

**Clinical prognostic factors and investigation of  
chemoresistance in testicular cancer**

**Lajos Géczi M.D.**

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Department of Medical Microbiology  
Faculty of Medicine  
University of Szeged

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## 1. INTRODUCTION

**1.1.** Testicular tumors are rare, making up only 1 per cent of all cancers in men, however this tumor has become an extremely important oncological disease.

1. Testicular germ cell tumor is the most common carcinoma in young men aged 15-35 years and thus has the potential to greatly shorten productive years of life.
2. Available serum markers (alfafetoprotein, AFP and human chorionic gonadotropin, HCG) allow the clinician to make important and accurate treatment related decisions.
3. Testicular cancer has been a model for multidisciplinary care, as surgical resection of postchemotherapy radiographically persistent disease can improve the cure rate.
4. Germ cell tumors have become an excellent testing ground for active experimental drugs (i.e., cisplatin, etoposide, and ifosfamide), all of which were approved primarily on the basis of data from studies of testicular cancer.

The prognosis of germ cell tumor cases has improved markedly following the introduction of cisplatin-based chemotherapy. In parallel with advances in the management of testicular cancer, growing concern about late adverse effects of treatment has been expressed.

The risk-adapted treatment strategy focuses on reducing toxicity in good-risk patients, while preserving treatment efficacy. However, more intensive chemotherapy regimens and treatment policies used in poor risk and relapsed patients. The goal of chemotherapy for patients with germ cell tumors is never merely palliation or prolongation of survival, but cure. The chance of cure for patients with poor prognosis - those with relapse or resistance to cisplatin based chemotherapy - is low because no standard treatment is available.

**1.2.** In clinical oncology, chemoresistance means that tumor cells avoid cytotoxic damage and no clinical response can be achieved despite chemotherapy being used. Two types of chemoresistance are distinguished: primary or intrinsic, and secondary or acquired resistance. Drug resistance (DR) can arise as a consequence of various mechanisms, however the following types can be considered when investigating chemoresistance in clinical oncology: (i) pharmacokinetic, (ii) pharmacogenetic and (iii) pharmacogenomic.

Only a few studies have been dedicated to investigating these mechanisms in testicular cancer. The exploration of whether there are molecular as well as clinical differences that separate curable from incurable disease remains highly important. This thesis highlights some clinical and clinicopathological works dedicated to this field.

## 2. AIMS OF THE THESIS

**2.1.** Between 48-93% of patients with testicular cancer are cured following therapy in Europe, with the prognosis being less favorable in eastern European countries. In Hungary, the likelihood of patients not responding to cisplatin based chemotherapy may be higher than in developed countries because of the more advanced stages and the resultant diagnostic delay. It has been shown that a delay of more than 3 months is correlated with a decreased 5-year survival. For this reason we started an educational and early detection program for testicular cancer in 1995.

- The goal was to determine, by an analysis of the results of the first 3 years, the efficacy of such a program on the early detection of testicular cancer.

**2.2.** The risk of contralateral tumor among patients cured for testicular cancer is about 2.5-5%. No predictive clinical parameter is available for the development of a second tumor. Most second testicular tumors are discovered by the patients following the onset of symptoms. Regular follow-up may allow earlier diagnosis of a second tumor.

- To determine the incidence, prognosis, clinical and histological characteristics, treatment and outcome of patients with bilateral testicular cancer in Hungary.
- To determine which clinical parameters might predict a metachronous testicular tumor, and whether regular follow-up may help in the early diagnosis of second testicular cancers.

**2.3.** Identifying of molecular markers for genes associated with - or responsible for - individual resistance mechanisms helps to inform current clinical studies and provides insights into clinical results achieved. These mechanisms have been poorly studied in testicular cancer, which is why we started clinicopathological trials to investigate the **clinical relevance** of these markers in testicular cancer.

- To investigate ATP-dependent drug efflux transporter proteins and genes in testicular cancer (MDR1/Pgp, LRP).
- To investigate metallothionein expression in primary germ cell testicular cancer.
- To investigate p53 protein expression in testicular cancer.
- To investigate mdm-2 expression, because little data has been reported in the literature about its role in testicular cancer.
- To investigate Bcl-2 expression in testicular tumors, about which there are no current publications.

