

Endocrinological late effects following radiotherapy and chemotherapy of childhood brain tumours

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1. INTRODUCTION

During the last three decades the overall cure rate of children treated for brain tumours has increased from approximately 50 to 67% (Bleyer, 1990a) (de Nully Brown et al., 1995) (Bleyer, 1999b). Gradually, long-term survivors have become a significant group, however, the improvement in prognosis has been achieved at the expense of serious late effects, which have their onset months or years after treatment. Children treated for brain tumours with surgery, radiotherapy (RT) and chemotherapy (CTx) are at risk of developing late effects, which include both severe neurological deficits, cognitive difficulties, and endocrinological deficits and the development of second tumours may also occur (Duffner et al., 1985b). Endocrine deficits with growth hormone (GH) deficiency, gonadal and thyroid and adrenal dysfunction have been described (Brauner et al., 1989) (Clayton and Shalet, 1991) (Livesey et al., 1990) (Ogilvy Stuart et al., 1991) (Constine et al., 1993) article I (Schmiegelow et al., 1999), article II (Schmiegelow et al., 2000), article III (Schmiegelow et al., 2000), article IV (Schmiegelow et al., 2001), article V (Schmiegelow et al., 2003), article VI (Schmiegelow et al., 2003). Direct destruction of normal brain tissue by the tumour as well as by an increased intracranial pressure and/or surgical trauma may cause some degree of damage to the neural tissue (Sonderkaer et al., 2003), however, RT has been implicated as the chief cause of adverse long-term sequelae (Andersen et al., 2003), and about 55% of children with brain tumours still have to be treated with cranial radiotherapy (CRT) to achieve long term survival. RT especially in combination with CTx administered to eradicate brain tumours may result in serious cognitive and neuropsychological deficits (Lannering et al., 1990) (Ilveskoski et al., 1996) (Reimers et al., 2003).

Information concerning threshold doses of radiation capable of damaging the hypothalamic-pituitary (HP) axis is limited, and it has not yet been possible to define an exact radiation dose to the HP region above which endocrinological deficiencies ensue and below which no pituitary dysfunction follows. Therefore, we wanted to examine the dose received by the HP region and relate this to endocrine endpoints. The first obstacle was to measure the dose received by the HP region, and as the delimitation of the HP region is difficult to delineate we invented a model for specific dosimetry of the HP region by means of a computer-based treatment planning system calculating more precisely and in a standardised fashion the quantitative dose to the HP region through analyses of dose-volume histograms (DVH) (Goitein and Abrams, 1983) (Goitein, 1985)

(Chen et al., 1987) (Bentel, 1996), article I (Schmiegelow et al., 1999). Using this method would allow one to determine the dose to the HP region relating this to endocrine deficits. Secondly, the study design was made population-based and cross sectional and the patients were endocrinologically evaluated at intervals sufficiently long for the endocrine sequelae of radiation to become evident. Thirdly, in order to achieve a long follow-up, we had to go back in time, which implied that computer-based planning systems were not available. In order to obtain a more accurate estimation of the impact of RT on the HP axis we applied the linear quadratic (LQ) model to determine the biological effective dose (BED) (Barendsen, 1982) (Fowler, 1989), article II (Schmiegelow et al., 2000) to the HP region relating the BED to endocrine deficits.

The present thesis documents that endocrine late effects appear to have specifically detrimental effects to the central nervous system (CNS) of the growing individual – the child – due to development of growth hormone deficiency (GHD), thyroid and gonadal dysfunction as well as adrenal insufficiency and show the need for lifetime follow-up of cured patients.

Detailed knowledge of the RT and the CTx received by the child and the recognition of the relation to the endocrine sequelae in a population-based study would enable us to predict possible risk factors and through this put forward suggestions to improve quality of treatment in order to reduce treatment-related morbidity for long-term survivors.

This review will try to encompass the contribution of the thesis in relation to the present knowledge of endocrinological deficits to treatment of childhood brain tumours.

2. AIMS OF THE STUDY

1. to try explaining the large interindividual difference in time of onset of endocrinological late effects that has been observed in children, who presumably have received the same dose to the HP region.
2. to determine the impact of fractionation dose on endocrinological late effects by means of measuring the BED to the HP region and
3. to determine the incidence of endocrinological late effects following RT with or without CTx for childhood brain tumours in a population-based study.

3. CHILDHOOD BRAIN TUMOURS

3.1. INCIDENCE AND SURVIVAL RATES

Brain tumours, the second most frequent childhood cancer, constitute about 25% of all types of cancers in children and is the most common solid tumour in children. There is a trend within the last 30 years of an increase in the annual incidence rates reported from 24 to 53 per 10⁶ children less than 17 years (Aarimaa et al., 1997) (Gjerris et al., 1998) (Hjalmarsson et al., 1999) (Bleyer, 1999b). During the same period a marked increase in 5 – year survival rates has been documented from 50% to 67% at present (Bleyer, 1990a) (de Nully Brown et al., 1995) (Bleyer, 1999b) – a development which has contributed to focus on reducing sequelae to treatment.

3.2. STUDY DESIGN

Most research on long-term effects of childhood brain tumours has been comprised of single-institution case series (Livesey et al., 1990) (Clayton and Shalet, 1991) (Ogilvy Stuart et al., 1991) (Constine et al., 1993), because it has been the only possible way to reach patients for instance in the UK and in the USA. However, in Denmark as well as in other parts of Scandinavia we have a unique central registration of persons, which makes it possible to perform population-based research. To mention four of the most weighty studies on miscellaneous endocrinological late effects following treatment of childhood brain tumours the study by Ogilvy-Stuart (Ogilvy Stuart et al., 1991) comprised 134 children, who had been treated with RT at a single institution from 1960-1990 between the ages of 0.5-16.9 years for a brain tumour not involving the HP axis, while in the

Table 1. Survey of the cohort and the distribution of participants in the different articles

	Paper I	Paper II	Paper III	Paper IV	Paper V	Paper VI
Males	15	47	42	47	45	46
Females	4	26	20	0	26	27
Males < 18 years				16		
Males ≥18 years				31		
Excluded due to hypothyroidism detected at the time of stimulation		3				
Excluded due to cortisol insufficiency detected at the time of diagnosis						2
Excluded due to hypothyroidism detected at the time of diagnosis					2	
Excluded due to continued levothyroxine at the time of stimulation					2	
Excluded due to continued testosterone at the time of stimulation				1		
Excluded due to thyrectomy detected at the time of stimulation					1	
Non-acceptors of GHRH test			11			
Excluded due to continued hydrocortisone at time of stimulation						1
Total n = 76	19	73	62	30	71	73

study by Livesey (Livesey et al., 1990) 144 children were studied with a median age at start of RT of 6.7 years and a median follow-up of 9.6 years. They had been treated for brain tumours at two centres between 1972 and 1985. Clayton and Shalet (Clayton and Shalet, 1991) studied 82 children treated for a brain tumour at one centre with a median age at time of RT of 6.2 years and a length of follow-up of 4.3 years. In the study by Constine (Constine et al., 1993) 32 patients were treated for a brain tumour at a single institution with a mean age at time of RT of 19 years and a mean age at time of follow-up of 26 years. In our study we enclosed all surviving patients in the Eastern part of Denmark (which covers about half the population in Denmark = 2.5 mio) who were diagnosed with a brain tumour between January 1970 through February 1997 comprising a total of 188 persons alive at time of follow-up, who met the inclusion criteria: aged less than 15 years at the time of diagnosis of a primary brain tumour not directly involving the HP axis; treated with surgery including a biopsy only and cranial irradiation (CIR) or cranio-spinal irradiation (CSI) and with or without CTx; more than 1 year from end of treatment at the time of follow-up; and in remission at the time of follow-up. The patients were excluded at the time of follow-up if they had emigrated or had a residence at the Faroe Islands or in Greenland or if their case record was missing (n = 11). A total of 177 persons were included: 83 patients, who had been treated with surgery only including a biopsy only, 3 patients had received CTx only, and the remaining 91 patients had been treated with surgery including a biopsy only and with RT +/- CTx and constituted the present cohort in this thesis. Seventy-six patients (84%) of these 91 patients (49 males, and 27 females) accepted to participate in the study. The patients were identified from all the neurosurgical departments in the Eastern part of Denmark (Rigshospitalet, Hvidovre and Glostrup) and crosschecked with the Danish Cancer Registry, Copenhagen. The Danish Cancer Registry was established in 1942 and to this registry reports are notified on all brain tumours, however, one may assume that the efficiency of reporting to the registry has improved over the years due to knowledge of its existence as well as the increasing awareness of the importance of registration of patients – something which was evident in the present cohort. We constructed a database encompassing all data registered of the patients and collected detailed information from medical, histological, surgical and radiotherapeutic records. The data included basic information of diagnosis, treatment information including

surgical procedures and RT and CTx regimens, and consecutive registration of growth and results of bloodtests. The patients were interviewed and had a thorough physical and neurological examination carried out and participated in endocrinological stimulation tests on subsequent days. The thesis is based on 6 publications and in the survey of the cohort the distribution of patients in the respective articles are registered (Table 1).

3.3. HISTOLOGICAL DIAGNOSES

Childhood brain tumours have important properties: 1) They are highly invasive, even when histologically of low malignancy, 2) many are heterogeneous in composition with areas of mixed tumour types, 3) many are spreading to cerebrospinal fluid (CSF) pathways, and 4) tumour progression may occur from low to high grade tumours. From Bailey and Cushing's old histogenetic tumour classification, where tumour morphology was considered to mirror specific stages of normal neural tissue, the tumour classification systems of brain tumours have been in a continuous development. During 1970-1997 – the time interval in the present cohort – the Kernohan and the WHO classification systems were used. The Kernohan system, a numerical grading system, was based upon the hypothesis that prognostic differences within each cell type were related to the degree of anaplasia (Kernohan and Sayre, 1952), while in the WHO classification system (Rorke et al., 1985) – latest the second edition of the WHO classification system (Kleihues et al., 1993) – the tumour location and cellular differentiation are the basis of the diagnostic system. In general, nearly half of the paediatric brain tumours

Table 2. The tumour diagnoses

Diagnoses	n*
Astrocytoma	32
Medulloblastoma	23
Ependymoma	6
Germ cell tumour	4
Glioma	3
Pinealoma	1
Haemangiopericytoma	1
Primitive Neuroectodermal Tumour (PNET)	1
Non-histologically verified	5
Total	76

n* = number of patients

present as supratentorial tumours, whereas infratentorial tumours encompass 55%, and the most common childhood tumours are astrocytoma: 45%, medulloblastoma: 20-25%, gliomas: 18%, ependymoma: 6-9%, and craniopharyngioma 2-8%. The present cohort mirrors this with: astrocytoma 42%, medulloblastoma 30%, and ependymoma 8% and miscellaneous tumours 20% (Table 2).

