REFINEMENTS IN THE MANAGEMENT OF TESTICULAR GERM CELL TUMOURS

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Refinements in the management of testicular germ cell tumours

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There are some things which cannot be learned quickly, and time, which is all we have must be paid heavily for their acquiring. They are the very simplest things, and because it takes a man's life to know them, the little new that each man gets from life is very costly and the only heritage he has to leave.

Uit: "Death in the afternoon" van Ernest Hemingway

Aan Nicole
Aan mijn ouders

GENERAL INTRODUCTION AND OUTLINE OF THESIS

Based on:

"Important factors in diagnosis and staging of testicular germ cell tumours" by J.R. Spermon, F.M.J. Debruyne and J.A.Witjes in Current Opinion in Urology 2002 and "The management of testicular germ cell tumours" by J.R. Spermon and F.M.J. Debruyne in Annual European Course in Urology 2002, Rome, Italy.

I. Epidemiology and classification of testicular germ cell tumours

Introduction

Testicular germ cell tumours (TGCT), although an uncommon malignancy, evokes widespread interest in healthcare. Three decades ago, it was the most common solid tumour cause of death in young males. The development of a multi-modal treatment policy has resulted in revolutionary changes in the cure rate. Today, TGCT is one of the most curable solid malignancies and serves as a paradigm for the treatment of cancer.

Incidence

Testicular germ cell cancer represents 1% of all male neoplasms. The age-related incidence curve of testicular germ cell cancer has a bimodal distribution. The major peak occurs between the ages of 15 and 35. Non-seminomatous germ cell tumours represents the predominant histopathologic diagnosis up to the age of 35, while the peak age for seminomatous germ cell tumours is approximately 10 years older. The rates declining more rapidly beyond the age of 40 for nonseminomas compared to seminomas.[1] As a result, virtually all testicular tumours among older men are seminomas.

The incidence of the disease varies markedly based on geographic distribution, with the highest incidence in northern Europe and North America and the lowest in Africa and Asia.[2,3] Race is also of importance, with the highest incidence in Caucasian compared to black and Hispanic males.[4]

The incidence is rising in the United States and in parts of Western Europe.[2,3] Between 1973 and 1995 the incidence of testicular cancer in the United States increased 51% (3.6 to

5.4/100,000).[5] In the Netherlands the age-adjusted incidence increased from 4.8 to 6.6 per 100,000 between 1991 and 2002.[6]

Risk Factors

Risk factors can be classified into Epidemiological, Pathological and Clinical risk factors as shown in Table I. The strongest proven risk factor for developing testicular cancer is cryptorchism with a risk ratio between 2.5 and 11.4 in case-control studies. Approximately, 7-10% of testicular cancers develop in patients with a history of cryptorchism.[1]

Table I. Classification of risk factors for germ cell tumours

Epidemiological
Cryptorchism
Contralateral tumour
Geographic distribution
Race
Familial predisposition
Infertility
Klinefelter's syndrome
Pathological predictors for occult metastatic disease in clinical stage I disease[1]
Histopathological subtype
Vascular/Lymphatic invasion (only for non-seminoma testis)
Tumour size (only for seminoma testis)
Clinical prognostic markers for survival in metastatic disease[2]
Primary histology
Primary location
Elevation of serum tumour marker levels
Presence of non-pulmonary visceral metastases

1. See also Treatment of low stage testicular germ cell tumours pg 21-23.
2. See also Table V, pg 20.

For clinical stage I non-seminomatous disease, the most important predictors for micrometastatic disease are the presence of vascular invasion in the primary non-seminomatous tumour.[7] In contrast, in seminomatous disease, the size of the primary tumour at presentation is predictive for metastatic disease.[8]

Recently, the International Germ Cell Collaborative Consensus Group (IGCCCG) identified some clinical independent adverse factors for metastatic disease.[9] A prognostic based staging system was generated out of these data, in which a combination of histology, stage of disease and degree of elevation of serum tumour markers determined subgroups of good, intermediate and poor prognostic risk groups for NSGCT, and good and intermediate risk groups for SGCT (see also Table V).

Classification

The histological type of testicular tumours varies, although there is a clear predominance of germ cell tumours (Table II).[10] All germ cell tumours of the testis, with the exception of spermatocytic seminoma, are expected to originate from "intratubular germ cell neoplasia", otherwise called "carcinoma in situ" of the testis.[11] It is usually a diffuse disease, that might progress to invasive TGCT in as much as 50 % of cases within 5 years.[12]

Testicular germ cell tumours are divided into seminoma and nonseminoma types. There are four subtypes of nonseminomas: embryonal cell carcinomas; yolk sac tumours; choriocarcinomas and teratomas. These nonseminomas can sometimes occur in combination, and are then called mixed tumours. The combination of seminoma and one or more non-seminomatous components is considered a non-seminomatous tumour, because nonseminomas are generally more aggressive than seminomas. At the University Medical Centre Nijmegen, the modified classification of the World Health Organisation is used (Table II).

Table II. Histological Classification of Testicular Tumours

1.	**Germ cell tumours (96%)**	
-	Intratubular germ cell neoplasia (IGCN)	5 %
-	Seminoma	35 %
-	Spermatocytic Seminoma	5 %
-	Anaplastic Seminoma	5 %
-	Embryonal cell carcinoma	5 %
-	Yolk sac tumour	5 %
-	Choriocarcinoma	1 %
-	Teratoma (mature, immature, with malignant transformation)	4 %
-	Mixed germ cell tumours	31 %
2.	**Sex Cord-Stromal Tumours (3.5%)**	
-	Leydig Cell Tumour	2 %
-	Sertoli Cell Tumour	1 %
-	Granulosa (adult and juvenile)	0.5 %
3.	**Gonadoblastoma (0.5%)**	

Seminoma germ cell tumours (SGCT) occur generally in older individuals than do most other germ cell tumours.[1,10] SGCT usually spread via lymphatics, mainly to the para-aortic nodes and sometimes to mediastinal and supraclavicular nodes. Haematogenous spread is rare. The tumour is extremely responsive to both radiotherapy and chemotherapy. Two other histologic subtypes of seminoma have been described: anaplastic seminoma and spermatocytic seminoma.

In 4 to 5% of seminomas, areas of anaplasia have been found and were thought to be associate with a lower overall survival rate.[13] However, it has been demonstrated that the difference in survival reflected a tendency for the anaplastic variant to present at advanced stages, but that stage by stage there is no prognostic difference with pure seminoma.

Spermatocytic seminoma is a unique entity among testicular germ cell neoplasms. It never occurs mixed with other germ-cell elements but only occurs in its pure form. Other important clinical features further distinguish it from classic seminoma, including an average age at diagnosis above 50 years, a higher frequency of bilaterality and a rare occurrence of metastasizes.[14]

Non seminoma germ cell tumours (NSGCT) occur most often in men between 20 and 35 years. NSGCT are extremely heterogeneous. They metastasize via both the lymphatics and blood stream, especially to the lung. Liver, brain and bones are much less involved.

a) *Embryonal cell carcinoma* is the most undifferentiated tumour, and the purer it is, the more aggressive it is.[7] It is very responsive to chemotherapy. This tumour may be associated with an elevation in serum alpha-fetoprotein (αFP) and/or betachorion-gonadotropin (β-HCG).
b) *Yolk sac tumour* is the most frequent testicular tumour in children. In children, the tumour almost invariably occurs as a histological pure lesion, whereas in adults it occurs in combination with other elements. Also the natural behaviour is different between childhood and adult yolk sac tumours. Prior to the introduction of effective chemotherapy, the presence of yolk sac elements in adult tumours conferred a poor prognosis.[15] Similarly, children older than two years with yolk sac tumours had a poorer prognosis than children younger than 2 years.[16] Metastases from yolk sac tumours occur via both lymphatic and haematogenous routes. Distant metastases most often involve the lungs and liver. It produces αFP.
c) *Pure choriocarcinoma* is extremely rare. It occurs almost exclusively in young adults. Choriocarcinoma has the highest potential for organ confined metastases and yields a very poor prognosis.[10] It metastasizes diffusely via the blood stream, often skipping the

retroperitoneum. When it is mixed with other histologies, it becomes a potentially curable disease, in contrast to pure choriocarcinoma which is very difficult to cure. It produces β-HCG.

d) *Teratoma* is a germ-cell tumour which underwent somatic differentiation from all three germ cell layers: ectoderm, mesoderm and endoderm. Incomplete differentiation leads to immature teratoma and complete differentiation leads to mature teratoma. The degree of maturity is closely linked to both the patient age at time of presentation and to clinical behaviour, specifically malignant potential.[10] In early childhood, teratomas are almost invariable mature and carry a uniformly benign prognosis, even in the rare instances that immature elements are detected.[17] In adults, on the other hand, the presence of mature and immature elements have a metastatic potential and may undergo malignant transformation also into non germ-cell cancers (e.g., sarcoma, adenocarcinoma). Because teratomas are poorly responsive to both chemotherapy and radiotherapy, surgical removal is necessary.[18] Serum tumour markers are normal in patients with pure teratoma.

II. Signs, diagnosis and staging of testicular germ cell tumours

Signs and Symptoms

Testicular cancer generally occurs in young men. The most common sign of testicular cancer is a painless unilateral mass in the scrotum. In 20% of the cases the first symptom is scrotal pain, followed by back and flank pain in 11%. Gynaecomastia appears in 7% of the cases and is more common in non-seminomatous tumours.[19] Differential diagnosis must be established with any other intrascrotal mass or disease, but scrotal complaints at young age need to be thoroughly investigated to rule out cancer.

Diagnosis

The first step in diagnosing testicular cancer is usually made by self-examination. Ultrasound will confirm the presence of a testicular mass and can explore the contralateral testis.[20] The sensitivity of ultrasound in detecting testicular tumours is almost 100%.[21] Furthermore, ultrasound is able to distinguish intra-testicular from extra-testicular lesions and is able to detect microlithiasis. Microlithiasis should at least be cautiously followed up, or subjected to biopsy since it can be associated not only with intra-tubular germ cell neoplasia but also with testis cancer.[22]

Serum tumour markers not only contribute to the diagnosis, but also to staging, prognosis and follow-up.[23] Tumour markers are especially helpful to differentiate germ cell tumours from each other and from other malignancies. In clinical practice the most common markers for testis cancer consist of αFP, β-HCG and lactate dehydrogenase (LDH). AFP and β-HCG are produced by yolk sac elements and syncitiotrophoblasts, respectively. AFP has been increased in 50-70 % of NSGCT to β-HCG in 40-60 %.

Over all, 90% of non-seminomatous tumours present a rise in one or both markers. In 8 to 10% of patients with seminoma an elevated β-HCG is found because of the presence of syncitiotrophoblasts in the tumour. LDH is a non-specific marker, being an expression of tissue destruction grossly proportional to tumoural volume. It can be elevated in 80% of patients with advanced testis tumour. Importantly, negative markers do not exclude the diagnosis of germ cell tumour, and normalization of markers after treatment does not necessarily mean cure.

After a testicular tumour has been clinically diagnosed, the ablation of the testis is indicated. The incision is high inguinal and the whole spermatic cord should be removed up to the internal ring with separation of ductus deferens and gonadal vessels. In case of bilateral tumour (synchronous or metachronous) an organ-preserving procedure should be considered.

The operation should be performed in cold ischemia and biopsies must be taken from the tumour ground and periphery.[24]

Staging

As soon as the diagnosis of germ cell cancer has been confirmed, further staging examinations are warranted to examine the extent of disease. Clinical staging describes the extent of disease based on nonsurgical diagnostic modalities. Computerized tomography (CT) of abdomen and chest is the standard routine radiological staging technique.

The abdominal CT-scan offers a sensitivity of 30-35% in the evaluation of retroperitoneal lymph nodes in the landing zone by using a threshold of 1 cm.[25] Lowering this threshold results into an increased sensitivity, but a decreased specificity.[25,26] New generation CT scans do not seem to improve the sensitivity.[27]

Chest CT-scan is the most sensitive way to evaluate the chest. Although it is able to detect pulmonary lesions as small as 2 mm in size, up to 70% of these small lesions seem to be benign. When abdominal CT scan is positive, all patients should undergo a chest CT-scan.[28]

Anteroposterior and lateral *Chest X-rays* can be considered as the only mandatory exploration when retroperitoneal and pelvic CT scans are normal.[28]

CT examination of the brain, *bone scan or liver ultrasound* are advised only if there is any clinical suspicion of metastases.[29]

In Europe, the most commonly used staging system for the primary tumour is the TNM classification (Table III),[30] while the Royal Marsden Hospital Classification is used for the staging of dissemination (Table IV).[31] Today, staging represents the cornerstone on which treatment is based. Low stage disease refers to clinical stages I and IIA disease, whereas high stage disease encompasses stages >IIA disease. This distinction between low and high stage

disease is important, as it determines, to a large degree, the adjuvant therapy that the patient receives.

Table III. The TNM staging system for testicular cancer (1997)[30]

Primary Tumour (only pathologically defined)	
pTx	Radical orchiectomy not performed
pT0	Histological scar or no evidence of primary tumour
pTis	Intratubular germ cell neoplasia
pT1	Tumour limited to testis, including rete testis and albuginea
pT2	Tumour limited to testis with evidence of vascular (hematic or lymphatic) invasion; or tunica vaginalis involved by tumour
pT3	Tumour involving spermatic cord, with or without vascular invasion
pT4	Tumour involving scrotum, with or without vascular invasion

Table IV. The Royal Marsden Hospital Classification system for germ cell tumours[31]

Stage I		Tumour limited to testis
Stage II		Infradiaphragmatic lymph node involvement
	IIA	Metastases <2 cm in diameter
	IIB	Metastases 2-5 cm in diameter
	IIC	Metastases 5-10 cm in diameter
	IID	Metastases >10 cm in diameter
Stage III		Supradiaphragmatic lymph node involvement
	A-D	See stage II
Stage IV		Extralymphatic involvement of lung (L), liver (H), brain and bone
	L1	≤ 3 metastases; ≤2 cm in diameter
	L2	multiple metastases; ≤2 cm in diameter
	L3	multiple metastases; ≥2 cm in diameter
	H1	less than four, <2 cm in diameter
	H2	more than four, <2 cm in diameter
	H3	more than four, >2 cm in diameter

In addition, a prognosis based staging system has been developed to classify all patients with advanced disease into different risk groups, based on histology, extent of disease and level of tumour markers after orchiectomy. The International Germ Cell Cancer Collaborative Group (IGCCCG) determines subgroups of good, intermediate or poor prognosis for NSGCT and of good and intermediate risk for pure seminoma (Table V).[9]

Table V. A prognostic factor-based staging system for metastatic germ cell cancer[9]

Good prognosis group

Non-seminoma: 56% of cases 5 year PFS* 89% 5 year survival 92%	**Seminoma:** 90% of cases 5 year PFS 82% 5 year survival 86%
with all of testis/retroperitoneal primary no non-pulmonary visceral metastases AFP< 1,000 ng/ml and HCG < 5,000 mIU/I (1000 ng/ml) and LDH < 1.5 x upper limit of normal	*with all of* any primary site no non-pulmonary visceral metastases normal AFP any HCG any LDH

Intermediate prognosis group

Non-seminoma: 28% of cases 5 year PFS 75% 5 year survival 80%	**Seminoma:** 10% of cases 5 year PFS 68% 5 year survival 73%
with all of testis/retroperitoneal primary no non-pulmonary visceral metastases AFP ≥ 1,000 and ≤ 10,000 ng/ml or HCG ≥ 5,000 and ≤ 50,000 mIU/l or LDH ≥ 1.5 and ≤ 10 x upper limit of normal	*with all of* any primary site non-pulmonary visceral metastases normal AFP any HCG any LDH

Poor prognosis group

Non-seminoma: 16% of cases 5 year PFS 41% 5 year survival 48%	**Seminoma:** no patients classified as poor prognosis
with all of mediastinal primary non-pulmonary visceral metastases AFP ≥ 10,000 ng/ml or HCG ≥ 50,000 mIU/l (10,000 ng/ml) or LDH > 10 x upper limit of normal	

* PFS = Progression-free survival

III. Treatment of low stage testicular germ cell tumours

Treatment of low stage testicular disease results in an extremely high survival rate up to 100%, independent on the choice of treatment.

Low stage Seminomatous Germ Cell Tumour

Patients with low seminoma germ cell tumour (SGCT) have traditionally been treated by orchiectomy followed by adjuvant abdominal radiotherapy. Only 5% of these patients relapse post-radiotherapy and the majority of them can be salvaged by chemotherapy.[32]

To date, surveillance for stage I seminomatous disease is also possible. Surveillance studies have shown subclinical metastatic disease in 15%-20% of stage I seminoma patients, usually occurring in the retroperitoneum.[32-35] About 15% of the relapsed patients require salvage radiotherapy and 5% salvage chemotherapy.[32] The main advantage of preventing needless radiotherapy in 80% of the patients must be balanced against intensified cancer treatment at relapse and cost of long-term follow-up. Furthermore, good patient compliance is mandatory to surveillance policy.

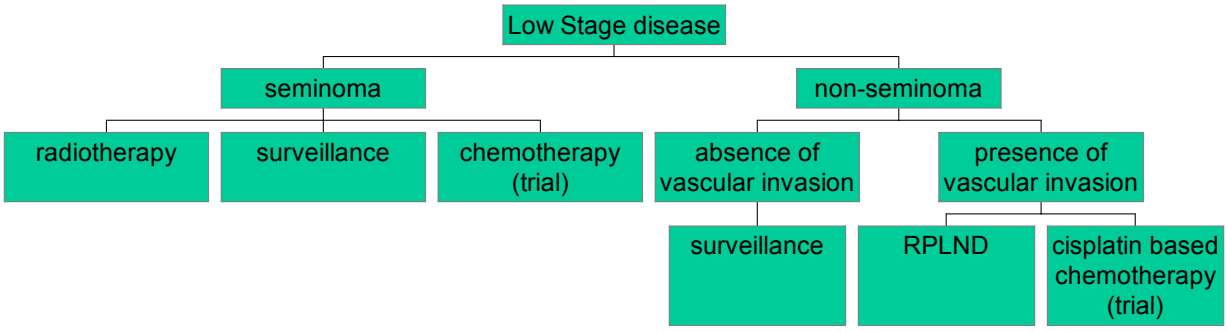
Seminoma cells are also clearly responsive to chemotherapy. Efforts are currently made to assess the use of prophylactic chemotherapy (two courses of carboplatin) as a treatment alternative. Two courses of carboplatin reduces the relapse rate to 0-1%.[36-38] Despite this favourable outcome, controversy exists, because the majority of patients are overtreated and the late sequelae of carboplatin treatment and quality of life compared to surveillance or radiotherapy are not known. To date, the prophylactic chemotherapy regimen should be investigated in randomized trials, before definitive incorporation into daily practice.

Low stage Non-Seminomatous Germ Cell Tumour

Patients with clinical stage I tumours have disease that is confined to the testis. However, approximately 30% of the clinical stage I non-seminoma germ cell tumour (NSGCT) patients are understaged by radiological imaging and are found to have metastatic disease at retroperitoneal surgery.[39] Primarily two strategies have been applied to treat clinical stage I NSGCT: primary retroperitoneal lymphadenectomy (RPLND) with or without adjuvant chemotherapy or watchful waiting with close surveillance and chemotherapy on relapse. The certainty of accurate staging by surgery must be balanced against overtreatment in 70% of the patients. Therefore, some advocate surveillance and only treat patients who develop metastatic disease. Currently, two courses of primary chemotherapy have also been used in treatment for clinical stage I NSGCT.

Today, treatment can also be adapted to prognostic factors for metastatic disease. The best predictor for metastatic disease is the presence of vascular invasion (VI) in the primary tumour.[40] For patients at high risk for metastases some investigators[39] advocate a RPLND with adjuvant chemotherapy in pathological stage (PS) II, whereas others recommend chemotherapy only.[41,42] In contrast, patients at low risk (absence of prognosticators) will enter a surveillance protocol. Independent of initial treatment, the survival rate for stage I NSGCT patients is near 100%.[43] So far, no consensus has been achieved about preferred treatment policy. The choice of treatment depends on a number of factors, such as risk factors for disease relapse, patient compliance and local expertise with respect to performance of the surgical procedure. Improving the clinical staging will ultimately result into better defined subgroup of patients and facilitates the choice of treatment.

Summary of primary treatment of low stage disease:



IV. Primary treatment of high stage testicular germ cell tumours

In general, high stage disease will be treated primarily with chemotherapy. The extent of treatment depends on the histology of the primary tumour and the prognostic risk groups as defined by the IGCCCG (Table V). The primary treatment of choice in high stage disease is combination of cisplatin based chemotherapy (BEP: Bleomycin, Etoposide, cisPlatin). While chemotherapeutic trials were being conducted, it became clear that prognoses varied and that patients should therefore be divided into low and high risk categories. The International Germ Cell Cancer Collaborative Group uses clinical parameters to divide patients with disseminated testis cancer into three risk categories: good prognosis, comprising 60% of germ cell tumours (GCT) with a 91% 5-year survival rate; intermediate prognosis, comprising 26% of GCT with a 79% 5-year survival rate; and poor prognosis, comprising 14% of GCT with a 48% 5-year survival rate.[9] Currently, three courses of BEP is the primary treatment for metastatic germ cell tumours with good prognosis and four courses remain the choice for intermediate and poor risk patients.[44]

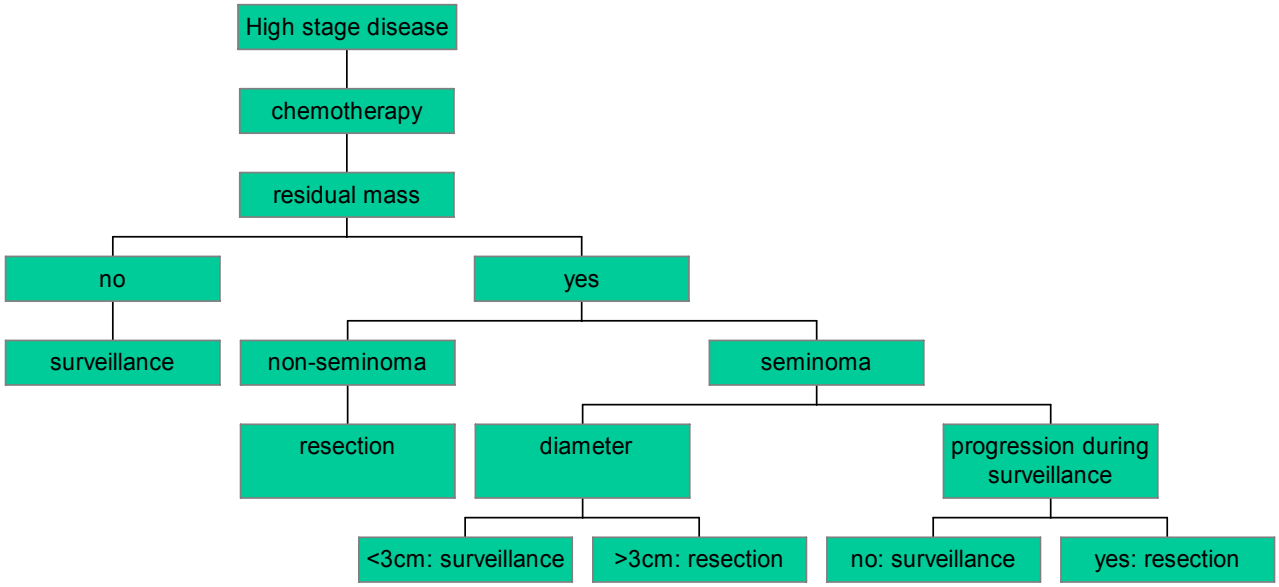
High stage Seminomatous Germ Cell Tumours

Advanced seminoma is highly responsive to chemotherapy. There are often residual masses after induction chemotherapy containing residual cancer (10%) or fibrosis (90%). Many authors suggest resecting residual masses over 3 cm in diameter, [45] but post-chemotherapy surgery in pure seminoma is usually very difficult and dangerous because of intense fibrosis. Another less frequently used option is surveillance of residual masses and reserving radiotherapy or chemotherapy for those patients who subsequently develop progressive disease. [46]

High stage Non-Seminomatous Germ Cell Tumours

Though chemotherapy plays a major role, postchemotherapy surgery has been complementary in the overall chance for cure of NSGCT. In general, residual mass after chemotherapy for advanced non-seminomatous disease has to be removed.[47] Three histological entities can be found in the residual mass: residual tumour (10%), teratoma (50%) and fibrosis (40%). Resection of fibrosis confers no benefit to the patient, however it is not possible to predict the histology of these masses by clinical means. Teratoma has to be removed as it is not sensitive to chemotherapy and consequently it may continue to grow or degenerate into non-germ cell cancers. The key to success is complete resection with histology being the second most imported prognostic factor: 50-70% disease free survival for radically resected residual cancer, 70-90% for mature teratoma and over 90% for fibrosis.[48] In case of residual tumourous disease, it is possible to give two adjuvant courses of chemotherapy. This policy is, however, not superior to careful observation and deferred treatment in case of relapse. [49]

Summary of primary treatment of high stage disease:



V. Follow-up in Testicular Germ Cell Tumours

Testicular germ cell tumour (TGCT) is unique compared with other urological malignancies in that recurrence is highly treatable, especially when detected early. [50,51] As a result, optimal follow-up seems critical to the care of TGCT patients. However, no prospective studies have been performed so far to justify any particular follow-up schedule. [51]

To date, patients are followed by regular outpatient visits, during which physical examination, serum tumour markers and radiological examinations are performed. In most commonly used schedules, patients will visit a medical specialist up to 30 times over a period of 10 years. Some advocate even lifelong follow-up as late relapses have been recorded.[52]

VI. Fertility aspects in Testicular Germ Cell Tumours

With the high cure rates in this young male population, fertility related aspects become increasingly important. For several reasons, fertility-related problems are common in patients with TGCT. Testicular cancer patients demonstrate decreased reproductive potential at baseline that might further deteriorate after treatment. Approximately a quarter of the patients remain azoospermic after treatment.[53] Furthermore, up to half of all the patients undergoing surgery may suffer from ejaculatory dysfunction after treatment.[54,55]

Today, the most effective way to preserve fertility potential in patients with TGCT is cryopreservation of sperm before any treatment is offered.[55]

Outline of this thesis

From the preceding review, it becomes clear that testicular germ cell tumours represent a type of malignancy that can be cured in a large number of patients. This success has become a "two-edged sword" in the contemporary urologic oncology. On one hand, the vast majority of patients will survive as a result of advances from effective chemotherapy. On the other hand, this spectacular success has led to a dichotomy of views in the management of clinical stage I nonseminoma germ cell tumours and post-chemotherapy residual masses. Furthermore, some complacency has taken place regarding follow-up and fertility related aspects after primary treatment. This thesis focuses on the fine-tuning of different aspects in the management of testicular germ cell tumours.

In the first part of this thesis, epidemiological risk factors have been evaluated. In daily practice, many patients and their relatives ask if testicular cancer is a familial related disease. In **chapter 1,** we evaluated if there is an increased incidence of cancer among relatives of testicular cancer patients. Possible familial clustering with other cancers may indicate a form of etiologic heterogeneity, which may facilitate the continued search for gene locations.

In **Chapter 2,** we compare the survival rate of young and elderly patients with a testicular germ cell tumour adjusted for stage and morphology. Data from the population based Cancer Registry in the Netherlands suggest that testicular cancer patients older than 50 years of age have a worse disease specific survival. We evaluated if the same trend is present in a population of 12.881 patients with testicular cancer selected from the public-domain data from the Surveillance, Epidemiology and End Results (SEER) registry in the U.S.A.. If so, special attention has to be given to treatment and long-term care of elderly patients with testicular cancer.

The second part of the thesis deals with the management of clinical stage I nonseminoma germ cell tumours. In **chapter 3,** the current treatment options of clinical stage I non-seminomatous testicular germ cell tumours are reviewed. The choice of management in clinical stage I disease is one of the most controversial topics in urological oncology today. Interestingly, the different treatment options –surgical versus conservative approach- have geographic trends that are influenced by personal choice of the medical specialists. In the United States, there is a strong urological surgical tradition. In Europe, the conservative approach is generally favoured. In the Netherlands, all but one centre advocate surveillance for clinical stage I disease. **Chapter 4** presents a comparison of surveillance policy at the Antoni van Leeuwenhoek Hospital, Amsterdam versus retroperitoneal lymph node dissection at Radboud Nijmegen University Medical Centre, in which the advantages and disadvantages of both policies in the Netherlands will be outlined.

A more rational approach to treatment would be possible if the likelihood of micrometastasis could be determined. In case of metastasis surgery is needed, whereas surveillance is the first choice of treatment in localised disease. In **chapter 5,** several prognostic risk factors are evaluated to predict the presence of micrometastasis in clinical stage I non-seminomatous disease.

The third part of the thesis investigates the value of ^{18}Fluor-Deoxy-Glucose Positron Emission Tomography (^{18}FDG-PET) in the management of testicular germ cell tumours. ^{18}FDG-PET is a relatively new technique in which the increased metabolic activity of malignant cells is visualized by radioactive labelling of glucose. Imaging the cellular glucose uptake by FDG-PET has proven to be able to visualize tumour masses and seems to be a promising method for monitoring treatment response in other solid tumours, like breast cancer, and head and neck tumours.[56] To study the value of FDG-PET in detection of testicular metastasis, **chapter**

6 introduces and discusses the role of ^{18}FDG-PET in initial staging and restaging after chemotherapy for testicular germ cell tumours. In **chapter 7,** we evaluated if monitoring chemotherapy response in patients with high stage testicular germ cell cancer has additional value in differentiation of residual masses after chemotherapy.

In the fourth part of this thesis, the efficacy of follow-up examinations for different subgroups of patients has been evaluated. The efficacy of follow-up after primary curative treatment has been described in **chapter 8.** A retrospective study was performed to get insight into the recurrence rate, the interval between completion of primary treatment and recurrence and the way in which recurrence was detected. Recommendations are given to optimize the current follow-up strategy at our centre. In **chapter 9,** special interest was focused on clinical stage I NSGCT patients on surveillance. The timely diagnosis of a relapse is important for a surveillance protocol. However, no prospective studies have been performed so far to justify particular follow-up schedules. In this chapter, we evaluate the efficacy of different follow-up strategies by using a simulation method (Markov analysis).

Testicular cancer patients are young and have an excellent chance of survival including a long life expectancy, making (late) side effects on important topic. In the fifth part of this thesis, the effect of the disease per se and its' treatment on fertility has been examined. **Chapter 10** reports on the prevalence of (in)fertility in men before and after treatment for unilateral testicular germ cell cancer. It is yet not known whether cancer treatment implies an increased risk of sperm damage. In **chapter 11**, the sperm quality including DNA-analysis has been analysed in testicular cancer patients before and after chemotherapy.

In the last part of this thesis, all studies are summarised and future directions for the management of testicular germ cell tumours are suggested.

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PART I

Epidemiological factors for germ cell tumour of the testis

CHAPTER 1

Cancer incidence in relatives of patients with testicular cancer in the eastern part of The Netherlands.

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Abstract

Objectives. To investigate the incidence of malignant tumours in first-degree relatives of patients with testicular cancer.

Material and Methods. Information about the occurrence of cancer in relatives of patients treated for testicular germ cell cancer (TC) at the Department of Urology of the University Medical Centre Nijmegen from 1986 to 1997 was collected using postal questionnaires from 379 (72%) of 524 patients. The expected numbers of cancers in relatives were computed from age- and sex-specific incidence data in the Netherlands Cancer Registry. The observed/expected (O/E) ratios with 95% confidence intervals (CIs) were calculated using Byar's approximation of the exact Poisson test.

Results. The O/E ratio for developing cancer in the families of patients with TC was 1.2 (95% CI 1.0 to 1.3). Among first-degree relatives of patients with TC, more TC was observed than expected (O/E 3.3; 95% CI 1.4 to 6.9). The risk for brothers of patients with TC increased 5.9-fold (95% CI 2.2 to 12.8). Both the risk of developing lung cancer (O/E 1.5) and malignancy of the female genital tract in sisters (O/E 2.8) was slightly increased. In contrast, the risk of urinary tract malignancies (O/E 0.3) and other and unknown primary tumours (O/E 0.2) had a lower incidence among relatives. However, both the increased and decreased risk of nontesticular cancer for first-degree relatives may have been caused by misclassification.

Conclusions. TC clusters in families were more pronounced among brothers than among fathers and sons. This study supports previous reports that families of patients with TC do not seem to be prone to nontesticular cancer. Additional investigations in families with TC are recommended to map candidate genes for TC.

Introduction

Nonseminomatous germ cell tumours (NSGCTs) and seminomatous germ cell tumours (SGCTs) of the testis are the most common malignant tumours in young adults. The etiology is still poorly understood. The incidence of testicular germ cell cancer (TC) has risen dramatically during the past century.[1] Important risk factors for TC are undescended testes, testicular dysgenesis, infertility, and an antecedent contralateral testicular neoplasm.[2] Several studies have focused on the possibility of a familial predisposition to TC. For brothers of patients with TC, a 3 to 12-fold increased risk was calculated.[3-7] This familial relative risk is much higher than that for most other types of cancer. After earlier inconclusive results,[8,9] recently a candidate gene location for TC was found with linkage analyses.[10] Possible familial clustering with other cancers may indicate a form of etiologic heterogeneity, which may facilitate the continued search for gene locations. Two previous studies have reported an increased risk of breast cancer (odds ratio 4.4, P <0.05).[11,12] The aim of this study was to investigate the risk of malignant tumours in relatives of patients with TC.

Material and methods

Family data were collected using postal questionnaires sent to all patients (n = 524) treated for TC at the Department of Urology, University Medical Centre Nijmegen from 1986 to 1997. All surviving patients who could be located were asked to complete the questionnaire asking for information about the occurrence of cancer in their first-degree relatives. Only first cancers occurring in the relatives were considered. When a patient had died (n = 42), the patient's partner or close relative was asked to complete the questionnaire.

We asked the participants to list, if applicable, the date and side of an inguinal hernia, undescended testis, and/or orchitis in their history, and to list the year of birth, year of diagnosis, type of cancer, and year of death if applicable for all of his first-degree relatives.

Only the familial TC, brain tumour, and bone tumour stated in the questionnaires were verified by pathology or hospital reports or by contacting the general practitioner.

At Statistical analysis, the observed numbers of cancers were compared with the expected numbers based on the population rates specific for age and sex. To compute the expected numbers of a specific type of cancer, we calculated each relative's time at risk as the number of years from his date of birth to the date of data collection, cancer diagnosis, or death, whichever came first. We then stratified the total person-years at risk by sex and age (5-year categories). The expected numbers were obtained by multiplying the person-years at risk in each category by the age and sex-specific incidence rates for the type of cancer obtained from the Netherlands Cancer Registry (1990 to 1995).[13] The incidence rates of cancer in the eastern part of The Netherlands are similar to other parts of the country.[14] Finally, the observed/expected (O/E) ratios with 95% confidence intervals (CIs) were calculated. The 95% CIs were calculated using Byar's approximation of the exact interval for the Poisson distributed variables.[15]

Results

In total, 379 (72%) of the 524 questionnaires were returned. Of the remaining 145 patients, 10 patients refused to take part, 41 patients had moved to an unknown address, and 94 questionnaires were not returned for unknown reasons.

The 379 patients had 2270 first-degree relatives and were observed for 85,375 person-years (data not shown). Seven (1.8%) of 379 families could be classified as having first-degree familial TC (FTC). The O/E ratios for TC among first-degree relatives are given in Table I.

There were four SGCTs and three NSGCTs among the probands with first-degree TC. Among the sporadic cases, 110 (29.6%) of the 372 tumours were SGCTs and 262 (70.4%) were NSGCTs. The distribution of the histologic types in the probands with a positive family

history was not different from that of the sporadic TC cases ($P = 0.20$, Fisher's exact test). The mean age at diagnosis of the patients with FTC and those with sporadic TC was 36.1 years (range 27 to 51) and 32.4 years (range 1 to 77), respectively. The difference in age was not statistically significant.

TABLE I Observed/Expected Ratios for first-degree Familial Testicular Cancer

	Relatives (n)	Person years at risk	Observed (O)	Expected (E)	O/E	95% CI
All men	1180	44.352	7	2.12	3.30	1.4-6.9
Fathers	379	21.877	1	1.04	0.96	0.0-5.4
Brothers	580	20.007	6	1.02	5.88	2.2-12.8
Sons	221	2.468	0	0.06	0.00	0.0-61.4

Key: CI = confidence interval

In the present study, one of seven probands with FTC (14.3%) had an undescended testis compared with 10.5% of the patients with sporadic TC, and two had bilateral disease compared with 6 of 372 of the sporadic cases.

In total, the first-degree relatives of patients with TC had a 1.2-fold increased risk of developing any cancer (95% CI 1.0 to 1.3). A statistically significant excess of respiratory tract cancer was found Table II. In contrast, other smoking-related cancers, such as urinary tract and mouth and pharynx malignancies, were observed less frequently than expected. Also, the number of cases in the group of other and unknown primary malignancies was observed significantly less frequently than expected. Stratification by histologic groups (i.e., SGCT and NSGCT) did not materially change these results, although the risk of TC seemed to be somewhat higher among the relatives of patients with SGCTs.

TABLE II	Observed/Expected Ratios for selected cancers in all first-degree relatives of testicular cancer patients				
Site	**Histologic type**	**Observed (O)**	**Expected (E)**	**O/E**	**95% CI**
Mouth and pharynx	NSGCT	0	2.6	0	0.0-1.4
	SGCT	2	1.4	1.4	0.2-5.2
	Total	2	4.0	0.5	0.1-1.8
Digestive tract	NSGCT	25	20.1	1.2	0.8-1.8
	SGCT	13	11.7	1.1	0.6-1.9
	Total	38	31.8	1.2	0.8-1.6
Respiratory tract	NSGCT	25	17.3	1.4	0.9-2.1
	SGCT	17	10.2	1.7	0.97-2.7
	Total	42	27.5	1.5	1.1-2.1
Bone and soft tissue	NSGCT	2	2.0	1.0	0.1-3.7
	SGCT	1	1.0	1.0	0.0-5.8
	Total	3	2.9	1.0	0.2-3.0
Skin	NSGCT	10	7.8	1.3	0.6-2.3
	SGCT	3	4.2	0.7	0.1-2.1
	Total	13	12.1	1.1	0.6-1.8
Testis	NSGCT	3	1.4	2.1	0.4-6.1
	SGCT	4	0.7	6.0	1.6-15.3
	Total	7	2.1	3.3	1.4-6.9
Prostate	NSGCT	11	7.0	1.6	0.8-2.8
	SGCT	3	4.7	0.6	0.1-1.9
	Total	14	11.7	1.2	0.7-2.0
Breast	NSGCT	22	18.1	1.2	0.7-1.8
	SGCT	11	9.1	1.2	0.6-2.2
	Total	33	27.2	1.2	0.8-1.7
Female genital system	NSGCT	9	6.7	1.3	0.6-2.5
	SGCT	4	3.4	1.2	0.3-3.0
	Total	13	10.2	1.3	0.7-2.2
Urinary tract	NSGCT	3	6.0	0.5	0.1-1.5
	SGCT	0	3.5	0	0.0-1.0
	Total	3	9.5	0.3	0.1-0.9
Central Nerve System	NSGCT	4	3.0	1.3	0.4-3.4
	SGCT	1	1.5	0.7	0.0-3.8
	Total	5	4.5	1.1	0.4-2.6
Endocrine glands	NSGCT	1	1.0	1.0	0.0-5.4
	SGCT	1	0.5	2.0	0.0-11.1
	Total	2	1.5	1.3	0.1-4.8
Haematolymphopoietic system	NSGCT	12	9.1	1.3	0.7-2.3
	SGCT	5	4.8	1.0	0.3-2.4
	Total	17	13.9	1.2	0.7-2.0
Unknown primary	NSGCT	1	3.9	0.3	0.0-1.4
	SGCT	0	2.2	0	0.0-1.6
	Total	1	6.1	0.2	0.0-0.9
Total	NSGCT	128	106.4	1.2	1.0-1.4
	SGCT	65	59.0	1.1	0.9-1.4
	Total	193	165.4	1.2	1.0-1.3

Key: CI= confidence interval; NSGCT= non-seminoma germ cell tumour; SGCT= seminoma germ cell tumour.

Sisters, but not mothers, had a significant excess of malignancy of the genital system (data not shown). The incidence of other hormone-related cancers, such as breast and prostate cancer, was not significantly different.

Discussion

We found a modest increase of 16% in the total cancer incidence in the families of patients with TC. The risk of TC among first-degree relatives was increased. For nontesticular tumours, an increase in risk for first-degree relatives was found for respiratory tract cancers and female genital system malignancies in sisters. To verify the familial cases of TC, the pathology records of all first-degree affected relatives were obtained from the institution at which they had undergone orchiectomy. After comparing the obtained pathology records with the family questionnaires, no difference in reporting was noticed. Among the nontesticular tumours, misclassification could have taken place, because the occurrence of cancer in family members was based solely on the questionnaire, with the exception of brain and bone tumours. For example, metastasized cancers may have been reported instead of the primary tumour, leading to overreporting of, for instance, lung cancer. During the first evaluation of the unverified data, we also found a remarkably increased incidence of bone tumours among relatives (n = 8). After verification, however, five of eight bone tumours initially reported appeared to be metastases of other primary tumours. In addition, the small numbers of expected cases of some cancers (e.g., endocrine gland, bone, and soft tissue cancer) make the risk estimates highly sensitive to chance effects. Finally, families with TC and other cancers were more willing to take part in the study than families without cancer. Consequently, the questionnaires of the affected families were more completely filled out than those from families with sporadic cases. However, the O/E ratios of the separate malignant tumours were close to those of previous studies.[6,12]

To assess the quality of the data concerning the occurrence of cancer among relatives, a positive and negative verification was performed by linking the questionnaire data of a similar study from our department on familial urothelial cell carcinoma to the population-based cancer registry. Three hundred thirty-nine relatives (case and control relatives) who lived in the catchment area of the Comprehensive Cancer Centre South (CCC South) were reported to have at least one malignancy diagnosed after 1975. This is the starting date of the cancer registry held by the CCC South. Three hundred one relatives could be linked to the cancer registry. In 77% of all tumours, the site registered by the CCC South and the site mentioned in the questionnaire were identical. For the 38 who could not be linked, additional information was obtained (through the proband or family). Fifteen relatives appeared to have been diagnosed and/or treated in hospitals outside the catchment area of the CCC South. Thus, the definitive positive linkage percentage was 93% (301 of 324). The remaining 23 relatives may not have been linked for several reasons, such as an inaccurate date of birth on the questionnaire, patient death within a few days after diagnosis (no pathology report), or the diagnosis was made in a nursing home (also no pathology report). A negative verification was performed as well. The 1940 relatives whom the probands reported as not having any malignancy were born before 1955 and lived in the catchment area of the CCC South. These relatives were also linked to the cancer registry, yielding a positive linkage percentage of 2.8% for relatives who were reported to have no malignancy. It is possible that some of these patients were not informed that they had cancer (e.g., pTa bladder cancer) or that the involved relatives did not mention their diagnosis to their family. In that study, we also evaluated the reasons for no response. The reasons for not returning or not completing the questionnaire were stated to be no contact with relatives, too much effort, too emotional, too complicated to retrieve all data on relatives, and no permission of relatives to give any information. However,

it appeared that the percentage of a positive family history of urothelial cell carcinoma was 8% in both responders and nonresponders, indicating an absence of response bias.[16]

In the present study, we were able to demonstrate the familial aggregation of TC in 7 of 379 families. The relative risk for first-degree relatives of patients diagnosed with TC was 3.3. Assuming a lifetime risk in the general population of 0.33 for TC,[13] the lifetime risk for first relatives was approximately 1.1%. Brothers of patients with TC were noted to have the highest risk of developing TC. This corresponded with a 3 to 12-fold increased risk in previous studies. The actual proportion of FTC of 1.8% is consistent with other studies, which reported first-degree FTC in 1.0% to 2.8% of cases.[3-7]

In two of our FTC families, lung cancer was diagnosed in a mother and a brother. In one family, the father developed prostate cancer at 84 years of age. In a fourth family, cancer of the digestive tract (brother) and breast cancer (mother) were found. Of interest, in all four families, the proband had a SGCT, but the numbers were too small to draw firm conclusions about the occurrence of nontesticular cancer in FTC families.

