Immunohistological determination of proliferative activity in seminomas

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SUMMARY The proliferative activity and growth pattern of 20 seminomas were determined immunohistologically with the monoclonal antibody Ki-67. A growth fraction of tumour cells between 50 and 80% was found in seminomas with an almost even distribution of proliferating cells in all sections, regardless of tumour size. There was a slight tendency towards a greater growth fraction in tumours at an advanced histopathological stage. No positive correlation could be found between growth fraction and tumour size or lymphocytic infiltration.

The results confirm the well known sensitivity of seminomas to radiation and chemotherapy and show that the determination of proliferative activity should be included in the histopathological routine diagnosis of malignant tumours with regard to systemic treatment and prognosis.

Pure seminomas comprise about 40% of malignant testicular germ cell neoplasms.¹⁻³ The incidence peaks in the third to fifth decade and has continued to rise over the past 20 years.^{3,4} Histopathological methods should therefore be used to integrate the assessment of relevant biological variables into the routine diagnosis of seminomas. This study investigated the association between the proliferative behaviour of seminomas with other histomorphological as well as clinical variables. The use of immunohistochemistry in conjunction with the monoclonal antibody Ki-67 facilitates determination of the proportion of proliferating tumour cells, which is important in view of the implications for the prognosis with and without treatment and to explain cases of unexpected brevity of survival.

Material and methods

Twenty seminomas surgically removed between 1984 and 1987 were subjected to immunohistological determination of the growth fraction of tumour cells. Histologically the tumours were typical seminomas—that is, anaplastic or spermatocytic seminomas were not included. No other tumour component was present. The age of the patients ranged between 26 and 54 years; tumour size ranged from 1 to 12 cm in diameter and from 1 to 972 cm³ in volume (table 1).

For immunohistological analysis, several unfixed samples from each tumour were stored at -80°C

immediately after orchidectomy or biopsy. The highly sensitive alkaline phosphatase-antialkaline phosphatase (APAAP) staining procedure,⁵ modified by Stein *et al*⁶ was applied to cryostat sections. In cases in which endogenous alkaline phosphatase could not be blocked sufficiently the immunoperoxidase technique was applied to prevent the nuclear reaction product from being covered by cytoplasmatic phosphatase.

The monoclonal antibody Ki-67⁷ detects a proliferation-associated antigen in the nucleus which is expressed in all states of the cell cycle except G₀. With the APAAP technique, the reaction product is red in colour and can easily be detected regardless of the tissue being counterstained with haematoxylin.

The fraction of proliferating cells was determined by counting the tumour cells stained by Ki-67 in five high power fields at a magnification of 420 and the unreactive tumour cells in the same fields for each case (about 700 to 900 tumour cells). Lymphocytic infiltration was likewise determined in formaldehyde fixed and paraffin embedded tissue by counting lymphocytes in five representative high power fields ($\times 420$) for each tumour and calculating the average number of lymphocytes.

All patients were staged histopathologically according to the International Union against Cancer classification system.⁸

Results

Histopathological staging confirmed four cases at stage pT1, two at stage pT2, and 14 cases at stage pT3;

thus 70% of our cases showed infiltration of rete testis. Fourteen cases were classified as N₀, three as N₂, and three as N₃; one of the patients with lymph node stage N₃ also had metastases in the liver, defined as stage M1 (table 1).

Case 8 had evidence of focal selections of epitheloid cells as well as a considerable number of Langhans' giant cells. Varying amounts of lymphocytes ranging from about 40 to 500 per high power field were seen, with formation of lymph follicles in cases 16 and 18. The lymphocytic infiltration was mostly concentrated in the supporting stroma and sometimes scattered in clumps throughout the tumours. Extensive necrosis was found in cases 3, 5, 6, 10, and 17 and circumscribed necrosis in well restricted areas in cases 13 and 19. In these, lymphocytic infiltration was not determined in

areas adjacent to tumour necrosis. Tumour diameter and volume varied considerably (table 1).

