English summary
Appendices
Thyroid problems in pediatric oncology
damage, prevention and consequences

The thyroid gland is an endocrine organ, which lies ventrally in the neck and is responsible for the production of thyroid hormone. For children, an adequate amount of thyroid hormone is essential for (mental) development and growth into adulthood. The thyroid gland requires iodine for the production of thyroid hormone. The production of thyroid hormone is regulated by the hypothalamus-pituitary-thyroid-axis in which the hypothalamus produces thyrotropin releasing hormone (TRH), which stimulates the pituitary gland to produce thyroid stimulating hormone (TSH) which, in turn, stimulates the thyroid gland for production of thyroxine ($T_4$) and triiodothyronine ($T_3$). Next to TSH, the thyroid is regulated by the amount of circulating iodine. To maintain a normal hormonal balance in case of fluctuating iodine concentrations (iodine excess or iodine deficiency), the thyroid has an auto-regulatory system which responds to the intra-thyroidal concentration of iodine. This mechanism is often referred to as the Wolff-Chaikoff effect and its escape-mechanism.

In the treatment for childhood cancer, the thyroid gland or its regulation can be distorted due to different reasons. In chapter 1 an overview is given on the thyroid gland, on the most important pediatric malignancies that are involved in this thesis (neuroblastoma (NB) and thyroid carcinoma) and on possible factors which may be of influence on the thyroid gland or its function during or after treatment for childhood cancer. The best well known and studied cause of damage to the thyroid gland is radiation-exposure. Radiation can be given as external beam (XR) or as $^{131}$I. Radiation damage may result in thyroid dysfunction or in thyroid cancer. Hypothyroidism can occur after radiation doses of 27 Gy, but also after lower irradiation doses hypothyroidism has been described. An eight-fold risk for hyperthyroidism exists after radiation. Thyroid cancer can be induced already from exposure after 0.1-2 Gy, with a latency time up to 40 years. Next to radiotherapy, also chemotherapy can influence thyroid hormone determinants during treatment and there are some reports about effects of chemotherapy on the thyroid function after treatment. During treatment, the thyroid function may also be altered due to the phenomenon of non-thyroidal illness.
In Part 1, thyroid damage in children with NB who are treated with $^{131}$I-Metaiodobenzylguanidine (MIBG) is evaluated. MIBG is a compound that is selectively taken up by tumor cells of NB. By coupling this compound to $^{123}$I$^-$(γ-irradiation) it can be used for diagnostics, to localize NB. By coupling MIBG to $^{131}$I$^-$ (γ & β-irradiation), it is a very elegant way of targeted radiotherapy. Because every gift of MIBG contains some free $^{131}$I$^-$ (2 to 5%), the thyroid gland must be protected against uptake and radiation damage by radio-iodide. In chapter 2, all children that were treated in the period 1989 to 1999 in Emma Children’s Hospital according the MIBG-de-Novo protocol were evaluated, retrospectively. To protect the thyroid from radiation, patients had received 100 mg KI for 3 days during use of (diagnostic) $^{131}$I-MIBG and for 14 days during treatment with (therapeutic) $^{131}$I-MIBG. Of 73 children, data was available of 42 children. Mean follow-up was 2.3 yrs (range 0.1-8.5). Of 428 scintigrams, uptake of $^{131}$I$^-$ in the thyroid was visible in 92 (21.5 %). Twenty-two patients (52.4 %) presented with an elevated TSH (≥ 4.5 mU/L) after a mean period of 1.4 years (range 0.1–5.8 yrs). Clinical signs of hypothyroidism were not observed. Eight patients received supplementation therapy with $T_4$. TE was transient in 4. Of 25 survivors, with a mean follow-up of 3.5 yrs, 16 (64 %) developed TE, of which in 14 (56 %) it was permanent. From these results, it was concluded that the occurrence of thyroid dysfunction after treatment with $^{131}$I-MIBG for NB is high, in spite of KI prophylaxis. After this first retrospective evaluation, some questions remained. Was this thyroid dysfunction transient or permanent, did the thyroid function affect growth or have other consequences? Did the radiation damage also result in structural abnormalities of the thyroid gland? Since these children are given multi-modality treatment, how are their other endocrine functions? To answer these questions, a cross-sectional evaluation of a cohort of NB survivors was performed which is described in chapter 3. Twenty-five NB survivors, with a mean age of 8.5 years, off therapy for a mean period of 6.2 years (range 1.3-11.1), were evaluated. Medical history, physical examination, fasting cholesterol profile and determinants of the thyrotropic-, corticotropic-, gonadotropic-, lactotropic- and somatotropic function were obtained. Seven survivors who used $T_4$ were withdrawn of medication for three months, after which a second evaluation was performed. Of the 25 patients, 14 (56 %) had permanent TE and 9 received $T_4$
supplementation. Two patients had a small thyroid volume, and in 6 patients thyroid nodules or cysts were found. Two boys showed hypergonadotropic hypogonadism, after treatment with alkylating agents. IGF-1 levels were all within the normal range, however growth was affected in 39 % of children. Mean Target Height Standard Deviation Score of patients with thyrotropin elevation was lower than those without (p=0.019). These results demonstrate that children treated for NB with $^{131}$I-MIBG (and given KI for thyroid protection), chemotherapy and surgery are at risk for developing irreversible thyroid function loss, thyroid nodules, hypergonadotropic hypogonadism, and growth retardation.

