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Cellular differentiation and prognosis in embryonal rhabdomyosarcoma

A report from the Cooperative Soft Tissue Sarcoma Study 1981 (CWS 81)*,**

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Summary. Sixty-four cases of embryonal rhabdomyosarcoma (eRMS) were investigated for cellular differentiation by light microscopy. Of these 64 cases 20 were studied by means of immunohistochemistry. Histologically, three subgroups could be distinguished: primitive (<10% rhabdomyoblasts), intermediate (10–50% rhabdomyoblasts) and well differentiated (>50% rhabdomyoblasts) eRMS. Vimentin-positive cells predominated in the primitive eRMS. Intermediate eRMS showed large proportions of desmin-positive cells but vimentin containing cells were also numerous. Myoglobin could only be demonstrated in well differentiated eRMS. Primitive and well differentiated eRMS mainly occurred in the head and neck area, whereas intermediate eRMS were predominantly located in the abdomen. Stage III and IV tumours predominated in cases of primitive eRMS, whereas lower stages were noted in cases of intermediate and well differentiated eRMS. Response to chemotherapy, evaluated after seven weeks of treatment, was achieved in 10/15 (66%) cases of primitive, in 16/19 (84%) cases of intermediate and 5/5 cases of well differentiated eRMS. It is concluded from the current study that the three subgroups of eRMS differ not only by cytological differentiation but also by site of predilection, stage at time of diagnosis and response to chemotherapy.

Key words: Embryonal rhabdomyosarcoma – Histology – Immunohistochemistry, Differentiation

* Dedicated to Professor Karl Lennert, Kiel, on the occasion of his 65th birthday

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I. Introduction

Prognosis in rhabdomyosarcoma depends upon various factors including the extent of disease, site of origin, histological appearance and treatment. The influence of histology on prognosis is well documented by the fact that alveolar rhabdomyosarcoma has a worse prognosis than the embryonal subtype (Maurer and Ragab 1984). By contrast, sarcoma botryoides, which is considered a variant of embryonal rhabdomyosarcoma, has a more favorable outcome (Gaiger et al. 1981). A different classification system with prognostic relevance was recently proposed by Palmer et al. (1981).

It is well known from light microscopic and ultrastructural studies that cellular differentiation in embryonal rhabdomyosarcoma may vary considerably. Recently, these differences in differentiation have also been documented by immunohistochemical studies. Thus, neoplastic cells in poorly differentiated embryonal rhabdomyosarcoma contain vimentin-type intermediate filaments predominantly, whereas better differentiated cells express desmin (Altmannsberger et al. 1983; Molenaar et al. 1985), and in some cases myoglobin (Tsokos et al. 1984). Despite obvious differences in differentiation no attempts have as yet been made to correlate results from light microscopic and immunohistochemical studies with prognosis.

The current study was performed on 64 patients with embryonal rhabdomyosarcoma, which are enrolled in the Cooperative Soft Tissue Sarcoma Study (CWS 81) of the (German) Society of Pediatric Oncology (GPO). All patients have therefore been treated uniformly.

It has been shown by light microscopy and immunohistochemistry that embryonal rhabdomyosarcoma can be subdivided into three subgroups. Our results suggest that these subgroups differ in site of predilection, stage at time of diagnosis and response to chemotherapy.

II. Materials and methods

During the period from August 1981 to April 1985 115 children with embryonal rhabdomyosarcoma or sarcoma botryoides were entered into the Cooperative Soft Tissue Sarcoma Study 1981 (CWS 81). Sixty-four of these cases, in which material was available for histological examination, form the basis of the current study. All cases were evaluated for age and sex of the patient, location of the tumour, extent of disease, treatment and survival. The last follow-up was performed in April 1985, and the median survival period is 23 months. Statistical evaluation was performed by Chi-Square test. Because of the relatively small number of cases no multivariate analysis of prognostic factors was performed.

