Virchows Archiv A Pathol Anat (1989) 414:243–251

Virchows Archiv A
Pathological Anatomy and Histopathology

Ewing's sarcoma

A retrospective study of histological and immunohistochemical factors and their relation to prognosis

S. Daugaard[1], C. Kamby[2], L.M. Sunde[3], O. Myhre-Jensen[4], and T. Schiødt[1]

The Bone and Soft Tissue Tumour Centres of Copenhagen and of Aarhus: Departments of [1] Pathology; [2] Oncology ONA and Orthopaedics, The Finsen Institute/Rigshospitalet, Copenhagen; [3] Hospital of Orthopaedics and Department of Oncology, The Municipal Hospital, Aarhus; [4] Department of Pathology, The County Hospital, Aarhus, Denmark

Summary. Histological and immunohistochemical features of 87 patients with conventionally diagnosed Ewing's sarcoma were studied retrospectively on routinely processed material and evaluated with regard to prognostic significance. 74% were convincingly positive when stained for vimentin, 13% were doubtful, and 13% were negative. A varying degree of positivity for neuron-specific enolase (NSE) was found in 15%; these cases all co-expressed vimentin. A single tumour contained scattered cytokeratin-positive cells. Positivity for the leukocyte common antigen (LCA) could be demonstrated in three cases; these were excluded from the statistical analysis of prognostic factors.

Growth pattern, soft tissue invasion, monomorphic or dimorphic cell population, and PAS-, NSE- or vimentin-positivity did not influence survival significantly. However, prognosis was increasingly poor with increasing degree of necrosis: median survival was 28 months for grade I necrosis (<10%), 16 months for grade II (10–50%), and 11 months for grade III (>50%), $p<0.0005$. A mitosis count of <1 per high-powerfield (HPF) was correlated to a median survival of 26 months, ≥1 per HPF to 12 months, $p<0.05$.

The findings indicate some degree of heterogeneity in Ewing's sarcoma which may be related to primitive peripheral neuroectodermal tumours (PNETs), or be a true blastoma. In future trials, diagnostic criteria (including immunohistochemistry) should be clearly defined and materials should be large enough to allow for stratification according to prognostic factors.

Key words: Ewing's sarcoma – Histology – Immunohistochemistry – Prognostic factors

Offprint requests to: S. Daugaard, Knabrostraede 9, DK-1210 Copenhagen K., Denmark

Introduction

Ewing's sarcoma (ES) is a rare, highly malignant tumour which occurs primarily in the bones of children and young adults. Until recently the prognosis was uniformly poor, but the introduction of adjuvant chemotherapy has improved survival markedly, with an increase in long-term survival rates from about 10% to 35–74% (Pomeroy and Johnson 1975; Nesbit et al. 1981; Sauer et al. 1987). These advances have spurred a renewed interest in identifying clinical and pathological factors of prognostic significance, since knowledge of these is a prerequisite for the proper evaluation of results from different clinical trials (Glaubiger et al. 1980).

The present study was undertaken in order to investigate the prognostic importance of various histopathological features, including immunohistochemical results, in a series of conventionally diagnosed ES. The clinical features and their prognostic significance have been reported elsewhere (Daugaard et al. 1987).

Materials and methods

In the period 1962–1983, our two tumour centres received 96 patients with a diagnosis of ES. Histological slides and/or paraffin blocks were retrieved from our archives, or borrowed from the referring institutions. New sections were cut and stained when necessary, the aim being that a HE, reticulin and PAS stain should be available. In some cases only the original slides were available, or there was no or little residual tumour tissue left in the blocks. In three cases, no histological material was available for review. The clinicopathologic review led to a revision of the diagnosis in six cases: three were neuroblastomas, one a malignant lymphoma, one a lymphoblastic leukaemia, and one an unclassifiable malignancy. In the remaining 87 cases, the diagnosis of ES was confirmed by two of the authors (TS and OMJ), who are experienced in this field of pathology.

Conventional light microscopic criteria were used (Ball et al. 1977; Kissane et al. 1981; Dahl et al. 1986; Triche et al. 1986).

The following histological variables were evaluated: the *growth pattern* which was categorised as showing diffuse/organoid (incl. the presence of "filagree pattern", Kissane et al. 1983) or pseudorosette pattern, *soft tissue invasion*, the *type of cell* which predominated (clear-cell/small dark cell/atypical large cell), the variability of the *cell population* (monomorphic/dimorphic, slight/pronounced), the degree of *nuclear pleomorphism* (subjectively graded as slight, moderate or severe) the *mitosis count per high-power field (HPF)* (when possible, counted in 10 HPFs using a Leitz Orthoplan, $\times 400 = 2.5\ mm^2$), the presence of *PAS-positivity* (subjectively graded $0/+/++/+++$) and the degree of *necrosis* ($<10\%/10$–$50\%/>50\%$ of tumour tissue, grade I, II, and III, respectively).

Available material was stained with antibodies directed against leucocyte common antigen (LCA), vimentin, neuron-specific enolase (NSE), desmin and cytokeratin, using the immunoperoxidase technique.

Monoclonal antibodies against LCA and vimentin (Dakopatts A/S) were used in a dilution of 1:5 in a three-layer procedure (Christensen and Strange 1987). No enzyme pretreatment was found to be necessary. In order to achieve optimal reaction for vimentin, slides were incubated for 6 days at 4 degrees Celsius. Polyclonal antibodies against NSE (Dakopatts A/S) were diluted 1:50, and slides incubated for 30 minutes; blocking of endogenous peroxidase was found to diminish staining intensity and was therefore omitted. Monoclonal antibody against desmin (Dakopatts A/S) was diluted 1:50; slides were pretreated with pronase for 10 min and incubated for 30 min. Monoclonal anti-cytokeratin (Becton Dickinson Ltd.; clone CAM 5.2, specificity human cytokeratin 39, 43 and 50 K daltons, Moll's catalog Nos. 8, 18, and 19) was used in a dilution of 1:10 following pretreatment with pronase for 5 min. All washing was done with Triton X-100.

