7

Hypogonadism After Childhood Cancer Treatment

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1. Introduction

Long-term survival of children with cancer has greatly improved in the last decades due to effective treatment, especially multiagent chemotherapy (ChT). The chief concern is now being directed toward the late effects of treatment. Endocrine glands, gonads in particular, are very susceptible to damaging effects of anticancer therapy. The damaging effect of both ChT and radiotherapy (RT) on gonads is well known (Cohen 2003, Diamond et al. 2001, Spoudeas 2002). In a study of 2283 long-term survivors of childhood cancer Byrne and colleagues found that RT below the diaphragm depressed fertility in both sexes for about 25%, ChT with alkylating agents (AA) with or without RT below the diaphragm depressed fertility by 60% in men, in women, however, AA therapy administered alone had no apparent effect on fertility (Byrne et al. 1987). Hypogonadism is most often due to direct damage by ChT, RT and/or surgery, rarely is due to damage to the hypothalamus and/or pituitary gland (Cicognani et al. 2003, Müller 2003).

The gonads have two main functions, the production of sex hormones (estrogens and testosterone) and germ cells (ova and sperm). Both of them depend on a normal function of the hypothalamic-pituitary-gonadal axis. Long term survivors of childhood cancer are at risk of hypogonadism related to gonadotropin secretion, but more frequently hypogonadism is caused by direct damage of testes or ovaries. In human testis two functions are combined: sex steroid production and sperm production. Germ cells form sperm, Sertoli cells support and nurture the developing germ cells and Leydig cells produce testosterone. These three cell types are organized into two functional compartments: germ cells and Sertoli cells form the seminiferous tubules where spermatogenesis takes place, and the network of Leydig cells are responsible for the production of testosterone, which is necessary for normal spermatogenesis. These two compartments are under separate controls and affected in different ways by cancer treatments (Meistrich 2009, Shalet 2009, Sklar 1999).

In the ovary, follicle is the site where the production of sex hormones and germ cells takes place. As a result, when ovarian failure occurs, both sex hormone production and fertility are disrupted. Germ cells in the ovary unlike spermatogonial cells lack the ability of repopulation. Preliminary stages of oogenesis are completed shortly after birth, the dominant part of the cell population in ovary being oocytes in the stationary stage of prophase. Older age is an important risk factor for ovarian failure following childhood

cancer and its treatment, given the progressive decline in oocyte reserve with increasing age (Johnston & Wallace 2009, Sklar 1999). If ovarian function is lost prior to the onset of puberty, it results in delayed puberty and primary amenorrhea. If ovarian function is lost during or after pubertal maturation, arrested puberty, secondary amenorrhea, and premature maenopause are observed. In the adolescent and young adults with ovarian failure increased plasma concentrations of gonadotropins and reduced levels of estradiol are typically found.

1.1 Toxic effects of ionizing radiation on testes

1.1.1 Germ cell epithelium

The sperm-producing cells are more vulnerable to cancer treatment than Leydig cells, and are frequently impaired by radiotherapy and different types of chemotherapy. Even small doses of ionizing radiation can damage germinal epithelium of testes. Among the germ cells, type A spermatogonial cells are the most sensitive (especially more differentiated stages A2-4, which are at the stage of mitosis) and type B spermatogonial cells (Ash 1980, Greiner 1985, Lu & Meistrich 1979, Meistrich et al. 1982, Rowley et al. 1974). These sensitive cells can be destroyed with a single dose of radiation as low as 15 cGy. Germ cells in later stages of spermatogenesis, eg. spermatocytes, and spermatides, are less sensitive to ionizing radiation (destroyed by a single dose of 200 cGy or more) (Ash 1980, Lushbaugh & Casarett 1976). After irradiation, the surviving germ cells (early type A spermatogonial cells) develop into more radiosensitive germ cells. Therefore, fractionated RT (administered in several small doses) may be more harmful because it empties the storage of germ cells (Ash 1980, Greiner 1982). Only type A spermatogonial cells can repopulate. If a sufficient number of these cells survive, there is recovery of spermatogenesis, even after several years (Hahn et al. 1982). The rate of damage of spermatogenesis and time in which there's a full recovery depends on the size of RT dose to the testes. Duration of azoospermia is likely to depend on the number of destroyed germ cells. After a single dose of less than 100 cGy to the testes, recovery of spermatogenesis occurs in 9-18 months, after a dose of 200 to 300 cGy in 30 months and after a dose of 400 to 600 cGy in more than 5-years (even after more than 10-years) (Lushbaugh & Casarett 1976, Sandeman 1966, Sanders et al. 1991, Rowley et al. 1974). Single doses greater than 600 cGy cause irreparable damage of spermatogenesis. After fractionated RT a total dose of more than 150 to 200 cGy cause irreversible azoospermia (Greiner 1982, Sandeman 1966). Oligospermia or azoospermia may occur during treatment with RT or mostly during the 2- 3-months from the start of RT (Sandeman 1966). During radiation treatment testes are rarely directly exposed to ionizing radiation, but they are exposed to indirect radiation (e.g. abdominal RT). Several studies reported radiation doses the testes receive at the spillage of ionizing radiation during RT of areas under the diaphragm. This dose to the testes following RT of abdominal areas may be as high as 7 to 13% of the total dose (i.e. the order of 100 to 300 cGy) (da Cunha et al. 1984, Kinsella et al. 1989, Lushbaugh & Casarett 1976, Whitehead et al. 1982). The dose of this size can cause irreversible azoospermia (Greiner 1982, Sandeman 1966). This dose to the testes can be reduced by an additional lead shielding of testis to the level bellow 50cGy, which is not harmful for spermatogenesis (Kovač et al. 1990, Whitehead et al. 1982). Germ cell dysfunction with azoospermia is present in essentially all males treated with TBI (Sanders et al. 1991). Recovery of germ cell function has occurred rarely and primarily following single-dose irradiation (Sanders et al. 1991, Sklar et al. 1984). Germ cell dysfunction with resultant

infertility is often associated with reduced testicular volume, increased FSH concentrations, and reduced plasma concentrations of inhibin B.

1.1.2 Leydig cells

Leydig cells are less sensitive for damaging effect of RT than germ cells, requiring higher dose of ionizing radiation (more than 1500 cGy) for failure, therefore only direct testicular irradiation can cause significant damage of LC. LC are the most sensitive for damaging effect of RT in prepubertal period (Castillo et al. 1990, Shalet et al. 1985). The probability of radiation induced Leydig cell failure is directly related to the dose delivered and inversely related to age at treatment (Leiper et al. 1986, Sarafoglou et al. 1997, Shalet et al. 1989). In the majority of males who receive 2000 cGy fractionated radiation to the testes there is no impairment of testosterone production, but after 2400 cGy of fractionated irradiation as therapy for young males with testicular relapse of ALL there is a very high risk for Leydig cell damage (Sklar 1999). The majority of boys who are prepubertal at the time of treatment, will develop Leydig cell failure after 2400 cGy testicular irradiation and require androgen replacement (Leiper et al. 1986, Shalet et al. 1985). Low doses of ionizing radiation but above 75 cGy can lead to dysfunction of the LC (compensated insufficiency of LC with normal levels of testosterone) (Rowley et al. 1974).

Unlike germinal epithelium LC impairment may develop several years after RT and is usually irreparable (Shalet et al. 1985). Treatment-induced Leydig cell failure and testosterone insufficiency following cancer tretament are relatively uncommon compared with germ cell dysfunction and infertility. Leydig cell failure results in delayed/arrested puberty and lack of secondary sexual characteristics if it occurs before onset of puberty. If it occurs following the completion of normal pubertal development, it can result in reduced libido, erectile dysfunction, decreased bone mineral density, decreased muscle mass, and other metabolic disturbances (Sklar 1999). Increased plasma concentrations of LH combined with low levels of testosterone are characteristic for Leydig cell dysfunction, but these changes may not become apparent until the individual has reached mid-adolescence (Shalet et al. 1985).

1.2 Toxic effects of cytostatic agents on testes

1.2.1 Germ cell epithelium

The chemotherapeutic agents most commonly associated with impaired male fertility are alkylating agents (AA). These citostatic agents are used in the treatment of many types of childhood cancer. Agents in this group are: cyclophosphamide (CY), busulfan, melphalan, nitrogen mustard (NM), DTIC, nitrosoureas (CCNU, BCNU), procarbazine, chlorambucil, ifosfamide. Alkylating agents damage especially late (differentiating) spermatogonial cells and early spermatocytes, and less mature spermatozoa (Meistrich et al. 1982). In the treatment of childhood cancer many cytostatic agents are used at the same time, making it difficult to identify gonadotoxic effect of individual cytostatic. The toxic effect of CY has been studied most. After the cumulative dose of CY of less than 7.5 g/m2 males may retain normal sperm production, after a dose between 7.5 and 22.5 g/m2 oligo-and azoospermia are observed, but the dose greater than 25 g / m 2 causes azoospermia (Kenney et al. 2001). It seems that the threshold dose of CY for azoospermia is around 10 g / m2 (Aubier et al. 1989, Casteren et al. 2009, Relander et al. 2000). Patients treated in prepubertal period have a lower risk for germ cell damage than those treated in postpubertal period (Aubier et al. 1989, Brämswig et al. 1990, Pennisi et al. 1975). Procarbazine, another alkylating agent, commonly used in the treatment of

Hodgkin's disease, can also induce impaired sperm production in a dose-dependent fashion. MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) or MOPP-like combinations, such as MVPP (mechlorethamine, vinblastine, procarbazine and prednisone) induce azoospermia in 90-100 % of pts with a 10-20% chance of recovery even 10 years after treatment (Chapman et al. 1979, Diamond & Bercu 2001, Viviani et al. 1985, Whitehead et al. 1982). Recovery of spermatogenesis following MOPP therapy appears to be dose-related, 3 courses of MOPP representing a limiting gonadal exposure for recovery, suggesting only a partial killing of germinal stem cells (da Cunha et al. 1984). Patients with Hodgkin's disease who received three cycles of MOPP alternating with three cycles of ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) suffer less testicular damage than patients who received 6 cycles of MOPP (Berg et al. 2004, Mackie et al. 1996). Chemotherapy with COPP (CY, VCR, PBZ, prednisone) or OPPA (VCR, prednisone, procarbazine and adriamycin) can cause azoospermia in 50% of patients. ABVD (Adriamycin, bleomycin, vinblastine,DTIC) protocol is less gonadotoxic, usually causing transient germ cell impairment with total recovery (Berg et al. 2004, Santoro et al. 1987, Viviani et al. 1985). CY and cytarabine were reported the most damaging antileukemic drugs for spermatogenesis (Lendon et al. 1978). There has been a report that cytarabine in cumulative doses greater than 1 g/m^2 is correlated with a decreased tubular fertility index in boys (Lendon et al. 1978). There are reports that vincristine also might have important role in causing azoospermia, when administered in childhood or adolescence (Waxmann et al. 1982).

Chemotherapy regimens containing cisplatin or carboplatin can induce germ cell damage with a different rate of recovery of spermatogenesis (Lampe et al. 1997). Recovery of spermatogenesis may be lower in those who received ChT with vinca alkaloids (vincristine and vinblastine) as well. Antimetabolites usually do not cause irreversible damage to testes, but can cause temporary oligospermia. (Sussman & Leonard 1980).

1.2.2 Leydig cells

Leydig cells are less vulnerable to damage from cancer therapy than germ cells, and chemotherapy-induced dysfunction of Leydig cells requiring testosterone replacement therapy is rare (Blatt et al. 1981, Sklar 1999). Leydig cell dysfunction may be observed following treatment with alkylating agent regimens. Ten to 57% of male patients can develop elevated serum concentrations of LH following treatment, but chemotherapy-induced Leydig cell dysfunction is generally subclinical (Bramswig et al. 1990, Kenney et al. 2001, Mackie et al. 1996, Relander et al. 2000, Romerius et al. 2009, Sklar 1999).