For immunohistological determination of proliferating tumour cells, the APAAP technique was successfully applied in 17 of our 20 cases. The remaining three cases were examined by the immunoperoxidase technique.

Use of Ki-67 showed a fraction of proliferating tumour cells ranging between 50 and 80% (table 1). In most cases, the nucleoli in particular stained strongly (figure), in an intranuclear pattern of granules and clots of irregular density. Despite varying intensity the reaction product was clearly visible. Thus reactive and non-reactive tumour cells—that is, proliferating tumour cells and those in the G₀ state—could easily be distinguished. The distribution of proliferating cells

Table 1 Summary of pathological, immunohistological and clinical data

Case No	Age	Percentage of Ki-67 positive cells	Staging	Volume (cm ³)	Lymph* cells	Necrosis	Treatment	Outcome (survival in months)	
1	44	50	pT3 N0 M0	10	170	None	Semicastration, radiotherapy	No evidence of disease	7
2	46	50	pT3 N2 M0	128‡	260	None	Semicastration, radiotherapy	No evidence of disease	19
3	43	50	pT3 N3 M0	630	81	Extensive	Semicastration, chemotherapy	No evidence of disease	14
4	29	50	pT1 N2 M0	37	97	None	Semicastration	Died of other causes	11
5	36	55	pT2 N0 M0	98	314	Extensive	Semicastration, radiotherapy	No evidence of disease	7
6	29	55	pT3 N0 M0	45	399	Extensive	Semicastration, radiotherapy	No evidence of disease	21
7	36	55	pT1 N0 M0	19	36	None	Semicastration, radiotherapy	No evidence of disease	12
8	26	60	pT1 N0 M0	4	500	None	Semicastration, radiotherapy	No evidence of disease	10
9	49	60	pT3 N0 M0	15	135††	None	Semicastration, radiotherapy	No evidence of disease	7
10	54	60	pT3 N2 M0	9	300	Extensive	Semicastration, radiotherapy	No evidence of disease	20
11	30	60	pT3 N0 M0	6	290	None	Semicastration, radiotherapy	No evidence of disease	26
12	30	60	pT3 N0 M0	1	460	None	Semicastration, radiotherapy	No evidence of disease	20
13	44	65	pT1 N3 M0	972	90	Circumscribed	Semicastration, chemotherapy, radiotherapy	No evidence of disease	19
14	45	70	pT2 N0 M0	154	83	None	Semicastration, radiotherapy	No evidence of disease	12
15	41	70	pT3 N0 M0	144	350	None	Semicastration, radiotherapy	No evidence of disease	20
16	29	70	pT3 N3 M1†	1	250	None	Semicastration, radiotherapy, chemotherapy	Died of disease	28
17	47	70	pT3 N0 M0	429	115	Extensive	Semicastration, radiotherapy	No evidence of disease	35
18	38	75	pT3 N0 M0	180	310	None	Semicastration, radiotherapy	No evidence of disease	4
19	32	75	pT3 N0 M0	7	240	Circumscribed	Semicastration, radiotherapy	No evidence of disease	23
20	31	80	pT3 N0 M0	180	180	None	Semicastration, retroperitoneal lymphadenectomy	No evidence of disease	4

*Average number of lymphocytes per high power field.

†tumour metastases in the liver;

‡only one diameter reported, the others set to 50% of the largest;

††tumour with granulomatous reaction.

Figure Immunohistological staining of proliferating seminoma cells by monoclonal antibody Ki-67. Intranuclear reaction product is clearly visible in about 50% of tumour cells.

Table 2 Tumour stage v average proportion of proliferating tumour cells

Stage (pT)		Stage (N)	
pT1	57.5%	N0	63.9%
pT2	62.5%	N1-3	57.5%
pT3	63.2%		

was almost uniform throughout our samples, the edge length being about 0.9 cm; cryostat sections were 7 µm thick.