The intensity of the reaction was graded subjectively $0/+/++/+++$, and the number of positive cells was estimated as $0/<10\%/10$–$50\%/>50\%$. Tumours were regarded as positive when more than 10% of their cells exhibited a staining of moderate $(++)$ or strong $(+++)$ intensity. Tumours which expressed only a slight $(+)$ positivity in more than 10% of the cells, or a moderate $(++)$ positivity in less than 10% were considered "equivocally positive". There were no instances of less than 10% strongly $(+++)$ reacting cells. The rest were rated negative.

Survival rates were calculated according to the Kaplan Meier method, and survival curves were compared using the logrank test (Peto et al. 1977). For the comparisons of qualitative data the Chi-square test and the Mann-Whitney *U*-test for unpaired samples were applied. A two-tailed significance level of 5% was chosen for all statistical calculations.

Results

The 87 patients (60 males, 27 females) had a median age of 16 years (range 1–62). The primary tumours were located in the pelvis/sacrum in 31%, proximally in the extremities in 33%, and distally in 20%. 77% had a palpable tumour at presentation, and 13 patients (15%) had distant metastases at the time of diagnosis. 32 patients received adjuvant chemotherapy, while almost all (95%) received radiotherapy (Daugaard et al. 1987).

Histopathology

The available material was rather sparse and traumatized. In many cases it had been decalcified. Thus, it was not possible to evaluate all variables in each case.

Growth pattern could not be assessed in 14 (16%). A purely diffuse growth pattern was found in 28/73 cases (38%). A similar number (28) exhibited the so-called filagree pattern as described by Kissane et al. (1983) in some areas. The remaining cases had various organoid structures, most commonly ill-defined trabeculae, or nests. Small pseudo-rosettes without central fibrillar material were seen in two cases. In more cellular areas, some cases exhibited irregular plasma lakes. In one case, where the primary lesion was diffuse, a later metastasis showed an organoid pattern.

Soft tissue invasion was demonstrated in 38 specimens (44%). In two cases, where the tumour invaded skeletal muscle, small intramyofiber metastases were seen. There was no obvious relationship between growth pattern and soft tissue invasion; 17 of the cases with soft tissue invasion exhibited the filagree pattern, corresponding to 45% (95% confidence limits 29–62%). This was not significantly different from the occurrence in the overall material: 38% (95% confidence limits 27–62%). Soft tissue invasion was neither correlated to the presence of metastases at the time of diagnosis, nor to tumour site.

The evaluation of *cell type and population* was rendered difficult in some cases by artefacts, notably the phenomenon of cell crushing in traumatized biopsies. This creates a picture of "combed hair" – as in small cell carcinoma of the lung – which has also been termed "Azzopardi phenomenon" by Kissane et al. (1983). Moreover, material that had been frozen before routine processing exhibited homogenous dark nuclear staining with consequent loss of morphologic detail. The dominant cell type had round or oval nuclei with finely granular chromatin and inconspicuous nucleoli and pale or sometimes clear cytoplasm. The secondary cells had condensed, dark nuclei and sometimes a more eosinophilic cytoplasm. The nuclear diameter of the two cell types was about 8 and 5 micrometer, respectively. Of 76 evaluable cases, 26 showed a purely monomorphic pattern while the remaining 50 exhibited a dimorphic cell population, 24 to a lesser, 26 to a higher degree. The secondary cells were randomly distributed.

Three cases had somewhat larger cells and nuclei (diameter 9–10 micrometer) as well as a slightly more pronounced pleomorphism. These were thought to represent the "atypical" or large cell

variant of ES (Llombart-Bosch et al. 1978; Nascimento et al. 1980; Navas-Palacios et al. 1984). Two of them turned out to be LCA-positive (see below).

Nuclear pleomorphism was slight to moderate; none of the cases exhibited pronounced variations in size/shape of cells or nuclei, and a reproducible subclassification was not possible.

Seventy six cases were evaluated for *mitosis*. The mean count per HPF was 0.98, median 0.4, ranging from zero to 5.5.

Seventy seven cases were evaluable for *PAS positivity*. Fourteen were negative, 15 were + positive, 22 + + positive, and 26 + + + positive. In one case, the primary tumour was PAS-negative, while a metastasis (removed less than a month after diagnosis) was + positive.

Grade I *necrosis* (<10%) was found in 42 cases (54%), grade II (10–50%) in 17 cases (22%), and grade III (>50%) in 19 cases (24%). 9 cases were not evaluable. When necrosis was marked, a surviving cuff of tumour cells was often seen around blood vessels, creating a pattern vaguely resembling that of a haemangiopericytoma.

In pelvic tumours, necrosis was somewhat more pronounced than in the other sites ($p<0.05$, Wilcoxon test). Also, there was a tendency for necrotic tumours to be less PAS-positive than non-necrotic ones ($p<0.05$, Kruskal-Wallis test), but otherwise no correlations could be demonstrated between the above-mentioned factors, symptoms, or signs.

Of 76 tumours tested for *vimentin* 56 (74%) were positive, 10 (13%) were negative, and 10 (13%) were doubtful. No non-specific reactions were seen; necrosis and decalcification did not influence staining pattern or intensity. Proliferating fibroblasts (in cases with soft tissue invasion) exhibited a strong positivity.