1.3 Toxic effects of ionizing radiation on ovaries

Ionizing radiation causes ovarian function impairment as a function of cumulative dose and age. Ovaries are frequently inside RT field or in its immediate vicinity during pelvic or abdominal RT. Preservation of ovarian function depends on the ability of single oocyte to repair damage. That is why RT with multiple small fractions is less toxic for ovaries than RT with one larger fraction, due to greater potential of damage repair during two smaller fractions (Greiner 1985). Females receiving abdominal, pelvic, or spinal irradiation are at increased risk of ovarian failure, especially if both ovaries are within the treatment field (Hamre et al. 1987, Horning et al. 1981, Sklar et al. 2006, Thibaud et al. 1992, Wallace 2005). However, when ovarian transposition is performed prior to RT, ovarian function is retained in the majority of young girls and adolescent females (Ortin et al. 1990, Sklar 1999, Thibaud

et al. 1992). In women over 40 years of age radiation dose of 400 to 700 cGy is sufficient for the sterilization, in younger women the dose from 1250 to 1500 cGy is necessary for sterilization (Ash 1980), and for those treated at the age of 10 years or less even dose of 2000 cGy is necessary for permanent ovarian damage (Lushbaugh & Casarett 1976, Sanders et al. 1991, Wallace et al. 2005). Nevertheless doses of less than 1000 cGy are capable of inducing ovarian damage in patients who have additional risk factors, such as concomitant exposure to alkylating agents and older age at diagnosis. In a report from the CCSS, doses of radiotherapy to the ovary of at least 2000 cGy were associated with the highest risk of ovarian failure; more than 70% of patients exposed to such doses developed ovarian failure, with higher rates in older individuals (13–20 years) when compared with those who were younger (0–12 years) at the time of treatment (Chemaitilly et al. 2006). Moreover, if radiation is given in association with alkylating agents, ovarian dysfunction may occur despite the use of lower doses. In the report from CCSS acute ovarian failure occurred in 6.3% of eligible survivors, exposure of the ovaries to high-dose radiation (especially over 1000 cGy), alkylating agents and older ages being significant risk factors for ovarian failure (Chemaitilly et al. 2006). Premature nonsurgical menopause occurred in 8% of participants versus 0.8% of siblings. Risk factors for premature menopause included attained age, exposure to increasing doses of radiation to the ovaries, increasing alkylating agent score, and the diagnosis of Hodgkin's lymphoma. The cumulative incidence of premature menopause in individuals treated with both alkylating agents and abdominal– pelvic radiation was in the range of 30% (Sklar et al. 2006). Offspring of women who received uterine radiation doses of more than 500 cGy were more likely to be small for gestational age, but there was no evidence for an increased risk of congenital malformations (Green et al. 2002). These studies demonstrated that women treated with pelvic irradiation and/or high alkylating agent doses were at risk for acute ovarian failure, premature menopause, and small-for-gestational-age offspring.

1.4 Toxic effects of cytostatic agents on ovaries

Ovaries are less sensitive to harmful effect of cytostatics than germ cells of testes. Biopsy of the ovary in girls after treatment with chemotherapy showed decreased number of primordial and antral follicles (even more pronounced after treatment with multiagent ChT and RT), decreased follicular maturation, cortical and stromal fibrosis, with / without proliferation and thickening of blood vessels (Chapman et al. 1979, Nicosia et al. 1985). Most sensitive for damaging effect of ChT are growing and preovulatory follicles, therefore ovaries of prepubertal girls are less sensitive to injury after ChT exposure (Stillman et al. 1982). Another reason for higher resistance of ovaries of prepubertal girls to ChT is their greater follicular reserve when compared with the ovaries of adults (Chemaitilly et al. 2006, Grigg et al. 2000). Among chemotherapeutic agents, alkylating agents, which prevent cell division by interacting with DNA, are known to be associated with the occurrence of ovarian failure (Brydøy et al. 2007, Chematilly et al. 2006, Green et al. 2009, Ortin et al. 1990, Zacharin et al. 2010), ovarian failure being dependent on the cumulative dose of cytostatic and age of patients during treatment. In CCSS-study, it was reported that alkylating agents cyclophosphamide and procarbazine were significant risk factors for ovarian failure (Chematilly et al. 2006). Although exposure to procarbazine was an independent risk factor for ovarian failure, regardless of age at treatment, cyclophosphamide significantly increased that risk only in subjects treated at an older age. As the number of oocytes declines with

advancing age, the ovaries of older individuals become more vulnerable to gonadal toxins compared with that seen in younger subjects (Sarafoglou et al. 1997, Sklar 1999). In the study of Ortin and colleagues 10% of girls were amenorrhoic after receiving 6 or more cycles of MOPP and none of those who received other regimes of ChT (e.g. MOPP / ABVD, ABVD). After combined treatment with ChT (6 or more cycles of MOPP) and pelvic RT at a dose of 2000 to 4400 cGy (with or without ovaropeksy) incidence of ovarian failure was about 50%. None of the girls who received 3 or less cycles of MOPP had ovarian failure (Ortin et al. 1990). Females who received, both before and after pubertal development, high-dose myeloablative therapy with alkylating agents such as busulfan, melphalan, and thiotepa in preparation for bone marrow transplantation are at high risk of developing ovarian failure (Michel et al. 1997). Recovery of function has been recorded only rarely (Michel et al. 1997, Thibaud et al. 1998). However, the majority of girls receiving standard chemotherapy maintain or recover ovarian function during the immediate posttreatment period (Horning et al. 1981, Sklar 1999). Histologic examination of ovarian tissue in prepubertal and postpubertal girls treated for solid tumors or leukemia nevertheless revealed a decreased number of ovarian follicles and inhibition of follicular growth compared with age-matched controls (Himelstein- Braw et al. 1978, Larsen et al. 2003). Therefore, among women who retain or recover ovarian function following treatment with ChT, a subset will experience premature menopause when they reach their 20s and 30s (Byrne et al. 1992, Sklar et al. 2006). In a report from the CCSS, female survivors with a history of exposure to high doses of alkylating agents, to lomustine or to cyclophosphamide were less likely to become pregnant when compared with sibling controls (Green et al. 2009). No adverse pregnancy outcomes were identified, however in a large study conducted within the framework of the CCSS (Green et al. 2002).

The aim of our study was: to establish the incidence of hypogonadism and the risk factors for its developement in childhood-cancer survivors in Slovenia and define the highest respective the lowest risk groups.

2. Patients and methods

2.1 Patients

In Slovenia, 1474 children were treated for cancer under the age of 16 years from 1.1.1965 to 31.12.1995 at the University Clinical Hospital Ljubljana and/or Institute of Oncology Ljubljana. At the time of our study 712 patients were alive, 460 of them were more than 16 years of age and were at least 3 years off treatment. Of those patients 390 were regularly followed at the outpatient clinic at the Institute of Oncology in Ljubljana (Jereb 2000, Zaletel 2004). We included in our study 297 consecutive patients in whom endocrinological evaluation was performed until 1.1.2003. Ninety-three patients refused examinations. There were 115 females and 182 males. They were 0-16 (median 9 yrs) years of age at the diagnosis of malignancy and had endocrinological evaluation 3-32 (median 11,5) years after the end of treatment at age of 14-42 (median 20) years. All pts were pubertal or postpubertal when studied. Distribution of diagnosis among patients included in our study is shown in table 1.

The majority (90%) of patients had combined treatment including 2-3 modalities, surgery (S), ChT, RT, a quarter of them had all 3 modalities, while 41% had combined ChT and RT. To evaluate the risk factors for hypogonadism after treatment for childhood cancer, we used a multivariate analysis method of the classification trees.

DIAGNOSIS	Males	Females	All	
	n	n	n (%)	
Leukemia	30	37	67	(22.5)
Hodgkin's disease	40	24	64	(21.5)
Brain tumor	30	18	48	(16)
NHL	35	3	38	(13)
Soft tissue sarcoma	16	8	24	(8)
Wilms' tumor	11	7	18	(6)
Bone sarcoma	6	6	12	(4)
Germ cell tumor	4	6	10	(3.5)
Neuroblastoma	4	1	5	(1.5)
Retinoblastoma	2	3	5	(1.5)
Carcinoma of nasopharynx	2	1	3	(1)
Other ♣	2	1	3	(1)
All	182	115	297	(100)

♣ retroperitoneal paraganglioma, hepatoblastoma, invasive adenoma of suprarenal gland, one each

Table 1. Diagnosis in 297 patients

2.2 Methods

2.2.1 Assessment of gonadal function

The patient´s data regarding diagnosis and treatment were collected from medical files, information concerning quality of life including attained educational level, marital status, employment and social life, past and present menstrual histories, the course of puberty and fertility histories was ascertained by interview. General physical examination was performed in terms of recording height, weight, clinical abnormalities and Tanner stages of pubic hair and genital development were recorded. Each patient´s blood samples were analysed for basal concentrations of testosterone (RIA, IMUNOTECH), estradiol (DELFIA-LKB) and prolactin (DELFIA-LKB). Concentrations of LH (DELFIA-LKB) and FSH (DELFIA-LKB) were determined before and 10, 20, 30, 60 minutes after i.v. administration of gonadotropin releasing hormone (50 mcg/m^2) (LH-RH). Primary hypogonadism (PH) was defined as basal serum FSH and/or LH level above the normal upper limit and exaggerated response after stimulation with LH-RH. In men, elevated basal serum FSH levels indicated germinal epithelium damage (GE-DA), while elevated LH levels (with/without reduced testosterone levels) indicated Leydig cells (LC) damage (LC-DA). Normal basal values of LH and/or FSH and exaggerated response after LH-RH stimulation were considered as subclinical impairment (SIG). Exaggerated response of FSH after LH-RH was considered as dysfunction of germinal epithelium (GE-dys), while exaggerated response of LH after LH-RH was considered as dysfunction of LC (LC-dys). PH and SIG together were named gonadal impairment (GI). Low serum basal FSH and LH levels with poor response after i.v. bolus of LH-RH was considered as secondary hypogonadism.

2.2.2 Classification tree analysis

Classification tree analysis is a multivariate analysis method that allows for studying of simultaneous influence of a series of independent variables on a single dependent variable (Jereb 1973). The output of the analysis is a classification tree, read from the root node, through the internal nodes all the way to the leaves. In each internal node, a test on the value of a single independent variable for the given case is being performed. Based on the outcome of the test, we follow one of the branches originating from the node. Following the branches in that manner, we arrive in one of the leaf nodes of the tree that provides a classification, i.e., the predicted value of the dependent variable, of the case at hand. In addition to predicting the value of the dependent variable for a given case, the structure of the classification tree also reveals the influence and relative importance of the values of independent variables on the dependent one.

Classification tree is being constructed by successive divisions of the original group of cases into pairs of subgroups, where each division is based on the value of a single independent variable. For each division (often referred to as a split), the variable is being selected that produces "pure" subgroups; the purity being measured as a fraction of cases with the same value of the dependent variable. In ideal case, a completely "pure" group of cases that share the same value of the dependent variable is obtained. Each of the subgroups generated in the process becomes a parent group in the next step of the analysis and is further divided in the same way. The division of cases stops when the group of cases is completely pure or when it contains less than a user-defined minimal number of cases. In our study, the C4.5 (Quinlan 1993) program for constructing classification trees was used. C4.5 allows the setting of several parameters that influence branching and quality of the final classification tree: most notably there is one parameter that determines the smallest number of cases to be included in a single group, and another parameter that determines the degree of the tree post-pruning performed. For details please refer to the description in (Quinlan 1993). The optimal values of these parameters were determined using a standard cross-validation method (Jazbec et al. 2007, Macedoni-Lukšič et al. 2003, Velensek et al. 2008). The usual performance measure for classification trees is the accuracy of the tree when predicting the outcome (the value of the dependent variable) on samples not seen during the process of tree building.

Note finally, that since we use an alternative performance criterion, the classification tree obtained the cross-validation procedure outlined above is not expected to provide accurate classification of cases into hypogonadism and non-hypogonadism classes. Instead of using the tree as an accurate predictor, we were interested in analyzing the tree structure and identifying the risk group where incidence of hypogonadism is significantly higher than the one observed in the population of 297 patients included in the study. Multivariate analysis with classification tree was not done when specific abnormalities were found in less than ten percent of examinated childhood cancer survivors.

Multivariate statistical analysis with classification tree analysis was performed with two groups of independent variables and their values. The first group included six independent variables:

- gender (female, male)
- age at diagnosis (in years),
- type of malignancy (1-12, see Table 1),
- surgery (yes, no)
- radiotherapy (yes, no)
- chemotherapy (yes, no).