### **3. PATIENTS AND METHODS**

**3.1.** Information describing the early signs of testicular cancer, the risk factors, the correct method of testicular self examination and the importance of early detection was disseminated through the media. Men who responded were given an appointment to have a medical examination. The medical examination consisted of physical and ultrasound examination of the testicles and in any case of suspicious findings tumor markers were also checked. An Acuson 128 PX ultrasound device, with a 7 MHz linear transducer was used for the testicular ultrasound examination. Between April 1995 and April 1998 5056 volunteers participated in the program.

Findings were analyzed according to the volunteers classification, who were divided into two main groups based on the presence or absence of complaints. The first group was subdivided according to the nature of the complaint observed through testicular self-examination i. e. pain, sensitivity to palpation of the testicle, palpable lump, swelling of the testicle or a complaint unrelated to the testicle, such as dysuria, impotence etc.

The clinical details of patients with a testicular tumor detected are also presented. The delay in the diagnosis of patients treated by chemotherapy in our Department in 1994 and in 1998 was also retrospectively analyzed and compared to measure the educational impact of the program.

The mortality rate of testicular cancer patients in Hungary between 1994 and 1998 was also analyzed.

**3.2.** Bilateral testicular cancer patients were retrospectively explored among the 2386 testicular tumor patients who were registered in our Department between November 1988 and November 1998. Detailed information on patient characteristics was obtained from the patients' hospital records – such as time of original surgery, location and histology of primary tumor, extent of the disease, serum concentration of hCG, AFP and LDH, history of testicular abnormalities, treatment, response to treatment, follow-up period, data on second (non germinal) carcinoma. Pathology reports of all 72 patients were reviewed in our Institute. The patients were divided into two main groups based on the synchronous or metachronous appearance of bilateral testicular cancer.

Patients with metachronous tumors were further subdivided into two subgroups, group A (patients who were followed in the institutional surveillance policy after treatment of the first cancer) and group B (patients who were lost to follow up or patients who did not participate in the last two scheduled follow-up appointments before diagnosis of second testicular cancer). In the groups with synchronous and metachronous testicular cancers we analyzed the distribution of the following parameters: main histological subtypes, clinical stages, treatments, survival and risk factors (cryptorchidism, infertility, atrophic testis, familial history of testicular tumor).

In the first and second metachronous cancers we investigated the histological characteristics, such as the proportion of pure seminoma, the presence of vascular invasion, and the presence of embryonal components in the tumors and the effects of previously applied treatment on the incidence of the secondary tumors.

The interval between tumors was analyzed in relation to the patient's age, previously applied treatment and histological subtypes of tumors.

**3.3.** Monoclonal antibodies were used for immunohistochemistry in paraffin section of primary testicular germ cell tumors, according to the protocol of the manufacturer (Pgp (C 219, Centocor USA), p53 (Dako clone DO-7), MT (Dako MT E-9), mdm-2 (clone IB10, Novocastra, UK), bcl-2 (clone 124, Ig G1; Dako) LRP (clone LRP-56, Santa Cruz Biotech. Inc. California, USA). Specimens were obtained by semicastration of 75 (for Pgp), 77 (for p53), 77 (for MT), 70 (for bcl-2) and 70 patients (for LRP) with testicular cancer, respectively. No prior chemotherapy and radiotherapy were used. Statistical test was used for evaluation of the relationship between the extent of target expression, i.e. Pgp (+, -), p53 (high vs. low), MT (high vs. low), mdm-2 (+, -), bcl-2 (+, -), and LRP (+, -), and histological subtype (seminoma vs. nonseminoma), metastatic potential (no metastasis vs. metastatic tumors) clinical stage (early stage group, I, II/A vs. later-stage group, II/B, II/C and III) of tumors and response to chemotherapy (sensitive vs. resistant tumors).

#### **3.4. Statistical analyses**

Categorical variables were compared by the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared by the Wilcoxon Mann Whitney test. Binominal related variables were compared by McNemar's test. The Kaplan-Meier method was used to evaluate survival. A difference was regarded as significant if the P value was  $\leq 0.05$ .