Seventy-five tumours were tested for *neuron-specific enolase (NSE)*. Sixty-four, (85%) were considered negative, 4 positive, and 7 equivocal. With this antibody, some nonspecific staining was encountered: Muscle cells were often – but not always – slightly (+) positive, and reaction was also seen in necrotic areas (since the endogenous peroxidase had not been blocked). Moreover, irregular staining of the edges of the sections was often seen; this phenomenon was not a problem with the other antibodies used in this study. The 4 NSE-positive, as well as the 7 "equivocally positive" tumours, did also express vimentin.

Leucocyte common antigen (LCA) proved very reliable with no nonspecific staining and a strong reactivity in the normal lymphocytes encountered sporadically within the tumour tissue or stroma, thus acting as built-in positive controls. 74 tumours were tested; 2 turned out to be strongly (+ + +) positive, while one showed a slight (+) staining of 10–50% of the cells. The rest were negative.

Thirty-two tumours were tested for *desmin*, all were negative, and no nonspecific staining was encountered.

Twenty-eight tumours were tested for *cytokeratin*; 27 were negative. In one tumour, about a quarter of the cells were moderately (+ +) positive. There was no unspecific staining. The tumour was strongly vimentin-reactive and negative for NSE.

Survival

The median over-all survival for all patients was 19 months and 5-year survival rate was 17%. Metastatic disease at the time of diagnosis, pain, and objective impairment of movement were all correlated with a significantly poorer prognosis. Tumour site did not exert any statistically significant influence, although pelvic localization was associated with a shorter median survival. Adjuvant chemotherapy prolonged recurrence-free survival, but overall survival was not improved significantly (Daugaard et al. 1987).

The three LCA-positive cases mentioned above were excluded from the statistical analysis of prognostic factors (see Discussion). The two patients with strongly positive tumours had survivals of 106+ months and less than one month, respectively; only the former received adjuvant chemotherapy. The patient whose tumour was slightly positive for LCA did not receive adjuvant chemotherapy, but was still alive at 68+ months. The single patient with a partly cytokeratin-positive tumour had a survival of 21 months and also received adjuvant chemotherapy.

Two of the histological variables were significantly correlated with survival: Patients with less than 10% necrotic tumour tissue (grade I) had a median survival of 28 months, while those with 10–50% (grade II) and ≥50% (grade III) had median survivals of 16 and 11 months, respectively (Fig. 1). The logrank test yields a p-value of less than 0.0005, and a test for trend (Peto et al. 1977) is also highly significant ($T=15.31$, $p<0.0005$). The median survival for patients with a mitotic index below 1 per HPF was 26 months, while 12 months was the median value for those with 1 or more mitoses per HPF (Fig. 2). The difference is significant ($p<0.05$). The other variables tested for prognostic influence are listed in Table 1. Growth pattern, soft tissue invasion, PAS-, vimentin, and NSE-positivity did not significantly influence survival. Patients with a dimorphic cell population

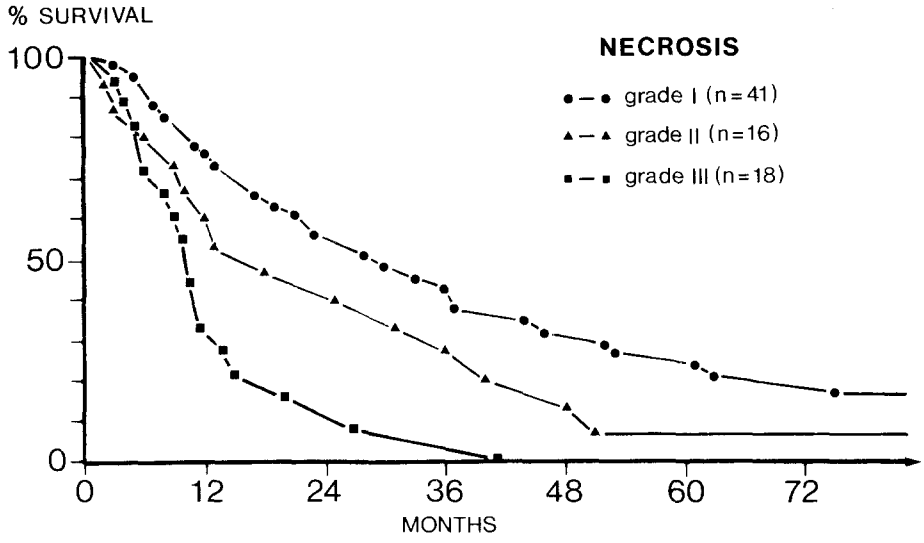

Fig. 1. Survival curves as a function of necrosis. Kaplan Meier method, $p<0.0005$. Test for trend: $p<0.0005$