To improve the thyroid protection during $^{131}$I-MIBG, in chapter 4 a prospective cohort (34 children with NB receiving MIBG) is described in which thyroid protection was done by the administration of T$_4$ (100 $\mu$g/m$^2$), methimazole (0.5 mg/kg per day) and KI (3 times a day 0.3 cc 10 % solution). Protection started one day before the diagnostic $^{123}$I-MIBG, until four weeks after the last therapeutic $^{131}$I-MIBG dose. The results were compared to the group of children presented in chapter 2. After a mean follow-up of 19 months, 23 patients could be evaluated. Fourteen % of survivors had TE compared to 56 % of the historic controls (p=0.011). Scintigraphic visualization of the thyroid diminished substantially after the new protection: 21.5% versus 5.3% respectively (p<0.000). From these results, it was concluded that T$_4$, methimazole and KI protect the thyroid more effectively against radiation damage from $^{123/131}$I$^{-}$ during MIBG administration in children with NB than KI alone.

For the survivors of this new protection, it was advised to check thyroid function every three months the first two years after the last $^{131}$I-MIBG administration, and hereafter every 6 months. Because thyroid dysfunction after treatment with MIBG was shown to increase in time (chapter 2), an update was performed in July 2004 of the 23 included children, which again showed significant reduction in thyroid dysfunction compared to the results of chapter 2 (addendum 1). Because of the frequent elevated TSH concentrations during DBR, it was advised to increase the dose of T$_4$ to 125 $\mu$g/m$^2$. In addendum 2 the clinical protocol for the new thyroid protection is given. To evaluate the implementation of this new protocol and evaluate the possibility that the positive results were influenced by the presence of a researchers-
bias, in addendum 3, an evaluation was performed of all children (n=31) that were given DBR, with increased dose of $T_4$, after closure of the study. Of 160 MIBG-images, in 3 (1.9 %) uptake in the thyroid gland was seen. Three children had elevated TSH values during thyroid protection, with a maximum value of 16.8 mU/L. Of 9 children whose thyroid function was followed in time, one child developed TE. From these updates, it may be concluded that $T_4$, methimazole and KI protect the thyroid more effectively against radiation damage from $^{123/131}$I during MIBG administration in children with NB than KI alone.