In the second group of independent variables, variables from the first group were further broken down (type of surgery, parts of the body, which was irradiated, type of ChT), because we wanted to determine the effect of various treatments on the gonadal function. The observation time, i.e. the time from the end of treatment to the gonadal evaluation, was added as a new variable. Variables of the second groups were:

- gender (female, male)
- age at diagnosis (in years),
- type of malignancy (1-12, see Table 1),
- surgery (no, outside the abdomen, abdominal surgery, orchidectomy, ovariectomy),
- radiotherapy (no, brain RT, RT above the diaphragm except the brain, RT of the upper abdomen, pelvic RT, testicular RT)
- chemotherapy (no, ChT without AA, ChT with AA),
- observation time.

We analyzed the influence of both groups of independent variables to each of the two dependent of PH and GI (i.e., PH and/or SIG) in three different groups of patients: all patients, females and males (impairment of LC, LC-DA and LC-dys as well as impairment of germinal epithelium, GE-DA and GE-dys).

3. Results

Primary hypogonadism was found in 76 (26%) adolescents, in 62 (34%) males and 14 (12%) females. Gonadal impairment was found in 114 (38%) adolescents, in 89 (49%) males and 25 (22%) females (Table 2).

Diagnosis	Males		Females		All	
	All (n)	Pts with PH n (%)	All (n)	Pts with PH n (%)	All (n)	Pts with PH n (%)
Leukaemia	30	5 (16.5)	37	1 (2.7)	67	6 (9)
Hodgkin's disease	40	26 (65)	24	6 (25)	64	32 (50)
Brain tumor	30	3(10)	18	2 (11)	48	5 (10)
NHL	35	12 (34)	3	0	38	12 (32)
Soft tissue sarcoma	16	7 (44)	8	1 (12.5)	24	8 (33)
Wilms' tumor	11	2 (18)	7	0	18	2 (11)
Bone sarcoma	6	2 (33)	6	1 (16.5)	12	3 (25)
Germ cell tumor	4	2 (50)	6	3 (50)	10	5 (50)
Neuroblastoma	4	2 (50)	1	0	5	2 (40)
Retinoblastoma	2	1 (50)	3	0	5	1 (20)
Carcinoma of nasopharynx	2	0	1	0	3	0
Others ♣	2	0	1	0	3	0
All	182	62 (34)	115	14 (22)	297	76 (26)

♣ retroperitoneal paraganglioma, hepatoblastoma, invasive adenoma of suprarenal gland, one each
PH - primary hypogonadism

Table 2. Primary hypogonadism versus diagnosis and gender in 297 patients.

All but one male subjects with PH had damage of germinal epithelium (15 of them at the same time damage of LC, 30 of them at the same time dysfunction of LC), with one failure, we found LC and DKE. In 12 of the 61 patients with germinal epithelium damage semen analyses was performed; in 11 patients azoospermia was found, one had normal spermiogram. Dysfunction of LC was detected in 54 patients (in 21 patients the only finding), dysfunction of germinal epithelium was found in 9 patients. All 14 female patients with PH had elevated basal FSH and FSH after stimulation, 5 of them had elevated basal LH and LH after stimulation, four had increased LH after stimulation, 6 had decreased levels of estradiol. Among 4 patients with PH who were treated in prepubertal period, two had delayed puberty. Ovarian dysfunction was detected in 11 patients, all had elevated levels of LH after stimulation, 6 had elevated FSH after stimulation as well. All had normal serum estradiol. The highest incidence (50%) of PH was found in those patients treated for Hodgkin's disease (HD) or germ cell tumor (GCT), the lowest incidence (10%) was found in those treated for brain tumors, leukemia and Wilms' tumor (Table 2). The incidence of PH depended on type of treatment as well. The highest proportion of PH (26 - 40%) was found in patients treated with combined treatment, t.i. ChT and RT with / without surgery (Table 3).

Type of treatment	All	Pts with PH n (%)
RT + ChT	121	31 (26)
S + RT+ ChT	76	31 (40.5)
OP + RT	38	4 (10.5)
OP + ChT	31	5 (16)
ChT	14	2 (14)
S	9	0
RT	8	2 (22)
All	297	76 (26)

PH - primary hypogonadism, RT - radiotherapy, ChT- chemotherapy, S – surgery

Table 3. Primary hypogonadism versus type of treatment in 297 patients.

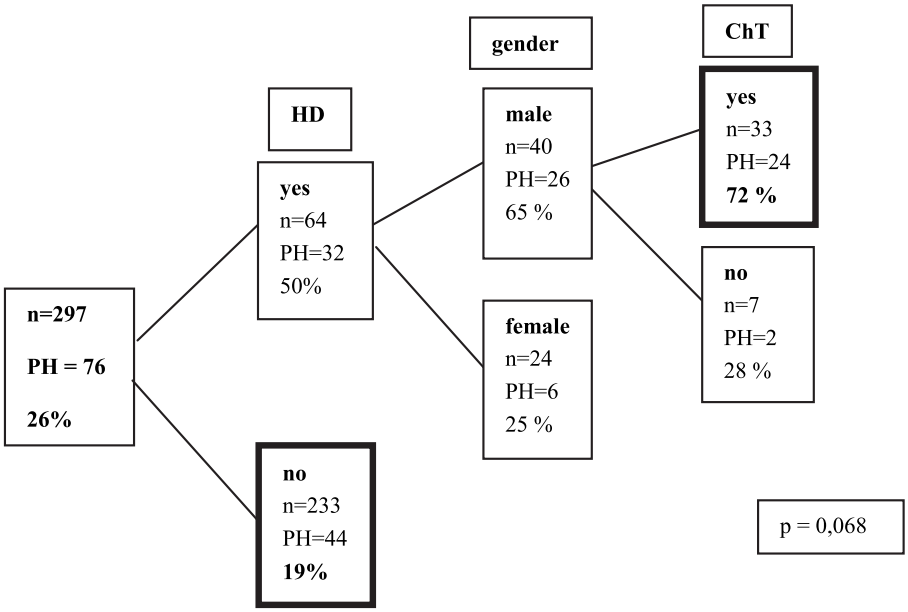
Secondary hypogonadism was found in 6 patients. Three of them had panhypopituitarism after treatment of hypothalamic tumor (2 patients) or orbital tumor (one patient) with surgery and RT (from 4400 to 5000 cGy), 2 patients were treated for brain tumors with surgery and RT (5500 or. 6500 cGy), 1 patient was treated for leukemia with ChT and brain RT (3000 cGy).
Sixty-seven patients had whole brain irradiation with a dose of 1200 - 4000 (median 2400) cGy in prepubertal period. Six patients had precocious puberty; 5 girls after treatment of leukemia and 1 boy after treatment of NHL. Those 6 patients were treated with ChT and brain RT at the age of 5 to 8 years. In all 6 the dose of ionizing radiation to the brain was equal to or greater than 2400 cGy (2400 - 3400, med. 2400 cGy).

3.1 Results of classification tree analysis
3.1.1 First group of independent variables

3.1.1.1 Dependent variable – primary hypogonadism, all patients

PH was found in 76 (26%) of 297 patients. The most important risk factor for PH, which divided the basic group into two subgroups, was diagnosis of HD (Figure 1). The second

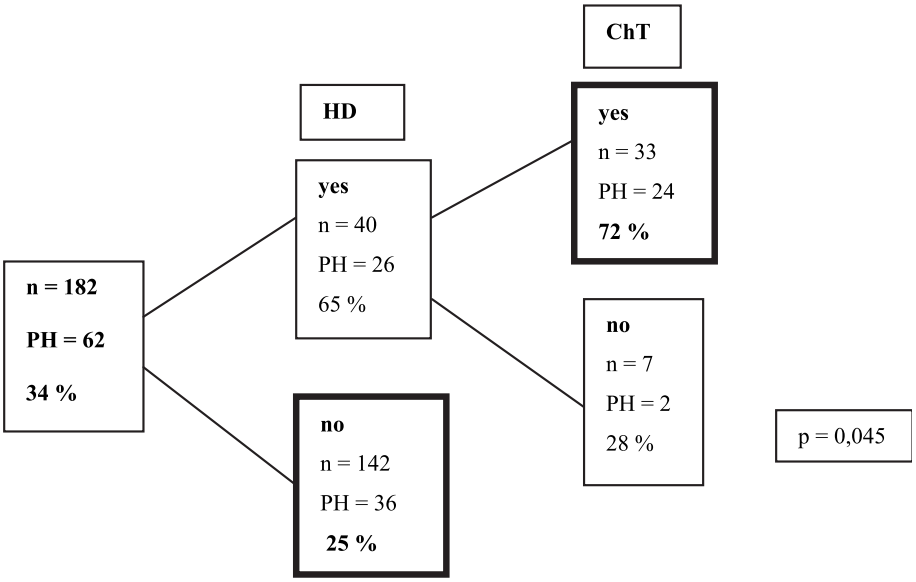
most important factor which divided a group of adolescents, treated for HD, was gender, and the next still important independant variable was therapy with ChT. Other independent variables from the first group didn't emerged as important risk factors for PH. Therefore, with this analysis we defined a group of 33 (11% of all) patients with the highest (72%) risk of PH; these are men treated for HD with ChT. Two hundred thirty-three patienst, who did not have HD, had low (19%) risk of PH. Low risk (25%) of PH was found also in the group of female patients, treated for HD.



PH – primary hypogonadism, ChT – chemotherapy, HD – Hodgkin's disease

Fig. 1. Classification tree analysis with first group of independent variables and PH as dependent variable in 297 patients

Statistical significance of this analysis was borderline (p = 0,068). We performed the analysis with the same independent variables only for males and it confirmed the results of previous analysis with statistically significance p = 0.045 (Figure 2). Namely, once again in the group of patients with the highest risk of PH (72%) were those treated for HD with ChT. Patients treated for other types of cancer, had a risk of PH of only 25%. Of the seven male patients treated for HD without ChT, only two had PH. Both were treated with pelvic RT.



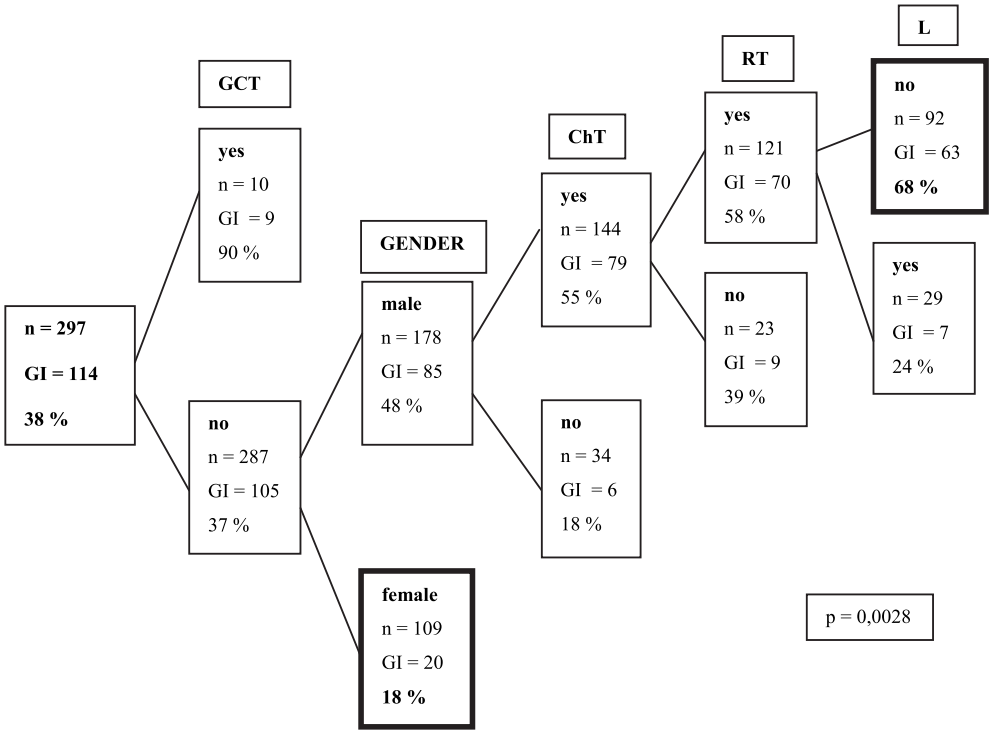
PH – primary hypogonadism, ChT – chemotherapy, HD – Hodgkin's disease

Fig. 2. Classification tree analysis with first group of independent variables and PH as dependent variable in 182 male patients

3.1.1.2 Dependent variable – gonadal impairment (GI, gonadal damage and subclinical impairment) in all patients

GI was found in 114 (38%) adolescents. Independent variable type of diagnosis, germ cell tumor, turned out as the most important risk factor for GI. Namely, 9 out of 10 patients treated for GCT had GI. In the remaining 287 patients the most significant risk factor for GI stood gender (Fig. 3). Males had 48% and females had 18% risk of GI. The group of male patiens further divided by important risk factors for GI: ChT, RT and diagnosis of cancer other than leukemia. With this analysis, we therefore defined the group of patients at highest, 68%, risk of GI: 92 male patients who were treated with ChT and RT for cancer other than leukemia. The lowest, 18%, risk of GI, had two groups of patients; group of females (excluding GCT) and group of 34 male patients who did not receive ChT.

