THYROID CANCER IN UKRAINE AFTER CHERNOBYL

M Tronko
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GA Thomas
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Editors

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dosimetry, epidemiology,
pathology, molecular biology

NASHIM

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2014

ISBN 4-931481-08-6

Printing and binding: IN-TEX, Nagasaki, Japan. Printed in Japan

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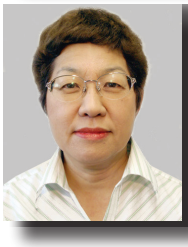

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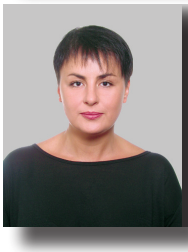

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Acknowledgements

The authors gratefully acknowledge financial support provided by the following bodies since 1994: The European Commission (Grant numbers ECP8, ERB15CT97 1100, FIR1200120123, FP7 211712), the National Cancer Institute of the USA (grant number 5U24CA082102), the Sasakawa Memorial Health Foundation of Japan and the World Health Organisation. We acknowledge the commitment of our colleagues at the Institute of Endocrinology and Metabolism (IEM) in Ukraine, especially the staff of the laboratories of Morphology and of Radiation Damage of Endocrine System who collected pathological material and frozen samples, extracted DNA and RNA for different scientific studies, and of Surgery Department who operated on most patients with thyroid tumors included in the present analysis. We thank Pathology Panel of the Chernobyl Tissue Bank (CTB): Professors D Williams (UK), V Livolsi (USA), J Rosai (USA), M Ito (Japan), J Hunt (USA), G Fadda (Italy), A Abrosimov (Russian Federation), and T Bogdanova (Ukraine) for consensus pathological diagnoses of the CTB cases. Support of our colleagues, members of panels overseeing the CTB in the past and at present, is also gratefully acknowledged, as is the generosity of patients who have donated their tissue specimens to the CTB.

We would also like to thank Nagasaki Association for Hibakushas' Medical Care (NASHIM) for financial support of this publication and all our colleagues from Belarus, Russia and different countries of Europe, USA and Japan who worked with us and/or performed research projects on thyroid cancer after Chernobyl. Our personal acknowledgements to A Statsenko (IEM, Ukraine), T Rogounovitch, A Bychkov, J Takahashi, and Y Kawaguchi (Nagasaki University, Japan) for their essential assistance in manuscript preparation.

Preface

The population living in areas exposed to radioiodine in fallout from the Chernobyl accident has shown a significant rise in thyroid cancer, particularly in those who were children and adolescents at the time of the accident. This rise in thyroid cancer is the only scientifically proven effect of the accident on health of the local population. The temporal and geographic distribution of thyroid cancer cases diagnosed in young patients is suggestive of a common causative event, i.e. internal exposure of the thyroid gland to radioiodine. Thyroid dose per unit intake of iodine isotope is higher for children then for adults mostly due to the smaller size of the thyroid in children. The background rate of childhood thyroid cancer is very low in unexposed populations; the additional thyroid cancer incidence in young patients is therefore particularly striking.

The VP Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine was a leading research center for study and treatment of thyroid diseases in Ukraine, and took a principal role in treating these patients as soon as the rise in cases became apparent. A specialized Clinical-morphological Registry of thyroid cancer of subjects at high risk was established in the Institute in 1992 and is still maintained today. The Histological archive stores pathological material from cases, most of which have been reviewed by the international experts since 1994.

The Ukrainian cases account for over 60% of the international Chernobyl Tissue Bank (CTB). For more than 70% of them matched pairs of samples from tumor and normal tissue are available. These are widely used by the leading research centers in the world to study various aspects of thyroid carcinogenesis. Recent efforts to supplement the CTB database with calculated individual thyroid exposure dose estimates have made it possible to search for markers of thyroid cancer with a radiation etiology.

Investigations performed on the same pathologically verified samples allow the comparison of the results from different projects as well as an in-depth integrative analysis.

The international team of authors of this book attempts to provide an overview of the large number of unique materials obtained from studies of Ukrainian thyroid cancer that have been accumulated over the years after the Chernobyl accident. Comprehensive scientific analysis is now expected to be useful for future studies of radiation-associated thyroid cancers.

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Chapter 1

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Overview of the Chernobyl accident and its consequences

The accident at Chernobyl nuclear power station was the worst technogenic catastrophe of the last century that involved radiation. Massive releases of radioactive substances led to radioactive contamination of territories surrounding the accident site through fallouts. Before Chernobyl, the only experience of massive radiation exposure known to humankind was the A-bombings of Hiroshima and Nagasaki in 1945. The character of radiation exposure of population after Chernobyl was principally different from that in Japan: protracted versus acute single dose, mostly internal versus external irradiation, influence of the different spectra of isotopes, irregular and patchy radioactive contamination of the environment, radiation exposure of millions of all ages. That is why many consequences related to health, radioecology and society could not be anticipated or reliably estimated quickly.

In this chapter we overview some technical aspects of the accident and information on radioactive releases that caused contamination of territories in the former Soviet Union countries – Ukraine, Belarus and Russia. We also describe the major groups of population affected by the accident and consider their dosimetric information. Epidemiological and medical studies from the early stages of the accident until present and their most salient results will be described. Finally, the comparative epidemiological information on thyroid cancer, one of the major health consequences of the accident, particularly focusing on the resident of contaminated territories will be given to compare the three affected countries. Note that the detailed epidemiological analysis of thyroid cancer in Ukraine after Chernobyl is presented in Chapter 3 of this book.

The accident at Reactor Number 4 of the Chernobyl Nuclear Power Plant (CNPP) located at the north of Ukraine close to the junction of the borders of the three states, Ukraine, Belarus and Russia, took place shortly after midnight on April 26, 1986. According to UNSCEAR [1], the course of events could be briefly summarized as follows. Due to some reactor design drawbacks and human errors during experimental operations immediately preceding the accident, irregular fuel overheating and fragmentation in the active zone led to the rapid transfer of excessive heat to the coolant water and induced the shock wave breaking the primary coolant system pipeline joints. Leaked water instantaneously turned to steam; this first explosion caused the reactor core displacement during which the remaining cooling water flew out of the system. Without coolant, part of the fuel vaporized because of overheating, eventually resulting in a large explosion that destroyed the reactor and

the building, and dispersed reactor debris and radioactive materials to CNPP, plant vicinity and into the environment. The initial fires that occurred after the major explosion were put under control by the end of the night of the accident. However, fuel materials remaining at meltdown site grew hot, ignited combustible products formed in the disrupted core milieu and caused an explosion-like fire. Tremendous efforts have been made to extinguish this fire, including dumping of various fission- and fire-control materials from helicopters, but the radioactive releases continued for approximately 10 more days [2,3].

There were 7 deaths during the first night of the accident: two staff members and five firemen in fire fighting actions. Among 237 firemen and CNPP employees examined within several next days for acute radiation sickness, manifestations of such of varying degrees of severity were found in 134 individuals. Despite the intensive therapy, including 13 bone marrow transplantations, 28 patients died within 4 months after the accident for various causes of death among which myelosuppression was the major reason. Nineteen more deaths were registered until 2004; in these cases bone marrow failure was unlikely the underlying cause [4].

According to the estimates, the release of radioactivity from the destroyed reactor totaled to about 13 EBq (1EBq=10^{18} Bq) [1,5,6]. The main radionuclides are listed in Table 1.1 of which ^{131}I and ^{137}Cs are radiologically most significant.

Radioactive emissions from CNPP were characterized by a wide spectrum of physicochemical forms and composition: gaseous, steam aerosol, aerosol mixtures, fuel particles, mineral particles with entrapped radionuclides, aggregates of different mineral particles, and organic compounds. The composition varied from monoelement noble gases and atomic iodine or ruthenium, to multi-element compounds and aggregates, fuel components, graphite, silicates and others, each with different radionuclide proportions [5].

Over 90% of ^{90}Sr, ^{141,144}Ce, and isotopes of Pu and ^{241}Am were released in the form of fuel particles measuring 10 μM and less [5]. 75% of ^{137}Cs contamination within the exclusion zone (the 30-km zone around CNPP) could be attributed to this physical form. At longer distances, contamination of the territories in European countries was due to steam-aerosol and gaseous mixtures, and to the particles of submicron size, containing ^{103,106}Ru, ^{131,133}I, ^{132}Te, ^{134,137}Cs and radioactive noble gases. The same isotopes were also detected in Pacific and Atlantic Oceans, and even in fallouts in Americas and Asia thus displaying the global scale of the accident. After a protective sarcophagus (object «Shelter») was upbuilt around the destroyed reactor and the building in November 1986, active emissions into the environment were not practically observed [1,2].

The dynamic meteorological conditions, including the wind, cloudiness, temperature, humidity, precipitations and their intensity in combination with varying physicochemical characteristics of radioactive materials released at different times after the reactor destruction defined the inhomogeneous pattern of the ground contamination [7,8,9]. Figure 1.1 demonstrates reconstructed plume traces over the part of Europe.

Further monitoring of the territories allowed establishing contamination pattern based on average ^{137}Cs deposition densities (this isotope is easy to measure, has a long half-life and is radiologically significant) as shown in Figure 1.2 for the territories around Chernobyl. The highest density of contamination is observed in CNPP vicinity; however the levels exceeding expected background could be detected as far as up to 3000 km from the accident site.

Table 1.1
Principal radionuclides released due to the Chernobyl accident*

Radionuclide	Half life	Activity released, PBq
Noble gases		
^{85}Kr	10.72 y	33
^{133}Xe	5.25 d	~6,500
Volatile elements		
^{129m}Te	33.6 d	240
^{132}Te	3.26 d	~1,150
^{131}I	8.04 d	~1,760
^{133}I	20.8 h	~2,500
^{134}Cs	2.06 y	~47
^{136}Cs	13.1 d	36
^{137}Cs	30.0 y	~85
Elements with intermediate volatility		
^{89}Sr	50.5 d	~115
^{90}Sr	29.12 y	~10
^{103}Ru	39.3 d	>168
^{106}Ru	368 d	>73
^{140}Ba	12.7 d	240
Refractory elements (including fuel particles)		
^{95}Zr	64.0 d	84
^{99}Mo	2.75 d	> 72
^{141}Ce	32.5 d	84
^{144}Ce	284 d	~ 50
^{239}Np	2.35 d	400
^{238}Pu	87.74 y	0.015
^{239}Pu	24,065 y	0.013
^{240}Pu	6,537 y	0.018
^{241}Pu	14.4 y	~2.6
^{242}Pu	376,000 y	0.00004
^{242}Cm	18.1 y	~0.4

* Decay corrected to 26 April 1986
Data are inferred from refs. [2,8,17,20]

Territories of Belarus, Ukraine and Russia were affected by the accident most heavily, as specified in Table 1.2. From the total ^{137}Cs activity of about 64 TBq (1.7 MCi) deposited in Europe in 1986, Belarus received 23%, Russia - 30% and Ukraine - 18% resulting in radioactive contamination of approximately 3% of the European part of the former Soviet Union [10]. There were also contaminated areas in Austria, Finland, Germany, Norway, Romania and Sweden.

Table 1.2
European countries contaminated by Chernobyl fallouts in 1986*

	Area with ^{137}Cs deposition density range (per km^2)			
	37-185 kBq/m^2	185-555 kBq/m^2	555-1480 kBq/m^2	> 1480 kBq/m^2
Russian Federation	49800	5 700	2100	300
Belarus	29900	10200	4 200	2200
Ukraine	37 200	3200	900	600
Sweden	12000	-	-	-
Finland	11500	-	-	-
Austria	8600	-	-	-
Norway	5200	-	-	-
Bulgaria	4800	-	-	-
Switzerland	1300	-	-	-
Greece	1200	-	-	-
Slovenia	300	-	-	-
Italy	300	-	-	-
Republic of Moldova	60	-	-	-

* Based on refs. [1,9]

An important role in radioactive contamination of the environment was played by radioactive ^{131,132,133,135}I isotopes, which are short-lived radionuclides belonging to the group of light volatile substances. It is worth mentioning, however, that only ^{131}I has a high radiological significance. Among other isotopes, only ^{133,135}I could increase the general exposure dose, especially for the thyroid, but due to their short half-lives their effect is restricted to the areas within the near zone around CNPP.

