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Non-seminomatous Germ Cell Tumors of the Testis: Morphology of Retroperitoneal Lymph Node Metastases after Chemotherapy*

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Abstract—*Gross and histological examination of residual retroperitoneal mature teratoma revealed different findings in patients treated with PVB remission-induction chemotherapy with maintenance chemotherapy followed by an RLND* 4–6 months *after PVB chemotherapy (group I) as compared to patients who received PVB chemotherapy only and underwent an RLND after* 4–6 weeks *(group II). RLND specimens in group I predominantly contained mature teratoma with organoid differentiation, whereas the specimens in group II often consisted of small mature teratoma areas with less-differentiated structures next to large areas of fresh tumor necrosis. Our findings suggest that, either due to maintenance chemotherapy or due to a prolonged time interval between PVB remission-induction chemotherapy and RLND, mature teratoma grows and differentiates further from tissue level to organoid level.*

INTRODUCTION

COMBINATION chemotherapy consisting of *cis*-platinum, vinblastine and bleomycin (PVB) results in high complete remission and cure rates in advanced-stage non-seminomatous germ cell tumors (NSGCT) of the testis [1–6]. As a result of chemotherapy residual pulmonary or retroperitoneal tumor often consists exclusively of differentiated, mature teratoma [7–12]. We observed previously that mature teratoma in residual retroperitoneal deposits after PVB chemotherapy was nearly always associated with mature teratoma in the primary tumors [12]. Therefore we concluded that destruction of components other than differentiating or already differentiated somatic tissues takes place rather than *de novo* induction of differentiation [12].

Since 1978 patients with advanced stage NSGCT referred to the University Hospital of Groningen, The Netherlands, have been treated with PVB remission-induction chemotherapy with or without maintenance chemotherapy consisting of *cis*-platinum and vinblastine, as described earlier [2]. Patients treated with maintenance chemotherapy underwent a retroperitoneal lymph node dissection (RLND) 4–6 months after remission-induction chemotherapy, whereas in patients not receiving maintenance therapy an RLND was performed 4–6 weeks after remission-induction by PVB.

These two patient groups showed differences in the gross examination and histology of the retroperitoneal tumors after chemotherapy. These morphological findings are reported here.

MATERIALS AND METHODS

Patients

All patients had bulky non-resectable NSGCT retroperitoneal tumor, defined as retroperitoneal tumor mass diameter larger than 4 cm, often fixed to large vessels, for which complete surgical excision was considered not feasible [13]. After admission to our hospital, all patients received chemotherapy consisting of four cycles of *cis*-diamminedichloroplatinum, vinblastine and bleomycin [1]. From January 1978 until January 1980 16 patients (group I) received PVB

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chemotherapy with maintenance chemotherapy consisting of vinblastine alternating with vinblastine plus *cis*-platinum at 3-week intervals for 1 yr [2]. Retroperitoneal lymph node dissection RLND was performed 4–6 months after the fourth induction with PVB.

From January 1980 until December 1982 33 patients (group II) had been treated with PVB inductions only. In this patient group an RLND was done 4–6 weeks after PVB.

Histopathological evaluation

Multiple sections of the primary tumor were examined by University's Pathology Department at the time of patient's referral. The hematoxylin- and eosin-stained sections were examined by two or more pathologists until a full consensus was reached on the exact classification of the tumor. The primary tumors and retroperitoneal lymph node metastases were classified by listing all histological components present: seminoma (SE), embryonal carcinoma (EM), differentiated or mature teratoma (TD), immature teratoma (TI), yolk sac tumor (YO) and choriocarcinoma (CH). In addition we entered lesions consisting exclusively of necrotic (NE) and fibrotic scar tissue (FI), consistent with regressed tumor tissue. In defining our histological criteria for the aforementioned components we used standard texts [14, 15] and some of the more recent papers on the subject [16–19].

The statistical analysis of the data was done by the chi-square test.