In general, the clustering of cancer within a population may be the result of environmental and/or genetic factors. Various environmental factors such as prenatal exposure to estrogens and white collar or professional occupations have been associated with an increased risk of developing TC.[2,17] However, study results have shown little consistency. Furthermore, the environmental risk factors would have to be very potent to induce familial clustering in absence of genetic susceptibility, and such a potent factor has not yet been identified in TC.[18]

According to Knudson's two-hit theory, genetic susceptibility leads to an increased incidence of cancer at younger ages and bilateral involvement of paired organs.[19] In a study by Nicholson and Harland,[20] significantly lower ages at diagnosis among familial cases (29.1 years) and bilateral cases (30.1 years) were observed compared with sporadic cases (35.7 years). In the present series, the younger age among sporadic cases compared with familial

cases was due to a disproportionate number of NSGCTs among the sporadic cases. Analyzing the age differences stratified by histologic feature showed a slightly younger age at diagnosis among familial cases compared with nonfamilial cases within the group of patients with NSGCTs.

Our findings of a greater proportion of bilateral tumours in FTC compared with sporadic TC is in agreement with published reports.[4,5,20] We found a rate of bilateral involvement of 28.4% (2 of 7) in first-degree FTC.

Higher rates of urogenital developmental anomalies have been associated with sporadic[21] and familial TC.[3] In a case-control study by Tollerud *et al.,*[3] an increased risk of urogenital developmental anomalies was observed among familial cases compared with sporadic cases and controls (17% versus 2.7% and 5.3%, respectively). An undescended testis remains the most important risk factor, with a 2.5 to 11-fold greater risk of developing TC. Up to 10% of TCs are diagnosed in males with a history of an undescended testis. Possibly, the susceptibility to malignancy is determined by prenatal factors that cause both cryptorchidism and raise the risk of malignancy rather than by the position of the undescended testis.[2] In our study, one of the FTC patients (14.3%) had an undescended testis compared with 10.5% of the sporadic cases.

At present, the mode of inheritance in FTC is unknown. The observation that brothers were more affected than fathers favours a recessive mode of transmission. However, this finding is susceptible to bias as well. The introduction of cisplatin-based chemotherapy has dramatically improved the survival during the last generation.[1] Before the introduction of cisplatin, there was essentially no effective treatment for metastatic TC. It is possible that only patients with late-onset disease were able to reproduce. In particular, the patients with NSGCT, which occurs 10 years earlier and has a worse prognosis than SGCT, may not have had a chance to reproduce. Consequently, more brother-brother pairs may be observed than father-son pairs.

In an effort to identify the susceptibility genes for TC, an International Testis Cancer Linkage Consortium has been formed to collect data on families with TC worldwide and to investigate candidate regions for a TC susceptibility gene. Candidate regions on chromosomes 3, 4, 5, 12, and 18 have been revealed, although the linkage was not strong.[8,9] Recently, a genome-wide linkage search on X-suggestive families (no father-son transmission) showed a suspect region on chromosome Xq27, with a significantly increased presence of bilaterality and undescended testis among FTC. These results provide evidence of a germ cell susceptibility gene on chromosome Xq27 that may also predispose to an undescended testis.[10]

In total, the families of patients with TC had a 1.2-fold increased risk of developing any cancer. As SGCTs and NSGCTs have different biologic and clinical aspects, it seems appropriate to analyze the cancer incidence among the relatives of patients with TC stratified by the histologic features. We did not find a difference between these groups. For nontesticular tumours, an increase in risk for first-degree relatives was found for respiratory tract cancers and female genital tract malignancies in sisters. In a study by Swerdlow *et al.,*[22] a nonsignificant increase in lung cancer incidence in both mothers and fathers of patients with TC was found. Heimdal and coworkers[12] found a significant increased risk for mothers of patients with TC, but this was based on a few cases. In contrast to lung cancer, other smoking-related cancers such as urinary tract and mouth and pharynx malignancies were observed less frequently than expected. A decreased risk was also found for other and unknown primary malignancies. The finding of an increased O/E ratio for lung cancer may have been because of the misclassification of metastases. Moreover, underreporting of superficial bladder cancer, which could be confused with benign papilloma, might have caused the low ratio for urinary tract malignancy.

Our finding of no increase in hormonally related cancers in parents is in contrast to previous studies. In a study by Moss *et al.,*[11] an increased incidence of breast cancer in the mothers of

patients with NSGCTs (odds ratio 4.4; P <0.05) was suggested. Heimdal and coworkers[12] reported an increase in endometrial cancer in mothers and a decreased incidence of prostate cancer in fathers. Although the increased risk of endometrial malignancies was not significant, they suggested that TC might be partially caused by a de-arrangement of the estrogen/androgen-related pathway. We found only a 2.8-fold increased risk of malignancies of the female genital system in 7 sisters, consisting mainly of uterine cancer (n = 3).

Conclusion

Brothers of patients with TC have an increased risk of developing TC. Among first-degree relatives of patients with TC, no strong clustering occurred with other cancers. Additional investigations in families with TC are recommended to map the candidate genes for TC.

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CHAPTER 2

Difference in stage and morphology-adjusted survival between young and elderly patients with a testicular germ cell tumour.

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Abstract

Objective. To compare the relative survival in men younger and older than 50 years with a testicular germ cell tumour.

Methods. Data on patients with testicular cancer diagnosed between 1973 and 1997 and registered by one of the nine population-based Surveillance, Epidemiology, and End Results (SEER) cancer registries in the United States were obtained from the National Cancer Institute public domain SEER*Stat 3.0 package. Survival rates adjusted for mortality owing to other causes (i.e., relative survival) were calculated for men within each category of the American Joint Committee on Cancer stage groupings.

Results. Patients who developed a germ cell tumour before the age of 50 years had a better 10-year relative survival (90.8%, 95% confidence interval [CI]: 90.6% to 91.0%) than those who developed one after the age of 50 years (84.0%, 95%CI: 81.9% to 86.1%).

This difference remained after stratification by histologic type and stage, except for patients with localized seminomatous disease (97.9% versus 98.0% for men younger and older than 50 years, respectively). The largest difference in 10-year relative survival was found in men with metastasized disease: seminomatous disease, 89.7% versus 69.6% and non-seminomatous disease, 76.9% versus 57.0% for men younger and older than 50 years, respectively.

Conclusions. Lower stage and morphology-adjusted relative survival rate was observed among patients older than 50 years of age with testicular cancer. This difference was more evident in metastasised disease. Whether the worse prognosis in testicular cancer can be explained by a lower tolerance to chemotherapy and/or to sub-optimal treatment in the elderly has to be established.

Introduction

Testicular germ cell cancer accounts for only 1% of all cancers in males.[1] Once the leading cause of cancer deaths in men between 15 and 35 years of age, it has now proved to be a model of success with an overall 5-year survival rate exceeding 95%.[2] This success is primarily attributed to the development of effective chemotherapy for advanced disease.

Data from the population-based Cancer Registry in the Netherlands[3] suggest that testicular cancer patients older than 50 years of age have a worse disease-specific survival. The 5-year relative survival rate was approximately 94% in men younger than 50 years versus 75% in men older than 50 years.[3] This finding encouraged us to examine whether the same trend was present in a larger population.

The aim of this study was to compare the relative survival rate in men younger and older than 50 years with testicular germ cell cancer using the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute in the United States.

Material and methods

Data on survival of patients with testicular cancer diagnosed in the United States were obtained from the public-domain computer package Surveillance, Epidemiology, and End Results (SEER) * Stat 3.0 (National Cancer Institute, 2000; see http://www-seer.ims.nci.nih.gov/). This package contains data on the cancer incidence and survival of nine population-based cancer registries belonging to the SEER program. The SEER Program of the National Cancer Institute collects and publishes cancer incidence and survival data from population-based cancer registries covering approximately 10% of the U.S. population. The selected database was the August 1999 submission, with data on 2,440,153 patients diagnosed between 1973 and 1997. For this study, we selected 12,881 patients with testicular cancer. In subsequent analyses, tumours were classified according to morphology and only

the seminomatous (morphology codes 9061-9063) and non-seminomatous germ cell tumours (morphology codes 9060 and 9064-9102) were taken into account. For staging of the tumours the 4th edition of American Joint Committee on Cancer classification was used.[4] Patients with regional or distant disease were classified as having metastasized disease. Unfortunately, complete data on treatment of testicular cancer patients were not available.

In the public-domain database, no information is provided on the cause of death. Instead, observed survival can be adjusted for the expected probability of survival. The expected probability of survival was generated from age, race, and gender-specific population statistics from 1970, 1980, and 1990 from the U.S. Bureau of the Census. These data are also available in the SEER*Stat 3.0 package. Subsequently, the relative survival was calculated as the ratio between the crude and expected survival.[5] All analysis were performed with the SEER*Stat 3.0 package.

Results

The study population consisted of 12,811 patients with testicular cancer, of which 12561 had testicular germ cell cancer (Table I). Of these patients, 11,515 (91.7%) were younger than 50 years of age. 5,890 (51.2%) of the tumours in the younger patients were non-seminomatous germ cell tumours (NSGCTs) and 5,625 (48.8%) were seminomatous germ cell tumours (SGCTs). The patients older than 50 years of age accounted for 8.3% of the study population, of whom 292 had a NSGCT (27.9%) and 754 a SGCT (72.1%).

Patients who developed testicular cancer before the age of 50 years had a better 10-year relative survival than those who developed one after the age of 50: 90.8% (95% confidence interval [CI]: 90.6% to 91.0%) versus 84.0% (95%CI: 81.9% to 86.1%). The largest difference in relative survival between patients younger and older than 50 years was seen within the first year after diagnosis (96.9% and 92.5%, respectively). After stratification by

histologic type, this difference in relative survival remained. After stratification by stage, no difference was seen in 10-year relative survival rate between men younger and older than 50 years in localized SGCT (97.9% versus 98.0%, respectively). By contrast, a significant difference was observed among men with localized NSGCT at 10 year after diagnosis (95.1% versus 88.9%). In patients with metastasized disease, the 10-year relative survival rate between both age categories was significantly different for both SGCT (89.7% versus 69.6%) and NSGCT (76.9% versus 57.0%).

Table I	**Relative survival at distribution of stage and morphology by age**			
Group (N)	**1-year (%)**	**3-year (%)**	**5-year (%)**	**10-year (%)**
All testicular germ and non-germ cell tumours (N=12,811)	96.5	92.5	91.6	90.1
< 50 (N=11,686)	96.9	92.9	92.0	90.8
≥ 50 (N=1,125)	92.5	88.0	86.6	84.0
Seminoma				
< 50 (N=5,625)	99.0	97.6	97.3	96.0
≥ 50 (N=754)	96.1	94.4	93.7	90.6
Non-seminoma				
< 50 (N=5,890)	95.2	88.9	87.6	86.4
≥ 50 (N=292)	88.5	78.5	76.4	74.3
Seminoma, localized				
< 50 (N=4,312)	99.9	99.3	99.0	97.9
≥ 50 (N=538)	99.5	98.7	98.6	98.0
Seminoma, metastasized				
< 50 (N=1,185)	95.8	92.0	91.5	89.7
≥ 50 (N=200)	87.5	83.3	81.3	69.6#
Non-seminoma, localized				
< 50 (N=3,041)	99.1	96.8	95.8	95.1
≥ 50 (N=154)	95.2	90.9	90.9	88.9#
Non-seminoma, metastasized				
< 50 (N=2,679)	90.8	80.1	78.6	76.9
≥ 50 (N=127)	80.9	65.0	59.5#	57.0#

Significant

Discussion

In this study, we confirmed the observation of the Dutch Cancer Registry that elderly patients with testicular cancer have a worse disease-specific prognosis. To our best knowledge, published studies have not presented findings about age-related and survival differences in men with testicular germ cell tumours.

The elderly represent a group of patients that is more difficult to treat because of alteration in organ function and presence of concomitant diseases.[6-9] In cancer treatment, it has been reported that the elderly are more vulnerable to both surgery[10] and chemotherapy[11] compared with younger patients. In contrast, radiotherapy is equally well tolerated.[12,13] The observed differences in relative survival in testicular germ cell cancer might be (in part) explained by the tolerability to different therapy modalities and/or by inadequate treatment.

Firstly, patients with proven metastasized testicular disease receive chemotherapy (cisplatin, etoposide and bleomycin). All three chemotherapeutic drugs have shown to be associated with increased toxicity in the elderly.[11] It is possible, that elderly patients have received dose reduction as result of toxicity,[14] and/or that they have been under treated for fear of treatment-related toxicity.[7,8] The latter has resulted in a more than twofold increased death rate among elderly patients with breast and colorectal cancer.[9] The evaluation of the effect of first-line chemotherapy in the older patient with testicular cancer is hampered because patients older than 50 years are sometimes excluded from participating in clinical trial on the basis of age.[15] Furthermore, the role of disease sensitivity to chemotherapy has to be taken into account. It is known that SGCTs are more sensitive to chemotherapy than NSGCTs. As a consequence, patients with SGCTs are subdivided into good or intermediate-risk groups, but NSGCTs contain also a poor-risk group.[16] The difference in chemosensitivity might explain the overall difference in 10-year survival rate between metastasized SGCTs and NSGCTs. Among other diseases (acute myeloid leukemia, large cell non-Hodgkin's lymphoma and coelomic

carcinoma of the ovaries), resistance to chemotherapy has been reported to intensify with increasing age of the patient.[14] Although age was not an exclusion criterion for participation in studies on refractory testicular germ cell tumours (second-line treatment), no patient older than 50 years was included.[17-20] Therefore, it is not possible to evaluate age as a prognostic factor for resistance to chemotherapy.

Chemotherapy regimes have evolved considerably between 1973 and 1997.[21] Patients treated before 1985 received different chemotherapy regimens (e.g., vincristine, actinomycin D, and bleomycin regimens, early vincristine and prednisone, single agents protocols, and cisplatin, vinblastine and bleomycin) than patients diagnosed thereafter. Since the mid-1980s, cisplatin, etoposide and bleomycin PEB has been used as first choice of first-line chemotherapy. It is possible that this somehow confounded our results. Therefore, we re-analyzed the data after stratification by calendar period of diagnosis. We assumed that only the patients with metastasized disease received full doses of chemotherapy and that if there a difference in relative survival was found, it would be most striking in those patients. As expected, the patients treated before 1985 had a poorer 5-year relative survival than those treated thereafter (Table II). In both periods, however, the difference between younger and older patients remained. Therefore, it may well be possible that the poorer prognosis among elderly testicular cancer patients is related to less than optimal treatment for the elderly.

Second, in low-stage disease a difference was only observed among patients with NSGCT. One explanation might be that non-seminomatous disease is treated by surgery plus two courses of adjuvant chemotherapy in case of metastatic disease, and seminomatous disease is treated by radiotherapy. It has been stated that the elderly are more vulnerable to complications (cardiopulmonary conditions and sepsis) in the early postoperative period[10,21] and that the time to full recovery after surgery becomes more prolonged with advancing age.[10]

Table II	**5 year-relative survival in patients with metastasized testicular disease in the period before and after the first of January 1985 in relation to the survival among patients diagnosed during the total period (1973-1997)**			
Histology	**Age**	**Period**	**5 year-relative survival**	**5 year-relative survival (1973-1997; table I)**
Seminoma, metastasized	<50	1973-1985	89.9	91.5
	<50	1985-1997	92.6	
	>50	1973-1985	75.4	81.3
	>50	1985-1997	86.5	
Non-Seminoma, metastasized	<50	1973-1985	69.9	78.6
	<50	1985-1997	86.9	
	>50	1973-1985	59.5	59.5
	>50	1985-1997	60.4	

It is unknown whether adjuvant chemotherapy in the elderly has influenced the prognosis. By contrast, radiotherapy schedules seem to be equally well tolerated, independent of patient age.[12,13] However, if coexisting diseases are present in the radiation field (e.g., pulmonary and cardiac), these may deteriorate further and cause problems for continuing therapy. Patients with low-stage SGCT receive only radiation to a limited part of the retroperitoneum, and, incidental (1%) gastrointestinal tract toxicity results in treatment deviation.[22] Consequently, the same relative survival among all patients may be explained in that they are treated equally well by radiotherapy.

Some confounding by stage might have taken place. We were only able to stratify by localized and non-localized disease, and not by the extent of disease in combination with serum tumour markers, which are of prognostic significance.[16] It is known that older patients

have a delay in diagnosis.[9,23] Consequently, they might have presented with more advanced disease (poorer prognosis) than younger patients.

The main issues concerning cancer management in the elderly are related to treatment strategy and tolerance. It is generally believed that tolerance to treatment might be compromised in older patients. Moreover, the true effect of age on tolerance is often obscured by the presence of comorbidities.[24] As a consequence, the elderly might be under treated, although any scientific evidence for reduced treatment is lacking. To date, most of what we know about the diagnosis and treatment of cancer originates from studies in younger populations. Therefore priority must be given to define the optimal treatment strategy in elderly patients, perhaps with some selection criteria, that would discriminate between elderly patients who would benefit from 100% treatment and those who would be better treated less aggressively.

Conclusions

A lower stage and morphology-adjusted relative survival rate was observed in patients older than 50 years of age with testicular cancer. This difference was more evident in metastasized disease. Whether the worse prognosis in testicular cancer can be explained by a lower tolerance to chemotherapy and/or to reduced treatment in elderly has to be established.

To define the optimal treatment regimen among the elderly, it will be necessary to include them into future clinical trials.

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PART II

Different aspects in stage I non-seminoma germ cell tumour of the testis

CHAPTER 3

Clinical Stage I Non-Seminomatous Germ Cell Tumours

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Abstract

For patients with a clinical stage I nonseminomatous germ cell tumour of the testis cure rates should be close to 100%, whether surveillance, primary surgery, primary chemotherapy or a combination is chosen. The identification of patients with microscopic metastasis is difficult. Even with the best predictive factors currently available (vascular invasion and percentage embryonal cell carcinoma in the primary tumour), the identification of micro-metastases is no better than the flip of a coin. Several additional prognostic factors have been studied, but none is yet applicable in daily practice.

Introduction

Since the application of *cis*-platinum-based chemotherapy, patients with testicular cancer are cured in the majority of cases, even in cases of disseminated disease. On the basis of histology (seminoma versus nonseminoma), the tumour stage according to the Royal Marsden Hospital staging system and the serum tumour markers, a risk group classification was defined at a consensus meeting of the International Germ Cell Cancer Collaborative Group in 1994.[1] In the low-risk group 56% of patients with a nonseminoma are identified and 90% of those with a seminoma. In this risk group the 5 year tumour related survival is 92% and 86% respectively. In the intermediate risk group 28% of the nonseminoma patients are found (5 year survival 80%), compared with 10% of the seminoma patients (5 year survival 73%). In the high-risk group, 16% of the nonseminoma patients are identified, with a 5 year survival of 48%. Looking at these figures, it is obvious that in low- and intermediate-risk patients the survival is good to excellent. In this vast majority of patients, improving the efficacy of the therapy is not the main goal, but a reduction of therapy-related morbidity and/or individualization of the therapeutic schedule has become more and more important. A good example of this strategy is a recent European Organisation for Research and Treatment of Cancer, (Genitourinary) Group study number 30941, co-ordinated by de Wit. In that study three and four courses of BEP (bleomycin, etoposide and *cis*-platinum), administered for 2 or 5 days, were compared in low- and intermediate-risk patients, with special attention to the quality of life. In high-risk patients, unfortunately, survival results are still to be improved.

Clinical stage I nonseminomatous germ cell tumours

The question of cure is even less relevant for patients with clinical stage I non-seminomas. In this patient group, 5 year survival should be close to 100%. After the initial clinical evaluation has excluded metastases, approximately 25-30% of these patients will develop metastases.

The majority will develop metastasis in the retroperitoneal space, whereas only a small percentage will have lung metastasis.[2] Without further discrimination between the real stage I patients and the microscopically stage IIa patients, 3 strategies are possible, each with their own advantages and disadvantages.

Surveillance.

The advantage is that in the real stage I patients no unnecessary therapy is given.[2-4] Although this advantage seems obvious, this is only possible on the basis of meticulous follow up. This not only includes regular CT scan's, but most importantly, during the 1-2 years of follow up there is a constant psychological threat to the patient whether he is cured or not.

Furthermore, meticulous follow up is only possible when the patient's compliance (intelligence, travel distance) is guarantied. The disadvantage is that patients with microscopic disease, once they have been diagnosed with macroscopic disease, usually need a full course of chemotherapy, being three or four courses of BEP. In all, the cure rate is close to 100%.

Primary retroperitoneal lymph node dissection (RPLND)

When patients are operated primarily, no microscopic metastasis will be found in 70%. In this group of patients, there remains a 5-10% risk of subsequent lung metastasis, which has to be treated with full-dose chemotherapy.[2,4] All other patients are cured, unfortunately after unnecessary surgery. Although the short- and long-term morbidity could be limited with the use of laparoscopic RPLND, this seems restricted to experienced centres, and the long-term disease specific outcome remains unknown.

The remaining 30% of patients have microscopic-positive lymph nodes. In that case two options are possible. In the case that no additional chemotherapy is given, approximately 70% of these patients will indeed be cured with surgery for microscopically metastasised disease.[5]

On the other hand, however, 30% will still develop recurrent disease, although seldom in the retroperitoneum.[2,5] These patients would still need full-dose chemotherapy, thereby wasting the potential advantage of surgery. With lymph node dissection and additional chemotherapy the cure rate is also (close to) 100%, whereas not all patients are cured with surgery and chemotherapy on indication. In the case of a negative RPLND or adjuvant chemotherapy after microscopic disease, retroperitoneal relapses are extremely rare.[4]

Primary chemotherapy

Several reports have shown that primary chemotherapy again results in a high cure rate. Studer *et al.*,[6] for example, found only 3.4% relapses in 59 high-risk (see risk factors in clinical stage I nonseminoma at page 68) stage I patients after two courses of chemotherapy. An interesting (partly) historical comparison between primary chemotherapy in high-risk patients and surveillance in stage I patients was presented at the 2001 EAU meeting in Geneva.[7] In the historical series 83 patients were in a surveillance strategy. As expected 30 (36%) relapsed and received chemotherapy. Of this group, one died of the disease, and one of heart failure. After 1985, this group used three primary chemotherapy protocols (one or two courses) in 123 high-risk patients. In this group, only five relapses were seen, of which two patients died of the disease. In the same period 124 low-risk patients, or those unwilling to receive chemotherapy, entered a surveillance protocol. In all, 33 relapses were seen (27%) and were treated with chemotherapy. Four patients died of the disease, and 1 of heart failure. Although four deaths out of 33 relapsing patients seems somewhat high, this study clearly illustrates that primary chemotherapy seems safe with regard to cure and long-term toxicity. However, even in high-risk stage I patients the rate of unnecessary chemotherapy is approximately 50%, and cure rates are, just as in the other two options in these patients, not 100%. Moreover, the long-term toxicity of chemotherapy remains a potential problem

(fertility, coronary artery disease, neuro-toxicity, pulmonary fibrosis and secondary cancers). In case of teratoma, long-term follow-up and imaging of the retroperitoneum is mandatory to detect slowly growing teratoma recurrences.

Risk factors in clinical stage I nonseminoma

The problem in clinical stage I non-seminomas would be solved if we could identify those patients with (a high risk of subsequent) microscopic metastases. Well-established risk factors for metastatic disease are blood and lymph vessel involvement and the percentage of embryonal cell carcinoma (ECC) in the primary tumour.[5,8] Additional histological factors are the absence of yolk sac tumour, a primary tumour greater than pT1 and less then 50% teratoma in the primary tumour. The presence of vascular invasion (VI) and ECC increases the risk of microscopic metastatic disease to 40-70% (i.e., high risk patient) whereas absence of these factors implies a risk of metastatic disease of around 10% (i.e., low risk patient).[5,8] In our daily practice vascular invasion is used to advise a clinical stage I non-seminoma patient for wait and see or primary RPLND, with subsequent chemotherapy in case of positive nodes. Although many other factors have been studied for their prognostic value in testicular cancer patients, this has been predominantly for the reaction to chemotherapy or survival. Only a limited number of studies particularly looked at the potential prognostic factors in clinical stage I non-seminomatous patients.

PET (positron emission tomography) has proven to be of use to identify vital tumour in residual masses after chemotherapy.[9] Other imaging studies, such as CT scanning, rely on size, whereas in PET scanning tissue metabolism is taken into account. It has also been studied in stage I patients. Although with PET scanning normal sized nodes with abnormal metabolism (cancer!) can be detected, unfortunately the lower limit of detection is around 5 mm, which is not significantly better as compared to modern CT scans.

The detection of circulating tumour cells with reverse transcriptase-polymerase chain reaction (RT-PCR) techniques seems to be related to survival in advanced disease, but again no additional value could be detected in stage I disease over the presence of ECC.[10,11]

Heidenreich *et al.*[8] found that p53, bcl-2, MIB-1, cathepsin D and E-cadherin expression did not have additional value over percentage ECC and VI in high-risk stage I patients.

With the use of micro-satellite markers, the loss of heterozygosity at candidate regions for chromosomal loss have been studied. Apart from a better insight into the development of seminoma and non-seminomatous tumours from precursor cells, these techniques might also give more insight into which genes play a crucial role in the metastatic process of these tumours.[12] So far, however, these techniques are not applicable in clinical practice.

A new marker that has been studied is telomerase activity. The quantification of human telomerase messenger RNA expression in testicular germ cell tumours appeared to correlate inversely with tumour differentiation.[13] This might give information with regard to the response to *cis*-platinum-based chemotherapy and the presence of mature teratoma components.

Conclusion

Except for high-risk nonseminomatous germ cell tumours, cure rates are excellent in patients with testicular tumours. Less treatment-related morbidity and a better quality of life, therefore are important goals in these patients. For patients with a clinical stage I non-seminomatous testicular tumour principally three approaches can be chosen: surveillance, primary surgery and primary chemotherapy. Even with the best prognostic factor available at present (vascular invasion and percentage ECC in the primary tumour) patient selection can be made with a 50% change of microscopic disease. This means that a substantial percentage of these patients will always be under- or overtreated. The search for additional factors to predict the presence

or absence of microscopic metastasis is ongoing. PET scanning might give some additional information. The identification of circulating tumour cells or tumour markers is possible with RT-PCR techniques, but so far no correlation with clinical outcome has been found. Several other molecular markers have unfortunately not given the answer.

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CHAPTER 4

Comparison of Surveillance and Retroperitoneal Lymph Node Dissection in Clinical Stage I Non-Seminomatous Germ Cell Tumours

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Abstract

Objective. To compare retrospectively the treatment results of surveillance and primary retroperitoneal lymph node dissection (RPLND) of patients with clinical stage I non-seminomatous germ cell tumours of the testis (NSGCT) in two institutions in The Netherlands.

Material and Methods. From 1982 to 1994, 90 consecutive patients with clinically stage I NSGCT were prospectively entered in a surveillance protocol in Amsterdam (group 1). In the same period, 101 patients with clinical stage I NSGCT underwent primary RPLND in Nijmegen (group 2). Both patient populations were comparable for patient age, presence of vascular invasion and embryonal cell components in the primary tumour. All patients in group 1 with relapse, except for two, were treated with cisplatin-based chemotherapy. All patients in group 2 with vital tumour in the RPLND specimen were treated with two adjuvant courses of combined chemotherapy (cisplatin, etoposide, and bleomycin).

Results. In group 1, at a median follow-up of 7.7 years, 23 patients (26%) relapsed. The median time to relapse was 12 months. Relapses were located retroperitoneally (n=18, 78%), in the lung (n=3, 13%), scrotum (n=1, 4%) and combined in the liver, lung and pleura (n=1, 4%). After treatment of relapses (chemotherapy in 21 and/or surgery in 11) only one patient died of disseminated disease. The disease-specific survival rate was 98.5% at the median follow-up. The main toxicities consisted of short-lasting leucopenia, accompanied by infection (13%). Four patients reported cardiovascular and four neuropathy complaints. In group 2, the median follow-up was 6.9 years. In 31 patients (30.7%), vital tumour was found retroperitoneally. After two courses of combined chemotherapy, none of them had a relapse. Seven patients with pathological stage I disease (6.4%) had a pulmonary relapse within one year after surgery. No retroperitoneal relapses were found. After chemotherapy six patients with relapse were salvaged, and one died of disseminated disease. The disease specific

survival in group 2 was 98% at the median follow-up. The most frequent surgical complications were abdominal pain (n=6), hydrocele (n=5) and lymfocele (n=3). The antegrade ejaculation rate was 94%.

Conclusions. Excellent treatment results in terms of overall survival can be achieved in stage I NSGCT with both surveillance and primary RPLND. The choice of treatment should be based on balanced information and not on dogmatic principles.

Introduction

Today, germ cell tumours are highly curable. Independent of initial treatment, the survival rate for patients with stage I nonseminomatous germ cell (NSGCT) patients is near 100%.[1,2] However, the proper treatment after orchiectomy remains controversial. Primarily, two strategies have been applied: watchful waiting with close surveillance and chemotherapy on relapse, or primary retroperitoneal lymphadenectomy (RPLND) with or without chemotherapy.[2,3]

Today, treatment of clinical stage I disease can also be adapted to risk factors. [4-6] For patients at high-risk of metastases some investigators[2] advocate RPLND with adjuvant chemotherapy for pathologic stage II disease and others recommend chemotherapy only.[7,8] In contrast, patients at low-risk will enter a surveillance protocol.

The purpose of our study was to compare the policy of surveillance in patients with clinical stage I NSGCT with that of primary RPLND in two different institutions in The Netherlands.

Materials and Methods

Retrospectively, we compared the treatment results of patients with clinical stage I NSGCT who entered a surveillance protocol at the Departments of Urology and Oncology of the Netherlands Cancer Institute Amsterdam/Antoni van Leeuwenhoek Hospital, Amsterdam (group 1) with those who underwent RPLND at the Department of Urology of the University Medical Centre Nijmegen (group 2). Staging was done according to the criteria of the Royal Marsden Hospital.[9] Clinical stage I NSGCT was defined as no radiologic evidence of metastatic disease and normalization of lactate dehydrogenase and the tumour markers, alpha-fetoprotein (FP) and beta-human chorionic gonadotropin (HCG) according to their half-life kinetics after orchiectomy.

The following criteria were assessed: cancer-specific survival, relapse rate, treatment-related morbidity, complications, and prognostic factors indicating occult metastases. Complications were categorized as follows: minor complication requiring little if any additional treatments and no extended hospitalization beyond 2 days; major complication requiring additional treatment and at least 2 more days of hospitalization[10]; and long-term complications (more than 3 months after completion of treatment).

Between 1982 and 1994, 90 consecutive patients with a median age at presentation of 30,1 years (range: 16.9-60.0) entered group 1. In the same period 101 patients (median age at presentation 30,3 years; range 14.9-61.3) entered group 2 (Table I). The median follow-up for groups 1 and 2 was 7,7 years (range 1,0 – 14,9) and 6,9 years (range 1,0 – 15,1), respectively (follow-up until January 2000). The presence of vascular invasion (VI) and embryonal cell carcinoma (ECC) components in the primary tumour were comparable in both groups. Vascular invasion was defined as either venous or lymphatic invasion.

The protocol of surveillance in group 1 included clinical examination, measurement of tumour markers and chest X-ray monthly in the first year, every second month in the second year and at 3-4 monthly intervals in the third year. In addition, abdominal CT was repeated every 2 to 3 months in the first year, every four months in the second year, and biannually between the third and fifth year. After 5 years, the follow-up was at discretion of the clinician, but participants were encouraged to continue for at least 10 years. Most patients who relapsed were treated with platinum-based chemotherapy; residual masses were surgically removed.

In group 2, all patients underwent standard RPLND as previously described.[2] RPLND was performed unilaterally in 75 patients. The indication to perform a bilateral dissection was based on results of frozen section analysis during surgery. Bilateral dissection was done in 26 patients. Since 1989, a nerve-sparing technique has been used to preserve ejaculatory function

(n=73). Patients with histologically proven lymph node metastases received two additional cycles of chemotherapy with cisplatin, etoposide and bleomycin.

The follow-up procedure was comparable with that of the surveillance protocol; however, only one abdominal CT scan was performed in all patients 3 months after treatment completion.

Kaplan-Meier survival analysis was performed for each group. The overall survival was defined as that from the date of histologic diagnosis of testicular cancer to date of death or last follow-up. Finally, in both groups, an independent analysis of the prognostic factors was performed by univariate analysis (two-tailed Student's t-test; $p<0.05$).

Results

In group 1, disease relapse was recorded in 23 (26%) of the 90 patients; 16 (70%) were detected in the first year, four in the second year and the remaining 3 at 34 (n=2) and 44 months (n=1). Relapses were located retroperitoneally (n=18, 78%), lung (n=3, 13%), scrotally (n=1, 4%) and combined in the liver, lung, pleura (n=1, 4%). Evidence of relapse was detected in 15 (65.2%) of 23 cases by both tumour markers and imaging studies. The median time to relapse was 12 months (range: 3 to 44). In the remaining cases (34.7%) only the imaging study were the first indication of relapse. Of the 23 patients with relapse, 21 received combined chemotherapy. In 6 cases, RPLND for residual mass was performed (1 embryonal cell, 2 mature teratoma, 2 fibrosis and 1 normal lymph node tissue). One patient underwent surgery for scrotal recurrence (mature teratoma). Two patients with a small retroperitoneal mass underwent surgical resection alone (mature teratoma, embryonal cell). Complete remission was obtained in 22 patients. The patient with the combined relapse died of organ confined disease within 6 years of follow-up. Initially, this patient was admitted for a testicular tumour 2 cm in diameter and slightly increased alpha-fetoprotein (10 ng/ml).

Histologic examination revealed mixed germ cell tumour containing seminoma, (im)mature teratoma and vascular invasion. At recurrence, the alpha-fetoprotein was increased (490 ng/ml). In total, a disease-specific survival of 98.5% (95% confidence interval [CI]: 95.6-100.0%) was achieved in group 1 at a median follow-up of 7,7 years (Table I). After treatment, 86% of the patients (n=77) completed the follow-up for at least 5 years.

TABLE I. Characteristics of patients with clinical stage I disease.

	Surveillance (%)	RPLND (%)
Patients (n)	90	101
Mean age (yr)	30.1	30.3
Median follow-up (yr)	7.7	6.9
Medical History		
Undescended testicle	7 (7.8)	15 (13.8)
Raised initial tumour markers*		
beta-HCG	22/86 (25.6)	35/88 (39.7)
alpha-FP	60/86 (69.8)	71/90 (78.9)
LDH	15/86 (17.4)	14/71 (19.7)
Primary tumour side		
right	50 (55.6)	57 (52.3)
left	40 (44.4)	52 (47.7)
Primary tumour characteristics		
Embryonal cell elements	73 (81.1)	85 (78.0)
Teratoma elements	65 (72.2)	68 (67.3)
Chorioncarcinoma elements	27 (30.0)	21 (19.3)
Yolk sac elements	25 (27.7)	14 (12.8)
Seminoma elements	28 (31.1)	26 (25.7)
Vascular invasion*	33/79 (41.8)	34/81 (41.9)
Pathologic Stage IIa	-	31 (30.7)
Relapse	23 (25.6)	7 (6.9)
Survival at median follow-up	98.5%; 95%CI:95.6-100.0%	98.0%; 95%CI:96.0-100.0%

Key: RPLND:retroperitoneal lymph node dissection; beta-HCG: beta human chorionic gonadotrophin; alpha-FP: alpha-fetoprotein:; LDH: lactate dehydrogenase; CI: confidence interval.
* when available

Fifteen of the 23 patients had a chemotherapy-related complication (Table II). The main toxicities observed during chemotherapy consisted of short-lasting leucopenia, nausea, vomiting and temporary alopecia. Two patients were treated with intravenous antibiotics for pneumonia that developed during a period of leucopenia. One patient who received high-dose chemotherapy with autologous bone marrow transplantation was admitted twice with *Staphylococcus epidermidis* sepsis. After treatment, he had persistent polyneuropathy. Four

patients reported cardiovascular toxicity (Raynaud's phenomenon and hypertension), and three had temporary tinnitus complaints. One patient was successfully treated with prednisone for osteomyelofibrosis and one with testosterone suppletion for 1.5 years. Two patients needed treatment for post-treatment depression.

All patients who underwent a RPLND for residual mass experienced antegrade ejaculation. The average postoperative hospital stay was 7 days (range 5-10). One patient had a wound infection that was treated conservatively. One surgeon performed all procedures (mean operative time: 4 hours, range 3 to 6).

In group 2, surgical staging confirmed that 69% of patients (n=70) with clinical stage I disease had pathologic stage I disease, and 31% (n=31) had positive nodes at operation (30 patients with stage IIa and 1 patient with stage IIb). Frozen section histologic analysis was falsely negative in 5 cases. No false-positive findings occurred. All patients with pathologic stage II disease received two additional courses of combined chemotherapy and were salvaged. Pulmonary relapses were only found in patients with pathologic stage I disease (n=7, 10.0%) and all were identified within 12 months by chest x-ray. In three of them, rising tumour markers were also present. Treatment at relapse involved four courses of combined chemotherapy, without further intervention in 6 of these cases. One patient had a repeated relapse in the chest and died of disseminated disease at seven years of follow-up. Initially, this patient was admitted for a testicular tumour 5 cm in diameter and increased serum tumour markers (AFP: 3000 ng/ml and bHCG: 36 ng/ml). Histologic examination revealed mixed germ cell tumour containing (im)mature teratoma and vascular invasion. At both recurrences, the beta-human chorionic gonadotrophin level was increased (8.5 and 26 ng/ml). Two contralateral clinical stage I seminomatous germ cell tumours were detected at 22 and 70 months of follow-up. The patients were treated successfully with orchiectomy and abdominal radiation. In total, the disease-specific survival rate is 98% (95%CI: 96-100.0%) at a median

follow-up of 6.9 years (Table I). After treatment, 87% of the patients (n=88) completed the follow-up for at least 5-years.

The operative time ranged from 2 to 6 hours (mean 2,). No intraoperative complications occurred, and the blood loss ranged from minimal bleeding to 1500 ml. The average postoperative hospital stay was 6 days (range 4-32). One surgeon performed 85% of the procedures.

With respect to the ejaculatory function, 87 patients (86%) reported antegrade ejaculation. Of the 15 patients, reporting absence of ejaculatory function postoperatively, 6 underwent a full bilateral, 6 an unilateral and 3 a nerve-sparing bilateral dissection, respectively. One patient experienced a delay of 12 months before return of antegrade ejaculation after nerve-sparing bilateral dissection. Finally, 3 patients (1 unilateral and 2 bilateral) are able to have antegrade ejaculation with imipramine.

After surgery, 6 major and 5 minor short-term complications occurred in 11 patients (Table II). Retroperitoneal lymphocele was the most frequent major complication postoperatively (n=3). It was treated by percutaneous drainage. One patient developed pulmonary embolism and was treated by heparinization. Three patients stayed longer at the hospital because of abdominal pain. In two of them, small bowel obstruction was diagnosed. All three patients were treated conservatively. In the long-term, 3 patients were readmitted for abdominal pain, caused by small bowel stenosis, necessitating resection of small bowel in one patient. The other two were treated conservatively, after which the pain disappeared. Five patients underwent hydrocele correction and 2 surgical reconstruction of a cicatricial hernia. In 3 patients, scar tissue was removed for pain/traction (n=2) and cosmetic reasons (n=1). Two patients with, respectively, urinary bladder retention and urinary tract infection during the hospital stay developed recurrent urethral strictures in the long term, necessitating surgery. Moreover, five patients had chemotherapy-related toxicities: respectively, haematological

toxicity, ototoxicity, Raynaud's phenomenon, neuropathy and a combination of haematologic and neuropathologic toxicity.

Table II. Complications of chemotherapy and retroperitoneal lymph node dissection

Complications	Minor (n)	Major (n)	Long-term (n)
Surveillance at Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands			
After 4 cycles of chemotherapy (or high dose*)			
Polyneuropathy			1*
Tinnitus			3
Raynaud			2
Hypertension			2
Leucopenia (pneumonia & sepsis)		2 &1*	-
Osteomyelosclerosis			1
Low testosteron level			1
Post-treatment depression			2
After secondary RPLND			
Wound infection		1	
RPLND at University Medical Centre, Nijmegen, the Netherlands			
After primary RPLND			
Urinary tract infection	1		
Urinary bladder retention	1		
Gastro-enteritis	1		
Ulcer of the heel	1		
Lymfocele (symptomatic)		3	
Pulmonary embolism		1	
Abdominal pain	1	2	3
Hydrocele			5
Cicatrical hernia			2
Correction of scar tissue			3
Urethral stricture			2
After 2 cycles of chemotherapy			
Raynaud's phenomenon			1
Hearing loss			1
After 4 cycles of chemotherapy (or high dose*)			
Polyneuropathy			1+1*
Raynaud			1
Leucopenia	1+ 1*		
Osteomyelosclerosis			1*

Key: RPLND: retroperitoneal lymph node dissection; *High-dose chemotherapy

In group 1, the percentage of embryonal cell components and the presence of vascular invasion were significant risk factors associated with relapse. Vascular invasion was found in

33 of 79 primary tumour specimens (Table I). The relapse rate in 33 patients with vascular invasion was 51%, significantly greater than 11% in 46 patients without vascular involvement ($P<0.0001$). In 32 (84%) of the 38 patients with less than 50% embryonal cell components, no signs of recurrent disease were found, and in 17 (33%) of 52 patients with more than 50% embryonal cell components had a relapse ($p<0.05$). In group 2, only vascular invasion was associated with pathologic stage II disease. In 17 (50%) of 34 patients with vascular invasion, positive lymph nodes at RPLND were found, and in 35 (74%) of 47 patients without vascular invasion had pathologic stage I disease ($p<0.05$).

