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Is Neuro-ectodermal Differentiation of Ewing's Sarcoma of Bone Associated with an Unfavourable Prognosis?

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Among Ewing's sarcoma (ES) of bone and related entities are tumours with neuro-ectodermal features that could represent a biologically distinct type. In order to assess the prognostic significance of the various forms of ES, a retrospective joint study involving three cancer centres in Europe and the U.S.A. was initiated. The material from 315 primary ES was reviewed by a panel of five pathologists and classified as typical ES (220 cases), atypical ES (48 cases) or ES with neuro-ectodermal features (47 cases). Prognostic factor analysis on treatment failure-free survival was performed using the Cox model. It included histopathological classification, initial patient characteristics, clinical presentation and treatment type. After multivariate analysis, in addition to treatment type ($P < 0.001$), metastases ($P = 0.003$) and proximal tumour location ($P = 0.006$), two histopathological parameters correlated with poor treatment failure-free survival, the presence of filigree pattern ($P = 0.044$) and dark cells ($P = 0.043$). We conclude that ES with neuro-ectodermal features does not appear to have a different outcome to the other subtypes.

Key words: Ewing's sarcoma, bone, neuro-ectodermal differentiation, prognosis
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INTRODUCTION

THE SO-CALLED small round cell tumours of bone are composed of a heterogeneous group of neoplasms. Among them, the use of specialised diagnostic methods [1–12] has helped to distinguish separate entities, such as Ewing's sarcoma (ES), small cell osteosarcoma, primitive sarcoma of bone, primary non-Hodgkin's lymphoma of bone, metastatic rhabdomyosarcoma, and synoviosarcoma, involving bone by direct extension.

The histological diversity of ES has been discussed at length in several reports, and documented by electron microscopic and immunocytochemical techniques [1]. Currently, several atypical forms have been described, including large cell ES, ES with neuro-ectodermal features (or PNET of bone), and ES with endothelial features [2–8]. Despite this morphological diversity, there are no generally accepted histopathological prognostic criteria applicable to all cases of ES [13–17].

It has been proposed that ES with neuro-ectodermal features (PNET) represents a biologically identical, but prognostically distinct entity [8–11]. The negative prognostic impact of neuro-ectodermal differentiation in ES has been reported in several publications [18–24], although others have disputed this [25–27]. The reasons for these discrepancies are multiple, because of factors such as: insufficient numbers of cases reported to achieve statistical significance, particularly on multivariate analysis; anatomical location (distal extremity versus central axial tumours) [23]; and limited scope of a study, such as the Askin tumour of chest wall [27].

Impressive advances in combination chemotherapy of ES with improved survival have occurred since the original description of this tumour with virtually no long-term survivors [28]. However, poor response in at least 40% of patients demands that clinically relevant histopathological prognostic factors be identified that could be utilised at diagnosis, prior to treatment allocation, because it is important to determine which patients might benefit from more aggressive therapy. Given the disparate results reported in several studies to date, it was clear that only a large-scale study of all evaluable cases treated uniformly since the advent of multi-agent chemotherapy, would be likely to identify such prognostic factors. To this end, a study was undertaken to analyse all evaluable cases from the National Cancer Institute in the U.S.A. and the Institut Gustave Roussy and the University of Valencia in Europe.

PATIENTS AND METHODS

Patients

The series was composed of 457 patients treated at the three institutions from 1958 to 1984. Of these 457 cases (252 from the Institut Gustave Roussy, Villejuif, France; 179 from the National Cancer Institute, Bethesda, Maryland, U.S.A.; and 26 from the Medical School, University of Valencia, Spain), pathological material was available in 387 (85%) cases. All specimens were reviewed by at least three of the five pathologists in two successive sessions. The final diagnosis resulted from a consensus and cases were excluded if no consensus was reached. All pathologists were blind to any information regarding previous classification, initial clinical presentation, and patient's outcome. After review, 38 (8%) cases were identified as being inconsistent with ES of bone (18 small round cell tumours other than ES, and 20 primary 'blastemic' mesenchymal sarcoma of bone). Among the remaining *true* ES cases, 34 (7%) had no available clinical data. Therefore, this study included 315 (69%) cases for whom all pertinent information was available, i.e. 157 cases treated at the Institut Gustave Roussy, 136 at the National Cancer Institute, and 22 at the University of Valencia.