Histological specimens from sixty-four cases of embryonal rhabdomyosarcoma were retrieved from the files of the Central Paediatric Tumor Registry, Kiel, which serves as pathology referral center for the CWS 81-Study. Evaluation of the histological specimens was done independently by three of the authors (D.H., D.S., O.R.) without information on clinical follow-up. In the vast majority of cases multiple paraffin blocks of formalin fixed tissue and sections stained with haematoxylin and eosin, Giemsa, periodic-acid-Schiff (PAS), Bielschowsky's reticulin stain and Goldner stain were available. The proportion of undifferentiated and differentiated (round or elongated rhabdomyoblasts with or without cross-striation) tumour cells was recorded. Based on the proportion of rhabdomyoblasts three subgroups of embryonal rhabdomyosarcoma were distinguished:

Table 1. TNM-staging system for rhabdomyosarcoma

Tumour	Nodes	Metastasis
T_0 no tumour	N_0 regional lymph nodes are not involved	M_0 no distant metastasis at onset
T_1 tumour confined to the organ of origin a) diameter less than 5 cm b) diameter more than 5 cm, less than 10 cm	N_1 regional lymph nodes are involved	M_1 distant metastasis at onset
T_2 tumour involving contiguous tissue or organs a) diameter less than 5 cm b) diameter more than 5 cm, less than 10 cm c) diameter more than 10 cm	N_x the minimum requirements to assess the regional lymph nodes cannot be met	M_x the minimum requirement cannot be met

primitive (P):
predominantly undifferentiated cells, less than 10% rhabdomyoblasts
intermediate (I): between 10 and 50% rhabdomyoblasts
well differentiated (W): more than 50% rhabdomyoblasts

Twenty cases in which paraffin blocks from formalin fixed tissue were available were studied by immunohistochemical methods. Vimentin and desmin intermediate filaments and myoglobin were demonstrated by means of the peroxidase-antiperoxidase (PAP) method of Sternberger et al. (1970). Briefly, paraffin sections were dewaxed in xylene, rehydrated in alcohol and incubated with the specific antibody, followed by incubation with the linking antibody (swine antirabbit Ig). Concentrations of the specific antibody were: vimentin (1:10), desmin (1:50) and myoglobin (1:40). The PAP-complex (Dakopatts, Hamburg, FRG) was applied in a concentration of 1:40 (vimentin) and 1:100 (desmin, myoglobin), respectively. Negative controls were performed using normal swine serum instead of specific antibody. Polyclonal antibodies were purchased from Euro Diagnostics, Apeldoorn, Holland (Anti-vimentin and anti-desmin) and Miles, Munich, FRG (antimyoglobin).

Two different staging systems were used to assess the extent of disease. The TNM-system refers to the preoperative assessment of the disease state (Table 1). The staging system of the CWS 81 is based on the primary surgical intervention and the result of the histological examination:

Stage I: Localized disease, complete excision of tumour
Stage II: Incomplete excision of tumour, microscopic residual tumour tissue
a) regional lymph nodes not involved
b) regional lymph nodes involved and resected
Stage III: Non-resectable tumour, incomplete excision or biopsy with gross residual disease
Stage IV: Distant metastatic disease at onset

The allocation of patients to a treatment protocol was determined by stage of disease based upon the CWS 81-staging system. Treatment is stratified according to the post-surgical state of the disease. The regimen of the treatment consists of a combined chemotherapy followed by second look surgery 16 weeks after initation of treatment. Chemotherapy using vincristin, actinomycin D, adriamycin and cyclophosphamide is identical for all patients with stage I–III. In stage IV cases cyclophosphamide is replaced by ifosfamide in the same therapy cycle. Chemotherapy duration for stage I/IIA is 35 weeks and for stage IIB/III/IV 56 weeks. Radiation therapy is stratified depending on grade of response after initial chemotherapy: stage I pc (post chemotherapy) no irradiation, stage II pc irradiation with 40 Gy, stage III irradiation with 50 Gy.

