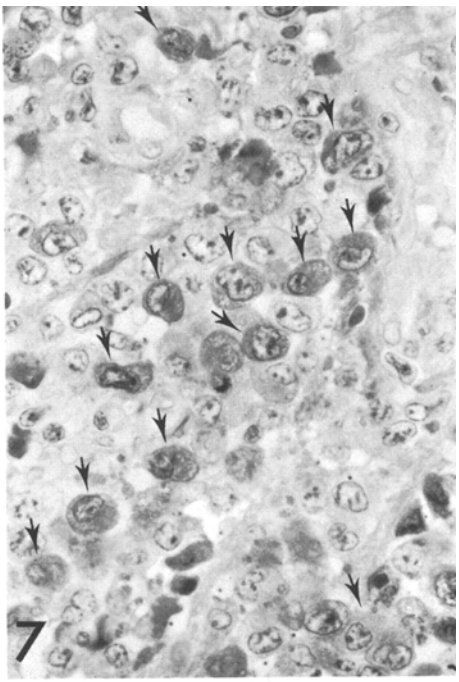
Immunohistochemistry and Immunofluorescence. The results of these investigations are summarized in Table 2. In general the two techniques gave concordant results. In some tumors (K, H, W, H, d.J, v.O) the immunoperoxidase technique showed weak intracytoplasmatic staining for both kappa and lambda light chain and for IgA, IgG, and IgM in a varying degree, whereas immunofluorescence was completely negative in all cases. This finding was, therefore, probably a fixation artefact. All tumors contained lysozyme (Fig. 6), but alpha$_1$-antitrypsin staining was more variable. Tumor cells from patients d.J, B, P-H and T clearly showed alpha$_1$-antitrypsin staining (Fig. 7). In patient v.O's tumor the cells were also positive, though staining was not as intense as in the former. Patients H, W, v.Z-R and K locally showed cells with weak alpha$_1$-antitrypsin activity. In patient K-H no activity could be demonstrated. Alpha$_1$-antichymotrypsin was demonstrated by the immunoperoxidase technique alone. Tumor cells of patients B and W did not show alpha$_1$-antichymotrypsin, in four patients (K-H, v.Z-R, K and v.O) staining was weak and in the three reamining tumors (T, P-H and d.J) a strong positive staining was observed. Staining for this protease inhibitor was not possible in the case of patient H.

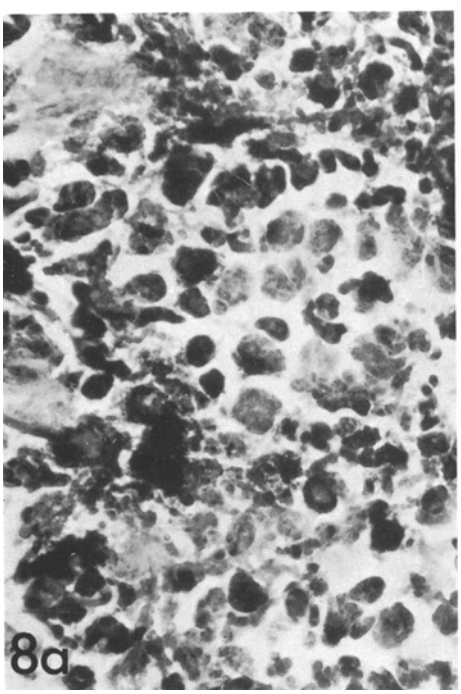
Enzyme Histochemistry. A constant finding in our cytochemical analysis was the strong acid phosphatase and alpha-naphtyl acetate esterase activity (Fig. 8a and b). Alkaline phosphatase was positive in one case (K). Adenosine triphosphatase was negative in one case (d.J), weakly positive in two (B, T) and positive in the remaining cases. 5-Nucleotidase activity was demonstrated in six cases (W, v.Z-R, K-H, H, K and P-H). The absence of alpha-naphtol-ASD-chloroacetate esterase and peroxidase activity excluded a myeloid origin of the tumor cells. The main results are summarized in Table 2.