## 4. RESULTS

**4.1.** 5056 volunteers participated in the program and 32 tumors were diagnosed in 30 patients (0.6%). Among the 5056 volunteers, 2714 were complaint-free and 2342 patients presented different complaints.

In the complaint-free population 1323 men had no physical or radiology findings (49%), but in the remaining 1391 men, 1599 different findings were detected by physical examination and/or TUS. No tumors were found in the complaint-free population (Table 1).

Of the 2342 men with different complaints, 532 (23%) had no detectable findings, but in the remaining 1810 men 2194 findings were discovered. The incidence of patients with tumors in this group was 1.66%, representing 1.5 % of the findings detected. The incidence of men having tumor in the group of 2342 volunteers with complaints was 1.28 %.

Table 1. Distribution of findings in the complaint-free population and in the population with complaints

Findings	Complaints-free population 2714 volunteers		Population with complaints 2342 volunteers		P	Complaints-free population 1599 findings %	Population with complaints 2194 findings %
	No	%	No	%			
Epididymal and testicular cyst	526	19.4	676	28.9	<0.001	32.9	30.8
Testicular atrophy	124	4.6	136	5.8	0.06	7.8	6.2
Hydrocele	480	17.7	585	25.0	<0.001	30.0	26.7
Epididymitis	39	1.4	232	9.9	<0.001	2.4	10.6
Varicocele	399	14.7	497	21.2	0.10	25.0	22.7
Tumor	0	0.0	30*	1.3	<0.001	0.0	1.5
Microcalcification	11	0.4	11	0.5	0.73	0.7	0.5
Others	20	0.7	25	1.1	0.22	1.3	1.1

\*30 patients with 32 tumors

The volunteers with complaints were subdivided according to the main symptoms as follows: 464 patients (20%) had a palpable lump, 228 (10%) had a swollen testicle, 472 (20%) had testicular pain, 897 (38%) had sensitivity to palpation of the testicle and 281 patients (12%) had symptoms unrelated to the testicles (Table 2).

No tumors were found in the group with pain, sensitivity, or complaints unrelated to the testicle. The percentages of men with various abnormalities in these groups were 79% (373), 71% (363) and 67% (186) respectively, and consisted mainly of cysts, hydroceles and varicoceles.

Of the 464 men who palpated a lump, 64 (14%) had no detectable abnormalities, but in the remaining 400 men, 477 abnormalities were discovered, among them 22 tumors. The incidence of men with tumors in this group was 4.5%, and these represented 4.6% of all abnormalities. In men with a palpable lump, cysts and varicoceles were observed most frequently.

Among the 228 men whose main complaint was a swollen testicle, no abnormalities were detected in 13 (5.4%), but in the remaining 215 men there were 249 findings, and 10 tumors were detected. The incidence of patients with tumor was 3.9% in this group, representing 4% of all detected abnormalities. Hydrocele was the most frequent finding in men (56%) with a swollen testicle.

In the group with complaints, the occurrence of testicular cancer was most frequent (1.6%) in the 15 to 40 age group. Only 3 testicular cancers were detected in men over the age of 45 (0.3 %), two of these being seminomatous tumors.

Table 2. Findings according to the volunteers' main complaint

Findings	Pain 373 men 457 findings	Sensitivity 636 men 782 findings	Palpable lump 400 men 477 findings	Swollen testicle 215 men 249 findings	Unrelated complaints 186 men 229 findings
Epididymal and testicular cyst	125 (27.4%)	246 (31.5%)	207 (43.4%)	46 (18.5%)	52 (22.7%)
Testicular atrophy	20 (4.4%)	63 (8.1%)	11 (2.3%)	10 (4.0%)	32 (14.0%)
Hydrocele	103 (22.5%)	209 (26.7%)	75 (15.7%)	139 (55.9%)	59 (25.8%)
Epididymitis	62 (13.6%)	88 (11.2%)	50 (10.5%)	19 (7.6%)	13 (5.7%)
Varicocele	141 (30.9%)	169 (21.6%)	98 (20.6%)	21 (8.4%)	68 (29.7%)
Tumor	0 (0%)	0 (0%)	22 (4.6%)	10 (4.0%)	0 (0%)
Microcalcification	2 (0.4%)	4 (0.5%)	2 (0.4%)	0 (0.0%)	3 (1.3%)
Other	4 (0.8%)	3 (0.4%)	12 (2.5%)	4 (1.6%)	2 (0.8%)