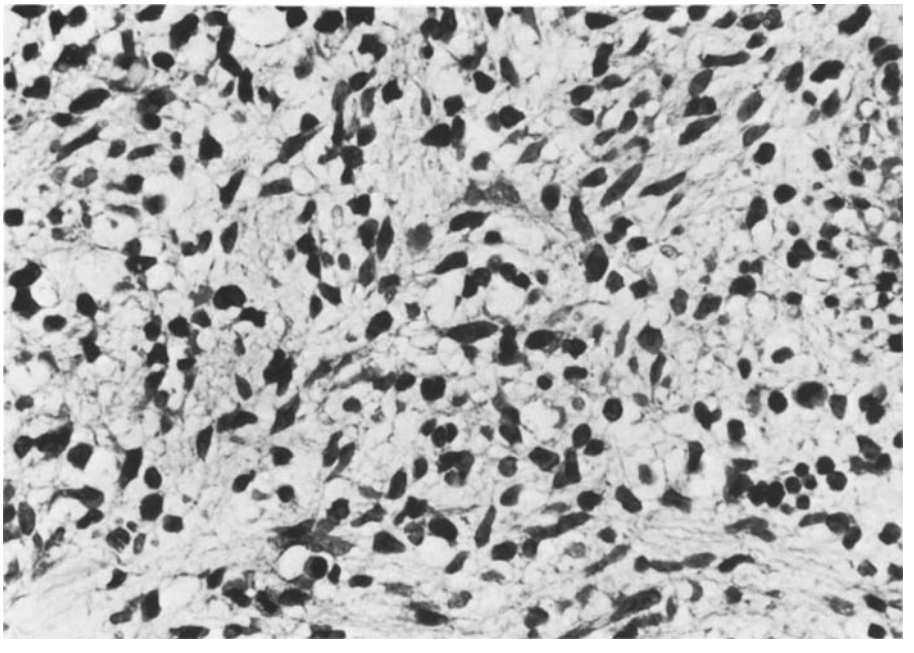

Fig. 1. Embryonal rhabdomyosarcoma of primitive type (HE, ×350)

Response was defined as a reduction of tumour volume of more than one third, *complete response* was used when there was no evidence of neoplastic disease and *no response* when there was progression of tumour growth, persistence or reduction of less than one third of the tumour volume within seven weeks after start of chemotherapy. *Local recurrence* described the reappearance of tumour tissue following a period of complete response.

III. Results

The median age of the sixty-four patients was 5 years and 11 months (range: six months to 18 years). There were 43 male and 21 female patients (ratio: 2:1).

There were 25 cases of primitive (Fig. 1), 29 cases of intermediate and 10 cases of well differentiated embryonal rhabdomyosarcoma (Fig. 2).

Most cases of primitive and well differentiated embryonal rhabdomyosarcoma occurred in the head and neck area (Table 2). Embryonal rhabdomyosarcomas of intermediate grade were predominantly located in the abdomen. Well differentiated embryonal rhabdomyosarcoma did not occur in the orbit, abdomen and trunk.

In seven of the 64 children, tumours were confined to the organ of origin and could be completely resected (stage I). Six of the 64 patients had locally confined disease, but the tumour was not completely resected (microscopic residual disease, stage II). Forty-three of the 64 children had local disease, the tumour was partially resected or merely biopsied (stage III). Eight of the 64 children had generalised disease, i.e. distant metas-

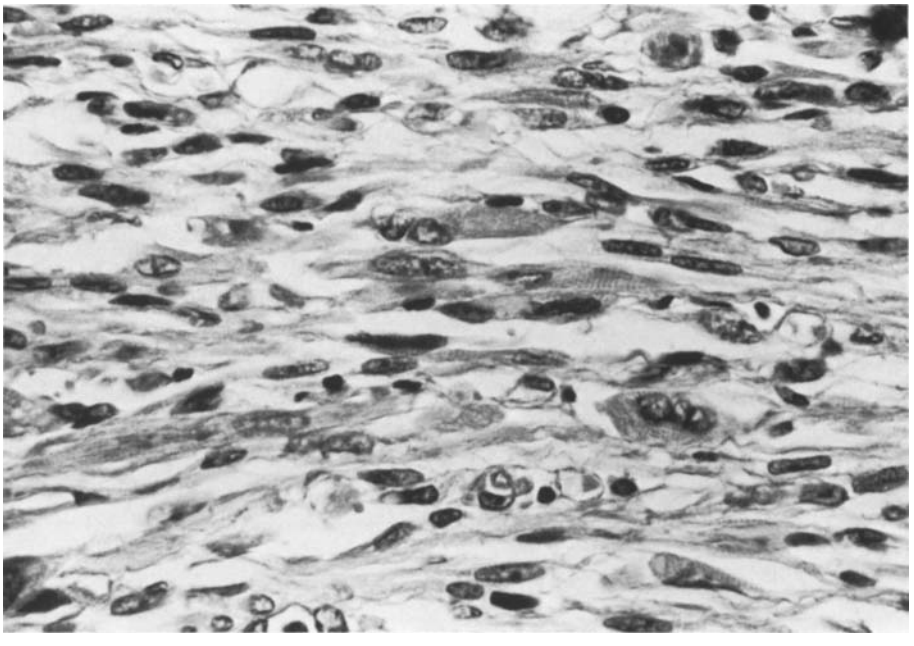

Fig. 2. Well differentiated embryonal rhabdomyosarcoma. A number of cells display cross striation (HE, × 560)