Discussion

This study compared routine management in two institutions in The Netherlands for clinical stage I NSGCT. In one institute this comprises surveillance and in the other RPLND. The two groups of patients were well balanced for clinical and pathologic parameters, enabling a comparison of the treatment results.

Despite advances in diagnostic radiology (CT-scan[11]/PET-scan[12]) the controversy concerning the treatment of stage I NSGCT is mainly caused by the inaccuracy of clinical staging.[5] In general, the rationale behind the surveillance strategy is that 60 to 70% of patients are cured by orchiectomy alone.[1] In 30% to 40%, a potential delay in treatment there exists after clinical identification of metastases. RPLND allows careful pathological staging and offers a therapeutic benefit in about 30% of the patients with clinical stage I tumour. On the other hand, 60% to 70% will have undergone unnecessary surgery.[13] Independent of both treatment options, the survival rate for patients with clinical stage I NSGCT is near to 100%.[1,2]

In agreement with former studies, 26% of the patients on surveillance had a relapse and 31% of the patients who underwent a RPLND had metastatic disease. The pattern of relapse in the surveillance study was similar to that in published studies (local, retroperitoneal and

pulmonary relapses).[1,14] In agreement with Donohue *et al*.,[2] no relapses were seen among patients with pathologic stage II disease adjuvantly treated with chemotherapy. In our series, the survival rate at 5 years of follow-up was 100% for both groups. In both groups, 1 patient died of disseminated disease at 5.9 years (group 1) and 6.9 years (group 2) of follow-up. Both patients were at high risk for metastatic disease. In conclusion, high-risk patients stay at high risk even after RPLND.

The pattern of relapse supports a policy of intensive follow-up for at least 2-years after orchiectomy, irrespective of the treatment policy. Tumour marker determination and imaging are necessary in both protocols. However, in the surveillance protocol a CT abdomen and chest x-ray are recommended; after RPLND a chest x-ray only is probably sufficient for follow-up, especially in patients with pathologic stage I disease.

Finally, surveillance will only work if patients are compliant and if follow-up is done rigorously within a centre dedicated to surveillance.[3] In a Canadian study, the compliance of 76 patients with clinical stage I NSGCT was evaluated.[15] Non-compliance was defined as missing more than one CT scan or two consecutive visits, including marker determination and radiographs. The compliance with clinical visits in the first and second year was 61% and 35%, but for CT scanning, it was only 25% and 12%, respectively. The only two deaths were in non-compliant patients. Although the study was too small to draw firm conclusions on non-compliance and survival, it is more likely that poor compliance results in detecting relapses at a more advanced disease stage. In our series, most patients completed the minimum required follow-up time of 5 years. The small travel distances within The Netherlands and the health insurance policy can explain this. Compliance to follow-up after RPLND is also mandatory. Although RPLND should eliminate retroperitoneal relapses, such relapses have been reported[16], and, irrespective of RPLND, in 7 to 11% of patients with pathologic stage I disease, pulmonary relapses will occur.[2,5]

Because both treatment regimens have the same capability for cure, treatment-related morbidity is important. The morbidity of a surveillance policy is particularly related to chemotherapy for patients with relapse and possible additional treatment.[17] In patients without progression, no treatment-related morbidity occurs. However, frequent follow-up visits are essential. Although they have been described as stressful,[18] this was never prospectively assessed. In both groups, most complications were fully reversible. The main long-term complication of RPLND is loss of antegrade ejaculation, which is strongly related to the extent of the template and the surgical skills.[19] The ejaculatory function can be preserved in most patients with stage I NSGCT, because the tumour load is limited and the dissection can be carried out unilaterally above the level of the inferior mesenteric artery. In accordance with published studies,[19] the antegrade ejaculation in group 2 has been retained after almost all nerve-sparing procedures (97%). Moreover, approximately 15% (n=15) of the patients had complications that required secondary surgical treatment**.**

Numerous studies have been reported identifying prognostic factors that predict metastatic disease in clinical stage I NSGCT.[4,5] None of these studies has prospectively identified a cohort of patients with a significantly greater than 50% chance of recurrence on a surveillance regimen or metastasis to the retroperitoneum or chest when treated with RPLND. As of today, embryonal cell components and vascular invasion are still the best prognostic factors to identify patients with clinical stage I disease at low and high risk for metastasis. In group 1, a significant association was found between vascular invasion or the percentage of embryonal cell components and an increased risk of relapse. In group 2, only vascular invasion was associated with metastatic disease. In Nijmegen, the management has been changed since 1994. Vascular invasion is used to select patients for RPLND and patients without adverse risk factors are put in a surveillance program. Between July 1, 1994 and December 31, 1998, 46 patients with clinical stage I NSGCT were treated. Seventeen patients without vascular

invasion underwent surveillance. One patient experienced a retroperitoneal metastasis at 18 months of follow-up. Two patients with vascular invasion preferred surveillance and developed a retroperitoneal or pulmonary relapse at 3 and 7 months of follow-up, respectively. Twelve of 27 patients with vascular invasion had pathologic stage II disease and were cured by two courses of combined chemotherapy. Two of the 15 negatively staged patients had a pulmonary relapse at 7 and 17 months. All patients with a relapse were successfully treated with four courses of combined chemotherapy. At last follow-up, all were free of disease (median follow-up 43 months, range 19-70).

The role of primary chemotherapy has also been investigated. This strategy will work in approximately 50% of all patients at high risk of metastases and will prevent relapses that would require a higher dose of chemotherapy. In contrast, the remaining 50% will be treated needlessly, with the potential risk of toxicity. Therefore, primary chemotherapy would only be a realistic alternative treatment option if occult metastases could be identified. The objective in patients with clinical stage I disease is to reduce treatment toxicity and maintain high survival rates. Before that, any toxicity in patients without metastatic disease is significant.[7,20]

Conclusions

Surveillance and RPLND with adjuvant chemotherapy for stage I nonseminomatous germ cell tumours show comparable cancer-specific survival figures. The debate on the most appropriate management is still unsettled and awaits refinement of prognostic factors and/or more reliable imaging technique enabling detection of microscopic metastasis. Patients should be offered a choice, based on balanced information on treatment-related morbidity.

Today, the choice of treatment will depend on the local expertise and treatment- and patient-related factors.

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CHAPTER 5

Alpha-catenin expression pattern and DNA image analysis cytometry have no additional value over routine histology in clinical stage I non-seminomatous testicular cancer

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Abstract

Objective. To determine whether alpha-catenin expression pattern and DNA content have additional value over routine tumour histology, including information on vascular invasion and tunica albuginea invasion in detecting occult metastasis in patients with clinical stage I non-seminomatous germ cell tumours of the testis (NSGCT).

Patients and Methods. Fifty consecutive patients with clinical stage I NSGCT underwent retroperitoneal lymphadenectomy (RPLND) between 1986 and 1992. The orchidectomy specimens were histopathologically reviewed and immunohistochemically stained with mouse monoclonal anti-alpha-catenin antibody. The presence of an aberrant or negative staining in more than 10% of the malignant cells was defined as abnormal; in all other cases tumours were classified normal. Furthermore, intact nuclei were isolated from 50 μm thick paraffin sections of the primary tumour, Feulgen stained, and analysed with the Discovery image analysis system.

Results. Of the 50 patients, 14 had positive retroperitoneal nodes (stage IIa, 28%), one pathologically staged I patient developed a lung metastasis (stage IV) within three months after RPLND. Univariate analysis revealed that presence of embryonal cell carcinoma, vascular invasion and tunica albuginea invasion were predictive for occult metastases. In multivariate logistic regression analysis only vascular invasion and tunica albuginea invasion were significant. All patients with no embryonal cell carcinoma in the primary tumour (n=11) were classified as having pathologic stage I disease. Also, the tumours which were DNA-diploid (n=3) or DNA-polyploid (n=2) were pathologically stage I. In screening for occult metastases the DNA content and the alpha-catenin expression pattern did not have additional value.

Conclusions. Vascular invasion and tunica albuginea invasion have prognostic value in identifying patients with clinical stage I NSGCT at high risk for occult retroperitoneal disease.

In contrast, the absence of embryonal cell carcinoma could predict all patients at low risk for metastasis. The DNA-ploidy also identified patients at low risk. Other DNA-analyses and the alpha-catenin expression pattern provided no additional information. Further studies are recommended to identify patients who are at low or high risk for metastasis.

Introduction

Testicular germ cell tumors are highly curable: independent of initial treatment, the survival rate for patients with stage I nonseminomatous germ cell (NSGCT) patients is near to 100%.[1,2] However, the proper treatment after orchiectomy remains controversial. Primarily, two strategies have been applied; watchful waiting with close surveillance and chemotherapy on relapse, or primary retroperitoneal lymphadenectomy (RPLND) with or without chemotherapy.[2,3] Currently, the treatment of clinical stage I disease can also be adapted to risk factors.[4-6] For patients at high risk for metastases some investigators[2] advocate RPLND with adjuvant chemotherapy in pathological stage II disease, whereas others recommend chemotherapy only.[7,8] In contrast, patients at low risk enter a surveillance protocol.

The morbidity of a surveillance policy is low for those patients with no progression, but the frequent essential follow-up visits may be stressful. A surveillance policy in case of occult metastasis might result in a treatment delay, with the potential of a more extensive disease than would be identified by surgical exploration. However, about half of patients with clinical stage I disease at high risk for metastases who undergo RPLND have negative lymph nodes.[9] Although the morbidity of RPLND has significantly decreased through modifications in technique, efforts should be made to minimise over- or under-treatment.[2,5] The role of primary chemotherapy has also been investigated. Again, this strategy will work in about half of all patients at high risk for metastases, but it prevents relapses that would require a higher dose of chemotherapy.[7] In contrast, the remaining half will be treated needlessly, with the potential risk of toxicity. Primary chemotherapy would be a realistic alternative treatment option if occult metastases could be identified. Before that any toxicity in patients without metastatic disease is significant.[7,9]

Currently, the objective in clinical stage I patients is to reduce treatment toxicity, while maintaining high survival rates and therefore a more rational approach to the treatment would

be possible if the likelihood of metastasis could be determined. Studies have been conducted to identify histopathological risk factors for occult retroperitoneal lymph node metastases. The presence of vascular invasion (VI) and both the presence and the percentage of embryonal cell carcinoma (ECC) in the primary tumour are of prognostic value.[4,6,10-12] The combination of these parameters may have a predictive value of 86% for the presence of occult metastatic disease.[4,12] However, because of contradictory results[13] and the lack of sufficient specificity and sensitivity, the existing predictive markers need to be improved and/or new markers are needed in order to detect patients at low and high risk for metastases. The role of adhesion molecules (cadherin-catenin complex) has been examined in other types of cancer[14-19] and in TGCT.[20] Loss of those molecules results in an increase in the invasive ability of tumour cells. No studies of the loss of alpha-catenin expression in clinical stage I NSGCT have yet been conducted. Flow cytometric analysis of nuclear DNA content has important prognostic information for several genito-urinary tumours.[21,22] In previous studies, relatively new variables like flow cytometry and single-cell cytophotometry have been used in CS I NSGCT[23-30], but the findings were inconclusive. In the present study, we evaluated the additional prognostic value of the immunohistochemical expression of alpha-catenin and DNA image analysis cytometry over routine tumour histology. All results were composed in univariate and multivariate analysis.

Patients and Methods

Between 1986 and 1992, 50 consecutive patients with clinical stage I NSGCT underwent a nerve sparing RPLND. Clinical staging included measurements of serum tumour markers (-foetoprotein, -human chorionic gonadotropin and lactic acid dehydrogenase) and CT of the abdomen and chest. All patients had undergone previous radical orchiectomy. Archival

material (formalin fixed, paraffin embedded tissue from the orchiectomy specimens) of all patients was obtained from the institution at which they had undergone orchiectomy.

Pathological stage IIa patients were treated with two additional cycles of bleomycin, etoposide and cisplatin. The median follow-up time after last treatment was 6.3 years (range, 2.3-11.1 years). Regular follow up data were available for all patients (physical examination, chest radiographs, abdominal CT and serum tumour markers).

Paraffin embedded blocks (1-10, mean 2) of 50 patients were assessed; from each block a 5-µm section, followed by a 50-µm section and finally a 5-µm section, were cut. The first and the last 5-µm sections were stained with hematoxylin and eosin (H&E), and used to ensue the presence of tumour tissue and for histopathological evaluation. Only blocks containing NSGCT were analysed: care was taken to avoid sampling necrosis, fibrosis and haemorrhage. All H&E slices were reviewed by one pathologist (H.E.S.), who was unaware of the clinical status of the patients.

The presence of VI, tunica albuginea invasion (TI), ECC, choriocarcinoma, yolk sac tumour, (im)mature teratoma and seminoma was recorded. There was no formal quantitative assessment of each separate histologic cell type. VI was defined as either venous or lymphatic invasion and TI as tumour extension into the tunica albuginea. The tumours were staged according to the TNM 1997 pathologic staging system.[31]

Immunohistochemistry for alpha-catenin was carried out on 5-µm tissue sections obtained from each of the tumour blocks present in a given case. The immunohistochemical staining protocol was conducted essentially as recently described [32]. Briefly, the monoclonal antibody clone HECD-1 (Takara, Berkeley, USA) at a dilution of 1:50 was applied at 4^0C, overnight. A Vectastain Elite kit (Vector laboratories, Burlingame, CA) was used for peroxidase visualization.

For alpha-catenin, non-malignant epithelium present in the tumour sections was used as an internal positive control: discrete staining should be present at cell-cell contacts only. Because the most malignant tumour cell population will ultimately determine the tumour's biological behaviour, the presence of an aberrant or negative staining in more than 10% of the malignant cells was defined as abnormal.[14] In all other cases, tumours were classified as normal.

The preparation procedure for DNA image-analysis cytometry is described elsewhere in detail.[33] In short, 50 m thick paraffin-embedded tissue sections were used to dissect selectively the areas in the section that contained tumour, using a preparation microscope. As a method for orientation and control of tissue composition, a 5- m thick, H&E-stained adjacently parallel tissue section was used. After deparaffinzation and rehydration, the 50- m thick microdissected tissue sections were placed in a centrifuge tube and incubated in phosphate-buffered saline (PBS, 137 mM NaCl, 13 mM $Na_2HPO_4.2H_2O$, 3 mM KH_2PO_4, pH=7.4) with 0.1% protease (type XXVII (Nargarse), Sigma Chemical CO, St. Louis, USA) at 37º C for 5-30 min. Incubation was terminated by adding 4 to 5 ml cold (4º C) PBS, and putting the tubes on ice. The nuclei were washed twice with PBS using intermediate centrifugation steps. About 30,000 nuclei were counted with a Coulter Counter, centrifuged and 200 l fetal calf serum was added. Subsequently, the nuclear suspension was centrifuged to a glass slide using a cytocentrifuge for 10 min at 450 rpm, briefly air-dried, and fixed in a mixture of methanol, 37% formaldehyde, and acetic acid (85:10:5 by volume) for 60 min. Subsequently, the specimens were stained with Feulgen-pararosaniline.[34] The Discovery image analysis system (Cellular Imaging Systems, Beckton and Dickinson, Leiden, The Netherlands) was used to measure the DNA content of tumour cells and the internal control cells (lymphocytes or neutrophylic granulocytes). The mean number of tumour cells per patient that was measured was 570 with a range of 136-872 nuclei. All measurements were taken by the same experienced cytotechnician. The DNA content of nuclei of tumour cells

was expressed as the DNA index (DI), defined as the nuclear integrated optical density of a nucleus divided by the median integrated optical density of the control cells (lymphocytes or neutrophilic granulocytes).

The DNA histograms were visually classified as DNA-diploid, DNA-polyploid or DNA-aneuploid according to the definitions given elsewhere.[35] In a diploid DNA histogram, a distinct G_0/G_1 peak was found in the diploid region (2C; DI=1.0 0.1) with a small proportion of tumour cells in S and G_2/M phases. In a polyploid DNA histogram, distinct peaks were present in the diploid (2C; DI=1.0 0.1) and tetraploid (4C; DI=2.0 0.2) regions, or in the diploid, tetraploid and octaploid (8C; DI=4.0 0.4) regions. DNA histograms were considered to be aneuploid in all cases with scattered DI distributions, or uni-, bi- or multimodal distributions, that were not diploid or not polyploid. In this group, a separate entity was defined as a DNA-triploid pattern, which has a distinct G_0/G_1 peak in the triploid (3C; DI=1.5 0.15) region, and a small proportion of tumour cells in the S and G_2/M phases (6C; DI=3.0 0.3). In addition, the 2.5c exceeding rate (DI>1.25), 3.5c exceeding rate (DI>1.75), and 5c exceeding rate (DI>2.5) were calculated for each DNA histogram. One pathologist (P.C.deW.) who was unaware of the clinical status of the patients classified the DNA-histograms.

Univariate logistic regression model was used to determine whether the presence of histological variables, the expression pattern of alpha-catenin, the exceeding rates (2.5c, 3.5c and 5c), the DNA-ploidy and the DNA-index were each a significant risk factor associated with stage IIa disease.[36] The univariate two-tailed Student-t-test and the Wilcoxon-test (for comparing square means) were calculated. If possible, the odds ratio with a 95% confidence interval was calculated to determine potential risk factors. All potential risk factors were considered in a multivariate logistic model to confirm the best set of risk factors. Differences between both groups were deemed statistically significant at a $p< 0.05$.

Results

All 50 patients were clinical stage I NSGCTs: according to the RPLND specimen, 14 (28%) had histologically confirmed pathological lymph-node(s). These patients are finally staged as pathological stage IIa NSGCT. One pathological staged I patient developed a lung metastasis within three months after orchiectomy and was included for statistical analysis in the pathological stage II group. The histopathological parameters are listed in Table I.

TABLE I **The univariate analysis of variables predicting occult metastatic disease in 50 patients clinical staged I NSGCT.**

Variable	No. of Patients	Pathological stage 35 stage I (%)	15 stage IIa (%)	p-value[1]	Odds ratio or mean (CI:95%)
Histology, n (%)					
Embryonal cell carcinoma	39	24 (69)	15 (100)	0.02	*
Yolk sac tumour	18	14 (40)	4 (27)	0.52	0.55 (0.14-2.06)
Mature teratoma	11	9 (26)	2 (13)	0.47	0.44 (0.084-2.36)
Immature teratoma	9	8 (23)	1 (7)	0.25	0.24 (0.027-2.13)
Seminoma	11	8 (23)	3 (20)	1.00	0.84 (0.19-3.75)
Vascular invasion	18	7 (20)	11 (73)	0.001	11.0 (2.68-45.18)
Tunica albuginea invasion	18	7 (20)	11 (73)	0.001	11.0 (2.68-45.18)
Abnormal alpha-catenin expression[2]	28	21 (62)	7 (47)	0.36	1.85 (0.54-6.30)
Ploidy					
Diploid	3	3 (9)	0	0.55	*
Polyploid	2	2 (6)	0	1.00	*
Aneuploid	22	14 (40)	8 (53)	0.54	1.71 (0.51-5.80)
Triploid	23	16 (46)	7 (47)	1.00	1.04 (0.31-3.50)
Mean (SD)					
DNA index	-	1.46±0.29	1.38±0.21	0.32	0.082 (-0.083 - 0.25)
Exceeding Rate					
2.5c-exceeding rate	-	47.94±23.28	42.86±18.15	0.46	5.09 (-8.51 – 18.68)
3.5c-exceeding rate	-	19.84±15.19	15.81±7.62	0.34	4.02 (-4.31 - 12.36)
5c-exceeding rate	-	8.87±8,85	7,07±5.98	0.48	1.81 (-3.23 – 6.85)

*Not available, division by zero; [1] Student's *t*-test for histology and ploidy, Wilcoxon test for DNA and exceeding rate; [2] 49 specimen available for analysis.

Univariate logistic regression showed a statistically significant correlation between occult metastases and the presence of ECC, VI and TI. Interestingly, the absence of ECC identified a subgroup of patients (n=11) at extremely low risk for occult metastatic disease (negative predictive value of 100%). However, this negative predictive value precluded a multivariate analysis on EC (because a zero is present): thus for the analyses we assumed each of the stage I patients in turn with no ECC in the primary tumour was stage II. With this modification, VI or TI were the only significant parameters in multivariate analysis (p=0.001), with a sensitivity of 87.5% and specificity of 80.0% in predicting the final pathologic stage (Table II).

TABLE II **Predictive values of histological factors in identifying occult metastases in clinical stage I NSGCT (univariate analysis).**

	Embryonal cell carcinoma (%)	Vascular invasion (%)	Tunica albuginea invasion (%)
Sensitivity	100	87.5	87.5
Specificity	31.4	80.0	80.0
Predictive value +ve	38.5	61.1	61.1
Predictive value - ve	100	73.3	73.3

On immunohistochemistry, in one of the 50 specimens the expression pattern of alpha-catenin was unclear: 49 specimens could thus be evaluated and in 28 there was an abnormal alpha-catenin expression pattern with positive staining for benign testicular tissue (internal control; Figure 1). Seven of these had pathological stage IIa and 21 pathological stage I disease. A division between metastatic and nonmetastatic tumours was not possible from the alpha-catenin staining results (Table I).

On DNA image cytometry, of the 50 testicular tumours, 3 were uniformly DNA-diploid (6.3%), 2 DNA-polyploid (4.2%) and 45 DNA-aneuploid (89.6%). The DNA-aneuploid

tumours were subdivided into 22 DNA-aneuploid and 21 DNA-triploid. Of interest, the DNA-diploid and DNA-polyploid tumours were all pathological stage I. Neither the ploidy parameters nor the DNA-index and the exceeding rate values were significantly correlated with stage IIa disease (Table I).

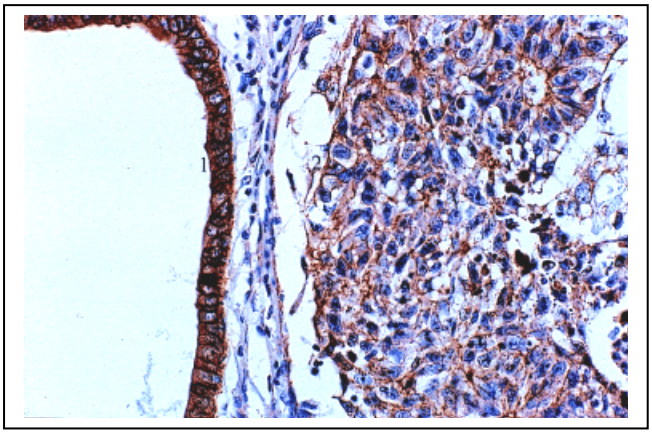

Figure 1. The immunoreactivity of α-catenin in nonseminomatous testicular cancer. Paraffin sections were stained with antibodies against α-catenin. Normal tissue on the luminal side of the rete testis (1) stained positively for α-catenin, whereas the tumorous part (embryonal call carcinoma, 2) had aberrant or negative staining for α-catenin. (x200)

Discussion

The main problem in the treatment strategy for clinical stage I NSGCT is detecting occult retroperitoneal metastases. The 28% incidence of positive nodes found at RPLND in the present series of patients is in accordance with published results (30-35%).[10,11] The identification of prognostic factors could be of great value for choosing the best therapeutic option. To date, the most important prognostic factors for occult retroperitoneal disease appear to be VI and the presence and/or percentage of ECC.[4,6,10-13] VI in the primary tumour correlates with an increased risk of retroperitoneal lymph node metastases for clinically staged I NSGCT patients. Former studies show that 36-88% of these patients had metastases or recurrences, compared with 10-24% of patients with no VI.[37] In a retrospective study of 92 clinical stage I patients, Moul *et al.*[12] found VI, with a sensitivity of 76.3% and a specificity of 83.3%, to be the best prognostic factor for pathological stage II disease. In the present study,

VI was, with TI, the best risk factor (87.5% sensitivity and 80.0% specificity). However, identifying VI is difficult: retraction artefacts in stroma may mimic lymphatic spaces and the stromal cells could be misinterpreted as lining endothelial cells.[37] Artefacts due to knife implantation into vascular spaces can be misconstrued on histology, resembling pseudoinvasion.[38] Therefore, some studies underline the importance of consulting experienced reference pathologists.[11]

In contrast to VI, the results for ECC are contradictory: in the largest study of prognostic risk factor analysis for clinical stage I NSGCT (75 pathological stage I, 204 pathological stage II) ECC could not be confirmed as an important prognostic factor (p=0.08), whereas VI was shown to be a significant risk factor in uni- and multivariate analysis.[13] Analysing the primary tumour for quantitative histopathology could increase the predictive value.[4,6,12] Heidenreich *et al.*[4] predicted in 85% of 48 selected clinical Stage I patients the correct pathological stage IIa, using an ECC threshold of 80%. A threshold of 45% ECC in the primary tumour of 77 clinical stage I patients predicted pathological stage I with 88% accuracy. These predictive values were improved by combining the presence or absence of VI with ECC, respectively. However, prospective trials have failed to establish any specific level of ECC to be accurately predictive for metastasis.[5,13] An explanation of these contradictory results might be that the pathological assessment of the percentage of ECC is subjective and depends on interpretation by the observer. As to quantitative histology, difficulties in recognising and distinguishing yolk sac tumour from ECC were reported.[37,39] For instance, in mixed germ cell tumours the components are arranged diffusely, and pathological review requires an estimate of the percentage of each component on each slide. This easily causes inter- and intra-observer variation. Therefore, in the present study only the presence or absence of ECC was sought: all stage IIa patients (including the one with a lung metastasis) had ECC components. In contrast, all 11 clinical stage I patients with no ECC components in the primary tumour were correctly

classified as pathological stage I NSGCT. The absence of ECC independently improved the prediction of patients at extremely low risk for metastatic disease.

The 1978 TNM classification, used in previous studies, revealed that the primary tumour stage (pT) 2 (meaning TI) was associated with a higher risk of metastases.[10,11,13] Fung *et al.*[40] reported, in 60 clinical stage I NSGCT patients, that 14 of the 20 pathological stage II patients had a pT of 2. In the present classification system (1997), TI has been lowered to a pT1 lesion, and VI has been added and classified as a pT2 lesion. The present results are comparable with former studies, as in all specimens TI coincided with VI. In 11 of the 15 specimen TI was positively correlated with pathological stage II: in three invasion in the epididymis was also found. TI was, together with VI, the best prognostic indicator in the present study.

The cadherin-catenin complex is essential for the adhesion and detachment of cells. This complex, consisting of E-cadherin and -, - and -catenins, is located in the cell membrane. Loss of those adhesive molecules results in an increase in the invasive ability of tumour cells. Cadherin expression is lost in advanced prostatic, breast, oesophageal, gastric, colonic, and testis carcinomas.[14-20] In prostate cancer, E-cadherin probably uses -catenin for anchorage to the cytoskeleton.[14] Studies on oesophageal and gastric cancer suggested that aberrant -catenin expression might be a better indicator of tumour invasion and metastasis than E-cadherin.[17,18] Recently, Saito *et al.*[20] reported aberrant E-cadherin expression in three of 16 seminomas and in 10 of 16 NSGCT, which was not expressed on normal germ cells. An aberrant expression of -catenin was detected in four of the NSGCT. They suggested that E-cadherin-catenin complex might not be functional in testis tumours, as most E-cadherin-positive testis cancers had no (or little) -catenin expression. However, the functionality might be best tested by correlating the expression pattern with clinical and pathological

variables, e.g. the stage of disease and/or survival. In a retrospective study of 149 clinical stage I NSGCTs, 78 specimens could be evaluated for E-cadherin expression. Nineteen had a loss of E-cadherin expression, subdivided into 17 pathological stage II and 2 pathological stage I NSGCT ($p<0.001$).[4] To our knowledge, the present study is the first to investigate the -catenin expression in clinical stage I NSGCT: -catenin was expressed in virtually all the specimens examined (98%). Unfortunately, we were unable to obtain any evidence that down-regulation of the -catenin expression was involved in the process of metastasis to retroperitoneal lymph nodes in clinical stage I NSGCT.

New methods (e.g. DNA-analyses) have been developed to improve the assessment of risk factors in cancer.[21] The hypothesis is that tumour cells have a higher amount of DNA than normal cells, which correlates to a more aggressive growth pattern, in terms of invasion and spread. To date, the results of these studies have been inconclusive.[23-30] Consequently, DNA-analyses have not yet been used routinely for staging clinical stage I NSGCT. Some investigators reported an abnormal DNA stem-line in NSGCT.[23,24,27] Moul *et al.*[24] suggested that if one sampled area of a NSGCT was DNA-diploid then other areas should be examined, and DNA-aneuploidy and DNA-multiploidy would be found. In contrast, in the present study, uniformly DNA-diploid and DNA-polyploid tumours were present only in pathological stage I patients. Moreover, all samples of pathological staged II patients were DNA-aneuploid: diploidy and polyploidy identified patients at low risk but a larger cohort is necessary to draw firm conclusions. In concordance with other studies, DNA-diploidy and the DNA-index had no predictive significance in detecting occult metastatic disease in NSGCT.[23,24,27]

Image analysis can used to detect nuclei with a very high DNA content. Tumours with DNA-hypertetraploid components are more likely to be pathologic stage II.[25,29] In a retrospective study of 74 clinical staged I patients, 23 of the 33 staged II patients with a 5c-ER above 3.1% had metastatic disease.[30] Unfortunately, this outcome is not useful a clinical setting

(sensitivity: 69.7%, i.e. 30.1% pathological stage IIa NSCGT were misclassified as stage I): the exceeding rates according to our study could not be used to predict the pathological stage of a tumour.

Conclusion

Although the primary goal of the present study was to evaluate the additional value of -catenin and DNA-markers over routine histology, we found VI and TI to be the most important predictors for occult metastatic disease. With a sensitivity of 87.5% and a specificity of 80%, VI and TI were useful for identifying clinical stage I NSGCT patients at high risk for retroperitoneal disease. In contrast, the absence of ECC in the primary tumour exactly predicted patients at low risk for metastasis. A DNA-diploid or DNA-polyploid primary tumour also identified patients at low risk.

To differentiate patients at low and high risk for metastases, independent, reliable and reproducible prognostic factors are needed. Therefore, we suggest that an experienced pathologist, who is able to analyse tumour histology, should assess TI, VI and ECC. Further study is necessary to clarify the best set of prognostic factors for evaluating clinical stage I NSGCTs.

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PART III

The evaluation of (treatment response in) germ cell tumour of the testis by Positron Emission Tomography

CHAPTER 6

The Role of ^{18}fluoro-2-deoxyglucose positron emission tomography in initial staging and re-staging after chemotherapy for testicular germ cell cancer.

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Abstract

Objective. To investigate the role of ^{18}fluoro-2-deoxyglucose positron emission tomography (^{18}FDG-PET) in the initial staging of clinical stage I and II nonseminomatous germ cell tumours (NSGCTs) and in re-staging of (non)seminomatous GCTs after chemotherapy.

Patients and methods. ^{18}FDG-PET studies were undertaken in 50 patients. FDG uptake was interpreted visually and when possible the standardized uptake value was determined. A ^{18}FDG-PET scan was taken in 5 patients with clinical stage I and in 7 with stage II NSGCT. The scans were validated by histology. Stage I patients underwent a retroperitoneal lymph node dissection because of vascular invasion in the primary tumour.

Thirty-eight scans were taken after the completion of chemotherapy (28 NSGCTs, 10 seminomatous GCTs), and validated by histology or clinical follow-up.

Results. In stage I NSGCT, ^{18}FDG-PET staging was equivalent to computed tomography (CT) staging. One small lesion, consisting of mature teratoma, was missed by both ^{18}FDG-PET and CT scan. In stage II NSGCT, ^{18}FDG-PET missed two lesions (mature teratoma and retroperitoneal mass with a small component of embryonal cell carcinoma) whereas CT correctly classified all.

In 20 of 28 patients with NSGCT, histology was obtained after chemotherapy. In 1 of 3 patients with viable tumourous residual mass the ^{18}FDG-PET scan was clearly positive; in 4 of 12 patients with mature teratoma and inflammation components retroperitoneally, the ^{18}FDG-PET was also positive. In contrast, 8 patients with solitary mature teratoma had a negative PET result. In 4 of 5 patients with necrosis after chemotherapy the PET result was correctly negative. All 8 patients on surveillance had a negative PET scan and are free of disease at median of 14 months (range 8-18). Interestingly, of the 12 patients with a correct negative PET result, 11 had no mature teratoma in their primary tumour.

Nine of 10 (90%) patients with seminomatous GCT (SGCT) were correctly staged. Two ^{18}FDG-PET studies showed increased uptake: in one, a viable seminomatous mass was found and in the other there was inflammation in the residual mass. In all other patients the ^{18}FDG-PET scan correctly predicted absence of viability in the residual mass.

Conclusions. In primary staging, ^{18}FDG-PET has no benefit over CT. In re-staging, a negative ^{18}FDG-PET result predicts fibrotic residual mass in SGCT. Moreover, it could be useful to predict fibrotic residual mass in NSGCT in those patients with no teratoma component in their primary tumour.

Introduction

As most testicular germ cell tumours (GCT) are curable, different therapeutic strategies have been evaluated to reduce the morbidity of treatment without affecting the survival rate. Currently, staging in GCT is used to determine the extent of disease and to facilitate the choice of treatment. However, clinical stage (CS) I nonseminomatous GCTs (NSGCT) are understaged in 30% of patients[1], despite improvements of CT technology.[2] Consequently, the optimal treatment after orchiectomy remains controversial. Primarily, two strategies have been applied, i.e. watchful waiting with close surveillance and chemotherapy on relapse, or primary retroperitoneal lymphadenectomy (RPLND) with or without chemotherapy.[1,3,4] The morbidity of a surveillance policy is low for those patients without progression, but the frequent follow-up visits may be stressful. Moreover, a surveillance policy if there is occult metastasis might result in a treatment delay with the potential for more extensive disease. Conversely, approximately 70% of the patients who undergo RPLND have tumour-negative lymph nodes.[1] Independent of initial treatment, the survival rate for stage I NSGCT patients is close to 100%.[4]

At present, treatment can also be adapted to prognostic factors. The best predictors for metastatic disease (50%) are the presence of vascular and lymphatic invasion (VI) and embryonal cell carcinoma (ECC) components in the primary tumour.[5] For patients at high risk for metastases some[6] advocate RPLND with adjuvant chemotherapy in those with pathological stage II, whereas others recommend chemotherapy only.[7] In contrast, patients at low risk will enter a surveillance protocol. The role of primary chemotherapy has yet to be defined, as half of the patients will be treated needlessly, with the potential risk of toxicity. In our centre RPLND is undertaken in CS I patients with vascular invasion in the primary tumour and those with no invasion are followed closely. However, the best choice of treatment is only possible if the presence of metastasis can be determined.

Another problem is the presence of indeterminate residual masses on CT after the completion of chemotherapy for disseminated GCTs. In general, the residual mass in NSGCT consist of viable tumour (10-20%), mature teratoma (30-40%) and necrosis or fibrosis (40-50%).[8] Thus, half of the patients with residual mass undergo unnecessary surgical treatment. For seminomatous GCT (SGCT), two-thirds of the patients have a residual mass after chemotherapy and finally only 12-15% contain viable tumour.[9] Some investigators have recommended resection of post-chemotherapy residual masses in SGCT[10], others advocate surveillance.[11] The response to therapy in SGCT can be assessed by the size of the residual mass.[12] In our centre, patients with SGCT and a residual mass of < 3cm in maximal transverse diameter on CT will enter the surveillance protocol, and those with >3cm will have surgery. However, in both NSGCT and SGCT the optimal method for differentiating patients with a post-chemotherapy tumourous residual mass from those with necrosis/fibrosis has yet to be defined.

A relatively new method of staging, ^{18}fluoro-2-deoxyglucose positron emission tomography (^{18}FDG-PET), might be useful to overcome the diagnostic problems in (re)staging GCTs. The glucose analogue FDG is a radiopharmaceutical that allows *in vivo* evaluation of glucose metabolism with PET. Most malignancies, including GCT, show increased glucose use.[13] In this setting, functional imaging may provide a more accurate means of delineating malignant versus benign tissue. Moreover, in other malignancies ^{18}FDG-PET seem to be more accurate in identifying metastatic disease in lymph nodes that are not enlarged.[14] As ^{18}FDG-PET can assess the metabolic activity of tissue it might be able to differentiate between different histological entities. We investigated the current applications and the added value of ^{18}FDG-PET over CT in patients with GCTs, reviewed our experience of ^{18}FDG-PET in CS I and II NSGCT and evaluated post-chemotherapy residual masses in (non)seminomatous disease.

Patients and methods

From November 1998 to July 2001, 50 men (mean age at diagnosis: 30 years, range 17-61), with CS I/IIa NSGCTs (n=12, Table I) or more disseminated GCTs (n=38, Table II) were studied. Patients were staged using abdominal and chest CT scan, determination of serum tumour markers and histopathological verification before the start of therapy. Primary staging was conducted according to Royal Marsden Hospital Classification.

Five patients with CS I at high risk of metastases (vascular invasion in the primary tumour) and seven with CS IIA NSGCT underwent ^{18}FDG-PET scan on the day before surgery. The results were compared with staging spiral CT. Solitary lymph nodes of < 1.0 cm in maximal transverse diameter were judged negative on CT. An experienced radiologist who was unaware of the disease status of the patient interpreted the CT images.

Additionally, 38 patients with stage IIb-IV GCTs were included (28 NSGCT and 10 SGCT). All patients underwent CT and ^{18}FDG-PET after the completion of first-line chemotherapy (cisplatin, etoposide and bleomycin). In all cases, FDG-PET imaging was taken within one month of CT. Histological verification of the residual tumour was obtained in 20 NSGCTs and 2 SGCTs. The median time from ^{18}FDG-PET scans to surgery was 21 days (range 1-60). Verification of the nature of the residual tumours in the remaining patients was based on information from serum tumour markers, CT and the duration of the event-free follow-up after the FDG-PET study (median 12 months, range 2-18). The study was approved by the local ethical committee and informed consent was obtained from all patients.

For the FDG-PET scan protocol, a dedicated, rotating half-ring PET-scanner (ECAT-ART, Siemens/CTI, Knoxville, TN, USA) was used for data acquisition. Before injection with ^{18}FDG the patients fasted for at least 6 hours although sugar-free liquids were permitted. Immediately before the procedure the patients were hydrated with 500 ml of water. One hour after intravenous injection of 200-220 MBq ^{18}FDG (Mallinckrodt Medical, Petten, The

Netherlands) and 20 mg furosemide, emission and transmission images of the area between proximal femora and the base of the skull were acquired (10 minutes per bedposition). The images were corrected for attenuation and reconstructed using the ordered-subsets expectation maximization algorithm. The reconstructed images were displayed in coronal, transverse and sagittal planes. All scans were interpreted by two nuclear medicine physicians (W.J.G.O., L.F.deG.O.) unaware of all clinical and pathological data. Tumour metabolism was evaluated using visual analysis and calculation of standardized uptake value (SUV). The visual scoring model was graded as suspect for carcinoma (++); increased uptake (+); negative (-). The SUV was calculated for the region of maximal FDG uptake.[15]

Results

Five patients with CS I and seven stage IIA underwent a FDG-PET scan before RPLND (Table I).

Table I **^{18}FDG-PET results in clinical staging non-seminoma testis stage I and IIa**

Patient	Primary Histology	Tumour markers (ng/ml)*	Lymph node Stage CT (cm)	^{18}FDG-PET (visual)	SUV	RPLND
1	ECC, SEM	N	<1	-	N.D.	Negative
2	ECC, SEM	N	<1	-	N.D.	Negative
3	MT	N	<1	-	N.D.	Negative
4	ECC	N	<1	-	N.D.	Negative
5	MT, SEM	N	<1	-	N.D.	MT
6	ECC, YST, MT	N	2	++	14,8	ECC
7	ECC, SEM	N	2	++	5.5	Histiocytosis
			1.5	++	4.3	ECC
8	ECC, MT, YST	N	1,4	++	5	ECC
9	ECC, YST, MT	N	1,2	++	4,8	ECC
10	MT, ECC	N	1,3	++	3,8	ECC
11	ECC	N	1,2	-	N.D.	ECC[1]
12	ECC, YST	bHCG:6	1,5	-	N.D.	MT

Key: *1 day before RPLND; ECC, embryonal cell carcinoma; SEM, seminoma; MT, mature teratoma; YST, yolk sac tumour; N.D, not done; ++clearly positive; - negative; N, normal; [1] microscopic disease (3 mm)

In CS I NSGCT, ^{18}FDG-PET staging was equivalent to CT staging. One small lesion, consisting of mature teratoma (patient 5), was missed by both ^{18}FDG-PET and CT. The remaining patients were correct negatively staged. In CS II NSGCT, the ^{18}FDG-PET missed two lesions whereas CT was able to classify all correctly. One lesion seems to be necrotic tissue of 1.2 cm in which ECC (approximately 0.3 cm in diameter, patient 11) was found and the other lesion was a mature teratoma of 1.5 cm in transverse diameter (patient 12). The remaining patients were correctly positively staged (Figure 1).