During the 3-year period, 4 benign testicular tumors were discovered among 5056 volunteers (0.08%). The histological findings were: cavernous hemangioma, dermoid cyst, Leydig cell tumor and adenomatoid tumor. Among the 26 men with germ cell testicular tumors, 19 stage I tumors were detected. The median age was 33 years (range of 20-48 years) and the overall median duration of complaints was less than 12 weeks (range 1-48 weeks). Fifteen seminomas (2 of them bilateral), and 13 non-seminoma tumors were diagnosed. The clinical stages were: 9 I/A, 9 I/B, 1 I/S, 3 II/A, 1 II/B, and 2 III/B. One patient refused further treatment and was lost to follow-up.

According to the IGCCCG classification, all patients belonged to the good prognostic group, except for the patient who was lost to follow-up after orchidectomy. All of our treated patients are in CR and are probably cured of their disease. The median follow up time of patients in December 1999 was 36 months (16-49 months).

Concerning the educational aspect of the program we did not observe a significant decrease in the diagnostic and medical delay in the patient population treated by chemotherapy in our department between 1994 and 1998 ( $p=0.58$ ). The impact of our educational and early detection program on testicular cancer mortality can not be ascertained between 1994 and 1998.

**4.2.** Using a database of 2386 patients treated with testicular cancer 72 patients were found to have bilateral testicular carcinoma (3%); 19 cases (0.8%) with synchronous and 53 cases (2.2%) with metachronous tumor.

The median age of the 19 synchronous tumor patients was 38 years at the time of castration. Three patients (16%) had a history of cryptorchidism and hypoplasia, 3 patients

were younger than 30 years at the time of castration. No family history was recorded. Stage I tumors were diagnosed in 13 (68%) cases. After primary treatment 17 (90%) CR and one PR was obtained. One patient was lost to follow-up after castration. Fifteen patients (79%) are alive with no evidence of disease. The 5 year overall survival rate was 84%, three patients died, 2 due to tumor progression. The median follow-up time was 93 months (range: 38-150 months).

Fifty-three of 73 patients (74 %) had metachronous testicular cancer. The median time to the development of second tumor varied from 18 to 203 months (median, 76 months). The median age at the time of first and second tumor diagnosis was 28 and 35 years , respectively. Of the 53 patients, 2 patients (4%) had a family history of testicular cancer, 5 (9%) testicular maldescent, 7 (13%) testicular atrophy, and one (2%) azoospermia. Thirty patients (57%) were younger than 30 years at the time of first tumor diagnosis.

The clinical stage I tumor was more frequent in the case of second tumors than in cases of first tumor, but the difference was not significant,  $p < 0.23$ . The clinical stage I tumor was statistically more frequent in group A than in group B,  $p < 0.01$ , suggesting that regular follow-up might improve the early diagnosis of metachronous germ cell testicular tumor.

The distribution of the two main histological subtypes did not differ between the first and second tumor,  $p < 0.48$ . The prevalence of carcinoma embryonal component and vascular invasion in the first and second tumor did not differ,  $p < 0.52$  and  $p < 0.18$ . Nonseminoma as first tumor was diagnosed in an earlier age (median age: 27 years) than seminoma (median age: 31 year)  $p < 0.05$ . Seminoma as second tumor was diagnosed in a later age group (median: 38 years) than nonseminoma (median: 32 years),  $p < 0.045$ . The proportion of metachronous seminoma was 19%, 37% and 60% in the 5, 10, and 15-year periods after the initial castration.

The cancer interval between metachronous tumors did not differ significantly according to the first treatment. There is a tendency for a longer interval after chemotherapy (ChT) and radiotherapy (Rt) , but the number of patients is too low to provide statistically significant data.