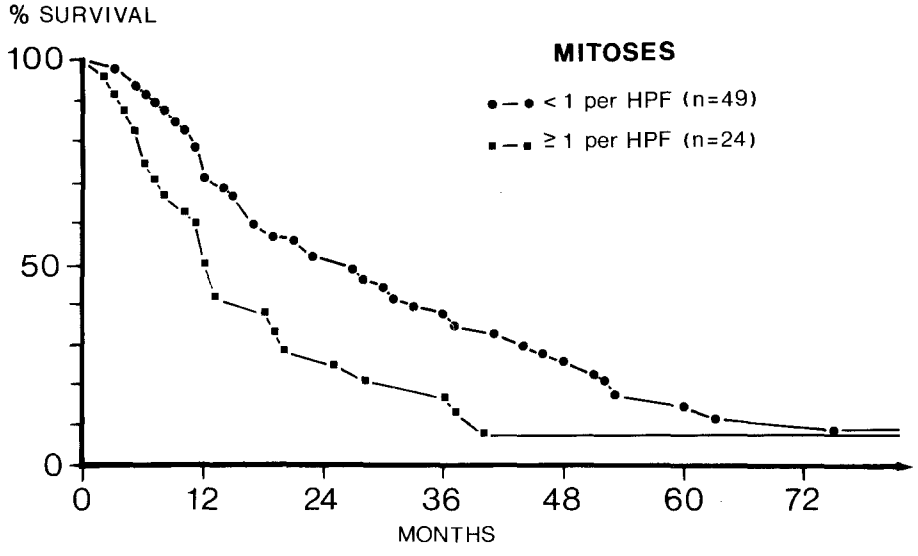

Fig. 2. Survival curves as a function of mitotic index. Kaplan Meier method, $p<0.05$

lived slightly longer than those with a monomorphic picture, but the difference did not reach significance.

Discussion

The histogenesis of ES remains obscure, and its cell of origin has been debated ever since Ewing described his "diffuse endothelioma of bone" in 1921. Advances in ultrastructural pathology and the introduction of immunohistochemical techniques have not solved the issue although they are helpful in the differential diagnosis towards other small, round, blue cell tumours (Triche et al. 1986). Most commonly favoured is the concept of the ES cell as a primitive, non-comitted mesenchymal cell (Dickman et al. 1982; Navas-Palacios et al. 1984; Löning et al. 1985). Following the observation of a reciprocal translocation involving chromosome 22, t (11; 22) (q 24; q 12) (Aurias et al. 1984; Maletz et al. 1986), Dehner (1986) and Triche (1986) have discussed the possibilitity that ES belongs to the group of peripheral neuroectodermal tumours (PNETs), which exhibit the same abnormality. However, Moll et al. (1987) conclude that ES is derived from a pluripotential primitive cell and thus represents a blastoma rather than a true sarcoma on the basis of immunocytochemical findings. The findings of Cavazzana et al. (1987) – that neural differentiation could be induced in vitro in ES cell lines – are not at variance with this latter theory.

These considerations are not wholly academic since conventional diagnostic criteria seem to be

Table 1. Histological variables tested for prognostic significance

		Survival (months):			
		No.	median	range	p^a
Filagree growth pattern	present	26	13	3–154+	0.79
	not present	58	20	2–196+	
Diffuse growth pattern	present	28	24	2–98	0.48
	not present	56	16	2–196+	
Soft tissue invasion	present	37	18	3–159+	0.87
	not present	47	17	2–196+	
Cell population	monomorphic	26	14	2–98	0.06
	dimorphic	47	23	2–159+	
PAS-positivity	0/+	26	14	2–98	
	++	22	22	5–159+	0.15[b]
	+++	26	25	2–154+	
Vimentin	positive	56	20	2–154+	0.55
	doubtful or negative	17	13	4–159+	
Neuron-specific enolase	negative	61	20	2–159+	0.76
	doubtful or positive	11	12	4–98	

[a] logrank test; [b] test for trend: $p=0.08$

incapable of separating the different categories of small, blue, round-cell tumours with different prognosis and treatment with sufficient reliability. Immunohistochemical techniques for the demonstration of various markers may serve to illustrate the heterogeneity of the present diagnostic classification and, at least theoretically, improve diagnostic accuracy, but their prognostic implications are rarely discussed. They were therefore included in the present study, which joins the few carried out, with discordant results. The most prominent recent works are those of the Intergroup Ewing's Sarcoma Study (IESS) by Kissane et al. (1983), a Uruguayan study by de Stéfani et al. (1984) and a French-Spanish one by Llombart-Bosch et al. (1986). Wilkins et al. (1986) from the Mayo Clinic merely conclude that no pathological variables of prognostic importance were found in their material; it is not stated which were tested.

In the IESS study (303 cases), the presence of a filagree-like growth pattern was associated with a poor survival rate; however, the site of primary involvement remained the most important prognostic factor. In their retrospective study of 40 cases of ES, de Stéfani and co-workers found necrosis to be most strongly correlated to survival, while the influence of primary site was insignificant. Llombart-Bosch et al. (1986) performed a multifactorial analysis on 261 cases of ES and, after adjustment for therapy and site, found both the presence of filagree growth pattern and the presence of necrosis to be correlated with a shorter survival; both were found to be of poor prognostic value, however.

Our results confirm that survival is increasingly poor with increasing degree of necrosis. The exact importance is difficult to assess, particularly since the surgeon is expected to take a biopsy from a vital area of tumour tissue, necrosis thus probably being underestimated by the pathologist. In soft tissue sarcomas, necrosis seems to be a prognostic factor independent of size and location (Costa et al. 1984). Nevertheless it seems plausible to postulate a correlation between the degree of necrosis and tumour size (volume). This latter feature was not evaluated in the present study, but the findings of Sauer et al. (1987) indicate that it is a major prognostic factor in ES, more important than tumour site and type of local therapy.

We could not confirm that a filagree growth pattern implies a shorter survival, nor that it is associated with soft tissue invasion. The latter feature, albeit evaluated roentgenographically and defined as a soft tissue mass extending at least 1 cm outside of the affected bone, may be of prognostic importance (Mendenhall et al. 1983). However, Wilkins et al. (1986) could not confirm these findings, and the microscopic equivalent did not affect survival nor incidence of local recurrences in our study.

The demonstration of glycogen in the tumour cells is not a *sine qua non* for the diagnosis of ES,

when the material has been processed routinely (Kissane et al. 1983). Its presence has been tested for prognostic value by Pomeroy and Johnson (1975); like us, they were not able to demonstrate any significant correlation.