4. TREATMENT OF BRAIN TUMOURS

Neurosurgery is the mainstay of treatment for most brain tumours, followed by RT, which about 55% of children with a brain tumour receive, while the role of CTx remains unsettled.

4.1. SURGERY

The goals of neurosurgical treatment are to potentially remove the disease, to excise or reduce the tumour volume, and to establish a tumour diagnosis since accurate histological classification of the tumour is crucial for the planning of further therapy.

4.2. RADIOTHERAPY

4.2.1. The hypothalamic/pituitary axis and irradiation

The pituitary gland was in early reports considered to be resistant to the effects of external irradiation (Kelly et al., 1951), while it was speculated that the hypothalamus was more sensitive to irradiation (Arnold, 1953). Several authors concluded that a dose of 30-60 Gy to the HP region was safe, and it was not until the mid 1960s that reports of deficiency of one or more anterior pituitary hormones following therapeutic external CIR were published (Tan and Kunaratnam, 1966). In the 1970s it was suggested that patients were at risk of developing anterior pituitary hormone deficiencies if the HP region was within the treatment field, and that the damage of irradiation was of hypothalamic origin (Larkins and Martin, 1973). The nuclei of the hypothalamus are responsible for the secretion of the different releasing hormones: growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH), and the portal hypophyseal vessels form a direct link between the hypothalamus and the anterior pituitary gland with transfer of the releasing hormones to act directly on the cells producing the anterior pituitary hormones. The releasing hormones are regulated by neurophysiological and endocrine feedback mechanisms and by means of neurotransmitters and hereby the hypothalamus serves as a link between different regions of the brain by afferent and efferent nerve pathways.

Children at risk of developing endocrine deficiencies include among others those irradiated for brain tumours, acute lymphoblastic leukaemia (ALL) or those who receive total body irradiation (TBI) in preparation for a bone marrow transplant (Ogilvy Stuart et al., 1992) (Leiper, 1995), as well as endocrine deficiencies have also been reported in patients who received RT for a nasopharyngeal carcinoma (Lam et al., 1986) (Samaan et al., 1987). Deficiency of one or more anterior pituitary hormones may ensue radiation to the HP axis with growth hormone deficiency (GHD) as the first and frequently the only manifestation of radiation-induced hypopituitarism during childhood (Duffner et al., 1985a) (Brauner et al., 1989) (Darendeliler et al., 1990). Life-table analysis in a large series of 165 adult patients (Littley et al., 1989), who underwent surgery and RT for tumours of the pituitary or closely related anatomical sites had a multistimulation test performed before RT. This was repeated six and 12 months later and subsequently annually and the order of development of hormone deficiencies was GHD as the first, followed by gonadal dysfunction and dysfunction of the HP-adrenal (HPA) axis with thyroid dysfunction as the least likely to emerge. These results were contradictory to the findings by Constine (Constine et al., 1993), who demonstrated a high incidence of thyroid dysfunction and a low incidence of corticotropin deficiency. Some prospective studies have shown that GHD was not present immediately after surgery of a childhood brain tumour prior to CIR, but was revealed

as early as 3 months post CIR (Shalet et al., 1978) (Duffner et al., 1985a). Low dose CIR (18-24 Gy) to the HP axis in the treatment of ALL often leads to isolated GHD (Brennan et al., 1998), however, radiation induced GHD generally does not become evident until 2 or more years after RT following treatment with doses in excess of 30 Gy to the HP axis (Clayton and Shalet, 1991) article I (Schmiegelow et al., 1999). There has been a demonstration of gonadal dysfunction with both hypogonadism (Rappaport et al., 1982) due to gonadotrophin deficiency after high-dose CIR exceeding 50 Gy to the HP axis as well as premature activation of the HP gonadal axis after CIR of childhood brain tumours (Ogilvy Stuart et al., 1994) (Oberfield et al., 1996), as well as an increased secretion of prolactin (Constine et al., 1987). Precocious puberty, predominantly in girls, has been reported in children receiving low doses of CIR (18-24 Gy) in the management of ALL (Leiper et al., 1987) (Quigley et al., 1989), and in either sex following CIR for a brain tumour (Ogilvy Stuart et al., 1994). However, as our study design was cross sectional and the retrospective data of registered time of menarche were incomplete, instead we tested the gonadal function in male patients ≥ 18 years in order to obtain a gonadal status independent of puberty, article IV (Schmiegelow et al., 2001).

5. ENDOCRINOLOGICAL DEFICITS IN THE PRESENT COHORT

In the cohort 71 patients could be compared with regard to the growth hormone, thyroid and adrenal axes because they all had the axes tested as published in article II (Schmiegelow et al., 2000), article V (Schmiegelow et al., 2003), and article VI (Schmiegelow et al., 2003). Fifty-eight had GHD, 21 were hypothyroid and 14 had an insufficient HPA axis. The distribution of patients insufficient at all 3, 2 and 1 or none of the HP axes were as follows: 5 had 3 insufficient axes, 23 had 2 insufficient axes, 30 had one, and 13 had normal axes (Figure 1).

6. RELATION OF TIME OF FOLLOW-UP TO ENDOCRINE DEFICITS

Ionizing radiation causes double-strand DNA lesions, which result in loss of ability for sustained cell division of proliferative cells. The side effects to irradiation of the normal tissue that surrounds the tumour depend on the tissue in question. The pathogenesis of CNS damage is only partially understood, but evidence suggests that radiation damage of neural tissue is produced by some combination of parenchymal cell loss and injury to the intracranial endothelial cells with arteriocapillary fibrosis which accentuates the cellular depletion of the parenchyma (Casarett, 1972). Late responding tissues to irradiation – like neural tissue – are signified by cell populations proliferating so slowly that they do not renew for several months or even years, which is why late effects have their onset from months or years following the cessation of treatment. This is because cells do not die after irradiation until they try to divide. We demonstrated

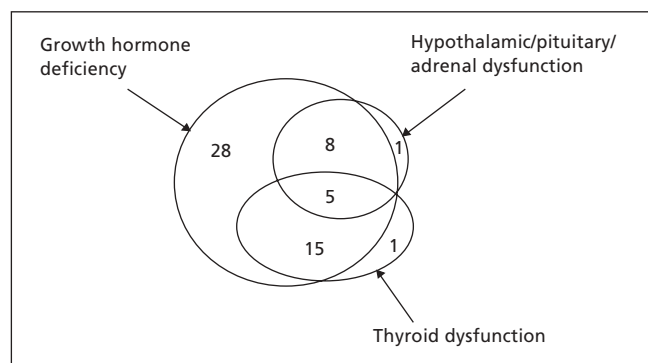


Figure 1. Distribution of the endocrinological deficiencies, which could be compared for the 71 patients. Thirteen patients had normal axes and 58 patients had deficiencies. Each area gives the number of patients having the hormone deficiencies in question.

that this pathogenesis is reflected clinically in the fact that the degree of pituitary deficiency following RT could be demonstrated as a function of the length of time after irradiation, article II (Schmiegelow et al., 1999), article III (Schmiegelow et al., 2000), article IV (Schmiegelow et al., 2001), article V (Schmiegelow et al., 2003), article VI (Schmiegelow et al., 2003). We demonstrated significant negative correlations between all tested endocrine hormone deficiencies and time of follow-up implicating effects of therapy on growth, thyroid and adrenal and gonadal function, however, no significant relations between the endocrine hormone deficiencies and age at RT. With a median length of follow-up in the present study of 12 years we would expect to have established the number of patients with endocrine hormone dysfunction. The clinical implications are that endocrine function should be followed life long after both CIR and craniospinal irradiation (CSI) of childhood brain tumours.

7. GROWTH HORMONE DEFICIENCY AND DOSIMETRY OF THE HYPOTHALAMIC/PITUITARY AXIS

In the present cohort the children had been treated with ^{60}Co units or with external conventional 4, 6, or 8 megavoltage RT delivered by a linear accelerator. To provide homogeneous dosage and to encompass the subarachnoid space a parallel opposed pair of treatment fields were used in whole brain RT, and very often a boost dose was delivered to the tumour bed. Brain tumours expected to potentially metastasize (medulloblastoma, high-grade ependymoma, germ cell tumours) were treated with CSI, while smaller tumours were treated with focal irradiation sometimes, but not always using a three-field technique. Some children treated in the 1990s had more complex planning and delivery of RT trying to maximize tumour dose and minimize dose to adjacent normal neural tissue at risk. This conformal therapy requires three-dimensional imaging using all appropriate techniques (Goitein and Abrams, 1983) (Bentel, 1996) (Smitt et al., 1998).