The age of patients at the time of first and second tumor castration had no influence on the interval,  $\gamma = 0.104$  (Pearson regression model). The interval did not depend on the presence (median: 78 months,) or the absence (median: 83 months) of testicular hypoplasia in the patients history. The relationship between tumor histology with cancer interval is shown in Table 2. The first testicular cancer was followed by a statistically significant longer interval in case of seminoma (median: 121 months) than in patients with nonseminoma (median: 50 months),  $p < 0.002$ .

Table 3. Cancer intervals between metachronous tumors according to the main histological subgroups (in months)

No of patients (%)	Primary tumor	Second tumor	Median interval (range)
9 (17 %)	nonseminoma	nonseminoma*	45 (21-180)
17 (32 %)	nonseminoma	nonseminoma**	50 (21-140)
7 (13 %)	seminoma	nonseminoma	86 (31-183)
9 (17 %)	seminoma	seminoma	120 (39-170)
11 (20 %)	nonseminoma	seminoma	121 (18-203)

Remarks: \* concordant  
\*\* discordant

The treatments of first tumor yielded 52 CR (98.1%). One patient with stage II/B seminoma progressed after retroperitoneal radiotherapy, and further supraclavicular and upper mediastinal field irradiation resulted SD. Chemotherapy was applied in 26 out of 53 patients (49.0%). No relapse or progression between metachronous tumors was detected.

The primary treatment of second testicular cancer resulted in CR in 52 patients. One patient was cured by second line chemotherapy. Chemotherapy was used in 38 cases (71.7%), and a wait-and-see policy in 14 out of 53 patients (26.4%) according to the institutional policy. Of the 53 patients, 25 (75.8%) were treated with chemotherapy in group A, and 13 (65.0%) in group B. During the 42 months median follow-up time one patient died 30 months after diagnosis of the second testicular tumor, due to testicular cancer. One patient relapsed 6 months later of a CR obtained by primary treatment and was saved by second line chemotherapy and surgical resection of the residual tumor. One patient whose first testicular tumor (II/B) was treated by radiotherapy as reported above, and by chemotherapy and radiotherapy because of second testicular seminoma (III/B), died due to pancreatic cancer 50 months after second testicular tumor diagnosis. The 5-year median overall survival rate was 93%. Median follow-up was 42 months (range: 27-121 months).

**4.3.** Pgp positive reaction was detected in two (8%) of the 25 seminomatous germ cell tumors and 23 (48%) of the 50 nonseminomatous cancer. The incidence of Pgp was significantly higher in the advanced stages ( $p=0.000$ ), than in the early stages. There was a tendency towards poor response rate in tumors which are positive for Pgp immunostaining. However, this association between Pgp expression and clinical chemoresistance was not statistically significant ( $p=0.16$ ).

Seminomas and nonseminoma all expressed MT irrespective of their subtype. There was no significant difference between the two major subtypes. Ninety percent of cases with early stage disease, compared to 70% at a later-stage, showed high MT staining. This represented a tendency towards a decrease in MT expression in more advanced stages, but the difference was not statistically significant ( $p=0.24$ ). Thirty-one out of 77 patients received curative chemotherapy in advanced stages. Nine cancers showed chemoresistance (7 died, 2 had stable disease) and 7 of them (86%) expressed low levels of MT, or none at all. In contrast, 19 of 22 sensitive tumors (86%) expressed high MT levels ( $p=0.0013$ ).

Out of 77 tumors, 70 (91%) were immunoreactive for p53 to a some extent (ranging -,  $\pm$ , +, ++). Five teratomas, 1 embryonal carcinoma, and 1 mixed tumor were negative. The incidence of p53++ immunostaining was higher in seminomas (74%) than in nonseminoma (40%).

P53 expression showed significant inverse correlation with the stage of disease. There was a significant positive relationship between p53 immunoreactivity and response to treatment. High level of p53 expression correlated with better response to chemotherapy  $p=0.0012$ .

We demonstrated a statistically significant inverse relationship between p53 and Pgp immunoreactivity ( $p=0.005$ ). Furthermore, we established a significant association between p53 and MT immunostaining ( $p=0.0002$ ).