In this multivariate analysis the highest (90%) risk of GI had a group od 10 patients (4 males, 6 females) treated for GCT; 5 patients had PH, 3 females after bilateral ovariectomy abdominal RT (one) and 2 males, one after unilateral orchidectomy and ChT with AA for testicular GCT, and the second after ChT with AA for mediastinal GCT. Four patients (2 females and 2 males) had SIG – all having been treated by unilateral removal of the ovary or testis and ChT with AA. The only patient of this group with normal function of the gonads was a female treated for GCT by unilateral ovariectomy and ChT (including bleomycin, etoposide, cisplatin and ifosfamide) at the age 13. Cumulative doses of AA were comparable to those received by the girls with GI following unilateral ovariectomy (ages 9 and 14 years). So, in this group of patients at high risk of GI gonadal surgery and ChT with AA seems to be important risk factors for gonadal impairment.



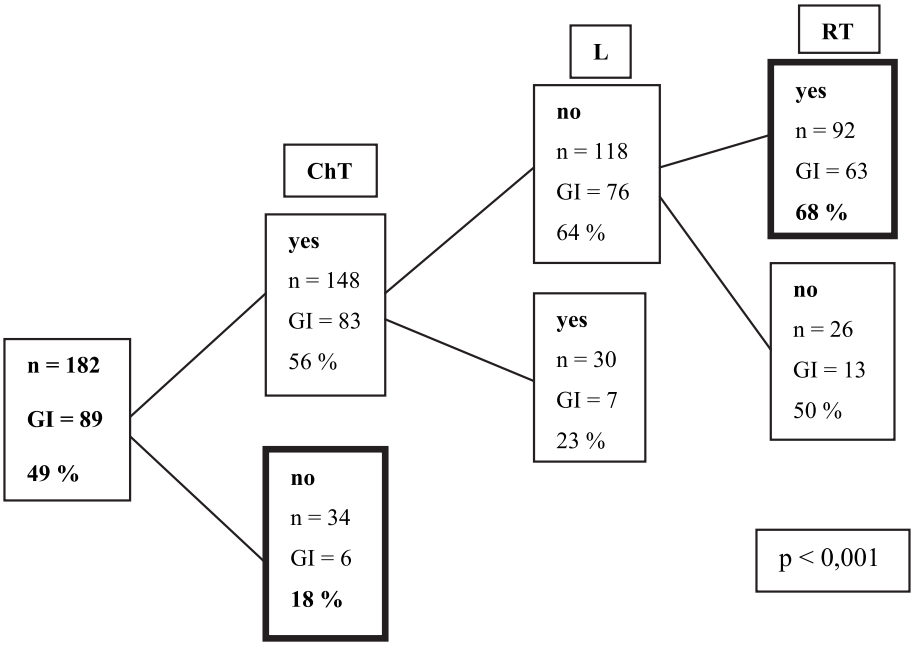
GI – gonadal impairment, GCT – germ cell tumor, L – leukemia, ChT – chemotherapy, RT – radiotherapy

Fig. 3. Classification tree analysis with first group of independent variables and GI as dependent variable in 297 patients

We looked more in detail at the group of 29 male patient, after the last division in the classification tree analysis, the risk factor being diagnosis: leukemia. Less than half of them received ChT with AA, two of them had testicular RT, none had pelvic RT. In 5 patients PH was diagnosed; 2 after testicular RT, 3 after ChT with AA - 2 received the highest cumulative dose of CY and cytarabine in their group (7 g/m2 and 9.5 g/m2). Two patients had subclinical gonadal impairment (SIG) after ChT with AA and/or cytarabine. On the contrary, in the other group of 92 patients, treated for other malignancies, with the highest proportion of GI, as many as 90% received ChT with AA and a quarter of them had pelvic RT. Thirty-one of 46 patients with PH received ChT with AA, 14 ChT with AA and pelvic RT (1500 to 4000 cGy), one was treated with ChT without AA and RT to the whole abdomen (1400 cGy). Fourteen out of 17 patients with SIG received ChT with AA (one pelvic RT as well), 3 patients received ChT without AA, but had RT of the whole abdomen. Therefore the most significant risk factors for GI in our patients were beside the diagnosis of GCT male gender and therapy with ChT and RT. In the group with the highest risk of GI among risk factors has stood out ChT with AA and pelvic RT (and gonadal surgery and ChT with AA in the group of patients with GCT). In the group with low risk of GI mainly ChT with AA and testicular RT emerged as risk factors.

3.1.1.3 Dependent variable – gonadal impairment (GI) in male patients

The analysis confirmed the results of the previous analysis. The largest, 68%, risk of GI, had a group of men, who were treated for cancer other than leukemia with ChT and RT (Fig. 4).



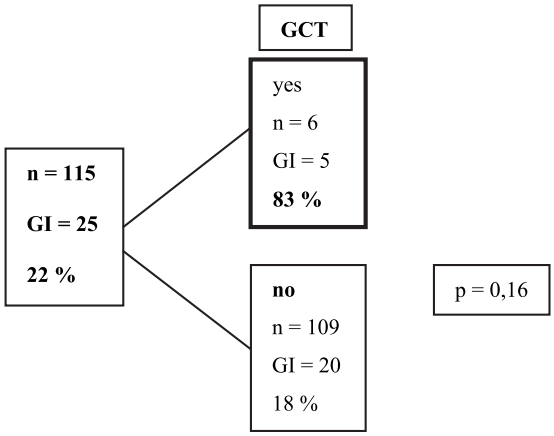
GI – gonadal impairment, L – leukemia, ChT – chemotherapy, RT – radiotherapy

Fig. 4. Classification tree analysis with first group of independent variables and GI as dependent variable in 182 male patients

We looked more in detail at the group of 34 males who did not receive ChT. Six patients had GI. Three of them had PH, all after pelvic RT (3000 - 4000 cGy). Three patients had SIG after being treated for brain tumors, 2 with surgery only, one with brain RT. Of the remaining 28 patients of this group (with normal gonadal function six had pelvic RT (2800 to 4800 cGy). Therefore, in the group of patients with low risk of GI (without therapy with ChT) mainly pelvic RT emerged as risk factor.

3.1.1.4 Dependent variable – gonadal impairment (GI) in female patients

Classification tree analysis with first group of independent variables and GI as dependent variable was performed for the cohort of 115 female patients as well. It had only one division, the only risk factor being diagnosis GCT. Six patients treated for GCT were at high risk (83%) for GI. The other group of 109 patients was not further divided (Fig. 5). This tree was not significantly different from random predictions, probably because of a very small number of positive outcomes and some other independent variables relevant for ovarian failure, which are not yet known.



GI – gonadal impairment, GCT-germ cell tumor

Fig. 5. Classification tree analysis with first group of independent variables and GI as dependent variable in 115 female patients

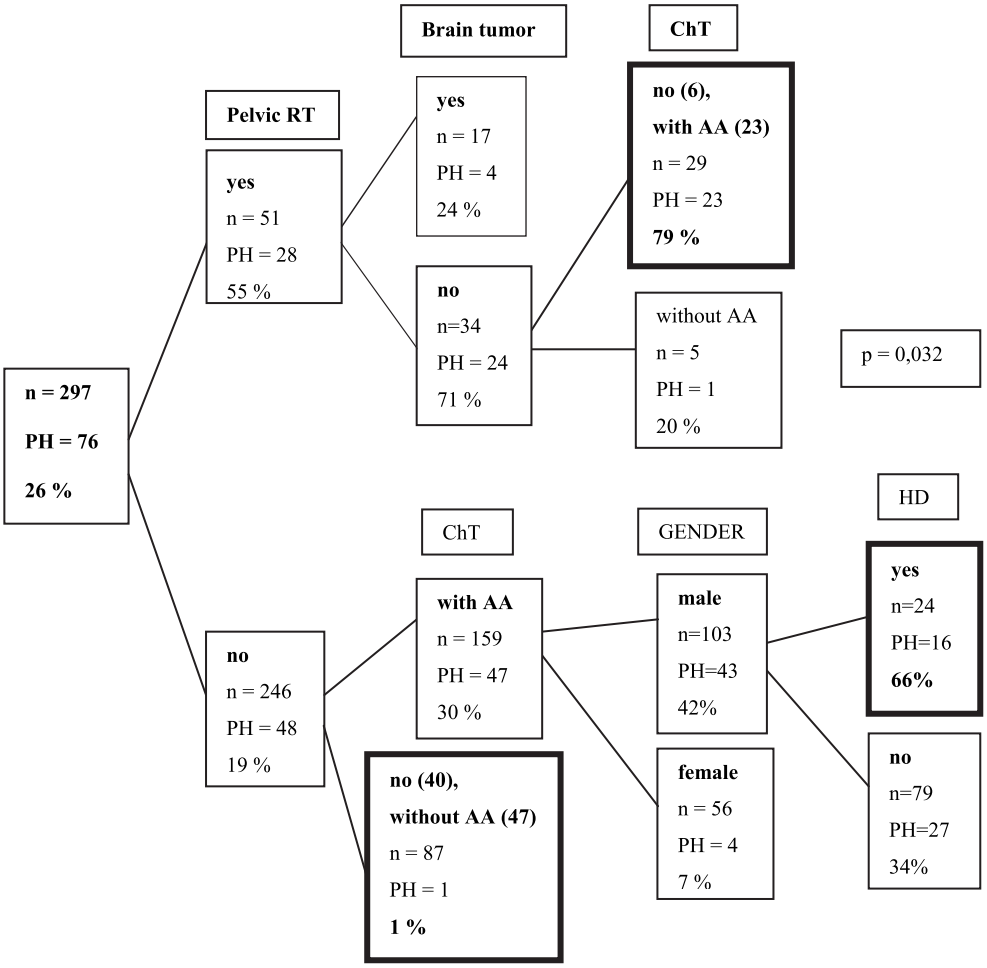
3.1.2 Second group of independent variables

3.1.2.1 Dependent variable – primary hypogonadism in all patients

Again, all patients were included in the analysis.. The most important risk factor for PH turned out to be pelvic RT (Fig. 6). Risk for PH in patients, treated with pelvic RT, was 55% and 19% only in the other group. The second most important factor which divided the group of 51 patients, who had pelvic RT, was type of diagnosis, and the third one treatment with ChT.

We defined a group of 29 patients with the highest, 79%, risk of PH, namely those who had pelvic RT, didn't have diagnosis of brain tumor and were treated with ChT including AA or did not receive ChT at all. In the group of 246 patients who didn't have pelvic RT, ChT with AA emerged as the most important risk factor for PH, followed by male gender and diagnosis of HD. Similar to the analysis with the first group of independent variables (Fig.1) we identified a group of 24 patients with the highest risk of PH (66%) among those patients who didn't have pelvic RT; t.i. males treated for HD with ChT including AA. We defined a group of patients with the lowest, 1%, risk of PH. Those were 87 patients who had neither pelvic RT nor ChT with AA (PH was found in one patient only after being treated with testicular RT). Low risk of PH (7%) had as well a group of 56 females who received ChT with AA, but didn't have pelvic RT. Males treated with ChT with AA but without pelvic RT, had much higher risk of PH (42%), suggesting that ChT with AA presented greater risk factor of PH in males than in females.

