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Original Paper

Cisplatin-based Chemotherapy in Advanced Seminoma: the Institut Gustave Roussy Experience

S. Culine,[1] L. Abs,[1] M.J. Terrier-Lacombe,[2] C. Théodore,[1] P. Wibault[3] and J.-P. Droz[1]

[1]Departments of Medicine, [2]Pathology and [3]Radiotherapy, Institut Gustave Roussy, 94805-Villejuif, France

The aim of this study was to report the results of cisplatin-based combination chemotherapy for patients with pure seminomatous tumours. 72 patients with advanced seminoma were treated with various cisplatin-based chemotherapy regimens. 61 (85%) patients achieved a sustained durable response. 11 relapses were observed with a median time to failure of 6 months. Overall, 60 (83%) of the 72 patients remain alive and free of disease after a median follow-up of 64 months. Initial clinical (age, site of primary, prior radiotherapy, extent of disease) and biological (serum human chorionic gonadotrophin levels, serum lactic dehydrogenase levels, p53 immunostaining) features which could be of predictive value for survival, were analysed in a univariate analysis. No variable retained statistical significance. High cure rates are expected after chemotherapy with standard cisplatin-based combinations in advanced seminoma. Renewed efforts are required to identify markers of chemosensitivity.

Key words: advanced seminoma, chemotherapy, prognostic factors
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INTRODUCTION

UNLIKE NON-SEMINOMATOUS germ cell tumours, pure seminomatous tumours of the testis have some unique features, including a low metastatic potential, an indolent course and an exquisite radiosensitivity. After radiotherapy, more than 90% of patients with clinical stage I (testis alone) and approximately 90% of those with non-bulky stage II disease (retroperitoneal lymphadenopathy < 5 cm) will be cured [1, 2]. However, in patients with advanced stages, i.e. bulky stage II (retroperitoneal lymphadenopathy ≥ 5 cm), supradiaphragmatic nodal metastases, extranodal metastases or extragonadal tumours, the percentage of survivors after radiotherapy alone is only 20–60% [3, 4]. Therefore, chemotherapy for pure seminomatous tumours has been reserved for the small percentage of patients who present with extensive disease or relapse after initial radiotherapy.

Because of the low incidence of advanced seminoma, most publications on the results of chemotherapy have included small numbers of patients (< 50). Most authors have reported high cure rates (60–95%) using two- to five-drug regimens including cisplatin or carboplatin [5]. These studies also suggested that extent of disease, prior radiotherapy or chemotherapy and elevated serum tumour markers could be significant adverse prognostic factors. The aims of the present study were, first, to report the results of cisplatin-based combination chemotherapy in 72 patients with pure seminomatous tumours treated at the Institut Gustave Roussy (IGR) and, second, to focus on the identification of clinical and biological pretreatment prognostic factors predictive of survival following chemotherapy.

PATIENTS AND METHODS

Patients

From June 1978 to December 1993, 72 patients with pure seminomatous tumours received cisplatin-based chemotherapy at the IGR. Initial clinical evaluation included a complete history and physical examination, chest X-ray, biochemical screening profile, review of the pathology of the primary tumour, measurement of serum tumour marker alphafetoprotein (AFP), serum free human chorionic gonadotrophin β subunit (β-hCG), serum lactic dehydrogenase (LDH) and computerised tomography of the thorax, abdomen and pelvis. Bone scans and brain computerised tomography were only carried out in case of clinical symptoms. Only patients with a confirmed histological diagnosis of pure seminoma, with or

Correspondence to S. Culine. CRLC Val d'Aurelle, 34298-Montpellier, France.
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without syncytiotrophoblastic cells, and normal serum AFP levels were retained in the present study.