Even though it was expected that the children had received the same dose to the HP region, a large interindividual difference in time of onset of endocrinological late effects had been observed. Our objective was to find a possible explanation for this discrepancy and we tried, using analyses of DVHs (Chen et al., 1987), to analyse in a standardised fashion the dose actually received by the HP region in a total of 19 children, who had received RT following three-dimensional treatment planning, article I (Schmiegelow et al., 1999). We explored a model for specific dosimetry of the HP region trying to predict the risk of developing GHD in children, who had been treated with CIR for a brain tumour. All the treatment fields for the 19 patients, whom it was possible to evaluate by means of dosimetry, were reestablished in the three-dimensional treatment planning system evaluating the total dose received by the HP model calculating the cumulative DVHs (Chen et al., 1987). When the RT consists of one set of fields covering the whole brain, the HP region lies within these fields and thus receives the full prescribed dose. If the RT consists of two sets of fields, a primary set covering the whole brain and a set of boost fields covering for instance the posterior fossa, the total dose to the HP region depends on whether this region is totally or partially included in the boost fields. Since the delimitation of the HP region is difficult to delineate it was not apparent how much of the volume of the HP model would provide the best description of the dose received by the HP region. A cumulative DVH is interpreted by looking at different percentages of the volume of interest and shows what doses these percentages of the volume receive. Due to both hot and cold spots within the treatment volume the dose received to a particular volume at some points differs from the prescribed dose and the consequence is dose inhomogeneity within the volume. DVHs are based on the concept that there is a volume effect for normal tissue damage, and therefore increasing the volume, which is receiving the same dose will lead to increased damage dependent on dose. There is a steep dose gradient on the geometrical border of a radiation field, and if the border is

within the HP region it has a significant effect on the DVH. Therefore we calculated the DVH in 10% steps from 10% to 100% of the volume relating the significance between dose and risk of GHD at each volume and found that the 90% volume was the strongest predictor of GHD. Whether this was the right interpretation of the model could be questioned, but Cox regression analysis verified the 90% volume of the HP region to be the strongest predictor of GHD, and thus, probably the best description of the HP region. We demonstrated that the speed of onset of pituitary dysfunction is dose-dependent. Children who had received ≥ 37.5 Gy to the HP region by the 90% volume had a significant risk of GHD of 87% 2.5 years after RT vs 33% for children receiving < 37.5 Gy (Figure 2), article I (Schmiegelow et al., 1999) – demonstrating a dose dependency of time of onset of GHD. This is in accordance with a study by Clayton and Shalet (Clayton and Shalet, 1991), embracing 82 survivors of childhood malignant disease. They estimated the dose to the HP region from the original prescription plans and verification films to be in the range of 27.0 to 47.5 Gy and concluded that children who had received a dose to the HP region ≥ 30 Gy ($n = 36$) compared to children receiving < 30 Gy ($n = 46$) developed GHD more rapidly. Our results indicate that the observed huge interindividual difference in time to onset of GHD in children presumably treated equally could be explained by interindividual differences in the degree of irradiation to the HP region, and the fact that there is a steep dose gradient on the border of the radiation field underlines the necessity of very precise planning of the treatment fields.

In our study the presence of GHD was tested if growth disturbances had been suspected. All 19 children had had at 3 month intervals from time of diagnosis until the end of follow-up their height determined and levels of insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) measured by radioimmunoassays (RIA) (Juul et al., 1994) (Juul et al., 1995). IGF-I and IGFBP-3 are regulated by GH and exhibit only minor diurnal variation with lower values during night (Juul et al., 1998). All IGF-I and IGFBP-3 measurements were performed at the same laboratory, which had well-defined assay-, age- and sex-related normal ranges for IGF-I and IGFBP-3 (Juul et al., 1994) (Juul et al., 1995). Several papers have documented that IGF-I levels are low in children with GHD (Furlanetto et al., 1977) (Juul and Skakkebaek, 1997), but doubt has been raised to the reliability of IGFBP-3 in diagnosing GHD (Sklar et al., 1993) (Tillmann et al., 1997) (Achermann et al., 1998) (Boquete et al., 2003). However, levels of s-IGF-I and s-IGFBP-3, height, and height velocity were transformed to standard deviation scores (SDS) using Danish reference values (Andersen, 1982) (Juul et al., 1994) (Juul et al., 1995). If there had been a fall in IGF-I, IGFBP-3 and/or in height velocity SDS to less than -2.0 SDS and/or in height SDS of more than 1 SDS, a possible GHD was evaluated testing the pituitary somatotrophic function by a GH

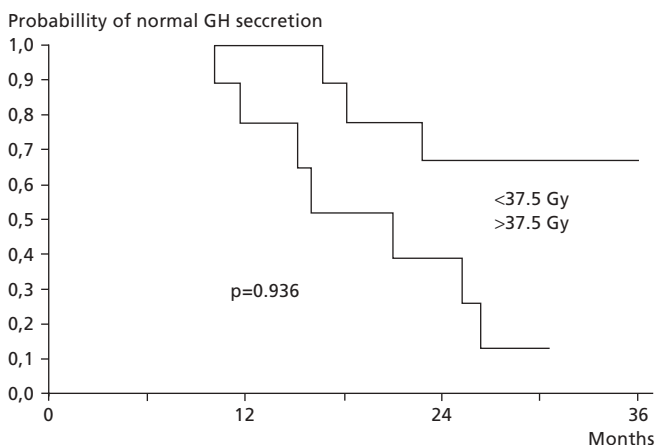


Figure 2. Growth hormone deficiency for patients receiving $>$ or $<$ than 37.5 Gy to the 90% dose-volume of the HP box. Medical Pediatric Oncology. 33(6): 564-72. 1999.

provocative test using arginine. As different provocative stimuli (insuline, arginine, clonidine etc), may result in different GH responses (Ghigo et al., 1996), and different assays may give different results, it is very important that each laboratory has its own cut-off limit. The GH response to the arginine test was evaluated in relation to an arbitrary cut-off value of 15 mU/L – a cut-off value which had been compared to other laboratories and previously published based on studies of normal and growth-retarded children (Andersson et al., 1995). Our criteria were consistent with the “Consensus guidelines for the diagnosis and treatment of GHD in childhood and adolescence” published by the Growth Hormone Research Society (GRS) (Growth Hormone Research Society, 1998) namely that one GH provocation test is sufficient in patients who have received CIR to support the diagnosis of GHD together with IGF-I and IGFBP-3 values below a cut-off limit less than –2SD.

Naturally, adequate coverage of the tumour must have first priority, but the present study showed a noticeable difference in dose-distributions. The number of patients available for the present study were limited, and our findings need to be confirmed in larger cohorts of patients. Few other reports have addressed the value of dosimetry to predict the risk of side effects following cranial RT. Slater could not in a somewhat similar study from 1988 (Slater et al., 1988) find a significant correlation between the risk of GHD and the dose delivered to the pituitary at the 50% dose-volume level. However, they studied only 14 patients, all were adults, and they only provided data on the dose that 50% of the pituitary received, and they did not attempt to include the hypothalamus.

The exact threshold dose has, however, not yet become evident and prospective studies with dosimetry of the HP region prior to the delivery of RT need to be performed followed by a long time of follow-up. It has been claimed that irradiation of the hypothalamus with focal RT of the posterior fossa, e.g. in children with medulloblastoma cannot be avoided due to the very close relation of the anterior limit of the posterior fossa radiation field extending to the posterior clinoid, and hence encompassing the posterior part of the hypothalamus (Duffner et al., 1985a) (Duffner and Cohen, 1991). However, since there is a steep dose gradient on the geometrical border of a radiation field, any limitation of the field based on accurate quality control of the boost fields could mean sparing a significant part of the hypothalamus and hence preserve endocrine hormone secretion, even if the border of the radiation field is within the HP region.

8. BIOLOGICAL EFFECTIVE DOSE AND GROWTH HORMONE DEFICIENCY

The first therapeutic use of x-rays is reported to have taken place in 1896, but as the treatment often involved single massive exposures aimed at the eradication of tumours and as the patients who survived the immediate postirradiation period often developed major complications, the use of x-rays to treat tumours would probably have been abandoned had it not been for the work on the relative radiosensitivity of different tissues in the 1920s and 1930s by Claude Regaud and Henri Coutard (Coutard, 1934), who found that by administration of fractionated doses of radiation they could achieve the same tumour response without serious side effects to adjacent normal tissues resulting in the development of different time-dose fractionation schemes (Juil, 1936). In the mid 1930s Strandqvist was the first to try to establishing a mathematical equation of overall treatment time and response to RT (Strandqvist, 1944), and in the 1960s and 1970s the Ellis Nominal Standard Dose (NSD) formula (Ellis, 1969) and variants of the NSD formula were presented, which predicted the total dose that could be given safely if either number of fractions or treatment time were changed. These formulae have been abandoned and instead equations based on the linear quadratic (LQ) cell-survival model were introduced in the 1980s by Barendsen (Barendsen, 1982) and Fowler (Fowler, 1989), who suggested the term BED. The LQ model includes the recognition that

different kind of tissues react differently to changes in fractionation. The LQ cell survival model describes the relationship between total isoeffective dose and dose per fraction. Using the BED makes it possible to quantify the biological effects of RT and even comparing different treatment regimens using altered fractionation schedules something which made it very interesting for us to use in the evaluation of a dose-response relationship in a cohort treated with very different RT regimens. A keypoint for this cell-survival theory was the demonstration of differences in fractionation sensitivity between early and late responding normal tissues. Both the fractionation size, the total dose of radiation, and the irradiated volume may determine the incidence of late effects of neural tissue. Radiobiologic approaches to the understanding of the long-term side effects of late responding tissues have shown that the biological effects depend for any given total dose on the fractionation size and on the α/β ratio, where α and β are the radiobiological cell survival parameters for the particular tissue within the treatment volume and specific for the tissue under consideration describing the fractionation sensitivity (Thames and Hendry, 1987) (Fowler, 1992). The α/β ratio describes the shape of the fractionation response: a low α/β ratio (about 3 Gy) is characteristic of late responding normal tissues and indicates a rapid increase of total dose with decreasing dose per fraction and a survival curve for the target cells that is significantly curved, in contrast to a high α/β ratio (about 10 Gy) in acute or early responding tissues like epithelia, haemopoietic or in many tumour tissues (Joiner and Van der Kogel, 1999). Radiobiologically, this difference may be related to slow cell turnover in late responding tissues, allowing the neural cells to remain in resting states where they are proficient in the repair of the damage caused by small doses. Consequently, late responding tissues are particularly sensitive to changes in fraction size, larger fractions being more damaging to the neural tissue. Late responding tissue probably does not experience repopulation during a course of RT, which is why neural tissue is relatively unaffected by the overall treatment time (Joiner and Van der Kogel, 1999). Thus, the normal tissue reaction of the neural tissue is dependent on the BED received. We determined the BED received by the HP region studying the original prescription plans and simulator films to estimate whether the HP region fell within the fields of radiation. In the present cohort the children had been treated with very different treatment schedules. The overall treatment time differed according to different treatment schedules used from 1970 to 1997 from 1 fraction every second day to 1 fraction per day, 5 times a week, and the dose per fraction differed from an increment strategy starting at very low doses increasing over the treatment period to the administration of uniform fractions during the whole treatment period. The physical dose was transformed to BED using the equation:

$$BED = D \times (1 + d/(\alpha/\beta))$$

where D is total dose, d is the fraction size, and α represents the linear non-reparable component of cell killing and thus the initial slope of the survival curve, and β represents the reparable quadratic component of cell killing. The dose at which the two components of cell kill are equal constitutes the α/β ratio of the tissue within the treatment volume. The usual form of the LQ model, which is used in the present study, does not include time factors, since late reacting tissues by definition are tissues whose cell populations proliferate very slowly, and as the cell kill caused by irradiation is due to mitotic death, changes in overall treatment time is of minor importance with respect to late complications (Fowler, 1989) (Joiner and Van der Kogel, 1999). For ^{60}Co γ rays or high-energy x-rays produced by linear accelerators at energies of 4, 6, or 8 megavoltage the biological effect per unit dose is similar (Joiner, 1999). If there had been a change in fraction size during the same schedule, which proved to be the fact especially for those patients, who had been treated during the seventies and in the beginning of the eighties the

partial BEDs could be added as they related to the same α/β , and the total BED was an addition of the partial BED values. This method has been proven correct and convenient (Barendsen, 1982) (Fowler, 1989) (Van Kogel, 1999):

$$\text{BED} = \text{BED}_1 + \text{BED}_2 + \text{BED}_3 + \text{BED}_4 + \dots + \text{BED}_n$$