Table 2. Site and grade of differentiation in 64 cases of embryonal rhabdomyosarcoma treated according to protocol of CWS 81

Site	Grade			Total
	P	I	W	
Head and neck	10 (40%)	7	4 (40%)	21
Orbit	6	4	—	10
Abdomen	1	8 (28%)	—	9
Trunk	2	1	—	3
Genitourinary tract	3	6	2	11
Paratesticular	2	2	3	7
Extremities	1	1	1	3
Total	25	29	10	64

tases at onset (stage IV). In only six cases was there involvement of regional lymph nodes without evidence of systemic spread. In the group of primitive embryonal rhabdomyosarcomas, patients with stage III and IV predominated, whereas lower stages were observed in intermediate, and notably, in cases of well differentiated embryonal rhabdomyosarcoma (Table 3).

In general, results of the immunohistochemical stains were in accordance with the light microscopic findings. Only three cases showed deviate results

Table 3. Stage and grade of differentiation in 64 cases of embryonal rhabdomyosarcoma treated according to protocol of CWS 81

Stage	Grade			Total	All cases of RMS in CWS 81
	P	I	W		
I	1	3	3	7 (11%)	15 (13%)
II	1	4	1	6 (9%)	13 (11%)
III	18 (92%)	20 (76%)	5 (60%)	43 (67%)	69 (60%)
IV	5	2	1	8 (13%)	18 (16%)
Total	25	29	10	64	115

Table 4. Results of immunostainings in 20 cases of embryonal rhabdomyosarcoma of various grades of differentiation

Grade	*n*	Vimentin	Desmin	Myoglobin
P[a]	6	+++	+−++	−
I[b]	6	++−+++	+−++	−
W[c]	8	++−+++	+++	+−+++

[a] one case negative for desmin
[b] one case positive for myoglobin
[c] one case pos. for desmin and negative for myoglobin

(Table 4). Most cells in primitive embryonal rhabdomyosarcoma stained positively for vimentin. Only a few cells were positive for desmin. Myoglobin was consistently absent. In embryonal rhabdomyosarcoma of intermediate type, vimentin containing cells were slightly less numerous than in primitive embryonal rhabdomyosarcoma (Fig. 3). The number of cells with desmin intermediate filaments varied, although they were more numerous than in the primitive subgroup (Fig. 4). Myoglobin could not be demonstrated. In well differentiated embryonal rhabdomyosarcoma, most cells reacted positively for desmin. Myoglobin was found in all tumours (Fig. 5).

All seven patients in stage I and four of six patients in stage II are living without evidence of neoplastic disease for periods ranging from seven to 48 months (median 24 months). Two of the six patients in stage II are living with locally recurrent tumour 24 months after diagnosis (local recurrence after 16 and 17 months).

From the definition of "response to chemotherapy" it is apparent that this term can only be applied to patients with stage III and IV tumours, since only these patients have gross residual tumour which could respond to postoperative treatment.

Forty of the 51 patients with stage III and IV tumours responded to chemotherapy. Thirty of these 40 patients are living without evidence of disease from periods ranging from seven to 56 months (median 17 months)