The mitotic index is one of the most important factors in grading systems of soft tissue sarcomas (Myhre-Jensen et al. 1983; Costa et al. 1984; Trojani et al. 1984). However, the feature does not appear to have been tested for significance in ES, at least it is not specifically mentioned in the studies referred to above. In our material a count of ≥ 1 mitosis per HPF was significantly correlated to a poorer survival.

The occurrence of small, dark or "secondary" cells in ES has become a well recognized, but not specific cytological and ultrastructural feature of this neoplasm (Llombart-Bosch et al. 1978; Mahoney and Alexander 1978; Kissane et al. 1983; Dahl et al. 1986). They are considered to represent regressive tumour cells, a view supported by autoradiographic findings (Roessner 1984). They are randomly distributed and are not related to the occurrence of necrosis or to mitotic activity. Their presence or absence did not influence survival significantly in our material.

Vimentin is a filament (polypeptide) of intermediate size (7–12 nm) which primarily occurs in cells of mesenchymal origin, including lymphocytes (Miettinen et al. 1984; Du Boulay 1985; Triche et al. 1986). Its uniform presence in ES has been confirmed by several investigators (Miettinen et al. 1982; Schulz et al. 1984; Meister 1984; Löning et al. 1985; Moll et al. 1987). We were not able to demonstrate vimentin in 13% of the tumours. This may be due to tumour heterogeneity or to less than optimal fixation and processing; fixation with formalin reduces vimentin reactivity to about 60% as compared with alcohol (Azuma and Battifora 1987). In any case, survival was not influenced significantly. However, vimentin remains a valuable tool in the diagnosis of ES, especially since neuroblastomas – an important differential diagnosis – are generally reported to be negative for this intermediate filament (Osborne et al. 1982; Kahn et al. 1983; Roholl et al. 1985). Occasionally, however, vimentin or other filaments are demonstrated in neuroblastomas (Azumi and Battifora 1987). However, neuroblastomas are always positive when stained for the presence of neuron-specific enolase (NSE) (Meister 1984; Tsokos et al. 1984; Vinores et al. 1984; Triche et al. 1985; Perentes and Rubinstein 1987). This glycolytic enzyme occurs principally in neurons and neuroendocrine cells, and its presence in tumour cells has been interpreted as evidence of neuronal origin/differentiation; however, it has also been detected in cells of non-neural derivation (Perentes and Rubinstein 1987). Furthermore, some NSE-antibodies may cross-react with the enolase present in muscle cells, and some rhabdomyosarcomas have been reported to be NSE-positive (Triche et al. 1985; Blatt et al. 1986). Staining of normal myoepithelial cells and (non-argyrophilic) carcinomas of the breast has also been described (Bussolati et al. 1987). Nevertheless, the prevailing opinion is that most ES are NSE-negative (Dhillon et al. 1982; Meister 1984; Tsokos et al. 1984; Triche et al. 1985).

In our material we found a varying degree of NSE-positivity in 11 tumours (13%). All of these co-expressed vimentin. From the considerations above it is evident that caution is warranted when interpreting these results. This is underlined by the fact that 7 out of these 11 tumours exhibited a staining reaction that was difficult to interpret and therefore scored as "equivocally positive". Moreover, none of them contained rosettes. This being said, the possibility remains that they represent PNETs. This concept has been discussed in detail by Dehner (1986), who also mentions that these tumours contain vimentin; a case of PNET in a rib of a boy was reported to be moderately NSE-positive and vimentin-negative (Haas et al. 1987). Immunoreactivity for vimentin was not investigated in the NSE-positive cases of ES-like bone tumours reported by Jaffe et al. (1984) and Kawaguchi and Koike (1986). Llombart-Bosch et al. (1988) found five vimentin-positive and 11 NSE-positive cases among their 14. PNETs are thus not clearly defined, and grey areas exist between this entity and ES as well as neuroblastomas (Dehner 1986). Anyway, there were no demonstrable differences in our material between the NSE-positive cases and the other patients; in particular, their overall survival did not differ significantly.

Metastatic rhabdomyosarcoma of the most undifferentiated type may also create differential diagnostic problems. No reliable marker for this neoplasm has been found so far; desmin (an intermediate filament of smooth and striated muscle cells) is one of the best, although it is not specific (Osborn and Weber 1983; Miettinen et al. 1985; Variend 1985; Triche et al. 1986). Vimentin is often co-expressed together with desmin (Osborn and Weber 1983; Kahn et al. 1983; Löning et al. 1985). As mentioned above, some rhabdomyosarcomas will react with antibodies against NSE.

The co-expression of vimentin with other intermediate filaments is a well-recognized phenomenon (Osborn and Weber 1983; Miettinen et al.

1984; Perentes and Rubinstein 1987). Tumours considered to be representative of ES are generally reported to be negative for other intermediate filaments than vimentin (Miettinen et al. 1982; Meister 1984; Schulz et al. 1984; Löning et al. 1985). Recently, Moll et al. (1987) and Miettinen and Herva (1987) have reported the occurrence of scattered cytokeratin-positive cells within some of these neoplasms. Both groups used frozen sections, Moll and co-workers also gel-electrophoresis and immunoelectron microscopy; with these methods they were in addition able to demonstrate reactivity for neurofilament proteins in a few cases. Miettinen and Herva (1987) found formalin fixation and paraffin embedding abolished staining for cytokeratin. Nevertheless, we found one tumour to contain cytokeratin-positive cells; the true incidence of this phenomenon remains to be established, especially with regard to different methods of fixation. Its clinical significance is unknown.