Chemotherapy regimens consisted of various cisplatin-based combinations. VAB-like (AVAB or VAB-6) regimens were given to 26 patients. 10 of them received the AVAB regimen which included actinomycin-D 0.01 mg/kg/day intravenously (i.v.), cyclophosphamide 300 mg/sqm/day i.v. on days 1–5, vincristine 2 mg i.v. on day 28, doxorubicin 60 mg/sqm i.v. on day 28, bleomycin 20 mg/day on days 28–30 and cisplatin 100 mg/sqm on day 30. The other 16 patients were treated with the VAB-6 regimen which included actinomycin-D 1 mg/sqm i.v., cyclophosphamide 600 mg/sqm i.v., vinblastine 4 mg/sqm i.v. on day 1, bleomycin 30 mg i.v. push on day 1 and 20 mg/sqm/day in continuous infusion days 1–3, cisplatin 120 mg/sqm i.v. on day 4. Whichever VAB-like regimen was used, each cycle was repeated every 4 weeks for a total of between four and six cycles. BEP or EP regimens consisted of etoposide 100 mg/sqm/day i.v. days 1–5, cisplatin 20 mg/sqm/day i.v. days 1–5 ± bleomycin 30 mg/day i.v. days 1, 8 and 15. Three to four cycles of BEP were applied every 3 weeks in 11 patients. Four cycles of EP were given every 3 weeks in 18 patients. 11 patients received the HOP regimen (ifosfamide 1200 mg/sqm/day, vincristine 2 mg day 1, cisplatin 20 mg/sqm/day days 1–5, applied four times every 3 weeks). Other cisplatin-based combinations were used in 6 patients.

The evaluation of response was performed 4 weeks after the last cycle of chemotherapy. Patients with complete disappearance of metastatic sites and tumour marker normalisation were considered in clinical complete response (cCR). A partial remission (PR) was defined as a 50% decrease or more in the sum of the products of the largest diameter and its perpendicular for all measurable sites, lasting for at least 4 weeks without development of new lesions. Patients with PR after chemotherapy were considered for surgical excision when the residual mass diameter was > 3 cm. A pathological complete response (pCR) was defined as the complete resection of necrotic debris or fibrosis without any evidence of viable seminoma. A surgical complete response (sCR) was defined by the complete excision of all residual masses containing viable seminoma. Additional chemotherapy and/or radiotherapy were administered if any resected mass contained seminoma. Patients with PR and negative markers who did not undergo surgery of residual masses were considered as $PRMq^-$. Other situations were judged as treatment failures.

After the end of treatment, all patients were followed every 2 months during the first 2 years and at gradually increasing intervals thereafter. The relapse-free survival was measured from the end of treatment to the date of relapse or 1 January 1997. No patient was lost to follow-up. Overall survival was measured from the first day of chemotherapy to the date of death or 1 January 1997.

Immunohistochemistry

Haematoxylin–eosin stained slides of available cases were reviewed and representative paraffin-embedded blocks were selected for study. Immunohistochemical analyses were performed after antigen retrieval on microwave oven heated sections using the LSAB (labelled streptavidin–biotin) kit (Dakopatts, Copenhagen, Denmark). The following primary monoclonal antibodies (MAbs) were used: anticytokeratin AE1/AE3 (Boehringer), anti-CD30 (BerH2; Dako, Santa Fe, California, U.S.A.), anti-c-erbB2 (Dako) and anti-p53 (D07, Dako). Negative controls consisted of immunostaining with monoclonal antithyroglobulin. Microscopic examination for the reaction products was scored as follows: negative = < 5% of cells staining; + = 5–50% of cells staining; + + = > 50% of cells staining.

Statistical methods

Survival curves were generated using the Kaplan–Meier method [6]. The outcome variable for the survival calculation was from the first day of chemotherapy to the date of death or last follow-up. The univariate analysis for comparisons between subgroups was conducted using the log-rank test [7].

RESULTS

Patient characteristics

Patient characteristics are listed in Table 1. There were 71 males and 1 female. The median age was 39 years. The primary site of disease was gonadal in 57 patients and extragonadal in 15 patients. Histology was pure seminoma in 64 patients, pure seminoma with syncytiotrophoblastic cells in 6 patients and anaplastic seminoma in 2 patients. 31 patients with gonadal tumour had previously been treated with infradiaphragmatic prophylactic (22 patients) or curative (9 patients) radiotherapy. 14 (19%) patients were retrospectively assigned in the intermediate-risk group of the international classification [8]. Pretreatment serum β-hCG and LDH levels were elevated in 22 of 48 patients and in 23 of 41 patients with available determinations, respectively.