The pathological material used for this review consisted of the primary untreated tumour obtained by surgical biopsy. Haematoxylin and eosin (HE) or haematoxylin safranin eosin (HSE) sections were available in all cases. Glycogen detection was carried out using periodic acid-Schiff (PAS) or a Best's carmin staining in 263 (83%) cases. Additional stains such as reticulin, silver or trichrome, were available in 270 (86%) cases. Immunocytochemical staining for neuron specific enolase, Leu7 (HNK-1), vimentin, desmin, myoglobin, F.VIII-related antigen, protein S100, leucocyte common antigen, and keratin were available in 46 (15%) cases only, and therefore not used in this study.

Pathology review

The classification used in this study was based upon previous experience [16]. Three variants of ES were defined: typical ES; atypical ES with either large cells or endothelial-like cells; and ES with neuro-ectodermal features.

The histology of typical ES has been published by several authors since first described by James Ewing in 1921 [29]. It usually presents with a homogeneous, diffuse pattern. Architectural configurations such as a cohesive, dissociated and chessboard type (or filigree pattern as shown in Figure 1a), depending on the extent of infiltration into the bone marrow, cortical bone or soft tissue, are observed alone or in association. The cell population usually consists of principal cells and dark cells. Principal cells are small round blue cells, presenting with round or elongated nuclei, finely dispersed chromatin, and one or two inconspicuous nucleoli. Their cytoplasm is poorly delineated owing to the presence of glycogen. Cell size averages 12–14 μm. Dark cells show a denser elongated nuclear contour, unidentifiable nucleoli and a tendency to aggregate. Some glycogen may be present within a barely visible cytoplasm. Necrosis is present to various extents. There is no reticular network supporting the neoplastic cells.

The categorisation of atypical ES is based mainly upon cytological criteria and some complementary architectural features. Cell size irregularities in the principal cell type may lead to a histiocytoid appearance with larger nuclei and irregularly condensed chromatin. Nucleoli are more prominent and the cytoplasm acidophilic, with better delineated contours. Cell size varies from 19 to 24 μm. Glycogen is present but less abundant than in typical ES cells. Included within this category is the endothelial-like cell, either isolated or delineating vascular-like spaces. Association of small round blue cells with large cells and endothelial-like cells can occasionally be seen within the same tumour. The reticular network acquires progressive density without reticular fibres around isolated cells. Necrosis is seen to a variable extent and a filigree pattern is present within the soft tissue invasion.

The diagnosis of ES with neuro-ectodermal features (ES/PNET) is made only when typical Homer-Wright rosettes or pseudo-rosettes are detected (Figure 1b,c,d). A lobular pattern is observed in the majority of these tumours (basket-like arrangement of the reticular fibres) and a fine fibrillary background occasionally isolates cells. Neovascularisation and other morpho-