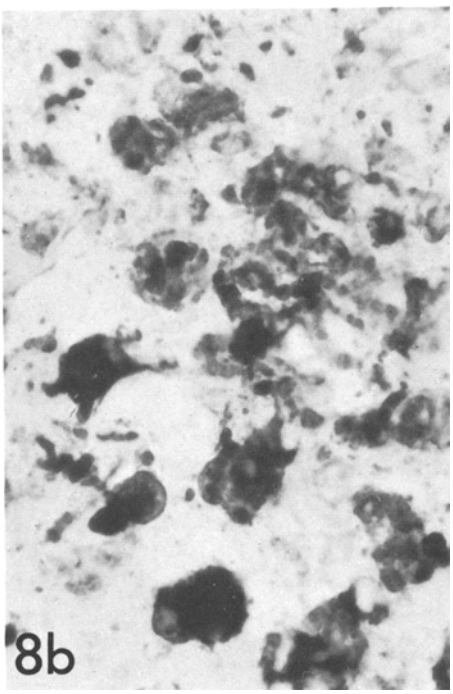
Rosette Assay. All tumors showed receptors for the activated third component of complement and the Fc portion of IgG (C3 and Fcγ receptors) (Figs. 9a, 9b, 10). However, the degree of adherence was variable (Table 2). Control sections overlayered with uncoated sheep erythrocytes or IgM coated sheep erythrocytes never showed erythrocyte adherence.

Electron Microscopy. Ultrastructurally, all tumors consisted of large cells with moderate to abundant cytoplasm. Frequently, villous cytoplasmic extensions