Because of the rapid decay of ^{131}I, collection of a large number of samples for detailed analysis was difficult [11]. However, the results of model calculations based on the limited number of measurements and determinations of ^{131}I/different radionuclides ratios, especially ^{137}Cs (which varied 5-60-fold in different measurements), allowed reconstruction of contamination density maps [1,5,12]. The most contaminated in Ukraine, are 6 northern and central regions: Cherkasy, Chernihiv, Kyiv, Rivne and Zhytomyr regions, and Kiev-city (Fig. 1.3). In Belarus, the 3 regions in the east and south-east: Brest, Gomel and Mogilev; and in Russia 4 south-western regions: Bryansk, Kaluga, Tula and Orel. The refined ^{131}I contamination data were considered by UNSCEAR and enabled calculation of thyroid dose estimates [13]. This is essential for radiation epidemiology and public health assessment of health consequences of the accident.

Most radionuclides released by the accident have already decayed. Attention over the next few decades will likely to be paid to ^{137}Cs and ^{90}Sr; the latter remains more important in the areas close to CNPP [5].

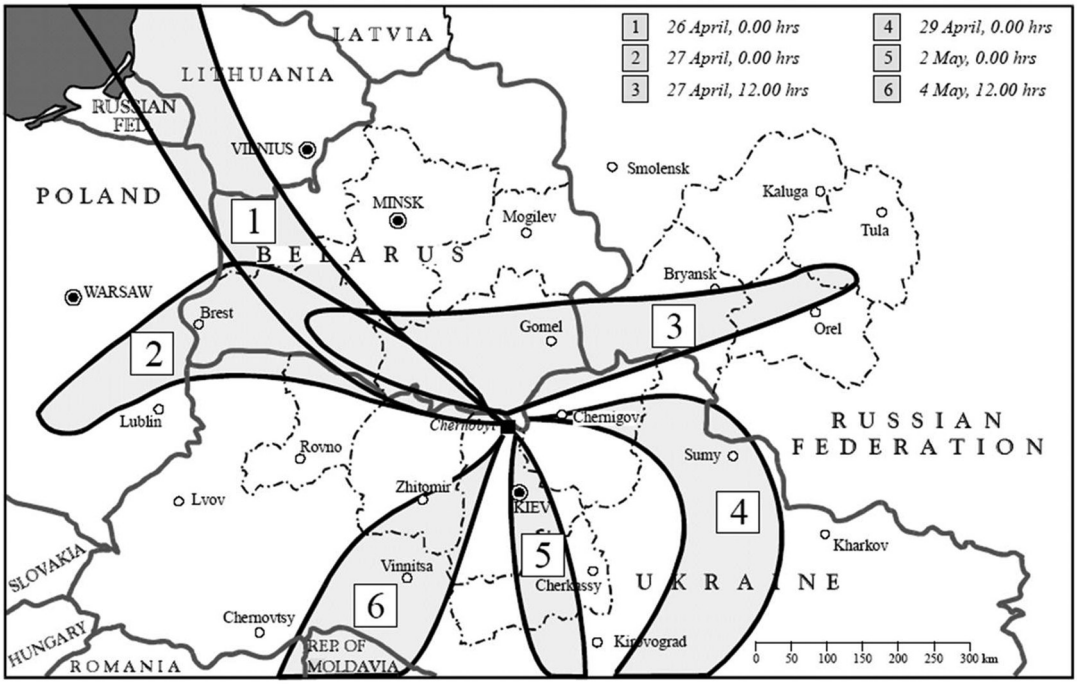

Figure 1.1. Calculated plume formation according to meteorological conditions for radioactive releases on corresponding dates just after the Chernobyl accident [7].

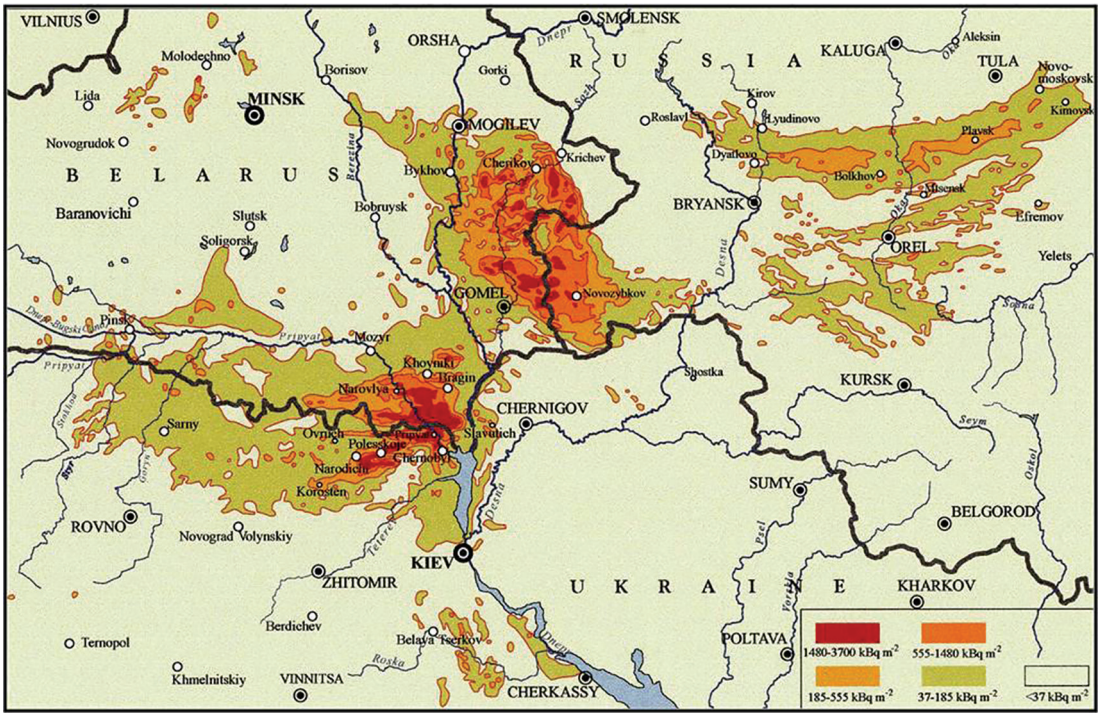

Figure 1.2. Ground deposition of ^{137}Cs in Ukraine, Belarus, and Russia around the accident site [1].

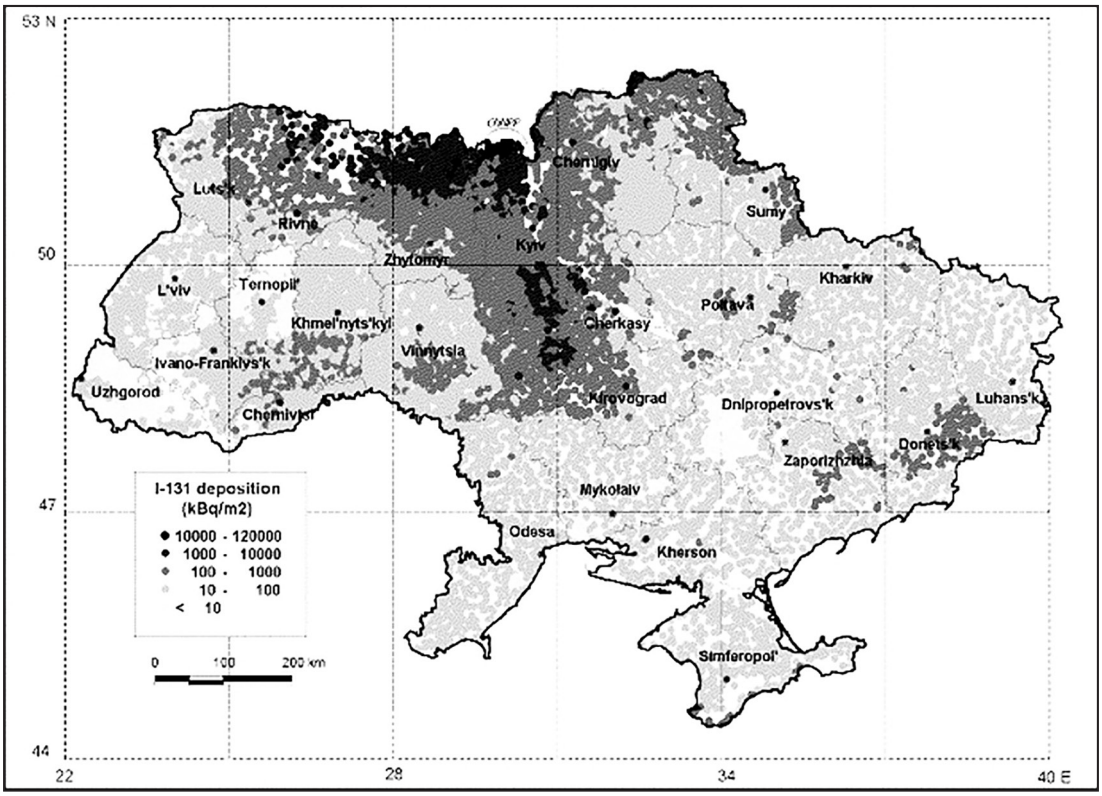

Figure 1.3. Cumulative ^{131}I surface ground deposition in Ukraine (kBq/ m^2) due to the Chernobyl accident [12].

There are three major categories of individuals considered for estimation of radiation health effects after Chernobyl. These are the workers involved in the actions during the accident or in the aftermath mitigation, evacuated persons and residents of contaminated territories. They all were exposed to radiation at different time after the accident, under different circumstances and to different spectra and amounts of radioactive elements. Thus, accumulated effective doses are quite different between the groups and furthermore there are large uncertainties in dose estimates.

The first category is further subdivided into those who were at CNPP during the first day of the accident and took part in emergency measures, and those who were engaged into recovery operations from 1986 to 1990. In the literature these workers are often and collectively referred to as "liquidators", the term officially introduced in the former Soviet Union. There were about 600 emergency workers at CNPP during May 26, and about 600,000 of liquidators including both civilians and servicemen until 1990. Estimated external doses in 134 emergency workers with acute radiation sickness manifestations ranged 0.8-16 Gy being noticeably higher than internal doses calculated to be between 0.021 and 4.1 Gy for the thyroid in 23 firemen who died of bone marrow failure [14]; it was suggested that the lower thyroid doses might have been due to stable iodine pills taken by emergency workers. Among liquidators, the average effective doses ranged from 15 mSv to 170 mSv with individual variations from <10 mSv to >500 mSv in 1986-87 [1]. Internal exposures to

the thyroid might have ranged from <0.15 Gy to 3 Gy with an average of 0.21 Gy in those who took part in the activities in and around CNPP during the first few months after the accident [15] as short-lived radioiodine isotopes largely decayed after that.

There has been also the massive evacuation of residents of the nearest settlements depending on radiological situation and the distance from CNPP [16-19]. On April 27, about 50,000 persons were evacuated from the town of Pripyat located 3 km from CNPP, where most employees and their families resided before the accident. During 10 days after the accident, through May 7, 1986, a similar number of persons who lived inside the 30-km zone surrounding CNPP were evacuated in Ukraine and Belarus. Active evacuations continued until September, 1986 and totaled in about of 116,000 relocation, mostly from Ukraine and Belarus. Estimates of external effective doses reconstructed for about 30,000 residents of the 30-km zone indicate the range from 0.1 mSv to 380 mSv with an average of 17 mSv [20]. Mean thyroid doses from ^{131}I, based on about 5,000 direct measurements and about 10,000 questionnaires collected from Ukrainian evacuees were 0.11-3.9 Gy in children, 0.066-0.39 Gy in adolescents and 0.066-0.40 Gy in adults [21,22]. In Belarussian evacuees the estimates are 1-4.3 Gy, 1 Gy and 0.68 Gy, respectively [23]. Concordantly, these investigations have demonstrated an inverse correlation between thyroid dose and age at exposure.