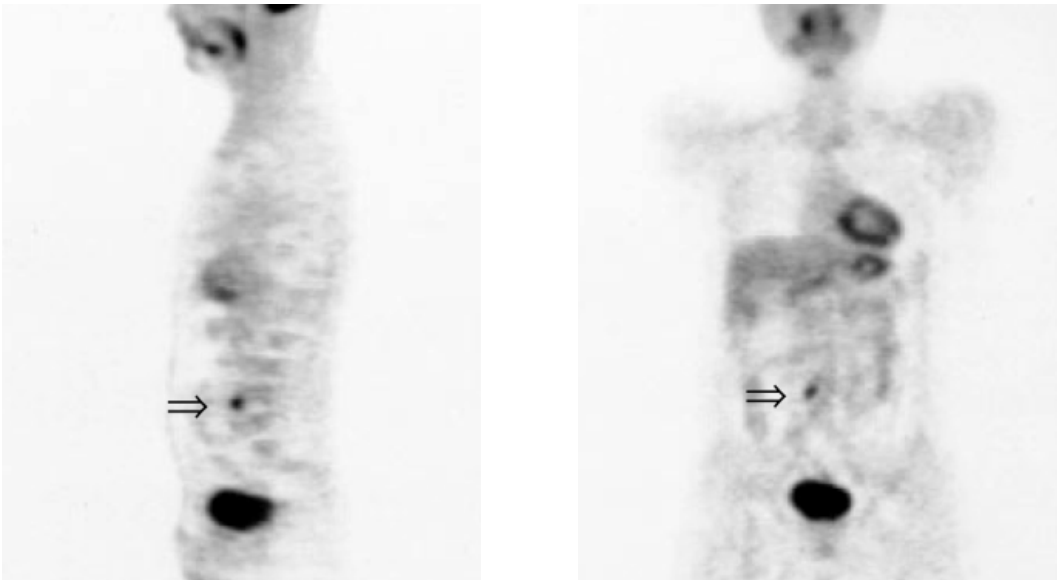

Figure 1. Sagittal and coronal PET scan with suspected retroperitoneal lymph node (arrow). The clinical diagnosis was stage IIa non seminoma testis.

Thirty-eight patients were evaluated for residual mass after chemotherapy (Table II). Twenty-eight patients with NSGCT were studied after chemotherapy; the surgical procedure was RPLND in 20, occasionally in combination with partial hepatectomy (patient 15) or thoracotomy (patient 16). The lesions detected by ^{18}FDG-PET were classified as viable tumourous (n=3), teratomatous (n=12) or as residual necrotic mass (n=5) after chemotherapy.

Table II The characteristics of patients evaluated with FDG-PET after chemotherapy for advanced germ cell tumour

	Initial Staging			After chemotherapy					
Patient	Primary Histology	Tumour stage	Tumour markers[1]	Region of interest	CT (cm)	FDG-PET (visual)	SUV	Histological diagnosis	Follow-up (months)
Surgery after chemotherapy for nonseminoma: viable cancerous disease									
13	MT, YST	IID	N	Retroperitoneum	18	++	3..5	Teratocarcinoma	14
14	ECC, CHO	IID	bHCG: 7	Retroperitoneum	8	-	NA.	ECC, CHO[2], N/ F	10
		IVL		Lung	NP	+	NA.	surveillance	
15	ECC, SEM	IID	N	Retroperitoneum	5.2	+	2.2	ECC[2], MT + His	17
		IVH1		Liver	2x2	-	ND	2X N/F	
Surgery after chemotherapy for nonseminoma: mature teratoma									
16	MT, ECC	IID	N	Retroperitoneum	1.5	-	NA.	MT	11
		IVL		Lung	0.5	-	NA.	MT	
				Paravesical	0.4	++	3.2	NO.	
17	MT, ECC	IID	N	Retroperitoneum	3	++	1.7	MT + His	12
		IIIn		Supraclaviculair		+	2.4	NO.	
18	MT, ECC	IID	N	Retroperitoneum	6	+	3.7	MT + His	13
19	MT, YST, SEM	IID	N	Retroperitoneum	6	+	1.8	MT + His	11
20	MT, SEM	IID	N	Retroperitoneum	9.5	+	1.9	MT + His	9
21	MT, ECC, CHO	IIB	N	Retroperitoneum	3	-	ND.	MT	13
22	MT, ECC	IIC	N	Retroperitoneum	7	-	NA.	MT	16
23	MT, YST	IIC	N	Retroperitoneum	5	-	N.D.	MT	2
24	MT, ECC	IIC	N	Retroperitoneum	4	-	ND.	MT	14
25	MT, SEM	IIC	N	Retroperitoneum	3	-	ND.	MT	10
26	MT, ECC, CHO	IID	N	Retroperitoneum	6	-	ND.	MT	18
27	IMT, SEM	IIB	N	Retroperitoneum	4	-	ND.	MT	9
		IV		Lung	NP	-	ND.	NO.	
Surgery after chemotherapy for nonseminoma: necrosis/fibrosis									
28	YST, SEM	IID	N	Retroperitoneum	8	+	4.2	N/F+ Gran. tissue	17
29	EC,C CHO, SEM	IID	bHCG: 3.6	Retroperitoneum	4	+	2.8	N/F + His	10
		IV		Lung	NP	-	ND.	NO.	
30	ECC, SEM	IID	N	Retroperitoneum	7.5	-	NA.	N/F	11
		IV		Lung	0.5 –2.5	++	NA.	NO.	
31	EC, CHO, YST	IIC	N	Retroperitoneum	2.5	-	ND.	N/F	13
32	MT	IIC	N	Retroperitoneum	3	-	ND.	N/F	12
Follow-up after chemotherapy for nonseminoma									
33	CHO	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	14
34	ECC	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	17
35	ECC, SEM	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	2
36	ECC	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	3
37	ECC, SEM	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	9
		IIIn		Mediastinum	<1	-	ND.	surveillance	
38	ECC	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	3
		IIIn		Mediastinum	NP	-	ND.	surveillance	
39	ECC	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	3
		IV		Lung	NP	-	ND.	surveillance	
40	ECC	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	3
		IV		Lung	NP	-	ND.	surveillance	
Surgery after chemotherapy for seminoma									
41	SEM	IID	N	Retroperitoneum	4	++	NA.	SEM +N/F	15
42	SEM	IID	N	Retroperitoneum	5.5	++	NA.	N/F + inflammation	11
Follow-up after chemotherapy for seminoma									
43	SEM	IID	N	Retroperitoneum	2.8	+ 02/2000	2.6	surveillance	18
				Retroperitoneum	2.0	- 03/2000	ND.		
44	SEM	IID	N	Retroperitoneum	3	-	ND.	surveillance	17
45	SEM	IIC	N	Retroperitoneum	2	-	ND.	surveillance	12
46	SEM	IID	N	Retroperitoneum	2	-	ND.	surveillance	8
47	SEM	IIC	N	Retroperitoneum	<1	-	ND.	surveillance	14
48	SEM	IID	N	Retroperitoneum	<1	-	ND.	surveillance	14
49	SEM	IIB	N	Retroperitoneum	<1	-	ND.	surveillance	10
50	SEM	IIC	N	Retroperitoneum	<1	-	ND.	surveillance	14

Key: abbreviations see Table 1; Cho, corioncarcinoma; His, histocytosis; NP, not present; N/F, necrosis,fibrosis; NA, not available; ND, not done; NO, not obtained, N, normal; 1, first response evaluation after chemotherapy; 2, microscopic disease

In 8 patients, the residual mass was evaluated as benign on the basis of an event-free follow-up after chemotherapy.

Three patients (13-15) had viable tumour in the residual mass. Patient 13 had a teratocarcinoma of 8 cm in the residual mass that showed intense ^{18}FDG uptake (SUV 3.5, Figure 2). In patients 14 and 15, the tumourous part was not (clearly) detected by ^{18}FDG-PET. In both, tumourous tissue was found in only one histological section, which is obviously below the detection limit of PET. In patient 14, small lesions in the lungs were also seen with moderate FDG uptake. However, CT of the chest showed no abnormalities and the patient has been followed up for 10 months with no evidence of disease.

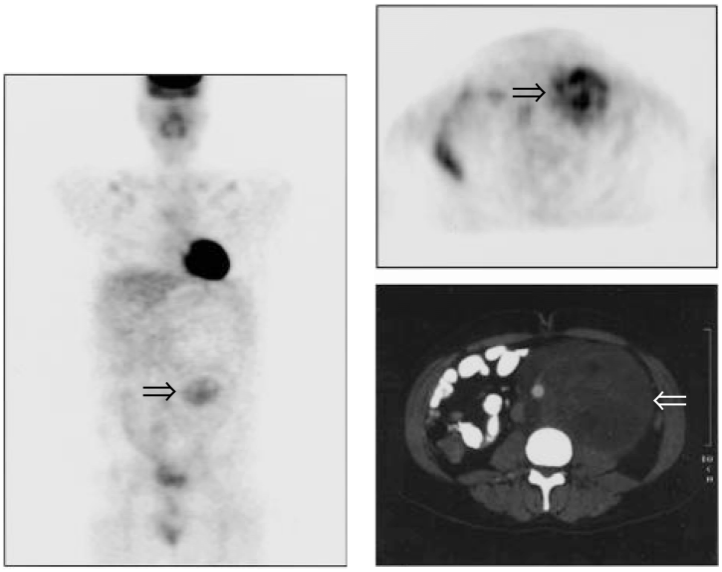

Figure 2. PET image (coronal and axial) and CT (axial) of post-chemotherapy residual mass. PET scan reveals focus of FDG uptake (arrow). Pathologic examination revealed terato-carcinoma.

In most patients with NSGCT mature teratoma was found in the residual mass (16-27). In 5 of them (16-20) ^{18}FDG uptake was found (SUV range: 1,7-3,7). On visual analysis, only patient 16 and 17 had a suspect lesion at PET. However, the lesion in patient 16 was not surgically removed as it was not suspect for disease on CT and it was located outside the suspected residual area (paravesical). In contrast, the residual lesions in retroperitoneum and lungs seen on CT were suspect and therefore removed. Histology revealed teratomatous residual mass in

both locations. In patient 17, ^{18}FDG-PET showed increased retroperitoneal and supraclavicular uptake. Only histology of the retroperitoneal mass was obtained, which showed a teratomatous mass with an inflammatory component (histiocytosis). The supraclavicular lesion detected was probably caused by reactive tissue after the primary diagnostic puncture. After chemotherapy, this lesion was not suspect on CT nor on physical examination and therefore not explored. Currently, patient 17 is free of disease 12 months after treatment.

Although patients 18-20 had increased SUVs, the lesions were not clearly suspect for metastatic disease on visual interpretation. All had ^{18}FDG uptake in the rim of the residual mass and histology showed inflammatory reactive tissue consisting of macrophages (histiocytosis). The remaining 7 patients with histologically mature teratoma (21-27) had negative findings at PET (Figure 3). In three of five residual masses with necrosis/fibrosis (28-32) there was ^{18}FDG uptake. The false-positive PET-scan (patient 28) was caused by benign granulomatous tissue**.** In patient 29, there was minor ^{18}FDG uptake at the border of the residual mass, not suspect for malignancy. At histology, macrophages (histiocytosis) were found in the rim of this mass. Patient 30 had also pulmonary lesions on PET, which were in regression on CT and therefore not removed. The lung metastases disappeared after four months on PET and CT scan. The remaining two patients (31&32) had negative findings at ^{18}FDG-PET in the retroperitoneum, which were in agreement with histological findings. Of interest, 3 patients (29-31) with no teratomatous component in the primary tumour were correct negatively staged by PET.

Eight patients (33-40) had no residual mass(es) after chemotherapy that were suspicious for malignancy and entered a surveillance protocol. Currently, all patients have no signs of disease at a median follow-up of 3 months (range 2-17).

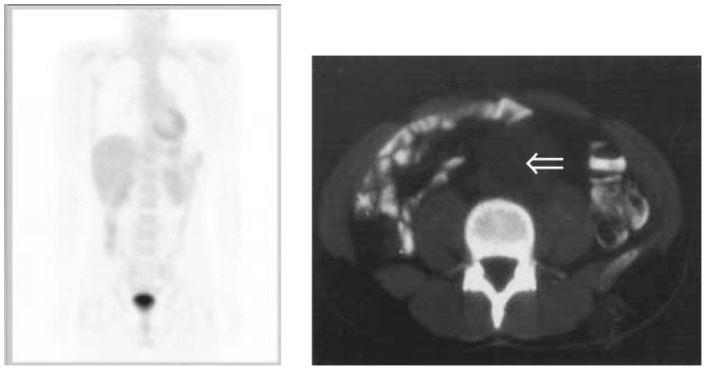

Figure 3. The PET scan shows no increased FDG uptake, whereas the CT-scan shows a post-chemotherapy residual mass (arrow). Histology revealed a mature teratoma.

Ten patients with SGCT received three or four cycles of cisplatin-based chemotherapy. Two patients (41 and 42) had a retroperitoneal residual mass of >3 cm. In both patients, the PET scan showed increased retroperitoneal uptake. At histology viable seminomatous tissue (no. 41) and necrosis with inflammatory components (no. 42) was found.

Eight patients (43-50) entered a surveillance protocol as the residual mass was <3 cm in transverse diameter on CT. Re-staging of patient 43 showed a residual mass of 2,5 cm on CT, which was positive at PET. ^{18}FDG-PET 1 month later was negative and consequently the patient continued follow-up. Four patients (44, 47-49) had positive findings at ^{18}FDG-PET outside the region of interest, probably caused by local activity at L5, bowel adenoma, oesophagitis and sarcoidosis, respectively. None of the patients with SGCT and on surveillance have relapsed during follow-up (median: 14 months; range 8-18 months).

Discussion

Currently, clinical staging of low-stage testicular GCTs is inaccurate; 30% of patients with CS I NSGCT are understaged.[1] As CT predominantly relies on size criteria and is not specific for lymph nodes <1 cm in diameter, clinical staging has not been improved by the newest

generation of CT scanners.[2] The new technique of ^{18}FDG-PET has been useful in localising small volume metastatic disease in comparison with CT in various malignant tumours.[17,18]

Another problem is evaluating residual mass after chemotherapy for disseminated (non)seminomatous disease. The visualization of the glucose metabolism by ^{18}FDG-PET might identify (viable) tumourous tissue and distinguish it from benign tissue.[19] Thus, we investigated the additional value of "whole-body" ^{18}FDG-PET scan in both the clinical staging of high-risk CS I or IIA NSGCT and evaluated residual mass after chemotherapy in disseminated (N)SGCT.

Albers *et al*.[20] assessed 24 patients with NSGCT stage I to IIB who underwent ^{18}FDG-PET for clinical staging. The ^{18}FDG-PET scan detected six of the nine metastatic lesions. The three false-negative findings were found in two small metastatic lesions (<0.5 cm.) and in one retroperitoneal mature teratoma of 4.8 cm in transverse diameter. On CT only both small nodes were judged negative. In contrast, CT was false-positive in 4 of 9 patients with lymph nodes >1.0 cm, whereas FDG-PET was completely conclusive. These authors stated that ^{18}FDG-PET may not be helpful in primary staging of stage CS I NSGCT, but it might have additional value in CS IIA patients, by omitting false-positive findings on CT. A limitation of ^{18}FDG-PET and CT in initial staging of low-stage nonseminomatous disease is its inability to detect very small lesions.[20] Perhaps malignant cells are already present in the lymph node before to nodal enlargement. Detecting even smaller lesions is an unending challenge since the detection of microscopic disease is probably impossible by any imaging technology. As ^{18}FDG-PET and CT miss microscopic disease, there may be no alternative but to submit high-risk CS I patients for surgery. Moreover, it will be difficult to detect mature teratoma by ^{18}FDG-PET since it is a benign condition with a (near) normal metabolism. However, mature teratomas must be resected because of their potential for malignant transformation.[21] In the

present series, ^{18}FDG-PET had no additional value over CT in clinical staging of NSGCT I and IIa.

As noted, it would be beneficial to have a predictive preoperative variable to eliminate unnecessary surgery after chemotherapy. In 30 patients with NSGCT and residual masses after chemotherapy, 11 had fibrosis, 15 a teratoma and 4 a viable tumour.[22] The ^{18}FDG-PET scan was able to differentiate viable tumour from residual necrosis/fibrosis or teratoma; unfortunately, it was unable to differentiate between necrosis/fibrosis and teratoma. In a similar study, Nuutinen *et al.*[23] showed that ^{18}FDG-PET scanning of residual mass could be hampered by false-positive findings. In three of nine positive scans inflammatory changes in benign tissue were found. Ganjoo *et al.*[24] evaluated post-chemotherapy residual masses in 29 patients with disseminated seminoma (19 first-line chemotherapy and 10 salvage chemotherapy). In only one patient the ^{18}FDG-PET scan was positive for a posterior mediastinal mass, but the pathologic diagnosis showed necrotic tissue. They concluded that ^{18}FDG-PET had no benefit in evaluating seminomatous residual mass. As no patients have relapsed after first-line chemotherapy, it can also be concluded that a negative ^{18}FDG-PET is useful for diagnostic surveillance.

In the present study 28 patients with disseminated NSGCT and 10 with disseminated SGCT were evaluated by ^{18}FDG-PET, to determine the ability to differentiate the histological content of residual masses after chemotherapy. In re-staging NSGCT after chemotherapy PET was hampered by false-positive lesions from inflammatory processes and by false-negative findings caused by mature teratoma residual masses after chemotherapy and microscopic viable lesions. However, ^{18}FDG-PET has additional value in patients with no teratoma component in their primary tumour: three of these patients with a residual mass of >1cm and eight with a residual mass of <1cm were correct negatively staged. In contrast, most patients with a teratoma component in the primary tumour also had a teratoma component in their

residual mass, which was not (clearly) suspected on ^{18}FDG-PET. As far as we aware this is the first study identifying a subgroup of patients with NSGCT in which ^{18}FDG-PET might be a valuable adjunct in determining post-chemotherapy resection; the results must be confirmed in a larger trial.

For SGCTs nine of ten patients were correctly staged; one false-positive result caused by an inflammatory process in the residual mass. In 8 patients the ^{18}FDG-PET scan predicted the absence of viable residual mass. As seminomas do not contain teratomatous elements, a negative ^{18}FDG-PET scan could be an argument for surveillance.

Currently, ^{18}FDG-PET scanning has been used in decision-making; one patient referred to our clinic had an undefined lesion in his right lung developed within 2 months after orchidectomy for SGCT. The CT scan of the thorax was inconclusive and all other parameters for metastatic disease were negative (clinical examination, serum tumour markers, abdominal CT-scan). He underwent PET, which was also negative and thus the presence of a metastasis could not be confirmed. This patient was staged as CS I SGCT and received radiotherapy on the retroperitoneum. Currently, he has no signs of metastatic disease at 11 months of follow-up.

Conclusions

In the primary staging of low-stage NSGCTs, ^{18}FDG-PET has no benefit over CT. In re-staging, it can be used to predict fibrotic residual mass in SGCTs. Moreover, it could be useful to predict fibrotic residual mass in a subset of patients with NSGCT and no teratomatous components in their primary lesion. A significant limitation of ^{18}FDG-PET FDG-PET is its inability to detect very small retroperitoneal lesions and mature teratoma components of any size. Moreover, there may be false-positive results caused by inflammatory processes.

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CHAPTER 7

The Role of ^{18}fluoro-2-deoxyglucose positron emission tomography for monitoring chemotherapy response in patients with high stage testicular germ cell cancer

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Abstract

Objective. Positron emission tomography with ^{18}F-fluorodeoxyglucose (^{18}FDG-PET) reflects tissue and tumour viability. We investigated if sequential ^{18}FDG-PET scans in patients with high stage testicular germ cell cancer provide early information on the efficacy of polychemotherapy.

Methods: Serial ^{18}FDG-PET studies were performed in 18 consecutive patients with advanced testicular cancer (14 non-seminoma and 4 seminoma), and who were subdivided into 15 and 3 patients with low and intermediate risk profile, respectively, according to the International Germ Cell Consensus Classification Group. The studies were performed before the start of chemotherapy, after the second chemotherapeutic cycle and 6 weeks after completion of chemotherapy. ^{18}FDG-PET images were interpreted visually and compared to CT-scans made pre-and post-chemotherapy. PET and CT were validated either by histology (n=5) or by clinical follow-up.

Results: Seventeen patients showed increased uptake before chemotherapy. The ^{18}FDG uptake decreased in all 17 patients during therapy. In one patient serial-PET did not show any uptake at all. Overall, the clinical course of disease after first-line treatment was correctly predicted by serial ^{18}FDG-PET in 71% of the patients (12/17) compared with 83% (15/18) by CT. PET was hampered by false-positive and negative findings caused by inflammatory processes and mature teratomatous tissue, respectively.

Of 18 patients enrolled, 16 were free of disease at a median of 10.3 months. Two patients with an intermediate risk profile relapsed at three months after discontinuation of chemotherapy.

Conclusion: This pilot study demonstrates that it is possible to monitor changes in ^{18}FDG uptake in testicular germ cell tumours. However, a decrease in ^{18}FDG uptake after start of therapy not necessarily predicts complete response in the further course of treatment. Serial PET-scanning in patients with low risk profile has no value over CT. Further studies in larger

series of patients are needed to estimate the additional value of serial-PET in patients with seminomatous germ cell tumours and in nonseminomatous patients with an increased risk profile.

Introduction

Since the introduction of cisplatin based chemotherapy, the prognosis of testicular germ cell tumour (TGCT) has dramatically improved, resulting in a long-term survival of more than 70%.[1] Despite this success, it is known that some patients with advanced testicular germ cell tumours fail to respond to first-line chemotherapy, while others benefit from a reduced chemotherapy regimen without compromising survival.[2,3] Thus, it is of great interest to have surrogate endpoints to distinguish responders from non-responders early after start of treatment.

In TGCT, serum tumour markers and radiological changes in tumour size have been used to monitor response to chemotherapy. Unfortunately, not all TGCT produce tumour markers and radiological changes do not necessarily reflect pathological response. General restrictions to the radiological assessment of tumour size include delay between initiation of therapy and tumour shrinkage[4] and the inability to differentiate malignant from non-malignant tissue in case of residual tissue after chemotherapy.[5] Because it is still common practice to excise residual masses, some patients will be operated needlessly for non-malignant residual mass. As a result, it would be of obvious benefit to have a test that accurately predicts the response after treatment and thereby avoiding morbidity due to over-treatment.

It has been demonstrated that malignant transformation of cells is frequently associated with increased metabolic activity and the use of glucose.[6] By imaging the cellular glucose uptake, ^{18}FDG-PET can visualize tumour masses. This method seems to be promising for monitoring treatment response in solid tumours, like breast cancer, glioma and head and neck tumours.[7,8] In breast cancer patients, ^{18}FDG-PET was able to differentiate responders from non-responders early in the course of primary chemotherapy.[7,9] Moreover, it has been stated that changes in metabolism often precede changes in tissue structure, which makes it possible to monitor the response to treatment earlier.[10,11]

The primary aim of this prospective pilot study was to evaluate the ability of ^{18}FDG-PET to monitor the response to chemotherapy in patients with disseminated testicular germ cell cancer. In addition, PET was compared with radiological imaging, the tumour marker decline and the risk profile for disseminated germ cell cancer according to the International Germ Cell Classification Consensus Group.[12]

Patients and methods

Patients. Serial ^{18}FDG-PET studies (n=54) were performed in 18 consecutive patients with disseminated TGCTs (at least stage IIB according to the Royal Marsden Hospital Staging system[13]). All patients were seen from January 2000 to December 2001. Details of the study were explained by a physician, and written informed consent was obtained from all patients. The study was approved by the ethics committee of the University Medical Centre Nijmegen. The chemotherapeutic regimen consisted of three courses of bleomycin 30mg (day 1-5, 8 and 15), etoposide (day 1-5) and cisplatin (day 1-5) and a final course of etoposide (day 1-5) and cisplatin (day 1-5). In total, all patients received four courses of chemotherapy. Five patients underwent surgery for residual mass after chemotherapy. Patients' characteristics are listed in Table I.

Tumour response evaluation. Verification of histology of the primary tumour was performed at our pathology department. All patients were staged using abdominal and chest CT scan, and determination of serum tumour markers (-human chorionic gonadotropin: HCG; -fetoprotein: AFP; lactate dehydrogenase: LDH). Baseline ^{18}FDG-PET imaging was performed the day before start of treatment. All pre-treatment staging procedures were performed within 5 days. Determination of tumour markers was repeated prior to each of the subsequent chemotherapy cycles. The second ^{18}FDG-PET scan was performed the day before start of the third course of chemotherapy. Since there is no consensus for treatment monitoring [8,14] and an

increase in ^{18}FDG-uptake has been reported after induction chemotherapy,[15,16] we decided to perform the second scan at mid-treatment. All patients were completely re-staged by serum tumour markers, CT scan and whole body ^{18}FDG-PET scan six weeks after completion of chemotherapy.

Table I.	**Patients Characteristics (N=18)**
Median age (years)	30.7
-range	23.1-50.0
Primary histology	
-seminoma	4
-non-seminoma	14
Location of metastases	
-abdominal mass	17
-nodes supra diaphragm	2
-lungs	3
-bone	1
-liver	1
No. of patients with elevated tumour markers	
-HCG	11
-AFP	10
-LDH	4
-None	4
IGCCCG risk profile	
-low	15
-intermediate	3
Response to therapy	
-complete response (90-100% reduction in size)	13
-partial response (<90% reduction in size)	2
-stable disease	3
Histology of residual mass	
-mature teratoma	4
-necrosis	1
Course of disease after completion of therapy	
-no disease progression (for at least 6 months)	16
-disease progression (within 6 months)	2
Median disease free follow-up (months)	9.6
-range	3.1-22.8

HCG: -human chorionic gonadotropin; AFP: -fetoprotein; LDH: Lactate dehydrogenase

CT scans were reviewed independently without knowledge of the clinical data. Response after chemotherapy was classified according to the modified WHO criteria.[17] A decline of less than 90% in transverse diameter was classified as a partial response whereas a complete response was defined as a more than 90% decline in diameter in combination with normalization of tumour markers.

Residual mass after chemotherapy was removed in all NSGCT and in SGCT in case the mass was larger than 3cm in transverse diameter.[18] Surgery was planned within eight weeks after completion chemotherapy.

All patients underwent monthly follow-up examinations including physical evaluation, evaluation of tumour markers levels, chest X-rays and a 3-monthly CT scan during the first year.

^{18}FDG-PET. A dedicated, rotating half-ring PET-scanner (ECAT-ART, Siemens/CTI, Knoxville, TN, USA) was used for data acquisition. Prior to ^{18}FDG-injection, patients were fasting for at least 6 hours. Intake of sugar-free liquids was permitted. Immediately prior to the procedure, the patients were hydrated with 500 ml of water. One hour after intravenous injection of 200-220 MBq FDG (Mallinckrodt Medical, Petten, The Netherlands) and 20 mg furosemide, emission and transmission images of the area between proximal femora and the base of the skull were acquired (10 minutes per bedposition). The images were corrected for attenuation and reconstructed using the ordered-subsets expectation maximization (OSEM) algorithm.

^{18}FDG-PET image analysis. The reconstructed images were displayed in coronal, transverse and sagittal planes. All scans were interpreted by two nuclear medicine physicians [W.J.G.O., L.F.deG.O.], blinded to all clinical and pathology data.

Tumour metabolism was evaluated using calculation of standardized uptake value (SUV) and visual analysis. In order to quantify ^{18}FDG uptake, 3-dimensional regions of interest were drawn around the metastases. Standardized uptake values were calculated using the concentration of ^{18}FDG in the metastases as measured by PET, divided by the injected dose and multiplied by body weight as a normalization factor. The visual scoring model was graded as suspect for carcinoma (++); increased uptake (+); or negative (-).

PET findings after chemotherapy were also correlated to histological findings of the resected residual mass. If no resection was performed, the clinical course of the patient was used. The absence of radiological tumour progression and negative serum tumour markers within 6 months after completion of therapy were considered markers of complete remission.

Statistical analysis. Because of the small number of patients available, we primarily used descriptive statistics in order to evaluate the value of PET. We evaluated the distribution differences of SUVs to differentiate between carcinomas with and without complete response after chemotherapy using the Receiver Operating Characteristic curve. We refrained from more elaborate analyses, especially the use of multivariable regression analyses to evaluate the independent value of PET, because of the limited power of the study.

Results

Eighteen patients receiving first-line treatment for testicular germ cell cancer were evaluated for treatment response by ^{18}FDG-PET. Baseline characteristics of the patients are listed in Table I. In 5 patients histology of residual mass was available, while the remaining 13 patients were evaluated by the clinical course at a median follow-up of 9.0 months (range 5.0-23.2). Overall, 16 patients remained free of disease at a median of 10.3 months after treatment, while two patients relapsed, both at 3 months after treatment.

Treatment response evaluation by ^{18}FDG-PET

Before the start of chemotherapy, 24 lesions in 18 patients could be identified by CT scan. Baseline PET prior to the start of chemotherapy was positive in all those lesions except in one patient (Table II, no.18). The retroperitoneal lesion of this patient could only be observed by CT-scan. After two cycles of BEP, only three patients had visible lesions and at completion of chemotherapy still two lesions could be detected by ^{18}FDG-PET (Table II).

Table II. Monitoring treatment response by ^{18}FDG-PET and conventional clinical staging methods.

Pt. no.	age	Histo-logy	Stage (1)	IGCCCG (2)	Diameter on CT [max in cm]		Serum Tumour Markers			Visual score (SUV max)			Follow-up of disease or resection of residual mass	Total follow-up time (months)
					pre	post	pre	during	post	pre	during	post		
1	26.1	NST	IIB	low	4.0	<1.0	E	N	N	++ (13.4)	clean	clean	Free of disease	9.5
2	42.3	NST	IIB	low	2.0	<1.0	E	N	N	++ (4.6)	clean	clean	Free of disease	9.0
3	36.9	NST	IIB	low	2..3	<1.0	E	E	N	++ (13.2)	clean	clean	Free of disease	7.1
4	25.9	NST	IIA IIIn	low	1.7 1..2	<1.0 clean	E	N	N	++ (4.5) ++ (4.8)	clean	clean	Free of disease	6.0
5	28.1	NST	IIB IIIn	low	4.0 1.0	<1.0 clean	E	E	N	++ (8.8) ++ (6.0)	clean	clean	Free of disease	16.5
6	23.0	NST	IIB IVlung	low	2.5 1.0	<1.0 clean	E	E	N	++ (17.0) ++ (3.2)	clean	clean	Free of disease	10.6
7	35.9	ST	IIC	low	7.0	3.0	E	N	N	++ (8.6)	+ (2.7)	Clean	Free of disease	18.5
8	32.6	ST	IID	low	> 10.0	<1.0	E	E	N	++ (18.6)	clean	clean	Free of disease	23.2
9	35.8	ST	IVother	Interme-diate	5.0	< 1.0	E	N	N	++ (6.3)	clean	clean	Free of disease	8.0
10	44.3	NST	IIB	low	3.0	<1.0	N	N	N	++ (5.9)	clean	clean	Free of disease	8.9
11	30.7	NST	IIB	low	3.5	<1.0	N	N	N	++ (5.0)	clean	clean	Free of disease	8.9
12	29.3	NST	IID	low	>10.0	<1.0	N	N	N	++ (7.3)	clean	clean	Free of disease	13.7
13	27.3	ST	IIB IVbone	Interme-diate	4.0 1.5	<1.0 <1.0	E	N	N	++ (11.1) ++ (3.2)	clean	clean	Relapse at follow-up (3 months after CTH)	5.0
14	26.7	NST	IID IVlung	Interme-diate	>10.0 2.0	4.5 clean	E	E	N	++ (14.6) ++ (4.9)	+ (2.9) Clean	+ (2.8) Clean	Necrosis & histiocytosis	5.0 (†)
15	39.2	NST	IID	low	10.0	9.5	N	N	N	++ (3.7)	++ (3.7)	+ (1.9)	Mature teratoma & histiocytosis	16.3
16	50.1	NST	IIC	low	7.0	3.0	E	N	N	++ (7.3)	clean	clean	Mature teratoma	19.7
17	26.3	NST	IIB IVlung	Low	4.0 1.0	4.0 clean	E	N	N	++ (9.5) + (1.4)	clean	clean	Mature teratoma	16.5
18	28.1	NST	IIC	low	5.0	5.0	E	N	N	clean	clean	clean	Mature teratoma	10.0

Abbreviations: (n)st: (non-) seminoma testis;; E: elevated; N: normal(ized); Cth: chemotherapy; IV-other: intra- and extracranially extension of metastatic tissue; ++: clearly positive; +: suspect; +/-: increased uptake; -: negative.;
1) Royal Marsden Hospital Staging system [11]; 2) International Germ Cell Consensus Classification System [14]; † patient relapsed at 3 months after surgery and finally died within two months of chemo-resistant disease.

Overall, the clinical course of disease after first-line treatment was correctly predicted by ^{18}FDG-PET in 12 patients (P1-12; Table III). In 11 of them the on-treatment PET scan was already negative. In the remaining 6 patients the ^{18}FDG-PET did not correlate accurately with clinical or pathology response (Table III).

Table III. Prediction of response to chemotherapy in testicular germ cell cancer

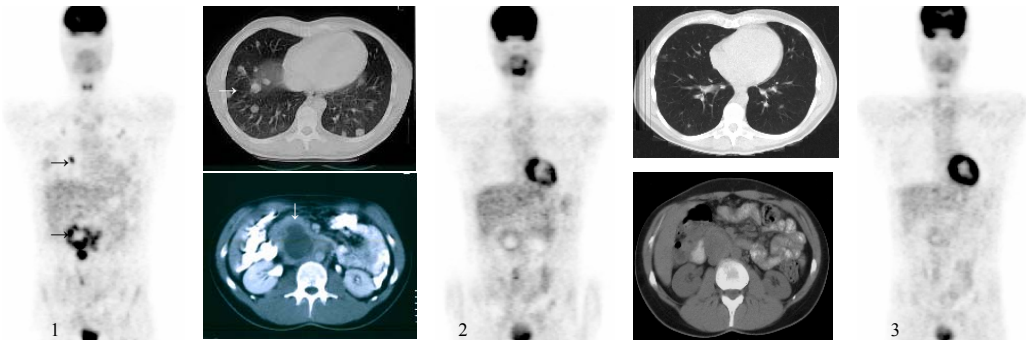
		Accurate outcome	Inaccurate outcome			
	Response evaluation	total	total	False-positive	False-negative	False-postive or negative
PET	17/18*	12/17	5/17	1/17 (15)	3/17 (16-18)	1/17 (14)
CT	18/18	15/18	3/18	1/18 (7)	1/18 (13)	1/18 (14)
STM	14/18	9/14	5/14		5/14 (13,14,16-18)	
		Complete Response		Incomplete Response		
IGCCCG						
-low risk		15/15				
-intermediate risk		1/3			2/3	

PET: positron emission tomography; CT: computed tomography; STM: serum tumour markers; IGCCCG: International Germ Cell Consensus Classification;* in 17 of 18 patients response evaluation by PET was possible; (..): refers to the patient number in Table II.

Although the CT and PET scan suggested a complete response, patient 13 developed a retroperitoneal relapse at 3 months after chemotherapy combined with radiation therapy for bone metastasis. In two patients (P14 and P15), the follow-up PET did not normalise after therapy. It is difficult to determine if these scans are false negative or positive. Despite the fact that patient no. 14 was macroscopically free of tumour after surgery (see Figure 1), he relapsed at the crux of the diaphragm. Three months after surgery he died of chemotherapy refractory disease. Most likely ^{18}FDG-PET detected histiocytosis (false-positive), but missed viable cells at the diaphragm (neither uptake found by PET nor suspect lesion seen on CT:

false-negative). In patient 15, the residual mass consisted of a combination of mature teratoma and histiocytosis. Because mature teratoma has a low metabolic rate, the detection of the residual mass by ^{18}FDG-PET was most likely caused by the histiocytosis component. In three other patients (P16-18) postchemotherapy residual mature teratomatous mass was not detected.

figure 1



Coronal ^{18}FDG-PET scan shows metastases of nonseminomatous testicular germ cell tumour in the retroperitoneum and in lungs with increased uptake of ^{18}FDG (arrow). The lesion in the retroperitoneum shows avital tissue (decreased uptake) in the center. The patient (no.14) showed partial radiological response during treatment: both decrease in ^{18}FDG uptake and volume reduction on CT scan retroperitoneally, and disappearance of lung metastases. Surgery of residual retroperitoneal mass showed necrotic tissue in the centre and inflammatory tissue at the rim of the retroperitoneal mass. Three months after surgery, he relapsed at the crux of the diaphragm and finally died of disease.

1) pre-chemotherapy
2) before third course of chemotherapy
3) six weeks after completion of chemotherapy

Seventeen patients had visible lesions at baseline PET. The quantitative ^{18}FDG uptake at baseline calculated by the standardised uptake value (SUV) is not predictive for complete or incomplete response after chemotherapy. The area under the Receiver Operating Characteristic curve is: 0.53 (95%CI: 0.21-0.84).

Comparison between ^{18}FDG-PET and CT-scan in evaluation of treatment response. Posttherapy CT scan correctly predicted the outcome in 15 of the 18 patients. In patient 7, the CT scan showed a posttherapy residual mass of 3 cm, while the second ^{18}FDG-PET scan showed a decreased intensity compared to the pre-treatment scan, and the third ^{18}FDG-PET was true-negative. Similar to ^{18}FDG-PET scanning, the CT scan of patient 13 suggested complete response and also showed no retroperitoneal mass at the crux of the diaphragm in patient 14. In contrast to ^{18}FDG-PET, the residual masses containing mature teratoma (P15-18) were clearly visualized by CT-scan.

Comparison between ^{18}FDG-PET and serum tumour markers in evaluation of treatment response. Since four patients (P10-12, P15) never had any elevated serum tumour markers, evaluation of the response was evaluated in 14 patients. In all patients, the serum tumour markers declined according to their half-life time to normal values posttreatment. In two patients (P13,14), although seemingly free of disease, the βHCG level started to rise 3 months after completion of treatment. In both patients a retroperitoneal relapse was found. On basis of the decline of serum markers it was not possible to differentiate mature teratoma (P16-18) from other histopathological entities. In total, the serum tumour markers correctly demonstrated complete response in 9 patients (P1-9).

Although three patients (P10-12) showed no increased tumour markers before chemotherapy, serial PET was also able to monitor response correctly in these patients.

Comparison between ^{18}FDG-PET and International Germ Cell Consensus Classification System. According to the International Germ Cell Consensus Classification System (IGCCCG),[12] three patients were classified as intermediate risk (P9,13,14) and the remaining 15 as low risk. All patients in the low risk group showed a complete response for more than

six months. Two of the three patients in the intermediate risk group relapsed after treatment (P13 and P14).

PET was correctly negative in eleven of fifteen patients and in one of three patients in the low and intermediate risk group, respectively. Consequently, the IGCCCG appeared to be more accurate to predict response than ^{18}FDG-PET.

Discussion

Metastatic germ cell cancer is a very heterogeneous group of tumours with respect to prognosis under cisplatinum-based chemotherapy. The main determinants of complete response are not only the extent of the metastases but much more the biology of the underlying tumour which is represented by the location of the metastases and the level of serum tumour marker elevation. To date, patients can be categorized into different prognostic risk groups on basis of those determinants.[12] This allows selection of patients for a risk-adapted treatment in the good prognostic group and intensified therapy for the high-risk group. However, the observed clinical response to chemotherapy does not always correlate with the pathologic response, which is known to be of considerable prognostic importance.[19] Since not all patients will benefit equally from first-line chemotherapy, early insight into treatment response would be helpful in individualising treatment regimen.

In other tumours, changes in ^{18}FDG uptake have been correlated with response to anti-tumour therapy. The ^{18}FDG signal appears to parallel the loss of tumour cells and to correlate with pathology.[20] It has also prognostic value.[9,21] To our knowledge, this is the first prospective study, which monitors first-line chemotherapy response in testicular germ cell tumours by serial PET. This study also compares ^{18}FDG-PET results with established criteria for response assessment in patients with metastatic germ cell cancer.

Active testicular germ cell tumours and their metastases are known to have increased ^{18}FDG uptake indicating high metabolic activity.[22] It has been hypothesized that tumours with higher glycolytic rates may achieve a superior response to antineoplastic therapy.[23] In that case, a single ^{18}FDG-PET evaluation before therapy may be sufficient to predict the response to treatment. In other tumours an association was found between tumour grade and pre-treatment ^{18}FDG-uptake, but no correlation to pathologic response was found.[24,25] In the present study, in all except one patient an increased ^{18}FDG uptake was observed at baseline. In this single patient the sequential scans remained negative and the CT-scan did not show any clinical response. At surgery mature teratoma was found. It suggests that only teratomatous tissue was present pre-treatment, which is known to have a reduced to normal metabolism. It seems that this patient needlessly received chemotherapy. For such patients, PET scanning before chemotherapy might be useful. In the other patients, the pre-treatment ^{18}FDG-uptake (visual score and SUV) was not associated with the level of serum tumour markers and the location of metastases at baseline, nor with response to treatment. We found a high degree of SUV overlap between patients with a complete and incomplete response to chemotherapy. As a result it was not possible to find a useful SUV cut-off value for predicting a complete response to treatment.

Changes in ^{18}FDG-uptake after start of treatment have been applied to assess tumour response and could be used to distinguish responders from non-responders.[7,9,26] Because many factors play a role in the ^{18}FDG uptake, the best protocol to monitor the response to chemotherapy has not been established yet.[8,14] Wilson *et al.* studied the usefulness of serial ^{18}FDG-PET scanning in five patients with TGCT.[27] Although the timing of the on-treatment scan (2 to 4 weeks after introduction of chemotherapy) and the administered first-line chemotherapy regimen varied, they found significant reduction of ^{18}FDG uptake in responders compared to non-responders. On the other hand, it has been reported that the rate of ^{18}FDG uptake

immediately after introduction of therapy may increase due to cellular repair processes.[15,16] Therefore, in this study it was decided to perform the mid-treatment scan halfway the treatment protocol.

All patients with an initially increased ^{18}FDG uptake showed a partial or complete remission on PET after two cycles of chemotherapy. In only 3 of 18 patients ^{18}FDG uptake was observed after two cycles. Of interest, in one patient the ^{18}FDG-uptake diminished to an undetectable level during therapy and on CT-scan the diameter of the seminomatous germ cell tumour shrunk from 7 to 3 cm post-treatment. Possibly, this tumour has an increased resistance to the antineoplastic effect of chemotherapy compared to those with negative mid-treatment PET-scan and small residual tissue (<1 cm). However, residual seminomas, do not correlate well with viable residual disease. Most residual seminomatous masses do not grow, and will gradually regress over a period of months after treatment. Subsequently, surveillance by frequent marker and CT scan evaluation is an option.[5,18,28] On the other hand, 10% of the residual masses do contain viable disease. ^{18}FDG-PET seems to be useful in discriminating between tumorous and benign residual disease. Because there is no residual teratoma in pure seminoma, a negative postchemotherapy PET-scan of a residual mass of 3cm or more is highly predictive for non-malignant residual mass.[29,30] To date, this patient is still free of disease at 6 months of follow-up. In two other patients with bulky retroperitoneal non-seminomatous disease the metabolic activity remained increased on post-therapy evaluation. Histology of these residual masses, revealed inflammation combined with either necrosis or mature teratoma. It is known that macrophages, present in inflammatory processes, also accumulate ^{18}FDG.[31] This is assumed to take place mainly in the early phase of therapy.[29] However, in TGCT it has also been reported in residual masses after chemotherapy.[32] The increased uptake of ^{18}FDG is probably not caused by the teratomatous tissue of the patient.