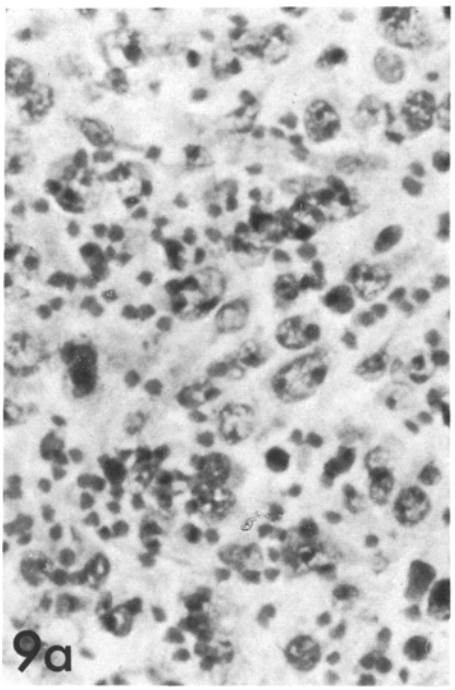


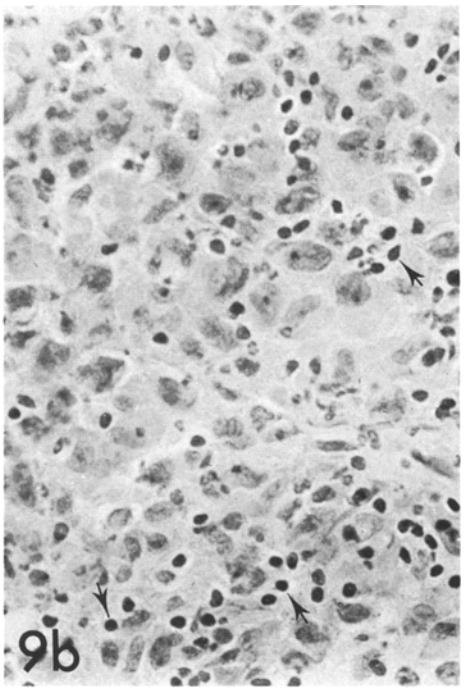


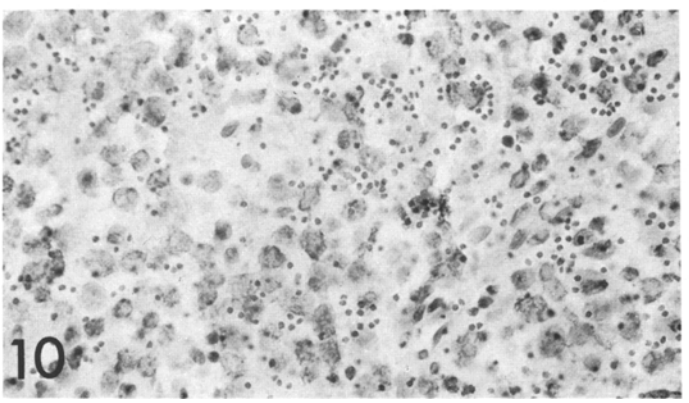




Figs. 6–8. Lysozyme staining of histiocytic sarcoma of patient d.J. Most tumor cells stain dark (*arrows*). ×450. **Fig. 7.** Alpha$_1$-antitrypsin staining of the same patient of Fig. 6. ×450. **Fig. 8a.** Acid phosphatase (patient K-H). **Fig. 8b.** Alpha-naphtyl acetate esterase (patient d.J). ×650