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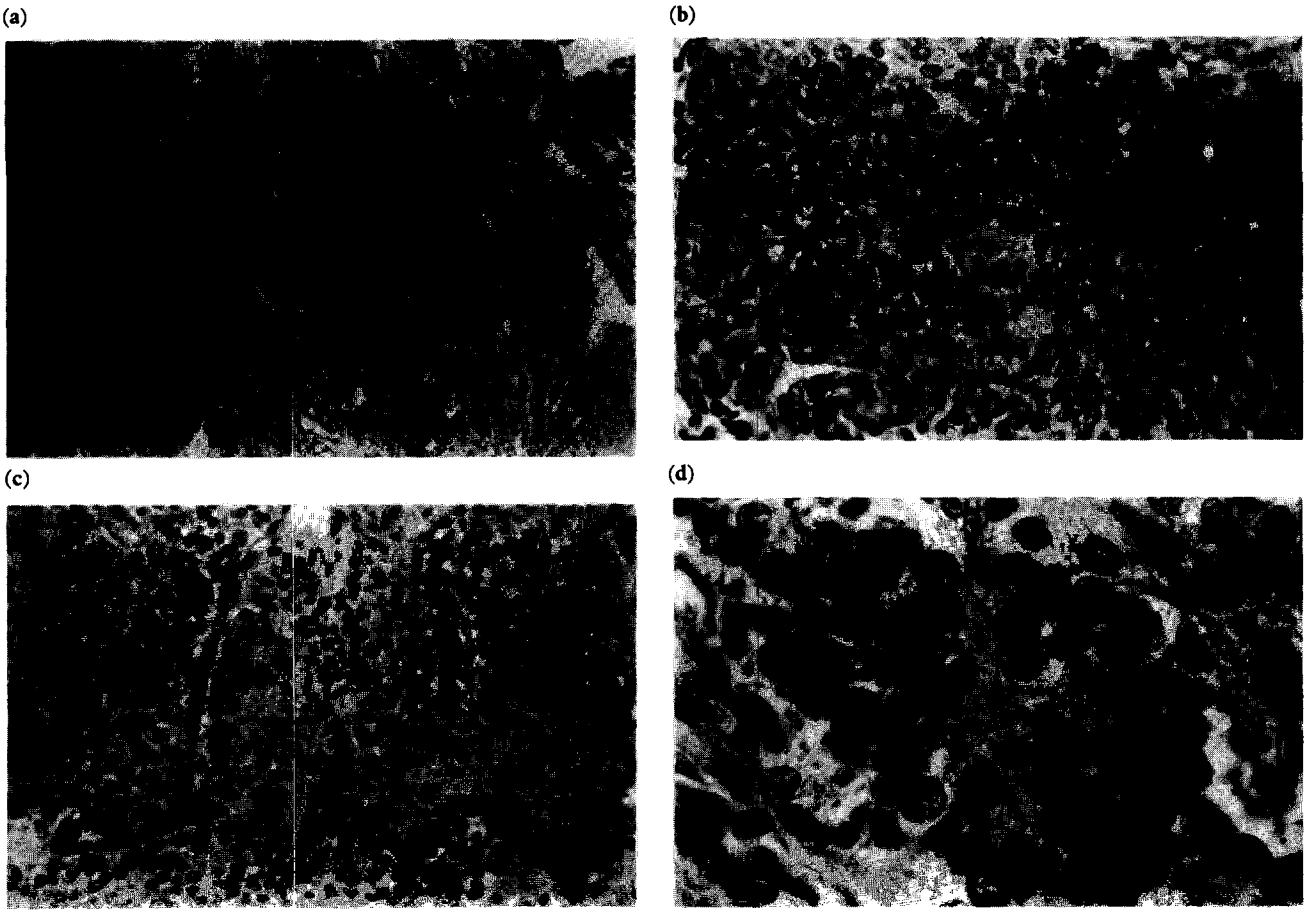


Figure 1. Illustration of the various appearances of Ewing's sarcoma of bone. (a) Typical Ewing's sarcoma with extension into soft tissue and filigree pattern (HES staining, × 100); (b) Ewing's sarcoma with neuro-ectodermal features, lobulated pattern and pseudo-rosettes (HES staining, × 400); (c) Ewing's sarcoma with neuro-ectodermal features, lobulated pattern and typical Homer-Wright rosettes (HES staining, × 400); (d) high-power view of a Homer-Wright rosette with fibrillary background (HES staining, × 1000).

logical parameters are somewhat similar to those seen in typical ES.

Clinical data

Clinical evaluation was based on routine clinical examination, chest CT and primary tumour CT (except for European patients treated before 1978 for whom chest X-ray and anteroposterior and lateral radiographs of the primary tumour were used), radionuclear bone scans, and bone marrow examination. Initial patient characteristics, clinical presentation, treatment administered, and follow-up were collected using a common summarised data sheet. It included the tumour site, the presence and site of metastases at onset, initial therapy (radical surgery, radiation therapy, and chemotherapy regimen), the date of initiation of therapy, the date of first treatment failure and the site of first relapse, the date of the last vital status and cause of death. Initial treatment consisted of radical surgery alone in 4 (1%) patients, surgery or radiation therapy, and single agent chemotherapy in 69 (22%) patients, intensive systemic multidrug chemotherapy not including doxorubicin in association with radiation therapy or, in few cases, surgery in 36 (12%) patients, and combination chemotherapy including doxorubicin in association with local irradiation at a dose $\geq$50 Gy in 206 (65%) patients [30–37]. The latter association, i.e. use of combination chemotherapy including doxorubicin and local irradiation $\geq$50 Gy, is referred to as "modern modality treatment" in the subsequent analysis.