We studied the BED to the HP region and analysed the relation to endocrine function trying to show a more accurate evaluation of the effects of RT to endocrine function, article II, (Schmiegelow et al., 2000), article III (Schmiegelow et al., 2000). We found that BED to the HP region was inversely significantly related to peak GH after an insulin intolerance test (ITT) or an arginine stimulation test ($r_s = -0.53$, $P < 0.0001$) (Figure 3), article II (Schmiegelow et al., 2000). Eighty percent of the patients in the cohort had GHD at the time of follow-up (median time of follow-up was 15 years (range: 2-28 years)). Comparing the GHD group with the group of patients without GHD (20%) there was a significant difference in the BED received by the HP region (77.5 Gy vs 54.0 Gy) ($P = 0.002$), and the importance of BED was underlined by the fact that the stepwise backward multiple linear regression analysis showed that the best-fit model to predict the peak GH release following an ITT/arginine stimulation test included BED ($P < 0.0001$). The BED was significantly correlated to increasing involvement of the HP axes ($r_s = 0.3$, $P = 0.01$), while the prescribed dose to the HP axis was not significantly correlated to increasing involvement of the HP axes (unpublished data).

The ITT is considered the most reliable GH provocative test for the assessment of GHD in adults and has thus remained the "gold standard" test for GHD according to the guidelines from the GRS (Growth Hormone Research Society, 1998). Provided adequate hypoglycemia is achieved (blood glucose of ≤ 2.2 mmol/L) the resulting neuroglycopenia activates the hypothalamus with secretion of GHRH resulting in secretion of GH from the pituitary gland. The ITT in adults is regarded a safe test in experienced hands (Jones et al., 1994) (Lange et al., 2002), however, case reports have demonstrated potential risks using the ITT in children (Burke, 1992) (Shah et al., 1992), the reason why we use the arginine or clonidine stimulation test for children. However, the ITT is contraindicated in patients with ischaemic heart disease or seizure disorders. Both the ITT and the arginine stimulation test induce GH release and the hypothesis is that the GH-releasing effect of both ITT and arginine is mediated both by activation of α -2-adrenergic receptors and by suppression of the growth hormone inhibitory hormone, somatostatin (SRIF) resulting in synthesis and release of GH from the somatotrophs (Alba-Roth et al., 1988) (Ghigo et al., 1990) (Ranke and

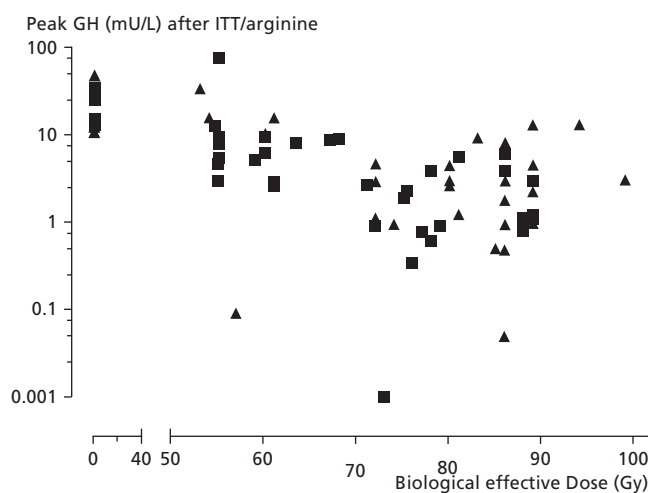


Figure 3. The relationship between the biological effective dose of irradiation (BED) (Gy) and peak GH (mU/l) in 73 patients ($r_s = -0.53$, $P < 0.0001$). Peak GH (mU/l) after ITT (\blacktriangle). Peak GH (mU/l) after arginine stimulation test (\blacksquare). Clinical Endocrinology. (2000) 53, 191-7.

Haber, 1996). A response to the ITT of less than $3 \mu\text{g/L} = 9 \text{ mmol/L}$ is defined as severe GHD, provided the cut-off value was defined in a GH assay employing a calibrated polyclonal competitive RIA (Growth Hormone Research Society, 1998). Patients treated with CIR or patients with one or more additional pituitary hormone deficits require only one provocative test of GH secretion for the diagnosis of GHD (Growth Hormone Research Society, 1998). The diagnosis of GHD was also confirmed by low median IGF-I SDS and IGFBP-3 SDS of -2.5 SDS and -1.7 SDS, respectively, and IGF-I SDS was significantly correlated to peak GH following ITT/arginine stimulation test, whereas median IGFBP-3 SDS and peak GH were not significantly correlated. As described in the former section 7. page 15 it has been documented that IGF-I levels are low in children with GHD, whereas IGFBP-3 has been questioned in diagnosing GHD. Although mean serum IGF-I levels are low in adults with GHD and very low IGF-I levels may indicate GHD, the IGF-I-IGFBP-3 complex probably can only assist diagnostic strategies in childhood onset GHD, while these markers probably are of limited predictive value in adult GHD (Gill et al., 1998) (Hilding et al., 1999) (Marzullo et al., 2001).

8.1 GROWTH HORMONE DEFICIENCY OF HYPOTHALAMIC ORIGIN AND BED

Clinical studies with small numbers of patients have implied that the hypothalamus rather than the pituitary is the site of radiation damage (Ahmed and Shalet, 1984) (Lustig et al., 1985) (Lam et al., 1986) (Lannering and Albertsson Wikland, 1987) (Constine et al., 1993). In the study by Lustig (Lustig et al., 1985) 5 children who received CIR for extrahypothalamic intracranial neoplasms or leukaemia and subsequently developed severe GHD were tested with GHRH in an attempt to distinguish hypothalamic from pituitary dysfunction as a cause of their GHD and a significant peak GH was reported. In the paper by Lannering (Lannering and Albertsson Wikland, 1987) GH secretion after administration of GHRH was compared to 24-hour GH profiles in 19 children, who had received RT to the brain including the HP region as part of the treatment for a tumour of the brain, eye or epipharynx. All children had disturbed profiles of spontaneous GH secretion and 16 children had a prompt rise in GH after GHRH was given indicating hypothalamic damage. In our study with a large sample size we tested the radiosensitivity of the hypothalamus vs the pituitary in 62 patients and compared the peak GH response to an ITT/arginine stimulation test vs the GH response to a GHRH stimulation test and showed that the peak GH response to the GHRH test was significantly higher than that of the ITT/arginine test, article III (Schmiegelow et al., 2000). These results underline a hypothalamic damage. We also demonstrated that the peak GH after the GHRH test was significantly inversely correlated to BED (article III) (Schmiegelow et al., 2000) underlining that the adverse effect of RT to the hypothalamus is directly related to BED.

8.1.2. BED and threshold doses to the HP region

Earlier attempts have been made trying to identify the threshold dose of radiation to the HP region for induction of endocrine late effects. Shalet (Shalet et al., 1976) studied the varying doses of irradiation received by the HP region from prescription plans and verification films in 39 children treated with RT for a brain tumour and 17 children treated for ALL. To ensure that all doses were biologically comparable they recalculated the doses by means of the Ellis NSD formula, which was the formula used at that time to evaluate the biological effect of radiation and related the doses to the GH peak response after an ITT. Even though the Ellis formula does not take the α/β ratio into consideration, they found an inverse significant correlation between dose and peak GH response after an ITT. The exact threshold dose of development of GHD could not be identified but was assumed to be > 29 Gy. Some other clinical studies have also observed the importance of total dose and fraction size. In

the study by Littley (Littley et al., 1989) radiation-induced hypopituitarism was studied prospectively for up to 12 years not in children but in 251 adult patients treated for a tumour of the pituitary gland or anatomically closely related structures (aged between 16 and 77 years at the time of RT) and in each patient there was normal function of at least one HP axis before RT and all patients had a full assessment of pituitary function undertaken after RT. One hundred and eighty-four patients underwent a surgical procedure before RT. All 251 patients were treated with external RT, ranging in dose from 20 Gy in eight fractions (2.5 Gy per fraction) to 45 Gy in 15 fractions (3.0 Gy per fraction). Ten further patients were studied 2-4 years after whole-body irradiation for haematological malignancies using 12 Gy in six fractions (2.0 Gy per fraction) over 3 days and 7 patients were studied 3-11 years after whole-brain RT for a primary brain tumour (30 Gy, eight fractions, 11 days) (2.5 Gy per fraction). They concluded that total radiation dose to the HP region is a major determinant of the speed of onset and incidence of anterior pituitary deficiencies and that the data indicated that fraction size was important. In the study by Shalet (Shalet et al., 1979) 14 out of 17 children treated with whole brain CIR for ALL with a dose of 25 Gy in 10 fractions (= 2.5 Gy per fraction) had a subnormal GH response to an ITT, compared with 1 out of 9 children who were treated with 24 Gy in 20 fractions (= 1.2 per fraction). Despite a nearly identical total radiation dose the incidence of GHD differed significantly due to change in fraction size.