RESULTS

Gross examination

The size of retroperitoneal tumors, surgically removed after chemotherapy, ranged from 1.5 to 22 cm. On cross-section residual retroperitoneal deposits usually had a thick fibrous capsule and either a cystic or necrotic fibrotic appearance. The size of the cysts ranged from microscopic to 3 cm in diameter. The cysts had a glistening lining and contained a clear mucinous fluid (Fig. 1). Sometimes cartilaginous structures were seen. Some residual deposits contained extensive necrosis and fibrosis either with or without smaller cystic, cartilaginous or bony structures (Fig. 2). The tumors in group I tended to be either predominantly cystic or consisted of relatively small areas of necrotic and fibrotic tissue (Fig. 1). Tumors in group II more frequently contained predominantly necrotic tissue with fibrosis with or without cystic areas (Fig. 2). The cystic part of the residual retroperitoneal tumors as well as the size of the cysts tended to be smaller in patient group II then in group I. The largest cystic area in group II measured 2 cm, compared with 8 cm in group I, whereas the largest cyst diameter in group II measured 1 cm compared with 3 cm in group I.

Histology

The histology of testicular and retroperitoneal tumors in groups I and II, as well as the tissue differentiation of mature teratoma in these tumors, is summarized in Tables 1 and 2 respectively.

Listing the different histological components of the primary tumors in groups I and II resulted in 11 tumors with one histological component (10 EM, 1 TD) and 38 tumors with two or more components. In the primary tumors both the incidence of TD (1/49, 2%) and of EM in combination with TD (31/49, 63%) approximated the figures reported by the British Testicular Tumor Panel (TD 4.9%, MTI 54%) [15]. The incidence of mixed choriocarcinoma in this study, however, was higher than that reported by the British Panel (respectively 11/49, 22.4% and 3.7%) [15], but approximated the incidence of choriocarcinoma in 154 autopsy cases of disseminated testicular germ cell tumors (25%), as reported by Bredael *et al.* [20].

The distribution of the specific histological components of the primary tumors in group I as compared to group II was similar, except for the higher incidence of embryonal carcinoma as the only component in the primary tumors in group II (9/33 vs 1/16).

Resected residual retroperitoneal deposits after chemotherapy consisted of necrosis and fibrosis only in 26 patients (53%), residual 'carcinoma' in 3 (6%) (embryonal carcinoma in 2 and yolk sac tumor in 1 case) and mature teratoma in 20 (41%).

With three exceptions in the whole patient group, metastases containing mature areas with or without other components always originated from primary tumors containing mature areas as well (Table 3): 34 primary tumors with mature areas gave rise to 18 metastases with mature tissues. On the other hand, out of 15 primary tumors without differentiated teratoma 3 showed mature tissue in its metastases ($P < 0.025$).

Mature somatic tissue were cystic structures lined by cuboid (cub), cylindrical (cyl), mucinous (muc) and squamous (sq) epithelium. Mesenchymal structures other than connective tissue (ct) were smooth muscle (sm), cartilage (cart) and bone (bo). Neuroglial tissue (ng) was occasionally found. Next to tissue level maturation, structures with organoid differentiation (OD) such as gut-like or bronchus-like cavities were also seen (Fig. 3).

Furthermore, the number of cases with necrosis and fibrosis only in the metastases was larger in