Eighty-one primary germ cell testicular tumors were investigated by immunochemistry for mdm-2 expression. Of these, 47 tumors were mdm-2 negative (58%), and 34 tumors stained positive for mdm-2. The incidence of mdm-2 immunostaining was significantly higher in nonseminomatous than in seminomatous tumors. The frequency of positive staining was higher in tumor from metastatic patients than in tumors from non-metastatic patients. Mdm-2 expression was detected significantly more frequently in advanced-stage tumors (II/B, II/C and III), as compared to tumors at early stages: I, II/A and



II/B ( $p=0.0098$ ). A significant difference in mdm-2 expression at different stages of disease was established ( $p=0.03869$ ).

Overall, out of 70 tumors, 41 carcinomas (58%) stained positively with anti-bcl-2 monoclonal antibody. The incidence of bcl-2 immunostaining was higher ( $p=0.05$ ) among nonseminoma than among seminomatous tumors. The expression of bcl-2 was more prevalent among tumors from patients with metastasis than among tumors from metastasis free patients ( $p=0.000$ ). Significant correlation between bcl-2 expression and Pgp immunostaining was established in 68 tumors investigated simultaneously ( $p=0.004$ ).

Previously we found that Pgp expression significantly correlated with advanced tumor stage and a poorer prognosis. Since the same specimens were screened for their bcl-2 immunoreactivity, we were able to demonstrate a significant correlation between these two markers, suggesting that Pgp and bcl-2 might not be independent of each other. As expected, a significant difference between the three stages of disease regarding the expression of bcl-2 ( $p=0.000$ ) was found. In this series, bcl-2 expression was clearly dominant in tumors of advanced stage.

Table 4. Correlation between LRP overexpression and response to chemotherapy in advanced stages (II/B, III).

Case	Stage	Histology	LRP +	LRP -	Response
1.	II/B	T	+		Progr., died
2.	III/B	CC+EC+S+T		-	CR
3.	III/B	T	+		MR, died
4.	II/B	EC	+		CR
5.	II/C	T		-	CR
6.	III/B	T	+		Progr., died
7.	III/B	T	+		CR
8.	III/B	T		-	Progr., died
9.	III/B	CC		-	CR
10.	II/C	T	+		CR
11.	III/B	S+T	+		CR
12.	III/A	EC		-	CR
13.	III/A	EC+T	+		PR→ + Surgery →CR
14.	III/B	S+T	+		PR, Progr, died
15.	III/B	T	+		NR, died
16.	II/B	EC+S	+		CR

Twenty-nine (41%) of 70 primary testicular cancers stained positive for LRP by immunohistochemistry: 6 out of 15 seminoma (40%) and 23 out of 55 nonseminoma (42%). Eight pure teratomas, 2 embryonic carcinomas and 13 out of 32 mixed tumors showed LRP expression. In the mixed-type tumors, besides 6 teratomas (T), in 3 cases seminoma (S), and in 4 cases embryonal carcinoma (EC) components of tumor were also stained by the LRP antibody. Pure teratomas showed significantly higher LRP expression compared to other types of germ cell tumors ( $p= 0.0418$ ). No positive reaction was found in the case of choriocarcinoma (CC) and in the choriocarcinomatous components of mixed tumor. No significant correlation between LRP expression and clinical staging was demonstrated, however LRP immunostaining was more frequent in advanced stage disease (50% versus 34%). The relationship between response to chemotherapy and LRP immunostaining in germ

cell testicular tumors at an advanced stage is demonstrated in Table 4. Out of 7 cases resistant to chemotherapy, 6 showed LRP expression in the primary testicular tumor, and of them, 5 died due to progression of the disease. The sixth was saved by the surgical removal of the remaining tumor, located in the left supraclavicular region (case 13 in table 4). Histological examination showed mature teratoma. One more patient without LRP overexpression in the primary tumor died despite chemotherapy being used.

No significant association between response to chemotherapy and LRP expression was demonstrated ( $p=0.30$ ).