With regard to the residents of contaminated territories, reconstructed maps of soil contamination with ^{137}Cs (Figure 1.2) taken together with demographic data for Belarus, Russia and Ukraine indicate that population of contaminated territories (i.e. with ^{137}Cs levels exceeding 37 kBq/m^2) was above 5 million at the time of accident, with about 1 million of children (<15 years old) and approximately 200,000 adolescents. Since the number of residents of contaminated territories is substantially greater than in the two categories of exposed persons described above, and also because the residents include individuals of all ages who might have been exposed to diverse radiological conditions at different geographical locations, dose estimates in them are more complicated and are intrinsically associated with uncertainties particularly seen in the differences between averaged collective and individual doses. Models of accumulated dose from external sources are based on soil ^{137}Cs contamination levels and are normalized to isotope deposition density. Estimates of external doses range from 11 μSv/kBq/m^2 to 24 μSv/kBq/m^2 in 1986 for contaminated territories of the three countries being higher in rural and lower in urban areas [1]. Study of external doses in one contaminated settlement in Russia in 1987 found individual external doses to be within 2-13 mGy range with a mean of 5 mGy [24]. Internal doses for the thyroid rely on direct thyroid measurements (several hundred thousand have been taken cumulatively after the accident), individual questionnaires and computer modeling. Estimates indicate that the doses varied in a wide range from <0.05 mGy to >2 Gy in Belarussian, Russian and Ukrainian individuals of all age groups with averages of <0.3-0.7 Gy in children and individual doses up to 10 Gy [25-31]. Thyroid doses exceeding 2 Gy were observed almost exclusively in younger children aged less than 4 years [31] and they usually were higher in the residents of rural than in urban areas with similar contamination level [30]. More detailed information on thyroid dosimetry is presented in Chapter 2 of this book.

Organized administration of prophylactic or thyroid-blocking doses of stable iodine was not common. According to some surveys, from1% to about 25% of the residents of contaminated territories reported taking KI pills shortly after the accident but the recall rate was low [30,32]. In part this was due to poor preparedness to large-scale accidents such as

one that happened at CNPP, and in part to inappropriate information from the authorities. An official announcement in mass media appeared only two days after the reactor was destroyed on April 28. The delay was caused initially by the insufficient understanding of the accident scale as well as apprehension of possible massive panic. It might be expected that if clear instructions on essential safety measures have been delivered swiftly and timely (e.g. taking KI pills, not consuming fresh milk and vegetables grown in the open plots, not going outside, etc.), health consequences, at least for the residents of contaminated territories, would be less dramatic. Cost-benefit analysis performed in Belarus for 2,566 thyroid cancers in children and adolescents diagnosed and treated during 1990-2005 showed that if potassium iodide prophylaxis had been done, budget expenditures would have decreased for $400,000 per 100,000 of population [33].

The scale of the accident and the number of people affected by it were unprecedented; therefore initially it was very difficult to predict possible health consequences. In 2002, S.Nagataki, evaluating state of knowledge about Chernobyl, designated the major post-accident periods as follows: 1986-1989 information difficult to obtain; 1990-1991 exchanges with other countries initiated; 1992 case report: childhood thyroid cancer; 1992-1994 period of ascertainment; 1995 ascertainment and search for causes; 1996-present investigations carried out to the future [34].

First health screenings in the most contaminated areas around Chernobyl were started shortly after the accident, mostly through local medical authorities. Only from 1990, after the request from the Government of the former Soviet Union in October 1989, international efforts were initiated to last until nowadays.

The first important collaboration was the International Chernobyl Project coordinated by IAEA. During 1990-91, the 200 experts from 25 countries examined health state including hematological, cardiovascular and thyroid disease, radiogenic cataract, cancer prevalence, fetal abnormalities and mental health for possible radiological consequences in a total of 825,000 people of 2,225 settlements in the three affected states [35]. One of the purposes was also to evaluate undertaken measures and to develop health-related advices for population residing in contaminated areas. The major findings of this project generally confirmed previously established surface contamination levels; the whole body lifetime doses were estimated not to exceed 160 mSv being several times lower than initial estimates of about 350 mSv; actual thyroid doses were difficult to confirm; stress and anxiety in the population were significant but apparently not radiation-related; no increase in leukemia or solid cancers was observed at that time; thyroid dose estimates in children were suggestive of the possible increase in thyroid cancer incidence in the future; extent of population evacuation and foodstuff restrictions appeared to be sometimes excessive.

In February 1990, the Government of the former Soviet Union appealed to Sasakawa Memorial Health Foundation (SMHF) of Japan to provide assistance specifically to the population of contaminated territories. SMHF in collaboration with the Japan Shipbuilding Industry Foundation (at present the Nippon Foundation) created a 5-year program at first entitled the "Chernobyl Sasakawa Health and Medical Cooperation Project". According to the report of experts who evaluated situation in Chernobyl areas, the major concerns were fear and anxiety among the residents, poor informational support, and insufficient knowledge of health problems in the population. Therefore, the direct health examination,

particularly in children, was identified as the highest priority task [36]. In May 1991, health checkups of children began in five health examination centers established in Kiev and Zhytomir (Ukraine), Gomel and Mogilev (Belarus), and Bryansk (Russia) with a special focus on direct thyroid dose measurement, thyroid examination and blood tests (also including hormone and antibody measurements) according to the unified protocol. To implement the project, SMHF donated to each center five mobile units equipped with whole body counters, ultrasound machines and blood analyzers, 10 buses for patients' transportation as well as other medical and diagnostic equipment, computers, supplies and medicines. Until April 1996, 158,995 children aged 0-10 years at accident were examined. The project also supported training in Japan and on-site, expert visiting of the five centers, and educational materials and lectures for the residents. Among 120,605 analyzed patients, 585 (4.85%, range 1.01-17.69%) patients with thyroid nodules and 63 (0.52%, range 0.22-1.92%) with thyroid cancer were found with the highest rate among the residents of the most heavily contaminated Gomel region in Belarus aged 0-3 years at accident [37]. The prevalence of goiter was 18-54% but no correlation with whole body ^{137}Cs count or the level of ^{137}Cs contamination at the settlement of residence was observed [38]. The frequencies of hematopoietic malignancies, abnormal hematological parameters and thyroid autoimmunity also did not correlate with whole body ^{137}Cs count or the level of ^{137}Cs contamination [39]. The results of the project, which was the most reliable study at the time, indicated a link between thyroid cancer in children and the Chernobyl accident and pointed at the need in further investigations.

In view of a high importance of the results obtained in 1991-1996, SMHF has extended the project for 5 more years focusing on Gomel region of Belarus. A comparative study of thyroid diseases in children born before and after the accident was designed to involve 21,601 persons examined from February 1998 to December 2000 using the approaches established during the first project [40]. A total of 32 (0.15% of examined children) thyroid cancers were diagnosed of which 31 was in the group of 9,720 children born before the accident, one in a child born during April 27 - December 31,1986 (i.e., possibly exposed *in utero*) while no thyroid cancers were detected in the group of 9,472 children born after the accident. The estimated odds ratios of the frequency of thyroid cancer in the group born before the accident and *in utero* exposed group were 121 and 11, respectively, as compared to those born after the accident. A conclusion about the likelihood of causal link between direct external or internal exposure to short-lived radionuclides including ^{131}I and ^{133}I was drawn.

The extended SMHF project provided a good opportunity for collaboration with the Belarus/Russia/EU/IARC epidemiological case-control study aimed at the evaluation of the risk of thyroid cancer after exposure to ^{131}I and elucidation of risk-modifying factors. In a united effort, which initially included all individuals with thyroid cancer aged less than 15 years at the time of accident from Gomel and Mogilev regions of Belarus and from Bryansk, Kaluga, Tula and Orel regions of Russia (a total of 276 at the end of study) and at least four closely matched population-based controls (1,300 persons) were analyzed. Individual thyroid doses were reconstructed and used to estimate dose-response relationship. It was found to be significant and linear up to 1.5-2 Gy [41]. Odds ratio for thyroid cancer varied from 5.5 to 8.4 for a dose of 1 Gy according to different risk models being generally comparable with risk estimates for external exposures [42]. Importantly, a strong modifying

effect of iodine deficiency was observed: relative risk for developing cancer was 3.2 in iodine deficient areas whereas a dietary supplementation with KI reduced the risk approximately 3-fold (relative risk of 0.34). This study was the largest population-based investigation in young people living in Chernobyl areas; it provided a strong definitive evidence of causal association between the risk for thyroid cancer and internal exposure to radioiodine at young age. The major route of ^{131}I ingestion by residents was its incorporation into food chains of pastured cattle, mostly cows, and consumption of fresh milk as well as from vegetables and fruits grown in open soil. Incorporation of ^{137}Cs may have contributed to dose formation. That is why both ^{131}I in the thyroid and in milk, and ^{137}Cs in soil, food and in the body are considered for dose reconstruction [43].

The World Health Organization (WHO) has been playing an active role in studying and managing health consequences of Chernobyl. One of the largest projects was the International Project on the Health Effects of the Chernobyl Accident (IPHECA) launched in May 1991 and completed in 1996 with international budget support primarily from the Government of Japan and with the contribution from Czech Republic, Slovakia, Switzerland and Finland [44]. IPHECA included a number of pilot projects: Brain damage *in utero*, Epidemiological Registry, Hematology, Medical and psychological rehabilitation of Chernobyl liquidators, Oral Health, Radiation Dose Reconstruction, and Thyroid. In collaboration with SMHF project, over 210,000 children were examined. The findings were in line with the earlier SMHF projects: by the end of 1994, 565 children (208 in Ukraine, 333 in Belarus and 24 in the Russian Federation) who lived in contaminated regions were diagnosed for thyroid cancer but no significant increase in the incidence of leukemia or other blood disorders were registered [45].

In February 1999, the WHO and SMHF started the Chernobyl Telemedicine Project whose aim was to improve early diagnosis, treatment, and follow-up of patients with thyroid cancer, primarily in Gomel region of Belarus. A satellite-based telematic system was established that allowed an exchange of thyroid ultrasound and cytology images, and of related information on the patients between Thyroid Oncology Center in Minsk, the Research Center for Radiation Medicine in Gomel and Nagasaki University School of Medicine with synchronized databases [46-49]. By September 2000, information on 330 cases was entered into the database and reviewed independently thus improving diagnosis.

Another important project was the establishment of the Chernobyl Tissue Bank (CTB) in October 1998 based on funding from the European Commission, WHO, SMHF and the U.S. National Cancer Institutes and approved by the Governments of Belarus, Russian and Ukraine [50]. CTB activities and projects are described in Chapter 6 of this book.

Even at present, when major causes of health consequences of the CNPP accident, at least with regard to thyroid cancer, are clarified, international activities continue. One of them is the Chornobyl Thyroid Diseases Study Group of Belarus, Ukraine, and the USA [51]. The study follows-up a cohort of 25,161 individuals (11,918 in Belarus and 13,243 in Ukraine) born between April 26, 1968 and April 26, 1986, with direct thyroid measurements available shortly after the accident to improve individual dose estimates and to collect health-related information based on bi-annual (or annual) screenings. The project was started in December 1996 in Belarus and in April 1998 in Ukraine.

During the first screening in 1998-2000, 45 thyroid cancers were detected in Ukraine [52]. An approximately linear dose-response relationship was found with excess relative risk estimate of 5.25 per 1 Gy. The older age tended to associate with the decreased risk of thyroid cancer. A fraction of cancers attributed to radiation was estimated to be 75% (95% CI 50-93%).

Reconstruction of thyroid doses in Belarus is now ongoing for the newly evaluation of the risk of radiation-associated thyroid cancer [53]. In Ukraine, there are extensive risk analyses of thyroid cancer and of other thyroid diseases among individuals exposed *in utero* to ^{131}I from Chernobyl fallout [54] as well as of that of non-cancer thyroid neoplasms [55] and autoimmune thyroiditis [56]. The results of this large-scale project are expected to further refine conclusions of the earlier, concurrent and ongoing studies.