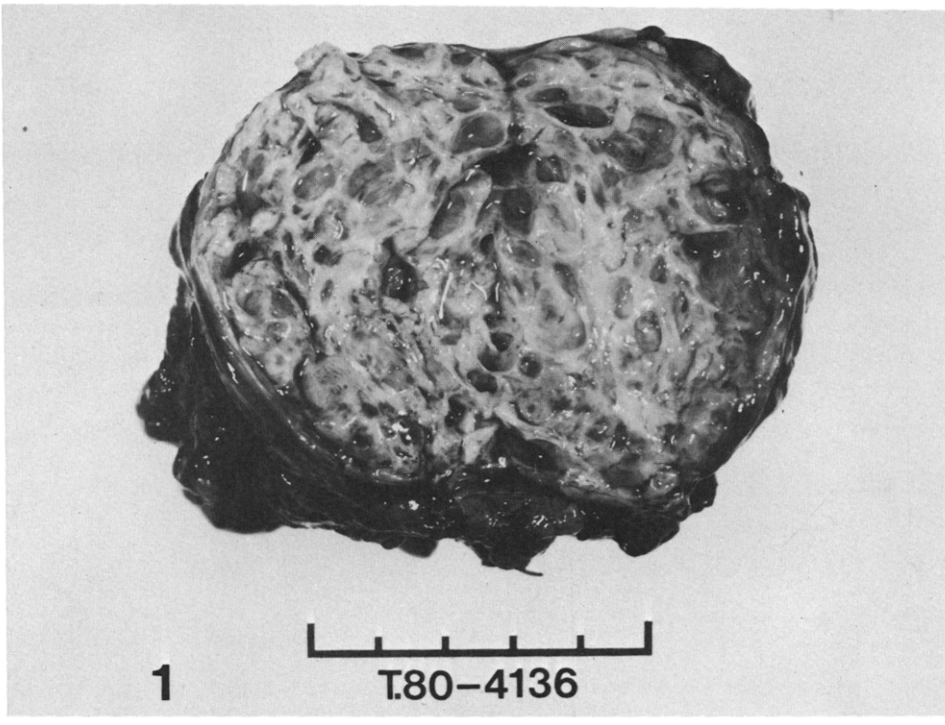

Fig 1. *Retroperitoneal tumor after PVB chemotherapy in group I with the characteristic appearance of cystic teratoma* (7 × 5 cm).