Responses to first-line treatment

68 of 72 patients were assessable for response. 4 patients (3 with gonadal and 1 with mediastinal primary) who were treated in an adjuvant setting were not assessable for response due to an absence of measurable disease. 1 patient was treated with EP after excision of spermatic cord and inguinal nodes. Another patient received EP after excision of a solitary axillary node. 1 patient was treated with EP after positive retroperitoneal lymph node dissection. Finally, the fourth patient was treated with adjuvant VAB-6 after complete resection of a mediastinal tumour.

2 patients progressed while on first-line chemotherapy (VAB-6 regimen). 66 (97%) of the 68 evaluable patients achieved a favourable response. 48 (71%) patients achieved a complete response, 15 of whom underwent surgical resection of residual masses without evidence of viable seminoma. 18 patients (26%) achieved a partial response with negative markers, 3 of whom received additional radiotherapy to residual masses. The responses according to regimens are listed in Table 2.

Failures and survival

After a median follow-up of 64 months (range 27–236 months), 61 (85%) of 72 patients maintained a durable response. 11 relapses were observed with a median time to relapse of 6 months (range 2–17 months). Relapses occurred in 5 (15%) of 33 patients who had achieved a CR status and in 5 (28%) of 18 patients with $PRMq^-$. It is noteworthy that relapses occurred in initial metastatic sites in 8 of 11 relapsing patients (Table 3). 2 patients who relapsed after CR were rendered free of disease after salvage ifosfamide and cisplatin-based chemotherapy. 9 patients died from progressive disease

Table 1. Patient characteristics

	Number (unless otherwise specified)	(%)
Age		
Median 39 years		
Range 18–57 years		
Primary site		
Testis	57	(79)
Mediastinal	8	(11)
Retroperitoneal	5	(7)
Pelvis	2	(3)
Prior treatment		
None	51	(71)
Radiotherapy	21	(29)
Sites of disease		
Retroperitoneum	47	(65)
Mediastinum	13	(18)
Cervical nodes	7	(10)
Lung	8	(11)
Bone	8	(11)
Liver	3	(4)
Brain	2	(3)
Other	10	(14)
Prognostic classifications		
Indiana University		
Minimal or moderate	42	(58)
Advanced	30	(42)
International		
Minimal	58	(81)
Intermediate	14	(19)
Serum tumour markers		
β-hCG		
Normal	26	(35)
N < β-hCG < 5N	13	(18)
5N ≤ β-hCG < 10N	6	(8)
≥ 10N	3	(4)
Not available	24	(33)
LDH		
Normal	18	(25)
1N < LDH < 5N	17	(24)
5N ≤ LDH < 10N	4	(6)
≥ 10N	2	(3)
Not available	31	(43)
Chemotherapy regimens		
VAB-like	26	(35)
EP	18	(25)
BEP	11	(15)
HOP	11	(15)
Others	6	(8)

hCG, human chorionic gonadotrophin; LDH, lactic dehydrogenase; VAB-like, vinblastine + bleomycin + actinomycin-D + cyclophosphamide + cisplatin ± doxorubicin; EP, etoposide + cisplatin; BEP, bleomycin + etoposide + cisplatin; HOP, ifosphamide + vincristine + cisplatin.

despite salvage therapy after a median follow-up of 18 months (range 12–30 months) after initiation of first-line chemotherapy. The 2 patients who failed initial chemotherapy died from progressive disease despite salvage therapy. The characteristics of the 13 patients who failed cisplatin-based chemotherapy are shown in Table 3. Finally, 1 patient died of a road accident. Overall, 60 (83%) of 72 patients remain alive and free of disease.