Clean-up workers is a group exposed to radiation at the accident site. Their thyroid doses are mostly attributed to external exposures although those who took part in mitigation efforts during the first two months might have received internal doses from inhaled radioiodines.

In 1997, based on a survey of 167,862 liquidators registered in Russian National Medical Dosimetric Registry, 47 verified thyroid cancers were reported. An excess relative risk (ERR) of thyroid cancer of 5.31 per 1 Gy and an excess absolute risk of 1.15 per 10^4 person-years/Gy were found [57]. By the end of 1998 a total of 58 thyroid cancers were diagnosed in a subset of 99,024 liquidators from 6 regions of Russia [58]. A statistically significant increase of standardized incidence rate (SIR) of thyroid cancer of 4.33 was found in this group as compared to the background rate in male population of Russia. However, dose-response relationship could not be established with ERR of -2.23 per 1 Gy (95% CI, -4.67; 0.22).

In Belarus, based on the information from National Cancer Registry, for about 120,000 liquidators in the country the standardized index of incidence for the period from 1993 to 2000 was reported to be 24.4 per 100,000 while in the adult population of the country aged more 30 years this index in 2000 was 5.67 [59].

Together these data indicate that thyroid cancer incidence is elevated in liquidators but radiation risk needs to be further clarified through continuous observations.

In exposed residents of all ages, which also include evacuated persons, an increased radiation-related risk was found in a number of epidemiological studies, as pointed above, for those who were exposed to radioiodines during very young ages. Descriptive epidemiology data on current situation with regard to thyroid cancer for the three countries is summarized below.

In Ukraine, as shown in a recent analysis of a sample set approaching in size to the whole population of the country, in 1989-2008 age-adjusted thyroid cancer incidence rate in female residents of highly exposed regions increased from 3.34 to 10.99 (3.3-fold), and from 2.51 to 5.69 (2.3-fold) in low-exposure regions per 100,000 individuals [60]. In males, age-adjusted incidence rate grew from 0.87 to 2.64 (3.0-fold) and 1.37 (1.6-fold) in highly exposed and low-exposure regions, respectively. For the patients diagnosed during childhood and adolescence, the analysis adjusted for screening and technological effects shows that incidence rate was significantly elevated in the residents of highly contaminated regions born before the accident as compared to those born after the accident while no similar difference was found for low-exposure regions. The average annual increase in

incidence was also significantly higher in the highly-exposed regions in all age subgroups in both males and females suggestive of the radiation excess of thyroid cancers not only in children and adolescents but also in adults. Interestingly, in 2006 mean incidence ratio in highly-exposed and low- exposure regions decreased in all female age subgroups and most male subgroups, which may imply that the peak of radiation excess of thyroid cancer in the country has been passed. These observations are important and need further dose-response and risk analysis for completeness.

Statistical data from the Clinico-morphological Registry at VP Komisarenko Institute of Endocrinology and Metabolism (IEM) of the National Academy of Medical Sciences of Ukraine in Kiev indicate that from 1986 to 2010 a total of 6,798 cases of thyroid cancer have been diagnosed, of which 5,044 (74.2%) in those who were children aged 0-14 years, and 1,642 (24.2%) adolescents aged from 15 to 18 years at the time of accident and also 112 (1.6%) cases of exposure *in utero* [ref. 61 and Chapter 3 of this book].

In Belarus, during the period from January 1985 to December 2006, 14,147 patients with primary thyroid cancer were diagnosed and treated in Thyroid Cancer Center in Minsk [62]. During over than 20 years of observations after the Chernobyl accident, the crude standardized incidence of thyroid cancer increased from 1.3 to 8.8 per 10^5 individuals. Within last five years, the number of primary thyroid cancers in patients aged 19-45 did not grow significantly. In contrast, patients above 46 years old demonstrate an increasing incidence. These tendencies are currently seen in both heavily and less contaminated regions of the country.

In Russia, according to currents data from the National Radiation Epidemiological Registry (former Russian National Medical Dosimetric Registry), a total of 9,120 thyroid cancer cases were registered for the period from 1981 to 2008 in the population of all ages from the four regions officially recognized as contaminated. In 1981-1986, 102 thyroid cancer cases were registered annually on average. By contrast, during 2002-2008 the number of detected thyroid cancer cases was growing with the maximum of 592 cases observed in 2008 thus displaying a 6-fold increase. During the pre-accident and latent periods, the crude incidence rate of thyroid cancer was 7.7 for males and 37.8 for females while for 2002-2008 it was 36.1 and 170.4, respectively, per 10^6 individuals. In a cohort of 309,130 residents of contaminated areas followed-up from 1991 to 2008 for whom individual thyroid doses were reconstructed, 993 cases of thyroid cancer were diagnosed. Of them 978 cases were histologically verified; 247 cases were in children and adolescents and 746 cases in adults [63]. Of note, the analysis in children and adolescent subgroup demonstrated a shift of the distribution functions towards the cases with the higher doses which may be indicative of radiation-related risk. Indeed, calculations indicated that a statistically significant ERR of thyroid cancer incidence is found in this subgroup with an estimate of 3.22 per 1Gy.

The tendencies in thyroid cancer incidence in different age groups of the residents of contaminated territories for Ukraine [61] and Belarus [64] are shown in Fig. 1.4. Despite the patterns between the countries may vary in details, common trends could be seen: peak incidence in childhood patients occurred in 1995-97, in adolescents in 2001-2004, and in adults a gradually increasing incidence is observed.

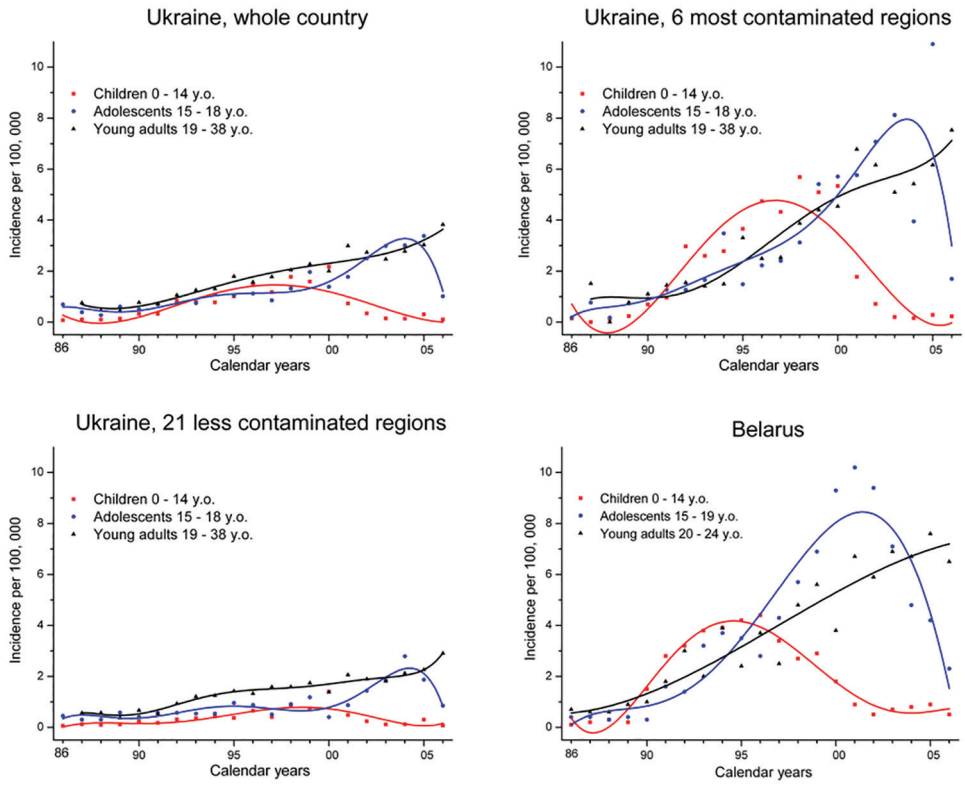

Figure 1.4. Incidence of thyroid cancer in Ukraine, in 6 most contaminated and 21 less contaminated regions of the country, and in Belarus (for comparison) in different age groups of patients diagnosed during 1986-2006. Data for Ukraine are from ref. [61], for Belarus data are derived from ref. [64]. Lines comprise polynomial best-fit of data for easier visualization.

Regarding radiation-related risk, it is well proven that such does exist in exposed children and adolescents while the question still remains open for exposed adults. It has been proposed, with reservations, that radiation excess of thyroid cancers may be observed in the group of population exposed at older ages as well as that latency of thyroid cancer may be proportional to age in adult cancer patients subjected to radiation therapy [65]. At present, however, there is no strong evidence of radiation-related excess risk in the residents of contaminated territories exposed in adult age.

To summarize the chapter, here we overviewed the major aspects of the accident at the CNPP, and its radiological and health consequences with a particular focus on thyroid cancer. As a result of massive radioactive releases, large groups of population received radiation doses. These include clean-up workers and general population which was either evacuated from the settlements in the vicinity of CNPP shortly after the accident or continued to live in the territories of Ukraine, Belarus and Russia which were contaminated by fallouts. Health consequences were initially difficult to forecast. Besides of deterministic effects of acute exposure to ionizing radiation in firemen, information about contamination levels of the affected territories, spectrum of pollutant radionuclides and doses accumulated by

the residents were completely unknown. That is why, after first several years of domestic efforts, large scale international collaborations were initiated to address nearly any related problem, involving many governmental and non-governmental organizations from a number of countries and from the world-wide community. Through cooperative investigations, health status and dosimetric data were obtained to provide a ground for assessing the consequences. First reports about the increase of thyroid cancer incidence in children and adolescents in Belarus and in Ukraine [66,67] were met cautiously by the experts because of doubts in the accuracy of diagnosis, too short period of latency (which would expected to be about 10 years as seen from A-bombing of Hiroshima and Nagasaki) and insufficient evidence of link between Chernobyl radiation and cancer outbreak. With time, however, essential proofs were found and efforts of both health authorities in the three most affected countries and of the international parties could be focused on the high-risk groups and through more specialized means.

The accident at CNPP allowed learning a number of lessons such as that a disaster in one country may affect other, that appropriate handling of vital information about and better preparedness to radiation-involving accidents may bring about less adverse consequences, that international collaboration even on delicate issues could be established and it can be effective. In the medical area, a large experience has been accumulated, including the understanding of the possibility of very short period of latency for thyroid cancer after internal exposure to radioiodine, and how to diagnose and treat young patients with thyroid cancer.

In conclusion, while major health effects of the Chernobyl accident have become clearer after 27 years that passed, we are still far from understanding of all the consequences, even applicably to thyroid cancer. As shown recently in A-bomb survivors of Japan, the excess thyroid cancer risk associated with irradiation in childhood remained detectable over 50 years after exposure [68]. It therefore is essential to continuously and consistently observe liquidators and exposed population to achieve higher level of knowledge of radiation effects after Chernobyl.

This chapter is an updated and modified version of our review article published earlier [69].

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Chapter 2

I Likhtarov, L Kovgan, S Masiuk, M Chepurny, O Ivanova, V Gerasymenko, M Tronko, T Bogdanova, GA Thomas

Different levels of thyroid dose individualization of the Ukrainian donors in Chernobyl tissue bank

In order to accumulate and centralize information on the radioinduced Chernobyl thyroid cancers, an international Chernobyl Tissue Bank (CTB) has been established. By 2012, it comprised 2,267 specimens of thyroid tumoral tissues from residents of Ukraine who had been operated on within the time period from 1990 to 2011 (see Chapter 6). These specimens were verified by experts in the course of 20 international panel meetings. The specimens accumulated in the CTB represent a material of paramount importance for various studies of the effects of ionizing radiation exposure at the molecular and genetic levels.

Over the last 25 years elapsed after the Chernobyl catastrophe, substantial experience was accumulated in the field of the individual thyroid and other organs doses reconstruction due to the accidental exposure. At that, in the ecological-dosimetry models, the reliability and adequacy of the resulting doses entirely depend on the available data (the levels and dynamics) of radioactive fallout on the soil, vegetation, milk, and other foodstuffs in different regions, as well as the information on the life style of residents during the time period of exposure (their movements over the radioactively contaminated area, diet composition, and amount of foodstuffs being consumed). The results of more than 150,000 direct measurements of ^{131}I activity in the thyroid of children and adolescents, which were performed in May-June of 1986 in the northern, most contaminated regions of Ukraine, are particularly important for the development of thyroid reconstruction models.