The results of the present study support the use of BED as a means of a more correct biological approach to estimate the risk of GHD after cranial irradiation and the adaptability of BED as a measure of the radiobiological impact of RT on the HP axis has been recognized in both textbooks (Reiter and Rosenfeld, 2003) as well as in clinical research (Darzy et al., 2003). However, when both the HP axis and the primary gland has been disturbed the degree of central hypofunction caused by CIR is very difficult to interpret. The interaction between central and primary hypofunction was probably also why BED was not significantly correlated to the endocrinological endpoints when we tried to evaluate the relation between the BED to the HP region and the endocrinological endpoints: gonadal, thyroid or adrenal function article IV (Schmiegelow et al., 2001), article V (Schmiegelow et al., 2003), article VI (Schmiegelow et al., 2003). Prospective studies need to be undertaken to determine the exact dose-response relationship trying to improve the therapeutic ratio, i.e. to reduce the level of normal-tissue damage while keeping tumour control.

9. CHEMOTHERAPY

9.1. CHEMOTHERAPY AND CHILDHOOD BRAIN TUMOURS

In general the criteria for the treatment of childhood brain tumours with CTx are i) preoperatively in order to provide tumour reduction, and ii) postoperatively to children < 3-5 years of age in order to postpone CIR until progression of tumour, because of the increased risk of CNS damage for the very young child (Syndikus et al., 1994), and iii) as primary treatment of inoperable or disseminated tumours.

There is doubt as to whether CTx improves the overall survival in brain tumours. The reasons for this are that i) the cytotoxic effect of antineoplastic drugs is exerted by interfering with the synthesis or function of DNA with drugs active in a specific phase of the cell cycle. However, most brain tumours have a low rate of cell turnover, and ii) the blood brain barrier prevents most of the cytotoxic agents at standard doses from penetrating the CNS, the reason why high-dose systemic therapy is used. Nevertheless, several different cytotoxic agents such as vincristine, procarbazine, the nitrourea BCNU and CCNU, etoposide, cisplatin, carboplatin, cyclophosphamide, high-dose Ara-C and methotrexate (MTX) are used either as monotherapy or in combination with different response rates reported (Evans et al., 1990) (Tait et al., 1990) (Bailey et al., 1995) (Taylor et al., 2003).

Children in the present cohort were treated during the late 1970s and 1980s with CCNU, and vincristine, and MTX as single drugs or in combination. Later in the nineties children with germinal cell tumour received cisplatin and bleomycin, and etoposide, and children with medulloblastoma received vincristine, carboplatin, endoxan, and etoposide according to the International Society of Paediatric Oncology (SIOP) II protocol (Bailey et al., 1995).

9.2. CHEMOTHERAPY AND GROWTH HORMONE DEFICIENCY

Endocrinological late effects of CTx in the treatment of brain tumours have only been investigated in few studies (Olshan et al., 1992) (Ogilvy Stuart and Shalet, 1995). The effect of CTx on growth has been suggested to potentiate the deleterious effects of RT. In the study by Olshan (Olshan et al., 1992) the growth of 38 prepubertal children treated for medulloblastoma postoperatively with CSI only (n = 15) or CSI + CTx (n = 23) showed that adjuvant CTx potentiates the radiation-induced growth failure effects. In the study by Ogilvy-Stuart (Ogilvy Stuart and Shalet, 1995) the impact of treatment with either CIR or CSI with or without CTx for a brain tumour distant from the HP axis was assessed in 29 children, who had reached final height. Both CSI and the use of CT resulted in a significant and equal reduction in final height and the effect was additive.

In the present cohort we investigated the possible influence of CTx on peak GH following an ITT/arginine stimulation test, article II, (Schmiegelow et al., 2000). Forty-three of the 73 children had been treated with CIR and 30 with CSI and 29 children were treated pre- and/or postoperatively with adjuvant CTx in addition to RT. We performed a multiple linear regression analysis of factors of possible correlation to log peak GH, but CTx did not have a significant impact on GH secretion.

10. GONADAL DYSFUNCTION

Gonadal dysfunction is the most common long-term side effect of CTx. One of the first reports on azoospermia after nitrogen mustard came in 1948 (Spitz, 1948) and subsequent reports suggested oligospermia and azoospermia after treatment with a number of drugs, particularly alkylating drugs and combination CTx with mustine, vincristine, procarbazine and prednisolone (Chapman et al., 1979) (Lentz et al., 1977) (Whitehead et al., 1982) (Charak et al., 1990) (Schmiegelow et al., 1995) (Howell et al., 1999). Gonadal dysfunction following treatment with CTx has been shown to be both drug-specific as well as dose-related (Watson et al., 1985) (Bramswig et al., 1990) (Pryzant et al., 1993). The impact of CTx on gonadal function has been reported especially in relation to the chemoregimens for Hodgkin's disease and several studies have reported oligospermia and azoospermia with reduced total testicular volume with raised or normal basal follicle-stimulating hormone (FSH) levels and raised FSH levels after a GnRH stimulation test indicating damage of the germinal cells (Whitehead et al., 1982) (Chapman et al., 1979) (Charak et al., 1990) (Bramswig et al., 1990) (Mackie et al., 1996). The germinal cells are regarded to be more sensitive to CTx than the Leydig cells (Whitehead et al., 1982) (Clayton et al., 1988). However, several reports have documented raised or normal basal luteinizing hormone (LH) levels or raised LH levels after a GnRH stimulation test suggesting Leydig cell impairment with normal or low levels of testosterone (Watson et al., 1985) (Charak et al., 1990) (Howell et al., 1999).

We assessed the effect of RT and CTx on gonadal function, article IV (Schmiegelow et al., 2001) and in order to avoid confounding from different pubertal stages we only included males ≥ 18 years at time of follow-up. Thirty males of the total cohort fulfilled the criteria.

FSH has previously been used as an indirect marker of spermatogenesis, since raised levels of FSH are seen as a negative feedback response to primary gonadal hypofunction. Serum inhibin B has lately been implemented as a more direct serum marker of sperma-

togenesis (Pierik et al., 1998) measuring the serum levels of the bioactive gonadal peptide hormone inhibin B by means of an enzyme-linked immunosorbent assay (Illingworth et al., 1996). Inhibin B is supposed to be produced in the Sertoli cells and the germ cells of the testes and directly reflects the degree of seminiferous tubular damage (Andersson et al., 1998), but as inhibin B (and inhibin A) in women vary during the menstrual cycle (Groome et al., 1996) (Sehested et al., 2000) and as at least some of our patients were suspected of having anovulatory menstrual cycles we excluded females from this population.

We evaluated the possible influence of CTx compared to RT on gonadal function comparing the group of patients who had been treated with both CTx and RT in relation to the group of patients who had been treated with RT only. As there was no significant difference in the two groups with regard to the BED to the HP region, we assumed that the same degree of RT-induced central hypogonadism could be expected and a possible difference in end-point parameters related to CTx. Since no strict normative data for the peak FSH and peak LH response to a GnRH provocative test exist we compared the two groups and found several indications strongly suggestive of primary gonadal dysfunction caused by CTx resulting in damage of the Sertoli cells and germ cells as well as the Leydig cells of the testes. We measured a significantly higher peak FSH response to the GnRH test and significantly lower levels of inhibin B in the group of patients, who had been treated with both RT + CTx compared to the group of patients, who had been treated with RT only (Figure 4). In addition there were significant correlations between peak FSH and inhibin B (Figure 5) as well as between inhibin B and the total testicular volume (Figure 6). A stepwise backward multiple linear regression analysis showed that the best-fit model to predict inhibin B included CTx in the model in contrast to spinal irradiation. This also indicated that even if a scatter dose from the spinal fields reached the testes, the amount of irradiation was probably of minor importance. This is in accordance with a former study by Ahmed (Ahmed et al., 1983) with a small number of children with medulloblastoma, who had all been treated with either lomustine or carmustine + CSI or CSI alone in which it was demonstrated that CTx resulted in a primary gonadal dysfunction, while spinal irradiation had no influence.

Since inhibin B is known to be produced mainly in the Sertoli cells and/or the germ cells of the testes in the adult male (Anawalt et al., 1996) (Andersson et al., 1998) and since we found a significant correlation between inhibin B and the total testicular volume our results further underline the fact that adjuvant CTx in the treatment of brain tumour results in reduced spermatogenesis with reduced total testicular volume. Inhibin B is a direct serum marker with the opportunity to assess the testicular damage without semen analyses, which was not found to be feasible in this cohort of patients because of the psychological impairment of the individuals. There was no significant difference between the RT + CTx group and the RT only group regarding the levels of testosterone, but as the levels of testosterone in both groups were significantly lower compared to the controls this probably indicated RT-induced central damage leading to decreased LH and thereby low serum testosterone. The fact that the GnRH test provoked significantly elevated levels of peak LH in the group of patients treated with RT + CTx compared to the group of patients who had been treated with RT only may suggest subtle damage to the Leydig cells, and we speculate that the Leydig cells are sensitive, yet, less sensitive to CTx compared to Sertoli cells and germ cells. This is in consistence with other reports that CTx has an influence on Leydig cell function (Tsatsoulis et al., 1990) (Wallace et al., 1997) (Howell et al., 1999).

In conclusion, these data suggest that CIR for a childhood brain tumour may affect the HP axis resulting in secondary hypogonadism, and adjuvant CTx may have a deleterious affect upon the seminiferous epithelium of the testes leading to reduction in inhibin B indicating primary gonadal damage. Thus, such patients may have

normal or even low levels of FSH despite damage to the seminiferous epithelium, and as the fertility status by a semen analysis for psychological reasons can be difficult to obtain in this group of patients, we suggest inhibin B as the most useful direct serum marker of spermatogenesis in the follow-up of individuals who have received both CIR and gonadotoxic CTx. However, since the number

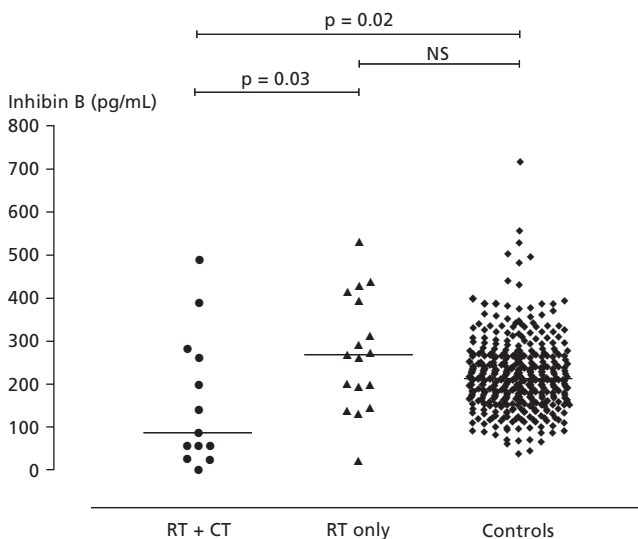


Figure 4. Levels of inhibin B in patients treated with RT + CT and patients treated with RT only compared to control. *J Clin Endocrinol Metab* 86: 2446-52, 2001.