Table 2. Outcome after cisplatin-based chemotherapy by regimen

	VAB-like	EP	BEP	HOP	Various cisplatin-based regimens
Number of patients	26	18	11	11	6
Adjuvant	1	3	0	0	0
cCR + pCR	16	10	6	10	6
$PRMq^-$	7	5	5	1	0
Failures	2	0	0	0	0
Relapse	6	1	3	0	1
Long-term status					
Free of disease	18	17	9	11	5
Dead of disease	8	1	2	0	0
Dead without disease	0	0	0	0	1

VAB-like, vinblastine + bleomycin + actinomycin-D + cyclophosphamide + cisplatin ± doxorubicin; EP, etoposide + cisplatin; BEP, bleomycin + etoposide + cisplatin; HOP, ifosfamide + vincristine + cisplatin; cCR, clinical complete response; pCR, pathological complete response.

Immunohistochemical analysis

Adequate material for immunohistochemistry was available in 34 patients. Immunostaining with monoclonal antibodies anticytokeratin and anti-c-erbB2 was clearly negative in all patients studied. Only 1 patient had positive cells (+) with anti-CD30. In 11 patients, a positive immunostaining was observed with anti-p53 antibodies (+ in 2 cases, ++ in 9 cases).

Prognostic factor analysis

Survival comparisons were performed by selected pretreatment clinical or biological characteristics (Table 4). Regarding clinical variables, the proportion of patients alive was less in patients with testicular primary or prior radiotherapy. However, the differences did not show statistical significance. Likewise, no prognostic value was detected in studying the pretreatment serum LDH or β-hCG levels. A trend to better survival was noted in patients with p53 positive immunostaining but no statistical significance was reached.

DISCUSSION

With the success of cisplatin-based combination chemotherapy in non-seminomatous germ cell tumours, investigators have been encouraged to treat patients with advanced seminoma using similar regimens [9–13]. Table 5 summarises the results reported so far, in series including more than 50 patients. The efficacy of cisplatin-based chemotherapy in advanced seminoma is undeniable, with survival rates ranging from 62–92%. The optimal combination chemotherapy remains a matter of debate. An important issue is chemotherapy-related toxicity. Toxic death rates up to 10–15% have been reported by several groups, with bleomycin lung toxicity accounting for approximately 50% of lethal complications. In this regard, the two-drug regimens of cyclophosphamide/cisplatin or etoposide/cisplatin seem to offer some advantage.

In spite of the excellent efficacy of cisplatin-based chemotherapy, some patients failed during or after first-line treatment and required salvage therapy. Investigators at the Indiana University recently reported the long-term results of a combination of vinblastine, ifosfamide and cisplatin (VeIP)

Table 3. Characteristics of patients who failed first-line chemotherapy

Patient no.	Prior radiotherapy	Metastatic sites	Initial chemotherapy	Response	Time to relapse (months)	Site of relapse	Salvage chemotherapy	Status
1	Yes	Lung	VAB-6	PD	—	—	Non-cisplatin-based	DOD
2	Yes	Mediastinum	VAB-6	PD	—	—	Cisplatin-based	DOD
3	Yes	Retroperitoneum	VAB-6	$PRMq^-$	2	Retroperitoneum	VIP	DOD
4	No	Retroperitoneum	EP	$PRMq^-$	2	Retroperitoneum + bone	VeIP	DOD
5	No	Retroperitoneum	BEP	$PRMq^-$	2	Epidural	Cisplatin-based	DOD
6	No	Retroperitoneum + cervical nodes	VAB-6	$PRMq^-$	3	Retroperitoneum + liver	VeIP	DOD
7	No	Retroperitoneum	VAB-6	$PRMq^-$	5	Retroperitoneum	VIP	DOD
8	Yes	Retroperitoneum	VAB-6	cCR	6	Retroperitoneum	VIP	DOD
9	Yes	Mediastinum + lung	VAB-6	cCR	6	Lung	None	DOD
10	Yes	Retroperitoneum + lung	VAB-6	pCR	7	Lung	VIP	DOD
11	Yes	Retroperitoneum	BEP	cCR	4	Liver	None	DOD
12	Yes	Liver	PVB	cCR	6	Pleura + Bone	VIP	NED
13	Yes	Retroperitoneum	BEP	cCR	29	Retroperitoneum	VeIP	NED