This observation is further exemplified by three other patients with solitary teratomatous residual mass and a negative PET scan.

In total, a negative mid-treatment PET-scan correctly predicted complete response in 11 patients (61%). Posttherapy CT imaging showed a maximum volume decrease in those patients. In contrast, in patients without a large volumetric decrease on CT-scan, teratomatous residual disease was found. It is known that mature teratoma is not very sensitive to chemotherapy. Furthermore, it has to be regarded as a pre-malignant condition, because it might transform into malignancy or degenerate into non-germ cell cancers over time. In order to reduce false-negative PET findings, it might be useful to correlate the PET results with primary histology. It has been suggested that when a mature teratoma is present in the primary tumour it is also frequently found in the retroperitoneal mass.[5] Subsequently, PET-scanning in those patients may not be reliable in detecting retroperitoneal teratomatous lesions.[30,33] In our series, three patients with retroperitoneal teratomatous disease, also had a teratoma component in the primary tumour, suggesting some benefit of including primary histology.

Irrespective of monitoring treatment response, the pre-treatment clinical findings, as used in the IGCCCG classification, seem to have the best prognostic value. All low risk patients remained free of disease after treatment, while two of three patients at intermediate risk relapsed at 3 months after therapy.

This study population served as a model for the evaluation of the ability of PET to predict response to treatment already during the course of therapy. It shows that it is possible to monitor changes in ^{18}FDG uptake in testicular germ cell tumours. However, ^{18}FDG-PET has a limited role, if any, in the therapeutic monitoring of low risk testicular cancer and does not provide significant information to structural imaging and measurement of serum tumour markers. Although the results are discouraging due to the high response rates in this group of patients, it supports further study in targeting patients with increased risk profiles.

Furthermore, seminomatous germ cell cancer is worth for targeting because it does not contain teratomatous elements and consequently the outcome is less hampered by false-negative results.

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PART IV

The efficacy of follow-up in germ cell tumour of the testis

CHAPTER 8

Efficacy of routine follow-up after first-line treatment for testicular cancer.

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Abstract

Objective. To define guidelines for the follow-up management of patients treated for testicular germ cell tumour this study assessed characteristics of patients with recurrent disease.

Methods. The charts of 505 patients with testicular cancer treated and followed-up at the University Medical Centre Nijmegen between 1982–2000 were reviewed retrospectively.

Results. In 42 patients disease recurrence was found during routine follow-up. In a subset of patients no recurrences were seen after first-line treatment: (a) pathological stage IIa nonseminoma patients who were adjuvantly treated with chemotherapy and (b) histologically confirmed complete responders after primary chemotherapy. Furthermore, in low-stage disease no intra-abdominal recurrences were seen in (a) pathological stage I nonseminoma patients and (b) low-stage seminoma patients who received radiotherapy.

Conclusions. The risk of recurrent testicular cancer depends on primary therapy and efficacy of it; these results indicate a limited role for follow-up in pathological stage II nonseminoma patients adjuvantly treated with chemotherapy and in histologically confirmed complete responders after chemotherapy. Abdominal computed tomography does not appear necessary in routine follow-up of patients treated for low-stage testicular cancer.

Introduction

After treatment of testicular germ cell cancer patients are followed up by regular outpatient visits, during which physical examination, serum tumour marker studies, and radiological examinations are performed. Testicular germ cell tumour (TGCT) is somewhat unique among urological malignancies in that recurrence is highly treatable, especially when detected early.[1,2] Optimal follow-up is therefore critical to the care of TGCT patients. No prospective studies have yet been performed to justify particular follow-up schedules.[3] Most commonly used schedules call for patients to visit a medical specialist up to 30 times over a period of 10 years. Some advocate even life-long follow-up, as late relapses have been recorded.[4] Since the vast majority of men are relatively young and show an excellent response to treatment, routine follow-up consumes considerable time and money.

The purpose of this study was to assess the efficacy of routine follow-up of patients treated with curative intent for testicular cancer in a single institution in order to examine the recurrence patterns and if possible to determine an optimal follow-up policy for testicular cancer patients.

Material and Methods

A retrospective study was performed of the medical records of all 535 patients, primarily treated for testicular germ cell tumours at the Department of Urology of the University Medical Centre Nijmegen between January 1982 and January 2000. Patients were considered eligible for the study if they were in complete remission for at least 3 months after first-line treatment and were routinely followed-up at our Department; the period of 3 months was chosen to distinguish recurrent disease from refractory disease. Thirty patients were not eligible (22 progressive disease, 8 recurrence within 3 months after primary treatment), and

therefore 505 patients were finally eligible for the study. Table I presents patients' characteristics.

Table I **Study population by histology (N=505)**

	Seminoma	Non-seminoma
Number of patients	145	360
Median age at diagnosis (range)	37.3 (14.7-79.6)	28.9 (14.9-77.6)
Clinical Stage at diagnosis		
I	68	184*
IIa	31	39
IIb-IId	34	65
III	6	8
IV	6	64
Initial increased markers at diagnosis **		
AFP	0	70% (177/252)
bHCG	30% (32/108)	62% (147/237)
LDH	24% (24/102)	24% (52/216)
Primary treatment after orchiectomy		
surveillance	4	24
radiotherapy	96	-
RPLND	-	93
RPLND and chemotherapy	-	106
chemotherapy	34	56
chemotherapy and surgery	10	82
Median routine follow-up in months (range)	60.0 (4-165)	64.7 (4-175)
Status at last follow-up		
Alive without disease	140	346
-still in follow-up	68	233
-completed follow-up	57	94
-lost to follow-up	15	19
Dead of disease	0	9
Dead of other disease	5	5

key: AFP: alpha-fetoprotein; bHCG: beta-choriongonadotropin; LDH: lactate dehydrogenase; RPLND: retroperitoneal lymph node dissection; *24 patients on surveillance and 160 patients underwent RPLND: 93 pathological stage I; 67 pathological stage II; ** information on the value of serum tumour markers was not available in all files.

All patients underwent treatment as described by the EAU guidelines.[5] Briefly, all orchiectomized patients were screened for metastases by computed tomography and staged according to the Royal Marsden Hospital classification.[6] The vast majority of patients with

low-stage (stage I or IIa) seminoma germ cell tumours (SGCT) were irradiated, but some patients were only followed up for clinical stage I SGCT.

Between 1982 and April 1996 all low-stage SGCT were irradiated on the iliac and para-aortal lymph nodes with 26–30 Gy. Thereafter the extension of the radiation field was reduced to the para-aortal lymph nodes only (20 Gy). Patients with low-stage (stage I or IIa) nonseminomatous disease (NSGCT) were treated with nerve-sparing retroperitoneal lymph node dissection (RPLND). Since 1994 only patients at high risk for metastasis (vascular invasion in the primary tumour) have undergone RPLND. In the case of lymph node metastases (pathological stage II) patients were adjuvantly treated with two cycles of bleomycin, etoposide, and cisplatin chemotherapy. Patients at low risk for metastasis were followed-up. Patients with either SGCT or NSGCT of high stage (>IIa) received cisplatin-based chemotherapy after inguinal orchiectomy. Residual mass after chemotherapy was removed in all NSGCT and in SGCT if the mass was larger than 3 cm in transverse diameter.[7] In the case of teratomatous tissue or viable tumour in residual mass patients were carefully followed up and were treated by chemotherapy at relapse.

The follow-up schedule consisted of outpatient visits every month during the first year, every 2 months in the second year, every 3 months in the third year, every 6 months in the fourth and fifth years, and thereafter once per year for at least 10 years of follow-up, after which the continuation of follow-up was at the discretion of the clinician and patient's wish (Table II). For this study the follow-up period was defined as the time elapsed between completion of treatment and 31 December 2001. Routine follow-up was stopped in the event of tumour recurrence, severe other diseases, death, end of follow-up protocol, end of study period, or on the patient's demand, and was calculated by the Kaplan-Meier method. The overall median follow-up period was 60.0 months (range 4–175).

Recurrence was defined as any clinically, radiographically, or histologically confirmed tumour relapse, contralaterally or distant. The contribution of each investigation to the initial identification of recurrence was examined. Recurrence was considered asymptomatic if the physician found it during regular follow-up examination in a patient with no relevant complaints. It was considered symptomatic if it was detected by the patient himself before the physicians' examination. The time from orchiectomy to death or date of last observation was defined as the survival time. The survival curves were calculated by the Kaplan-Meier method. Survival differences between groups were evaluated by the log-rank test.

Table II. Follow-up protocol for testicular germ cell tumours by stage and histology

Histology and Stage	1st Year (monthly)	2nd Year (2-monthly)	3nd Year (3-monthly)	4th and 5th Year (6-monthly)	6th –10th Year (once)
Low stage					
stage I NSGCT at surveillance	CE, Lab, CXR and 3 monthly CT	CE, Lab, CXR and 4 monthly CT	CE, Lab, CXR and 6 monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR and once a CT
stage I and IIa NSGCT: RPLND with or without CTH & SGCT:RT	CE, Lab, CXR and 6 monthly CT	CE, Lab, CXR and 6 monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR	CE, Lab, CXR
High stage					
stage > IIa NSGCT & SGCT: CTH with or without RPLND*	CE, Lab, CXR and 3 monthly CT	CE, Lab, CXR and 4 monthly CT	CE, Lab, CXR and 6 monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR and once a CT

Key: NSGCT: non-seminoma germ cell tumour; SGCT: seminoma germ cell tumour; RPLND: primary retroperitoneal lymph node dissection; CTH : chemotherapy ; RT: radiotherapy; CT: computed tomography of abdomen and chest; CE: clinical examination; Lab: serum alfa-fetoprotein, beta-human chorionic gonadotrophin, lactate dehydrogenase; CXR: chest x-ray.

*Residual masses of pure seminoma less than three cm in diameter post-chemotherapy are followed by CT scanning at 3 monthly intervals for the first year, unless regression occurs. Masses that do not regress over the first year are followed by further CT scans at 6 monthly intervals for 3 years after completion of chemotherapy.

Results

Recurrences. Forty-two patients developed recurrent disease during the routine follow-up period (recurrence percentage at 5 and at 10 years of follow-up: 8.2% and 10.1%). Seven

patients had a recurrence in the contralateral testis (Table III), and 35 experienced systemic recurrent disease (Table IV). The median period between completion of first-line treatment and contralateral recurrence was 48 months (range 15–85). Contralateral recurrence was detected by a programmed follow-up in three patients and by self-examination in four. In six patients the contralateral recurrence was the isolated site of relapse at the time of detection, and all were free of disease after treatment of relapse. In one patient metastasized disease was already present, for which he received chemotherapy. He withdrew from follow-up 12 months after completion of chemotherapy. Thirty months later he was readmitted for general malaise due to advanced pulmonary metastases and finally he died from disease.

The median period between completion of first-line treatment and the detection of systemic recurrence was 6 months (range 4–89). Of the 35 systemic recurrences 31 were detected during the regular follow-up visits (i.e., asymptomatic). The four symptomatic recurrences were detected by self-examination of supraclavicular lymph nodes (*n*=3) or by visiting the hospital because of neurological signs (*n*=1).

Table III **Contralateral recurrence by primary stage and histology**

Initial Stage	Primary histology	First-line treatment after orchiectomy	Interval to contralateral recurrence (months)	Number of recurrences per number of similarly treated patients	histology and stage of recurrence	Primary detection of recurrence	Treatment of recurrence after orchiectomy	Disease free follow-up after first recurrence (months)	Outcome at routine follow-up
I	SGCT	radiotherapy	44	2 of 64	SGCT I	SE	surveillance	48	FOD
	SGCT	radiotherapy	85		SCGT IIa-IVlung	SE	chemotherapy	12	FOD*
	SGCT	surveillance	49	1of 4	SGCT I	SE	radiotherapy	70	FOD
	NSGCT	RPLND	71	1 of 93	SGCT I	CE	radiotherapy	37	FOD
IIb-d	SGCT	chemotherapy	15	1 of 35	NSGCT I	CE	radiotherapy	49	FOD
	NSGCT	chemotherapy + RPLND	48	2 of 82	NSGCT I	CE+ marker	radiotherapy	75	FOD
	NSGCT	chemotherapy + RPLND	35		NSGCT I	SE	surveillance	126	FOD

key: (N)SGCT: (non)-seminomatous germ cell tumour; CE: clinical examination; SE: self-examination; FOD: free of disease; DOD: dead of disease; other abbreviations as in table I.
*Twelve months after completion of chemotherapy this patient withdrew from further follow-up. Thirty months later he was readmitted for general malaise, due to advanced pulmonary metastases, from which he finally died.

Table IV Detection of systemic recurrences adjusted for primary histology and stage

Primary histology & stage	First-line treatment (histology)	Interval to recurrence (months)	Stage of recurrence	Primary detection recurrence	Therapy of recurrence	Follow-up after first recurrence (months)	Outcome
SGCT I	surveillance	17	IIa	CTabd	RT	4	fod
SGCT IIa	RT	4	IIIn	CTth + marker	CHT	66	dood
	,,	9	IIIn	marker	CHT	44	fod
SGCT IIb-d	CHT	4	IIb-IVH	CTabd	salvage CHT + RT	113	fod
	,,	7	IIIn-IVl	SE	salvage CHT + RT	38	fod
	CHT+RPLND (vi)	5	IIC	CTabd+marker	salvage CHT + RT	65	fod
NSGCT I	surveillance	4	IVl	CXR	CHT	31	fod
	,,	4	IIB	CTabd +marker	CHT	32	fod
	,,	4	IIB	CTabd +marker	CHT	36	fod
	,,	4	IVl	CXR	CHT	46	fod
	,,	9	IVl	CXR	CHT	30	fod
	,,	17	IIB	marker	CHT + RPLND	25	fod
	RPLND	4	IVl	CXR + marker	CHT + RPLND	31	fod
	,,	4	IVl	CXR	CHT	52	fod
	,,	4	IVl	CXR	CHT	61	fod
	,,	4	IVl	CXR	CHT	62	fod
	,,	6	IVl	CXR	CHT	21	fod
	,,	6	IVl	CXR + marker	CHT	44	fod
	,,	6	IVl	CXR+ marker	CHT	69	dod
	,,	8	IVl	CXR	CHT	31	fod
	,,	8	IVl	CXR	CHT	121	fod
	,,	18	IVl	CXR	CHT	60	fod
NSGCT IIb-d	CHT	4	IIa	marker	surgery + salvage CHT	118	fod
	,,	6	IVl	CTth + marker	salvage CHT	7	dod
	,,	36	IVl	marker	CHT	62	dod
	CHT+RPLND (mt)	5	IIB-IVH	Ctabd + marker	salvage CHT + surgery	15	dod
	,, (vi)	5	IVL	marker	salvage CHT	46	dood
	,, (vi)	12	IVL	marker	salvage CHT+ surgery	24	dod
	,, (mt)	19	IIIn	marker	salvage CHT + surgery	6	dod
	,, (mt)	52	IIIn-IVL	SE	salvage CHT	55	fod
	,, (mt)	89	IIIn	SE	surgery + RT	48	fod
NSGCT IIn-IV	CHT	13	IVB	SE	palliation	1	dod
	CHT+RPLND (vi)	5	IVL	marker	salvage CHT	12	dod
	,, (mt)	12	IVL	marker	salvage CHT	53	fod
	,, (mt)	37	IVL	marker	salvage CHT	14	dod

Key: RT = radiotherapy; PRPLND = primary retroperitoneal lymph node dissection; CHT = chemotherapy. Tumour stage was according to the Royal Marsden Hospital Staging classification: I, no metastases; IIa, retroperitoneal lymph nodes smaller than 2 cm; IIb-d, retroperitoneal lymph nodes at least larger than 2 cm; III lymph nodes above diafragm IIIn; IVL, lung metastases; IVH, liver metastases; IVB, brain metastases. (..): residual mass after chemotherapy: mt: mature teratoma; vi: viable disease; FOD = free of disease; DOD = dead of disease; DOOD = dead of other disease.

Recurrence and first-line treatment. Recurrences were also analyzed according to first-line treatment given to patients with a particular stage of disease and histology (Tables III,IV). There was no recurrence in the 106 patients with low-stage NSGCT (39+67; Table II) who were adjuvantly treated with two courses of bleomycin, etoposide, and cisplatin chemotherapy for retroperitoneal metastatic disease, (95% confidence interval, CI: 0.0–2.8%; median follow-up 71.5 months). In contrast, at 5-years of follow-up 10.8% of the patients (95% CI: 4.5–17.1%) who were negatively staged for retroperitoneal metastatic disease by surgery developed a recurrence (ten pulmonary metastasis (Table IV) and one contralateral SGCT (Table III). All patients except one, who developed multiple recurrent pulmonary metastases, survived. Furthermore, 6 of the 24 patients (25.2%; 95% CI: 7.7–42.7%) who were enrolled into a surveillance program (no vascular invasion in primary tumour) developed a retroperitoneal (*n*=3) or pulmonary recurrence (*n*=3) within 2 years of follow-up. All are free of disease at a median of 31.5 months after treatment for recurrence (Table IV).

In low-stage SGCT no metastatic disease was found retroperitoneally after radiation treatment in 95 patients (95% CI: 0.0%–3.1%, median follow-up 63.0 months). Recurrences were found outside the radiation field (contralateral, *n*=2, Table III; supraclavicular, *n*=2, Table IV). One of the four patients on surveillance after orchiectomy for stage I SGCT developed a retroperitoneal recurrence (Table III).

In high-stage disease pathologically incomplete responders after chemotherapy have a higher risk of systemic recurrent disease than complete responders. None of the 35 NSGCT patients with necrotic residual disease have relapsed since surgery at a median of 70 months (range 12–175; 95% CI: 0.0–8.6%; Table II), whereas 6 of 39 patients with residual teratomatous tissue (recurrence proportion 15.4%; 95% CI: 2.7–28.1% at 5 years of routine follow-up), and 3 of 7 patients with viable tissue relapsed (43%; 95% CI: 5.5–79.5% at 1 year of routine follow-up). The same observation was seen in SGCT patients: all ten patients with necrotic

residual disease were free of recurrent disease (95%; 0.0–0.3%; median follow-up 99 months), whereas one patient with viable disease relapsed retroperitoneally at 5 months after surgery (Table IV).

Recurrence and serum tumour markers. The correlation of marker levels at the time of diagnosis and recurrence for each specific tumour marker is shown in Table V. An elevated α-fetoprotein level at first recurrence was observed in 7 of 14 NSGCT patients (50%) with elevated levels at the time of diagnosis. Choriongonadotropin levels at recurrence were elevated in 8 of 14 NSGCT patients (45%) and in 2 of 3 SGCT patients with elevated levels at diagnosis. Serum lactate dehydrogenase was elevated at the time of recurrence in 1 of 6 NSGCT patients (17%) and in 1 of 3 SGCT patients (33%) who had elevated levels at the time of diagnosis. A normal tumour marker at presentation was associated with a normal marker at first recurrence.

Table V **Correlation between specific tumour makers at diagnosis and systemic recurrence**

tumour maker at diagnosis	tumour marker at first recurrence	Seminomatous germ cell tumour			Non-semonimatous germ cell tumour		
		Low stage (n=3)	High stage (n=3)	Totaal (n=6; %)	Low stage (n=11/16)*	High stage (11/13)*	Totaal (n=22; %)
AFP +	AFP +	n.a.	n.a.	n.a.	4	3	7 (32)
AFP -	AFP -	n.a.	n.a.	n.a.	3	5	8 (36)
AFP +	AFP -	n.a.	n.a.	n.a.	4	3	7 (32)
AFP -	AFP +	n.a.	n.a.	n.a.	0	0	0
BHCG +	BHCG +	1	1	2 (33)	2	6	8 (36)
BHCG -	BHCG -	1	2	3 (50)	6	2	8 (36)
BHCG +	BHCG -	1	0	1 (17)	3	3	6 (27)
BHCG -	BHCG +	0	0	0	0	0	0
LDH +	LDH +	1	0	1 (17)	0	1	1 (0.5)
LDH -	LDH -	1	2	3 (50)	11	5	16 (73)
LDH +	LDH -	1	1	2 (33)	0	5	5 (23)
LDH -	LDH +	0	0	0	0	0	0

key: AFP: alpha-fetoprotein , BHCG: beta-choriongonadotropin, LDH: lactate dehydrogenase; n.a.: not available; - serum tumour marker within range of normal levels, + increased serum tumour marker level.
*information on the value of serum tumour markers was not available in all files.

Survival data. The 5-year disease-specific survival for the entire population was 98.4% (95% CI: 97.2–99.6%). The only patients who died of testicular cancer during routine follow-up were those with systemic recurrence. The chance of dying was highest in patients with initial systemic NSGCT and subsequent systemic recurrence: 62% (8 of 13) died of disease at a median follow-up of 22.5 months after orchiectomy. There was a significant difference in survival between NSGCT patients with complete and those with incomplete histological response after chemotherapy (Fig. 1). There was no significant survival benefit in patients with teratomatous residual mass compared to those with a viable residual mass (P=0.15).

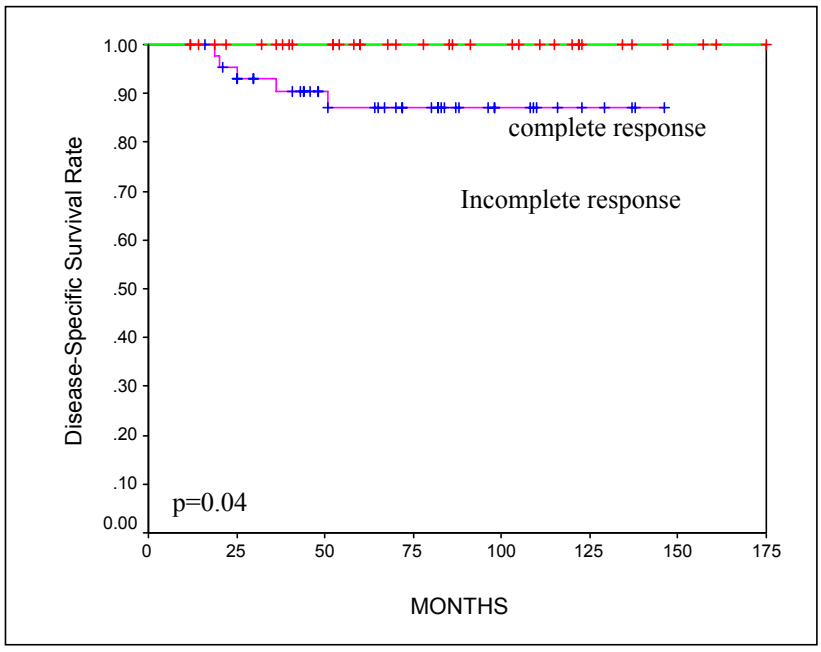

Figure 1. Disease-specific survival for non-seminomatous germ cell patients with histologically confirmed response (necrosis) after chemotherapy compared to those with incomplete histological response (mature teratoma, viable cancer).

Detection of second primary non-testicular tumours. During follow-up, 8 new malignancies developed in the 505 patients (1.6%): gastrointestinal cancer (n=2), leukemia, prostate, renal cell carcinoma and cancer of lung and breast (median: 73.5 months; range: 38-119).

Discussion

Although no randomized studies have been performed, routine follow-up of all patients is recommended after definitive treatment for testicular germ cell cancer. Specific programs of follow-up have been suggested by stage of disease and nature of treatment.[5] We evaluated our follow-up protocol to identify subsets of patients at increased or decreased risk for relapse to determine a more optimal follow-up regimen for testicular cancer patients. In our patient series contralateral recurrence developed later in time and was more frequently detected by the patient himself than systemic recurrences. The overall risk of 2.6% of contralateral disease at 10 years of follow-up in the present study is in accord with earlier findings.[8,9] All contralateral tumours were found by physical examination. Although one patient who withdrew from further follow-up died of systemic disease originating from a contralateral disease, most patients with bilateral disease have had a favourable outcome.[8,9] Furthermore, four patients found a contralateral relapse by self-examination after completion of routine follow-up. The second tumour was found at a median of 120 months (range 100–140) after first diagnosis (3 seminomas stage I and one pathological stage I nonseminoma). All patients are free of disease at a median follow-up of 39 months after bilateral orchiectomy (range 7–101).

Carcinoma in situ has been reported to be an important predictor for contralateral disease, being present in 5% of contralateral testis and leading in 50% of cases to invasive cancer within 5 years' time if left untreated.[10] However, our own and other researchers' data[11] do not support the routine use of contralateral biopsy for the early detection of carcinoma in situ. In our series physical examination is sufficient for detecting contralateral tumours, and life-long use of testicular self-examination must therefore be recommended to each patient. Remarkably, contralateral recurrence (n=11) seems to occur more frequently in patients who had not been treated systemically with chemotherapy (n=7). The very low recurrence rate

after two courses of carboplatin in stage I SGCT support this finding of a decreased risk for contralateral recurrence after primary systemic treatment by chemotherapy.[12,13] Today it is also feasible to perform a partial orchiectomy and adjuvant local irradiation in select patients, thereby minimizing the recurrence rate and maintaining a normal testosterone level in the majority of the patients.[14] In low-stage testicular cancer there was a stage- and treatment-dependent risk of developing recurrent disease. In agreement with the literature,[15,16] none of the NSGCT patients who received two cycles of adjuvant chemotherapy for confirmed retroperitoneal disease experienced a relapse. In contrast, approximately 10% of pathological stage I patients developed a pulmonary relapse and another 1% a contralateral tumour. In agreement with other studies, the elimination of thoracic computed tomography would have not changed the detection of pulmonary recurrences.[17,18]

Since 1994 we have performed only RPLND in patients at high risk for metastatic disease. Patients at low risk are put on surveillance. Of the 24 patients at low risk three have experienced a retroperitoneal and three a pulmonary relapse. Analysis of the data of patients with stage I NSGCT disease treated before and after 1994 suggest that the three patients with retroperitoneal relapse while on surveillance could have been free of disease if a primary lymph node dissection had been performed. In contrast, the remaining 18 patients still benefit from surveillance, being free of disease at a median of 32.5 months (range 12–56). Furthermore, the three patients who developed lung metastasis would not have profited from retroperitoneal surgery.

A wide range of risk factors have now been determined to distinguish cases that are truly clinical stage I from those that appear on the basis of investigation to be clinical stage I but are actually pathological stage II or higher. In all studies performed today vascular invasion is the most reliable and reproducible prognosticator for predicting metastatic spread in 50% of the cases.[19] For low-stage SGCT the same remarks can be made. None of the patients who

underwent radiotherapy experienced a retroperitoneal relapse. Although the 0% recurrence rate in stage I SGCT might result from overtreatment with regard to the extension of the both radiation field to the iliacal lymph nodes, no recurrences have been reported since the limitation of radiation of the para-aortic nodes only. A review of published reports revealed less than 1% infradiafragmatic recurrences after radiation treatment for low-stage SGCT.[20] In contrast, review of postorchiectomy surveillance series reports that approximately 20% of the patients relapse, but most of them have been cured by radiation or chemotherapy.[21]

In high-stage disease none of the patients with necrotic tissue in their retroperitoneal residual mass experienced a relapse, whereas those with residual teratomatous and viable disease had an increased risk of relapse. It is known that the key to success in managing residual masses is the absence of viable tissue and complete resection of the residual mass.[22] Although surgical resection of necrotic tissue after chemotherapy has no therapeutic benefit and confers good prognosis without relapse on the short term, follow-up is still advised for the long-term. In fact all high-stage patients are advised to be followed-up by a combination of diagnostic follow-up modalities.[5] According to our data, long-term results must be awaited to definitively resolve the necessity of follow-up in histological complete responders. In our series two patients relapsed retroperitoneally within 6 months after lymph node dissection for postchemotherapeutic viable tissue, suggesting incomplete resection. In the literature there is an ongoing debate as to whether two additional courses of chemotherapy for viable residual disease have a survival benefit over our policy of careful observation and deferred treatment in cases of relapse.[23-25] Despite all therapeutic options only 20–30% of these patients with relapse will be free of disease at the long-term.[26]

In accordance with others series, serum tumour markers have more diagnostic value in detecting recurrence in high-stage than low-stage disease,[27] and the marker status at time of recurrence was poorly related to marker status at the time of initial diagnosis.[20,27] Although

tumour markers are of incalculable value in detecting recurrent disease,[28,29] we believe that radiological exploration is still needed during follow-up, as markers are only presumptive evidence of recurrence.

Although it is generally accepted that routine follow-up needs to be more intense initially with gradually increasing periods between the appointments, there is no consensus over the optimal frequency.[30] In low-stage disease comparison of survival rates between different protocols shows no substantial differences. However, it must be kept in mind that a difference between one or two dead patients might not seem significant in terms of survival rate, but it is clinically relevant in potentially curable disease. Also the length of follow-up is not clear. Generally the yield with respect to recurrences is low if continued for more than 2 years, which is in agreement with our results. However, some relapses [5] and second malignancies may occur over a longer period of time.[31] Although some authors feel that 2 years of follow-up in low-stage patients is sufficient,[32] only long-term follow-up of those patients will ultimately resolve the problem of appropriate length of follow-up in low-stage disease. In high-stage disease it is more difficult to justify a particular follow-up protocol. Our results suggest a limited role of follow-up in histologically confirmed responders. However, both the small number of patients in this group and in the literature means that conclusions might be considered tentative.

Table VI proposes a recommended follow-up protocol according to our results. This protocol is for the greater part in line with the EAU guidelines on testicular cancer and the protocol of the German Testicular Cancer Study Group,[5,30] with the exception that our results suggest less radiological follow-up of low-stage testicular cancer patient posttreatment.

Table VI Proposed follow-up protocol with regard to stage and treatment performed

Histology and Stage	1st Year (2-monthly)	2nd Year (3-monthly)	3rd Year (4-monthly)	4th and 5th Year (6-monthly)	6th –10th Year (once)
Low stage SGCT					
Surveillance (CS I)	CE, Lab, CXR and 3-monthly CT	CE, Lab, CXR and 4-monthly CT	CE, Lab, CXR and 6-monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR
Radiation (CS I & IIa)	CE, Lab, CXR once CT after 3 months*	CE, Lab, CXR	CE, Lab, CXR	CE, Lab, CXR	CE, Lab, CXR
Low stage NSGCT					
Surveillance (low risk)	CE, Lab, CXR and 3-monthly CT	CE, Lab, CXR and 4-monthly CT	CE, Lab, CXR and 6-monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR
RPLND only (PS I)	CE, Lab, CXR once CT after 3 months*	CE, Lab, CXR	CE, Lab, CXR	CE, Lab, CXR	CE, Lab, CXR
RPLND and adjvant CTH (PS II)	CE, Lab, once CT after 3 months*	CE, Lab	CE, Lab	CE, Lab	CE, Lab
High stage SGCT & NSGCT (> IIa)					
Primary CTH only	CE, Lab, CXR and 3-monthly CT	CE, Lab, CXR and 4-monthly CT	CE, Lab, CXR and 6-monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR
Primary CTH with secondary RPLND and					
- non malignant residual disease	According to our results, a less intensified protocol might be possible, however more long-term data on this category of patients are needed to justify a particular protocol. Up till then, we propose the same protocol as for malignant residual disease.				
-malignant residual disease	CE, Lab, CXR and 3-monthly CT	CE, Lab, CXR and 4-monthly CT	CE, Lab, CXR and 6-monthly CT	CE, Lab, CXR and once a CT	CE, Lab, CXR

key: (N)SGCT: (non)-seminomatous germ cell tumour; CS: clinical stage; PS: pathological stage; RPLND: retroperitoneal lymph node dissection; CHT: chemotherapy; CT: computed tomography; SE: self-examination; CXR: chest X-ray.
* after RT and RPLND, a baseline CT scan is obtained and repeated if clinically indicated thereafter.

In conclusion, the risk of recurrent testicular cancer depends on primary therapy and response to it, and it subsequently influences the follow-up protocol. In routine follow-up of patients with low-stage testicular cancer adjuvantly treated at the retropritoneum abdominal computed tomography is not necessary and computed tomography of the chest can be replaced by chest radiography.

Routine follow-up of pathological stage II patients adjuvantly treated with chemotherapy or of histological complete responders after primary chemotherapy has limited value, as no recurrences were found in these subgroups of patients. However, only randomized studies will determine the optimal follow-up protocol. Until these studies are carried out, follow-up must be based upon the known natural history of the malignancy and the physicians' assessment of the relative costs and benefits involved. Regardless any modification in follow-up regimen, life-long self-examination should be advocated.

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CHAPTER 9

The efficacy of different follow-up strategies in clinical stage I non-seminomatous germ cell cancer. A Markov simulation study.

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Abstract

Objective. There is no universally accepted standard protocol for surveillance of patients with clinical stage I Non Seminomatous Germ Cell Tumours (CS I NSGCT). Prospective studies to compare different follow-up policies have not been performed, even though a great deal of time and resources is spent in surveillance. In this study, we constructed a Markov model to evaluate the impact of different follow-up strategies on disease-specific mortality (DSM) and life expectancy (LE) of patients with CS I NSGCT.

Methods. A discrete time non-homogeneous semi-Markov model was used to simulate different follow-up strategies for a hypothetical population of CS I NSGCT patients. Estimates of the model parameters were based on the literature. Output parameters were DSM and LE. Three different strategies were compared: 1) the intensive The Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital (NCI/AvL) protocol; 2) the European Association of Urology (EAU) protocol; and 3) a hypothetical minimal protocol (*i.e.* follow-up limited to the first two years). Furthermore, we evaluated the impact of abdominal CT scans and chest X-rays on DSM.

Results. Comparing with the EAU protocol (DSM: 3.05%; LE: 53.3 years), the intensive NCI/AvL protocol leads to a 1.2% lower DSM and a 6 months higher LE (DSM: 1.81%; LE: 53.9 years). The hypothetical follow-up scenario during the first two years shows a DSM of 6.83% and an LE of 51.4 years. Abdominal CT scans of the retroperitoneal lymph nodes appear to be important, while chest X-rays have little impact on DSM.

Conclusions. A follow-up policy limited to the first two years will result in an unacceptable high percentage of death from disease (6.83%). The benefit of an intensive follow-up protocol as proposed by the NCI/AvL, compared to that of the EAU, must be weighed against its economic and psychological costs. Our model suggests that CT-scanning is essential for a low DSM, whereas the large number of X-rays seem to have little additional effect.

Introduction

Before the 1980s, patients with non-seminomatous germ cell tumour (NSGCT) without clinical evidence of metastatic disease (*i.e.,* clinical stage I) were treated with either retroperitoneal lymph node dissection (RPLND) or external beam irradiation to the iliac and para-aortic nodes. Histopathology of these nodes shows that approximately 70% of patients have no retroperitoneal metastases.[1] Furthermore, up to 10% of patients will develop relapses outside the retroperitoneum.[2] Despite the overtreatment of the majority of patients, this policy was justified by the need to cure at first attempt because of the lack of alternative treatment options. The introduction of cisplatin-based polychemotherapy for disease relapse, together with improvements in clinical staging by computed tomography (CT) and serum markers, has prompted a reassessment of adjuvant treatment after orchiectomy.[3] Surveillance was introduced as an alternative management option. Interestingly, in Europe surveillance is now generally favored, while in the United States RPLND remains the standard approach. Abdominal external beam irradiation has been abandoned almost entirely. Regardless of the initial choice of treatment the 5-year relative survival of this group of patients is over 98%.[1,4]

The first line treatment of clinical stage I NSGCT is one of the most controversial topics in urological oncology today. Unfortunately, the debate is hampered by the lack of comparative empirical data. The main advantage of RPLND is proper pathology staging, while at the same time being therapeutic. Clearly, the advantage of surveillance is treatment of the recurrent disease only. It relies on the detection of recurrent disease at an early stage. In comparison with follow-up schedules after RPLND, surveillance protocols are more intensive, especially during the first two years after orchiectomy, when the risk of recurrence is highest. Inevitably, surveillance will result in treatment delay, as some patients will have more extensive disease than they would have had after immediate surgical exploration. Because treatment efficacy is

related to disease extent at time of recurrence, optimal follow-up is critical to the care of patients on surveillance.[5]

There is no universally accepted standard protocol for surveillance of patients with stage 1 NSGCT. Prospective studies to evaluate particular follow-up policies have not been performed,[6] even though a great deal of time and resources is spent in surveillance.[7] The optimal design to study the efficacy of different follow-up policies is a randomized trial. However, such a trial would require both physicians and patients to be compliant during a long period of follow-up while less intensive policies may be considered unethical without clear evidence that these are equally effective. Alternatively, mathematical decision models can mimic randomized trials in order to estimate the value of different follow-up strategies without being hampered by the practical disadvantages of those strategies.

The purpose of this study was to develop a Markov model to evaluate the impact of different follow-up strategies on disease specific mortality (DSM) and life expectancy (LE) in patients on surveillance for clinical stage I NSGCT.

Materials and Methods

Decision analysis was used to simulate three different protocols (Table I): 1. the intensive follow-up protocol currently used by The Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital (NCI/AvL[8]); 2. the protocol recommended by the European Association of Urology (EAU 2002[9]); and 3. a hypothetical minimum follow-up policy (*i.e.* follow-up limited to the first two years after orchiectomy). For the analyses, we constructed a discrete-time non-homogeneous semi-Markov model. Calculations were carried out using MATLAB software [MathWorks Inc., Natick, Massachusetts, 1999].

Table I **Follow-up schedules (times per year) for patients on surveillance for clinical stage I non-seminomatous germ cell tumours**

Year	Follow-up modality	NCI/AvL	EAU	MFP
1	Physical examination	12	6	12
	Tumour markers	12	6	12
	Chest X-ray	12	6	12
	Abdominal CT-scan	4	4	4
2	Physical examination	6	6	6
	Tumour markers	6	6	6
	Chest X-ray	6	6	6
	Abdominal CT-scan	3	2	3
3	Physical examination	4	2	-
	Tumour markers	4	2	-
	Chest X-ray	4	2	-
	Abdominal CT-scan	1	1	-
4	Physical examination	3	2	-
	Tumour markers	3	2	-
	Chest X-ray	3	2	-
	Abdominal CT-scan	1	1	-
5	Physical examination	2	2	-
	Tumour markers	2	2	-
	Chest X-ray	2	2	-
	Abdominal CT-scan	1	1	-
6-10	Physical examination	2	1	-
	Tumour markers	2	1	-
	Chest X-ray	2	1	-
	Abdominal CT-scan	1	-	-

NCI/AvL: Surveillance protocol used by The Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital[8]

EAU: Surveillance protocol recommended by the European Association of Urology, 2002[9]

MFP: Minimal follow-up policy: follow-up limited to the first two years (identical to NCI/AvL).

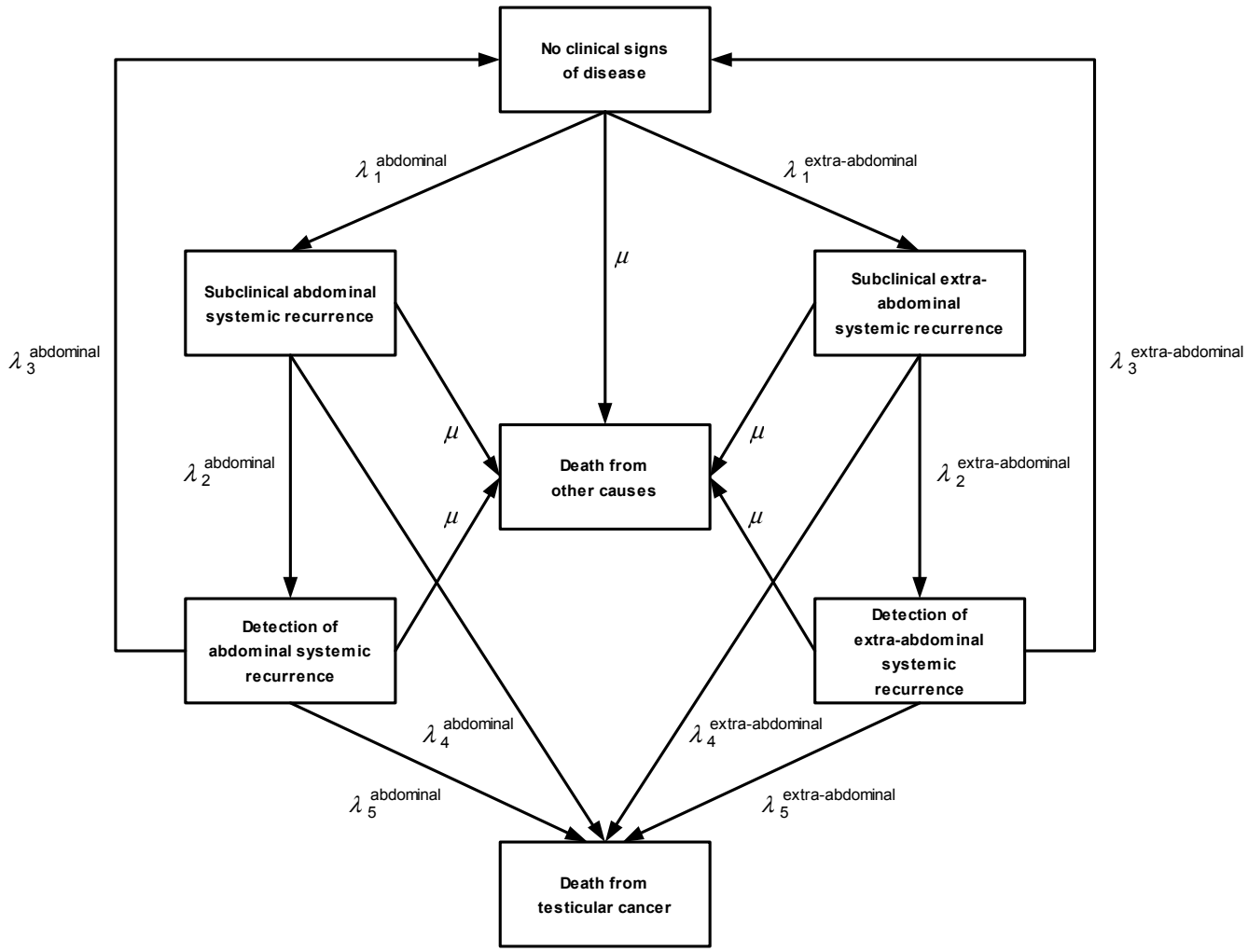
Structure of the Markov model

Markov models can be used to characterize the progression of a disease as a finite sequence of discrete states of illness. The course of the disease develops at certain transition rates between

the different states in fixed intervals.[10,11] At the end of each interval (*i.e.,* at discrete time moments), the patient either has made a single transition from one state to another or has remained in the same state. The final state is an absorbing state, usually associated with death. According to the fundamental Markov property,[10] the probability of transition from one state to another is independent of disease history and the transition rates are constant at all times (*i.e.,* homogeneous Markov model). Since the holding times in each state are exponentially distributed, the transitions between states are independent of the time spent in a particular state. Clearly, this approach is unrealistic, since the longer an individual has spent in a state of illness, the higher the probability that the next transition is to the absorbing state of death. Therefore, we used a semi-Markov model, where arbitrary hold times for each state can be applied and the transitions may depend on holding times.[12] Because we allow some transition rates to vary with time, the model becomes non-homogeneous.