Leucocyte Common Antigen (LCA) is a reliable and sensitive marker of lymphocytes and cells of lymphoid derivation; 93–100% of non-Hodgkin lymphomas are reported to be positive (Kurtin and Pinkus 1985; Michels et al. 1987). Very poorly differentiated lymphoblastic leukaemias and lymphomas may, however, be negative (Kurtin and Pinkus 1985; van Eyken et al. 1987). Even its specificity is remarkable; we are aware of only a single report of LCA-positivity in a non-lymphoid malignancy, a primitive sarcoma (McDonnell et al. 1987). Thus, the demonstration of LCA in three of our cases speaks in favour of a lymphoid derivation, and it was therefore decided to withdraw them from the statistical analysis of prognostic factors (incidentally, the only effect of this was to produce an apparent increase in the significance of the importance of mitotic index). Reviewing their morphological features, it was noted that all these cases were PAS-negative, and that two exhibited the features of atypical large-cell ES with large and often grooved nuclei (Llombart-Bosch et al. 1978). Some caution thus seems to be warranted when this diagnosis is contemplated. The third tumour was morphologically indistinguishable from the rest of the material. These findings are in accordance with those of Ball et al. (1977), who in their attempt to draw a purely morphological distinction between ES and lymphomas (reticulum cell sarcomas) reported some tumours to be "not typical of either group". They also serve to underline the inadequacy of conventional histological criteria in defining ES.

Other markers and methods may prove to be more suitable in defining and differentiating these tumours, e.g. desmoplakin (Moll et al. 1987), tetanus toxin (Berliner and Unsicker 1985), nerve growth factor receptor (Perosio and Brooks 1988), lectin and collagen typing (Dickman et al. 1982; Gabius et al. 1986), or counting of nucleolar organizer regions (Egan et al. 1987). The possibility remains – to some degree supported by the finding of common chromosomal abnormalities and common surface antigens like N-CAM – that neuroblastomas, PNETs, and ESs are histogenetically related, with ES representing the most undifferentiated end of the spectrum (Dehner 1986; Lipinski et al. 1987). However, immunohistochemically demonstrable heterogeneity, in the view of Moll and co-workers (1987), indicates that ES is derived from a pluripotential, primitive cell with a potential for differentiation into mesenchymal, epithelial, and neural direction. This idea of ES as a blastoma suggests a relationship to Jacobson's concept of 'polyhistioma': an ES-like neoplasm with light microscopically recognizable differentiation into various, primarily mesenchymal, elements, but also with occasional pseudorosettes or epithelial-like structures (Jacobson 1977).

The only histopathological factors of significant prognostic importance in our study were the degree of necrosis, and the mitosis count. The former may be a prognostic factor itself, but it is also possible that it is correlated with tumour volume, which in a recent study was found to be even more important than site (Sauer et al. 1987). We could not confirm the importance of growth pattern, nor were PAS-positivity, soft tissue invasion, cell population, and immunohistochemical staining patterns found to be of prognostic significance.

The recent advances in the treatment of ES, with more long-term survivors, make knowledge of prognostic factors important: poor-risk patients may be identified and aggressively treated, while patients with a good prognosis may be considered for less mutilating or intensive therapy. Moreover, patient materials should be large enough to allow for stratification. Diagnostic criteria (including immunological and other techniques) should be clearly defined – as our study shows, there is some heterogeneity in a conventionally diagnosed material. These goals can only be reached with centralized diagnosis and treatment and participation of multiple centres.

References

Aurias A, Rimbaut C, Buffe D, Zucker J-M, Mazabraud A (1984) Translocation involving chromosome 22 in Ewing's