Statistical analysis

Relationships between clinical and pathological features were estimated using the Fisher exact test and the analysis of variance, as appropriate. Time at risk for treatment failure was calculated from the date of the start of the initial therapy to the date of the first treatment failure, date of death if the patient died from disease progression or a treatment-related death, date of the last examination, or 1 April 1990, whichever came first. Treatment failure-free survival curves were calculated using the Kaplan and Meier method. The impact of initial patient characteristics, clinical and pathological features, and treatment modality (listed in Tables 1 and 2) on treatment failure-free survival was analysed using the log rank test. Variables which correlated with treatment failure-free survival were then included in a proportional hazards regression model to assess their independent prognostic value [38, 39].

Data stored at the three institutions were transfered to the Institut Gustave Roussy, Department of Biostatistics and Epidemiology, to be analysed by a common statistical design. A general database management system developed at the Institut Gustave Roussy was used [40] as well as the STATA® statistical software [41].

RESULTS

Initial patient characteristics are given in Table 1, both for the overall cohort and by histological type. There were 192 males

Table 1. *Initial clinical characteristics in* 315 *patients included in the study*

	Typical ES	Atypical ES	ES with neuro-ectodermal differences	All cases
Numbers at risk	220 (70%)	48 (15%)	47 (15%)	315
Centre				
IGR	48%	50%	58%	157 (50%)
NCI	46%	38%	38%	136 (43%)
Valencia	6%	12%	4%	22 (7%)
Male/female ratio	1:1.7	1:1.2	1:1.4	1:1.6
Mean age				
In years (SE)	13.7 (6.9)	13.7 (6.4)	15.5 (9.6)	14.0 (7.3)
Range				1–56
Site				
Limbs	50%	46%	53%	157 (50%)
Pelvic girdle	26%	29%	21%	82 (26%)
Rib, vertebra	16%	19%	17%	53 (17%)
Others	8%	6%	9%	23 (7%)
Distal	30%	12%	32%	86 (27%)
Central or proximal	70%	88%	68%	229 (73%)
Metastases present	21%	27%	19%	69 (22%)
Treatment categories				
Modern, standard-like	65%	63%	72%	206 (65%)
Others	35%	37%	28%	109 (35%)

IGR, Institut Gustave Roussy, Villejuif, France; NCI, National Cancer Institute, Bethesda, Maryland, U.S.A.; Valencia, Medical Schools, University of Valencia, Spain.
Central sites include skull, face, mandible, vertebra, rib, sternum, clavicle and pelvic girdle. Proximal sites include humerus, scapula and femur. Distal sites include forearm, hand, leg and foot.
Modern treatment, standard-like: use of combination chemotherapy including doxorubicin and local irradiation to a dose $\geq$ 50 Gy.

and 123 females. Overall, 73% of tumours were central (skull or face, $n = 5$; mandible, $n = 2$; vertebra, $n = 20$; rib or sternum or clavicle, $n = 40$; and pelvic girdle, $n = 82$) or proximal (humerus or scapula, $n = 38$; and femur, $n = 42$) and only 27% were distal (forearm, $n = 9$; hand, $n = 1$; leg, $n = 68$; and foot, $n = 8$). Metastases at onset were confined to bone (6%), lung (7%) or other locations (2%), as well as in multiple forms (7%). Metastases correlated with tumour location, with 25% present when proximal versus 14% when distal ($P = 0.035$). Initial treatment was completed in the 1958–1969 period for 57 (18%) patients; in the 1970–1979 period for 180 (57%) patients; and in the 1980–1984 period for 78 (25%) patients.

After review, 220 (70%) cases were classified as typical ES, 48 (15%) cases as atypical ES, and 47 (15%) cases as ES/PNET (Table 1). None of the initial characteristics or treatment categories differed within the three subtypes except the tumour location, atypical ES presenting with significantly ($P = 0.033$) fewer distal sites than other subtypes. ES/PNET patients were diagnosed when 2 years older on average, than those presenting with tumour of other histological subtypes, but the difference was not statistically significant.