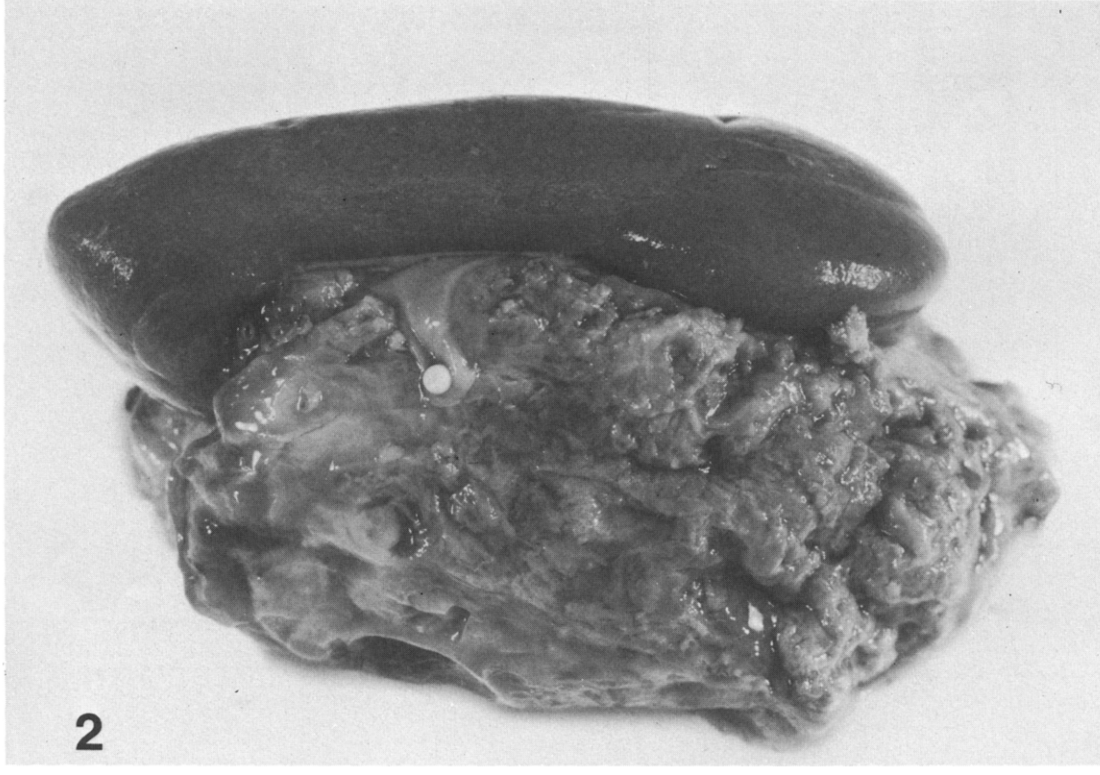

Fig. 2. *Retroperitoneal tumor in the renal hilus after PVB chemotherapy in group II consisting predominantly of necrotic tumor tissue* (14 × 7 cm).

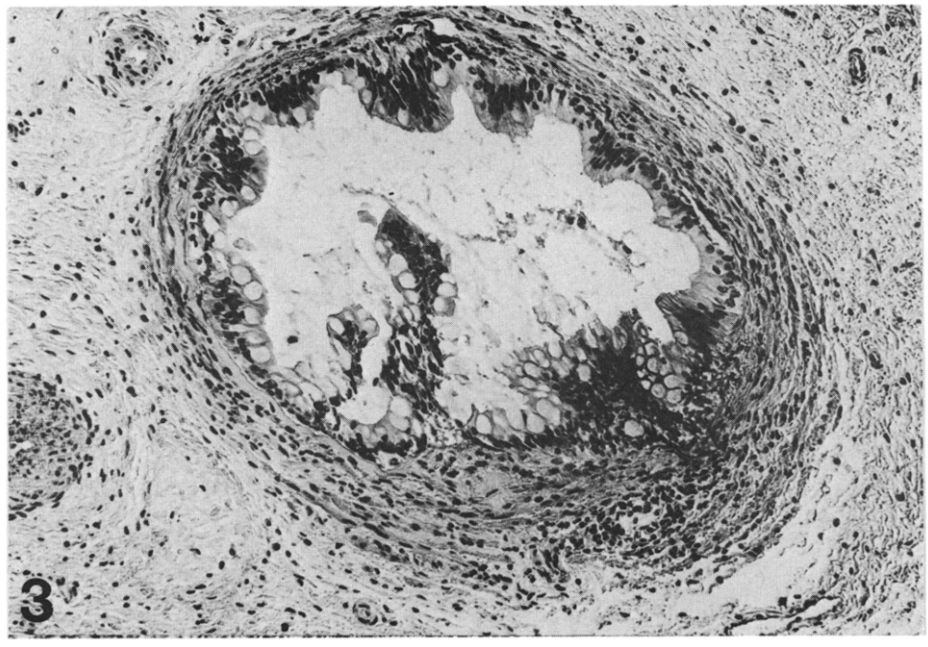

Fig. 3. Retroperitoneal mature teratoma after PVB chemotherapy (group I): gut-like cavity as an example of organoid differentiation (H & E: ×140).