sarcoma. A cytogenetic study of four fresh tumors. Cancer Genet Cytogenet 12:21–25
Azumi N, Battifora H (1987) The distribution of vimentin and keratin in epithelial and nonepithelial neoplasms. Am J Clin Pathol 88:286–296
Ball J, Freedman L, Sissons HA (1977) Malignant round-cell tumours of bone: An analytical histological study from the cancer research campaign's bone tumour panel. Br J Cancer 36:254–268
Berliner P, Unsicker K (1985) Tetanus toxin labeling as a novel rapid and highly specific tool in human neuroblastoma differential diagnosis. Cancer 56:419–423
Blatt J, Russo P, Taylor S (1986) Extrarenal rhabdoid sarcoma. Med Pediatr Oncol 14:221–226
Bussolati G, Papotti M, Sapino A, Gugliotta P, Ghiringhello B, Azzopardi JG (1987) Endocrine markers in argyrophilic carcinomas of the breast. Am J Surg Pathol 11:248–256
Cavazzana AO, Miser JS, Jefferson J, Triche TJ (1987) Experimental evidence for a neural origin of Ewing's sarcoma of bone. Am J Pathol 127:507–518
Christensen L, Strange L (1987) Universal immunoperoxidase staining protocol to optimize the use of polyclonal and monoclonal antibodies. J Histotechnol 10:11–15
Costa J, Wesley RA, Glatstein E, Rosenberg SA (1984) The grading of soft tissue sarcomas. Cancer 53:530–541
Dahl I, Åkerman M, Angervall L (1986) Ewing's sarcoma of bone. A correlative cytological and histological study of 14 cases. Acta Pathol Microbiol Immunol Scand (A) 94:363–369
Daugaard S, Sunde LM, Kamby C, Schiødt T, Jensen OM (1987) Ewing's sarcoma. A retrospective study of prognostic factors and treatment results. Acta Oncol 26:281–287
Dehner LP (1986) Peripheral and central primitive neuroectodermal tumors. Arch Pathol Lab Med 110:997–1005
Dhillon AP, Rode J, Leathem A (1982) Neurone specific enolase: an aid to the dianosis of melanoma and neuroblastoma. Histopathology 6:81–92
Dickman PS, Liotta LA, Triche TJ (1982) Ewing's sarcoma. Characterization in established cultures and evidence of its histogenesis. Lab Invest 47:375–382
Du Boulay CEH (1985) Immunohistochemistry of soft tissue tumours: A review. J Pathol 146:77–94
Egan MJ, Raafat R, Crocker J, Smith K (1987) Nucleolar organizer regions in small cell tumours of childhood. J Pathol 153:275–280
Ewing J (1921) Diffuse endothelioma of bone. Proc NY Pathol Soc 21:17–24
Gabius H-J, Engelhardt R, Sartoris DJ, Cramer F (1986) Pattern of endogenous lectins of a human sarcoma (Ewing's sarcoma) reveals differences to human normal tissues and tumors of epithelial and germ cell origin. Cancer Lett 31:139–145
Glaubiger DL, Makuch R, Schwarz J, Levine AS, Johnson RE (1980) Determination of prognostic factors and their influence on therapeutic results in patients with Ewing's sarcoma. Cancer 45:2213–2219
Haas OA, Chott A, Ladenstein R, Gadner H (1987) Poorly differentiated, neuron-specific enolase positive round cell tumor with two translocations t(11;22) and t(21;22). Cancer 60:2219–2223
Jacobson SA (1977) Polyhistioma. A malignant tumour of bone and extraskeletal tissues. Cancer 40:2116–2130
Jaffe R, Santamaria M, Yunis EJ, Tannery NH, Agostino RM, Medina J, Goodman M (1984) The neuroectodermal tumor of bone. Am J Surg Pathol 8:885–898
Kahn HJ, Yeger H, Baumal R, Thom H, Phillips JM (1983) Categorization of pediatric neoplasms by immunostaining with antiprekeratin and antivimentin antisera. Cancer 51:645–653
Kawaguchi K, Koike M (1986) Neuron-specific enolase and Leu-7 immunoreactive small round-cell neoplasm. The relationship to Ewing's sarcoma in bone and soft tissue. Am J Clin Pathol 86:79–83
Kissane JM, Askin FB, Nesbit ME Jr, Vietti TJ, Burgert EO Jr, Cangir A, Gehan EA, Perez CA, Pritchard DJ, Tefft M (1981) Sarcomas of bone in childhood: Pathologic aspects. Natl Cancer Inst Monogr 56:29–41
Kissane JM, Askin FB, Foulkes M, Stratton LB, Shirley SF (1983) Ewing's sarcoma of bone: Clinicopathologic aspects of 303 cases from the Intergroup Ewing's Sarcoma Study. Hum Pathol 14:773–779
Kurtin PJ, Pinkus GS (1985) Leukocyte common antigen – a diagnostic discriminant between hematopoietic and nonhematopoietic neoplasms in paraffin sections using monoclonal antibodies: Correlation with immunologic studies and ultrastructural localization. Hum Pathol 16:353–365
Lipinski M, Braham K, Philip I, Wiels J, Philip T, Goridis C, Lenoir GM, Tursz T (1987) Neuroectoderm-associated antigens on Ewing's sarcoma cell lines. Cancer Res 47:183–187
Llombart-Bosch A, Blache R, Peydro-Olaya A (1978) Ultrastructural study of 28 cases of Ewing's sarcoma: Typical and atypical forms. Cancer 41:1362–1373
Llombart-Bosch A, Contesso G, Henry-Amar M, Lacombe MJ, Oberlin O, Dubousset J, Rouëssé J, Sarrazin D (1986) Histopathological predictive factors in Ewing's sarcoma of bone and clinicopathological correlations. A retrospective study of 261 cases. Virchows Arch [A] 409:627–640
Llombart-Bosch A, Lacombe MJ, Peydro-Olaya A, Perez-Bacete M, Contesso G (1988) Malignant peripheral neuroectodermal tumours of bone other than Askin's neoplasm: characterization of 14 new cases with immunohistochemistry and electron microscopy. Virchows Arch [A] 412:421–430
Löning Th, Liebsch J, Delling G (1985) Osteosarcomas and Ewing's sarcomas. Comparative immunocytochemical investigation of filamentous proteins and cell membrane determinants. Virchows Arch [A] 407:323–336
Mahoney JP, Alexander RW (1978) Ewing's sarcoma. A light- and electronmicroscopic study of 21 cases. Am J Surg Pathol 2:283–298
Maletz N, McMorrow LE, Greco MA, Wolman SR (1986) Ewing's sarcoma. Pathology, tissue culture, and cytogenetics. Cancer 58:252–257
McDonnell JM, Beschorner WE, Kuhajda FP, deMent SH (1987) Common leukocyte antigen staining of a primitive sarcoma. Cancer 59:1438–1441
Meister P (1984) Immunhistochemische Methoden bei der Diagnostik von Weichgewebstumoren. Pathologe 5:90–98
Mendenhall CM, Marcus RB Jr, Enneking WF, Springfield DS, Thar TL, Million RR (1983) The prognostic significance of soft tissue extension in Ewing's sarcoma. Cancer 51:913–917
Michels S, Swanson PE, Frizzera G, Wick MR (1987) Immunostaining for leukocyte common antigen using an amplified avidin-biotin-peroxidase complex method and paraffin sections. Arch Pathol Lab Med 111:1035–1039
Miettinen M, Lehto VP, Virtanen I (1982) Histogenesis of Ewing's sarcoma. An evaluation of intermediate filaments and endothelial cell markers. Virchows Arch [B] 41:277–284
Miettinen M, Lehto VP, Virtanen I (1984) Antibodies to intermediate filament proteins in the diagnosis and classification of human tumors. Ultrastruct Pathol 7:83–107
Miettinen M, Lehto VP, Virtanen I (1985) Antibodies to inter-