The highest risk for PH (79%) had the group of patients treated for cancer other than brain tumor, with pelvic RT and ChT with AA (23 patients) or without ChT (6 patients). Only 6 patients in this group didn't develop PH; 2 females treated with unilateral RT to iliaco-inguinal region (2400 cGy) and ChT with AA for HD (one female with 2 relapses 6 cycles of LOPP (chlorambucil, vincristine, procarbazine and prednisone), 6 cycles of MOPP-ABV and 6 cycles of ABVD). Among 4 males without PH one received RT to both iliacal regions (3000 cGy) and 2 cycles of MOPP ChT, one received RT to the left femur and iliac bone (4800 cGy) for hondrosarcoma of the iliac bone, and 2 were treated for NHL of the caecum with



PH – primary hypogonadism, AA – alkylating agents, ChT – chemotherapy, RT – radiotherapy, HD – Hodgkin's disease

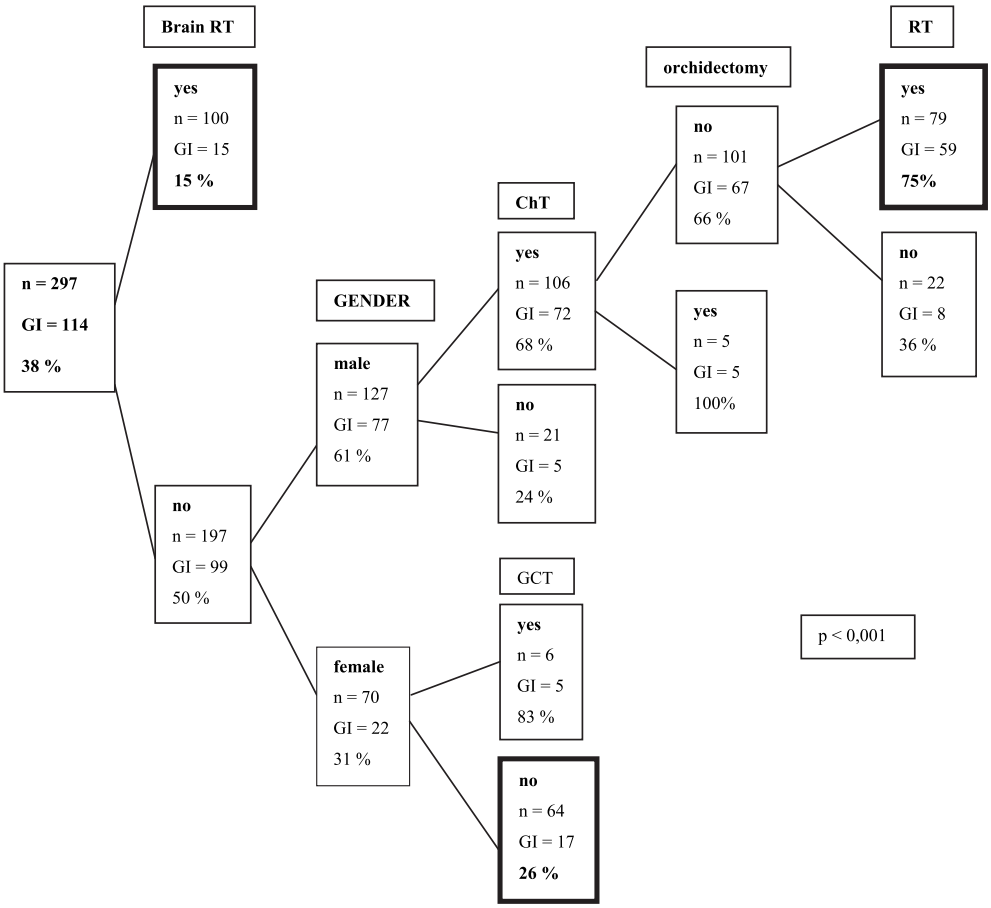
Fig. 6. Classification tree analysis with second group of independent variables and PH as dependent variable in 297 patients

surgery, ChT with AA and RT (one had 900 cGy to the whole central nervous system, the other had abdominal RT with 3000 cGy). Of the 6 patients in the group not receiving ChT with AA, as many as five had PH; 3 of them had whole abdominal RT, 2 RT of bilateral iliacoinguinal regions. Among patients who had pelvic RT, a group of 17 patients with low proportion of PH emerged. They were treated for brain tumor by craniospinal RT (600-4400 cGy), 7 of them also received ChT with AA. All 4 patients with PH were treated for medulloblastoma, 3 of them received, in addition to craniospinal RT, ChT with Procarbazine or CCNU. The risk of PH in patients treated with craniospinal RT and ChT with AA was therefore 43%, while in those treated with craniospinal RT without ChT with AA, was only

10%. With this statistical analysis we found that the significant risk factors for PH were pelvic RT, ChT with AA, male gender and diagnosis of HD. In the group with a lower risk of PH craniospinal RT and ChT with AA emerged as important risk factors for PH.

3.1.2.2 Dependent variable – gonadal impairment (GI) (t.i. gonadal damage and subclinical impairment)

Gonadal impairment was found in 114 (38%) adolescents. The most significant risk factor for GI was RT (Fig. 7). The group of 100 patients who had brain RT only had the lowest (15%) risk of GI, in the group of the remaining 197 patients, who were irradiated to any other part of the body or had no RT, the risk of GI was 50%. Similar to the analysis of the first set of variables male patients treated with ChT and RT stood out as a group with the highest (75%) risk of GI.



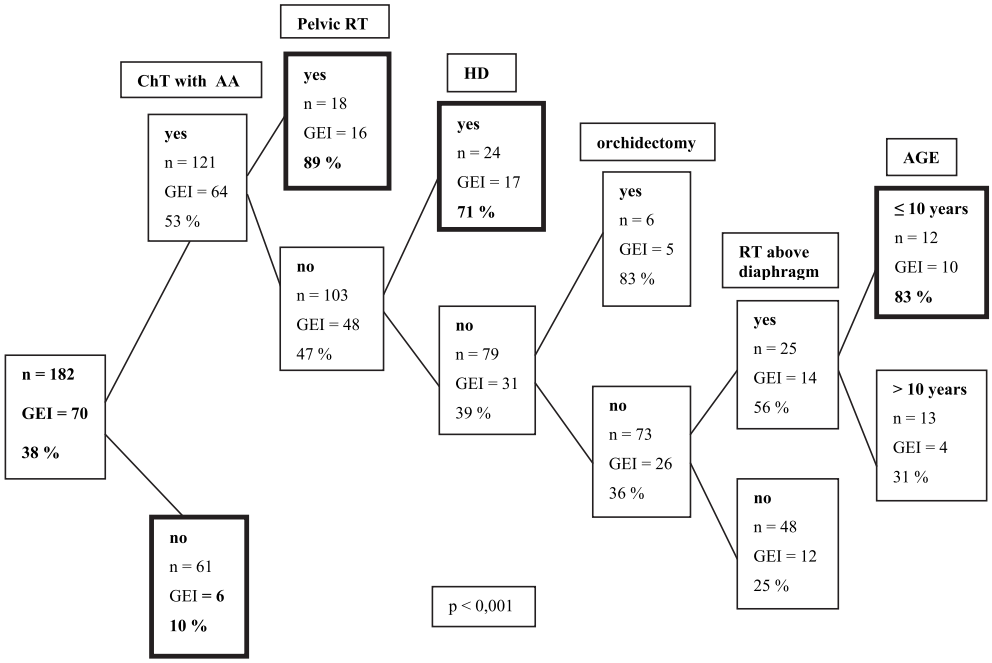
GI – gonadal impairment, AA – alkylating agents, GCT – germ cell tumor, ChT – chemotherapy, RT – radiotherapy

Fig. 7. Classification tree analysis with second group of independent variables and GI as dependent variable in 297 patients

We looked more in detail at the group of 100 (63 males, 37 females) patients with the lowest risk of GI who had only brain RT; 69% of them were treated for ALL, 27% for brain tumors, 9% for NHL, 4% for soft tissue sarcoma. Only 45% of this group of patients received ChT with AA. All 7 males who had PH, received ChT with AA, as did also 6 of 8 patients (3 females, 5 males) with SIG. In the second division of decision tree the subgroup of male patients had 61% risk of GI and the subgroup of females only 31%, although a similar proportion of patients in both subgroups received ChT with AA (72%: 75%) or did not receive ChT (28%: 25%) and the same (27%) proportion of patients had pelvic RT.
Therefore, in this statistical analysis the most important risk factors for GI turned out to be: male gender, treatment with ChT with AA and RT and orchidectomy.

3.1.2.3 Dependent variable - impairment of germinal epithelium GEI, t.i. germinal epithelium damage (GE-DA) and germinal epithelium dysfunction (GE-dys) in male patients

In this analysis as the most important risk factor for GEI emerged ChT with AA (Fig. 8). Only 10% of males who didn't receive ChT with AA had GEI (all 6 patients in this group who had GEI, had pelvic or testicular RT). The second most important factor that divided the group of 121 males who received ChT with AA, was pelvic RT. The risk of GEI in the group of 18 patients who had pelvic RT was as high as 89%. The next most important risk factor, which divided the group of patients who received ChT with AA and didn't have pelvic RT, was diagnosis HD. It is the same result as above when analyzing all patiens (with dependent variable PH), a group of 24 patients treated for HD with ChT with AA but without pelvic RT (71% risk of GEI). In the other group of patients treated for other malignancies than HD were at greater risk of GEI those who had RT above the diaphragm and were at the age of 10 years or less at the time of treatment (83% risk of GEI). In this analysis the most important risk factors for GEI turned out to be: ChT with AA and pelvic RT.
In the group of 18 patients who received ChT with AA and had pelvic RT, only 2 patients had normal gonadal function; one patient received 2 cycles of MOPP and RT of iliac regions with a dose of 3000 cGy for HD, the other had craniospinal RT with 900 cGy and ChT following BFM protocol (including 7 g/ m 2 of CY and 3, 2 g/m2 of cytarabine) for NHL. High, 71% the risk of GEI, had a group of 24 patients with HD treated with ChT with AA without pelvic RT. Seven patients of this group didn't have GEI. They received ChT following protocol LOPP (6 cycles), MOPP (1 to 4 cycles) or OPPA (2 cycles).
At the last division of this tree patients age at treatment emerged as risk factor for GEI. The group of 25 patients who were treated for HD with ChT with AA and RT above the diaphragm, was divided into those who were 10 or less years of age at diagnosis (83% risk of GEI), and into the group of older patients (31% risk of GEI). The younger patients received a slightly higher cumulative dose of CY (2.8 - 40, med. 10 g/m2) than the group of older patients (1.4 - 16, med. 5 g/m2), doses of cytarabine were approximately equal in both groups. Among patients without GEI in the group of younger patients there was one who received CY 15 g/m2. Taking in account that the two age groups have different cumulative doses of AA, we can not consider patient's age at treatment as an important risk factor for GEI.
Therefore, in this analysis the most important risk factors for damage or dysfunction of germinal epithelium were ChT with AA, pelvic RT, diagnosis HD and orchidectomy.