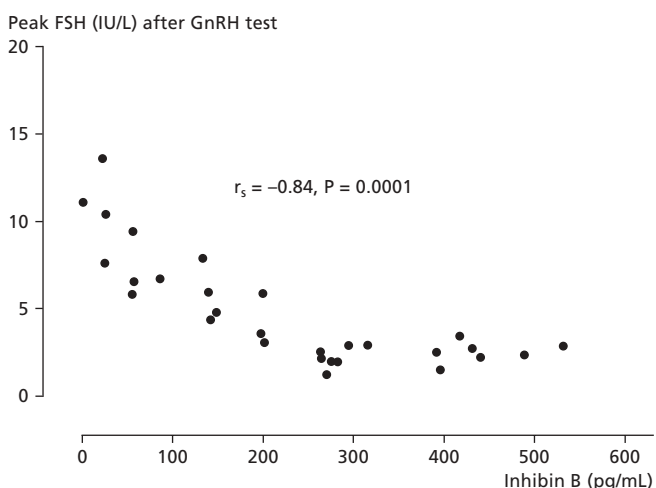


Figure 5. Correlation between peak FSH and inhibin B. *J Clin Endocrinol Metab* 86: 2446-52, 2001.

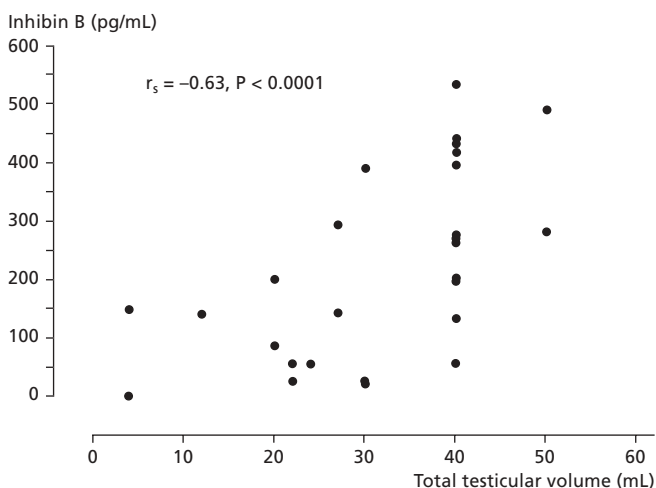


Figure 6. Correlation between inhibin B and total testicular volume. *J Clin Endocrinol Metab* 86: 2446-52, 2001.

of patients with RT + CT and RT only are small these data have to be confirmed in further studies.

11. THYROID DYSFUNCTION

11.1 HYPOTHYROIDISM

Data about radiation-induced thyroid damage include reports from studies on Hodgkin's disease (Shalet et al., 1977) (Schimpff et al., 1980) (Constine et al., 1984) (Hancock et al., 1995) and tumours of the head and neck (Kaplan et al., 1983) (Samaan et al., 1987) (Nishiyama et al., 1996) (Tell et al., 1997) while more seldomly about children who have been treated with TBI in preparation for bone marrow transplantation (Sklar et al., 1982) (Boulad et al., 1995) or children who have been treated with CSI for childhood brain tumours (Oberfield et al., 1986) (Livesey and Brook, 1989) (Ogilvy Stuart et al., 1991) (Constine et al., 1993). CIR may cause TRH secretory abnormalities if the hypothalamus, in which TRH is synthesized and secreted, lies within the fields of radiation. Central hypothyroidism may thus occur due to an inadequate stimulation of TSH resulting in low levels of TSH and as a result low levels of thyroxine from an otherwise normal thyroid gland. Direct or scattered irradiation from the spinal fields are received by the thyroid gland during CSI administered to treat certain childhood brain tumours, and often the first detectable abnormality of the thyroid axis is compensated thyroid dysfunction (mild primary hypothyroidism) with increased levels of TSH and normal thyroxine levels. Further deterioration of thyroid function results in overt primary hypothyroidism with high levels of TSH and low thyroxine levels. In our study compensated thyroid dysfunction was defined as a TSH value greater than or equal to 4 mU/L and a T₄ value within the normal range. Overt primary hypothyroidism was defined as an elevated TSH value (>4 mU/liter) in the presence of a subnormal free T₄ value (<9 pmol/liter).

We wanted to assess the incidence of primary and secondary hypothyroidism following RT and evaluate the impact of both CTx and CIR only versus CSI on the HP-thyroid axis, which has been less well documented. Our hypothesis was that CIR cannot be regarded as negligible since CIR may cause a minor scattered dose from the cranial fields to the thyroid gland and hereby contribute to damage of the thyroid gland. We demonstrated significantly elevated basal TSH levels in the CSI group compared with the CIR only (Figure 7), article V, (Schmiegelow et al., 2003). This was probably due to scat-

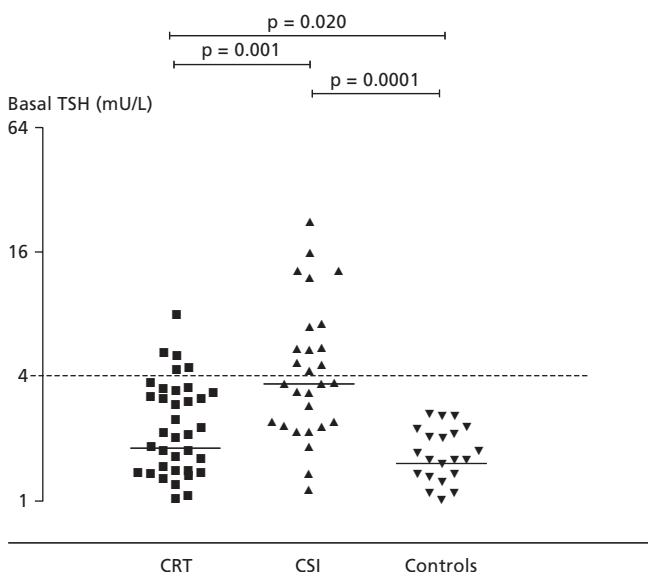


Figure 7. Levels of basal TSH showing significant differences, when comparing patients treated with craniospinal irradiation CSI (▲; median: 3.65 mU/L, range: 0.87-22.5 mU/L), vs cranial irradiation CIR (■; median: 1.78 mU/L, range: 0.56-8.99 mU/L), vs controls (▼; median: 1.51 mU/L, range: 0.53-2.61 mU/L), respectively. The dashed line of 4 mU/L indicates the upper normal limit of serum TSH levels. *J Clin Endocrinol Metab* 88: 136-40, 2003.

tered irradiation to the thyroid gland from the spinal fields resulting in a primary hypothyroid state, which, however, was compensated in most cases. Since patients treated with CSI had received different degrees of irradiation both to the HP axis and to the thyroid gland the degree of central hypothyroidism caused by CIR was very difficult to interpret. However, we also found that the CIR only group had significantly higher median basal TSH levels compared to controls, and we speculate that this was probably due to scattered irradiation from the cranial fields to the thyroid gland. The hypothesis of significant scattered irradiation from the cranial fields was strengthened by the fact that we found significantly lower levels of free T₄ in the CSI group and the CIR only group compared to controls, however, no significant difference between free T₄ in the CSI group compared to the CIR only group. The results emphasize that the dose to the thyroid gland in those receiving CIR is not negligible and that the thyroid should be shielded during both CSI and CIR due to the scattered irradiation from the cranial fields. A few studies using thermoluminescent dosimeters have reported the amount of irradiation to the thyroid from CIR during treatment of childhood leukaemia (Rogers et al., 1982) (Bessho et al., 1994) (Stevens et al., 1998), however, without any evaluation of a possible correlation to endocrine endpoints. In the study by Bessho (Bessho et al., 1994) it was demonstrated that the absorbed dose to the thyroid gland ranged from 0.7-7.3% (= 0.13-1.32 Gy) of the dose delivered to the cranium compared to 1-5% (= 0.20-0.40 Gy) in the study by Stevens (Stevens et al., 1998).

We found hypothyroidism in 24% of the total cohort. Seventy-three percent had mild or compensated hypothyroidism and 27% had overt primary hypothyroidism. Central hypothyroidism was found in 6% of the cohort. Of the patients with mild or overt primary hypothyroidism 71% had been treated with CSI, whereas 29% had been treated with CIR only. The high incidence of hypothyroidism in children who were treated with CSI was expected, because their thyroid gland was irradiated to some degree, however, the fact that we also found hypothyroidism in 1/3 of the primary hypothyroid patients due to CIR only was inconsistent with some and consistent with other reports (Livesey et al., 1990) (Ogilvy Stuart et al., 1991). In the study by Livesey (Livesey et al., 1990) on long-term endocrine effects following RT for childhood brain tumour in 144 children with a median follow-up of 9.6 years (2-26 years) primary thyroid dysfunction was found in 23% treated with CSI but in none treated with CIR only. In the study by Ogilvy-Stuart (Ogilvy Stuart et al., 1991) 134 children had been treated for a brain tumour not involving the HP axis and thyroid function was assessed up to 24 years after treatment with RT. Thirty-five percent of children treated with CSI developed abnormalities compared to 20% of those treated with CIR only – very similar to our results. Possible explanations for these apparent inconsistencies might be less sensitive and specific laboratory assessments used in the past. In our study we used an ultrasensitive third-generation TSH assay measuring the TSH levels and we had a median length of follow-up of 12 years (range: 2,0-28,0 years). We therefore expect to have established the prevalence of thyroid dysfunction in this cohort of children treated for a brain tumour not involving the HP axis. Central hypothyroidism is considered less common and a consequence of higher doses of irradiation, however, a dose response relation has not yet been possible to establish. We also could not show a significant difference between the CSI and the CIR only group with respect to the BED to the HP axis, indicating that the same degree of RT induced central hypothyroidism would be expected in the two groups. The interaction between central and primary hypothyroidism is probably also why BED was not significantly correlated to the endocrinological endpoints.