The results of direct measurements of thyroid activity that were performed in May-June 1986 are very helpful both to estimate the individual thyroid doses for the subject under the measurement and to development and parameterization of the reconstruction models. Hence, the *four-level system of thyroid dose reconstruction* was developed so that to estimate the internal thyroid doses for individuals or population groups of all regions of Ukraine. This system has been applied for the CTB donors' thyroid doses reconstruction. The decision on which levels of the four-level system is to be used for a CTB subject depends on the available information on the existence of direct measurement in 1986 and place of living (settlement, raion and oblast) in 1986. In all cases, the thyroid dose estimates either directly used the result of measurement performed for the subject, or indirectly used the results of thyroid measurements performed for other inhabitants in the settlements of raion in the form of relationships between the result of thyroid measurements in different age-gender groups and environmental characteristics.

Individual thyroid doses were estimated in 2009-2011 for 1,933 of 2,267 Ukrainian CTB subjects [1] in the framework of EU Contract 211712 *"CTB - The Chernobyl Tissue Bank-Coordinating International Research on Radiation Induced Thyroid Cancer"*. For each subject the central (deterministic) value of the thyroid dose was estimated and also the uncertainty of such estimates, which are due to different types of errors in the parameters of the ecological and biokinetic models that were used.

The objectives of this chapter are to present:

- the subdividing CTB cohort over the Groups and subgroups depending on the available individual data and characteristics;
- a brief description of the dosimetry models that were used for the reconstruction of individual thyroid doses of Ukrainian CTB subjects of different Groups and subgroups, as well as a description of the approaches used to assess the uncertainties attached to the dose estimates for the subjects of the different groups;
- the generalized results of the dose estimates provided for the Ukrainian CTB subjects.

Geographical distribution of the subjects

It was found that in May-June of 1986, the CTB subjects were distributed over 24 oblasts of Ukraine and Crimea autonomous republic (Fig. 2.1). At that about 60% of the exposed UkrCTB subjects resided in areas of highly-contaminated northern oblasts (Kyiv, Chernihiv, and Zhytomyr), whereas 16% of CTB-subjects resided in areas with moderate levels of radioiodine fallout, and 23% of Ukrainian CTB subjects resided in areas with low levels of radioiodine fallout. In addition, there were 9 subjects who did not reside in Ukraine at the time of the accident.

Types of exposure condition

Ukrainian CTB cohort may be subdivided according to the following exposure conditions:

- Subjects aged *one year or more* at the time of the accident exposed *as a result of radioactivity-contaminated foods consumption* in 1986.
- Subjects aged *less than one year* at the time of the accident exposed as a result of *breast feeding* in May-June 1986.
- Subjects born in May-June 1986 who have been exposed partly *in utero,* and partly as a result of *breast feeding*.
- Subjects born within the time period from July 1986 to March 1987 exposed *in utero*.
- *Non-exposed subjects* born after March 1987.

Distribution of the UkrCTB subjects by age at the time of the accident

For a unit of ^{131}I intake due to the consumption of contaminated foods the dose to the child's thyroid exceeds the dose to adults. This is mainly because the significantly smaller size of children's thyroid gland, in comparison to that for adults [2]. Thus, the *age* at which child's exposure occurred is one of the critical parameter in the dose reconstruction. Table 2.1 shows the distribution of the UkrCTB subjects by age at the time of accident.

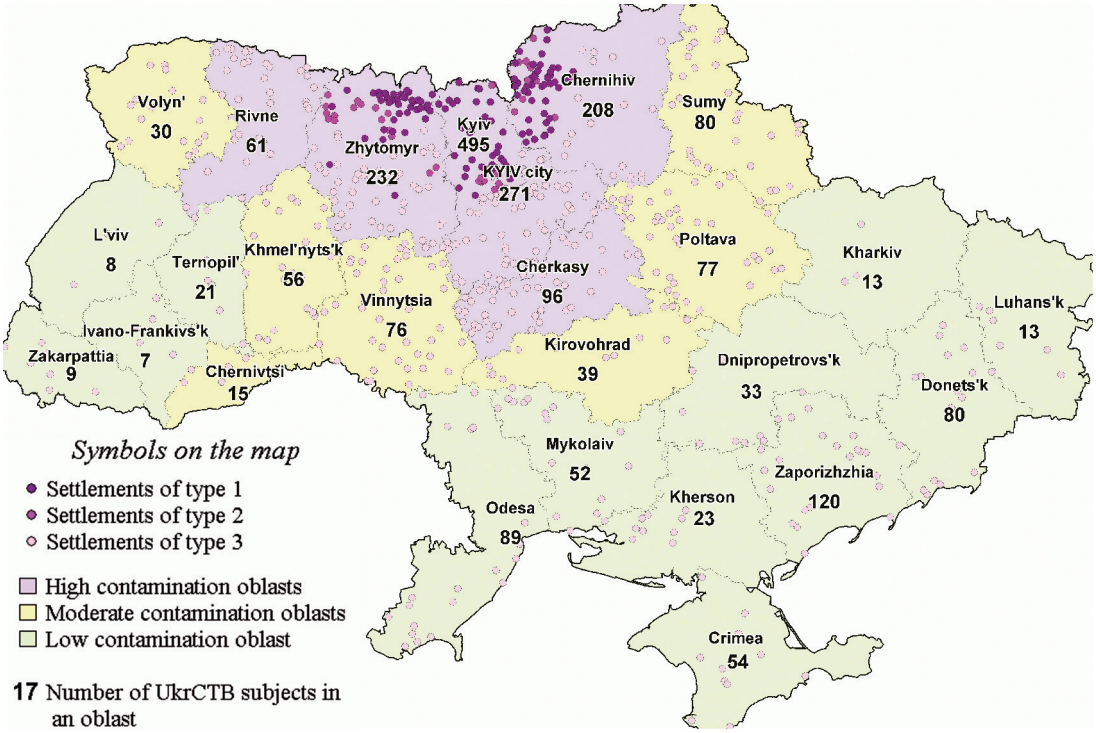

Figure 2.1. Geographical distribution of the Ukrainian CTB subjects over the settlements of different types in the oblasts with high, moderate and low ^{131}I Chernobyl ground deposition.

Table 2.1
Distribution of Ukrainian CTB subjects by age at the time of the accident

Age, years	Number of UkrCTB subjects	
	boys	girls
< 1	87	39
1 – 4	327	84
5 – 9	366	73
10 -14	433	127
15 – 17	256	59
18	32	10
Total exposed	1501	392
Born after March 1987	294	80
Total for the UkrCTB cohort	1795	472

In Ukrainian CTB cohort 64 subjects were exposed *in utero*. The thyroid dose to the fetus depends to a large degree on the level of ^{131}I intake by the mother due to consumption of food, as well as on the embryo and fetus gestational age (period of mother's pregnancy) during exposure. The greater was the gestational age during the exposure period (from 26 April to 30 June 1986), the higher was the fetal dose per unit of ^{131}I intake by the mother [3]. Table 2.2 presents the distribution of the Ukrainian CTB subjects who were exposed *in-utero* according to the period of mother's pregnancy at the time of accident.

Table 2.2
Distribution of *in utero* exposed UkrCTB subjects according to the period of mother's pregnancy at the time of the accident

Period of mother's pregnancy on 26 April 1986 (days)	**Number of subjects**
≤ 0	9
1-30	5
31-60	7
61-90	5
91-120	4
121-150	6
151-180	9
181-200	2
> 200 (subjects born before July 1986)	17
Total	*64*

Distribution of Ukrainian CTB subjects by method of thyroid dose reconstruction

In the *thyroid dose system* that is used for the CTB (*TDS-CTB*) all CTB subjects were subdivided into several groups depending on the availability of direct measurements in 1986 performed either directly on the subject, or on other persons in the region of his (her) residence at the time of accident.

Group 1. CTB subjects who had a *direct thyroid measurement* performed in May-June 1986 and were administered a *special interview* concerning the consumption rate of contaminated foods (essentially cow milk and leafy vegetables), as well as their possible changes of residence in May-June 1986 [4]. The interviews of these subjects were performed in framework of Ukraine-U.S. cohort study [4,5].

Group 2. CTB subjects who had individual direct thyroid measurements carried out in May-June 1986 [6], but without personal interviews.

Group 3. CTB subjects with neither individual direct thyroid measurements in May-June 1986 nor personal interviews. These subjects are further subdivided into three subgroups depending on their residence or non-residence in the raions of different oblasts where direct measurements were performed among the local population.

Subgroup 3.1 CTB subjects who resided in May-June 1986 in the settlements where direct thyroid measurements were performed on some inhabitants (*settlement of type 1*).

Subgroup 3.2 CTB subjects who resided in the settlements where no direct thyroid measurements were performed, but such measurements were performed on some inhabitants of neighboring settlements of the raion (*settlement of type 2*).

Subgroup 3.3 CTB subjects who were residents of the regions (raions, oblasts) where direct thyroid measurements were not carried out at all (*settlement of type 3*).

Group 4. CTB subjects who were exposed *in utero*. This group includes those born within the period from April 26, 1986 to March 31, 1987.

The distribution of the CTB subjects into the four groups is presented in Table 2.3.

Table 2.3
Distribution of CTB subjects by the groups and subgroups

Dosimetry group and subgroup	Description of dosimetry group	Number of UkrCTB subjects*
Group 1	CTB subjects who had direct thyroid measurements and personal interviews	**165**
Group 2	CTB subjects who had direct measurements of ^{131}I in the thyroid in May-June of 1986 but had no personal histories	**19**
Group 3	CTB subjects who had neither personal histories nor direct measurements of ^{131}I activity in thyroid in April-June of 1986:	**1685**
Subgroup 3.1	- residents of settlements of *type 1*	*613*
Subgroup 3.2	- residents of settlements of *type 2*	*43*
Subgroup 3.3	- residents of settlements of *type 3*	*1029*
Group 4	CTB subjects who were exposed *in utero*	**64**
Non-irradiated		**310**
Total		**2243**

*For 24 CTB-subjects the settlement of their residence in 1986 could not be identified

Table 2.4 shows the distribution of the Ukrainian CTB subjects of four above-mentioned groups according to date of birth: children, adults, and exposed *in utero*.

As shown in Tables 2.3 and 2.4, Group 3, consisting of 1,685 CTB subjects (mainly children) is the most numerous.

Table 2.4
Distribution of the CTB subjects of different groups according to date of birth

Dosimetry groups of CTB cohort	Date of birth				Total Number
	before 26.04.1968	26.04.1968-26.04.1986	May-June 1986	July 1986 - March 1987	
	Adults	Children	Exposed *in utero* + postnatal	Exposed *in utero*	
Group 1	-	165	-	-	165
Group 2	-	19	-	-	19
Group 3	43	1642	-	-	1685
Group 4	-	-	17	47	64
Total	43	1826	17	47	1933

Members of Groups 1 and 2 (~9.5% of all irradiated Ukrainian CTB subjects), for whom information on direct measurements of radioiodine activity in the thyroid in May-June 1986 is available, have the highest degree of dose individualization.

Because the members of Group 3 had no direct thyroid measurements in 1986, a mean settlement-age-gender specific dose is assigned to subjects of these groups. The adequacy of estimate of mean group-specific dose depends whether the direct thyroid measurements were provided in the settlement of residence of the subject or in a neighboring settlement. Thus, the highest degree of adequacy of an individualized dose estimations have the members of subgroup 3.1 residing in 52 settlements of type 1, and then, in the descending order, the members of subgroups 3.2 and 3.3 who were residents of 27 and 486 settlements of types 2 and 3, respectively. The distribution of settlements of different types in which CTB subjects were residing in 1986, for different oblasts of Ukraine, is shown on Figure 2.1. Settlements of type 1 and 2 are located predominantly in the most contaminated northern oblasts, while settlements of type 3 are located in the less contaminated southern oblasts.