mediate filaments proteins. The differential diagnosis of cutaneous tumors. Arch Dermatol 121:736–741
Miettinen M, Herva R (1987) Cytokeratin in skeletal and extraskeletal Ewing's sarcoma. Pathol Res Pract 182:526
Moll R, Lee I, Gould VE, Berndt R, Roessner A, Franke WW (1987) Immunocytochemical analysis of Ewing's tumors. Patterns of expression of intermediate filaments and desmosomal proteins indicate cell type heterogeneity and pluripotential differentiation. Am J Pathol 127:288–304
Myhre-Jensen O, Kaae S, Madsen EH, Sneppen O (1983) Histopathological grading in soft-tissue tumours. Relation to survival in 261 surgically treated patients. Acta Pathol Microbiol Immunol Scand (A) 91:145–150
Nascimento AG, Unni KK, Pritchard DJ, Cooper KL, Dahlin DC (1980) A clinicopathologic study of 20 cases of large-cell (atypical) Ewing's sarcoma of bone. Am J Surg Pathol 4:29–36
Navas-Palacios JJ, Aparicio-Duque R, Valdés MD (1984) On the histogenesis of Ewing's sarcoma. An ultrastructural, immunohistochemical, and cytochemical study. Cancer 53:1882–1901
Nesbit ME Jr, Perez CA, Tefft M, Burgert EO Jr, Vietti TJ, Kissane J, Pritchard DJ, Gehan EA (1981) Multimodal therapy for the management of primary, nonmetastatic Ewing's sarcoma of bone: An intergroup study. Natl Cancer Inst Monogr 56:255–262
Osborne M, Altmannsberger M, Shaw G, Schauer A, Weber K (1982) Various sympathetic derived human tumours differ in neurofilament expression. Virchows Arch [B]40:141–156
Osborn M, Weber K (1983) Biology of disease. Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. Lab Invest 48:372–394
Perentes E, Rubinstein LJ (1987) Recent applications of immunoperoxidase histochemistry in human neuro-oncology. An update. Arch Pathol Lab Med 111:796–812
Perosio PM, Brooks JJ (1988) Expression of nerve growth factor receptor in paraffin-embedded soft tissue tumours. Am J Pathol 132:152–160
Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG (1977) Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1–39
Pomeroy TC, Johnson RE (1975) Combined modality therapy of Ewing's sarcoma. Cancer 35:36–47
Roessner A (1984) Zur Zyto- und Histogenese der malignen und semimalignen Knochentumoren. Cytogenesis und histogenesis of malignant and semimalignant bone tumors. Veröffentlichungen aus der Pathologie. In: Progress in Pathology, vol. 122, Gustav Fischer Verlag, Stuttgart New York, pp 83–101
Roholl PJM, De Jong ASH, Ramaekers FCS (1985) Application of markers in the diagnosis of soft tissue tumours. Histopathology 9:1019–1035
Sauer R, Jürgens H, Burgers JMV, Dunst J, Hamlicek R, Michaelis J (1987) Prognostic factors in the treatment of Ewing's sarcoma. Radiother Oncol 10:101–110
Schulz A, Jundt G, Alles JU, Altmannsberger M (1984) Ewing-Sarkom des Knochens. Stand der morphologischen Diagnostik. Pathologe 5:63–72
de Stéfani E, Carzoglio J, Pellegrini HD, Olivera L, Cendan M, Kasdorf H (1984) Ewing's sarcoma: Value of tumor necrosis as a predictive factor. Bull Cancer (Paris) 71:16–21
Triche TJ, Tsokos M, Linnoila RI, Marangos PJ, Chandral R (1985) NSE in neuroblastoma and other round cell tumors of childhood. Prog Clin Biol Res 175:295–317
Triche TJ (1986) Neuroblastoma – biology confronts nosology. Arch Pathol Lab Med 110:994–996
Triche TJ, Askin FB, Kissane JM (1986) Neuroblastoma, Ewing's sarcoma, and the differential diagnosis of small-, round-, blue-cell tumors. In: Finegold MJ, Bennington JL (eds) Pathology of neoplasia in children and adolescents. Major problems in pathology, vol. 18. W.B. Saunders Company, Philadelphia, pp 145–195
Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, de Mascarel A, Goussot JF, David M, Bonichon F, Lagarde C (1984) Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int J Cancer 33:37–42
Tsokos M, Linnoila RI, Chandra RS, Triche TJ (1984) Neuron-specific enolase in the diagnosis of neuroblastoma and other small, round-cell tumors in children. Hum Pathol 15:575–584
Variend S (1985) Small cell tumours in childhood: A review. J Pathol 145:1–25
Van Eyken P, de Wolf-Peeters C, Van den Oord J, Tricot G, Dessmet V (1987) Expression of leukocyte common antigen in lymphoblastic lymphoma and small noncleaved undifferentiated non-Burkitt's lymphoma: An immunohistochemical study. J Pathol 151:257–261
Vinores SA, Bonnin JM, Rubinstein LJ, Marangos PJ (1984) Immunohistochemical demonstration of neuron-specific enolase in neoplasms of the CNS and other tissues. Arch Pathol Lab Med 108:536–540
Wilkins RM, Pritchard DJ, Burgert EO Jr, Unni KK (1986) Ewing's sarcoma of bone. Experience with 140 patients. Cancer 58:2551–2555

Received May 13, 1988 / Accepted November 18, 1988