Pathology analysis (*Table* 2)

The typical ES entity showed a predominant cohesive architecture (82%) with the presence, in a low percentage (16%), of a lobular pattern, and the presence of filigree structures in 36% of cases, which denote extension into surrounding soft tissues. Atypical ES and ES/PNET presented with a significantly less cohesive pattern ($P = 0.068$), but a more lobular pattern ($P < 0.001$). The proportion of cases displaying filigree structures was similar. Although typical ES was never associated with the presence of pseudo-rosettes, this pattern was nearly always present (94%) in ES/PNET. Among the latter, all cases had a cohesive architecture and a fibrillar background; 10 cases showed typical Homer-Wright rosettes with a central fibrillar core.

Analysis of cytological features showed typical ES to be almost entirely composed of small round blue cells (97%) and dark cells (91%). In contrast, small round blue cells were significantly less abundant in atypical ES while, in ES/PNET, their proportion was similar to that of typical ES (89%). Dark cells were less represented in both entities (75 and 76%, respectively, $P = 0.0012$). Atypical large cells or endothelial-like cells were poorly represented (5%) in typical ES while they were nearly always present (98%) in atypical ES. In ES/PNET, their proportion was intermediate (36%) ($P < 0.001$).

Glycogen was detected in, at least, 80% of the cases. Necrosis was present in 41–56% of the cases, with no statistical significant difference between the three entities. However, dark cells and necrosis were linked ($P = 0.089$); necrosis was present in 55% of cases presenting with dark cells while it was present in 40% of cases presenting without dark cells.

Prognostic factors analysis on treatment failure-free survival

Overall, treatment failure was observed in 74% of the patients with a 5-year treatment failure-free survival rate of 29% and a 10-year rate of 25%. Most patients who failed presented initially with metastases or did not receive modern modality treatment, i.e. without the use of combination chemotherapy including doxorubicin and local irradiation $\geq$50 Gy. Patients presenting without metastases and treated with modern modality treatment ($n = 158$) had better treatment failure-free survival, with a 10-year rate of 36% compared with 14% in the other groups of

Table 2. Pathological features by ES subtype

	Typical ES	Atypical ES	ES with neuro-ectodermal differences	*P* value
Numbers at risk	220 (70%)	48 (15%)	47 (15%)	
Architecture				
Cohesive pattern	82%	73%	68%	0.068
Lobular	16%	44%	57%	<0.001
Filigree pattern	36%	38%	28%	>0.20
Rosettes pattern or pseudo-rosettes pattern	1%	0%	94%	<0.001
Cytology				
Small round blue cells	97%	46%	89%	<0.001
Dark cells	91%	75%	76%	0.0012
Atypical large cells or endothelial-like cells	5%	98%	36%	<0.001
Glycogen present	80% (184)*	85% (39)	83% (40)	>0.20
Necrosis present	56%	41%	51%	0.12

*Numbers in parentheses correspond to the cases with available information.

patients (n = 157). Among the former, no statistically significant difference was observed between either modern modality treatment protocols or institution.

Univariate analysis. In a first step, clinical and pathological characteristics were analysed individually. Clinical characteristics studied were sex; age at diagnosis; tumour site including specific bone of origin and a more generalised grouping into distal (forearm, hand, leg, foot) or proximal location; presence or absence of metastases at diagnosis; and treatment modalities. Factors which significantly correlated with poor outcome were treatment without combination chemotherapy including doxorubicin and local irradiation to dose ≥50 Gy ($P < 0.001$), presence of metastases ($P < 0.001$) at diagnosis, and proximal tumour location ($P < 0.001$).

Pathological characteristics studied were: cohesive, lobular, filigree, and rosette or pseudo-rosette patterns; presence or absence of small round blue cells, dark cells, and atypical or endothelial-like cells; presence or absence of glycogen and necrosis. The three histological subtypes were also compared. Of these 10 factors, only 2 correlated with poor outcome, i.e. presence of filigree pattern ($P = 0.008$) and presence of dark cells ($P = 0.04$).