General structure of models in TDS-CTB

Figure 2.2 shows the structure of the four-level *TDS-CTB* used to calculate the thyroid doses for the subjects of each of four groups as well as the common and group-specific initial data sets used in the calculation models of each level.

As shown in Figure 2.2, the data on the daily fallouts of ^{131}I on the ground in the settlements of residence are used as input in the *TDS-CTB* models of each of four groups. Daily fallouts over the 10 days of accidental release for each settlement are estimated using a model of atmospheric transfer [7, 8], allowing to calculate the daily dynamics of ^{131}I concentrations in vegetation and milk during May-June 1986.

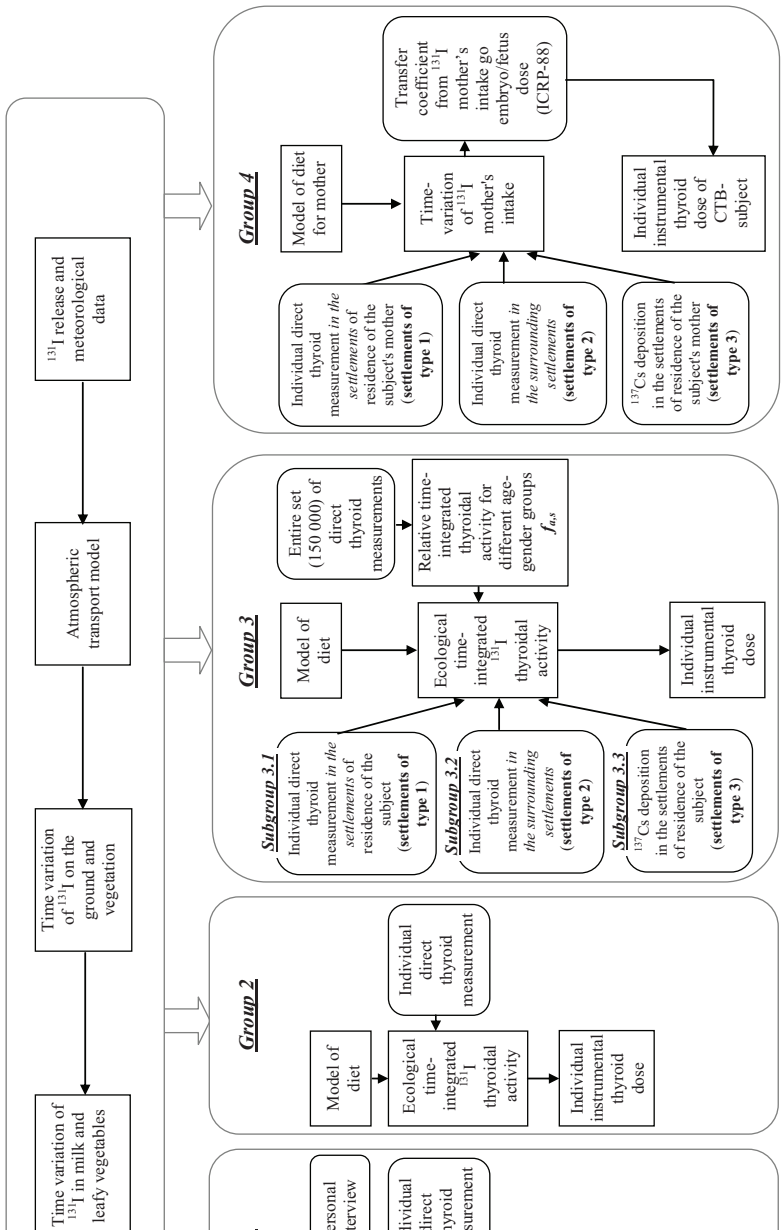

Figure 2.2. General scheme of models and initial data sets used in the different dosimetry groups in TDS-CTB.

Table 2.5
Reference daily consumption rates of milk and leafy vegetables used in the *TDS-CTB* for Groups 2, 3, and 4; AM and STD are the arithmetic mean and standard deviation, respectively

Age	Rural areas				Urban areas			
	Boys		Girls		Boys		Girls	
	AM	STD	AM	STD	AM	STD	AM	STD
	Milk consumption rate ($L\ d^{-1}$)							
1	0.67	0.42	0.62	0.46	0.48	0.33	0.40	0.32
2	0.66	0.44	0.57	0.38	0.33	0.27	0.33	0.28
3	0.64	0.46	0.52	0.38	0.34	0.28	0.29	0.28
4	0.63	0.39	0.58	0.37	0.36	0.38	0.30	0.25
5	0.69	0.47	0.52	0.41	0.39	0.41	0.30	0.22
6	0.69	0.47	0.57	0.40	0.31	0.28	0.29	0.21
7	0.65	0.45	0.50	0.36	0.32	0.23	0.30	0.25
8	0.70	0.55	0.49	0.36	0.35	0.35	0.29	0.35
9	0.73	0.61	0.52	0.41	0.30	0.33	0.21	0.16
10	0.79	0.60	0.45	0.39	0.37	0.39	0.21	0.18
11	0.77	0.58	0.40	0.33	0.46	0.57	0.25	0.28
12	0.72	0.57	0.48	0.50	0.47	0.41	0.23	0.21
13	0.78	0.65	0.47	0.51	0.47	0.62	0.27	0.23
14	0.78	0.65	0.47	0.41	0.44	0.35	0.20	0.19
15	0.72	0.62	0.43	0.36	0.44	0.50	0.20	0.29
16	0.83	0.74	0.48	0.40	0.44	0.50	0.20	0.29
17	0.83	0.66	0.44	0.37	0.44	0.50	0.20	0.29
18	0.83	0.66	0.44	0.37	0.44	0.50	0.20	0.29
	Consumption rate of leafy vegetables ($kg\ d^{-1}$)							
1	0.011	0.014	0.010	0.011	0.014	0.017	0.007	0.006
2	0.015	0.021	0.013	0.019	0.013	0.017	0.015	0.017
3	0.019	0.020	0.017	0.020	0.012	0.013	0.014	0.018
4	0.021	0.018	0.022	0.024	0.020	0.024	0.017	0.020
5	0.025	0.022	0.017	0.017	0.019	0.017	0.017	0.016
6	0.029	0.025	0.023	0.021	0.025	0.024	0.024	0.028
7	0.027	0.025	0.027	0.022	0.020	0.026	0.025	0.021
8	0.029	0.028	0.029	0.026	0.020	0.020	0.018	0.017
9	0.028	0.020	0.026	0.027	0.024	0.030	0.023	0.019
10	0.037	0.032	0.033	0.025	0.031	0.031	0.020	0.018
11	0.036	0.031	0.032	0.025	0.032	0.036	0.037	0.038
12	0.035	0.027	0.036	0.031	0.028	0.026	0.034	0.028
13	0.039	0.029	0.030	0.023	0.026	0.018	0.038	0.037
14	0.036	0.032	0.031	0.030	0.035	0.031	0.042	0.036
15	0.044	0.031	0.036	0.027	0.027	0.022	0.033	0.032
16	0.038	0.028	0.044	0.041	0.027	0.022	0.033	0.032
17	0.046	0.032	0.035	0.028	0.027	0.022	0.033	0.032
18	0.046	0.032	0.035	0.028	0.027	0.022	0.033	0.032

The "ecological" time-integrated thyroid activity of ^{131}I is calculated for the subjects of Groups 1, 2 and 3 using the time-variation of ^{131}I in leafy vegetables and milk and also the model of diet.

For the subjects of Group 1 the model of diet was developed using the information from the personal interviews [4] on the daily consumption of different components of diet: cow milk, goat milk, private milk, shop milk, sour milk, kefir, and leafy vegetables. The information on the settlements of residence in May and June 1986, duration of his (her) stay in each residence, dates of arrival/departure from each settlement of residence was also clarified during the personal interviews of the CTB subjects of Group 1.

Due to the absence of personal dietary data for the subjects of Groups 2, 3, and 4, the reference age- and gender-dependent consumption rates of milk and leafy vegetables were used for the calculation of the ^{131}I daily intake (Table 2.5). It was also assumed that the subjects of Groups 2, 3, and 4 were not relocated from their settlements of residence in April-June 1986.

Group-specific modifiers are established for the ecological time-integrated ^{131}I thyroidal activity are also shown in Figure 2.2 for each group.

The following initial data sets were used for establishing the modifiers:

- *subjects of Group 1 and 2*: the results of direct thyroid measurements of ^{131}I activity for the CTB subject in May-June 1986;
- *subjects of subgroup 3.1:* the results of direct thyroid measurements of ^{131}I activity performed in May-June 1986 among the inhabitants (not the CTB subjects) in the settlement of staying of the CTB subject (settlement of type 1);
- *subjects of subgroup 3.2:* the results of direct thyroid measurements of ^{131}I activity performed in May-June of 1986 among the inhabitants in the settlements of the raion surrounding the settlement of residence of the CTB subject in 1986 (settlement of type 2);
- *subject of subgroup 3.3*: data on ^{137}Cs ground deposition in the settlement of residence of the CTB subjects (settlement of type 3).

The modifiers of subgroups of Group 3 are developed by analyzing the results of ~150,000 direct thyroid activity measurements taken in May-June 1986 in the Northern oblasts of Ukraine.

The model of thyroid dose reconstruction applied for the subjects of Group 4 (exposed *in utero*) includes the calculation of time-variation of ^{131}I intake for mother. At that the modifiers are dependent on the type of settlement of a CTB-subject's mother's residence in 1986.

Using the ecological time-integrated ^{131}I thyroidal activity corrected by the correspondent modifiers and age-gender dependent thyroid mass [9], the individual "instrumental" thyroid doses (in deterministic and stochastic modes) are calculated for each CTB subject of Groups 1, 2 and 3. The individual "instrumental" thyroid doses for the subjects of Group 4 are calculated using the transfer coefficients from ^{131}I mother's intake to embryo-fetus dose, which are significantly changing with the period of mother's pregnancy [3].

Parameters of models in TDS-CTB

The central estimates and uncertainty distributions that were selected for the main parameters of the ecological and dosimetry models in *TDS-CTB* are presented in Table 2.6, with the exception of age-dependent lung ventilation rate and biological half-time of iodine excretion from the thyroid, which are provided in Table 2.7.