## 5. DISCUSSION

**5.1.** The results of the early detection program confirm that the screening of asymptomatic patients does not necessarily lead to the detection of tumors, and the incidence of detected tumors is low even in volunteers with complaints, in spite of the growing incidence of testicular cancer.

No tumors were found in the group with pain, sensitivity, or complaints unrelated to the testicle. From our results we can conclude that physical examination alone appears to be sufficient for the first medical consultation in an early detection program in cases of men with pain, with sensitivity to palpation, and with symptoms unrelated to the testicle. In the case of a palpable lump and/or a swollen testicle, testicular ultrasound examination is obligatory to aid the physical examination at the time of the first consultation, especially in young men.

Of the 26 germ cell tumor patients, 13 had seminomas and all had stage I tumors. Among the 12 nonseminoma patients, 4 had clinically detected regional metastases, and 2 had hematogenous dissemination. This data suggests that seminoma is detected more frequently and earlier in an early detection program than nonseminoma. The low incidence of detection in the age range of 15-40 years (1,6%) and the detected rate of seminoma in our patient population (48%) does not justify an early detection program even in this age group, in spite of the increasing incidence of testicular cancer.

In most cases the diagnosis was based on the physical and ultrasound examination, confirmed by histology. Because of the early stages and the high percentage of seminoma tumor markers, had a limited role in an early detection program.

During the 3-year period, only 3-4% of the estimated testicular tumors (about 400-420 new patients) in Hungary were discovered by the program. Although early detection might help in the identification of some testicular cancers, the efficiency of the program is limited. The effect of the program on outcome is uncertain since the contribution of early detection to the probable 100% cure rate can not be estimated. The majority of diagnosed testicular cancers were stage I tumors, and all of the treated patients belonged to the good prognostic group: this fact made it possible to apply less aggressive treatment and improve the patients' quality of life.

Concerning the educational aspect of the program we did not observe a significant decrease in the diagnostic and medical delay in the patient population treated by chemotherapy in our department between 1994 and 1998. The impact of this educational and early detection program for testicular cancer mortality can not be justified, but the 3-year study interval may be too short.

Early diagnosis should be based on an educational program for the population at risk, the appropriate training of doctors and staff engaged in the health care of the young, and the use of early ultrasound examination for men with palpable lumps and swollen testicles, especially in young men.

**5.2.** An increase in the incidence of bilateral testicular cancer following the improvement in survival rates for testicular cancer has been reported. The prevalence of bilateral testicular

cancer published from 1990 to 1999 was 2.8% (range 1.0% to 4.0%), which corresponds with our data.

We could not identify clinical factors which predicted patients at risk for metachronous testicular cancer even though a higher risk has been reported by others in cases of cryptorchidism, atrophic testis, infertility, and familiar testicular cancer. These findings are in line with the data of Tekin, who concluded that every patient with unilateral testicular cancer has a risk of developing a contralateral tumor in the remaining testis.

In our series the aggressiveness of the first and second metachronous tumors did not differ significantly, which corresponds to the observation of Alberts et al, although they used other parameters besides vascular invasion to evaluate aggressiveness.

The prevalence of seminoma as second tumor did not differ according to applied treatment, but was more frequent after chemotherapy and radiotherapy. Publications have suggested a decreased incidence as well as a delay in the appearance of metachronous testicular cancer after chemotherapy and an increased incidence of asynchronous seminoma, however, the data are controversial, and further follow-up is essential.

Our results showed that the cancer interval of a metachronous testicular cancer depended on tumor histology and patient age and the probability of an early stage seminoma increased with follow-up time. Regular follow-up might improve the early diagnosis of metachronous testicular cancer, but we found no difference in survival between patients who underwent regular surveillance and those who did not.

Because of the relatively early stages distribution and the high percentage of seminoma, the prognosis for cases of contralateral testicular cancer was not worse than those with single tumors. Among the 53 patients with bilateral tumors only one patient died due to testicular cancer (1,9%). The first line treatment was highly effective, and second line therapy showed a response rate of 50% in metachronous tumors. The prognosis was good for both seminoma and nonseminoma, and in metachronous tumors did not depend on the patients' age at the time of first tumor diagnosis.