VAB-6, vinblastine + bleomycin + actinomycin-D + cyclophosphamide + cisplatin; EP, etoposide + cisplatin; BEP, bleomycin + etoposide + cisplatin; PVB, bleomycin + vinblastine + cisplatin; VIP, etoposide + ifosfamide + cisplatin; VeIP, vinblastine + ifosfamide + cisplatin; PD, progressive disease; $PRMq^-$, partial response with normal serum tumour markers; cCR, clinical complete response; pCR, pathological complete response; DOD, died of disease; NED, no evidence of disease.

in this setting. Among 23 patients who were treated with the VeIP regimen, 13 (56%) were rendered free of disease after a median follow-up of 58 months [14]. Therefore, salvage VeIP chemotherapy appears to have a greater curative potential in seminomatous than in non-seminomatous tumours, as the cure rate of the latter is only 25% [5]. However, the prognosis of recurrent seminoma remains poor and further improvements are required in initial management.

Table 4. Analysis of prognostic factors for survival

Characteristic	No. of patients	Alive		*P*
		No.	(%)	
Age				
< 35 years	29	24	(83)	0.75
≥ 25 years	43	37	(86)	
Primary				
Testis	57	47	(82)	0.56
Extragonadal	15	14	(93)	
Prior radiotherapy				
No	41	30	(73)	0.53
Yes	31	23	(74)	
No. of metastatic sites				
1	46	38	(83)	0.79
≥1	26	21	(81)	
Indiana classification				
Good/intermediate risk	42	35	(83)	0.43
Poor risk	30	27	(90)	
International classification				
Good risk	58	48	(83)	0.55
Intermediate risk	14	11	(78)	
Serum β-hCG levels				
Normal	26	22	(84)	0.30
Elevated	22	21	(95)	
Serum LDH levels				
Normal	18	16	(89)	0.67
Elevated	23	20	(87)	
p53 immunostaining				
Negative	23	17	(74)	0.46
Positive	11	9	(82)	

hCG, human chorionic gonadotrophin; LDH, lactic dehydrogenase.

In contrast to patients with non-seminomatous tumours [15], prognostic factors have been more difficult to identify in patients with advanced seminoma because of the rarity of the disease and the high efficacy of cisplatin-based chemotherapy. Prior radiotherapy and extent of disease were the main clinical variables associated with an inferior survival in most series (Table 5). In the recently published international consensus prognostic classification for metastatic germ cell tumours, two groups of patients were identified according to the absence or presence of non-pulmonary visceral metastases. The 3-year overall survival was 86 and 77%, respectively. However, the good-risk group included 90% of the whole population [8]. Regarding serum tumour markers, elevation of hCG and LDH reached borderline statistical significance in the experience of investigators at MSKCC [13].

The weakness of clinical features for predicting outcome prompted us to seek biological prognosticators which could give insight into the mechanisms involved in tumour behaviour and sensitivity to chemotherapy. Immunostaining with MAbs anticytokeratin and anti-c-erbB2 was negative in all 34 patients studied. These results are in accordance with previous results suggesting negative expression of c-erbB2 in seminomas and embryonal carcinomas [16]. Expression of the CD30 antigen using the MAb Ber-H2 was only positive in 1 case in our series. This patient with metastatic retroperitoneal lymph nodes achieved a sustained complete response after four cycles of VAB-6. The failure of expression of Ber-H2 has been reported in seminoma, as well as in intratubular germ cell neoplasia, and contrasts with the positive expression observed in most cases of embryonal carcinomas [17].