GEI - impairment of germinal epithelium, AA – alkylating agents, ChT – chemotherapy, RT – radiotherapy, HD – Hodgkin's disease

Fig. 8. Classification tree analysis with second group of independent variables and GEI (impairment of germinal epithelium) as dependent variable in 182 male patients

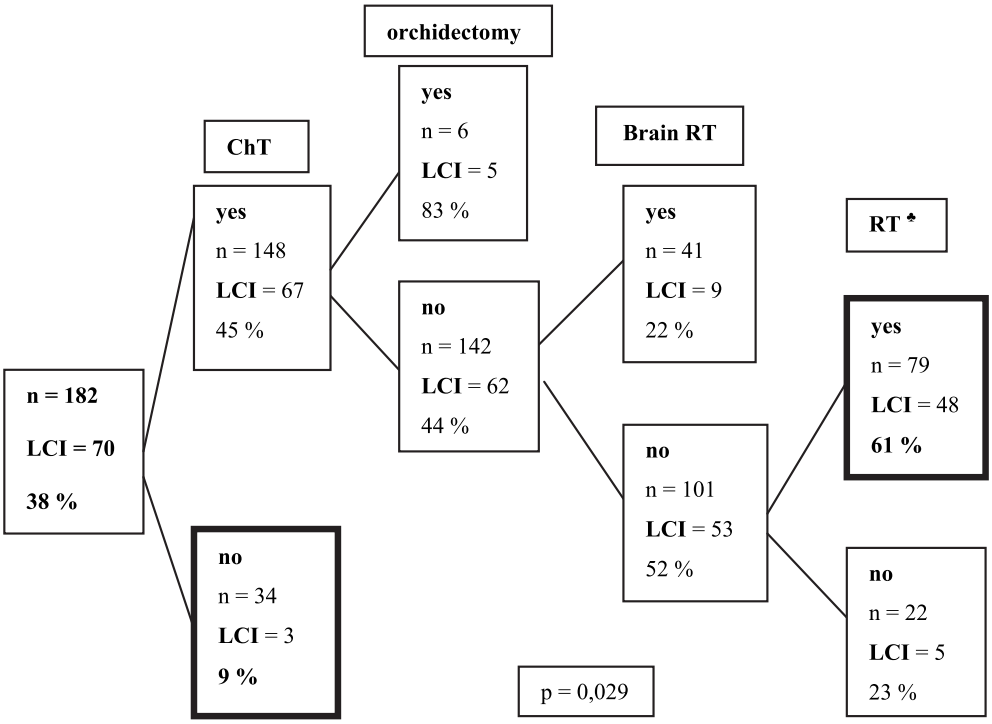
3.1.2.4 Dependent variable - impairment of Leydig cells (LCI), t.i. damage of LC (LC-DA) and dysfunction of LC (LC-dys) in male patients

In this analysis as the most important risk factor for LCI stood out treatment with ChT (Fig. 9). All but three patients with LCI received ChT. In the group of 148 patients who received ChT, 67 patients had LCI; all but the 6 of them received ChT with AA (13 of them also had pelvic RT, one testicular RT). Five of the 6 patients not receiving ChT with AA had abdominal RT.

The highest, 83%, risk of LCI was in the group of patients, who received ChT and had orchidectomy. Similar to the above analysis (GI in all patients), we defined a group of 79 patients with high risk for LCI (61%); namely patients treated with ChT and RT of other regions than brain. Eleven patients in this group had both, damage of GE and damage of LC (2 after testicular RT, 3 after ChT with AA and pelvic RT, 6 after the ChT), 37 patients had dysfunction of LC; 24 of them with the damage of GE as well (15 after ChT with AA, 8 after ChT with AA and pelvic RT, one after pelvic RT), 13 patients had an isolated finding (10 after ChT with AA, 3 following abdominal RT).

In the group of 22 patients treated with ChT, but without RT (with 23% risk of LCI), all 5 patients with LCI had only dysfunction of LC and all received ChT with AA. In the group of 34 patients with the lowest, 9%, risk of LCI, patients didn't receive ChT; only 3 patients had LCI (one patient following abdominal RT, 2 patients after brain RT).

Therefore, in this analysis orchidectomy and therapy with ChT and RT emerged as the most important risk factors for LCI.



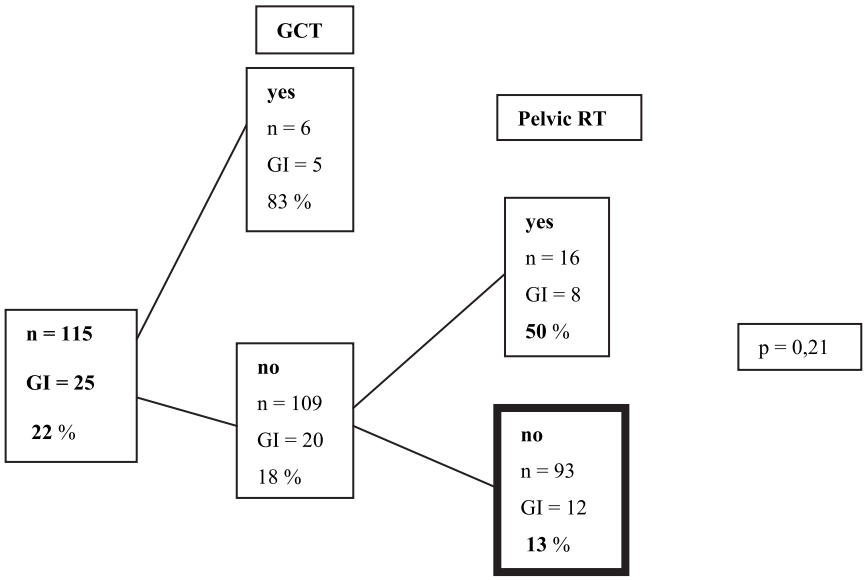
LCI - impairment of Leydig cells, ChT – chemotherapy, RT♣ – radiotherapy to other region than brain

Fig. 9. Classification tree analysis with second group of independent variables and impairment of Leydig cells (LCI) as dependent variable in 182 male patients

3.1.2.5 Dependent variable –gonadal impairment (GI) in female patients, t.i. damage of ovarian function and dysfunction of ovarian function in female patients

The decision tree with the second group of independent variables (Fig. 10) was more branched than the one using the first group of independent variables (Fig. 5), although this tree was not significantly different from statistical random predictions. Similar to the previous tree the highest risk for GI was observed in the group of females treated for GCT (GI in 5 out of the 6 patients). The remaining group of 109 patients with 18% risk of GI, treated for other malignancies, was further divided by the most important risk factor - pelvic RT. In the group of 16 patients treated with pelvic RT risk of GI was 50%. As many as 7 out of the 8 patients with GI had damage of ovarian function, t.i.PH (one following RT of the whole abdomen (2000 cGy) and ChT with AA for leukemia, 2 after craniospinal RT (3400 and 3600 cGy) and ChT with AA (only one) for medulloblastoma, 2 after RT of unilateral iliac region (3000 and 2400 cGy) and ChT with AA for HD, 2 following pelvic RT (3000 and 4000 cGy) and ChT with AA for sarcoma. One of the patients in this group had only ovarian dysfunction after craniospinal RT (2400 cGy) and ChT without AA for leukemia. The risk of

GI in the subgroup of 93 female patients without pelvic RT was as low as 13%. Only 4 out of 12 patients with GI in this group had damage of ovarian function (all after ChT with AA), 8 patients had only ovarian dysfunction (4 following ChT with AA).
In the group of 6 females treated for GCT at the age of 9 to 15 (med. 13) years, 5 had GI; 2 female patients had bilateral ovariectomy, one had ovarian damage after unilateral ovariectomy and abdominal RT, two females had dysfunction of ovarian function after unilateral removal of the ovary and ChT with AA.
In females, therefore, the most important risk factors for ovarian failure turned out to be pelvic RT, ChT with AA and ovariectomy.



GI – gonadal impairment, GCT – germ cell tumor, RT - radiotherapy

Fig. 10. Classification tree analysis with second group of independent variables and gonadal impairment as dependent variable in 115 female patients

General observations

Forty-six patients (21 males, 25 females) in our study had no PH, despite treatment with either ChT including AA or pelvic or testicular RT. Thirty-four (19 males, 15 females) of them were treated with ChT, which contained the antimetabolites methotrexate and 6-mercaptopurine, VCR, corticosteroids with or without L-asparaginase and adriamycin. Eleven patients (2 males and 9 females) received ChT containing actinomycin D (AMD) and vincristine (VCR) with or without adriamycin. One girl received ChT with vinblastine. Six patients (3 males and 3 females) out of 46 in this group of patients had subclinical impairment of the gonads (males had dysfunction of LC only) and as many as 4 patients (2 males and 2 females) had ChT with AMD and VCR. The impact of antimetabolite cytarabine on gonadal function could not be assessed because this cytostatic was administered to patients as part of ChT protocols, which contained AA (especially CY). So, we can conclude on the basis of our cohort of patients that ChT with antimetabolites (other

than cytarabine), antibiotics and vinca alkaloids wasn't toxic to germinal epithelium of testes, but primarily AMD and VCR may cause mild failure of ovaries and LC.
Chemotherapy regimens usually include several cytostatics, to study the toxic effect of each one on the gonads was therefore not possible. In our study, one male patient received the largest cumulative dose of cyclofosfamide 24 g/m2 at the age of 5 for retinoblastoma by ChT protocol containing vincristine and Adriamycin as well. In this patient we discovered damage of GE and LC-dysfunction. On the other hand male patient treated at the age of 9 years with ChT containing cumulative dose of CY 20 g/m2 for bone sarcoma had normal gonadal function. Normal functioning of the testes after treatment with combined ChT was found in another male patients after receiving a cumulative dose of CY of 15 g/m2, and other 3 males after receiving 11 -12 g/m2.
One male patient received the highest cumulative dose of cisplatinum (1 g/m2) at the age of 16 years for nasopharyngeal cancer with local RT and ChT, containing platinol, vinblastine, methotrexate and bleomycin. He maintained normal gonadal function.

4. Discussion

We found primary hypogonadism (PH) in 76 (26%) long-term survivors, 62 (34%) males and 14 (12%) females. High incidence (50%) of PH was found in those treated for Hodgkin's disease (HD) or germ cell tumor (GCT). A group of males, treated for HD with ChT was at high risk (72%) for PH as was a group treated with RT to the pelvis and ChT with AA, 79% (Fig. 1). At high risk of damage to and dysfunction of the germ epithelium of the testes were those treated with pelvic RT and ChT with AA. At high risk for damage to and dysfunction of ovaries were those treated for GCT (89%) and those treated with pelvic RT (50%).

4.1 Gender

Primary hypogonadism was detected in one third of males and in 12% females, indicating a greater susceptibility of male gonads for the deleterious effects of cancer treatment in childhood. In the multivariate analysis, 3 trees showed gender as the second most important risk factor for PH or GI (Fig.1,3,7); in the analysis of risk factors for PH in patients treated for HD the risk for PH in males was 65%, in females 25% (Fig. 1), in the analysis of risk factors for GI after exclusion of patients treated for GCT, the risk for GI in males was 48%, in females 18% (Fig. 3) and in the analysis of risk factors for GI (the second group of independent variables) in patients who had brain RT the risk of GI was 61% in males and 31% in females (Fig. 7). This is consistent with observations of other authors (Byrne et al. 1987, Kinsella et al. 1989, Rivkees & Crawford 1988).

4.2 Age at diagnosis

In multivariate analysis, age at treatment did not turned out as a significant risk factor for hypogonadism. Only in the analysis of risk factors for germ epithelium impairment at the last division patients age at diagnosis emerged as a risk factor for GEI. Namely, the group of 25 patients who were treated for HD with ChT with AA and RT above the diaphragm, was divided into those who were 10 or less years of age at diagnosis (83% risk of GEI), and into the group of older patients (31% risk of GEI). But the cumulative doses of AA were different in the two age groups and we can not consider patient's age at treatment as an important risk factor for GEI.

In our study, therefore, we didn't observe the impact of age at diagnosis or pubertal status of patient during treatment on severity of gonadal damage. The results of various studies regarding age of male patients at diagnosis as independent variable are contradictory. Some authors reported lesser degree of testicular damage in those patients treated in prepubertal period in comparison with those treated in postpubertal period (Rivkees & Crawford 1988, Waxman et al. 1982), while others didn't confirm that observation (Aubier et al. 1989, Casteren et al. 2009, Hoorweg -Nijman et al. 1992, Lendon et al. 1978, Mustieles et al. 1995). However, in very few studies took into account the size of cumulative doses of AA, that patients in different age groups received (Lendon et al. 1978, Rivkees & Crawford 1988).
On the other hand there are numerous studies reporting reduced susceptibility of ovaries to deleterious effects of cancer therapy in prepubertal period, when the number of oocytes is larger and they are in the "peaceful" phase (Chapman et al. 1979, Chemaitilly at al. 2006, Lushbaugh & Casarett 1976, Sanders et al. 1991, Wallace et al. 2005, Waxman et al. 1982).