Table 2 shows the correlation between the fraction of proliferating tumour cells detected by Ki-67 and the pathological stage. There was a slight tendency towards a greater growth fraction the more advanced the pT stage, the proportion of proliferative cells ranging from about 57 to 63%. Cases with lymph node stage N0 tended to have a smaller growth fraction in the primary tumours compared with stage N₁₋₃ seminomas (table 2).

There was no positive correlation between growth fraction and density of lymphocytic infiltrate. Almost all percentiles of the growth fraction comprised specimens with both dense and scanty infiltration. The one case with a granulomatous reaction fell within the intermediate range of proliferative activity.

It is obvious that there was no clearcut association between growth fraction and volume of tumour mass, but most of those tumours with a larger volume were in the upper range of proliferative activity.

The time from initial surgery up to the date of this study ranged between four and 35 months. Thus

comparability of the follow up data is somewhat limited. Case 16, with seminoma metastases in the liver, died of the disease 27 months after diagnosis, and case 4, 11 months after diagnosis of other causes. All other patients were still alive without recurrent disease at the time of writing.

Discussion

This study shows that the overall growth fraction of seminomas ranges from 50 to 80% with an average of over 60%. This finding agrees with the proliferative activity in seminomas detected by ³H-thymidine labelling in vivo^{9,10} and in vitro.¹¹ A homogeneous pattern of proliferating cells was found in all of our specimens, which agrees with the findings of Silvestrini *et al.*¹⁰

There was no positive correlation between proliferative activity and tumour size at the time of diagnosis,^{4,12} because rapidly growing tumours are detected and removed at an earlier stage of growth.¹³ The assumption that proliferative activity and invasive behaviour may be divergent attributes of tumour cells¹⁴ is corroborated by the negative correlation shown between lymph node stage and growth activity in seminomas. This tendency, however, was barely perceptible and needs to be confirmed in a greater number of cases. Silvestrini *et al* found an opposite tendency, but no significant association between tumour growth and lymph node stage by H³-thymidine labelling.¹¹

No association was observed between grade of

atypia and growth fraction. Hence proliferative activity determined by immunohistological examination is not a reliable criterion for defining anaplastic seminoma,^{9 15 16} an aspect already noted by Hochstetter¹⁷ in relation to the mitotic index.

In accordance with Rabes *et al* this study could find no correlation between lymphocytic infiltration and proliferative activity.⁹ Some studies^{4 12 15} have found an association between lymphocytic infiltration and prognosis, but infiltration has not been positively correlated with tumour growth.

Only one patient in our series died of a seminoma. Thus at present no convincing conclusions can be drawn from this study in respect to the prognostic importance of rate of growth. The association with prognosis was not examined in the studies quoted above.^{10 11} Determination of mitotic index is the only method for assessing the proliferative activity that has been correlated with the prognosis of seminomas, but the results have been inconsistent.¹ Survival alone cannot be regarded as a criterion for the determination of prognosis as the survival of treated patients is more than 90%. Thus the term "prognosis" has to be redefined for seminomas.¹³

Proliferating cells are much more sensitive to radiotherapy and chemotherapy than non-proliferating cells.¹⁸⁻²⁰ Hence the high proliferative activity not only confirms the data yielded by ³H-thymidine labelling but also explains the well documented sensitivity of seminomas to radiotherapy^{18 21-24} and chemotherapy.²⁴⁻²⁷

Determining the growth fraction may, in selected cases, influence therapeutic decisions—for example, in favour of forced systemic treatment in cases with extremely high proliferation, and surgery in those with low proliferative activity. The use of the monoclonal antibody Ki-67 is—as has recently been stated by Gatter *et al*²⁸—a reliable and easy method for routine histopathological diagnosis and is thus also a useful part of the diagnostic panel in seminomas.

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