In Part 2, ways to prevent thyroid damage due to external radiation is evaluated in rats. For prevention of thyroidal radiation damage, it has often been suggested that lowering the stimulation of the gland, by lowering the concentration of plasma TSH may be beneficial. To study ways of prevention, *in vivo*, firstly an animal model had to be developed, which is described in chapter 5. Because the concentration of TSH is necessary to interpret thyroid histology, a model was developed in which radiation-induced effects were quantified, using thyroid morphology and plasma TSH. Thirty-five Wistar rats, 5 weeks old, were X-irradiated on the ventral side of the cervical region, with a single dose varying from 0 to 20 Gv. After 6 weeks TSH, $T_4$ and $T_3$ values were determined and thyroid glands were processed for histological examination. A histological classification scale was developed, using follicular size, colloid density and cell height of thyrocytes to measure hyperplasia and hypertrophy. By the sum of these scores a cell-activity index was calculated, which was related to plasma TSH. Also numbers of PAS-positive droplets and epithelial desquamation were counted. Intraclass Correlation Coefficients (ICC) were calculated to assess interobserver reliability. Good to very good reliability was found for scores of follicular size, colloid density and cell height. Significant increase of cell-activity index was found after 10, 15 and 20 Gv. The concentration of plasma TSH was positively correlated to the cell-activity index increasing with radiation-doses up to 15 Gv. The number of desquamated cells was significantly increased after radiation doses $> 10$ Gv, with moderate reliability. This model was subsequently used for pre-clinical studies of prevention of radiation-induced thyroid damage (chapter 6). Of 80, 5-week old Wistar rats, 64 received 15 Gv
X-radiation (XR) (single dose). During XR, endocrine intervention was given to protect the thyroid gland from radiation damage using T₄ (20 μg/100 g), T₄ & NaI (10 mg $^{125}$I) or NaI alone, compared to placebo. T₄ was administered from one week before XR, until two weeks after. NaI was administered from 1 day before XR until 6 days after. Histological evaluation of thyroids, pituitary glands or the hypothalamus and any suspect lymph nodes, lungs and liver was performed after 6 and 54 weeks. No significant reduction in hypothyroidism or thyroid carcinoma was found between the different groups of rats given any endocrine intervention or no intervention. A significant higher number of adenoma was found in rats given NaI during XR after 54 weeks. From these results it was concluded that the administration of T₄, NaI or the combination during X-irradiation does not prevent against radiation-induced thyroid damage.

In **Part 3**, the adverse events for children with thyroid carcinoma are described. In **Chapter 7**, it was shown that, although the prognosis for children with differentiated thyroid carcinoma (DTC) is very good (no mortality in our treated cohort), frequent adverse events are seen. Twenty-five of 26 children treated between 1962 and 2002 were evaluated. Mortality was 0. Seven developed recurrent disease, 2 developed a 3rd recurrence. Twenty-one (84%) had ≥ 1 adverse event. Eight had permanent hypoparathyroidism (PH), 6 permanent recurrent nerve paralysis (PRNP) and 2 Horner's syndrome. Risk factors for PH and PRNP were total thyroidectomy with lymph node dissection (RR: 6.45, p=0.015) and recurrent nerve tumor encasement (RR: 8.00 p=0.001), respectively. Other adverse events were fatigue (n=5), scar problems (n=4) and chronic myeloid leukemia (n=1). It was concluded that the treatment strategies need to be improved. In **Chapter 8**, a case report of a boy, with the diagnosis Multiple Endocrine Neoplasia (MEN)-2A syndrome and disseminated medullary thyroid carcinoma (MTC), is reported which demonstrates that in some situations, when prophylaxis of MTC has failed, it may be necessary to withdraw from further invasive medical treatments to prevent iatrogenic damage to the patient ("secondary prevention").

Because it can be expected that children with cancer who are in a bad clinical condition and receive treatment with many cytotoxic drugs develop the syndrome of non-thyroidal
illness, the thyroid function was evaluated in all children admitted to the pediatric oncology ward for chemotherapy in a three-month period (part 4, chapter 9). During 46 courses of chemotherapy, mean concentrations of TSH, T₃, Tg and cortisol decreased to 53, 67, 69 and 15% of the baseline value respectively. Mean plasma rT₃ increased to 217% of baseline. In 87% of all courses, > 1 thyroid parameter(s) was aberrant. Small changes were seen in scores for clinical condition and none were related to a change in thyroid function determinant. Most changes in thyroid hormones could be attributed to the use of dexamethasone.

Although the long term negative effects of radiation treatment on the thyroid gland have been described extensively, it was still unclear whether chemotherapy has an additional detrimental effect on the thyroid gland. For this reason, in chapter 10, the thyroid axis was evaluated in 205 childhood cancer survivors of a broad spectrum of childhood cancers in relation to former use of chemotherapy and radiotherapy (cranial, cranio-spinal, cervical, mediastinal or thoracic). After a mean follow-up time of 17.5 years, damage to the thyroid axis was found in 55 patients (26.8%), of which 37 (18%) had thyroidal disease. Diagnoses varied from TSH elevation to papillary carcinoma. After multivariate analysis, high risk radiation field, irradiation dose and the diagnosis non-Hodgkin's lymphoma/Hodgkin's disease were found to be significant risk factors for developing thyroid disease. Treatment with chemotherapy did not have an additional negative effect on the thyroid axis. It can be concluded that chemotherapy for childhood cancer, does not contribute to the damage on the thyroid axis inflicted by radiotherapy during young adulthood.