4.3 Type of malignancy

The largest, 50%, incidence of PH was observed in patients treated for HD and germ cell tumors and the lowest, 9-11 %, in those treated for leukemia, brain tumors and Wilms' tumor (Table 2). In multivariate analysis diagnosis of GCT and HD repeatedly turned out to be important risk factors for PH and GI (Fig. 1,2,3,5,8,10). This is related to the nature of the disease (germ cell tumors of the gonads) and the type of treatment. Majority of patients with HD were treated with combination therapy (ChT and RT with / without surgery), in which the largest, 26 to 40%, proportion of patients with PH was observed (Table 3). In addition, ChT for HD usually contains more than one gonadotoxic AA simultaneously in the same protocol. As many as 65% of male subjects with diagnosis of HD had PH (Fig. 1,2).
Another reason for high incidence of PH in males treated for HD is preexistent impairment of spermatogenesis before treatment. Indeed, some authors have observed reduced number and / or reduced sperm motility in the ejaculate in as many as one third of adult males with HD prior to treatment (Vigersky et al. 1982, Whitehead et al. 1982). On the contrary, in adult women ovarian biopsy did not reveal any abnormalities prior to treatment of HD (Chapman et al. 1979).

4.4 Surgery

As for surgery in the multivariate analysis only orchidectomy stood as important risk factor for GI as well as germinal epithelium impairment (GEI) and LC impairment (LCI) (Fig. 7-9). In 6 of our male patients unilateral orchidectomy was performed during treatment (3 had testicular GCT, 2 paratesticular rhabdomyosarcoma, one leukemia). In all 6 gonadal impairment was observed, namely 4 had damage of germ cell epithelium and LC , one had dysfunction of germ cell epithelium and LC and one had dysfunction of LC only. It is true that all those 6 patients received ChT with AA as well but probably surgery itself also contributed to the testicular damage. Nijman et al. (1987) observed elevated levels of FSH, LH, and decreased levels of testosterone in adult patients undergoing unilateral orchidectomy. These findings were attributed to the LC insufficiency in the remaining testis.
Unilateral ovariectomy as independent variable itself didn't stand as an important risk factor in the analysis, but the diagnosis GCT was the most important risk factor for ovarian damage and dysfunction (Fig. 5, 10). In all 6 females with GCT ovariectomy was performed, in 3 bilateral, in 3 unilateral. All 3 girls with unilateral ovariectomy received also ChT with

AA without pelvic RT; in 2 ovarian dysfunction was observed, the third one had normal gonadal function. Unilateral ovariectomy is therefore compatible with normal gonadal function, which was also observed by other authors (Perrin et al. 1999).
In 12 patients within diagnostic procedures for HD staging laparotomy with transposition of ovaries to the posterior wall of the uterus was performed. Only 2 of these patients had pelvic RT (unilateral iliac region), all but one received ChT with AA. Ovarian damage was observed in 3 of those 12 females, namely in one following unilateral iliac RT with 2400 cGy and 6 cycles of LOPP at the age of 6 years (primary amenorrhea), the other 2 after RT of the upper abdomen with 3000 and 3600 cGy and 6 cycles of MOPP at the age of 11 and 14 years (both delivered healthy children). The remaining 9 patients had no signs of ovarian failure, six of them gave birth to healthy babies. Therefore, it seems that transposition of ovaries itself does not reduce the fertility. Indeed Thomas et al. (1976) didn't observe neither worsening of ovarian function nor interrupted gamets transfer after ovarian transposition in their patients with HD. Nevertheless, possible complications of this surgery, among others, are tubal obstruction and ovarian failure due to vascular damage (Thibaud et al. 1992).

4.5 Radiotherapy (RT)

RT has emerged as an important risk factor in the analysis of GI in males (Fig. 3,4) and pelvic RT was a major risk factor for PH in all subjects (Fig. 6). Risk for PH after pelvic RT was as high as 55%. With another division we identified a group of 17 patients treated with craniospinal RT for brain tumors, with low, 24%, incidence of PH. Addition of ChT with AA (mostly CCNU and PBZ) to RT, increased incidence of PH to 43% (being only 10% in those without ChT including AA). So, ChT with CCNU or PBZ in our subjects markedly increased risk of PH, especially in men, as 2 of the 3 males after craniospinal RT and ChT with AA had PH and none of the 6 males after only craniospinal RT had PH. One of 4 females had PH after RT alone. Higher gonadotoxicity of craniospinal RT in girls than in boys is in concordance with reports of other authors (Hamre et al. 1987, Sklar et al. 1990).
In the analysis of risk factors for ovarian impairment (Fig. 10) as the most important risk factor emerged GCT and in the second division pelvic RT. Similarly, other authors observed that pelvic RT is the most important risk factor for ovarian failure (Hamre et al. 1987, Stillman et al. 1981) observed that the only risk factor for ovarian failure in 182 long-term survivors of childhood cancer was ovarian position relative to the RT field and not ChT. Of course, ovarian failure may be caused by ChT with AA (Chapman et al. 1979, Nicosia et al. 1985, Ortin et al. 1990, Siris et al. 1976), what was observed in our study as well, namely all females with PH without receiving pelvic RT had ChT with AA.
Four females in our series had unilateral pelvic RT. Two had evidence of PH, but all received ChT with AA and PBZ as well. After RT of paraaortic lymph nodes the estimated ovarian dose is about 6 % of the prescribed dose (in the range of 100 cGy) and this dose of radiation can cause transient disturbances of menstrual cycle. Haie-Mader and colleagues (1993) analyzed ovarian function in 134 females treated for HD or gynecological cancer and showed that the age over 25 years, MOPP ChT and total dose to the ovaries higher than 500 cGy are important risk factors for ovarian castration.
In the analysis of risk factors for impairment of germ cell epithelium (GEI) the highest, 89%, risk of GEI, was observed in the group of males who received ChT with AA and had pelvic RT (Fig. 8). Only two of the 18 patients in this group did not have GEI, namely one patient after RT of bilateral iliac regions (3000 cGy) and two cycles of MOPP and the other following craniospinal RT (900 cGy) and ChT with AA (incl. 7 g/m2 CY).

This observation is consistent with reports of other authors (Byrne et al.1987, Casteren et al. 2009). In the group of 61 male patients who didn't receive ChT with AA, all 6 patients with GEI had pelvic or testicular RT (Fig.8).
By the last division in the analysis of risk factors for PH (Fig. 1), a group of 7 male patients, treated for HD without ChT, was identified. PH was observed in 2 patients only, in both following pelvic RT with 4000 cGy. Only one of our male patients had additional shielding of testes during pelvic RT (3600 cGy to bilateral iliacoinguinal regions) by a lead capsule, he received 6 cycles of LOPP. In spite laboratory testing showing damage of germinal epithelium and LC dysfunction he fathered 2 children, suggesting only a partial impairment of spermatogenesis. This is consistent with the study of Kovač et al. (1990) in which authors reported about significant reduction of testicular dose by an additional lead shielding of testis (Kovač et al. 1990).

4.6 Chemotherapy (ChT)

ChT emerged as an important risk factor in all multivariate analysis using the first set of variables. In the analysis of risk factors for PH as well as GI in male patients ChT turned out to be very important risk factor, immediately after the type of malignancy (GCT or HD) (Fig. 2,4).
In the analysis of risk factors for PH using a second set of independent variables (Fig. 8), a group of 246 patients who didn't have pelvic RT divided further by therapy with ChT. So, we identified a group of 87 patients neither receiving ChT with AA nor pelvic RT, with the lowest, 1%, risk of PH. The only one patient with PH in this group had testicular RT. Using multivariate analysis we, therefore, identified a group of childhood cancer survivors in whom gonadal testing could be omitted.
We observed greater impact of ChT with AA on gonadal function in boys than girls, as 42% males and only 7% females treated with ChT with AA without pelvic RT, had PH (Fig. 6). Similar conclusions were drawn in the study of Byrne et al. (1987) analyzing 2283 long-term survivors of childhood cancer and showed that RT under the diaphragm decreased fertility in both sexes for 25%, ChT with AA (with or without abdominal RT) decreased fertility only in boys for 60%, but not in girls.
This reflects in the analysis of risk factors for impairment of germ cell epithelium (Fig. 8) where the most important risk factor for GEI was ChT with AA, followed by pelvic RT. Males who received ChT with AA for HD without having pelvic RT, had incidence of GEI as high as 71%. Only 7 of 24 patients in this group of patients had normal testicular function, 4 patients after 6 cycles of LOPP cycles, 2 patients after 1 to 4 cycles of MOPP, one after 2 cycles of OPPA. On the other hand none of the male subjects after 6 or more cycles of MOPP ChT had normal function of germ cell epithelium. Our findings are in concordance with data from other studies establishing that MOPP or MOPP-like combinations, such as MVPP (mechlorethamine, vinblastine, procarbazine and prednisone) and COPP induce azoospermia in 90-100 % of pts with a 10-20% chance of recovery even 10 years after treatment (Chapman et al. 1979, Diamond& Bercu 2001, Viviani et al. 1985, Waxmann et al. 1982, Whitehead et al. 1982, Zaletel 2010). Recovery of spermatogenesis following MOPP therapy appears to be dose-related with 3 courses of MOPP representing a limiting gonadal exposure for the recovery, suggesting only partial killing of germinal stem cells (da Cunha et al. 1984). Indeed, in our study we found normal gonadal function in two males after having received 1 and 2 cycles of MOPP ChT. We found ChT according to the protocol LOPP less damaging for testicular function than MOPP, causing GEI in 5 of 10 males having

received 6 cycles, a finding not published elsewhere to our knowledge. It seems that chlorambucil inside LOPP protocol is more gonadotoxic than nitrogen mustard in MOPP protocol. Our subjects received many different chemotherapy regimens, so the comparison with the results of other studies is difficult.

Among patients who didn't receive pelvic RT, the highest incidence of PH was observed in the group of males treated for HD with ChT including AA (66%) (Fig. 6). Those males who were treated with ChT including AA for other malignancies, had much lower incidence of PH (34%). This finding is consistent with other studies reporting that ChT used in treatment of HD is more gonadotoxic from other ChT regimens (Müller 2003).

In women, in the analysis of risk factors for ovarian impairment pelvic RT emerged as the most important risk factor, immediately after diagnosis GCT (Fig. 10). But 6 of the 8 patients who had pelvic RT received ChT with AA as well. On the other hand, all females with ovarian impairment, who didn't receive pelvic RT, were treated with ChT with AA. So, we can conclude that ChT with AA contributed to development of ovarian impairment. ChT according to MOPP protocol is more toxic to the ovaries than other types of ChT. There are data of adverse effects of ChT that is in use for HD, on ovarian function in adult females, but very little on ovarian function in girls. In the study of Ortin and colleagues 2 of 18 girls were amenorrhoic after having received 6 or more cycles of MOPP (Ortin et al. 1990). In our study none of the 6 females was amenorrhoic after 6 or more cycles of MOPP, but 2 had evidence of ovarian damage while retaining fertility Probably in girls younger than 16 years ChT is less gonadotoxic because relative quiescence of stromal cells and oocytes in prepubertal period protects ovaries from cell cycle specific cytotoxic agents (Siris et al. 1976, Stillman et al. 1981).

Forty-six patients didn't receive neither testicular or pelvic RT nor ChT with AA. They were treated with ChT containing antimetabolites (except cytarabine), antibiotics and vinca alkaloids. None of them had PH. Other authors as well, didn't observe important role of this type of ChT in pathogenesis of gonadal failure (Blatt et al. 1981, Müller 2003), although they reported on transient oligospermia in adult patients following treatment with methotrexate (Sussman & Leonard 1980) and on possible role of VCR in pathogenesis of germ cell epithelium damage in childhood and adolescence (Rautonen et al. 1992). In the group of patients treated with ChT containing AMD and VCR, 2 female patients had ovarian dysfunction and 2 males had LC dysfunction, suggesting that therapy with these 2 chemotherapy agents can cause mild degree of ovarian or LC damage. To our knowledge there is no article reporting on the potential gonadotoxicity of AMD.