To describe the course of disease of patients on surveillance for clinical stage I NSGCT, we developed a model with five non-absorbing and two absorbing states (Figure 1). A hypothetical cohort of patients enters into the initial state of the model: no clinical signs of disease after orchiectomy. At the end of each 1-month interval a fraction of the cohort is partitioned among subsequent states according to the transition probabilities. In this way, patients may remain in the same state, develop abdominal or extra-abdominal (sub-) clinical recurrent disease, recover from recurrent disease, or die from testicular cancer or other unrelated causes. A simulation period of 100 years was chosen to assure that eventually all patients end up in the absorbing states of death. The transition probabilities were derived from data in the literature. If no direct estimates for these probabilities were available, rates were calculated based on specific assumptions (see Appendix).

Figure 1: Markov state-transition model of clinical stage I NSGCT on surveillance



Legend of figure 1: Transition rates used for the Markov model simulation

$_1$: Transition rate from *'no clinical signs of disease'* to *'subclinical systemic recurrence''*

$_2$: Transition rate from *'subclinical systemic recurrence'* to *'detection of clinical systemic recurrence'*

$_3$: Transition rate from *'detection of clinical systemic recurrence'* to *'no clinical signs of disease''*

$_4$: Transition rate from *'subclinical systemic recurrence'* to *'death from testicular cancer''*

$_5$: Transition rate from *'detection of clinical systemic recurrence'* to *'death from testicular cancer'*

$_1$: Transition rate from *'no clinical signs of disease'* to *'death from other causes''*

$_2$: Transition rate from *'subclinical systemic recurrence'* to *'death from other causes''*

$_3$: Transition rate from *'detection of clinical systemic recurrence'* to *'death from other causes''*

Hypothetical cohort

The hypothetical cohort of patients is assumed to be a representative sample from the 20-year old male population of The Netherlands, apart from being hemi-orchiectomized for NSGCT. At entry into the model, the patients are assumed to have no clinical signs of metastatic disease (*i.e.,* clinical stage I disease) and to comply with the surveillance regimen.

Disease Progression

At the end of each 1-month interval, patients may have developed disease progression. Such disease progression is assumed to occur in a sequence of non-absorbing states of illness. The onset of disease progression is not detectable, because of insensitive diagnostics (*i.e.,* subclinical disease). As disease progresses, the recurrence will be detected by the patient himself (*i.e.,* symptomatic clinical disease) or by the physician during a regular follow-up examination (*i.e.,* asymptomatic clinical disease). The percentage of patients in whom recurrent disease was diagnosed per unit of time depended on the frequency of follow-up examinations and the sensitivity of the follow-up modality used. We assumed that patients with recurrent disease are treated with chemotherapy. Ultimately, all patients will be absorbed by one of two states of death: either 'death from testicular cancer' or 'death from other causes' (Figure 1).

Follow-up

As mentioned before, we analyzed three different surveillance policies (Table I). The protocol proposed by NCI/AvL was considered as a 'maximum' schedule because it is the most intensive protocol used in current daily practice.[8] A hypothetical policy of follow-up limited to the first two years only was considered to be the 'minimum' protocol. The policy recommended by the EAU was chosen as an 'intermediate' policy.[9]

Sensitivity analysis

Sensitivity analyses were performed to assess the robustness of the model to changes in assumptions. For model parameters with uncertain values, different input values were used to estimate their impact on DSM and LE. Transition rates that were directly derived from the literature were not subjected to a sensitivity analysis. All other model parameters were varied one at a time. The effect of age at diagnosis on DSM and LE was analyzed over a wide range of ages (*i.e.,* 20, 30, 40, and 50 years).

For the NCI/AvL protocol, we also investigated the impact of the sensitivity of abdominal CT-scans and the number of chest X-rays on DSM.

Results

The effects of the three different follow-up strategies on diseases specific mortality (DSM) and life expectancy (LE) are presented in Table II. The more intensive follow-up regimen proposed by NCI/AvL compared to that of EAU results in a decrease of DSM from 3.05% to 1.81% for a 20-year old man. The potential gain from follow-up strategies was found to depend largely on the detection of subclinical abdominal disease. From Table II, it is clear that a more intensive follow-up program leads to a higher LE. The absolute gain in life expectancy for a 20-year old man who undergoes the intensive follow-up policy (NCI/AvL) compared to the minimum follow-up policy (MFP) is 2.5 years (53.9-51.4). The potential benefit in LE from an intensive strategy is less for older men, while the benefit in DSM is almost the same across age groups.

The analyses were repeated to assess the sensitivity of the model to changes in baseline parameters (Table III). Both the DSM and the LE estimates for the different protocols are quite stable for a variety of alternative parameter values. The changes in DSM and LE are most pronounced for the minimal follow-up protocol. For example, if the transition rate from

subclinical recurrence to symptomatic recurrence (lambda 2) is changed from 1% per month to 2% per month the LE increases with more than 1 year while the DSM decreases with more than 2%. In the NCI/AvL and EAU protocols, the corresponding LE increase is only 0.3 and 0.5 years, respectively, while the decrease in DSM is 0.6% and 1%, respectively. In the latter protocols, both the DSM and the LE seem to depend mostly on the doubling time of the serum tumour marker alpha-fetoprotein (component of lambda 4). For all three protocols, the model is less robust for DSM than for LE.

Table II	**Effect of different follow-up strategies on disease-specific mortality (%) and life expectancy (%) for different ages at onset of disease.**			
	AGE	**NCI/AvL**	**EAU**	**MFP**
-Subclinical abdominal recurrence	**20y**	1.71	2.86	5.95
-Clinical abdominal recurrence		0.06	0.06	0.05
-Subclinical extra-abdominal recurrence		0.03	0.12	0.82
-Clinical extra-abdominal recurrence		0.02 +	0.02 +	0.01 +
Disease Specific Mortality		**1.81**	**3.05**	**6.83**
Life expectancy	**55.5***	**53.9**	**53.3**	**51.4**
-Subclinical abdominal recurrence	**30y**	1.71	2.86	5.94
-Clinical abdominal recurrence		0.06	0.06	0.05
-Subclinical extra-abdominal recurrence		0.03	0.12	0.82
-Clinical extra-abdominal recurrence		0.02 +	0.02 +	0.01 +
Disease Specific Mortality		**1.81**	**3.05**	**6.82**
Life expectancy	**45.9***	**44.5**	**44.0**	**42.5**
-Subclinical abdominal recurrence	**40y**	1.70	2.84	5.90
-Clinical abdominal recurrence		0.06	0.05	0.05
-Subclinical extra-abdominal recurrence		0.03	0.12	0.81
-Clinical extra-abdominal recurrence		0.02 +	0.02 +	0.01 +
Disease Specific Mortality		**1.80**	**3.03**	**6.77**
Life expectancy	**36.2***	**35.1**	**34.7**	**33.6**
-Subclinical abdominal recurrence	**50y**	1.66	2.77	5.76
-Clinical abdominal recurrence		0.06	0.05	0.05
-Subclinical extra-abdominal recurrence		0.03	0.11	0.80
-Clinical extra-abdominal recurrence		0.02 +	0.02 +	0.01 +
Disease Specific Mortality		**1.76**	**2.95**	**6.62**
Life expectancy	**27.0***	**26.1**	**25.8**	**25.0**

Key : see Table 1

* Statistics Netherlands, Life Tables 1998: Life expectancy of general male Dutch population[39]

Table III **Sensitivity analyses: impact of variation in baseline parameters on mortality and life expectancy for 20-year old men on surveillance for clinical stage I non-seminomatous germ cell cancer.**

Transition rate	Baseline assump-tion	Alter-native values	Disease Specific Mortality (%)			Life Expectancy (years)		
			NCI/ AvL	EAU	MFP	NCI/ AvL	EAU	MFP
Simulation results (Table II)			1.81	3.05	6.83	53.9	53.3	51.4
2, symptomatic	0.01	0.005	2.20	3.71	8.34	53.8	53.0	50.7
		0.02	1.24	2.07	4.59	54.2	53.8	52.6
3, treatment time [days]	75	50	1.80	3.05	6.84	54.0	53.3	51.5
		100	1.83	3.06	6.83	53.9	53.3	51.5
4, die from subclinical disease								
-AFP (t=o)	0.00001	0.00002	1.92	3.17	6.92	53.9	53.3	51.4
		0.000005	1.71	2.94	6.74	54.0	53.4	51.5
-Doubling time of AFP [days]	40	50	1.15	2.40	6.20	54.3	53.8	51.8
		30	2.88	4.29	7.54	53.4	52.7	51.1
5, die from clinical disease	0.001	0.002	1.89	3.12	6.89	53.9	53.3	51.5
		0.0005	1.78	3.02	6.80	54.0	53.3	51.4

Key: see Table I
AFP: serum tumour marker alpha-fetoprotein

In all models, a lymph node of 10 mm or more located in the primary landing zone was considered as a positive CT scan. We examined the impact of different criteria for a positive CT scan in the most intensive follow-up strategy (see Table IV). If the cut-off point of CT scanning is decreased, its sensitivity will increase (while its specificity will decrease) leading to a net beneficial effect. For example, if the cut-off is lowered from 10 mm to 8 mm, the DSM during the first 10 years of follow-up will drop from 1.81% to 0.95%. The number of chest X-rays during follow-up appears to be less critical (Table V). Lowering the total number of chest evaluations with 25% will result in an increase of only 0.06% in DSM (from 1.81%

to 1.87%). A reduction of the number of chest X-rays with 50% results in an increase in DSM of only 0.16%.

Table IV **The impact of improved abdominal staging on disease-specific mortality for 20-year old men on surveillance for clinical stage I non-seminomatous germ cell cancer.**

Lymph node size in the primary retroperitoneal landing zone as cut-off point for a positive CT scan#	Sens CT#	Spec CT#	DSM (%)	Percentage death from abdominal disease	
			total	subclinical	clinical
≥ 10 mm	37%	100%	1.81	1.71	0.06
≥ 8mm	47%	100%	0.95	0.85	0.06
≥ 6mm	67%	83%	0.27	0.16	0.06
≥ 4mm	97%	58%	0.12	0.01	0.06

Key: Sens: sensitivity; Spec: specificity; DSM: Disease specific mortality
Quoted from Hilton et al.[14]

Table V **The impact of the number of chest X-rays on disease-specific mortality for 20-year old men on surveillance for clinical stage I non-seminomatous germ cell cancer.**

Protocol	DSM	Death from extra-retroperitoneal disease	
	Total (%)	Subclinical (%)	Clinical (%)
NCI/AvL at baseline	1.81	0.03	0.02
NCI/AvL – (75%)[1]	1.87	0.08	0.02
NCI/AvL – (50%)[2]	1.97	0.19	0.02

Key: 1) 75% of the number of chest X-rays, used in the standard NCI/AvL model
2) half of the number of chest X-rays, used in the standard NCI/AvL model
DSM: Disease specific mortality

Discussion

The efficacy of routine follow-up in cancer is debated for many tumour sites, especially because disease recurrence is frequently detected between follow-up visits and the prognosis of recurrences is usually poor. Clinical stage I NSGCT is probably one of the exceptions. Close patient monitoring might result in survival advantage because 1. approximately one-third of patients harbor micrometastatic disease; 2. most recurrences are diagnosed during follow-up visits; and 3. these recurrences can be treated effectively with chemotherapy.[13] Although there is general agreement on the benefit of follow-up, there is no universally accepted protocol for surveillance in clinical stage I NSGCT. The purpose of the present study was to design a decision model to quantify the value of follow-up in terms of DSM and LE of the various protocols.

The simulation results confirm that surveillance influences DSM in clinical stage I NSGCT. The differences in DSM between different protocols seem small at first sight. However, these small absolute differences should be interpreted in light of the excellent 98% 10-year survival of this group of patients.[2,4,5,7] The importance of follow-up is further underlined by the outcome of the minimal (2-year) follow-up policy: 6.8% of the patients will die from disease while the overall life expectancy is 2.5 years shorter than with the intensive NCI/AvL policy.

It is well known that surveillance compared to RPLND delays the recognition of occult metastases. The drawback of surveillance is that abdominal CT misses retroperitoneal metastases in approximately one-third of patients when using the conventional criterion for lymph node enlargement of 10 mm in the primary landing zone.[14] Previous studies indicate that the use of a smaller size criterion in this group of patients decreases the proportion of

false-negatives, although the downside is an increased number of false-positives.[14-16] Our model shows a strong effect of increased sensitivity of abdominal staging on DSM.

By contrast the number of chest X-rays is questionable.[17] Lowering the number of chest evaluations has little impact on DSM, whereas the number of these investigations has clear impact on financial costs and cumulative radiation dose to the patient. Nevertheless chest X-rays are still important in detecting relapse in the mediastinum and lungs.[7]

Munro and Warde used a Markov model to evaluate the cost-effectiveness of more than 20 different surveillance schedules for clinical stage I NSCGT.[18] Relevant differences in 2-year survival were not found, whereas the costs differed significantly especially because of the different number of abdominal CT scans. The authors suggested that a prospective trial would have to reveal the true value of CT-scanning during surveillance. However, the results from this study must be interpreted carefully because the patients were followed for two years only. Differences in policies may become apparent only after a longer time period.

Of course, the results of our study should be interpreted carefully as well. For example, in the absence of clinical data, we had to assume the transition rates to death from disease. The probability to die from subclinical disease was assumed to be related with the level of the serum tumour marker alpha-fetoprotein (AFP). Therefore, we chose a semi-Markov model, which emphasizes a time dependent transition rate. In other words, the longer time the patient has spent in this particular state of illness (*i.e.,* subclinical disease), the higher the level of AFP and the higher the probability of transition to the state of death from disease. By contrast, for sake of simplicity the chance to die during chemotherapy ($_5$) was assumed to be constant over time. In fact, this chance would reach its maximum just before the start of therapy and will be lower as treatment progresses. In the sensitivity analysis, only a small impact of different $_5$ was seen on DSM, which justifies the assumption of time independence of this parameter.

The rates of other causes of death were assumed to be equal in all health states and similar to those in the Dutch population. As patients with symptomatic recurrences will receive systemic chemotherapy, their risk of dying from other causes may be higher than that of the general population. We did not correct for this because the treatment-related mortality for chemotherapy for germ cell tumours is very low, particularly for previously untreated patients.[19,20]

The probability of detection of a recurrence by the patient himself will increase by a longer follow-up interval. In theory, this probability reaches its maximum value just prior to the next follow-up evaluation. Our simplifying assumption that this rate is constant between follow-up intervals somewhat biases the results in favour of the NCI/AvL model.

Survival is one parameter of the net benefit of oncologic follow-up. Effects on cancer-anxiety and quality of life are also important.[21] Reassurance of absent disease outweighs the negative impact of follow-up visits for most men. For others, the thought of a terrifying outcome or the repetitive character of follow-up visits, outweighs such reassurance, making them to discontinue visits. In our analysis, we assumed that all patients were compliant to follow-up. Hao et al. reported a compliance of 61% in the first year but only 35% in the second year.[22] In general, in patients in whom surveillance therapy is elected, this should be considered an active form of treatment with careful follow-up being mandatory as neglecting follow-up will result in lower LE. In addition, because we intended to show the more objective results of different follow-up protocols, we calculated LE unadjusted for Quality of Life.

The statistical significance of differences in LE and DSM could not be determined because our analyses did not yield measures of uncertainty (*i.e.,* standard errors). As an alternative for the cohort simulation, a Monte Carlo simulation would have allowed the calculation of standard errors, making statistical comparisons possible.[11] But even then, the transition rates

of individual patients may be highly variable making these models only general guides to decision making of a group of patients.

Until prospective randomized trials have been performed to evaluate the efficacy of different protocols, our model may help physicians to understand the anticipated effects of different follow-up protocols on long-term survival of clinical stage I NSGCT patients on surveillance.

Conclusions

The logistical and economical benefits of a less intensive follow-up protocol as proposed by the EAU compared to that of the NCI/AvL must be balanced against the increase in disease specific mortality rate from 2 to 3%. Follow-up limited to the first two years only will result in an unacceptably high percentage of death from disease (7%). Because of the excellent prognosis of clinical stage I NSGCT, follow-up schedules have limited impact on life expectancy. Our model suggests that CT-scanning is essential to disease-specific survival, whereas the large number of chest X-rays seems to have little benefit.

Acknowledgement

We thank all the clinicians involved in this study for providing the basis for our research.

Appendix: Transition rates

The transition rates indicated with and μ, along the arrows in Figure 1, were derived from the literature, or estimated by 'educated guess' if no direct figures were available. These rates describe the conditional rate of occurrence of events per unit of time.

Transition rates (and μ) may be converted to transition probabilities (p) taking the time interval (Δt) into account, according to the formula: $p = 1 - e^{-\Delta t}$. The monthly transition rates (-monthly) were approximated from the annual rates (-annual) using the formula: -monthly = 1/12 * -annual.[23]

LAMBDA 1 ($_1$): entering subclinical disease state

In the model $_1$ represents the rate at which patients develop a subclinical recurrence. The time course of this rate, $_1(t)$, was estimated from the database of The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital (NCI/AvL).[8] From this data we estimated a Kaplan-Meier relapse-free survival function, $\hat{S}(t)$, and subsequently calculated an estimate of the cumulative hazard function: $\hat{H}(t) = -\log \hat{S}(t)$.[24,25] Plotting $\log \hat{H}(t)$ versus time showed a strong linear trend (especially for large t, whereas for small t the trend was curved), suggesting a Gompertz distribution.[26] An almost perfect fit (r=0.98) was found by first fitting $\hat{H}(t)$ to a 3-parameter exponential association growth model and subsequently taking the derivative, yielding the hazard function: $_1(t) = e^{+t}$.

The values for and are constant: NCI/AvL data: = -3.823; = -0.069. In order to refine the model and bring it into accordance with the clinical situation, we divided the data of

NCI/AvL into abdominal ($_1{}^{abd}$) and extra-abdominal subclinical disease ($_1{}^{xabd}$). The time course of $_1(t)^{abd}$ and $_1(t)^{xabd}$ were estimated in the same way. The values for ^{abd} and ^{abd} are -4.06975 and -0.06354, and for ^{xabd} and ^{xabd} -5.18180 and -0.08308, respectively.

LAMBDA 2 ($_2$): transition to symptomatic disease state

The $_2$ represents the rate to develop clinical disease. It consists of an asymptomatic, $_2{}^{asym}$, (detection by follow-up examination) and a symptomatic, $_2{}^{sym}$, (detection by the patient between consecutive follow-up moments) component.

We assumed $_2{}^{asym}$ to be dependent of the accuracy of the imaging modality at follow-up, and to be zero between consecutive follow-up moments. Using the commonly accepted criterion of 10 mm for metastatic disease, the abdominal CT-scan has a sensitivity of 37% and a specificity of 100%[14] and the chest X-ray of 35% and 99%, respectively.[27] In our model, the specificity of the different imaging modalities was set to be 100% and the transition probability, p, to be equal to the sensitivity of the imaging modality. Consequently, the following formula was applied to find the transition rate: $_2{}^{asym}(t) = -\log(1-\sigma)/\Delta t$, where σ is the sensitivity of the imaging modality, and Δt is the 1-month Markov interval time.

The chance to detect a symptomatic recurrence is believed to be a function dependent on the time in-between two successive follow-up moments and therefore it was modeled to be inversely proportional to the frequency of the regular follow-up visits. Because both the published literature reported only a small percentage of symptomatically detected recurrences among patients on surveillance (0-2%)[8,28-35] and for sake of simplicity, we assumed $_2{}^{sym}$ to be constant over time and equal to: $_2{}^{sym} = 0.01$.

LAMBDA 3 ($_3$): transition to cured state.

Lambda 3 ($_3$) is related to the time needed to be cured after the 'detection of clinical systemic recurrence'. It was assumed that all patients received combined chemotherapy (three cycles of cisplatin, etoposide, bleomycin and a last cycle of etoposide, bleomycin during a 5 day regimen) and are regarded as cured after completion of therapy. In total, it would take 75 days (time between detection and start of chemotherapy is 7 days together with 68 days of treatment time) before a patient can leave the state 'detection of recurrence' and re-enter the state of 'no clinical signs of disease'. In this way the states "detection of clinical systemic recurrence" behave like a first-in-first-out queue with a fixed time spent in that state (*i.e.,* sojourn time).[10,11] During the course of treatment, $_3$ has a value of zero. Only patients having spent the total sojourn time are regarded to be cured, and $_3$ takes on such a value that only that fraction of all patients in treatment is released to the initial state. The following transition rate was applied: $_3 = -\log(1-N_1/N_{total})/\Delta t$, where N_1 is the number of patients leaving the treatment queue and N_{total} is all patients in the treatment queue.

LAMBDA 4 and 5 ($_4$, $_5$): failure rate

The transition rates $_4$ and $_5$ express the rate at which patients are transferred from the states "subclinical recurrent disease" and "clinical recurrent disease" to the absorbing state of "death from testicular cancer", respectively. In the literature, approximately 1% dies from testicular cancer at five years after diagnosis to 1-3% at 10 years of follow-up.[2,5] Clinical data on "death from disease" in the literature were sparse. Therefore, we assumed that all four transition rates ($_4^{abd}$, $_4^{xabd}$, $_5^{abd}$, $_5^{xabd}$) have the same impact on 'death from testicular cancer' and that combining these rates results into the overall percentage of death from disease in patients with clinical stage I NSCGT.

Literature data to calculate $_4$ are not available. Alternatively, we used a tumour growth model to estimate the natural course of developing a metastatic testicular tumour and to die from this metastasis without therapeutic intervention. In testicular germ cell tumours the prognosis in terms of survival seems to be more related to the level of the tumour markers and to the presence of metastasis in liver, bone and brain than to the growth of the pulmonary and retroperitoneal metastasis.[36] Because the vast majority of metastatic disease in clinical stage I disease is found in the retroperitoneum and in the lungs, we have focused on the growth pattern of the tumour markers. We used the tumour marker alpha-fetoprotein (AFP) as an example to calculate tumour growth.[37] The mean doubling time for AFP was 28.5 days. Because only 60% of the non-seminoma tumours produce AFP[38], we assumed the remaining tumours (40%) had a doubling time twice as long as the AFP-producing tumours (57 days). All together, the average doubling time in our model was 40 days (0.6*28.5 + 0.4*57). Furthermore, we assumed that the chance to be cured is zero with an AFP-value of 100,000 ng/ml and that the baseline value of AFP was 1/100,000 ng/ml. In the literature, most tumour growth models have shown an exponential growth pattern during the observation period.[39] Subsequently, the natural course to die from recurrent metastatic testicular disease will take 33.2 doubling steps ($0.00001 * 2^n = 100{,}000$ ng/ml). Thus, it will take approximately 44 months (33.2 * 40 days) to die from untreated recurrent systemic testicular cancer.

The hazard function $_5$ is the proportion of individuals alive and treated for recurrent testicular cancer who die from recurrent testicular germ cell cancer during treatment. We adopted a baseline rate for treatment-related mortality of 0.001 according to Munro *et al.*[18]

MU 1 ($_1$), MU 2 ($_2$) and MU 3 ($_3$): mortality rates

Transition rates ($_1$, $_2$, $_3$) that led to the state 'Death from other causes' were based on male mortality data acquired from Statistics Netherlands.[40] The 'age-specific mortality rate' for the

years 1990-1994 was averaged into an 'average age-specific mortality rate per year' which is the probability to die within one year given survival until a specific age. Subsequently, the 'average age-specific mortality rate per year' was converted to a 'monthly average age-specific mortality rate'.

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PART V

Fertility aspects in management of germ cell tumour of the testis

CHAPTER 10

Fertility in men with testicular germ cell tumour

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Abstract

Objectives. To assess the prevalence of (in)fertility in men before and after treatment for testicular cancer.

Methods. Information about fertility was collected using postal questionnaires from 226 out of 305 patients, treated for testicular germ cell cancer at the Department of Urology of the University Medical Center Nijmegen from 1982 to June 1999. Fertility was defined as the ability of a sexually active non-contracepting couple to achieve pregnancy within one year. The results were compared to the lifetime prevalence of infertility in the general population (20-28%).

Results. Before the cancer was diagnosed, 79 of 120 couples (66%) who attempted to conceive, succeeded within one year. After treatment 38 of 88 couples (43%) conceived within one year. Seven couples used cryopreserved sperm to conceive a child after treatment. The different treatment modalities did not significantly influence the outcome of patients' wish for children. Congenital malformations were recorded in approximately 4% of the children born before or after treatment.

Conclusions. Although the majority of the patients with testicular cancer have a fulfilled wish with regard to children, it seems to be more difficult to father a child after treatment compared with the case in the general population. Because it is not possible to predict which patient will have fertility problems after treatment, cryopreservation should be offered to every testicular cancer patient. An increased risk for congenital malformations was not observed.

Introduction

Although germ cell tumours of the testis (TGCT) account for only 1 percent of all tumours in males, it is the most common malignancy in males between 15 and 35 years of age.[1] The combination of surgery, radiotherapy and chemotherapy has made TGCT curable in approximately 90% of patients with newly diagnosed disease.[2] Since the majority of cured patients are young and have not started or completed families, fertility and sexual function are important issues.

For several reasons, subfertility is common in patients with TGCT. Firstly, three quarters of the patients have a decreased baseline spermatogenesis at the time of diagnosis.[3,4] Potential causes include a pre-existing defect in spermatogenesis, a history of cryptorchidism, hormone production by the tumour, antisperm antibodies, possible contralateral or intraepithelial germ cell neoplasia, and generalized stress associated with illness.[2,5] Among all, cryptorchidism has been proven to be associated with both testicular cancer (relative risk [RR]: 2.5-11; see Benson et al.[6]), and infertility (RR: 2-6; see Lee[7]).

Second, deterioration of spermatogenesis has been observed after adjuvant radio- or chemotherapeutic treatment in a dose-dependent fashion.[4,8] Approximately a quarter of the patients remain azoospermic after treatment.[2] Finally, up to half the patients undergoing surgery may suffer from ejaculatory dysfunction after treatment.[9,10]

Even though most studies in patients with TGCT have used sperm quality as indicator for fertility, it is not apparent from the published reports that paternity is compromised. Because fertility is defined as the ability of a sexually active non-contracepting couple to achieve pregnancy within one year[11], attempting paternity is a better measure for fertility.[3]

To put this study into perspective, it is important to note that up to one-third of the couples (in the general population of industrialized countries), attempting parenthood appear to be subfertile.[12]

The objective of this study is to assess the prevalence of fertility in patients curatively treated for TGCT and to evaluate fertility-related factors, the frequency of use of cryopreserved sperm, the health of offspring and patients' satisfaction with the given information on fertility and sexual dysfunction.

Patients and Methods

The medical records of testicular cancer patients treated at the department of urology of the University Medical Center Nijmegen between June 1982 and June 1999 were reviewed. Medical information was obtained from the patients' records.

Staging (Royal Marsden Hospital Staging; see Peckham[13]) and first-line treatment principles have been reported previously.[14] In short, all orchidectomised patients were screened for metastases using abdominal and thoracic computed tomography scanning at the time of the initial diagnosis. Of those in whom no metastases were found, patients with seminomas were irradiated on the iliac and para-aortal lymph nodes with 26 to 30 Gy. Of patients with no or only small (<2 cm) positive retroperitoneal lymph nodes on computed tomography scan (Stage IIa), those with nonseminoma germ cell tumour (NSGCT) were treated with retroperitoneal lymph node dissection (RPLND).

Since 1994, the decision to perform RPLND in patients with Stage I NSGCT was also dependent on the presence of vascular invasion in the primary tumour. RPLND was done in case of vascular invasion and was followed by two cycles of bleomycin, etoposide, and cisplatin chemotherapy in patients with lymph node metastases.

When metastases were present and the tumour was minimally Stage IIb, patients with either seminoma or NSGCT received three to four cycles of cisplatin based chemotherapy after inguinal orchiectomy. Residual mass after chemotherapy was removed in all

nonseminomatous germ cell tumours and in seminomatous germ cell tumours in case the mass was larger than 3cm in transverse diameter.

A questionnaire was sent to 305 patients who were assumed to live at the address given by the post office, did not relapse after first-line treatment and had been followed-up for more than 18 months. Because no validated questionnaire was available, we designed it ourselves in collaboration with the Departments of Epidemiology and Gynecology at our institution.

The questionnaire mainly consists of questions about pre- and post-treatment attempt for paternity, length of unprotected intercourse to conception or period of infertility, paternal and maternal age at attempts, health including (previous) testicular or gynecological disorder, patient lifestyle factors (duration and amount of using tobacco and alcohol), patient contact with chemical or other occupational hazards, health of offspring and the use of cryopreserved semen. Moreover, we asked if the patients were satisfied with the available information about fertility and sexual function after treatment.

According to the ethics committee for biomedical trials of the University Medical Centre St. Radboud, it was not necessary to approve this study, as long as only a questionnaire was used.

We have defined fertility as the ability of a sexually active noncontracepting couple to achieve pregnancy within one year.[11] The prevalence of fertility before and after treatment was calculated among those who stated that they had attempted fathering a child (population at risk).

The prevalence was expressed as percentages with corresponding 95% confidence intervals. Where applicable, differences in percentages were tested for statistical significance with Fisher's exact test and differences in continuous variables (e.g. age) was tested with T-tests.

Results

Patient characteristics. In total, 74% (226/305) of the questionnaires were returned. Of the remaining 79 patients, 10 patients refused to take part, 5 patients had moved to an unknown address, and 64 did not return their questionnaires for unknown reasons.

Fifty-four patients (24%) had been treated for seminomatous germ cell tumours and 172 (76%) for non-seminomatous germ cell tumours. The median age at initial diagnosis was 31.7 years (range 17.4 to 70.0 years). The median period between diagnosis and returning the questionnaire was 89 months (range 19-224 months).

The influence of patient characteristics on the probability of fathering a live-born child was investigated (Table I). There was no significant effect of histological type, prior cryptorchidism or other genital condition on success at conception before treatment. Also lifestyle factors (history of or currently smoking and/or drinking) and occupation (history of or currently exposure to chemicals) failed to show a significant difference (data not shown).

Fertility status before treatment. Before testicular cancer was diagnosed, 120 of 226 (53.1%) patients attempted to father a child. Ninety-tree patients (77.5%; 95%CI: 69.9-85.1) had fathered 194 live-born children. The majority of them succeeded in conceiving within 1 year (84.9%; 79/93). Six of 79 couples had initially experienced a miscarriage, but managed successful pregnancy within one year after their first attempt.

Of the 14 couples who did not conceive within one year, one couple needed assisted conception treatment, and in one couple, the partner had been treated successfully for hormonal dysbalance. The remaining 12 succeeded spontaneously after attempting for more than one year. The median age of father and mother, respectively, at first attempt pre-orchiectomy was 27.9 years (range: 20.0-36.0) and 24.8 years (range: 19.1-36.0).

Twenty-seven couples (22.5%), who attempted for more than one year, remained childless pre-treatment. Among them, 3 experienced a miscarriage after attempting for more than one year. Five couples sought specialized help: three patients appeared to have hypospermia, one woman had hormonal dysbalance and in one couple there was no explanation for childlessness. The median age of father and mother, respectively, at first unsuccessful attempt was 26.3 years (range: 19.1-35.0 years) and 23.4 years (range: 18.9-31.5 years).

Table I **Clinical characteristics and paternity in patients at diagnosis of testicular cancer**

	Fatherhood before treatment (n=93)	Unfulfilled wish after attempting > 1 year (n=27)	No attempt to father a child (n=106)
Patients' history before disease			
-cryptorchidism	14	8	27
-inguinal hernia	10	5	11
-varicocele	1	-	2
-orchitis	3	1	4
Paternity status before diagnosis			
Time to conceive			
<0.5 year	68	-	-
0.5-1 year	11		
>1 year	14		
Median age father (years)	27.9 (at birth)	26.3 (at first attempt)	-
-range	20.0-36.0	19.1-35.0	
Median age mother (years)	24.8 (at birth)	23.4 (at first attempt)	-
-range	19.1-36.0	18.9-31.5	
At diagnosis			
Median age at diagnosis (years)	36.0	32.1	25.9
-range	26.4-70.0	22.1-58.7	17.4-62.6
NSGCT/SGCT*	60/33	20/7	92/14
Stage†			
-I	37	13	35
-II	38	12	39
-III	-	1	1
-IV	13	1	21

Key: *(N)SGCT: (non)-seminomatous germ cell tumour
†according to Royal Marsden Hospital Staging system[13]

Fertility status after treatment. After treatment, 88 patients attempted to have a child. Fifty-four of them (62.5%; 95%CI: 52.5-72.5) had fathered a child (Table II), and of those 46 patients had fathered by natural intercourse (median time after diagnosis: 44 months, range 12 – 134 months) and 8 patients, by assisted reproduction (including two couples who used donor insemination). At the time of the questionnaire, one spouse was pregnant for 6 months after assisted help (see Table II).

The median ages of the father and the mother, respectively, at first attempt after completion of treatment were 32.0 (range: 22.1-41.0 years) and 29.1 years (range: 21.3-39.0 years). In total, 81 children were born post-treatment at a median of 46 months (range 12-143 months).

Thirty-eight of the 88 couples (43%) succeeded in conceiving within one year. Although in 7 of them a miscarriage occurred, 4 couples managed to conceive a successful pregnancy within one year after their first attempt.

Although not significant, the percentage of patients who fathered after chemotherapy and surgery was higher than those receiving chemotherapy or surgery alone (Table II). No pregnancies occurred during any cancer treatment.

Thirty-two of 33 patients attempted conception for more than one year at a median of 104 months (range 18-216 months) after completion of treatment (Table II). The median age of father and mother, respectively, at first attempt (unsuccessful) after treatment was 33.3 years (range: 22.1-41.0 years) and 30.2 years (range: 22.9-39.5 years).

Nine of the 138 patients, who did not attempt to father a child after treatment, reported a wish for children in the future.

Table II	Patient characteristics and paternity after treatment						
	All patients	**SV**	**RT**	**PRPLND**	**PRPLND and CT**	**CT**	**CT and SRPLND**
Fatherhood	54	3	8	9	17	7	11
Time to conceive							
<0.5 year	28	1	5	5	10	4	3
0.5-1 year	7	1	-	3	1	-	2
>1 year	19	1	3	1	5	3	6
pregnant at time of questionnaire	1	-	-	-	1	-	-
No Fatherhood* (attempting > 1 year)	33	3	2	9	7	7	4 (and 1†)
No attempt	138	14	26	26	18	30	24

Key: SV, surveillance; RT, radiotherapy; PRPLND, primary retroperitoneal lymph node dissection; SRPLND, secondary retroperitoneal lymph node dissection; CT, chemotherapy.
* Including 3 patients, who experienced a miscarriage within one year after attempt, but did not deliver a child at all.
† at time of questionnaire attempting 0.5-1 year

Association between fathering a child before and after treatment. Most of the patients who fathered a child before treatment did not attempt to do so after treatment (70/93; Table III). After having fathered a child before treatment, 23 patients also attempted to father a child after treatment. Sixteen of them succeeded.

Six of the 27 patients who did not succeed in fathering a child before treatment did succeed afterwards. Two of those 6 couples experienced a miscarriage before treatment, and in one, the spouse had had a hormonal dysbalance that was corrected after completion of testicular cancer treatment.

More than a quarter (60/226) of the patients did not attempt at all.

Table III	Association between fathering a child before and after treatment			
After treatment / Before Treatment	Fatherhood	No Fatherhood	No attempt	Total
Fatherhood	16	7	70	93
No fatherhood	6	13	8	27
No attempt	33	13	60	106
Total	55	33	138	226

Assisted reproduction after treatment. Between orchiectomy and adjuvant treatment, sperm cryopreservation was offered to 121 patients. Ninety-nine patients had accepted preservation, and in 78 patients, the quality of the semen was viable enough to preserve[15] (Table IV). According to Table IV, the percentage of patients who attempted preservation after request was significantly higher among patients who were unsuccessful in reproduction before disease (100%) than in those who were successful before disease (50%). The sperm quality revealed no significant differences between those who fathered a child or being childless both before and after treatment (Table IV and V).

After treatment 13 of 78 patients (17%) attempted artificial insemination with cryopreserved semen (Table VI). Seven of them had been successful (including a pregnancy of 6 months in one couple). Two azoospermic couples managed to have a child by donor insemination.

Table IV **Cryopreservation before adjuvant treatment in orchiectomized patients* for testicular cancer.**

	Fatherhood	Unfulfilled wish	No attempt	Total
offered	32	20	69	121
attempted	16	20	63	99
preservation†	15	11	52	78
normospermic‡	7	4	11	22
hypospermic	5	7	31	43
azoospermic	1	3	6	10

* one patient cryopreserved his sperm before orchiectomy of his single testicle (see also table VI).
† according to WHO guidelines (1999)
‡ at least $20x10^6$/ml; $40x10^6$; 50% motility

Table V **Distribution of pre-treatment sperm-analysis and wish for children after adjuvant treatment for testicular cancer.**

After treatment / Before treatment	Fatherhood	No Fatherhood	No attempt	Total
normospermic*	12	2	8	22
hypospermic	17	8	18	43
azoospermic	3**	6	2	10

*see Table IV
**2 patients used donor insemination

Table VI. Outcome of artificial insemination after treatment.

Histology and stage	Treatment*	Fertility problems	Assisted reproduction	Pregnancy	Birth
Non-seminoma IIa	PRPLND and CT	dry ejaculation	IVF	Yes	Yes (3 children)
Non-seminoma IIc	CT and SRPLND	dry ejaculation	IVF	Yes	Yes (1 child)
Non-seminoma IIc	CT and SRPLND	dry ejaculation	IVF	Yes	Yes (1 child)
Non-seminoma IId	CT and SRPLND	dry ejaculation	ICSI	Yes	Not yet, (6-months pregnancy)
Non-seminoma IIa	PRPLND and CT	dry ejaculation & azoospermic	DI	Yes	Yes (2 children)
Non-seminoma IIc	CT and SRPLND	anejaculation after therapy	ICSI	No	No
Non-seminoma I	SV	attempting > 1 year (irregular cycle of the partner)	IVF	Yes	Yes (1 child)
Non-seminoma I	PRPLND	Attempting > 1 year	IVF	Yes	Yes (twin)
Non-seminoma IV	CT	attempting> 1 year	IVF	No	No
Non-seminoma I	PRPLND	attempting> 1 year	IVF	No	No
Non-seminoma IIa	PRPLND and CT	attempting> 1 year	IVF	No	No
Non-seminoma IIa	PRPLND and CT	Attempting > 1 year: (azoospermic)	DI	Yes	Yes (twin)
Seminoma I	RT	bilateral orchidectomy†	ICSI	Yes	Yes (2 children)

Note: IVF: In-vitro fertilization; ICSI: intracytoplasmic sperm injection; DI: donor insemination.
Quality cryoperserved sperm: 1: normospermic; 2: hypospermic; 3:azoospermic
* See Table 2
†First orchiectomy for small cryptorche testicle

Ejaculatory function after treatment. Thirty-three patients were found to have ejaculatory problems after treatment (radiotherapy: 2; chemotherapy: 4; surgery: 9; combination surgery and chemotherapy 18). This dysfunction recovered spontaneously within one year in six patients and in one by using sympathomimetic drugs. Seventeen patients with an (history of) ejaculatory dysfunction after treatment reported a wish for children. Four patients managed to father a child by natural intercourse after spontaneous recovery, and 6 patients used assisted reproduction (Table VI).

Offspring. Two hundred seventy five children were born out of 320 pregnancies. One hundred ninety-four children and 81 children were born before and after fathers' diagnosis of TGCT,

respectively. The median time between discontinuation of treatment and birth of first child was 46 months (range 12-143 months).

A congenital defect was identified in 8 children born before the diagnosis (defect: schisis, 2; poly-dactyly, 1; bifid spine, 1; heart disease, 1; undescended testicle, 2; Werdnig-Hoffman disease,1) and in 3 who were born after the diagnosis (poly-dactyly, 1; heart disease, 1; Zellweger Syndrome, 1). No relation was found between the type of treatment of father and the congenital defect of the child. Also, no relation was found between the period after completion of treatment and the birth of child with congenital defect.

All except three children (Werdnig-Hoffman disease, Zellweger Syndrome and leukemia) have been reported to develop normally up to a median age of 181 months (range 1-582 months).

Evaluation of information on fertility and sexual function given before adjuvant treatment.

Most of the patients (63%) reported that the information given at our department was sufficient. In contrast, 27% considered the information poorly sufficient to insufficient, and 10% reported they were given no information on fertility and sexual function at all.

Most patients said that they were poorly or not informed about possible treatment-related and/or disease-related poor sperm quality. Consequently, three patients would have tried assisted reproduction when they had preserved sperm before therapy. In contrast, almost all patients where informed about possible ejaculatory dysfunction after surgery.

No correlation was observed between patients' content about the given information and their (un)fulfilled childwish (data not shown).

Discussion

The majority of studies that suggest a direct association between subfertility and TGCT, have used sperm analyses instead of paternity as a parameter for fertility.[3] These studies do not control for the contribution of the female factor in infertility and use sperm analyses as an algorithm for fertility.

In our series, several hypospermic patients were able to father a child, and even two patients who were diagnosed as azoospermic at time of diagnosis had fathered a child before or after treatment, respectively. Therefore, time to conception and rate of conception per unit time are more valid measures of fertility.[5] We evaluated the prevalence of fertility in 226 patients before and after treatment for TGCT and used paternity and time to conceive as indicators.