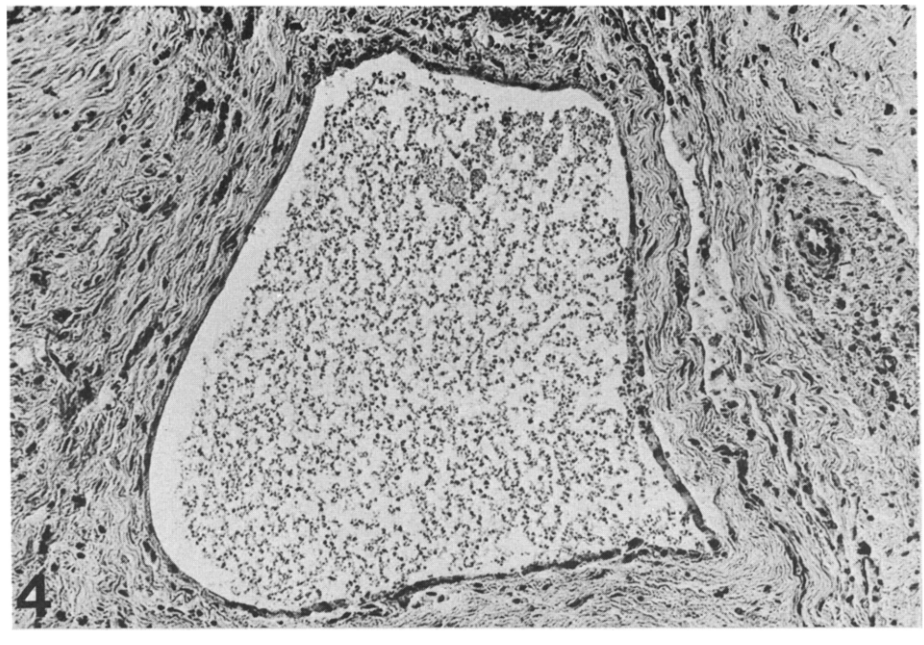

Fig. 4. Retroperitoneal mature teratoma after PVB chemotherapy (group II): cyst lined by simple cuboidal epithelium (H & E: ×140).

Table 1. NSGCT patient group I: histology of testicular and retroperitoneal tumors

Patient	Histology of testis	Tissue components of differentiated teratoma	OD	Histology RLND	Tissue components of differentiated teratoma	OD
1	EM/TD/TI	cub, cyl, muc, sm, ct	+	NE/FI		
2	EM/TD/TI/SE	cub, cyl, muc, sq, ct, cart, ng	+	TD	cub, cyl, muc, sq, sm, ct, cart, ng	+
3	EM/TD/TI/SE	cub, cyl, sq, ct	-	TD	cub, cyl, muc, sm, ct, cart	+
4	EM/TD/TI/CH	cub, cyl, sq, ct, cart	+	TD	cub, ct	-
5	EM/TD/TI	cub, cyl, muc, ct, cart	+	TD	cub, cyl, muc, sq, ct	+
6	EM/SE			TD	cub, cyl, muc, sq, ct, bo	+
7	EM			NE/FI		
8	EM/TD/CH	cub, cyl, muc, ct	-	EM/NE		
9	EM/TD/TI/SE	cyl, ct, sm, ct	-	NE/FI		
10	EM/YO			NE/FI		
11	EM/TI/CH			EM/NE		
12	TD	cub, cyl, muc, sm, ct, cart	+	TD	ct, sm, cart	-
13	EM/TD/TI	cub, cyl, ct	-	NE/FI		
14	EM/TD/TI	cub, sq, ct	-	TD	ct, sm	-
15	EM/TD/SE	ct, cart	-	NE/FI		
16	EM/TD/TI	cub, cyl, sm, ct	-	TD	cub, cyl, muc, sq, sm, ct	+

OD = organoid differentiation.

Table 2. NSGCT patient group I: histology of testicular and retroperitoneal tumors