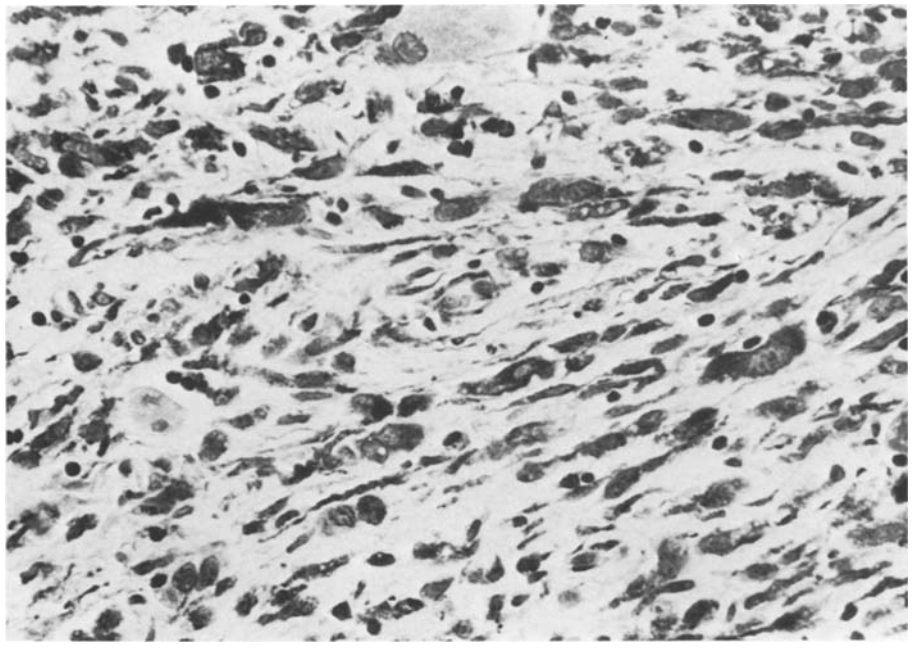

Fig. 3. Embryonal rhabdomyosarcoma of intermediate type. Most cells in this area stain positively for vimentin (PAP, ×350)

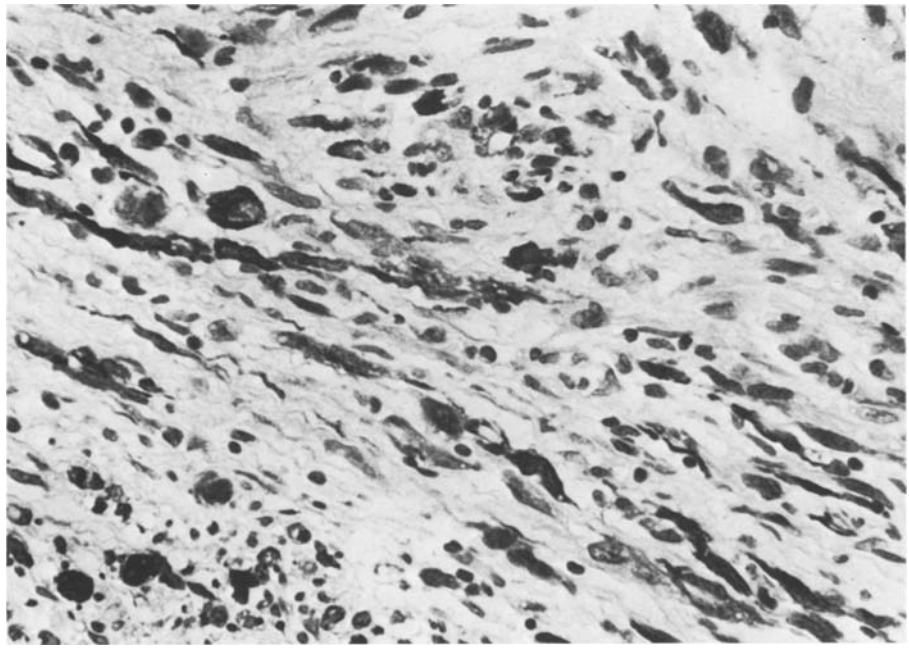

Fig. 4. Embryonal rhabdomyosarcoma of intermediate type. Many cells are positive for desmin (PAP, ×350)