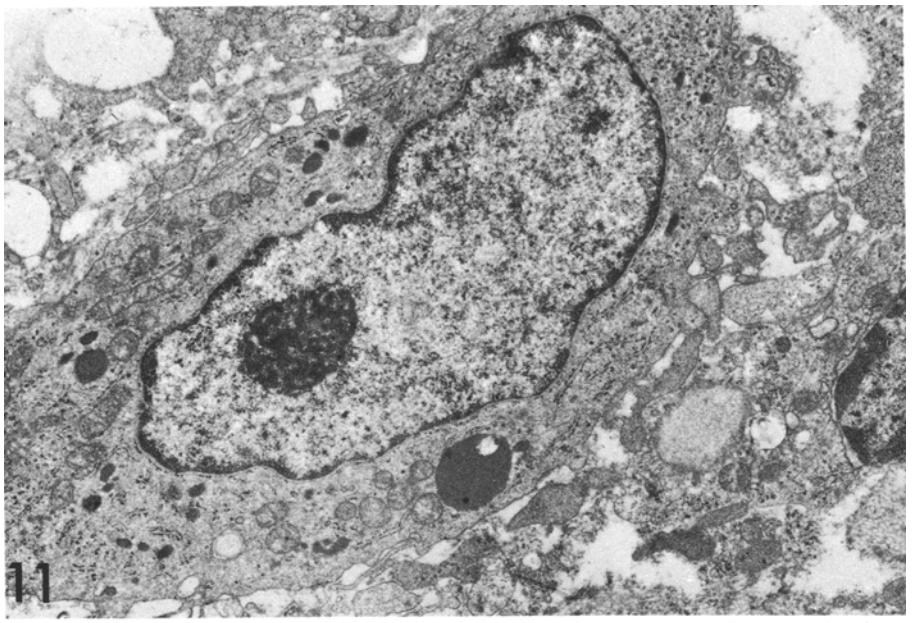


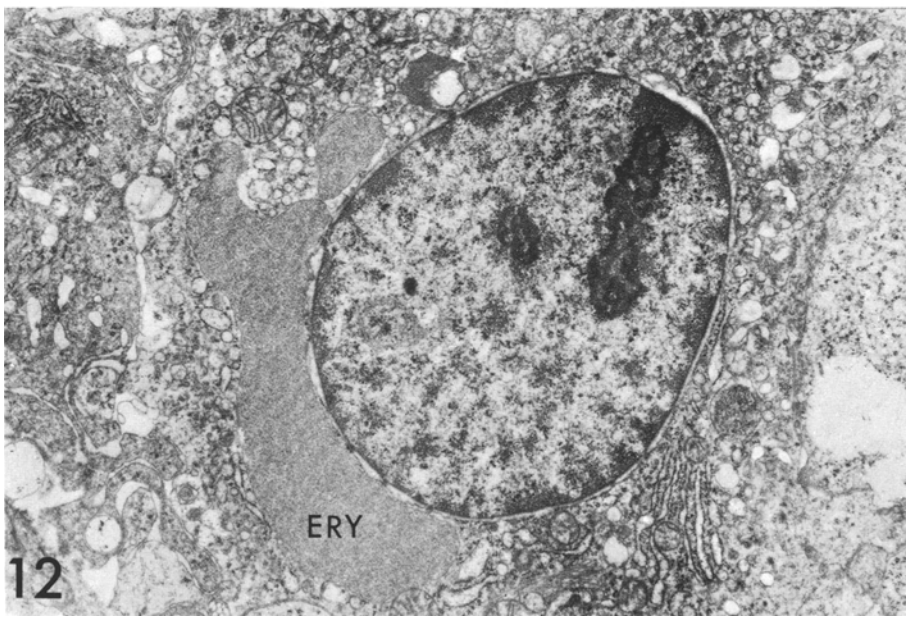




Figs. 9–10. Histiocytic Sarcoma. Rosette assays. **Fig. 9a.** Moderately strong adherence of EAC (C3 receptors) (patient P-H). ×420. **Fig. 9b.** Weak adherence of EAC (patient v.O). Eryhtrocytes (as indicated by arrows) are unevenly distributed over the section. ×400. **Fig. 10.** EA rosettes (Fcγ receptors), moderately strong adherence (patient d.J). ×275

could be seen and in two cases (H, v.O) the cells interdigitated with blunt cytoplasmic protrusions. No abortive junctions between cells were observed. The mostly exentrically located nuclei were round to oval, elongated or showing bizarre shapes, with deep indentations. Nucleoli were moderate to large and some heterochromatin was condensed at the nuclear membrane, occasionally in small clumps. The tumor cells of patient H were very rich in organelles





Figs. 11 and 12. Histiocytic Sarcoma. Electron microscopy. **Fig. 11.** Tumor cell with abundant lysosomes (patient W). ×7,750. **Fig. 12.** Tumor cell with scanty lysosomes (patient v.O). The cell has phagocytized an erythrocyte (ERY). ×7,000

with abundant lysosomes (±6–8 per cross sectional area of g-cell), polyribosomes, mitochondria and rough endoplasmatic reticulum; some phagosomes were observed. In patient K-H's tumor lysosomes were found in almost all cells, but in smaller numbers (±4–6 per cross sectional area of a cell). There were many mitochondria and some rough endoplasmatic reticulum. Most of the cells of patients W (Fig. 11) and v. Z-R showed some lysosomes (±2–5 per cross sectional area of a cell), mitochondria, polyribosomes and rough endoplasmatic reticulum.

Four patients had only few lysosomes in their tumor cells (±1–2 per cross sectional area of a cell) (Fig. 12). In addition to the scanty lysosomes, some mitochondria, Golgi complexes and smooth and rough endoplasmatic reticulum could be seen. In two of the tumors (v.O and T) erythrophagocytosis could be demonstrated ultrastructurally. In patient T's tumor numerous phagosomes were present.

The state of preservation of the tissue of one patient (P-H) did not allow any conclusions to be drawn.

Thus electron microscopy of the tumor cells was compatible with their histiocytic origin (lysosomes in all tumors investigated, phagosomes in patients T and H and erythrophagocytosis in patients v.O and T). Together with the absence of junctions between the cells this supported the diagnosis of histiocytic sarcoma.

Discussion

In this study we have presented *ten cases* of histiocytic sarcoma; this diagnosis was based on the following criteria: lysozyme, strong acid phosphatase and alpha-naphtyl acetate esterase activity in the cytoplasm, the presence of C3 and Fcγ receptors, the presence of lysosomes and the absence of junctions on ultrastructural level in eight out of ten cases and the absence of intracytoplasmatic or membrane immunoglobulins as demonstrated by immunofluorescence. The tumor cells, thus defined as histiocytes, showed fairly specific histological characteristics: they were large to enormous with abundant cytoplasm and with large, indented nuclei with coarse chromatin, conspicuous nucleoli and a sharply defined nuclear membrane. In methylmethacrylate embedded sections erythrophagocytosis and haemosiderin pigment could be observed in all cases but one. PAS staining showed granular (sometimes weakly diffuse) diastase resistant PAS positive material in the cytoplasm.