In accordance with Fossa *et al.*[16], 78% of the patients (93/120) with newly diagnosed testicular cancer had been successful in their attempts to father a child before orchiectomy. In the majority of the cases (66%) the partner conceived within one year (and thus were considered fertile). It has been stated that approximately one third of all patients seek paternity after treatment for TGCT and more than half of them finally succeeds.[4,8] This is in accordance with our figures: 88 of the 226 couples (39%) attempted to reproduce, and 55 couples succeeded. In total, 43% of the couples (38/88) conceived within one year after discontinuation of treatment. Compared with the prevalence figures of infertility in the general population (20-28%; Schimdt *et al.*[12]), it seems that patients who have been treated for testicular cancer have a higher risk of infertility.

However, it is also important to take into account that maternal age[17], and post-treatment gonadal and ejaculatory (dys)function may affect pregnancy-rate.[10] First, it is well known that the age of the spouse is an important determinant of the man's ability to initiate pregnancy because the length of time required to establish pregnancy increases with advancing maternal age. The decline in fertility starts in the mid-thirties.[18] In our series, the median ages of the

females who did not achieve pregnancy before and/or after their partners' treatment is 23.4 and 30.2, respectively. There was no significant difference in female age between the couples who conceived and who did not. Although some data might be not completely accurate due to recollection bias (the maternal and paternal age at successful attempt is easier to recollect than the age at first unsuccessful attempt), the contribution of maternal age to fertility related problems seems to be negligible in our series.

Second, it is well known that spermatogenesis is sensitive to the effects of chemotherapy and radiotherapy in a dose-dependent fashion. More than four cycles of cisplatin-based chemotherapy[4], and radiation doses of >30 Gy will result in long-term impaired spermatogenesis.[8] In our series, none of the patients received a dose that exceeded the above-mentioned doses because only those patients who were included were cured after first-line treatment.

Furthermore, ejaculatory dysfunction may result from retroperitoneal surgery, radiotherapy and/or chemotherapy. Whereas surgery may damage the postganglionic fibers of the lumbal sympathetic nerves, radiotherapy and chemotherapy may affect the vascular and nervous systems of the retroperitoneal area.[19] Since the introduction of nerve-sparing surgery, the incidence of ejaculatory dysfunction has reduced, without compromising the staging and therapeutic benefit.[3] Moreover, some patients may benefit from taking sympathicomimetic drugs. In our series, one in seven patients reported ejaculatory dysfunction. In some, assisted reproduction was the only option the overcome this dysfunction.

Today, the most effective way to preserve fertility in patients with TGCT is cryopreservation of sperm. Even extremely poor quality semen is worthwhile to cryopreserve with the availability of intracytoplasmic sperm injection techniques.[20] In accordance with Fossa *et al*.[15], the minority (17%) of the patients opting for sperm cryopreservation later makes use of the option. Moreover, most patients seem to discontinue sperm banking after treatment.[21] At

first sight, sperm-banking seems to be less effective; however, as it is not possible to predict the fertility status for the individual patient after treatment[22], it should be recommended to each patient who is adjuvantly treated after orchiectomy.

Less is known about the risk of congenital defects among offspring of male cancer survivors. So far, no study has reported an increased risk among the offspring of testicular cancer patients.[10,23,24] However, it must be kept in mind that most studies (including ours) are hampered by low power and lacking long-term results. In our series, the percentage of malformations was similar before and after fathers' treatment (4%). To put this into perspective, congenital defects were reported among 2.2% of live-born children in general population.[25] This difference is best explained by selection bias.

In addition, it is suggested that sperm should not be banked during chemotherapy and contraceptive intercourse is advised for at least 3 to 6 months after completion of chemotherapy.[26,27] Besides the teratogenic effect, we also advise use of contraception during the first year after completion of treatment for psychological reasons, because the risk of recurrent disease is highest during this period.[14]

In retrospect, 37% of the patients found the pre-treatment information on possible fertility problems, especially on the possibility of cryopreserving sperm insufficient. In our series, preservation was offered to approximately half of all patients. Moreover, three patients who are unfulfilled childless were very upset about not having had any information on preserving sperm before therapy. Today, we extensively inform every patient, and the majority opts for preservation.

According to the available evidence, it was impossible to predict the fertility outcome in patients with testicular cancer, but in general, they seem to have more difficulty fathering a child after treatment compared with the case in the general population. Despite fertility problems, a couple of patients fathered a child after using assisted-reproductive methods. In

accordance with results of previous studies, an increased risk for congenital abnormalities among the offspring of testicular cancer patients was not observed when starting to father a child one year after completion therapy.

Thus, before any treatment is offered, information must be given about possible fertility problems, and sperm banking should be strongly recommended for each patient, as it may represent an extra chance of fathering a child.

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CHAPTER 11

Sperm quality before and after chemotherapy in men with testicular germ cell cancer

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Submitted

Abstract

Objective. While (partial) recovery of spermatogenesis has been observed in most of the testicular cancer patients after chemotherapy (ciplastin), sperm genomic integrity and its implication for the patient's fertility is poorly understood.

Methods. Semen and serum from 22 patients treated for testicular cancer were analysed pre- and post-chemotherapy. Besides routine semen analysis, sperm samples were evaluated by computerized-karyometric-image-analysis (CKIA), chromomycin-A_3 assay (CMA_3, chromatin condensation) and TdT-mediated dUTP nick-end labelling assay (TUNEL, DNA-damage).

Results. External sperm characteristics (CKIA morphometry) and counts did not deteriorate after chemotherapy. An improvement in DNA condensation was assessed after chemotherapy (37% vs. 50% and 47.5% vs. 63.7% for CMA3 and CKIA respectively; $P<0.005$) but still a high percentage of TUNEL positive sperm was found in the samples (21% vs. 25% for pre- and post-chemotherapy samples respectively). Serum FSH and LH (IU/ml) increased after chemotherapy compared with pre-treatment levels (8.1 vs. 16.7 and 4.5 vs 6.8; $P<0.05$, respectively).

Conclusion. In spite of the improvement in sperm chromatin packaging after chemotherapy, still an abnormal percentage of DNA-damaged sperm was found in these samples. As sperm quality does not reach normal levels after treatment, it remains difficult to outline the best strategy and guidance concerning fertility potential of testicular cancer patients.

Introduction

The introduction of cisplatin-based chemotherapy has greatly improved the cure rate for testicular germ cell tumours.[1] Because the majority of men are in the prime of their reproductive lives, the long-term side effects become increasingly important.[2]

Gonadal dysfunction is one of the most common side effects of chemotherapy. Both the endocrine and exocrine compartments of the testis are affected by chemotherapy. The serum follicle stimulating hormone (FSH) levels seem to rise immediately after initiation of chemotherapy, indicating a dysfunction of the germinal epithelium.[3-7] Although the Leydig cells of the testes are more resistant to cytotoxic damage than germinal epithelium, the increased luteinizing hormone (LH) levels in men suggests an endocrine dysfunction.[3-7] This probably represents a compensatory mechanism resulting from reduced negative feedback by testosterone at the hypothalamopituitary level, thereby reflecting a degree of impairment of testosterone production by the Leydig cells. The increased levels of LH and FSH are suggested to maintain normal testosterone levels and support sperm production as well.[3-7]

Testicular exocrine function is even more affected by chemotherapy. The differentiating spermatogonia appear to be most vulnerable to the cytotoxic effects of chemotherapy.[2,7] Previous studies have shown that the majority of patients with testicular germ cell tumours have reduced sperm counts at diagnosis and this will further deteriorate during treatment.[4-16] The duration and severity of the spermatogenic depression depends upon the dose and duration of chemotherapy and the baseline testis function prior to therapy.[7,13] Despite an early depression in spermatogenesis, a reasonable number of patients show recovery within one to two years after treatment with variable sperm counts in their ejaculates.[3,6,9-11] On this point knowledge about the spermatogenesis and sperm integrity pre- and post chemotherapy is lacking. In the first place, it is not clear whether the cancer itself is also capable of inducing changes in the genomic integrity of the spermatozoon. Sperm DNA-breaks induced during

spermiogenesis or uncompleted maturated sperm with abnormal condensed chromatin may contribute to high rates of damaged sperm in the ejaculate. In the second place, it is doubtful whether classical semen analysis (WHO criteria[17,18]) gives the right information about the status of semen from men with testis carcinoma.

The present study was set up to obtain more information about these problems. We used both conventional and new methods to determine the changes in the quality of ejaculated spermatozoa and applied them in samples obtained before and after exposure to cisplatin-based chemotherapy. Sperm genetic integrity, defined as normal condensed, undamaged DNA, was measured using three techniques: computerized karyometric image analysis (CKIA), proved to be an objective method to study the morphometry, DNA density and chromatin texture of sperm samples and individual spermatozoa.[19] Chromomycin A3 (CMA_3) and the TdT-mediated dUTP nick-end labelling assay (TUNEL) were used to assess the DNA condensation (packaging) and damage rate (DNA breaks) respectively.[20-23]

Materials and Methods

Patients and controls. The subjects of this study were patients who had hemi-ochiectomy and chemotherapy in the past as a result of testicular cancer. Of these patients, frozen sperm samples and serum were available, both obtained between hemi-orchiectomy and subsequent chemotherapy. All included subjects received bleomycin, etoposide and cisplatin on a 5-day regimen for advanced stage of disease. All patients gave written informed consent before inclusion in the study. This research was reviewed and approved by the institutional ethics committee.

Semen samples of anonymous normospermic donors (controls, n=13) were used as control for CKIA, CMA3 and TUNEL assays.

Collection and work-up of samples. Semen and blood samples were stored before the start of chemotherapy treatment. For this study, a new blood sample (20 ml in EDTA) was taken and stored at -40^0C until assayed. Also a new semen sample was collected and cryopreserved in liquid N2 to avoid cryopreservation bias in the assessments. The paired (before and after treatment) semen and blood samples were analysed at the same time except for the routine semen analysis parameters (determined in the fresh samples). Cryopreserved control samples were treated similarly as for patients.

Assessment of semen parameters. Semen analysis was performed in fresh samples as described before.[18] Shortly, concentration and motility were measured using a Makler counting chamber. For morphology assessment, semen was mixed on a slide with methylene blue/eosin, smeared and flame-fixed. Only vital cells (eosin negative) were evaluated for morphology using WHO criteria (1992 for samples previous and 1999 for post chemotherapy samples)(WHO 1999). Furthermore, we used the computerized karyometric image analysis (CKIA) for the quantification nuclear sperm characteristics in the frozen samples. For a detailed description see Ramos *et al.*[19] The karyometric parameters were grouped into three categories: 1) morphometric parameters, which describe size, form and shape of the nuclei; 2) densitometric parameters, related to staining intensity of DNA content; 3) chromatin texture parameters, related to the stain distribution pattern.

Assessment DNA damage and chromatin condensation. Sperm DNA damage was evaluated by the TdT-mediated dUTP nick-end labelling assay (TUNEL) to evaluate the percentage of cells with DNA fragmentation in the total sample. The TUNEL reaction detects single and double DNA-strand breaks. Defects in chromatin condensation that increase the accessibility of the DNA to fluorochromes were detected by using chromomycin A_3 (CMA_3). The more

positive cells for CMA_3 the more poorly condensed sperm DNA was present (i.e., abnormal or immature cells). The results in this study are given as the percentage (and range) of normal condensed sperm (CMA_3 negative cells) in the samples. At least 200 cells were evaluated per sample for the TUNEL and CMA_3 staining. Our methods have been described in detail before. [22,23]

Assessment of testicular function. Testicular function was evaluated by measuring luteinizing hormone (LH), follicular stimulating hormone (FSH) and testosterone in serum. FSH and LH were determined with the fluorescence Immuno Enzymatic Assay (Abbott, USA) using the Random Access Analyser type AxSYM. Testosterone was measured after extraction using the direct Radioimmuno assay.[24]

Statistics. The paired t test was used if the differences in a paired set of data were normally distributed, if not normally distributed the Wilcoxon signed rank test was used. Correlations between continuous variables were estimated using Pearson product-moment correlation coefficients.

Results

Patients. From a total of 51 patients with stored sperm and serum obtained before the start of chemotherapy, 22 agreed to participate in the study. The relevant patient characteristics are summarized in Table I.

Table I Patient characteristics

Number of patients	22
Median age (range in years)	31.2 (22.2-41.6)
Median time between end of treatment and 2nd semen preservation (range in months)	48.2 (18.4-84.8)
Histology	
-non-seminoma	19
-seminoma	3

Routine semen analyses. The effect of chemotherapy on routine sperm parameters is shown in Table II.

Table II Routine semen analyses before and after chemotherapy

	Median before chemotherapy (range)	Median after chemotherapy (range)	p-value*
Volume (ml)	3.1 (0.8-7.2)	2.1 (0.5-7.0)	0.02
Sperm concentration (10^6/ml.)	24.5 (0.5-160.0)	30.0 (0-200.0)	0.33
Total sperm count (10^6)	57 (0.9-425.0)	65 (0.0-300.0)	0.94
% motile sperm	50.0 (10.0-75.0)	55.0 (7.0-80.0)	0.33
% normal morphology	41 (9.0-64.0)	16 (2.0-37.0)	**
% of patients with oligo-and azoospermia	50% / 0%	32% / 9%	**
% of patients with teratospermia	22%	47%	**

* All calculated by the Wilcoxon signed rank test
** Criteria for evaluation differed in time: 1992 and 1999 WHO criteria in samples before and after chemotherapy respectively

The only statistical significant difference found was a lower ejaculate volume after chemotherapy. Because of the different criteria used in the evaluation of morphology (shift to a more strict evaluation criteria for the post chemotherapy samples), no statistical evaluation for the conventional morphology was performed. The decrease in the percentage cells with normal morphology after chemotherapy is probably not only due to a poorer spermatogenesis but also to technical changes. Using the cut-off values for teratospermia considered in our centre at the time of evaluation, 22% and 47% of the samples presented teratospermia before and after completion chemotherapy respectively. Two patients became azoospermic after chemotherapy treatment (9%).

Sperm DNA analyses. In Table III, the results obtained by CKIA, CMA_3 and TUNEL are shown.

Table III. Semen damage before and after chemotherapy

	Median before chemotherapy (range in %)	Median after chemotherapy (range in %)	p-value*	Control Group (N=13)
CKIA				
normal morphometry	30.9 (17.0-53.0)	28.6 (18.0-56.0)	*0.78*	53.9 (41.1-66.0)
normal DNA condensation	47.5 (28.0-85.0)	63.7 (24.0-94.0)	*0.005*	81.3 (73.5-89.9)
normal chromatin texture	39.0 (19.0-88.0)	50.6 (15.0-89.0)	*0.23*	69.4 (53.1-88.6)
total normal cells	7.8 (0.8-81.2)	10.4 (0.0-60.0)	*0.40*	40.5 (29.6-53.1)
CMA_3				
defect chromatin condensation	63.0 (40.0-88.0)	50.0 (26.0-90.0)	*0.001*	30.2 (9.0-45.0)
TUNEL				
Damaged cells	21.0 (8.0-66.0)	25.0 (10.0-47.0)	*0.48*	9.7 (4.0-14.0)

CKIA: Computerized karyometric image analysis; CMA3: chromomycin A_3; TUNEL: TdT-mediated dUTP nick-end labeling assay.
*All calculated by the Wilcoxon signed rank test

The percentage of cells with normal morphometry by CKIA was equally distributed before and after treatment. Sperm DNA condensation improved significantly after chemotherapy according to CKIA and CMA_3 assays (the percentage of CMA_3 positive cells, i.e. abnormal condensed cells, was lower after chemotherapy). The number of DNA damaged cells (TUNEL positive) did not statistically change after chemotherapy treatment. Nevertheless, both percentages (chromatin condensation and DNA damaged cells) were significantly different (abnormal) for patients compared with controls.

Hormone analyses. Elevated serum FSH and LH was measured after chemotherapy compared with pre-treatment levels (Table IV) without a significant change in the testosterone level.

There was a positive correlation between LH and FSH level both before ($r = 0.99$; $P < 0.01$) and after treatment ($r = 0.87$; $P < 0.01$). No correlation between testosterone and LH or FSH was found.

Table IV. Hormone analyses before and after chemotherapy

	Median before chemotherapy (range)	Median after chemotherapy (range)	p-value
FSH (IU/L)[1]	8.1 (0.2-49.3)	16.7 (3.5-62.1)	0.003*
LH (IU/L)[2]	4.5 (0.2-27.0)	6.8 (1.3-32.5)	0.04*
Testosterone (nmol/L)[3]	18.7 (1.7-44.0)	15.8 (6.7-26.0)	0.15

Reference range in our laboratory: 1) 1.5 – 7.5 IU/L; 2) 1.4 – 8.5 IU/L; 3) 11 – 45 nmol/l.
*Calculated by the Wilcoxon signed rank test

Discussion

In this study, the effect of cisplatin-based chemotherapy (<400 mg/m2) was evaluated by using conventional semen parameters and DNA-integrity related tests in sperm samples of testicular cancer patients. It was found that treatment with cisplatin has no effect on sperm count and morphometric characteristics of spermatozoa. On the other hand, an improvement in sperm DNA condensation with elevated rates of DNA damage was observed after treatment, which was still abnormal compared to fertile semen samples.

Recent literature is in agreement with our findings. After completion of chemotherapy, (partial) recovery of spermatogenesis occurs within two years and may continue thereafter. [2,5-7,9-11,16] In our series, sperm concentration and total sperm count were not significantly affected after chemotherapy and approximately 50% of the patients still had at least $20*10^6$ sperm cells/ml, with only 2 (9%) patients presenting azoospermia. These observations are all made after using mild chemotherapy (<400 mg/m2). In patients receiving more than 400 mg/m2 total dose cisplatin a significant decrease in spermatogenesis has been described.[13]

Not only chemotherapy causes impairment of the spermatogenesis. In the literature has been described that 50-70% of the testicular cancer patients were subfertile or had impaired spermatogenesis before start of chemotherapy.[8,9] This impaired spermatogenesis was neither related to stage of disease nor with the duration and severity of symptoms attributed to testicular cancer.[4,9,10] This study confirms that a minority of patients who have been hemi-orchiectomized for testicular cancer are normospermic at the time of diagnosis. Berthelsen and Skakkebaek showed that in 24% of their cases there were irreversible changes such as spermatogenic arrest (azoospermia) and in over half of the remaining patients there was a potentially reversible depression of spermatogenesis.[25]

There are two major limitations of the routine semen analysis. In the first place there is a lack of standardisation and there is a significant observer bias.[26-28] In the second place, the standard morphology analysis does not describe the integrity of the sperm DNA. To bypass the observer bias in the morphology analysis, we evaluated sperm morphology with computer qualitative measurements: CKIA. CKIA also gives information about sperm DNA condensation and chromatin texture. With this technique we found that although the external features in the morphometric parameters were equally distributed before and after chemotherapy, there were changes in internal characteristics (DNA stainability and condensation). Abnormal sperm DNA condensation is known to be adversely correlated to male fertility potential.[21,29] In spite of the significant increase of cells with normal DNA condensation after chemotherapy, chromatin condensation as indicated by low CMA_3 values (<60% negative) and CKIA is still poor if compared with normal donors. With respect to DNA damage, no adversely effect was measured as a result of chemotherapy. For this result also counts that the patient group scored significantly higher than the fertile donors. These observations support the study by O'Donovan who used propidium iodide for DNA condensation measurements.[30] In our series none of the sperm samples had normal CMA_3

values before start of chemotherapy, in contrast to 8 of 22 patients after chemotherapy. Available literature does not offer an obvious explanation for this observation. Based on animal studies, we hypothesize that spermatogonia with abnormal chromatin, as a consequence of the disease, might be more susceptible to chemotherapy, thus being eliminated by treatment.[31] The remaining normal and more viable germ cells (spermatogonia) are responsible for the partial restoration of spermatogenesis after a recovery time. More research is necessary on this point, not in the last place to enable effective treatment: abnormalities in chromatin condensation may contribute to failures in sperm decondensation after penetration into the oocyte and subsequently result in fertilization failure.[21] In the literature controversial results has been published on sperm DNA damage in testicular cancer patients. Stahl et al reported a significantly lower DNA damage among 16 patients compared with controls (healthy males) after completion of chemotherapy (7.3% vs. 11%; P = 0.028) by the use of the sperm chromatin structure assay.[32] Gandini *et al.* reported a significant increase of DNA fragmentation in 30 testicular cancer patient postorchiectomy compared to healthy controls (11% vs 2.5%).[33] Despite the fact that chromatin is better packed after treatment, no decrease in the percentage of sperm DNA damaged cells was observed and remained high compared with healthy controls. This is in accordance with the results obtained by O'Donovan with use of the Comet assay.[30] Our results led us to postulate that there might be an intratesticular alteration in the apoptotic control system as reaction to neoplastic cell proliferation or that the chemotherapeutic treatment might affect removal of abnormal sperm by apoptosis. Consequently, damaged cells that should have been eliminated during spermatogenesis can be found in the ejaculates (abortive apoptosis).[20,34]

Normal spermatogenesis also depends on normal endocrinologic balance. Testosterone, regulated by LH, is an absolute requirement for normal spermatogenesis. Our study demonstrated that standard doses of chemotherapy do not lead to a significant decrease of the

testosterone level. However, the LH values were significantly elevated, indicating a compensatory reaction to decreased Leydig cell function and resulting in the mentioned constant testosterone level. [3-7] In agreement with others we found biochemical evidence of germinal epithelial failure of the contra-lateral testis, indicated by increased FSH levels after treatment.[3-7] The observed correlation between LH and FSH is not surprising given the relative susceptibility of Leydig cells and germinal epithelium to damage and suggests interaction between each other.[3]

There is no doubt that testicular cancer and its treatment have serious impact on gonadal function in these young patients.[35] Fertility is therefore a major concern, and health care providers are increasingly aware of the need to improve the quality of life of cancer patients by maintaining their reproductive capacity. In the past, recovery of high sperm counts were of main concern for effective treatment and this reduced the fatherhood chances of many patients.[6,11] With the advent of assisted reproduction by ICSI it is now possible to offer a good chance of conception in all men with a low sperm count. Now a second problem arises: theoretically there is a possible risk of genetically transferred disease in the offspring as a result of the selection of spermatozoa with increased DNA damage.[36] In animal models there is already evidence for this risk.[37] In humans, an increase in the number of autosomal and sex chromosome aneuploidy was reported in sperm samples after chemotherapy, but genetic consequences to offspring are not yet known.[38,39] In any case we have to conclude that the genetic effects of cancer and cancer treatment on sperm needs to be kept in perspective, meaning that we have to be careful in treatment and follow up of their offspring.

In conclusion, sperm count and morphometry (CKIA) was not affected in the majority of patients treated with chemotherapy for testicular cancer. Although an improvement in the chromatin condensation was found in sperm, the percentage of DNA damaged cells did not decrease after chemotherapy. Sperm DNA integrity in general remained poor compared to

healthy controls. It should be elucidated whether the observed changes in sperm integrity represents a real post-chemotherapy removal of an abnormal germ cell subpopulation or whether the post-chemotherapy matured sperm presents other types of genomic damage not detectable by the current methods. Although our results call for further investigations, it seems prudent to evaluate semen from cancer patients not only by routine analysis as external characteristics of spermatozoa do not necessarily correlate with their DNA integrity.

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GENERAL DISCUSSION

The management of germ cell tumours of the testis (TGCT) has proved to be a model of success in terms of survival. The improved prospects of cure are attributed to the appropriate integration of a better understanding of the natural history of germ cell tumours, improved diagnostic and surgical techniques and last but not least effective platinum-based combination chemotherapy. Because the majority of patients are cured by first-line treatment, attention has turned to reduction of morbidity by altering therapeutic protocols in selected subsets of patients. This thesis focuses on refinements in the management of germ cell tumours in the platinum era.

The **first part** of this thesis focuses on risk factors in the management of TGCT. Testicular cancer is most commonly diagnosed between the ages of 20 and 44 years and it is among this subgroup that the age-adjusted incidence has risen markedly in many Western populations over the past half-century.[1] Lately, much research has focused on familial and hereditary factors in search for risk factors for developing testicular cancer. **Chapter 1** presents the incidence of malignant tumours in first-degree relatives of patients with testicular cancer in the eastern part of the Netherlands. Brothers of testicular cancer patients seem to have a 5.9-fold increased risk for developing testicular cancer. In contrast, first-degree relatives of testicular cancer patients do not seem to be prone to non-testicular cancer. We recommend further investigations in testicular cancer families to map candidate genes for testicular cancer.

In contrast to the rise in incidence improved management has led to a markedly decline in age adjusted mortality rates. To date, the overall 5 year relative survival rate is in excess of 90%.[2] In **chapter 2,** we used the follow-up data from Surveillance, Epidemiology, and End Results cancer registries in the United States to examine whether testicular cancer patients older than 50 years of age have a worse disease-specific survival than patients younger than 50 years of age, as observed in The Netherlands. In the United States, a lower stage- and morphology-

adjusted relative survival rate was observed in patients older than 50 years of age with testicular cancer. This difference was more evident in metastasized disease. Whether the worse prognosis in testicular cancer can be explained by a lower tolerance to chemotherapy and/or to a reduced treatment in elderly has to be established. This study suggests that elderly patients might be undertreated one way or another, and therefore it justifies inclusion of elderly into future clinical trials and treatment in tertiary centres.

The **second part** of this thesis provides three studies regarding a rational approach in the management of clinical stage I non-seminomatous germ cell cancer (NSGCT). To date, patients with clinical stage I nonseminomatous germ cell cancer can expect survival to approach 100%.[3] The achievement of these excellent results has resulted into research for refinements in treatment without compromising the cure rates. In **chapter 3** an update is presented of the current available treatment alternatives for low stage nonseminomatous germ cell tumours: surveillance, primary surgery, or primary chemotherapy.

Today, there is an ongoing and increasing controversy regarding the management of these patients. Interestingly, the dichotomy of views toward the surgical versus the conservative approach has worldwide geographic trends. In the United States there is a strong urological surgical tradition, whereas in Europe the conservative approach is generally favoured. Unfortunately, this controversy about the optimal choice of management has been exacerbated by a lack of comparative data, mainly caused by geographic different choices in treatment. In the Netherlands, the University Medical Centre (UMC) in Nijmegen is the only hospital that performs primary retroperitoneal lymph node dissections in low stage NSGCT.

In **chapter 4**, we compared the results of the two currently available treatment options in the Netherlands: primary surgery at the Department of Urology at the University Medical Centre Nijmegen or surveillance at the Departments of Urology and Oncology of the Netherlands

Cancer Institute/Antoni van Leeuwenhoek Hospital Amsterdam. Although this study was performed retrospectively, we believe that both groups were well balanced not only for clinical and pathological parameters but also for the period of follow-up. Furthermore, the strength of this study was that the choice of treatment is independent on health insurance policy and the travel distance to the hospital (important for compliance, especially in surveillance protocol). Both options provided excellent overall survival close to 100%. Given these comparable outcomes, selection of the most appropriate treatment regimen could not be guided by survival considerations. The choice of treatment seems to depend on regional factors related to the local expertise and treatment availability and the patient-related factors.

In fact, the current controversy is driven by the performance characteristics of the presently available staging methods. About 20-30% of the patients with clinical stage I NSGCT are understaged by clinical staging and appear to have pathological stage II disease at lymph node dissection. The choice of treatment would be facilitated if the likelihood of presence of occult disease could be increased. In **chapter 5**, we evaluated prognostic risk factors in 50 consecutive clinical stage I NSGCT patients to identify patients at high and low risk for occult metastatic disease. The prognostic value of histological factors, such as vascular invasion, tunica albuginea invasion, presence of embryonal cell carcinoma, alpha-catenin expression and the tumour DNA-content were evaluated. In 14 patients (28%) occult metastatic disease in the lymph node specimen was detected, and one patient developed a lung metastasis within 3 months after diagnosis. In multivariable logistic regression analysis only vascular invasion and tunica albuginea invasion were predictive of occult disease. The absence of embryonal cell carcinoma (n=11), diploid (n=3) and polyploid (n=2) tumours correlated with pathological stage I disease. However, the numbers are small. Meticulous analysis of prognostic indicators, might individualize the management of stage I NSGCT. Nevertheless, for the group of low stage patients it is important to have independent, reliable and

reproducible prognostic markers. In the literature, vascular invasion is the best reproducible marker and is currently being used to stratify patients into low and high risk for occult disease.[4-6] The presence of vascular invasion in the primary tumour increases the risk for occult disease to 50%, whereas its absence implies a risk of approximately 10%. To date, we (UMC Nijmegen, the Netherlands) advise high-risk patients to undergo a retroperitoneal lymph node dissection, and surveillance to low-risk patients.

The **third part** of this thesis discusses the potential role of 18-Fluoro-Deoxy-Glucose Positron Emission Tomography (^{18}FDG-PET) in different staging aspects of testicular germ cell tumours. To date, the diagnostic work-up of patients with testicular tumours remains a difficult clinical challenge. Not only in primary staging of low stage non-seminomatous disease (see part II), but also in re-staging after chemotherapy for advanced testicular cancer, it is of great interest to identify patients who require surgery and those who do not in order to optimise individual treatment. After the completion of chemotherapy, residual viable tumour lesions are found in up to 15% of patients with seminomas,[7] to 20% of patients with NSGCT.[8] Furthermore, in 40% of the residual non-seminomatous masses, also mature teratoma is found.[8] Because radiologic criteria derived from the conventional imaging methods have failed to differentiate reliably between the histologic entities, definitive differentiation is possible only with histological examination of the resected specimen. Improvement of non-invasive methods would allow avoidance of unnecessary surgery.

Positron Emission Tomography imaging using ^{18}Fluoro-Deoxy-Glucose (^{18}FDG-PET) is a new diagnostic technique that has the potential to distinguish viable cancerous tissue from non-viable (fibrosed or necrotic) tissue. In general terms, tumour cells are characterized by a higher glucolytic rate than normal tissue cells. PET exploits this difference by assessing the rate of ^{18}Fluoro-Deoxy-Glucose uptake in the tumour. Because the metabolic activity alters in

cancer well before structural changes occur, ^{18}FDG-PET has proven to be useful both in detecting small nodules in non-small-cell lung cancer,[9] and monitoring the changes in treatment response before any changes become apparent with anatomy-based imaging modalities.[10]

In **chapter 6**, we evaluated the value of ^{18}FDG-PET in both primary staging of low stage NSGCT tumours and in re-staging of advanced germ cell tumours after chemotherapy. In primary staging of low stage non-seminomatous germ cell cancer ^{18}FDG-PET has no additional value over abdominal computed tomography, because small pathologic nodes and mature teratomas of any size are not detected. In re-staging after chemotherapy, PET results are hampered by false-negative and false-positive findings; a negative PET may represent either necrosis, mature teratoma or very small (<5 mm) viable carcinoma, whereas a positive PET represents viable carcinoma or inflammatory processes.

The main resource of false-negative findings in the residual mass was the presence of mature teratoma, probably caused by equal metabolism of the mature teratoma compared to normal tissue. In our series, the diagnostic efficacy of ^{18}FDG-PET could be improved when patients with no mature teratoma in the primary tumour were excluded from analysis. Unfortunately, this finding could not be confirmed in a larger series.[11] Because seminomatous germ cell tumours do not contain teratomatous elements, a negative PET-scan might have additional value in staging the residual mass after chemotherapy.[12,13] Secondly, a limitation of ^{18}FDG-PET is its inability to detect very small tumorous lesions in primary staging and in re-staging after chemotherapy.

Because ^{18}FDG is not a tumour specific agent, also false-positive findings might occur.[14] It is known that macrophages, present in inflammatory processes, also accumulate ^{18}FDG. To reduce this phenomenon, the interval between completion of treatment and PET examination was required to be at least three weeks.[15] However, in our series it even occurs after 6 weeks.

In conclusion, larger series have to elucidate the definitive clinical value of ^{18}FDG-PET in re-staging of residual seminomatous germ cell cancer. The first results are conflicting regarding a positive PET-scan, in contrast to a negative scan result which is suggestive for fibrotic residual mass.[12,13] There seems to be no benefit of ^{18}FDG-PET in any staging of non-seminomatous germ cell cancers.

Another utility of PET is monitoring response to treatment. Assessing the tumour response after the start of treatment might be a useful parameter to distinguish responders from non-responders: successful therapy can be maintained and ineffective treatment can be suspended and other options considered. To date, ^{18}FDG-PET has been applied to monitor response in other tumours such as lung, brain, breast and colorectal tumours, but has not yet been used in daily practice.[10] In **chapter 7**, we evaluated the value of serial ^{18}FDG-PET in monitoring the response of advanced testicular germ cell tumours to chemotherapy. This pilot study shows that ^{18}FDG-PET has a limited, if any role, in the therapeutic monitoring of low risk testicular cancer and does not provide significant incremental information to structural imaging and measurement of serum tumour markers.

The **fourth part** of this thesis embraces the different aspects of follow-up in management of patients with testicular cancer. For patients with testicular germ cell cancer, long-term, close surveillance has traditionally been the "*sine qua non"* of post-treatment care. In the past, these patients have usually been treated within the context of a clinical trial in which close follow-up protocols were used to evaluate the results. With the introduction of cisplatin-based combination chemotherapy regimens, dramatic improvements in survival have been seen and established the "Bleomycin, Etoposide and cisPlatin" regimen as the new standard.[16] Although a lot of effort has been taken to optimise the chemotherapy regimen for the different

risk-group of patients,[17-19] little attention has been paid to the role of follow-up in the management of testicular cancer.

In **chapter 8,** we evaluated the value of routine follow-up for the detection of recurrent disease after curative treatment of testicular cancer. This study clearly shows that the risk for recurrent testicular cancer depends on primary therapy and the response to it, and indicates a response-to-treatment guided approach of the follow-up protocol. Abdominal and thoracic computed tomography provided no additional value in patients, treated at the retroperitoneum for low-stage disease and followed by chest X-ray. In this group of patients, two years of routine follow-up seems to be sufficient. Furthermore, there seems to be a limited role for follow-up in patients, adjuvantly treated with chemotherapy for pathological stage IIa nonseminmatous disease, and in patients who are histologically proven free of disease after chemotherapy. Although these data advocate a less intensified follow-up protocol, we believe prospective randomised trials or mathematical decision models are needed to justify response-to-treatment guided follow-up schedules in testicular cancer.

In **chapter 9**, we evaluated the efficacy of different follow-up regimens on disease-specific mortality (DSM) and life-expectancy (LE) of patients on surveillance for clinical stage I nonseminomatous disease. The optimal design to study this issue would be a randomised trial. However, such a trial would require both physicians and patients to be compliant during a long period of follow-up while less intensive policies may be considered unethical without clear evidence that these are equally effective. Alternatively, mathematical decision models can mimic randomised trials without being hampered by its' practical disadvantages. We used a Markov model to compare the efficacy of the surveillance protocol of the European Association of Urology (EAU), the Netherlands Cancer Institute/Antoni van Leeuwenhoek hospital (NCI/AvL) and a hypothetical minimal protocol (*i.e.* follow-up limited to the first two years) with each other: comparing with the EAU protocol (DSM: 3.05%; LE: 53.3 years),

the more intensive NCI/AvL protocol leads to a 1.2% lower DSM and a 6 months higher LE (DSM: 1.81%; LE: 53.9 years). The hypothetical follow-up scenario during the first two years shows a DSM of 6.83% and a LE of 51.4 years. In conclusion, the logistical and economical benefits of a less intensive follow-up protocol as proposed by the EAU compared to that of the NCI/AvL must be balanced against the increase in disease specific mortality rate from 2 to 3%. In view of the high survival rate in clinical stage I NSGCT, this difference is enormous. Follow-up limited to the first two years only will result in an unacceptably high percentage of death from disease.

Until prospective randomized trials have been performed to evaluate the efficacy of different protocols, our model may help physicians to understand the anticipated effects of different follow-up protocols on long-term survival of patients on surveillance for clinical stage I nonseminomatous disease.

The **last part** of this thesis deals with fertility aspects in the management of germ cell tumour of the testis. Because of the long-term survival of these young man, limiting disease- and treatment-related complications such as infertility becomes increasingly important. Prior to the discussion of the relation between testicular cancer and infertility, it is salient that most studies have used semen analysis and not necessarily the definition of infertility as the gold standard. Infertility is defined by the American Society of Andrology as the inability to conceive after one year of unprotected intercourse. In the general population, the prevalence of infertility varies between 20- and 28%.[20] In **chapter 10,** we estimated the prevalence of infertility before and after treatment for testicular cancer. Infertility was reported by 34% of the patients before disease, whereas by 57% after treatment. Although it seems to be more difficult to father a child after treatment, none of the treatment modalities in particular influenced this outcome. Because it is not (yet) possible to predict which patient will have

fertility problems after treatment, cryopreservation should be offered to every testicular cancer patient before the start of treatment. In our series, 18% of the couples used cryopreserved semen to deliver a child after treatment. In accordance with other published series, no increased risk for congenital malformations was observed among the children before and/or after treatment for testicular cancer of their father.

In the **last chapter** we evaluated the influence of cisplatin-based chemotherapy on the quality of ejaculated spermatozoa. Serum hormone levels, routine semen sample including qualitative measurements by computer analysis (CKIA) and sperm DNA integrity (sperm DNA damage and condensation) were compared before and after exposure to chemotherapy.

Increased levels of LH are found after chemotherapy to maintain normal testosterone levels and the increased FSH suggests evidence of germinal epithelial failure of the contralateral testis.

The sperm count and morphometry (CKIA) was not affected in the majority of patients treated with chemotherapy for testicular cancer. Although an improvement in the chromatin condensation was found in sperm, the percentage of DNA damaged cells did not change after chemotherapy. Overall, the Sperm DNA integrity remained poor compared to healthy controls. It should be elucidated whether the observed changes in sperm DNA represents a real post-chemotherapy removal of an abnormal germ cell subpopulation or whether the post-chemotherapy matured sperm presents other types of genomic damage not detectable by the current methods. Although our results call for further investigations it seems prudent to evaluate semen not only by routine analysis in case of ICSI as external characteristics of spermatozoa do not necessarily hold the same internal structures post-chemotherapy.

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Treatment results of testis cancer have improved dramatically over the last 25 years, with overall cure rates for individuals with low stage disease approaching 100% and more than 80% for patients with advanced disease. The present cure rates illustrate that significant diagnostic, chemotherapeutic and surgical advances have transformed testicular cancer from a once fatal disease into an oncological success story. To date, the challenge is to pursue therapeutic methods that further increase the overall cure rates while decreasing the disadvantages associated with treatment.

The current controversy in the treatment of testicular germ cell tumours is mainly driven by the performance characteristics of presently available clinical staging methods. Clinical staging is hampered by both the inability to detect micrometastatic disease and the inaccuracy to distinct residual malignant from non-malignant disease.

Because 70% of patients with a clinical stage I non-seminomatous germ cell cancer have no metastatic disease, a central issue relates to the stratification into true stage I disease, which should be cured with orchiectomy alone versus true stage II disease requiring adjuvant treatment. In low stage non-seminomatous germ cell cancer, advances in technology are focused on lowering the false-negative rate of clinical staging. Because the sensitivity of conventional imaging studies is inversely proportional to tumour volume, other non-invasive staging methods have been developed to detect micrometastatic disease. The principle of ^{18}Fluor-Deoxy-Gluose Positron Emission Tomography has the potential to detect altered metabolic activity in cancer before structural changes occur. In low stage germ cell cancer, this technique did not have additional value over CT in the early detection of micrometastatic disease. To date, other ^{18}F-labelled tracers have been developed, but have not yet been tested in clinical staging.[1]

Another non-invasive staging method, that has been developed to detect micrometastatic disease but has not yet been implemented into testicular cancer research, is the combination of

magnetic resonance imaging with the intravenous injection of ultra-small super paramagnetic iron oxide particles. This technique has been shown to detect small metastatic lymph nodes in prostate- and urinary bladder cancer[2] and should, therefore, be tested in the detection of lymphogenic micrometastases in germ cell tumours.

To my opinion, the detection of even smaller lesions is an unending challenge, as detection of microscopic disease is probably not possible by any clinical imaging method. To date, retroperitoneal lymph node dissection still the most accurate staging method. In an effort to decrease the morbidity associated with open lymph node dissection, several centres have applied laparoscopic surgical techniques to assess the presence of micrometastatic disease.[3-6] Moreover, to locate the sentinel node more accurately during laparoscopy, lymphoscintigraphy using intratesticular radiocolloid administration might be feasible.[7,8] Although laparoscopy might replace open surgery as a staging tool, it has not yet proven its therapeutic capability. In all studies [3-6] chemotherapy had been administered at detection of stage II disease. Since deferred treatment of patients with pathologic stage II disease is usually successful, as employed in surveillance series, what would be the benefit of laparoscopy? In other words, if the intent is to treat retroperitoneal-only disease with chemotherapy, why not enter the patient into surveillance protocol and treat him with chemotherapy not before clinical relapse? The future will reveal the definitive place of the laparoscopy in management of low stage non-seminomatous disease.

Despite research in clinical staging, in 30% of the cases microscopic disease is still missed. Several studies have evaluated predictive variables in the primary tumour to predict the presence or absence of metastatic disease. The presence of embryonal cell carcinoma and vascular invasion in the primary tumour are established risk factors for micrometastatic disease. However, many of these studies have limitations, in that the populations studied were selected and the parameters used to predict metastasis may suffer from interobserver

variability. To define a paradigm that is more useful in the prediction of pathologic stage, prospective studies comparing consecutive, unselected patients managed by retoperitoneal lymphadenectomy versus surveillance is needed. Furthermore, it is unclear whether or not patients with such risk factors necessarily have poor outcome if they are managed aggressively at diagnosis (surgery or primary chemotherapy) as compared with their outcome if they are managed by surveillance.

As long as we are not able to identify patients with or without (increased risk of) microscopic metastasis, controversy in the management of low stage disease will persist. Consequently, the ultimate method to evaluate the best choice of treatment at the moment would be a randomised trial comparing surveillance, lymphadenectomy and primary chemotherapy by using proper long-term survival analysis as well as cost, toxicity, fertility, work-performance, and quality of life parameters.

