Urol Res (1995) 23:211–213

ORIGINAL PAPER

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Prognostic factors in patients with pathological stage I non-seminomatous testicular germ cell tumors and tumor recurrence during follow-up

Received: 22 November 1994 / Accepted: 15 February 1995

Abstract Clinical staging in patients with stage I non-seminomatous germ cell tumors (NSGCTs) of the testis fails in 30% to correctly assess pathological stage since microscopic and small-volume retroperitoneal disease is not detectable on computed tomography of the abdomen. Patients staged by retroperitoneal lymph node dissection as pathological stage I incur a distant (chest or serological) tumor relapse rate of 7–15% during follow-up. Recently, we reported on new risk factors as predictors of pathological stage by flow cytometric DNA analysis in clinical stage I patients. These same methods were applied to a group of 14 pathological stage I patients who subsequently had either chest or serological recurrence. The findings in this group of patients were compared with those in a group of 47 pathological stage I patients who did not experience recurrence. In pathological stage I NSGCT patients with distant (chest or serological) tumor relapse, we found by histological evaluation and DNA analysis of the original orchiectomy specimen proliferative tumor activity to be significantly predictive of relapse. Much as proliferative activity of the primary tumor is predictive of retroperitoneal metastasis, it may be a predictor of recurrence in pathological stage I patients.

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Key words Non-seminomatous testicular germ cell tumors · Tumor recurrence · Prognostic parameters · Flow cytometry

In 70% of patients presenting with clinical stage I non-seminomatous germ cell tumors of the testis, surgical staging by nerve-sparing retroperitoneal lymph node dissection shows no evidence of retroperitoneal disease. However, 7–15% of patients with pathological stage I will show tumor recurrence during follow-up [2, 5, 7]. Relapse occurs outside the operative field, mainly (70%) in the chest [2].

In patients who present as clinical stage I and have three or four histological risk factors as defined by the Medical Research Council (MRC) group (embryonal carcinoma, vascular invasion, lymphatic invasion, absence of yolk sac tumor), the risk of being pathological stage II is approximately 50% [3, 7]. Recently, Moul found percentage of embryonal carcinoma and vascular invasion to predict pathological stage [6]. In a series of 102 clinical stage I NSGCT patients, we used in addition to traditional histological parameters DNA analysis by flow cytometry to assess new risk factors for occult metastasis. In multivariate analysis, pathological stage II was correctly predicted in 91% of patients using either 100% embryonal carcinoma or a high proliferative activity of the tumor assessed by flow cytometric measurement of the G_2M+S fraction of the cell cycle as predictive factors [1]. Whether these new risk factors are able to predict tumor relapse in the small group of patients who initially had no node involvement at the time of retroperitoneal lymph node dissection has not been elucidated. Therefore, these methods were applied to pathological stage I patients who subsequently recurred to determine if this group of patients differed from pathological stage I patients who did not recur.

Material and methods

Primary orchiectomy specimens of 47 pathological stage I NSGCT patients without relapse and 14 pathological stage I patients with subsequent tumor recurrence were retrospectively analyzed. Tumor material was requested from referral hospitals who had performed the orchiectomy between January 1987 and December 1990. Group 1 (47 pathological stage I patients without relapse) consisted of the same patients we used for analysis in our previous study [1]; group 2 (14 pathological stage I patients with recurrence) consisted of unselected patients of the same time period. Both groups were not consecutive since requested tissue blocks were not returned from all hospitals. All returned blocks were included in this study. All patients without tumor recurrence had a follow-up of more than 2 years after surgery. A mean of 2.5 tissue blocks per patient (range 1–6) were histologically reevaluated. For flow cytometry a single-cell solution was prepared using a modified Hedley's technique of deparaffinizing [4] as previously described [1]. Staining was performed with propidium iodide. DNA histograms were evaluated using a Coulter Profile II flow cytometer (Coulter Electronics, Hialeah, Fla., USA) and Modfit software (Verity Software House, Topshaw, Me., USA). A total of 25 000–50 000 events were measured per sample and proliferative cell cycle phases were calculated using a trapezoid model with debris correction integrated in the software. Histopathological review and flow cytometry were performed independently without knowledge of clinical follow-up.