Patient	Histology testis	Teraromatus tissue components	OD	Histology RLND	Teratomatous tissue components	OD
17	EM/TI			NE/FI		
18	EM/TD/TI	cub, sq, ct, cart	+	TD	cub, cyl, ct	-
19	EM/TD/TI/CH	cub, cyl, muc, sm, ct	+	TD	cub, cyl, ct	-
20	CH/TD	cub, cyl, ct	-	TD	cub, cyl, muc, sq, sm, ct, cart	+
21	EM			NE/FI		
23	EM/TD/TI/SE	cub, cyl, sm, ct	+	NE/FI		
22	EM/TD/TI	cub, cyl, ct	-	NE/FI		
24	EM			TD	cyl, ct	-
25	EM/TD/TI	cub, cyl, sq, sm, ct	+	NE/FI		
26	EM/TD/TI/YO	cub, cyl, sq, ct, cart	-	NE/FI		
27	EM/TD/TI	cub, cyl, muc, sm, ct, cart	+	NE/FI		
28	EM			NE/FI		
29	EM/CH/SE			NE/FI		
30	EM/TD/CH/SE	cub, cyl, sm, ct	-	NE/FI		
31	EM/TD/CH	cub, cyl, sm, ct	-	TD	cub, cyl, sm, ct	-
32	EM			TD	cart, ct	-
33	EM/TD/TI	cub, cyl, muc, sm. ct, cart	+	TD	cub, cyl, muc, ct	-
34	EM			NE/FI		
35	EM/TD/TI	cub, cyl, muc, sq, sm, ct	+	NE/FI		
36	EM/TD/CH	cub, cyl, ct	-	NE/FI		
37	EM			NE/FI		
38	EM/TD/TI/YO	cub, cyl, ct, cart, bo	-	TD/YO	cub, ct, cart	-
39	EM/TD/TI	cub, cyl, sq, ct	-	TD	cub, cyl, muc, sm, ct	+
40	EM/TD	cub, cyl, muc, ct	-	TD	cub, cyl, muc, sm, ct, cart	+
41	EM/TD/CH	cub, cyl, muc, sm, cart	+	TD	cub, cyl, muc, cart	+
42	EM/TD/TI/YO	cub, cyl, muc, sq, sm, cart	+	NE/FI		
43	EM/TD/CH	cub, cyl, muc, sm	-	TD	cub, cyl	-
44	TD/YO	cub, cyl, muc, sm	+	TD	cub, cyl, sq	-
45	EM			NE/FI		
46	EM/TD/TI/SE	cub, cyl, sm, cart, ng	-	NE/FI		
47	EM			NE/FI		
48	EM/TD	cub, cyl	-	NE/FI		
49	EM			NE/FI		

OD = organoid differentiation.

group II than group I (20/33 = 61% vs 6/16 = 38%) and the number of metastases containing mature teratoma was somewhat larger in group I than group II (8/16 = 50% vs 13/33 = 39%).

Besides the different gross appearance of the retroperitoneal tumors in group I as compared to group II, we also found differences in the level of tissue maturation and organoid differentiation of

mature teratoma components as well as differences in the histological appearance of areas with necrosis and fibrosis. For instance, the metastases in group I contained more areas of mature teratoma with organoid differentiation than group II (respectively 5/8 = 63% and 4/13 = 31%, Tables 1 and 2). Moreover, 7/13 metastases with mature teratoma in group II (patients 18, 19, 24, 31, 33, 43 and 44 in Table 2) consisted almost entirely of small cysts lined by histologically benign, simple cuboid or cylindrical epithelium (Fig. 4). One retroperitoneal tumor in group II (patient 32 in Table 2) showed extensive necrosis and fibrosis together with some small mature cartilaginous structures. Histological examination of the (often large) areas with necrosis and fibrosis in the metastases of group II revealed necrotic tumor tissue, xanthogranulomatous reaction with fibroblastic proliferation, whereas the (often small) areas with necrosis and fibrosis in group I consisted microscopically of amorphic necrosis and scar tissue.

Gross examination and histology of the testicular and retroperitoneal tumors in group I compared with group II are summarized in Table 4.

DISCUSSION

Patients with advanced stage NSGCT of the testis can be cured with combination chemotherapy and aggressive surgery [1–7, 10, 11]. PVB chemotherapy for advanced-stage NSGCT of the testis results in a complete remission in about 70% of the patients [1–5]. A further 10% of the patients can be rendered disease-free with surgical removal of residual disease, so that an 80% overall disease-free status can be achieved [11]. Maintenance chemotherapy does not result in higher cure rates in disseminated tumors [3, 4].

Histological examination of most of the residual retroperitoneal deposits after PVB chemotherapy revealed mature teratoma only (20/49 cases, 41%) or necrosis and fibrosis only (26/49 cases, 53%). Residual carcinoma was seen in 3 cases (6%). Three mechanisms have been postulated for the frequent occurrence of mature teratoma only in treated metastases [7, 9]: (a) selective destruction of components other than mature teratoma; (b) spontaneous differentiation of totipotent embryonal carcinoma cells made possible or facilitated by chemotherapy; and (c) direct induction of differentiation of malignant cells.