The *p53* gene encodes a 53 kDa nuclear phosphoprotein which plays a critical role in several cellular functions. Documented effects include regulation of the mitotic G1/S

Table 5. Results of cisplatin-based chemotherapy in large (>50 patients) series in the literature

Reference	Chemotherapy	Number of patients	Long-term no evidence of disease (%)	Prognostic factors for survival
Logothetis and associates [11]	CP	52	48 (92)	Prior chemotherapy
Fossa and associates [9]	PVB or BEP	55	43 (78)	Prior radiotherapy
Loehrer and associates [10]	PVB (±A) or BEP	60	37 (62)	Extent of disease Prior extensive-field radiotherapy
Tjulandin and associates [12]	VAB-6 or CP	59	24 (66)	None
Mencel and associates [13]	VAB-6 or EP or ECa	140	120 (86)	Serum LDH, Serum hCG
Present series	VAB-like or HOP or EP (±B)	72	60 (83)	None

VAB-like, vinblastine + bleomycin + actinomycin-D + cyclophosphamide + cisplatin ± doxorubicin; EP, etoposide + cisplatin; BEP, bleomycin + etoposide + cisplatin; PVB, bleomycin + vinblastine + cisplatin; CP, cyclophosphamide + cisplatin; HOP, ifosfamide + vincristine + cisplatin; A, doxorubicin; ECa, etoposide + carboplatin; B, bleomycin; LDH, lactic dehydrogenase; HCG, human chorionic gonadotrophin.

checkpoint in the cell cycle and a role in determining cell death through apoptosis [18]. Cells harbouring *p53* deletions or mutations, or both, are unable to regulate apoptosis and, hence, have an increased potential for resistance to treatment with DNA-damaging agents. The presence of normal *p53* enables induction of apoptosis in cells with DNA damage and, hence, favourable response to treatment. These observations have raised the exciting prospect that *p53* alterations may be used to predict the responses of tumours to radiation therapy or chemotherapy [19]. All histological components of germ cell tumours have been reported to show immunohistochemically detectable expression of the *p53* gene product [20, 21]. Although a region closely contiguous with the *p53* gene has been involved in loss of heterozygosity [22], only rare mutations in the p53 gene itself have been described [23–25]. Cellular, for example post-translational, alterations rather than intragenic mutations are supposed to stabilise the p53 protein for immunohistochemical detection. These findings suggest that the basis for the high degree of responsiveness of germ cell tumours to chemotherapy is a result of p53-based apoptosis. In the present series, a trend for better survival was observed in patients with positive p53 immunostaining, but no statistical significance was reached. However, this immunohistochemical study, which was based on retrospective materials and consequently suboptimal fixation techniques, only involved 34 patients and no firm conclusion can be drawn.

Finally, it should be stressed that, apparently, not all seminomas are simply seminomas. Some seminomas explanted *in vitro* assume features of embryonal carcinomas and some embryonal carcinoma cells *in vitro* accumulate glycogen like seminomas *in vivo* [26]. Interestingly, investigators at the MSKCC have reported a subtype of seminoma, named atypical seminoma, with morphological features of seminoma but increased mitotic index, increased nuclear-to-cytoplasmic ratio, more aggressive clinical course and a distinct immunohistochemical staining pattern [27]. Indeed, classical seminoma lacks expression of both blood group antigens and low molecular weight keratin, as confirmed for the latter in the present series. In contrast, non-seminomatous tumours express the type 1 precursor to blood group antigens and prekeratin. Atypical seminoma is characterised by the surface expression of the type 1 blood group antigen but lacks cytokeratin. Atypical seminoma could represent a borderline histology between seminomatous and non-seminomatous tumours with a more aggressive clinical course and lower sensitivity to chemotherapy.

High cure rates are expected after chemotherapy with standard cisplatin-based combinations in advanced seminoma. Half the patients who fail first-line chemotherapy are expected to be cured after salvage treatment. There is no strong clinical or biological factor which could be used to predict a poor outcome. Renewed efforts are required to identify markers of chemosensitivity and distinguish borderline entities between 'true' seminomas and non-seminomatous tumours.

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