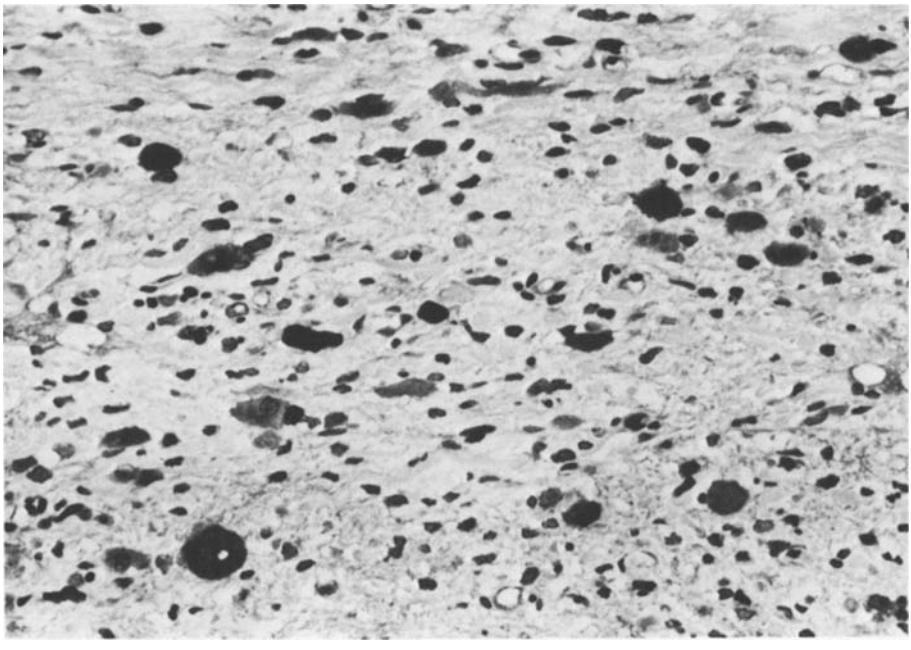

Fig. 5. Well differentiated embryonal rhabdomyosarcoma. A number of cells contain myoglobin (PAP, ×350)

Table 5. Response to treatment and survival in 39 patients with stage III or IV tumours evaluated at seven weeks after start of chemotherapy

Grade	Responder			Non-responder		Total
	CR	Loc. rec. and/or met.	Dead	Alive	Dead	
P	6	3	1	4	1[a]	15
I	15[b]	3[b]	1	2	1	19
W	5	–	–	–	–	5

[a] after cessation of therapy
[b] three patients identical (2 pat. following local reccurrence CR, 1 pat. following metastatic disease)

after diagnosis. Five of the 40 patients are alive twelve to 30 months (median 24 months) after local recurrence. One patient died after cessation of chemotherapy. Another patient died of epilepsy without evidence of neoplastic disease at autopsy.

Eleven of 51 patients did not respond to chemotherapy. Three of them are alive one to 27 months after radical second look operation with no evidence of disease. Three of the eleven patients developed distant metas-

tases. Two of them survive three and 16 months after development of metastatic disease. The outcome of one patient is unknown. Five of the eleven patients died of neoplastic disease.

Evaluation after seven weeks of treatment was possible in 39 patients (Table 5). In the group of primitive embryonal rhabdomyosarcoma, six of 15 patients achieved complete remission and three developed local recurrences or metastatic disease. Two patients died. Of 19 patients with embryonal rhabdomyosarcoma, intermediate subtype, 15 showed complete remission, three developed local recurrence and two died. None of the five patients with well differentiated embryonal rhabdomyosarcoma died. All patients in this group responded to chemotherapy. Differences in response to treatment between the three subgroups were not statistically significant.

IV. Discussion

Based on light microscopic and immunohistochemical findings three subtypes of embryonal rhabdomyosarcoma were distinguished in the current study. They were designated as primitive, intermediate and well differentiated embryonal rhabdomyosarcoma. The discriminating feature between these three subgroups was the number of rhabdomyoblasts. Primitive embryonal rhabdomyosarcomas were characterized by a small number of rhabdomyoblasts which did not exceed 10% of all neoplastic cells. Large areas in these tumours were occupied by small to moderately sized cells without recognizable differentiation, which made it difficult to distinguish them from other so-called "small, round, blue-cell" tumours. By immunohistochemistry, vimentin containing cells predominated. However, there were also some desmin-positive cells, more than expected from light microscopy. As in other studies (Corson and Pinkus 1981; Brooks 1982; Kagawa et al. 1983; Tsokos et al. 1984) no cells reacted positively for myoglobin. By contrast, Kindblom et al. (1982) found positive staining also in primitive embryonal rhabdomyosarcomas. It has been suggested that about one third of all embryonal rhabdomyosarcomas belong to this subgroup (Bale et al. 1983; Molenaar et al. 1984). In our material, primitive embryonal rhabdomyosarcomas account for 25 out of 64 cases (39%), thus being close to the estimated figure of about 30%.

Embryonal rhabdomyosarcomas of intermediate grade contained up to 50% rhabdomyoblasts. By immunohistochemistry, the proportion of desmin-positive cells was higher than in the primitive subtype. The number of vimentin-positive cells differed only slightly. As expected, this group of tumours comprised the largest subgroup, accounting for 29 of 64 (45%) cases.