In order to minimize sampling error, only those tissue blocks were taken for statistical analysis in which histological review and flow cytometry showed representative tumor tissue in at least 20% of the block. Of these, the most proliferative or highest index values were selected. Univariate two-tailed Student's *t*-test, chi-square tables or univariate logistic regression analysis was performed for all parameters in order to screen for variables for entry to multiple logistic regression analysis. For multivariate analysis all variables which were only marginally related were considered ($P < 0.1$). Differences were considered to be significant if $P < 0.05$.

Results

Histopathological review and univariate regression analysis revealed no correlation between tumor relapse and percentage of embryonal carcinoma in the primary tumor. Only 3 of 14 patients with tumor relapse were found to have more than 75% embryonal carcinoma, and 4 of 14 showed less than 25% embryonal carcinoma. Ten of 14 patients (71%) with subsequent tumor relapse were diagnosed with yolk sac tumor elements in their mixed germ cell tumor, which was considered in various studies (e.g., MRC) to be a prognostic parameter for low risk of recurrence [3, 7]. Eleven of 14 patients (78%) who recurred showed vascular invasion of the testicular tumor compared with 13 of 47 (28%) pathological stage I patients who did not subsequently recur (Table 1). In both groups lymphatic invasion was more frequent than venous invasion.

DNA analysis by flow cytometry showed in univariate analysis significantly higher proliferation rates in patients with tumor relapse. In 47 patients without relapse the mean fraction of aneuploid cells in G_2M+S-phase was 24.7% (SD 16.5) compared with 43.3% (SD 15.5) in relapsers. DNA indices were only marginally different in both groups (Table 2). Only 1 of 14 patients with relapse was found to have a diploid tumor.

Multivariate analysis showed aneuploid cells in G_2M+S-phase to be the best predictive parameter of tumor recurrence in pathological stage I patients ($P = 0.0006$). Vascular invasion ranked second in multivariate analysis ($P = 0.0291$). Using a "cutoff" of 31% for the aneuploid cell fraction in G_2M+S-phase, 13 of 14 (93%) patients with recurrence and 35 of 47 (74%) patients without recurrence were correctly classified (Table 3). Of the 12 misclassified patients without recurrence, 5 (42%) had vascular invasion.

Table 1 Vascular invasion (VI) as predictor of tumor relapse

	Nonrelapsers	Relapsers	
VI +	13	11	Positive predictive value: 11/24 (46%)
VI −	34	3	Negative predictive value: 34/37 (92%)
Total	47	14	
Sensitivity	78%		
Specificity	72%		

Table 2 Univariate analysis (two-tailed Student's *t*-test and chi-square test) of histopathological and flow cytometric parameters predicting tumor relapse in pathological stage I NSGCT patients (*SD* standard deviation, *NS* not significant). The remaining parameters (% yolk sac tumor, % mature or immature teratoma, % choriocarcinoma, extratesticular involvement, greatest tumor dimension, rete testis involvement) were not correlated to subsequent relapse in pathological stage I patients

Parameter	Mean pathological stage I without relapse (SD) ($n = 47$)	Mean pathological stage I with relapse (SD) ($n = 14$)	*P* value
Aneuploid G_2M+S (%)	24.7 (16.5)	43.3 (15.5)	0.0004*
Vascular invasion	13/47	11/14	0.0024*
DNA index	1.39 (0.33)	1.61 (0.39)	0.04*
Embryonal carcinoma (%)	38.7 (32.6)	44.3 (33.2)	NS

* Significant ($P < 0.05$)