Table 3. Advanced stage NSGCT: relationship between primary tumors and RLN metastases with and without differentiated teratoma (TD)

	n	Primary tumors		Retroperitoneal tumors		
Group I + group II	49	with TD	34	with TD	18	$P < 0.025$
				without TD	16	
		without TD	15	with TD	3	
				without TD	12	

Table 4. Advanced stage NSGCT: patient characteristics, macroscopy and microscopy of testicular and retroperitoneal tumors in patient group I compared with patient group II

	Group I	Group II
No. of patients	16	33
Chemotherapy schedule	PVB + maintenance	PVB only
Time of RLND	4–6 months after PVB	4–6 weeks after PVB
Macroscopy:		
testicular tumors	variegated appearance	variegated appearance
retroperitoneal tumors	predominantly cystic; small necrotic areas	predominantly necrotic; small cystic areas
Microscopy:		
testicular tumors	14 pluritissular tumors; 1 tumor with EM only	24 pluritissular tumors; 9 tumors with EM only
retroperitoneal tumors	TD in 50% of the tumors; NE/FI in 38% of the tumors; TD: more areas with organoid differentiation	TD in 39% of tumors; NE/FI in 61% of tumors; TD: more areas with less differentiated structures

Metastases with mature areas are usually derived from testis tumors with mature teratoma (Table 3). Chemotherapy seems to favor the survival of differentiating or differentiated cells in a metastasis by selectively killing the less-differentiated elements.

Three metastases with mature structures were derived from primary tumors with undifferentiated elements. In these cases small mature areas may have been missed in the primary tumor due to a sampling error. Alternatively, induction of differentiation of embryonal carcinoma cells by chemotherapy cannot be absolutely ruled out in these cases. Either due to maintenance chemotherapy or due to a prolonged time interval between remission-induction and RLND or both, differences in the gross and histological appearance of mature teratoma in the metastases of group I as compared to group II were seen: large areas of cystic teratoma often with organoid differentiation were found in the metastases in group I, whereas the metastases in group II contained large areas of necrosis and fibrosis with relatively small areas of mature teratoma. Although areas of organoid differentiation were also seen in mature teratoma in group II, many mature teratomas consisted of less-differentiated tissues.

Our findings suggest that two changes occur after PVB chemotherapy that are responsible for the morphological appearance of the metastases: (1) large amounts of tumor necrosis are cleared away by phagocytosis, resulting in small areas of amorphic necrosis after a certain period of time; inflammation and fibroblastic proliferation result in fibrotic scar tissue; and (2) in the course of time after chemotherapy mature teratoma can grow, giving rise to larger tumors, and can differentiate from tissue level to organoid level.

The growth potential of mature teratoma in metastases after chemotherapy has been reported by others. Carr *et al.* [21] have described this phenomenon in a case of testicular teratocarcinoma, and Logothetis *et al.* [22] described six patients with a so-called 'growing teratoma syndrome', occurring 2–7 months after chemotherapy, and associated with teratoma in the testicular tumors. Little is known of the malignant potential of unresected mature teratoma following chemotherapy because histological diagnosis is made on resected tissue [23]. Resected mature teratoma is histologically benign. Careful follow-up of patients with incompletely resected mature teratoma, e.g. for technical reasons, should provide information.

Another approach is the use of animal models of teratocarcinoma. Oosterhuis and Damjanov [24] have reported reappearance of malignant elements in benign teratomas after *cis*-platinum treatment in a primary embryo-derived teratocarcinoma model, illustrating the shortcomings of histologic examination of complex germ cell tumors. They urge caution in labeling all histologically mature tumor masses residual after chemotherapy in NSGCT as unequivocally benign [24].

In conclusion, gross and histological examination of residual retroperitoneal deposits excised 4–6 months as compared to 4–6 weeks after PVB chemotherapy suggests that residual retroperitoneal mature teratoma after chemotherapy can grow and further differentiate.

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