Multivariate analysis. All factors which significantly correlated with poor treatment failure-free survival were included in a proportional hazards regression model. The results are given in Table 3. Treatment, metastases, and tumour location were factors most significantly associated with poor survival. However, dark cells and filigree pattern also had a significant prognostic value on treatment failure-free survival.

A second multivariate analysis was performed on the subgroup of non-metastatic patients who were given modern modality treatment (n = 158). In these patients, the model used included tumour location and filigree pattern, which were the only factors which significantly correlated with poor treatment-free survival in univariate analysis. In the regression model, filigree pattern remained the most significant factor (Table 4). The use of these two parameters, filigree pattern and tumour location, allowed the definition of four subsets: proximal tumour location and presence of filigree pattern (G1, n = 44), proximal tumour location and absence of filigree pattern (G2, n = 62), distal tumour location and presence of filigree pattern (G3, n = 10), and distal tumour location and absence of filigree pattern (G4, n = 42). Failure-free survival curves of these four subsets are given in Figure 2 which highlights the worse outcome of group G1 compared with the other groups. The 5-year failure-free survival rates were 22, 43, 50 and 64% in the four groups, respectively. Relative to group G4, the relative risk (RR) of treatment failure of group G1 was 2.60 ($P < 0.001$) while those of groups G2 (RR = 1.36) and G3 (RR = 1.16) were not statistically increased.

Proximal location of the tumour being the most adverse factor in non-metastatic patients treated with modern modality treatment, it was anticipated that this factor would overshadow the influence of other factors, including histological subtype. Therefore, a separate analysis was performed on the subgroup of patients with distal tumour location (n = 52). This subgroup included 40 patients with typical ES, 5 patients with atypical ES and 7 patients with ES/PNET. Multiple regression analysis was unable to show any difference between typical ES and the other two histological subtypes combined, nor was it when filigree pattern and dark cells were included in the model.

DISCUSSION

The aim of this study was to assess the prognostic significance of specific histopathological and clinical features of ES of bone. Since cases were included from three different institutions, an extensive pathological review of all cases was made to ensure that all fulfilled the definition of ES of bone. In addition to confirmation of diagnosis, cases of primitive sarcomas of bone, small cell osteosarcomas, synoviosarcomas, lymphomas, rhabdomyosarcomas, and other non-ES cases encountered upon review were excluded from study, as well as those presumed ES with insufficient material for review. On this basis, 31% (142/457) of cases were excluded, leaving 315 evaluable cases. Three major groups were identified: typical ES (70%), atypical ES (15%) and ES/PNET (15%).

This study focused on two questions: (i) do atypical forms, particularly those displaying neuro-ectodermal differentiation, have poorer survival? and (ii) are some microscopic features, such as filigree [15] and necrosis [16], related to a more aggressive

Table 3. Relative risks (RR) of treatment failure. Cox regression analysis for a model allowing all variables simultaneously (overall series, n = 315)

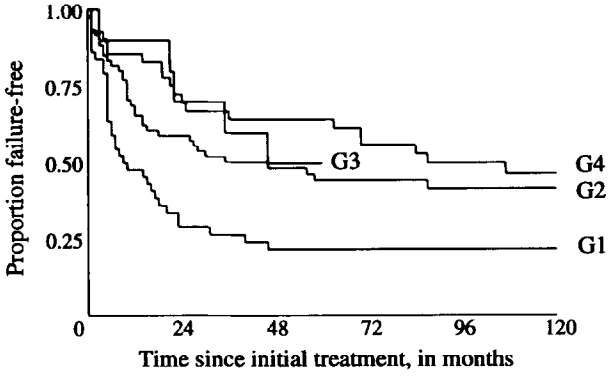
	Coeff/SE*	RR†	95% CL‡	*P* value
Location (central or proximal/distal)	0.439/0.158	1.55	1.14–2.12	0.006
Metastases (present/absent)	0.463/0.157	1.59	1.17–2.16	0.003
Modern CMT (no/yes)	0.622/0.137	1.86	1.42–2.44	<0.001
Pathology				
Filigree pattern (present/absent)	0.280/0.138	1.32	1.01–1.74	0.044
Dark cells (present/absent)	0.435/0.214	1.54	1.01–2.35	0.043

*Coefficient of risk and standard error; †RR, exp(coefficient of risk); ‡95% confidence limits of RR. Modern CMT: use of combination chemotherapy including doxorubicin and local irradiation to a dose ≥ 50 Gy.