Until recently it was thought that apart from Malignant Histiocytosis, tumors of true histiocytic origin were very rare. However, Isaacson (1979b) showed that histiocytic malignancies are probably much less rare than was previously accepted. Since we now know the histological criteria to identify this tumor by the combined histological, immunological, enzymehistochemical and ultrastructural study presented here, we have identified retrospectively in our files a considerable number (30) of these tumors. However, routine histological staining is often not conclusive enough to arrive at the correct diagnosis. Additional techniques are then necessary. In retrospective studies (Isaacson et al. 1979b; Meister et al. 1980a; Mendelsohn et al. 1980) staining for lysozyme, alpha$_1$-

antitrypsin and alpha$_1$-antichymotrypsin on paraffin embedded tissue sections proved to be very helpful in distinguishing Histiocytic Sarcoma from other malignancies (Meijer and Van der Valk, in preparation). Undifferentiated carcinoma can cause problems: its pattern of growth and nuclear polymorphism may account for mistakes. Moreover, alpha-naphtyl acetate esterase and acid phosphatase may be present on some epithelial tumors (Tubbs et al. 1979). However, lysozyme is not found in undifferentiated carcinoma, and electron microscopy will demonstrate junctions between the tumor cells. Likewise, some non-Hodgkin lymphomas can resemble histiocytic sarcoma closely in histological sections, especially B- and T-immunoblastic lymphomas, large cleaved follicle centre cell tumors and convoluted T-lymphoblastic lymphomas. Since these tumors consist of either B- or T-lymphoid cells, they can be distinguished by the presence of monoclonal immunoglobulins on their surface or in their cytoplasm, or their ability to form rosettes with uncoated sheep erythrocytes or specific T-cell antigens, respectively. Furthermore, there are histological differences: B-immunoblastic sarcoma often shows more plasmablasts, the cells show less nuclear indentations, and have less cytoplasm. Their cytoplasm is more basophilic. T-immunoblasts have coarser chromatin, the nuclear membrane is not as sharply defined and the cells have less cytoplasm. In follicle centre cell tumors, the blast cells frequently have more than one nucleolus, they are smaller and have less cytoplasm. Convoluted T-lymphoblasts are smaller, there is less variation in nuclear size, they have much less cytoplasm and the number of mitotic figures in T-lymphoblastic lymphoma is often higher. Any lymphoma with a great number of reactive histiocytes may cause a problem, but reactive histiocytes are smaller, show less variation in nuclear area (Table 2) and usually stain more intensely for lysozyme with the immunoperoxidase technique than do their malignant counterparts. On these ground a reactive histiocytosis can be excluded. It may sometimes be difficult to exclude Hodgkin's disease, with lymphocytic depletion. However, the typical Reed-Sternberg cell, the diagnostic criterion for this disease, is not seen in histiocytic sarcoma. Additionally some other disorders may be confused with histiocytic sarcoma, for instance Histiocytosis X. However, Histiocytosis X is an essentially benign looking tumor of Langerhans cells and can be differentiated by its characteristic ultrastructural features (Corrin and Basset 1979) and by enzyme histochemical criteria (Elema and Poppema 1978). Malignant Fibrous Histiocytoma must also be mentioned, as this tumor contains numerous histiocytes. The origin of this neoplasm is probably from an undifferentiated mesenchymal cell (Weiss and Enzinger 1978) that develops along both fibroblastic and histiocytic lines. Additional histological features, like presence of granulocytes, giant cells or a myxoid stroma determine the subtype of this tumor. In histiocytic sarcoma no development along a fibroblastic line is present, which make the two malignancies easily distinguishable.

Morphometry supplied yet another characteristic for the differentiation between histiocytic sarcoma and the non-Hodgkin lymphomas: no lymphoma had such enormous nuclei, or showed a nuclear indentation and variety in size as did histiocytic sarcoma. In particular, comparison of the number of cells with an area exceeding 80 μm^2 or an NCI exceeding 5.5 illustrated the difference between the tumors most clearly. However, the significance of these findings for diagnosis needs further evaluation on a larger number of patients.