Table 2.6
Central estimates and statistical characteristics of the distributions of the main parameters used in *TDS-CTB*. GM is the geometric mean; GSD is the geometric standard deviation [8,10-18]

Name of parameter	Notation	Unit	Central estimate (AM)	Distribution type	Distribution parameters
Radioactive decay constant for ^{131}I	λ^{r}	d^{-1}	$8.62 \cdot 10^{-2}$	Constant	—
Energy absorbed in thyroid per ^{131}I radioactive decay	$E^{I-131,th}$	*MeV*	0.20	Constant	—
^{131}I activity decrease factor in leafy vegetables due to culinary treatment	$K^{r,cul,gr}$	*Unitless*	0.8	Uniform	min=0.6, max=1.0
Effective transfer coefficient that describes excretion of iodine into human milk	TC_{ef}^{I}	$day \cdot L^{-1}$	0.4	Censored lognormal	GM =0.37, GSD = 1.4 min=0.25, max=0.89
Effective half time of iodine excretion into human milk	$T_{ef}^{I,m}$	*day*	0.58	Censored lognormal	GM =0.5, GSD =1.7 min=0.21, max=1.33
Transfer factor of ^{131}I from lungs to blood	$B^{r,inh}$	*Unitless*	0.61	Triangular	mode=0.58, min=0.40, max=0.85
Transfer factor of ^{131}I from blood to thyroid	$B^{I,th}$	*Unitless*	0.3	Triangular	mode=0.25, min=0.15, max=0.5
Deposition velocity of ^{131}I on the ground (dry conditions)	$V^{I,soil}$	$m \cdot d^{-1}$	600	Censored lognormal	GM=540, GSD=1.6, min=210, max=1380
^{131}I mass interception factor by vegetation (wet mass)	$K_{M}^{r,gr}$	$m^2 \cdot kg^{-1}$	0.25	Triangular	mode=0.2, min=0.1, max=0.45
Grass yield (wet mass)	M^{biom}	$kg \cdot m^{-2}$	0.75	Triangular	mode=0.75, min=0.5, max=1.0

Continuation of Table 2.6

Short half-time of elimination of ^{131}I from the grass surface by weathering	$T_1^{I,gr}$	*d*	7.0	Censored lognormal	GM=6.9, GSD=1.2, min=4.6, max=9.5
Long half-time of elimination of ^{131}I from the grass surface by weathering	$T_2^{I,gr}$	*d*	28.0	Censored lognormal	GM=27.5, GSD=1.2, min=12, max=37
Daily consumption of fresh grass by cow	I^{gr}	*kg d^{-1}*	45	Triangular	mode=45, min=30, max=60
Biological half purification period of milk from ^{131}I	$T^{I,cow}$	*d*	1.1	Censored lognormal	GM=1.0, GSD=1.4, min=0.5, max=1.96
Transfer factors for ^{131}I from daily cow's intake to concentration in cow's milk	TF^{I}	*d·L^{-1}*	0.01	Censored lognormal	GM=0.0065, GSD=2.5, min=0.00, max=0.04

Table 2.7
Central estimates and ranges of age-dependent parameters in *TDS-CTB*: lung ventilation rate and biological half-time of iodine excretion from the thyroid [2,12,19]

Age (years) a	**Lung ventilation rate ($m^3 \cdot d^{-1}$)**			**Biological half-time of iodine excretion from the thyroid (*d*)**		
	AM	min	max	AM	min	max
0	2.9	1.4	5.4	15	7.1	28
1	5.6	2.7	10.6	20	9.4	38
2	6.5	3.1	12.3	22	10.4	42
3	7.4	3.5	14.0	25	11.8	47
4	8.3	3.9	15.6	28	13.2	53
5	9.3	4.4	17.5	30	14.2	57
6	10.4	4.9	19.6	38	18.0	72
7	11.5	5.4	21.7	46	21.7	87
8	12.6	5.9	23.7	54	25.5	102
9	13.6	6.4	25.8	62	29.3	117
10	14.8	7.0	28.0	70	33.1	132
11	16	7.5	30.2	72	34.0	136

Continuation of Table 2.7

Age (years) a	Lung ventilation rate ($m^3 \cdot d^{-1}$)			Biological half-time of iodine excretion from the thyroid (*d*)		
	AM	min	max	AM	min	max
12	17.2	8.1	32.4	74	35.0	140
13	18.3	8.7	34.7	76	35.9	144
14	19.5	9.2	36.9	78	36.9	147
15	20.3	9.6	38.4	80	37.8	151
16	20.7	9.8	39.2	82	38.7	155
17	21.2	10.0	40.0	84	39.7	159
18	21.6	10.2	40.8	87	41.1	164

Main equations of TDS-CTB for the subjects of different groups

Groups 1 and 2

The following equation was developed to calculate the individual dose $D_{1,2}^{ind}$ for subject *i* of dosimetry group 1 or 2:

$$D_{1,2}^{ind} = z \frac{E^{I-131}}{M_{a,s}} A_i^{ecol} \cdot K_i^{sc}, \quad (2.1)$$

where:

$M_{a,s}$ is the thyroid mass of the subject of age *a* and sex *s*, *g*;

E^{I-131} is the energy absorbed by the thyroid per ^{131}I radioactive decay, *MeV per decay*;

A_i^{ecol} is the individual "ecological" time-integrated thyroid activity calculated using the models of environmental iodine transport and the biokinetic model, *kBq·d*;

K_i^{sc} is the *individual* "*scaling factor*" which adjusts the ecological integrated activity A_i^{ecol} according to the results of the direct thyroid measurement for the CTB-subject *i*, *unitless*;

z is a unit conversion coefficient, equal to:

$$13.82 \frac{Bq}{kBq} \cdot \frac{g}{kg} \cdot \frac{J}{MeV} \cdot \frac{s}{day} \cdot \frac{mGy}{Gy}.$$

In equation 2.1, the individual scaling factor K_i^{sc} for the subject *i* is estimated as:

$$K_i^{sc} = \frac{Q_i^{I,mes}}{Q_i^{I,ecol}(t^{mes})} \tag{2.2}$$

where:

$Q_i^{I,\,mes}$ is the measured ^{131}I activity in the thyroid of subject *i*, *kBq*;

$Q_i^{I,ecol}(t^{mes})$ is the estimate of thyroid activity at the time of measurement t^{mes} using the ecological environmental iodine transport model and the biokinetic model, *kBq*.

<u>*Groups 3*</u>

For the subjects in dosimetry Group 3, the individual thyroid dose D_i^{ind} is calculated as the *settlement-specific age-gender group averaged* thyroid dose $D_{a,s,j}$, where *a* and *s* are the age and the gender of the age-gender group the subject *i* belongs to, and *j* is the settlement of residence of the subject in April-June 1986.

Subgroup 3.1 (Settlements of type 1, j^*):

$$D_{3.1}^{ind} = D_{a,s,j^*} = z\frac{E^{I-131}}{M_{a,s}} A_{a_{ref},s,j^*}^{mes} \cdot f_{a,s}$$
$$A_{a_{ref},s,j^*}^{mes} = \frac{1}{N}\sum_{k=1}^{N} \frac{A_{k,a,s,j^*}^{ecol} \cdot K_k^{sc}}{f_{a,s}} \tag{2.3}$$

where

N is the number of residents *k* (not the subjects of Ukrainian CTB) with direct thyroid measurements in the settlement of residence j^* of the UkrCTB subject under consideration;

$f_{a,s}$ is the relative time-integrated thyroidal activity for the subjects of *a-s* group [20] (Table 2.8);

A_{a_{ref},s,j^*}^{mes} is the settlement-specific time-integrated thyroidal ^{131}I activity for gender *s* in the *reference age group* a_{ref} for the settlement j^*, *kBq·d*;

A_{k,a,s,j^*}^{ecol} is the ecological time-integrated ^{131}I thyroidal activity calculated for the resident *k* (not an UkrCTB subject) of age-gender group *a-s* the CTB-subjects belonged to in the settlement j^*, *kBq·d;*

K_k^{sc} is the individual scaling factor of subject *k* (not the subjects of Ukrainian CTB) with direct ^{131}I thyroid measurement in te settlement j^* of CTB-subject *i* staying.

Table 2.8
Parameters of the relative time-integrated thyroidal ^{131}I activity function $f_{a,s}$ for different age-gender groups. GM is the geometric mean; GSD is the geometric standard deviation

Age	Relative time-integrated thyroidal activity ($f_{a,s}$)							
	Urban areas				Rural areas			
	Girls		Boys		Girls		Boys	
	GM	GSD	GM	GSD	GM	GSD	GM	GSD
0	0.81	3.3	0.61	3.0	0.58	3.1	0.47	3.2
1	0.81	3.3	0.61	3.0	0.58	3.1	0.47	3.2
2	0.81	2.8	0.62	3.0	0.64	2.8	0.55	2.8
3	0.77	2.5	0.61	2.8	0.64	2.7	0.54	2.6
4	0.86	2.4	0.67	2.5	0.70	2.6	0.56	2.9
5	0.97	2.5	0.86	2.5	0.70	2.6	0.59	2.6
6	1.0	2.3	0.86	2.3	0.77	2.5	0.66	2.6
7	1.0	2.1	0.85	2.3	0.75	2.2	0.70	2.4
8	0.88	2.4	0.79	2.4	0.76	2.4	0.72	2.5
9	0.91	2.2	0.81	2.2	0.80	2.4	0.79	2.4
10	0.92	2.2	0.81	2.2	0.81	2.4	0.84	2.2
11	0.97	2.1	0.91	2.1	0.89	2.2	0.85	2.3
12	0.95	2.1	0.94	2.1	0.95	2.1	0.90	2.2
13	1.0	2.1	0.96	2.2	1.0	2.1	1.0	2.2
14	1.1	2.1	1.1	2.2	1.0	2.1	1.1	2.2
15	1.1	2.2	1.1	2.3	1.0	2.3	1.2	2.4
16	1.2	2.4	1.1	2.5	1.0	2.4	1.3	2.5
17	1.2	2.5	1.1	2.3	1.0	2.4	1.1	2.4
18	1.1	2.8	1.0	3.0	0.95	2.7	1.1	2.1

Subgroup 3.2 (Settlements of type 2, j^{**}):

$$D_{3.2}^{ind} = D_{a,s,j^{**}} = z\frac{E^{1-131}}{M_{a,s}} \cdot \frac{A_{a_{ref},s,j^{**}}^{ecol}}{K_{s,raion}^{scal}} \cdot f_{a,s}$$

$$K_{s,raion}^{sc} = \frac{1}{J}\sum_{j^*=1}^{J} \frac{A_{a_{ref},s,j^*}^{ecol}}{A_{a_{ref},s,j^*}^{mes}} \qquad (2.4)$$

where

j is the number of settlements j^* with direct thyroid measurements in the *raion* where the settlement $j^{**}$of CTB-subject is located.

A_{a_{ref},s,j^*}^{ecol} is the settlement-specific time-integrated thyroid ^{131}I activity for gender s in the reference age-interval a_{ref} in the settlement j^* estimated using the ecological model, *kBq·d*;

$K^{sc}_{s,raion}$ is the *raion-specific scaling factor* for the settlements of type 2 (j^{**}).

Subgroup 3.3 (Settlements of type 3, j^{***}):

$$D^{ind}_{3..3} = D_{a,s,j^{***}} = z\frac{E^{I-131}}{M_{a,s}}\frac{A^{ecol}_{a_{ref},s,j^{***}}}{K^{\sigma}_{s,j^{***}}}f_{a,s} \qquad (2.5)$$
$$K^{\sigma}_{s,j^{***}} = B(\sigma_{Cs,j^{***}})^{\beta}$$

where

$K^{\sigma}_{s,j^{***}}$ is the scaling factor for the settlements of type 3 (j^{***}) (Table 2.9);

$\sigma_{Cs,j^{***}}$ is the ^{137}Cs ground deposition in settlement j^{***}, *kBq·m^{-2};*

Table 2.9

Parameters used to estimate the scaling factors $K^{\sigma}_{s,j^{***}}$ (Subgroup 3.3). AM is the arithmetic mean, STD is the standard deviation, GM is the geometric mean, GSD is the geometric standard deviation.

$K^{\sigma}_{s,j^{***}} = B(\sigma_{Cs,j^{***}})^{\beta}$

Location	Gender	*B*		*β*	
		GM	GSD	AM	STD
Rural	Boys	0.59	1.1	0.36	1.03
	Girls	0.47	1.1	0.36	1.03
Urban	Boys	0.36	1.5	0.61	1.10
	Girls	0.34	1.5	0.58	1.10

Groups 4 (exposed in-utero)

As shown in Figure 2.2, the reconstruction of the thyroid dose for the CTB-subjects exposed *in-utero* [3] includes a model the CTB-subject's mother diet and CTB-subject's mother ^{131}I intake with food in May-June 1986. The data sets used as the modifiers in the model of thyroid dose reconstruction for the CTB-subjects of Group 4 ($K^{sc}_{i,mother}$) are identical to those developed in the reconstruction models for dosimetry subgroups 3.1, 3.2, and 3.3, and dependent on the type of settlement in which the mother resided in May-June 1986. Thus, the individual dose to CTB subjects *i* due to *in-utero* exposure ($D^{ind}_{i,F}$) is described by the following equation:

$$D_F^{ind} = K_{i,mother}^{sc} \times \int_0^T h_F(\tau(t)) \times q_{i,mother}^{ecol}(t)dt \quad (2.6)$$

where

$$K_{i,mother}^{sc} = \begin{cases} K_i^{sc} & \text{if the mother had a direct thyroid measurement in May - June 1986} \\ K_{j*}^{sc} & \text{if the mother resided in a settlement of type 1 (j*)} \\ K_{s,raion}^{sc} & \text{if the mother resided in a settlement of type 2 (j**)} \\ K_{s,j***}^{\sigma} & \text{if the mother resided in a settlement of type 3 (j***)} \end{cases} \quad (2.7)$$

$q_{i,mother}^{ecol}(t)$ is the daily intake of ^{131}I by the mother, estimated using the ecological ^{131}I-transport model, *Bq·d*$^{-1}$.