Table 4. Relative risks (RR) of treatment failure in the subgroup of non-metastatic patients treated with modern modality treatment (n = 158). Cox regression analysis for a model allowing all variables simultaneously

	Coeff/SE*	RR†	95% CL‡	*P* value
Location (central or proximal/distal)	0.460/0.231	1.58	1.00–2.50	0.048
Pathology				
Filigree pattern (present/absent)	0.534/0.210	1.71	1.13–2.58	0.012

*Coefficient of risk and standard error; †RR = exp(coefficient of risk); ‡95% confidence limits of RR. Modern modality treatment: use of combination chemotherapy including doxorubicin and local irradiation to a dose ≥ 50 Gy.



Patients at risk			
G1	44	5	4
G2	62	24	12
G3	10	5	-
G4	42	24	17

Figure 2. Treatment failure-free survival according to tumour location (proximal or distal) and filigree pattern (absent or present) in non-metastatic patients treated with modern modality treatment (i.e. use of combination chemotherapy including doxorubicin and local irradiation to a dose ≥ 50 Gy). G1 comprises patients whose tumour was proximal with filigree pattern (*n* = 44); G2, patients with proximal tumour location without filigree pattern (*n* = 62); G3, patients with distal tumour location with filigree pattern (*n* = 10); and G4, those with distal tumour location without filigree pattern (*n* = 42).

course? In addition to these parameters, factors already known to correlate with poor survival, such as the presence of metastases at diagnosis and proximal location of the tumour [17], were included in the statistical model. Initial treatment type (modern modality treatment versus other less aggressive treatments) was also evaluated.

With multivariate analysis, in addition to treatment, two initial clinical features significantly correlated with poor survival: the presence of metastases and a proximal location of the tumour, confirming previous reports (Table 3; refs [16, 17]). The analysis failed to demonstrate any prognostic value associated with any histological subtype (typical ES, atypical ES, or ES/PNET), even when the analysis was restricted to non-metastatic patients with distal location of their tumour and treated with modern modality therapy. However, two microscopic features did have adverse prognostic importance: both the filigree pattern and dark, or effete, cell content (indicative of impending cell necrosis) were predictive, independently of other prognostic factors. In addition, filigree pattern remained the only histological factor which correlated with prognosis in non-metastatic patients treated with modern therapy (Table 4). The above findings are similar, in part, to previous reports on non-metastatic patients, where necrosis and filigree pattern were associated with poor prognosis [17]. In our study, necrosis was not predictive, as reported by others [21, 42]. However, dark cells have been interpreted by many others as effete, or nascent necrotic cells, and are therefore likely to represent the antecedent lesion to the geographical necrosis typical of this tumour [4]. This widespread necrosis was used as the basis for "necrosis" in

previous studies. Here, the limited biopsy material in many cases may have precluded effective analysis of large-scale necrosis as a prognostic factor.

Our observations confirm the existence of histological heterogeneity within the family of tumours commonly known as Ewing's sarcoma. This morphological heterogenity supports the concept of a biological spectrum from typical ES to PNET, with intermediate 'atypical' forms, all of which share many non-morphological features in common. This also supports the concept of a common histogenesis for all members of this group, as recent cytogenetical, immunological, and molecular genetic findings clearly indicate [2–5, 18, 26, 43, 44]. Knowing this, the present study was constructed to determine whether morphological variability within a family of tumours correlates with different prognoses.

The results reported here clearly fail to substantiate a worse prognosis for patients whose tumours have neuro-ectodermal or atypical features; these two categories fared the same as typical ES. This is probably due to the fact that virtually all patients in this study received effective, multi-agent chemotherapy. Not all patients were treated on the same protocols, and the time period covered in this study approaches 20 years; these factors may have introduced additional variability in the results. Nonetheless, all forms of ES were distributed evenly over the time frame of the study, and the two histological features (i.e., filigree pattern and dark cells) were distributed uniformly among all three subtypes (Table 2). Thus, the survival differences ascribed to these two features cannot be explained by association with another prognostic clinico-anatomical variable, such as multi-agent chemotherapy, metastases at presentation, or central axial location.