Our data supports the favorable clinical behavior of most asynchronous testicular cancer with slow development and late onset of distant metastasis and symptoms. This finding underlines the importance of patient education and self examination of the remaining testis and long term follow-up.

**5.3.** In our study Pgp immunoreactivity is associated with an advanced stage and more aggressive tumor phenotype. Our findings suggest that the higher Pgp expression in nonseminoma might be one simple explanation for difference existing between the two main histological subtypes. However the association between Pgp expression and chemoresistance approached, but did not reach statistical significance. Our results show that there is no causal relationship between MT overexpression and cisplatin resistance. Furthermore, MT overexpression, being one possible factor correlated to chemotherapy sensitivity, may predict a better response to chemotherapy. P53 expression, like MT, was found to predict a better response to chemotherapy. High level of p53 expression correlated with better response to chemotherapy. Our results suggest that negative p53 or MT, and positive Pgp immunoreactivity, may identify a patient subgroup with less responsivity to standard chemotherapy, and thus a worse prognosis. It was also shown, that bcl-2 is expressed in testicular cancer and is associated with a more advanced malignant phenotype of this tumor.

The prevalence of p53 overexpression in the early stage tumors suggests that the overexpression of this protein is an early event in germ cell tumor progression. P53 expression is frequently found in the carcinoma in situ known as a precursor of germ cell testicular cancer.

The frequency of mdm2 immunostaining was significantly higher in tumors from metastatic patients, then in tumors from metastatic-free patients, and the incidence of mdm2

expression increased significantly with an increasing in stage, implicating the role of mdm2 in tumor aggressiveness and progression. Same results were reported in cases of human osteosarcoma and in breast cancer. Furthermore the mdm2 immunostaining may provide a tool to identify patients with clinical stage I testicular germ cell tumor who carry a high risk of developing disseminated disease and may benefit from adjuvant chemotherapy. To validate this hypothesis further clinical trial is essential.

Our results showed positive correlation between LRP expression and pure teratoma ( $p=0.04$ ). Similar observation was published by Mayer et al. In contrast to Zurita et al we could not recognize positiv LRP immunostaining in the more aggressive choriocarcinoma and choriocarcinoma component of the mixed tumors. We also did not observed significant correlation between LRP positivity, clinical stage and response to chemotherapy. Further studies is required to evaluate the exact clinical role of LRP expression is this rare tumors.

Whether the examined factors for chemoresistance will increase the predictive power of current clinically oriented prognostic models remains to be determined. The use of methods with the ability to investigate multiple factors at the same time are probably necessary in predicting the response of individual tumors to chemtherapeutic regimens.

## 6. CONCLUSIONS

1. Despite an increasing incidence of testicular cancer, the screening of asymptomatic volunteers does not necessarily lead to the detection of testicular tumors.
2. Despite the increasing incidence of testicular cancer the widespread use of an early detection program, the examination of patients who reveal symptoms through testicular self-examination, cannot generally be recommended.
3. Early diagnosis should be based on an educational program for the population at risk, the appropriate training of doctors and staff engaged in the health care of the young, and the use of early ultrasound examination for men with palpable lumps and swollen testicles, especially in young men.
4. The prevalence of synchronous and metachronous bilateral testicular cancer is 0.8 and 2.2 %, respectively.
5. We could not identify clinical factors for predicting which patients are at risk of developing metachronous testicular germ cell tumor.
6. The prognosis for bilateral testicular cancer patients is good. Regular follow-up might improve the early diagnosis of metachronous testicular cancer, but does not improve the prognosis or increase the survival rate of patients.
7. The interval between metachronous tumors depends on tumor histology and the age of the patient, and the probability of an early stage seminoma increases with follow-up time.
8. The ATP-dependent membrane transporter Pgp, and LRP may be possible clinical prognostic factors in cases of testicular tumor.
9. Expression of p53 and MT correlate with tumor sensitivity to chemotherapy, and bcl-2 and mdm-2 expression are related to tumor progression in testicular germ cell tumor.

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## **PUBLICATIONS RELATED TO THE THESIS**

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