Well differentiated embryonal rhabdomyosarcomas with more than 50% rhabdomyoblasts constituted the smallest group ($10/64 = 15\%$). In these tumours, cross-striations were easily detectable, and desmin-positive cells predominated. Moreover, all tumours contained cells positive for myoglobin. One case corresponded to a so-called rhabdomyoblastoma (Hajdu 1979).

Differences between the three subgroups of embryonal rhabdomyosarcoma related to the site of predilection, stage of disease and response to chemotherapy. Primitive tumours were located predominantly in the head and neck area. As in other studies (Kingston et al. 1983; Maurer and Ragab 1984) this was also the most common site for the whole group of tumours. Embryonal rhabdomyosarcomas of intermediate type were frequently located in the abdomen, and well differentiated tumours showed an almost equal distribution for the head and neck area and the genitourinary tract and paratesticular region. The predilection of primitive tumours for the head and neck area may be explained by the early detection of the neoplasms at this site. Because of the usually short duration of the disease process the neoplastic cells may not be able to differentiate into large rhabdomyoblasts. On the other hand, that about 50% of all well differentiated tumours in the present study occur in this location suggests that other factors, including the microenvironment, may determine the grade of differentiation. Age of the patients does not seem to play a role, since median age of patients in the three subgroups did not differ significantly.

As in IRS-I (Ragab et al. 1983) most patients in the current study had stage III tumours (43/64 = 67%). This number correlates very well with the percentage of grade III embryonal rhabdomyosarcomas (60%) in the whole group of 115 patients with embryonal rhabdomyosarcoma entered into the CWS 81. However, there is a marked difference in frequency between grade P and grade W embryonal rhabdomyosarcoma in stage III (72% vs. 50%). Also, stage IV tumours are mostly primitive embryonal rhabdomyosarcomas, while the proportion of well differentiated embryonal rhabdomyosarcomas in lower stages is higher than that of primitive or intermediate-grade tumours. It appears from these data that embryonal rhabdomyosarcomas of primitive histological grade tend to infiltrate locally and metastasize more frequently than those of intermediate grade or well differentiated embryonal rhabdomyosarcomas. This difference in biological behavior is well known from embryonal and alveolar rhabdomyosarcoma (Bale et al. 1983; Kingston et al. 1983; Ragab et al. 1983; Maurer and Ragab 1984). In the CWS 81 the proportion of stage IV alveolar rhabdomyosarcomas is 28.2%, whereas only 14.2% of patients with embryonal rhabdomyosarcoma have stage IV tumours (unpublished data). It is important to note that in contrast to Bale et al. (1983), who made a diagnosis of alveolar rhabdomyosarcoma only when 70% or more of the examined areas showed an alveolar pattern, we make a diagnosis of alveolar rhabdomyosarcoma irrespective of the proportion of alveolar areas. Therefore, in our opinion, a diagnosis of rhabdomyosarcoma of mixed histology should no longer be entertained.

When tested for response to chemotherapy after seven weeks of treatment, differences were noted between the three subgroups. The response rate of patients with stage III and IV embryonal rhabdomyosarcomas of primitive type was 40% compared with 79% in intermediate and 100% in well differentiated embryonal rhabdomyosarcoma. Moreover, in the whole group of patients 5/25 (20%) patients in the group of primitive and 3/29 (10%) in the group of intermediate embryonal rhabdomyosarcoma

died due to neoplastic disease, but 9/10 patients in the group of well differentiated tumours survive. The remaining patient died after cessation of therapy. Although survival periods for many patients in the current study are too short to draw definite conclusions, our data seem to be in contrast to those reported by Weichert et al. (1976) stating that partially matured tumours have a worse prognosis than undifferentiated lessions. Our experience is supported by Bale et al. (1983), who noted the highest mortality among patients with undifferentiated tumours. Since histological differentiation in the current study was related to stage, i.e. better differentiated tumours were found in lower stages, it may be assumed that lower mortality rates in grade I tumours and absence of tumour death in grade W tumours mainly result from more successful local control, which is still one of the major problems in the treatment of rhabdomyosarcoma.

In conclusion, although differences were not statistically significant, it appears from the current study that subdividing embryonal rhabdomyosarcoma into three subgroups with different grades of differentiation may be justified. There is a clear tendency for these subgroups to differ in site of origin, stage at time of diagnosis and response to chemotherapy.

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