The diagnostic work-up of patients with residual mass after chemotherapy remains another clinical challenge. Because it is not possible to discriminate between malignant and non-malignant residual disease on radiologic imaging, there is no alternative but to submit all these patients for surgery. It would be of utmost benefit, if we could distinguish responders from non-responders after chemotherapy. In this thesis, ^{18}FDG-PET has been applied to monitor response during and after chemotherapy for disseminated germ cell tumours. Although PET has the ability to monitor treatment response in low risk non-seminomatous germ cell tumours, it does not provide significant additional value to structural imaging. Further studies targeting patients with intermediate to high-risk profiles might have additional value, as the a priori likelihood of response is less favourable. Although the future applications of PET in cancer management seem to be promising, the additional value of ^{18}FDG-PET in testicular cancer is limited. Because FDG is not a tumour specific and sensitive

marker in testicular cancer, results were hampered by false-positive observations (inflammatory processes) and false-negative observations (mature teratoma, and micrometasis). Hopefully, new ^{18}F-ligands will improve the accuracy of PET in the management of testicular germ cell tumours.[1]

In general, follow-up after treatment for cancer is assumed to be beneficial. With the improved treatment possibilities in patients with testicular cancer, it is time to focus on the real value of follow-up in the management of these patients. In this thesis we have identified a subgroup of patients who might benefit from less extensive follow-up schemes. Furthermore, we have developed a computer model to evaluate the impact of follow-up on the overall survival rate of patients on surveillance for low stage non-seminomatous disease. This thesis provides data to evaluate the value of follow-up in low stage disease and in patients with complete response after chemotherapy in a prospective randomised trial. In addition, it is worth looking at psychological stress, as it is assumed to be related to follow-up, but this has never really been assessed prospectively.

There is no doubt that testicular germ cell cancer and the treatment of this disease both have serious impact on the gonadal function in these young patients. Further research is needed to elucidate the best objective parameter for semen quality. Our present knowledge should force us to counsel these men about fertility-aspects and the possibilities for cryopreservation.
With the increased possibilities of assisted reproduction, a multi-disciplinary approach is recommended for the testis cancer patient with fertility problems instead of treatment for cancer at the urology department and treatment for fertility at the departments of fertility and gynaecology.

Furthermore, the issue of a theoretical risk of inborn errors in children fathered by these patients after treatment has not yet been clarified. Clinical investigations do not indicate such a risk, but the present studies have been small and careful registration of sufficient numbers of cases and sufficient follow-up of children is needed to determine the real risk of inborn errors.

Finally, in the more distant future improved understanding of the biology and genetics of germ cell tumours will lead to new therapeutic targets and approaches. By discovering the genetic alternations involved in testicular cancer development, gene therapy and new molecular markers may become a reality. Hopefully, genetic-based therapy may lead to less toxicity while maintaining the same efficacy.

In the last three decades great strides have been made in treating germ cell tumours, but it is likely that future advances will occur in smaller steps that aim to fine tune the management of germ cell tumours of the testis. Despite the fact that a small percentage of the male population will develop germ cell cancer of the testis and the majority will survive today, we should continue research to further optimize the management of testicular cancer. In this way testis cancer could be a therapeutic milestone in treatment of other cancers. We encourage financial support to future trials.

The seeds of death have become the seeds of concern and will return the seeds of ……live?

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Deze samenvatting geeft een overzicht van de inhoud van dit proefschrift zodanig dat niet-medici een overzichtelijk en begrijpelijk beeld krijgen van zaadbalkanker.

Zaadbalkanker neemt slechts 1% van alle maligne aandoeningen bij de man voor haar rekening. Jaarlijks wordt in ons land bij ongeveer 500 mannen zaadbalkanker vastgesteld. In de leeftijdscategorie van 20 tot 35 jaar komt zaadbalkanker het meest voor.

De meeste mannen bij wie zaadbalkanker wordt vastgesteld, hebben zelf een verandering aan een zaadbal geconstateerd. Deze verandering bestaat uit een vergroting van de zaadbal en/of een verharding in de zaadbal, waardoor deze anders aanvoelt. Bij verdenking op zaadbalkanker dient zowel echografie van de zaadbal als bloedanalyse naar tumormakers (bèta-humaan choriogonadotrofine; alfa-foetoproteïne en lactaatdehydrogenase) te worden verricht. Als de uitkomsten van de echografie en het bloedonderzoek op zaadbalkanker wijzen, dient de zaadbal via het lieskanaal te worden verwijderd.

Het wegnemen van de zaadbal is het begin van de behandeling. Nader onderzoek wijst uit om wat voor type zaadbalkanker het gaat: een seminoom, een nonseminoom of een combinatie van beide. Daarnaast wordt de uitgebreidheid van de ziekte, met behulp van een CT-scan, vastgelegd in het zogenaamde stadium:

Stadium I: De ziekte zit alleen in de zaadbal.
Stadium II: De ziekte zit in de zaadbal en in de lymfklieren onder het middenrif.
Stadium III: De ziekte zit in de zaadbal en in de lymfklieren onder en boven het middenrif.
Stadium IV: De ziekte zit in de zaadbal en heeft zich uitgezaaid naar andere organen.
Grootte van de uitzaaiing: a: <2cm; b: 2 – 5cm; c > 5cm.

De combinatie van het type en het stadium van de kanker bepaalt de soort behandeling. Bij een seminoom in de stadia I en IIa wordt standaard radiotherapie gegeven. In de stadia groter dan IIa wordt standaard chemotherapie gegeven. Chemotherapie kan in sommige gevallen worden gevolgd door een verwijdering van het restant aan tumorweefsel. Bij een nonseminoom in stadium I bestaat de behandeling vaak uit 'wait and see' of preventieve verwijdering van de lymfklieren onder het middenrif. In geval van 'wait and see' wordt de patiënt geruime tijd zeer regelmatig gecontroleerd. Bij een nonseminoom in de stadia II, III en IV wordt vrijwel altijd chemotherapie gegeven en wanneer er dan nog restweefsel resteert, wordt dit operatief verwijderd.

De geneeskans bij zaadbalkanker hangt af van het type zaadbalkanker en van het stadium waarin de ziekte wordt ontdekt. De kans dat een patiënt met zaadbalkanker geneest, is tegenwoordig groot. Negentig procent van alle patiënten kan worden genezen en heeft vervolgens een normale levensverwachting. Na afloop van de behandeling wordt jarenlang gecontroleerd of de ziekte niet terugkeert.

Door de vaak jonge leeftijd van de patiënt en de goede prognose na behandeling heeft de kwaliteit van leven een steeds belangrijker plaats ingenomen in het management van deze ziekte. Met name de fertiliteit is een belangrijk aspect bij deze jonge mannen. Bij de behandeling met zowel radiotherapie, chemotherapie als operatieve verwijdering van tumorweefsel kan verminderde vruchtbaarheid optreden. Soms is dat blijvend. Patiënten die nog kinderen willen krijgen, kunnen het beste vóór het begin van de behandeling sperma laten invriezen.

Sinds de intrede van platinum-bevattende chemotherapie in de tachtiger jaren is zaadbalkanker, wat daarvoor nog voor de meeste patiënten het doodvonnis betekende, één van

de best te behandelen kankersoorten geworden. Door verbeterde overleving is de nadruk in de behandeling van zaadbalkanker meer komen te liggen op vermindering van de bijwerkingen zonder de goede prognose aan te tasten. Dit proefschrift beschrijft de zoektocht naar verfijningen in het management van zaadbalkanker in het chemotherapie tijdperk.

Het **eerste gedeelte van dit proefschrift** beschrijft een tweetal epidemiologische factoren in het management van zaadbalkanker. De incidentie van zaadbalkanker neemt toe in West-Europa. In Nederland steeg de incidentie met 40 procent in de periode 1990-2000. Tot op heden is er geen duidelijke verklaring voor deze stijging gevonden. Wel is bekend dat er een verhoogde kans op zaadbalkanker aanwezig is bij mannen van wie de testikels niet op natuurlijke wijze in de balzak zijn ingedaald. Mogelijk is er ook sprake van erfelijke aanleg. Het **eerste hoofdstuk** beschrijft de incidentie van kwaadaardige aandoeningen onder eerstegraads familieleden van patiënten met zaadbalkanker in Oost-Nederland. Broers van patiënten met zaadbalkanker hebben een 5.9 maal verhoogd risico op het ontwikkelen van zaadbalkanker. Daarentegen is er geen verhoogd risico op het ontwikkelen van andere vormen van kwaadaardige aandoeningen onder eerstegraads familieleden. Om nader inzicht te krijgen in de rol van erfelijkheid bij het ontstaan van zaadbalkanker is bestudering van de overerving van genen in deze ‘zaadbalkanker’ families noodzakelijk.

In diezelfde periode (1990-2000) is het aantal mannen dat aan zaadbalkanker overleed gedaald van 38 naar 25 per jaar. Uit gegevens van de Nederlandse Kankerregistratie blijkt dat patiënten met zaadbalkanker die ouder dan 50 jaar zijn een slechtere prognose hebben dan patiënten jonger dan 50 jaar. In **hoofdstuk twee** wordt gekeken of dit verschil ook aanwezig is in de grotere kankerregistratie database van de Verenigde Staten van Amerika (Surveillance, Epidemiology and End Results cancer registries). Ook hier werd een lagere overlevingskans (gecorrigeerd voor uitgebreidheid en type van zaadbalkanker) waargenomen

voor patiënten ouder dan 50 jaar. Met name oudere patiënten met uitzaaiingen bleken een slechtere prognose te hebben. Het is onduidelijk of dit het gevolg is van verminderde tolerantie voor chemotherapeutische behandeling of dat oudere patiënten een minder uitgebreide behandeling hebben ondergaan. In ieder geval worden oudere patiënten op een of andere manier onderbehandeld, wat mijns inziens pleit voor inclusie van oudere patiënten in toekomstig onderzoek.

In het **tweede gedeelte van dit proefschrift** wordt een drietal studies gepresenteerd betreffende een rationele benadering van klinisch stadium I nonseminoom. Ter verduidelijking, klinisch stadium I nonseminoom wil zeggen dat hoewel er bij screening met CT-scan geen lymfklieruitzaaiingen zijn aangetoond er bij 30% van deze patiënten toch micro-uitzaaiingen in de lymfklier aanwezig zijn.

Patiënten met klinisch stadium I nonseminoom hebben een te verwachte overlevingskans van bijna 100%, ongeacht keuze van behandeling. **Hoofdstuk drie** presenteert een uitgebreid overzicht van de drie huidige therapiekeuzes in klinisch stadium I nonseminoom: 'wait and see', lymfklierdissectie, of chemotherapie. De eerste twee behandelingmethodes zijn alom gerespecteerd. Chemotherapie wordt alleen nog in onderzoeksverband gebruikt. De tweedeling in therapiekeuze heeft een geografische verdeling. In de Verenigde Staten van Amerika wordt, als gevolg van de traditionele chirurgische benadering van ziekte, de operatieve benadering gepropageerd, terwijl in Europa met name conservatieve benadering ('wait and see') wordt nagestreefd. Door de geografische tweedeling is er een gebrek aan goede vergelijkende studies. In Nederland is het Universitair Medisch Centrum in Nijmegen het enige ziekenhuis dat lymfklierdissectie verricht in klinisch stadium I nonseminoom.

In **hoofdstuk vier** worden de huidige twee behandelingsmethoden voor klinisch nonseminoom stadium I in Nederland met elkaar vergeleken: lymfklierdissectie verricht door

de afdeling urologie van het Universitair Medisch Centrum in Nijmegen of 'wait and see' geadviseerd door afdeling urologie en oncologie van het Nederlands Kanker Instituut/Antoni van Leeuwenhoek ziekenhuis te Amsterdam. Alhoewel het een retrospectieve studie betreft, zijn beide groepen vergelijkbaar qua grootte, klinische parameters en follow-up periode. Onafhankelijk van therapiekeuze is de overlevingskans ook in Nederland bijna 100%. De keuze van behandeling lijkt met name te worden bepaald door de voorkeur van de behandelend arts, de lokale mogelijkheden voor behandeling en patiënt gerelateerde factoren.

In feite wordt de tweedeling in behandelingsvoorkeur veroorzaakt door de huidige stadiëringscapaciteit van de CT-scan. De keuze van behandeling wordt vereenvoudigd wanneer de kans op het waarnemen van aanwezigheid of afwezigheid van micro-uitzaaiingen kan worden vergroot. In **hoofdstuk vijf** wordt bij 50 patiënten met klinisch stadium I nonseminoom gezocht naar prognostische risicofactoren voor de aanwezigheid of afwezigheid van micro-uitzaaiingen in lymfklieren. In 14 patiënten (28%) werden micro-uitzaaiingen vastgesteld en 1 patiënt ontwikkelde binnen 3 maanden na zaadbalverwijdering een uitzaaiing in de long. Bij analyse (multivariate logistische regressie analyse) blijken vasculaire invasie en tunica albuginea invasie in de primaire tumor (d.w.z. de zaadbal) voorspellend te zijn voor micro-uitzaaiingen. De afwezigheid van enkele factoren (embryonaal cel carcinoom (n=11), diploïdy (n=3) en polyploïdy (n=2)) werd daarentegen geassocieerd met de afwezigheid van micro-uitzaaiingen.

Uit literatuurgegevens blijkt, overeenkomstig onze studie, dat vasculaire invasie de meest betrouwbare en reproduceerbare prognostische marker is. Bij aanwezigheid van vasculaire invasie in de primaire tumor is er 50% kans op micro-uitzaaiingen in de lymfklieren, tegen maar 10% bij afwezigheid. Het huidige beleid in Nijmegen is gebaseerd op de aan- of afwezigheid van vasculaire invasie in de primaire tumor. Bij aanwezigheid adviseren we een lymfklierdissectie, maar bij afwezigheid wordt een "wait and see" beleid gevolgd.

In het **derde gedeelte van dit proefschrift** wordt de rol van 18-Fluoro-Deoxy-Glucose Positron Emissie Tomografie (^{18}FDG-PET) in stadiëring van zaadbalkanker besproken. De stadiëring van zaadbalkanker is belangrijk aangezien dit de behandelingsstrategie bepaalt. Daarnaast vindt re-stadiëring na chemotherapie plaats, om het effect van chemotherapie te evalueren en te besluiten of er nog aanvullende chirurgie noodzakelijk is. Na chemotherapie wordt in 15% van de seminomen[7] en 20% van de nonseminomen[8] nog een restmassa met actieve tumorcellen gevonden. Nog eens 40% van de nonseminomen bevat bovendien matuur teratoom (premaligne aandoening).[8] Met andere woorden, na chemotherapie is 85% van de seminomen en 40% van de nonseminomen vrij van (pre)maligne cellen. Aangezien de CT-scan geen onderscheid kan maken tussen maligne en niet-maligne weefsel, wordt uit voorzorg restant weefsel na chemotherapie verwijderd. Een aantal patiënten wordt dus achteraf bekeken onnodig geopereerd.

^{18}FDG-PET is een nieuwe diagnostische techniek om actieve cellen (kankercellen) van niet-actieve cellen te onderscheiden. De werking van ^{18}FDG-PET is gebaseerd op glucoseverbruik van de cel. Cellen met een verhoogde stofwisselingsactiviteit, zoals kankercellen, zullen meer ^{18}FDG opnemen dan cellen met een lagere activiteit. Met behulp van een PET-scan kan de verhoogde activiteit worden waargenomen. Omdat uitzaaiingen in de lymfklier niet direct hoeven te leiden tot een kliervergroting, heeft visualisatie van het metabolisme een theoretische meerwaarde ten opzichte van een anatomische weergave, zoals bij de CT-scan.

^{18}FDG-PET heeft op deze manier een additioneel effect bij de stadiëring van kleincellig bronchuscarcinoom.[9] Daarnaast blijkt dat het behandelingsresultaat eerder wordt waargenomen dan met de CT-scan.[10]

In **hoofdstuk zes** wordt de waarde van ^{18}FDG-PET geëvalueerd in zowel primaire stadiëring van stadium I en IIa nonseninomen als in re-stadiëring van (non)seminomen (stadium >IIa) na behandeling met chemotherapie. In primaire stadiëring heeft ^{18}FDG-PET geen additionele

waarde aangezien noch kleine uitzaaiingen noch grotere uitzaaiingen met premaligne cellen (matuur teratoom) worden gedetecteerd. In re-stadiëring van nonseminomen na chemotherapie blijken de resultaten van ^{18}FDG-PET negatief te worden beïnvloed door foutpositieve en foutnegatieve bevindingen: een negatieve PET-scan kan geen onderscheid maken tussen necrose (littekenweefsel), matuur teratoom (premaligne weefsel), en erg kleine tumorcellen(<5 mm); een positieve PET scan daarentegen kan indicatief zijn voor zowel een actieve tumor als een actief ontstekingsproces.

De voornaamste oorzaak voor foutnegatieve bevindingen is de aanwezigheid van matuur teratoom. Matuur teratoom heeft een gelijke stofwisselingsactiviteit als normaal weefsel, en wordt daarom niet waargenomen. In onze serie kon de effectiviteit van ^{18}FDG-PET worden verhoogd door die patiënten met matuur teratoom in de primaire tumor (zaadbal) uit te sluiten van analyse (matuur teratoom in primaire tumor geeft ook vaak matuur teratoom in de uitgezaaide lymfklier). Deze subanalyse kon helaas niet worden bevestigd in een grotere serie.[11] Bij seminomen komt per definitie geen teratoom voor, en hier lijkt een rol weggelegd voor de PET-scan: een negatieve PET-scan bij restadiëring van seminoom na chemotherapie suggereert littekenweefsel.[12,13]

Een tweede oorzaak voor een foutnegatief resultaat is dat de ^{18}FDG-PET, net zoals de CT scan, niet in staat is om zeer kleine uitzaaiingen op te sporen.

Omdat ^{18}FDG niet tumorspecifiek is, kunnen ook foutpositieve waarnemingen plaatsvinden.[14] Het is bekend dat macrofagen, de zogenaamde afweersysteemcellen, ook ^{18}FDG stapelen. Verhoogde macrofaag activiteit wordt met name gezien bij afbraakprocessen, zoals afbraak van dode cellen na chemotherapie bijvoorbeeld. Om kans op foutpositieve waarneming te verminderen wordt geadviseerd PET-scanning tenminste drie weken na beëindiging van chemotherapie te verrichten.[15] In onze serie werd zelfs na 6 weken nog verhoogde afbraak waargenomen. Concluderend, grotere series zijn nodig om de definitieve rol van ^{18}FDG-PET

in stadiëring van zaadbalkanker (met name seminomen) te bepalen. Mijns inziens is er geen rol voor ^{18}FDG-PET weggelegd in primaire stadiëring en herstadiëring van nonseminomen.

Een ander aspect van PET scanning is de mogelijkheid van therapiemonitoring. Het meten van tumor respons na behandeling biedt de mogelijkheid om vroegtijdig een onderscheid te maken tussen responders en non-responders. In geval van respons wordt de behandeling voortgezet en bij een non-responder kan de ineffectieve behandeling worden gestaakt, waarna andere opties kunnen worden overwogen. In onderzoeksverband is therapiemonitoring met behulp van ^{18}FDG-PET reeds verricht bij andere tumoren zoals die van long, borst, hersenen en darm.[10]

In **hoofdstuk zeven** wordt de waarde van opeenvolgende ^{18}FDG-PET scans in chemotherapiemonitoring voor uitgezaaide zaalbalkanker geëvalueerd. Deze pilot studie toont aan dat een tussentijdse ^{18}FDG-PET scan nauwelijks van toegevoegde waarde is in het voorspellen van het therapieresultaat.

Het **vierde gedeelte van dit proefschrift** behelst verschillende aspecten van follow-up na behandeling van zaadbalkanker. Follow-up is in het algemeen bedoeld om een recidief in een vroeg stadium vast te stellen, om zodoende de behandeling bij een zo laag mogelijk tumorvolume te kunnen starten. Het is de "*sine qua non"* van nazorgse behandeling. In het verleden werden de follow-up schema's met name gebruikt om het resultaat (effectiviteit en bijwerkingen) van verschillende soorten chemotherapie te evalueren. Na de introductie van platinum-bevattende chemotherapie is de overlevingskans drastisch verbeterd, en werd de combinatie chemotherapie bestaande uit "Bleomycine, Etoposide and Platinum" de gouden standaard in de behandeling van uitgezaaide zaadbalkanker.[16] Nadien is er veel aandacht besteed aan het optimaliseren van dit chemotherapie schema,[17-19] daarentegen is er maar weinig aandacht besteed aan de rol van follow-up.

In **hoofdstuk acht** wordt de rol van routinematige follow-up in detectie van een recidief testis carcinoom bekeken. De kans op recidiverende ziekte blijkt afhankelijk van de ingezette primaire behandeling en de therapierespons op deze behandeling. Dit suggereert een "response-to-treatment" gerelateerde benadering van het follow-up schema. Een CT-scan van thorax en abdomen heeft geen toegevoegde waarde voor patiënten die behandeld zijn met radiotherapie of chirurgie voor zaadbalkanker stadium I of IIa en nadien vervolgd met röntgenfoto's van de longen. Voor deze groep van patiënten lijkt de follow-up duur van twee jaar voldoende. Daarnaast lijkt er maar een beperkte rol voor follow-up weggelegd voor die groep van patiënten die 1) nadat de kwaadaardige lymfklieren zijn verwijderd en aanvullend behandeld zijn met chemotherapie (nonseminoom IIA) of die groep van patiënten die 2) eerst chemotherapie hebben gekregen voor uitgezaaide ziekte en vervolgens een chirurgische verwijdering van wat naar achteraf bleek niet pathologische restmassa. In beide patiëntgroepen werd geen recidiverende ziekte ontdekt.

Hoewel deze gegevens een mindere stringent follow-up schema suggereren, denk ik dat alleen prospectieve gerandomiseerde studies of simulatie analyse een "response-to-treatment" gerelateerd follow-up schema kunnen rechtvaardigen.

In **hoofdstuk 9** wordt de effectiviteit van verschillende "wait and see" schema's in klinisch stadium I nonseminoom geëvalueerd ten aanzien van levensverwachting (LV) en ziektespecifieke sterfte (ZSS). Om de ware effectiviteit van follow-up te evalueren is een gerandomiseerd onderzoek nodig wat follow-up versus geen follow-up met elkaar vergelijkt. Maar dit stuit op ethische bezwaren aangezien follow-up niet bewezen ineffectief is. Bovendien vereist zo'n onderzoek compliante patiënten. Als alternatief kunnen computer modellen (simulatie analyse) gebruikt worden, die het effect van verschillende schema's kunnen nabootsen zonder belemmerd te worden door bovengenoemde nadelige effecten van gerandomiseerd onderzoek. Een Markov model werd ontwikkeld en gebruikt om de

effectiviteit van verschillende "wait and see" schema's met elkaar te kunnen vergelijken: schema van "the European Association of Urology" (EAU) versus schema van "Het Nederlands kanker Instituut/Antoni van Leeuwenhoek ziekenhuis" (NKI/AvL) versus een hypothetisch minimaal follow-up protocol gedurende de eerste twee jaar.

Het intensievere NKI/AvL schema (ZSS: 1.81%; LV: 53.9 jaar) leidt tot een 1.2% lagere ziektegerelateerde sterfte en een 6 maanden langere levensverwachting vergeleken met het EAU schema (ZSS: 3.05%; LV: 53.3 jaar). Het hypothetische minimaal follow-up protocol heeft een ZSS van 6.83% en een LV van 51.4 jaar. Concluderend, het voordeel van mindere intensive follow-up volgens de EAU, in vergelijking met NKI/AvL, moet worden afgewogen tegen een stijging van ziektespecifieke sterfte van 2% naar 3%. Follow-up alleen de eerste twee jaar heeft een onacceptabel hoog ziektespecifieke sterfte tot gevolg. Totdat gerandomiseerde onderzoeken zijn verricht, geeft dit model een indicatie van wat de gevolgen kunnen zijn van een veranderd follow-up schema in "wait and see" beleid van klinisch stadium I nonseminoom.

Het **laatste gedeelte van dit proefschrift** behelst fertiliteitaspecten bij zaadbalkanker. Doordat veruit de meeste patiënten genezen van zaalbalkanker worden ziekte- en behandelingsgerelateerde complicaties, zoals infertiliteit, steeds belangrijker. Een saillant detail is dat vele studies semenanalyse hebben gebruikt als indicator voor (in)fertiliteit bij patiënten met zaadbalkanker, terwijl volgens de "American Society of Andrology" infertiliteit wordt gedefinieerd als het onvermogen om binnen één jaar zwanger te raken bij onbeschermd seksueel contact. De prevalentie van infertiliteit onder de algemene bevolking varieert tussen de 20 en 28%.[20]

In **hoofdstuk tien** wordt de prevalentie van infertiliteit voor en na behandeling van zaadbalkanker geschat. Vóór de behandeling rapporteerde 34% van de patiënten infertiliteit,

tegen 57% na de behandeling. De infertiliteit na behandeling bleek onafhankelijk van behandelingskeuze. Na behandeling gebruikte 18% van de mannen ingevroren zaad voor bevruchting. In overeenstemming met literatuurgegevens werd er onder de nakomelingen van patiënten met zaadbalkanker geen verhoogd percentage aangeboren afwijkingen gevonden. Kortom, aangezien het niet mogelijk is te voorspellen welke patiënt fertiliteitproblemen zal hebben na de behandeling van zaadbalkanker, dient elke patiënt de mogelijkheid te worden geboden om van tevoren zaad te laten invriezen.

In het **laatste hoofdstuk** wordt de kwaliteit van geëjaculeerd sperma (inclusief serum hormoon analyse, routinematige en computergestuurde zaadanalyse (CKIA), sperma DNA schade en sperma DNA condensatie) voor en na de behandeling met platinum-bevattende chemotherapie met elkaar vergeleken. Tevens werden de uitkomsten vergelijken met de zaadkwaliteit van gezonde controlepersonen.

Verhoogd concentratie LH zorgt voor een compensatoir gelijkblijvende testosteron concentratie, en een verhoogde FSH waarde suggereert epitheelschade aan de gezonde contralaterale bal. Routinematige zaadanalyse en morfometrie volgens CKIA lieten geen veranderingen in zaad kwaliteit zien voor en na chemotherapie. Hoewel er een verbetering in chromatine condensatie van het zaad werd gevonden, was er geen afname in DNA beschadigde cellen. In vergelijking met zaad van gezonde controle personen blijft de sperma DNA kwaliteit matig. Het is onduidelijk of de verbeteringen in sperma DNA kwaliteit alleen het gevolg zijn van verwijdering van kwaadaardige cellen of dat er nog op andere niveaus schade aan sperma DNA aanwezig is dat we niet gedetecteerd hebben.

Niettemin lijkt het raadzaam om sperma ook op DNA niveau te bekijken met name in geval van geassisteerde voorplanting, aangezien de externe karakteristieken van sperma, gemeten bij routinematige zaadanalyse, niet overeenkomen met de interne karakteristieken (DNA analyse).

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De prognose voor patiënten met zaadbalkanker is enorm verbeterd in de afgelopen 25 jaar, waarbij bijna 100% van de patiënten met een laag stadium en 80% van de patiënten met een vergevorderd stadium de ziekte overleeft. Toekomstig onderzoek is gericht op enerzijds het vergroten van de totale overleving en anderzijds het verminderen van bijwerkingen van behandeling.

Een belangrijk aspect van de vermindering van de bijwerkingen van behandeling vormt de juiste keuze tot behandeling. De huidige controverse met betrekking tot behandeling van **laag stadium zaadbalkanker** wordt met name veroorzaakt door beperkingen in visuele diagnostiek, zoals het onvermogen om micro-uitzaaiingen te detecteren. Dertig procent van de patiënten met een klinisch stadium I nonseminoom blijkt stadium IIa te hebben. Aangezien de sensitiviteit van huidige beeldvormende diagnostiek omgekeerd evenredig gerelateerd is aan tumorvolume, zijn andere vormen van diagnostiek ontwikkeld om micro-uitzaaiingen op te sporen.

Het principe van ^{18}Fluor-Deoxy-Glucose Positron Emissie Tomografie (^{18}FDG-PET) is gericht op detectie van verhoogde stofwisselingsactiviteit nog voordat structurele veranderingen, zoals toename in weefselgrootte, plaatsvindt. Helaas bleek in laag stadium nonseminoom geen meerwaarde voor PET-scanning boven de conventionele CT-scan aanwezig. De meerwaarde van andere ontwikkelde tracers dan ^{18}FDG in PET-scanning zijn nog niet getest in primaire diagnostiek van laag stadium nonseminoom.[1]

Een andere niet invasieve stadieringmethode, die met succes ontwikkeld is om micro-uitzaaiingen van prostaat en blaaskanker op te sporen, is de combinatie van MRI en intraveneuze injectie van extreem kleine super paramagnetische ijzeroxide partikels.[2] Deze methodiek is nog niet toegepast bij zaadbalkanker.

Ongeacht welke klinische stadieringsmethode wordt gebruikt, denk ik dat het niet mogelijk is om microscopische ziekte te visualiseren. Ook heden ten dage blijft verwijderen van lymfeklieren en aansluitend histopathologisch onderzoek onder de microscoop de meest accurate stadieringsmethode. In een poging om de morbiditeit van open lymfeklierdissectie te verminderen, zijn een aantal centra begonnen met laparoscopische verwijdering van deze lymfeklieren, de zogenaamde kijkoperatie.[3-6] Om de betreffende afwijkende klier nog nauwkeuriger te kunnen opsporen wordt bovendien door een enkeling radioactieve stof in de primaire tumor gespoten wat vervolgens wordt opgenomen door de drainerende lymfeklier. [7,8] Hoewel laparoscopie de open chirurgie kan vervangen als stadieringsmethode, is het onduidelijk of het even effectief is in therapeutische zin. In alle tot nu toe uitgevoerde studies werd na de detectie van micro-uitzaaiingen een aanvullende behandeling met chemotherapie gegeven. [3-6] Het is bekend dat behandeling van stadium II zaadbalkanker goed te behandelen is met chemotherapie alleen, zoals blijkt uit de "wait and see" studies. Met andere woorden, als het de bedoeling is om pathologische lymfeklieren te behandelen met chemotherapie, waarom dan geen "wait and see" beleid voeren en chemotherapie starten in geval van klinisch bewezen uitzaaiing? De toekomst zal uitwijzen wat de rol van laparoscopie in laag stadium nonseminoom zal zijn.

Een andere methode om de huidige stadiering te verfijnen is het analyseren van verschillende prognostische markers in de primaire tumor. Deze manier van analyse stoelt op het concept dat primaire tumoren vaak al in een vroeg stadium van ontwikkeling alle eigenschappen bezitten die nodig zijn om te metastaseren. De aanwezigheid van met name vaatinvasie leidt tot een 50% kans op micro-uitzaaiingen. Veel reeds verrichte studies herbergen een selectie en interobserver bias. Om de definitieve rol van prognostische markers in laag stadium nonseminoom vast te stellen is een prospectieve studie nodig. Bovendien is het niet duidelijk of patiënten met een verhoogde kans op micrometastasen een slechtere prognose hebben

wanneer primair agressief behandeld (lymfeklierdissectie of chemotherapie) in vergelijking met het “wait and see” beleid.

Zolang we niet in staat zijn om patiënten op klinische gronden accuraat te onderscheiden in stadium I en II, zal controverse in behandeling blijven voortbestaan. De beste keuze van behandeling zal dan alleen tot uitdrukking kunnen komen door een gerandomiseerde trial waarin “wait and see”, lymfeklierdissectie en primaire chemotherapie onderling worden vergeleken op grond van levensverwachting, prognostische markers, bijwerkingen, kosten, en kwaliteit van leven.

Herstadiering na chemotherapie blijft ook een uitdaging. Aangezien het op radiologische gronden niet mogelijk is kwaadaardig restweefsel van niet-kwaadaardig restweefsel te onderscheiden, is er geen ander alternatief mogelijk dan bij alle patiënten het restweefsel te verwijderen. Een grote winst zou het opleveren wanneer we de responders van de non-responders konden onderscheiden. In dit proefschrift werd de aanvullende waarde van ^{18}FDG-PET in monitoring de response op chemotherapie bekeken. Hoewel ^{18}FDG-PET response kan meten, had het geen aanvullende waarde ten opzichte van de CT-scan. Misschien dat het enige waarde heeft in agressievere vormen van zaadbalkanker (dwz. zaadbalkanker met hoge concentratie serum tumormarkers of met uitgebreide uitzaaiingen). Hopelijk kunnen de nieuwe ^{18}F-gelabelde tracers de stadiering van zaadbalkanker verbeteren.[1]

Follow-up na behandeling van kanker wordt in het algemeen als nuttig ervaren. De vraag is echter, wat is de waarde van follow-up na behandeling van zaadbalkanker. Zoals eerder in dit proefschrift is beschreven is de effectiviteit van follow-up niet voor elke patiënt hetzelfde. De effectiviteit hangt vooral af van de soort en het stadium van de ziekte en de daarop toegepaste behandeling. Maar ook in dezelfde categorie van ziekte (bijvoorbeeld laag stadium

nonseminoom) blijkt verandering in follow-up schema, gesimuleerd door een computer-programma, grote invloed te hebben op de mortaliteit. Met andere woorden er is genoeg reden om een prospectief gerandomiseerd onderzoek te verrichten naar de effectiviteit van follow-up schema's, vooral in de groep van laag stadium nonseminoom en in de groep van complete responders na chemotherapie. Bovendien is het nuttig om eens prospectief de psychologische stress te evalueren van follow-up na zaadbalkanker, aangezien dit vaak wordt verondersteld maar nooit is bewezen.

Het is bekend dat zaadbalkanker en de behandeling ervan fertiliteitproblemen kunnen veroorzaken. Juist daarom moeten wij als artsen, ons ertoe dwingen patiënten niet alleen informatie te geven over de ziekte zelf maar ook over de fertiliteit aspecten en de mogelijkheid tot cryopreservatie. Door de toegenomen mogelijkheden van geassisteerde voortplanting denk ik dat we zaadbalkanker meer multidisciplinair moeten benaderen. Nu wordt nog de ziekte door de uroloog en de fertiliteitproblematiek door de gynaecoloog behandeld.

Daarnaast is het nog steeds niet duidelijk of er nu een verhoogde kans op afwijkingen aanwezig is bij het nageslacht van patiënten, die behandeld zijn voor zaadbalkanker. De onderzoeken, die tot nu toe zijn verricht, tonen geen verhoogd risico aan, maar daarbij dient de kanttekening te worden gemaakt, dat het kleine studies zijn met een korte follow-up tijd. Kortom, nader onderzoek is gewenst.

In de nabije toekomst zal voortschrijdend inzicht in biologie en genetica van zaadbalkanker mogelijk leiden tot een nieuwe therapeutische benadering en behandeling. Wanneer de lokalisatie van veranderingen in het DNA van zaadbalkanker kan worden opgespoord, is het misschien ook mogelijk nieuwe moleculaire markers en zelfs gentherapie te ontwikkelen.

Nieuwe markers zouden een bijdrage kunnen leveren aan risicogerelateerde behandeling en gentherapie is misschien minder belastend maar net zo effectief als de huidige chemotherapie.

Mijn conclusie luidt dat in de laatste drie decennia grote stappen voorwaarts zijn gemaakt in het management van zaadbalkanker, waarschijnlijk zullen de stappen in de toekomst kleiner zijn om het management te optimaliseren.

Ongeacht het feit dat zaadbalkanker relatief weinig voorkomt en het overgrote deel van de patiënten overleeft, moeten we onderzoek voortzetten om het management van deze ziekte verder te maximaliseren. Het management en de ontwikkeling van behandelingstrategie van deze ziekte kan als voorbeeldfunctie dienen voor de behandeling van andere tumoren. Daarom moedigen we financiële ondersteuning voor toekomstig onderzoek aan.

Het zaad des doods is geworden tot zaad vol met zorg en zal weer worden tot zaad des …levens?

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APPENDICES

Promoveren betekent letterlijk voortbewegen, voortgaan (pro – movere). De reis die ik heb gemaakt begon in 2000 en heeft me langs vele illustere personen gevoerd. Zij hebben mij kortere of langere tijd vergezeld en hen wil ik bedanken voor de geboden hulp, raad en levenswijsheid om dit boekje tot stand te brengen.

Prof. dr. J.A. Witjes, beste Fred, jij bent de grondlegger geweest van dit boekje. De twaalf losse regels die je me overhandigde op een half A viertje, met daarop enkele gedachtespinsels heb ik gekoesterd alsof het de twaalf geboden waren. En zie hier het resultaat: een elftal artikelen. Dank!

De reis die we samen maakten heeft ons langs Nijmegen by Night gevoerd. Samen op stap een drankje drinken en een hapje eten bij de Mc Donalds: De bediende vroeg als eerste aan mij "wat mag het zijn?", waarop ik antwoordde: "Voor mij graag een Big Mac en voor mijn vader ook één".

Prof. dr. L.A.L.M. Kiemeney, beste Bart, ik heb je als de best denkbare criticaster en ondersteunende begeleider ervaren, wanneer jij (uiteindelijk) goedkeuring gaf aan het manuscript streepte ik weer een van de twaalf geboden door.

Beste Bart, met jou heb ik een reis gemaakt op zoek naar de puurheid van het onderzoek waarbij jouw begrip en verstand van zaken zoveel sneller zijn dan het mijne, dat ik nog wel eens dreigde te verdwalen in het duistere woud van de statistiek. Dank!

Prof. dr. W.J.G. Oyen, beste Wim, we hebben vele vruchtbare gesprekken gevoerd over de rol van PET scanning bij testis tumor. Ondanks de indrukwekkende stapels werk op je bureau en de gecorrigeerde artikelen, die je nog moest verwerken, had je altijd tijd voor me.

De reis die we hebben gemaakt bestond uit spaarzame minuten relaxend, de boel de boel latend, achterover leunend in de stoel genieten van een dikke pluim. Vo.

Prof. dr. S. Horenblas, beste Simon, dank voor de interesse in mijn onderzoek en beoordeling van het manuscript.

Terwijl we buiten in de zon op een stoepje zaten en een tweetal uur over de behandeling van stadium I nonseminoma hadden gepraat, sprak je voor mij de memorabele woorden: "onderzoek: the less the much" (Horenblas, EAU Brussel, 2000).

Dr. J. van Moorselaar, beste Jeroen, jouw interesse in en enthousiasme voor de urologie werken aanstekelijk.

Ik waardeer zeer de moeite die je hebt genomen om naar Nijmegen te komen om ons onderzoek de laatste push te geven die het nodig had en ik zal de keren niet vergeten dat ik je bezocht in Utrecht met de zoveelste versie van mijn artikel: we begonnen met koffie en eindigden met filosoferen over de urologie en het gecorrigeerde artikel ontving ik enkele dagen later per post. Gezellig.

Aswin Hoffmann, dank voor de onvoorwaardelijke hulp bij het ontwikkelen van het follow-up model. Het project zou 3 weken gaan duren, maar door ons perfectionisme het zo realistisch mogelijk overeen te laten komen met de werkelijkheid, heeft het 2 jaar geduurd.

Ontspannend en vooral verhelderend waren de voettochten door de bossen van Malden; uiteindelijk werden daar de meeste analyse problemen geattackeerd.

Beste UIC/BME-ers: Tommy, Michiel, John, Jos, Bennie, Mark, Tjerk, Pim, Reza en Floor, dank voor de ontspannende lunches en voor jullie frisse kijk op aardse zaken.

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begrijpen wat sperma precies betekent: "*een kiemcel op wereldreis*". Dank voor de vele zaadanalyses.

Marc Kicken en Bob Gosselt, de dinsdagavonden in het café "St. Anneke" hebben een ontspannend en geestverruimend karakter gehad. Top!

Ik vraag me nog steeds af hoe we achter de bar zijn beland, de muziek regelden en de tap toucheerden. De gordijnen gingen dicht, de echte klanten naar huis, het café werd aan kant gemaakt en wij……wij bleven de tap toucheren. Galant hebben we de smeekbeden aangehoord en verlieten als Heeren de bar om 04:45. Memorabel.

Fredrik Ebbens, beste Fredje, ik ben je zeer dankbaar voor de coverpainting "Struggle for life". Does the germ cell become malignant or will it come alive, that is the question? Een perfecte samenvatting van dit proefschrift.

De Dom was een mooie beklimming (maakt de cover alleen maar mooier!).

De 'Paranifmen': Steven van Gaalen en Max van Leyenhorst. Heeren, ik weet hoe het voelt om aan jullie zijde getuige te mogen zijn, en ben zeer verheugd om nu door jullie geflankeerd te worden.

Lieve Ouders en Ouders, Broers en Broers, Zus en Zusjes. Jacques, Renee, Jan, Marianne, Jacco, Boaz, Mark, Erik, Naomi, Albertine en Luz Elena. Jullie zijn altijd een grote steun geweest en zullen dat ook blijven. Sinds 10 September is letterlijk alles verdubbeld. Het voelt verdomd goed. Hoop vanaf nu weer meer tijd met jullie te kunnen doorbrengen.

Lieve Niekje. Ik hou van jou. En dit gevoel van liefde is alleen maar meer geworden.

Frommel de pommel was de eerste bijnaam die ik je gaf, aangezien je zo knuffelgek bent. Ik hoop dat je elke dag tot in lengte der dagen het ritueel blijft herhalen dat wanneer we samen eten jij op mijn schoot komt zitten wanneer jij je bord als eerste leeg hebt.

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Niek ik hou van je zodanig dat ik wil je vragen of ik je huidige bijnaam: Mijn kleintje…… ……10 september 2005……..het was een superdag. Ik ben gelukkig.

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Jesse Roan Spermon werd geboren op 9 september 1972 te ‘s Hertogenbosch. In 1991 behaalde hij het Gymnasium- -diploma aan het Rooms-katholiek Gymnasium “Beekvliet” te st. Michielsgestel. Aangezien hij werd uitgeloot voor de studie Geneeskunde in Nederland volgde hij van 1991-1992 de studie geneeskunde aan de Katholieke Universiteit te Leuven, België. In 1992 werd hij wél ingeloot en verhuisde naar Utrecht om aan de betreffende Universiteit de studie Geneeskunde te volgen. Op 28 december 1998 legde hij het artsexamen succesvol af.

In januari 1999 werd hij werkzaam op de afdeling Urologie van het Universitair Medisch Centrum St Radboud als AGNIO (assistent geneeskundige niet in opleiding). Van januari 2000 tot december 2003, verrichte hij onderzoek naar verfijningen in het management van kiemceltumoren van de testis onder begeleiding van Prof. dr. J.A. Witjes, Prof. dr. L.A.L.M Kiemeney en Prof. dr. W.J.G. Oyen. In oktober 2002 werd aan hem door de Nederlandse Vereniging van Urologie (NVU) de Vlietstra prijs toegekend voor de beste wetenschappelijke voordracht, gepresenteerd tijdens de vergadering van de NVU.

In juni 2002 werd hij geselecteerd voor de opleiding Urologie (cluster Nijmegen, opleider Prof. dr. J.A. Witjes). Van begin 2003 tot eind 2004 werd de chirurgische vooropleiding in het Elizabeth Ziekenhuis te Tilburg (opleider: Dr. C.J.H.M. van Laarhoven) doorlopen, waarna in januari 2005 werd gestart met het perifere deel van de opleiding Urologie in Rijnstate Ziekenhuis te Arnhem (opleider: Dr. E.J. Barten).