11.2 THYROTROPIN-RELEASING HORMONE STIMULATION TEST

In healthy normal individuals, the intravenous injection of TRH is

promptly followed by an increase in TSH reaching a peak after 20 to 30 minutes and then declining to basal values after 4 hours. Patients with central hypothyroidism may show both an absent or exaggerated, prolonged TSH response indicative of HP dysfunction (Faglia, 1998). We measured an exaggerated TSH response to TRH comparing the TSH levels in the CSI group with the CIR only group and controls. The extent of increased serum TSH concentration after TRH administration correlates well with the basal serum TSH concentration and it has been shown that there appears to be no diagnostic advantage gained by TRH testing compared to an accurately measured basal TSH value (Spencer et al., 1993) (Hartoft-Nielsen et al., 2004). However, in central hypothyroidism the TRH test reflects partly or totally i) reduced mass of functioning thyrotrophs, ii) defect in TRH-stimulated TSH secretion, iii) reduced bioactivity of circulating TSH (Faglia et al., 1979). The TRH test allows the differentiation between pituitary (second) and hypothalamic (tertiary) hypothyroidism (Faglia, 1998) and at the time when this study was planned and carried out it still was a routine examination in patients with possible central hypothyroidism to use the TRH test. We found a significantly higher median peak response in the CSI group at TSH_{30 min}, and a significantly delayed decrease at TSH_{60 min}, and at TSH_{150 min}, respectively. Comparing controls with both the CSI and the CIR only group showed significantly lower median TSH_{0 min}. At 60 minutes the median TSH was significantly lower in controls compared to the CSI group, however, there was no significant difference between the CIR only group and controls. As a consequence of central hypothyroidism in both the CSI and CIR only group we found exaggerated and prolonged TSH responses following TRH stimulation indicating a hypothalamic origin of hypothyroidism likely to result from alterations in TRH secretion or transport to the anterior pituitary (Faglia, 1998) or due to the secretion of biologically inactive TSH (Faglia et al., 1979). The fact that the response to TRH was clearly more pronounced in the CSI group compared to the CIR only group and also compared to controls may reflect the higher basal TSH level in the CSI group compared to the CIR only group.

11.3 CHEMOTHERAPY AND THYROID FUNCTION

Whether or not CTx significantly compounds the risk of hypothyroidism is still a matter of debate (Constine et al., 1984) (Livesey and Brook, 1989) (Ogilvy Stuart et al., 1991) (Chin et al., 1997) (Paulino, 2002) (van Santen et al., 2003). In our study CTx did not seem to add to the damaging effect of RT on the thyroid axis neither in the CSI group nor in the the CIR only group. We found that patients treated with CTx in addition to RT (n = 30) compared to patients treated with RT only (n = 41) had significantly higher basal TSH levels, however, in the CSI group (n = 29) 22 patients had been treated with CTx in addition to CSI. If we compared these 22 patients with the remaining CSI patients without CTx (n = 7) we found no significant difference with regard to median TSH levels and patients treated with CTx in addition to CIR (n = 8) compared to patients treated with CIR without CTx (n = 34) also had no significant difference with regard to median TSH levels. This result is in accordance with several other reports (Constine et al., 1984) (Chin et al., 1997) (van Santen et al., 2003). Van Santen evaluated damage to the thyroid axis in 205 cancer survivors in relation to CIR, CSI and CTx and found that CTx did not have an additional negative effect on the thyroid axis. However, contrary to these reports are the study by Livesey and Brook (Livesey and Brook, 1989), which demonstrated that CTx further increased the incidence of thyroid dysfunction and the report by Ogilvy-Stuart (Ogilvy Stuart et al., 1991), who demonstrated that irradiation plus CTx was more damaging than irradiation alone. In the study by Paulino (Paulino, 2002) it was also demonstrated that low dose CSI + CTx in the treatment of medulloblastoma compared to standard CSI without CTx did not show a reduction in the incidence of hypothyroidism.

In conclusion, we have demonstrated a high incidence of thyroid dysfunction after both CSI as well as after CIR only and we recom-

mend that the thyroid gland should be shielded during both CSI and CIR. Our policy is prolonged surveillance of full pituitary thyroid function in long term survivors of a childhood brain tumor and institution of thyroid hormone replacement if the levels of TSH and free T₄ are above and below the normal range, respectively, to ensure normal growth and metabolism.

12. DYSFUNCTION OF THE HYPOTHALAMO/PITUITARY/ADRENAL AXIS

Overt adrenal insufficiency has rarely been described in patients following treatment for a childhood brain tumour with RT and CTx and data concerning the relationship between RT and CTx and dysfunction of the HPA-axis are limited. ACTH deficiency is quite rare as demonstrated in few studies in which only subtle abnormalities in adrenal function following RT for a childhood brain tumour were shown (Livesey et al., 1990) (Constine et al., 1993) (Oberfield et al., 1997) (Spoudeas et al., 2003). Livesey (Livesey et al., 1990) evaluated the HPA axis in 144 children treated for brain tumours with a median follow-up of 9.6 years (2-26 years) by means of the ITT and only found 4% with diminished cortisol responses. In the study by Constine (Constine et al., 1993), the HPA axis was evaluated 2 to 13 years after CRT in 32 patients treated for brain tumours by means of the ACTH test together with a CRH test and a metyrapone test. The CRH test is supposed to demonstrate evidence of hypothalamic dysfunction, while the metyrapone test works by stimulating the release of ACTH by lowering cortisol secretion. They found normal cortisol responses to the ACTH test as well as a mean peak ACTH response to the CRH test within the normal range and normal serum cortisol responses, however, 35% of the patients had a low 11-deoxycortisol response to the administration of metyrapone. In the study by Oberfield (Oberfield et al., 1997) they evaluated the HPA axis in 17 patients treated with CRT at a median of 5 years (0.1-20 years) before follow-up for acute leukaemia or a brain tumour distant from the HP region with a CRH test. They found significantly lower basal cortisol levels as well as peak cortisol responses, but the mean integrated values for cortisol (area under the curve) and the ACTH responses to CRH were not different from controls. They concluded that patients who are treated with CIR may be at risk for adrenal insufficiency and that unexplained fatigue in these patients could indicate mild adrenal insufficiency and might warrant treatment with glucocorticoids. In the study by Spoudeas (Spoudeas et al., 2003) they studied a small sample size (12 males, 4 females) with a follow-up of 11 years (6.8-21.4 years) treated for posterior fossa brain tumours with CRT. The HPA axis assessment was undertaken using the ITT and showed that 12,5% had ACTH insufficiency (defined as a peak serum cortisol less than 500 nmol/liter).

In our study, article VI (Schmiegelow et al., 2003), we demonstrated that 19% were insufficient of their HPA axis defined as basal cortisol levels below 500 nmol/liter and a response to either an ACTH test 30 minutes or 60 minutes or to an ITT with a peak cortisol below the cut-off (500 nmol/liter) level in all three tests. The optimal means of assessing a possible HPA insufficiency is controversial, but we had the opportunity to evaluating the cortisol response to both the ACTH test and the ITT in 33 patients of the total cohort of 73 patients. All had an ACTH test performed but only 33 also had an ITT performed as an ITT is contraindicated in patients with seizure disorders and regarded as potentially hazardous in children. We found 10 discrepancies = 30%, who passed the ACTH test and failed the ITT (Figure 8). By regarding the ITT as the golden standard it appeared that the ACTH test is less reliable in assessing the HPA axis in patients, who have been treated with CIR and therefore at risk of a hypothalamic damage. It is important to underline that no failure of the ITT to detect HPA axis impairment was proven, since we found no patients with a cortisol response to the ITT \geq 500 nmol/liter, who had a cortisol response to the ACTH test <500 nmol/liter. These results should rise the question whether the ACTH test should be abandoned as a first line test, since the 10 patients

who passed the ACTH test but failed the ITT might be at risk during fever episodes or in case of emergencies to endanger survival. These results are in line with some other studies, which concluded recommendation of the continuous use of the ITT for evaluation of the HPA axis in patients with pituitary disease (Ammari et al., 1996) (Streeten et al., 1996) (Shankar et al., 1997) (Erturk et al., 1998) (Mayenknecht et al., 1998). This is, however, contradictory to other studies, which have come to the conclusion that the ACTH test can substitute the ITT (Lindholm et al., 1978) (Lindholm and Kehlet, 1987) (Stewart et al., 1988) (Hurel et al., 1996) (Clayton, 1996) (Bangar and Clayton, 1998) (Weintrob et al., 1998) (Gleeson et al., 2003). An explanation for these contradictory reports could be that data from different centres have employed different assays, and different criteria used to define states of adrenal deficiency: different cut-off values, and mixed patient populations with regard to diagnosis and treatment. In our study we have been able to undertake a large homogeneous series of patients treated for a primary brain tumour not directly involving the HPA axis using the same assays tested in the same laboratories. It should also be mentioned that the conventional ACTH test incorporates 250 µg ACTH – a dose which is regarded as supraphysiological and several investigators have published evidence for a more sensitive ACTH stimulation test using a

lower dose of 1 µg ACTH (Thaler and Blevins, 1998) (Dickstein et al., 1991) (Abdu et al., 1999) (Tordjman et al., 2000). Hopefully, ongoing studies will determine whether this test could be the gold standard for assessing the HPA.

We also measured significantly lower basal cortisol levels as well as peak cortisol responses to the ACTH test at both 30 and 60 minutes in patients compared to controls. It should be noticed that no controls responded <500 nmol/liter at either 30 or 60 minutes (Figure 9). We found a significant correlation between the ACTH test (30 min.) and (60 min.), but 13 patients turned out to be what could be called Aslow responders@ in that their cortisol levels at 30 minutes were <500 nmol/liter, but raised at 60 minutes ≥500 nmol/liter. This implies that in patients, who have been treated for a childhood brain tumour with CIR and therefore at risk of being HPA insufficient, a 30 minutes value after the ACTH test with a cut-off value of 500 nmol/liter might give a misinterpretation of the cortisol capacity of the patient and a 60 minutes value should be obtained, otherwise a life-long substitution with hydrocortisone can be the consequence.