Clinically, the ten tumors presented here showed an interesting spectrum. There was one classical case (v.O), and one argueable case (d.J) of Malignant Histiocytosis. They presented with disseminated disease. On the other hand, five patients (K-H, W, v.Z-R, K, H) presented with a localized form of histiocytic sarcoma and reached complete remission on therapy. The three remaining cases presented with more advanced disease, but the progression in these cases was quite unlike the fulminating course Malignant Histiocytosis runs. This poses a problem for terminology. The name Malignant Histiocytosis is too closely associated with a grim clinical picture. The term histiocytic lymphoma according to Rappaport is confusing, since most of these tumors are of lymphoid origin. Malignant lymphoma, histiocytic type (Lukes and Collins 1975) is the same tumor as ours, but the name is a contradiction in terms, and the old term reticulum cell sarcoma also includes nonhistiocytic tumors. Our tumor is identical with the tumors Lennert describes as sarcomas of histiocytic reticulum cells (Lennert 1978). As we are dealing here with localized malignant proliferations of mononuclear phagocytes, having excluded monocytic leukemia, we propose the term histiocytic sarcoma for the tumors made up of these cells.

Although the follow-up of these patients is short and the number of patients small, the clinical data are of interest. Five from the ten patients showed a favorable clinical course and are at this moment free of disease, three of them longer than 17 months. Four of these five patients had stage I disease and reached a complete remission. Remarkably all these five patients had no or very little alpha$_1$-antitrypsin or alpha$_1$-antichymotrypsin, both protease inhibitors and described as markers for histiocytic cells (Isaacson and Wright 1978; Meister and Nathrath 1980b) and showed a high number of lysosomes. Furthermore, five of the six patients with 5-Nucleotidase on their surface had a favorable clinical course. However, the prognostic value of his enzyme, which is present on resident macrophages and histiocytes and disappears on activation of these cells (Van Furth 1979), must be evaluated further on a larger series of patients.

Our finding that abundance of lysosomes is apparently a prognostically favorable factor is in accordance with the findings of Mendelsohn et al. (1980) and Lombardi et al. (1978). These authors found a relationship between the stage of differentiation of the cells and the amount of lysozyme and the number of lysosomes in the cytoplasm. Thus, according to these authors, tumors with low amounts of lysozyme and low numbers of lysosomes are less differentiated and therefore have a poor response to therapy. Whether the presence of alpha$_1$-antitrypsin and absence of 5-Nucleotidase are characteristics of more differentiated histiocytes is not mentioned in the literature. Meister et al. (1980a) do mention the fact that alpha$_1$-antichymotrypsin activity disappears in undifferentiated forms of Malignant Histiocytosis. They did not, however, investigate the more localized forms of histiocytic tumors, as presented here. Nevertheless the variation of alpha$_1$-antitrypsin, alpha$_1$-antichymotrypsin and 5-Nucleotidase and the number of lysosomes in the cytoplasm of immunologically, enzymehistochemically and morphologically defined histiocytic sarcomas indicate that they constitute a spectrum not only clinically, but also pathologically.

A striking clinical aspect was the affinity of the tumor for bone and skin. The literature on true histiocytic disorders mentions skin lesions (Abele and

Griffin 1972), but reports on bone involvement are scarce. It is not unlikely, however, that in the group of primary histiocytic lymphoma of bone (Boston et al. 1974; Mahoney and Alexander 1980) a number of histiocytic sarcomas is included.

In conclusion: Histiocytic Sarcoma (lymphomas of true histiocytic origin) constitutes a pathological entity which may be defined by morphological, ultrastructural, immunological and cytochemical criteria. The need to differentiate these tumors from non-Hodkin lymphomas and undifferentiated carcinoma is emphasised by their different clinical behavior (frequent involvement of skin and localized bone destruction) and the different therapeutic approach that may be necessary, as these cells are not of lymphoid or epithelial origin.

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