4.7 Observation time

In none of the multivariate analysis observation time emerged as a significant risk factor for gonadal impairment. This is in accordance with expectations, as normally gonadal failure develops during or shortly after administration of toxic therapy and correction of gonadal damage eventually takes place within the first decade thereafter or so (Mustieles et al. 1995, Rowley et al. 1974, Viviani et al. 1985, Waxman et al. 1982). In 73 patients, we performed gonadal testing twice. Indeed, in 3 males the second testing showed normal functioning of the germ cell epithelium, after first testing showing damage of germ cell epithelium (4 to 16 years earlier). In female subjects as well the correction of the ovarian function was found. One of them had secondary amenorrhea after treatment and after 5 years menstrual cycle restored. The second one had primary amenorrhea till 16 years of age and then restored normal menstrual cycle. In contrast to the germ cell failure, damage of LC can develop

within a few years after treatment, and usually there is no correction of LC damage (Shalet et al. 1985). Indeed, in 4 male patients in our cohort we found LC impairment at the second testing 3 to 10 years after their first testing, when they had normal function or only dysfunction of LC.

4.8 Leydig cell damage

LC damage was detected in 16 adolescents. All who were on treatment in prepubertal period (10 patients) had normal pubertal development. Lowered testosterone level was observed only in one patient, who received ChT with AA and RT above the diaphragm at the age of 10 years for HD. At relapse, 5 years later, he had additional ChT with AA and RT to the upper abdomen (3400 cGy). So, the majority of these 16 patients had clinically insignificant impairment of LC function. Two of these patients had testicular RT (1200 cGy in 4 fractions). Other authors did neither observe clinically significant impairment of LC after the testicular dose of this size (Brauner et al. 1983, Castillo et al. 1999). Of the remaining 14 patients with LC damage, only 4 had pelvic RT with / without ChT with AA, 10 patients had ChT with AA without pelvic RT. So, failure of LC in these adolescents was not simply a consequence of RT, but was mainly caused by ChT with AA. Most studies after ChT with AA observed compensatory insufficiency of LC (normal testosterone level and elevated basal level of LH and / or elevated level of LH after stimulation) (Brämswig et al . 1990, Kenney et al. 2001, Meistrich 2009, Romerius at al. 2009, Sherins et al. 1978, Whitehead et al. 1982). However, in two studies LC dysfunction following ChT with AA was not identified (Pennisi et al. 1975, Shalet et al. 1981). The likely cause of this discrepancy lies in the fact that in one of these studies LC function was evaluated only by basal LH levels without GnRH-test, but LC dysfunction can reflect in increased LH response to GnRH (Pennisi et al. 1975). All our 16 patients with LC damage had damage of germinal epithelium as well.

LC dysfunction was observed in 54 patients. All but 6 received ChT with AA (9 of them had pelvic RT as well). Again, this result confirms that ChT with AA contributes in the pathogenesis of compensatory LC damage.

Incidence of LC damage is increasing in the years after treatment, therefore, patients with elevated basal or stimulated LH require annual monitoring of LH and testosterone and the timely introduction of hormone replacement therapy when reduced secretion of testosterone is discovered.

4.9 Secondary hypogonadism

Secondary hypogonadism was detected in two female patients with panhipopituitarism after combination therapy of GCT of hypothalamus, in three patients after treatment of brain tumors located outside the hypothalamus or pituitary gland with surgery and postoperative RT (5000 - 6500 cGy) and in one female after whole brain RT with 3000 cGy for leukemia. All patients with secondary hypogonadism had hiposomatotropism as well, which corresponds to reports of others (Constine et al. 1993, Gleeson & Shalet 2004).

4.10 Classification tree analysis

We analyzed our data by multivariate analysis method, classification tree model, which allows for studying of simultaneous influence of a series of independent variables on a single dependent variable. The main advantage of this method is its ability to detect the mutual effect of independent variables. The decision tree determines groups of subjects with

a set of specific values of independent variables, in which the risk of gonadal damage is the highest or the lowest. So, in analysis where we took damage and dysfunction of germinal epithelium of testes as dependent variable we identified a group of patients at highest risk of this outcome, namely males who were treated with ChT with AA and pelvic RT. In analysis of risk factors for primary hypogonadism (PH), we identified a group of patients with very low risk, 1%, for this outcome, namely, males who had neither ChT with AA nor pelvic or testicular RT as their treatment.

Classification trees in our study were mostly significantly different from random prediction, with the exception of those trees constructed on data from female patients, due to the low number of patients with "positive" outcomes (e.g. PH). Predictive accuracy of the trees was high and it would be even higher if the analysis included other independent variables that might further explain the difference between groups of subjects with different outcome regarding dependent variable. In our analysis, we wanted to include more independent variables, or several different values for independent variables (e.g. cumulative doses of various cytostatics), but, despite the relatively large number of subjects, we could not do it. Each multivariate analysis restricts the number of independent variables and their values. On the other hand, other risk factors for gonadal damage can exist which we haven't identified yet.

Maybe, a limitation of this method is that in some analysis, a larger group of patients is not further divided, e.g. a group of 233 patients who were treated for cancer other than HD (Fig. 1) and a group of 109 female patients who was not treated for GCT (Fig. 5). The reason for this was the small number of subjects with observed outcome (e.g. PH) in these groups of patient. So we could loose some information on potential risk factors for impaired gonadal function in these patients. Decision trees, which we got using the second group of independent variables, were more diversified and gave also more information (Fig. 6-10).

In the published articles studying gonadal function after childhood cancer treatment different multivariate analysis for analyzing risk factors were used, mainly logistic regression (Chematilly et al. 2006, Haie-Mader et al. 1993, Rautonen et al. 1992, Romerius et al. 2009, Stillman et al. 1981), linear regression (Siimes & Rautonen 1990), Cox regression analysis (Byrne et al. 1987). But no one used the decision tree classification model, therefore, not been able to identify links between risk factors for impaired gonadal function. But there are a number of articles published in medicine, in which a classification tree method was used (Jazbec et al. 2004, Jereb & Eklund 1973, Macedoni-Lukšič et al. 2003, Velensek et al. 2008).

4.11 Assessment of gonadal function

For assessment of gonadal function in long-term survivors of childhood leukemia we used, beside clinical evaluation, hormonal testing, which is an indirect measure of testicular and ovarian function. Several studies showed that in men basal FSH level and FSH response to GnRH correlate well with sperm production (Aubier et al. 1989, Hoorweg-Nijman et al. 1992, Kinsella et al.1989, Kirkland et al. 1976, Mustieles et al. 1995, Siimes & Rautonen 1990). An increased FSH response to GnRH can be the first manifestation of testicular damage, although normal FSH levels do not rule out the possibility of azoospermia (Aubier et al. 1989, Kenney et al. 2001). All our male patients with PH were advised to perform analysis of spermiogram, but only 12 of them decided to do so. In 11 of them azoospermia was found, confirming that GnRH-testing offers a good estimate of spermatogenesis. On the other hand 6 male patients with documented PH became fathers, suggesting that elevated levels of FSH do not rule out fertile ability. But on the other hand normal FSH levels do not exclude the

possibility of impaired spermatogenesis (Aubier et al. 1989, Kenney et al. 2001). We couldn't confirm that observation because none of our male patients with normal laboratory findings performed spermanalysis. But none of them had problems with fertile capability. We didn't use testicular volume for evaluation of gonadal function in male subjects. Indeed, some studies reported that testicular volume is not a reliable indicator of spermatogenesis (Kenney et al. 2001, Relander et al. 2000). There are reports on Inhibin B as a good serum marker which correlate well with sperm concentration (Beek at al. 2007, Casteren et al. 2009).

GnRH-test served us for the evaluation of LC function as well. Good test for the evaluation of LC function is HCG test, which measures testosterone levels after repeated administration of chorionic gonadotropin (Brauner et al. 1983). However, this test is difficult to implement as it lasts for several days. Anyway, Brauner (1983) found good correlation between GnRH-test and HCG-test in males if performed in postpubertal period. Actually, our subjects were tested in postpubertal period.

Five of 24 males with germ cell epithelium damage fathered children indicating that they are not azoospermic but possibly oligospermic and fertile. Hoorweg-Nijman and colleagues found elevated levels of FSH compatible with normospermia (Hoorweg-Nijman et al. 1992). FSH levels may provide an estimate of possible impaired spermatogenesis, however only semen analysis is confirmatory assessment of male gonadal function.

We used GnRH-test for evaluation of ovarian function as well. Primary hypogonadism was detected in 14 females, but only 6 of them are amenorrhoic, 3 after bilateral ovariectomy for GCT. Interestingly, one of our patients in spite of being amenorrhoic and having levels of gonadotropins in menopausal range, gave birth to a healthy boy. Of the remaining 8 female patients with PH, one had transient, secondary amenorrhea lasting for 5 years, one is in early menopause (at 38 years of age) after 2 deliveries, 6 of them have irregular periods, 2 after 1 to 2 deliveries. Indeed, in most studies the term ovarian failure was used in patients with amenorrhea, elevated levels of gonadotropins and lower levels of estradiol (Stillman et al. 1981, Chapman et al. 1979). Thus, ovarian failure, defined in such a way, was diagnosed in 6 of our female patients only. So we, maybe, slightly overestimated the rate of PH in female patients (as well as in male patients) taking under the cover of ovarian damage more subtile, clinically insignificant gonadal damage as well. But it is likely that these patients are at risk of early menopause which already happened in one of our female patients. After cancer therapy, indeed, the number of primordial follicles decreases further, increasing the "age" of ovaries and shortening fertile period (Larsen et al. 2003). Hyperexcitability of gonadal axis (elevated LH / FSH after stimulation with GnRH), t.i. ovarian dysfunction, was detected in 11 of our female patients. All have regular menstrual cycles and five of them gave birth to healthy children. However, ovarian hyperexcitability may indicate mild impairment of ovarian tissue and higher risk of early menopause in most subjects but does not appear to be clinically significant. Other comparable studies of ovarian function after cancer treatment in childhood authors have not reported on hyperexcitability.

5. Conclusions

With the presented population based study we confirmed several already known results of other studies, such as :

- a greater susceptibility of male gonads for the deleterious effects of cancer treatment in childhood,

- RT is an important risk factor for GI in males and pelvic RT as a major risk factor for PH in all, male as well as in female survivors,
- unilateral orchidectomy is an important risk factor for germinal epithelium impairment as well as LC impairment , which is attributed to the LC insufficiency in the remaining testis,
- unilateral ovariectomy is compatible with normal gonadal function,
- gonadal failure develops during or shortly after administration of toxic therapy and correction of gonadal damage eventually takes place in male as well as in female survivors. In contrast to the germ cell failure, damage of LC can develop within a few years after treatment, and usually there is no correction of LC damage therefore. Therefore, patients with elevated basal or stimulated LH levels require annual monitoring of LH and testosterone and the timely introduction of hormone replacement therapy when reduced secretion of testosterone is discovered.

But there was no multivariate analysis using the decision tree classification model, which is able to identify links between risk factors for impaired gonadal function. With this model we could also identify a group of patients with the lowest risk of gonadal impairment those who had neither pelvic or testicular RT nor ChT including AA. In those hormonal testing could be omitted.

6. Acknowledgements

The research was supported by Ministry of science and Ministry of health in Slovenia.

7. References

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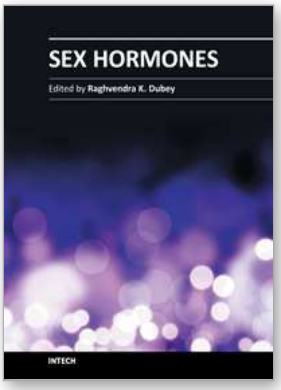

Sex Hormones

Edited by Prof. Raghvendra Dubey

ISBN 978-953-307-856-4
Hard cover, 430 pages
Publisher InTech
Published online 08, February, 2012
Published in print edition February, 2012

Sex Hormones not only regulate reproductive function, but they also play a prominent role in the biology and physiology of several organs/tissues and in the pathophysiology of several diseases. During the last two decades, the information on the mechanisms of action of sex hormones, such as estrogens and androgens, has rapidly evolved from the conventional nuclear receptor dependent mechanisms to include additional non-nuclear, non-genomic and receptor-independent mechanisms. This highlights the need to update the current knowledge on sex hormones and their mode of action. Increasing evidence that exogenous/epigenetic factors can influence sex hormone production and action highlights the need to update our knowledge on the mechanisms involved. This book provides a systematic and updated overview of the male/female sex-hormones and their impact in the biology and physiology of various organs. Additionally, the book discusses their positive and negative association with the pathophysiology of various diseases (e.g. osteoporosis, cardiovascular-disease, hypogonadism, reproduction, cancer) and their therapeutic potential.

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