The issue of neuro-ectodermal differentiation in these tumours bears additional discussion, as it alone has been described as an independent prognostic variable in several publications, a finding not substantiated here. Neuro-ectodermal expression seen in this group of tumours, manifest as Homer-Wright rosettes or pseudo-rosettes, fibrillary background, or a lobular pattern, did not correlate, in our series, with any difference in treatment failure-free survival compared with typical ES. At first glance, this finding appears to be discordant with other published studies, such as the Kiel Tumour Registry [24]. In the Kiel series of patients, disease-free survival at 7.5 years follow-up in ES patients was 60%, compared with 45% in patients with PNET. The criteria for a diagnosis of PNET in the Kiel series was similar to those in this study, plus two immunocytochemical markers for neural phenotype were required to be positive for a diagnosis of PNET.

A possible explanation for the disparate results reported by the Kiel group compared with ours is apparent when the anatomical distribution of ES and PNET is considered in the Kiel study. In these 117 patients, of whom 81 had ES and 36 had PNET, 75% of the PNETs were central axial in location, while only 57% of ES were in this poor prognostic group. Because central axial location is widely documented to be a poor prognostic feature, the PNET character of the Kiel patients is unlikely to be as predictive as the central axial location, since the difference in survival was only 15%, a difference easily attributable to anatomical location as opposed to histological appearance. In contrast, the striking difference in survival reported previously by Hartman and colleagues [23] was limited to patients with distal extremity lesions. This prognostic implication of neuro-ectodermal differentiation was also reported previously by some of the present authors in a limited study [18]. However, Ladanyi and associates [26] found no evidence of poor survival among 11 neuro-differentiated tumours when compared with typical ES. Similarly, no influence of histological subtype was observed in our study restricted to the 52 non-metastatic patients with distal tumour location, treated with modern modality therapy. Clearly, there is no unanimity of opinion on the prognostic significance of neuro-ectodermal differentiation in ES in recent studies. It is hoped that our results will provoke both caution in such claims, and a large-scale prospective randomised study of neural variables in ES, assessed by morphological and non-morphological means.

In contrast to the equivocal impact of neuro-ectodermal differentiation in ES, the present study clearly supports a strong independent impact of the filigree pattern on prognosis in ES. There has also been significant controversy regarding this prognostic variable since Kissane and associates showed a worse prognosis in patients treated on the Intergroup Ewing's Sarcoma Study (IESS) [15], and both supportive [16] and non-supportive [21, 23] studies have been published. Our study supports the prognostic importance of the filigree pattern in ES; such patients had significantly worse survival than those who did not, in all morphological patterns examined.

A similar controversy exists regarding tumour necrosis. In earlier analyses, we and several authors [16, 21, 42] demonstrated shorter survival among ES patients with large amounts of necrosis (geographical necrosis) by histological examination. In this study, necrosis of this type was not prognostic. However, the single-cell counterpart of necrosis, manifest as a high content of dark or effete cells, was significant, predicting a poor outcome. This unexpected result bears further study in future, prospective analyses of Ewing's sarcoma patients.

In conclusion, this study clearly shows that histopathological subclassification of ES into typical, atypical, and neuro-ectodermal variants was of no prognostic significance in this patient population. Today, the most adverse prognostic factors in patients treated with multi-agent chemotherapy, including doxorubicin, include the presence of metastases at presentation, a central axial location and the presence of a filigree pattern. However, one should remember that the present report is a retrospective study concerning patients with and without metastases, treated at three cancer centres over a long period of time according to various protocols. Therefore, our findings have to be confirmed in a large prospective study conducted over a shorter period of time before ascribing clinical utility to these prognostic variables. Certainly, the availability of a host of newer diagnostic methods to be applied to such cases should further refine the importance of these and other, as yet unidentified, prognostic variables in this disease. We view this as particularly important, given the problem of late relapses, second malignancies, and variable survival seen in patients with these tumours.

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