In part 5, all possible preventive actions for the thyroid gland are summarized and discussed. In chapters 11a and 11b, systematic searches are presented for studies that have been performed on prevention of the thyroid gland against radiation damage in humans (11a) and animals (11b).

Literature searches were performed using PubMed, Embase, OLD MEDLINE, and the Cochrane Central Register of Controlled Trials as primary source. Only empirical studies were included. Contents and limitations of all studies were appraised and discussed by two reviewers and consensus was reached on scientific validity, relevance and implications for research and practice.
Regarding the evidence of effective strategies in humans, 66 studies were reviewed. More than 30 different outcomes were used to evaluate thyroid radiation damage or uptake of radio-iodide. In 17% of studies the outcome measurements were either unclear or not valid, e.g. no reference value given or palpation of the thyroid or an outdated outcome used. The most frequently used protective intervention was the administration of excess cold iodide. In 80% of studies, patients developed hypothyroidism despite this prophylaxis. Only in one study, a 100% reduction of radio-iodine uptake was demonstrated after KI administration. The addition of T4 and methimazole improved thyroid function in children after 131I-MIBG treatment. Also, the combination of perchlorate and KI reduced thyroid uptake. Only one study was found that evaluated the effects of pharmacological intervention during X-irradiation: in all other studies interventions were started after radiation-exposure. No benefit from T4 administration during X-irradiation was found. T4 administration after X-irradiation resulted in a reduction of benign thyroid nodules. No evidence was found for a preventive effect of X-induced thyroid malignancies by the administration of T4. Effective physical intervention strategies for X-induced thyroid damage were the elimination of X-irradiation by replacement with chemotherapy, reduction of radiation dose and hyperfractionation.

For studies on thyroid radiation protection in animals, 61 studies were detected by the search. Thirty-three different thyroid outcomes were used to interpret radiation damage. Follow-up time of most studies was insufficiently short. The administration of T4 decreased the number of thyroid adenomas but did not prevent the occurrence of subsequent carcinomas. The administration of methylthiouracil (MT) briefly before radiation exposure resulted in less thyroid damage. The administration of free radical scavengers demonstrated a protective effect for the thyroid, however this was evaluated after a very short follow-up time. More than 45 different interventions were studied for the exposure of the thyroid to radio-iodine. The administration of propylthiouracil (PTU) or MT increased the number of thyroid adenomas and carcinomas. Hypophysectomy prevented the development of thyroid tumors. The administration of thyroid hormone reduced the occurrence of histological abnormalities reversibly, but did not prevent against the development of thyroid carcinoma. Rats given a low
iodine diet showed an increased uptake of radio-iodine. The administration of KI, briefly before radio-iodine exposure reduced thyroid uptake with 97%. Perchlorate (ClO₄⁻) had a stronger effect on reduction of uptake than KI. The administration of selenium protected against the development of thyroid adenomas in one study.

It was concluded that for humans, KI alone is insufficient for thyroid protection against radio-iodide and T₄ administration during or after X-irradiation cannot prevent the occurrence of hypothyroidism or thyroid malignancies. For animals, hypophysectomy is the only proven strategy to protect against the occurrence of thyroid malignancies, yet very hard to implement in clinical practice. For both searches, it was concluded that, given the current uncertainty regarding the effectiveness of preventive measures in this field, there is a need for well designed randomized controlled trials with well-defined, clinical relevant outcomes.

In chapter 12, the results generated in this thesis are discussed. Ways to improve the endocrine care for the child with cancer are discussed, with special attention for the preventive actions against the development and/or consequences of changes in thyroid function parameters caused by radiation or chemotherapy and thyroid carcinoma, resulting in implications for clinical practice and suggestions for further research, based on the results and questions that evolved during the different studies bundled in this thesis.