We tried to investigate the impact of CTx on the HPA axis and concluded that CTx did not have a significant influence since the stepwise backward multiple linear regression analysis did not include CTx in the model in contrast to length of follow-up as well as BED to the HP region. To our knowledge there has been no earlier reports on the possible contribution of CTx to adrenal dysfunction

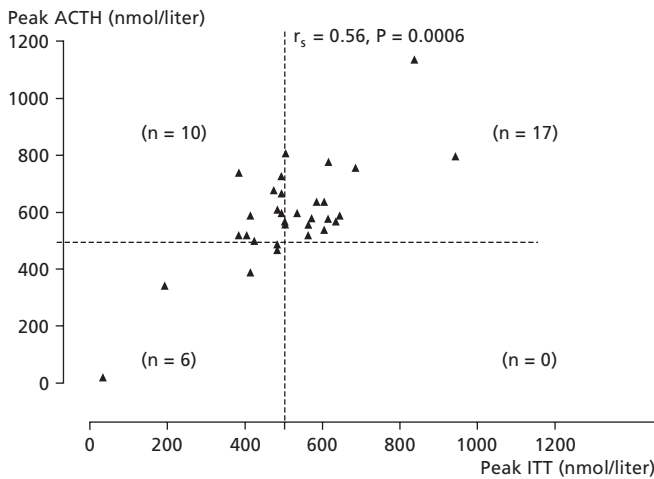


Figure 8. Thirty-three patients of the total cohort also had an ITT performed. Sixteen patients (48%) failed the ITT with a peak cortisol response less than 500 nmol/liter, whereas a peak cortisol of 500 nmol/liter or greater was reached in 17 patients. There was a significant correlation between the ITT and the peak ACTH test ($r_s = 0.56$, $P = 0.0006$). There was discordance in 10 patients of 33 (30%), who passed the ACTH, but failed the ITT.

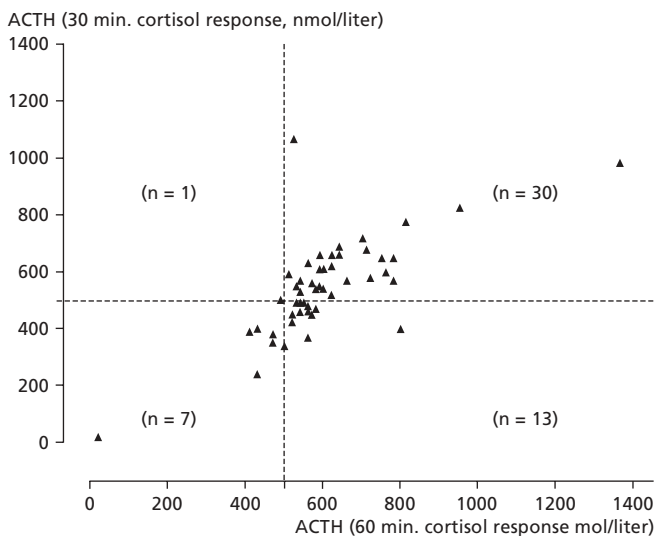


Figure 9. Thirteen patients failed the ACTH test (30 min) but passed the ACTH test (60 min). *J Clin Endocrinol Metab* 88: 3149-54, 2003.

13. CONCLUSIONS

As the overall cure rate of children treated for a brain tumour has grown during the last 3 decades from 50% to 67% at present, the number of long-term survivors are increasing. The improvement in prognosis has been achieved at the expense of serious late effects, which have their onset months or years after treatment. We demonstrated a significant relation between time elapsed from RT and development of GHD, thyroid, gonadal and adrenal dysfunction – the deficits being progressive over time – and no relation between age at the time of RT and endocrine deficits. This is in contrast to the well described relation between age at the time of RT and intellectual dysfunction following CIR for a childhood brain tumour (Duffner et al., 1991). However, the time of début of endocrinological deficits is very variable and by means of specific dosimetry of the HP region we demonstrated large interindividual differences in the dose received by the HP region and a significant relation between the dose and the time to onset of GHD. Until recently the total dose of radiation was considered to be significantly related to the incidence of endocrinological sequelae to RT. We determined the impact of fractionation dose on endocrinological late effects of neural tissue by application of the BED. We demonstrated a significant relation between the BED to the HP region and GHD implicating that the degree of endocrinological deficiencies following CIR depends on the dose of fractionation. This fact has been taken into account in the latest edition of Williams Textbook of Endocrinology (Reiter et al., 2003). In patients with GHD we found a significant increase in the peak GH response after the GHRH test compared to the peak GH response after the ITT/arginine stimulation test demonstrating that the damage seems to be primarily hypothalamic following CIR. Eighty percent of the patients had GHD determined by the ITT or the arginine stimulation test, and IGF-I SDS was significantly correlated to peak GH following the ITT/arginine stimulation test. Thyroid dysfunction could be demonstrated in 24% of which 71% had been treated with CSI and in 29%, who had been treated with CIR only demonstrating scattered irradiation from both the spinal and cranial fields to the thyroid gland implicating that children should be shielded during both CSI and CIR. In patients with thyroid dysfunction 73% had mild hypothyroidism and 27% had overt primary hypothyroidism while central hypothyroidism was found in 6%. We found dysfunction of the HPA axis in 19%, who demonstrated insufficiency of their HPA axis with basal cortisol levels below 500 nmol/liter, and who did not respond with a peak cortisol

above the cut-off level to neither an ACTH test (30 or 60 min.) nor an ITT. We could not demonstrate that CTx had a significant influence on development of GHD, or development of HP-thyroid dysfunction or dysfunction of the HPA-axis. This was in contrast to the demonstration of the impact of CTx on primary gonadal dysfunction resulting in low levels of inhibin B and reduced total testicular volumes due to the damage of the Sertoli cells and germ cells of the testes with reduced spermatogenesis. In the group of patients, who had been treated with both RT and CTx compared to the group of patients, who had been treated with RT only we found a significantly higher peak FSH response to the GnRH test implicating that even if a scatter dose from the spinal fields reached the testes, the amount of irradiation was probably of minor importance.

Thus, children treated for a brain tumour with RT and CTx are in great danger of development of hormone deficiencies and should be followed life-long and assessment of growth and serum concentrations of IGF-I and IGFBP3 should be monitored and if GHD is suspected an ITT/arginine stimulation test should be performed. Life-long surveillance of the pituitary-thyroid function should be carried out monitoring TSH and serum levels of thyroxine and life-long surveillance of the HPA-axis with basal serum cortisol levels and regularly ITTs performed as well as life-long surveillance of the gonadal axis including monitoring levels of inhibin A and B.

14. FUTURE PERSPECTIVES

Long-term survivors of childhood brain tumours have become a significant group and the necessity of using CIR to treat this group has resulted in CIR as an increasing cause of HP dysfunction. In this population-based study we have been able to undertake a large series of patients treated for a childhood brain tumour not directly involving the HP axis with RT and with or without CTx and demonstrated profound endocrine deficiencies on all tested axes with GHD, thyroid dysfunction, gonadal and adrenal insufficiency.

The results, using specific dosimetry of the HP region, indicate that the observed huge interindividual difference in time to onset of endocrine insufficiency, in children presumably treated equally could be explained by interindividual differences in the degree of irradiation to the HP region, and the fact that there is a steep dose gradient on the border of the radiation field underlines the necessity of very precise planning of the treatment fields and efforts should be made to quality securing the treatment planning using conformal RT.

The results also indicate that the impact of fractionation of RT on neural tissue resulting in endocrinological late effects should be taken into account and the present thesis demonstrates the clinical implications of using the BED as a means of a more correct biological approach to estimate the risk of endocrinological late effects following RT. Initiatives have been taken to incorporate the biological effect of fraction size as well as total dose by means of a radiobiological LQ transformation of DVHs of the dose distribution (Wheldon et al., 1997) however, future research needs to be undertaken to evaluate biologically based treatment planning with biological endpoints.

Prospective studies need to be undertaken to determine the exact dose-response relationship trying to improve the therapeutic ratio, i.e. to reduce the level of normal tissue damage while keeping tumour control.

Attempts have been made trying to use hyperfractionated radiotherapy (HFRT) in which treatment schedules with small fractions ending up with a high total dose take advantage of differences between the survival curves of cells in late responding tissues (low α/β ratio and fraction size sensitivity) and the survival curves in typical tumours (high α/β ratio and total dose sensitivity) giving a favourable therapeutic ratio. This has led to new treatment schedules with accelerated hyperfractionation and some reports with small sample size have demonstrated positive results (Chin et al., 1997) (Ricardi et al., 2001). In the study by Chin (Chin et al., 1997) and in the study

by Ricardi (Ricardi et al., 2001) they demonstrated a lower risk of developing thyroid dysfunction after HFRT compared to conventional fractionated radiotherapy in children treated for medulloblastomas.

Recent advances in radiotherapy techniques with stereotactic irradiation seek to improve the therapeutic ratio in childhood brain tumours by adding potentially more effective treatment in ways that will increase tumour control and limit radiation toxicity (Kortmann et al., 1998). However, stereotactic irradiation techniques can at present only be applied to tumours < 4 cm in diameter comprising single high-dose delivery or fractionated therapy and three dimensional conformal therapy focusing the dose to the tumour while sparing surrounding normal tissue. Whether stereotactic radiation therapy will add substantially to disease control and preserve endocrine and neurologic and neuropsychologic function remains to be established and should be part of future investigations.

LIST OF ABBREVIATIONS

ACTH	Corticotropin
ALL	Acute lymphoblastic leukaemia
BED	Biological effective dose
CCNU	Lomustine
CIR	Cranial irradiation
CNS	Central nervous system
CSF	Cerebrospinal fluid
CRH	Corticotropin-releasing hormone
CRT	Cranial radiotherapy
CSI	Craniospinal irradiation
CTx	Chemotherapy
CT-scan	Computerized tomography scan
DVH	Dose-volume histogram
FSH	Follicle-stimulating hormone
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone-releasing hormone
GnRH	Gonadotropin-releasing hormone
HFRT	Hyperfractionated radiotherapy
HP	Hypothalamic-pituitary
HPA	Hypothalamic-pituitary-adrenal
IGF-I	Insulin-like growth factor-I
IGFBP-3	Insulin-like growth factor binding protein-3
ICRU	The international commission on radiation units and measurement
ITT	Insulin tolerance test
LQ model	Linear quadratic model
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
MTX	Methotrexate
NSD	Nominal Standard Dose
PNET	Primitive neuroectodermal tumour
RT	Radiotherapy
SDS	Standard deviation scores
SRIF	Somatostatin
TBI	Total body irradiation
TRH	Thyrotropin-releasing hormone
TSH	Thyreotropin
VCR	Vincristine

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