Discussion

Traditional histopathological parameters fail to predict occult metastasis in 50% of clinical stage I NSGCT patients who are felt to be "high risk." In the MRC study, 40 of 83 patients with three or four classical risk factors showed tumor metastasis and these 83 high-risk patients represented only 23% of all investigated patients. The same study demonstrated a higher incidence of vascular invasion in clinical stage I patients who

Table 3 DNA analysis (proliferation rate $G_2M + S$) as predictor of subsequent tumor relapse

	Nonrelapsers	Relapsers	
$G_2M+S>31\%$	12	13	Positive predictive value: 13/25 (52%)
$G_2M+S\leq 31\%$	35	1	Negative predictive value: 35/36 (97%)
Total	47	14	
Sensitivity	93%		
Specificity	74%		

subsequently developed tumor metastasis outside the retroperitoneum [7].

Vascular invasion remained in our group of pathological stage I NSGCT patients the only histopathological risk factor for recurrence. These patients showed no higher percentage of embryonal carcinoma, and yolk sac elements were more frequently present than in non-relapsers. Using the MRC classification, our patients with subsequent distant tumor relapse would be classified as low risk (0–2 risk factors).

Interestingly, lymphatic invasion was more common than venous invasion. This may be due to the difficulties of discriminating small venous from lymphatic vessels. DNA analysis, however, aided in predicting recurrence. Thirteen of 14 patients with relapse showed a high tumor proliferation rate (sensitivity 93%). Using this risk factor alone, only one patient with subsequent tumor relapse was not detected (Table 3). The specificity of this risk factor was 74%; 12 of 47 patients without recurrence were found to have a high proliferation rate (Table 3).

Evaluation of a low G_2M+S proliferation rate ($\leq 31\%$) was able to correctly classify 35 of 36 nonrelapsers (negative predictive value 97%). High proliferation rates ($>31\%$), however, were less effective in predicting the relapsing patients (13 of 25 correctly classified, positive predictive value 52%).

Using the risk factor vascular invasion, 11 of 14 relapsers (sensitivity 78%) were correctly predicted; 13 of 47 patients were misclassified as high risk (specificity 72%) (Table 1). The positive predictive value of this parameter was 46% (11 of 24 patients with vascular invasion subsequently were found to have tumor recurrence). But vascular invasion was able to correctly predict disease-free follow-up in 34 of 37 patients (negative predictive value 92%). A combined approach of vascular invasion and high proliferation rate was not able to better classify relapsers and nonrelapsers.

In this study, DNA analysis contributed a new independent risk factor to detect subsequent relapse in pathological stage I NSGCT patients with a sensitivity of 93%. Remarkable is a high negative predictive value of both risk factors, proliferative activity as well as vascular invasion; patients with either low proliferation rates or absence of vascular invasion had an extremely low risk of relapse. In our previous study we demonstrated a correlation between occult retroperitoneal lymph node metastasis and tumor proliferation rate in clinical stage I patients [1]. Hence, tumor proliferation rates seem to play a role in metastatic tumor behavior. Vascular invasion may also be involved in tumor metastasis. However, the exact events leading to tumor metastasis are undoubtedly complex and in large part remain to be elucidated. Our data in this small group of nonconsecutive patients show that proliferation rates in early-stage testicular tumors may help to predict tumor behavior.

Certainly, no patient management issues have been resolved based on this pilot study. There is currently no clinical application for assessment of proliferation rates in pathological stage I patients since all patients enjoy a favorable prognosis even after subsequent recurrence and chemotherapy. However, as investigation into mechanisms of tumor behavior and recurrence proceeds, the proliferation rate of the aneuploid cell fraction should be evaluated as one factor related to tumor metastasis. If further studies show that absence of risk factors is able to predict a relapse-free course of pathological stage I patients at the 95% level, it might become possible to modify their follow-up.

Acknowledgements The authors thank Terry Reister, Department of Biostatistics, Indiana University, for assistance in statistical analysis and Edwin B. Walker, Laboratory for Diagnostic and Analytical Cytometry, Indianapolis, USA, for technical assistance in flow cytometry.

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