$\tau(t)$ is the stage of pregnancy of the mother of the CTB subject at time *t* counted from 26 April 1986, *days*;

$h_F(\tau)$ is the fetal thyroid dose per unit intake of ^{131}I by the mother [21] dependent on the stage of pregnancy τ *Gy·Bq*$^{-1}$;

Estimation of thyroid dose uncertainties for the UkrCTB subjects

The uncertainties of the thyroid doses estimations in the CTB subjects of different dosimetry groups were calculated using a Monte-Carlo procedure. For this purpose, probability distributions were assigned to most of parameter values. The types and characteristics of the distributions of some of the parameters are shown in Table 2.6. It was assumed in the Monte-Carlo procedure that all parameters were independent. One thousand values of the individual dose were calculated for every CTB subject. Finally, the arithmetic and geometric means of the 1,000 values of instrumental doses as well as the geometric standard deviation minimum, maximum and 25%, 50%, 75% percentiles of these distributions were estimated for every CTB subject.

The Monte-Carlo procedure used for estimating the dose uncertainties differed in some respect for the different groups and subgroups, since different parameters and functions were applied.

Estimates of individual thyroid doses for the UkrCTB subjects

Individual thyroid doses were estimated for 1,933 Ukrainian CTB subjects. Doses have not been reconstructed for 24 CTB subjects (because the settlements of their staying in 1986 could not be identified), and for 310 non-irradiated subjects born after March 1987.

For each of the subjects the thyroid dose and their uncertainties were estimated from the environmental transport and biokinetic models adapted to the information available for the different groups and subgroups (Figure 2.2) using a Monte-Carlo simulation procedure with 1,000 trials for every CTB subject.

Table 2.10 shows the arithmetic mean as well as the 50%, 25%, 75% percentiles and the minimum and maximum values of individual doses estimated for the subjects of different

age-groups in different dosimetry groups. For 15 of the 64 subjects of Group 4, the individual thyroid doses are estimated to be negligibly small, and taken to be equal to zero.

Table 2.10
General groups of individual thyroid doses estimated for the UkrCTB subjects of the different groups and subgroups

Age intervals, years	Number of subjects	Individual thyroid doses, Gy					
		Mean	Percentiles			Min	Max
			50%	25%	75%		
Group 1							
0-4	44	1.9	0.43	0.81	2.3	0.046	13
5-9	42	1.2	0.16	0.54	1.8	0.025	7.2
10-14	69	0.75	0.055	0.31	1.1	0.001	5.3
15+	10	1.6	0.045	0.25	2.5	0.023	8.5
All ages	165	1.2	0.13	0.48	1.4	0.001	13
Group 2							
0-4	13	2.7	0.43	0.72	3.8	0.008	13
5-9	3	0.033	0.028	0.032	0.037	0.027	0.039
10-14	2	0.33	0.021	0.33	0.63	0.021	0.63
15+	1	0.3	0.3	0.3	0.3	0.3	0.3
All ages	19	1.9	0.099	0.53	3.2	0.008	13
Subgroup 3.1							
0-4	164	0.26	0.1	0.15	0.23	0.04	5.2
5-9	159	0.096	0.039	0.051	0.068	0.021	2.7
10-14	186	0.049	0.024	0.03	0.034	0.013	1.1
15+	104	0.037	0.024	0.026	0.028	0.014	0.37
All ages	613	0.12	0.027	0.043	0.11	0.013	5.2
Subgroup 3.2							
0-4	16	2	0.09	0.15	0.91	0.04	24
5-9	5	1.4	0.017	0.2	3	0.016	4.1
10-14	13	0.1	0.021	0.075	0.14	0.014	0.44
15+	9	0.031	0.013	0.027	0.035	0.011	0.091
All ages	43	0.93	0.028	0.091	0.19	0.011	24
Subgroup 3.3							
0-4	293	0.3	0.036	0.11	0.21	0.001	1.9
5-9	223	0.061	0.014	0.034	0.068	0.002	0.8
10-14	283	0.033	0.008	0.017	0.037	0.001	0.45
15+	229	0.029	0.008	0.017	0.031	0.001	0.59
All ages	1029	0.09	0.012	0.031	0.08	0.001	1.9
Group 4							
All ages	49	0.1	0.001	0.014	0.08	<0.001	2.1

The results presented in Table 2.10 indicate that the highest doses were estimated for the CTB subjects of dosimetry groups 1 and 2, and also for the subjects of subgroup 3.2. The high doses in the subjects of these groups and subgroups are the consequence of their residence in May-June 1986 in the most radioactively-contaminated areas (Figure 2.1). The lowest doses are estimated for the subjects of subgroup 3.3 because these subjects were staying in the areas with moderate and low radioactive contamination in 1986. As shown in Table 2.10, the younger children (aged 0 to 4 years) received higher thyroid doses than any other age group in all dosimetry groups and subgroups.

The general characteristics of the uncertainties of the estimated individual thyroid doses are presented in Table 2.11 in terms of mean, min, max and geometric standard deviation (GSD) of individual geometric standard deviation for the different groups and subgroups. The data of Table 2.11 clearly show that the uncertainties in the reconstructed individual thyroid dose estimates increase from Group 1 to Group 4. In dosimetry Group 3, the highest uncertainties in the estimated individual doses were found for the subjects of subgroup 3.3. This is due to the fact that direct thyroid measurements of ^{131}I activity are not used to justify the ecological dose estimation for this subgroup. The substantial uncertainties in the thyroid dose estimates for the CTB subjects exposed *in utero* (Group 4) are related to: (1) the complexity of modeling the iodine metabolism in the embryo/fetus and the transfer of ^{131}I from the mother to fetus at different stages of pregnancy, and (2) a number of difficulties in modeling the mother's diet in May-June 1986.

Table 2.11
Average values of geometric standard deviation (GSD) of individual geometric standard deviation for the subjects of diferent groups and subgroups. N is the number of subjects

Dosimetry group and subgroup	N	GSD of individual thyroid doses		
		Mean	Min	Max
1	165	1.5	1.3	4.7
2	19	1.5	1.4	1.7
3-1	613	2.9	2.3	4.6
3-2	43	3.3	2.3	5.2
3-3	1029	3.6	3.1	4.5
4	49	3.8	2.8	8.7

Table 2.12 presents the distribution of the Ukrainian CTB subjects of different dosimetry group/subgroup over the intervals of individual thyroid dose irrespectively of the age. As shown in Table 2.12, the CTB members of Group 1 are distributed rather evenly by dose value within the interval up to 5 Gy. Individual doses for 17 of 19 members of Group 2 are also within the dose interval up to 5 Gy. In subgroup 3.1 for 99% of subjects the estimated individual thyroid doses do not exceed 1 Gy, and for about 55% of members of this group the thyroid doses do not exceed 0.05 Gy. In the subgroup 3.2 the relative distribution of the subjects by the dose interval is similar to that in subgroup 3.1. For 97% of members of subgroup 3.3 the individual thyroid doses were estimated to be less than 0.5 Gy, and for 79% as being less than 0.1 Gy. For 47 of 64 Ukrainian CTB members

of Group 4, i.e., those who were partly or entirely exposed *in utero*, individual thyroid doses were estimated to be less than 0.05 Gy. For one subject of Group 4 the individual thyroid dose estimation is greater than 0.5 Gy.

Table 2.12
Distributions of UkrCTB subjects according to the interval of estimated mean individual thyroid dose

Thyroid dose interval, Gy	Group and Subgroup					
	1	2	3.1	3.2	3.3	4
	Number of UkrCTB subjects					
<0.05	23	5	335	18	681	47
0.05-0.1	12	-	109	5	129	9
0.1-0.2	15	-	96	10	124	2
0.2-0.5	36	4	52	1	62	5
0.5-1	24	4	13	4	22	-
1-2	22	-	5	1	11	-
2-5	27	4	2	3	-	1
5-10	4	1	1	-	-	-
>10	2	1	-	1	-	-
Entire cohort	165	19	613	43	1029	64

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Chapter 3

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Epidemiology of thyroid cancer in Ukraine after Chernobyl

Thyroid malignancies are relatively rare worldwide, yet their incidence has increased over the last few decades [1,2]. Epidemiology of thyroid cancer in the population of Ukraine exposed to radiation as a result of accident at the Chernobyl Nuclear Power Plant in April, 1986 is of a particular interest. Although the relationship between the Chernobyl radiation and increasing incidence of thyroid cancer among exposed children and adolescents in Belarus, Russia, and Ukraine is established in a number of studies and is the main proven health consequence of the accident [3,4], a continued monitoring and analysis of temporal trends of this disease have important medical and scientific impacts [5-7].

The purpose of this Chapter is to overview data on thyroid cancer incidence for the period from 1986 to 2010 in specific groups of population of Ukraine exposed in childhood or adolescence and also prenatally (*in utero*). In addition, the group of subjects born after the accident is included in the study because it provides important information on thyroid cancer incidence in the population of Ukraine which had not experienced Chernobyl fallouts.

A tool for recording and storage of data on the incidence of any cancer are population-based registries [8-11]; information can be used for subsequent analysis of morbidity and mortality in certain age groups and geographical areas. Domestic sources of data on thyroid cancer incidence in Ukraine are the site-specific Clinico-morphological Registry (CMR) of the State Institution "VP Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine" (IEM) [12], and a classic cancer registry, which keeps records on all types of cancer, The National Cancer Registry of Ukraine (NCR) of the National Cancer Institute of the Ukraine Ministry of Public Health [13,14].

Specialized Clinico-morphological and National Cancer Registries of Ukraine

In the first half of the 1980-ies there were no personalized records of thyroid cancer cases in Ukraine [15]. Statistical summary of thyroid cancer could be derived from the archives of hospitals or from the reports of regional endocrinology services. After the Chernobyl accident in 1986, the Ministry of Public Health of USSR recommended to establish personalized registries of affected population and to improve recording system of patients with thyroid diseases which were anticipated to be one of the possible health effects of the Chernobyl accident [16,17]. In Ukraine, the collection of data on thyroid cancer incidence

was coordinated by the Institute of Endocrinology and Metabolism (IEM) with participation of regional endocrinology services.

In 1992, a personalized Clinico-morphological Registry was established at the IEM for the first time in Ukraine, into which all cases of thyroid cancer in patients whose age at the time of accident did not exceed 18 years (i.e., born in 1968 and later) were entered. Creation of the Registry was promoted by the initiative of the Ukraine Ministry of Public Health in the Order No. 12 dated January 20, 1992 "On the improvement of endocrinological care to children and adults with thyroid diseases" in which the need for an increased attention to thyroid disorders after the Chernobyl accident and compulsory referral of children and adolescents with such diseases for surgical treatment to the Clinic of the IEM was formally stated. In addition, regional and municipal endocrinology and oncology clinics were imposed to collect and report complete information about thyroid cancer cases in corresponding age group to IEM, and to provide histological slides of tumors for additional expertise of the quality of pathomorphological diagnosis. Thus, IEM managed to collect the necessary data for comprehensive registry functioning.

In the post-Chernobyl period, the CMR has been continuously providing statistics on the number of cases and incidence of thyroid cancer among children and adolescents to the Ministry of Public Health and Academy of Medical Sciences of Ukraine, and also served as a source of data for a number of analytical studies on thyroid cancer incidence and assessment of radiation risks of thyroid cancer in the younger age groups of Ukraine [3,5,6,12].

Statistical records of thyroid cancer as of a separate entity in the structure of oncological services of Ukraine were started in 1989 [14,18]. At the same time, the Institute of Oncology of the Academy of Medical Sciences of Ukraine launched an automated National Cancer Registry (NCR) that covered all regions of Ukraine. NCR was based on a network of specialized cancer institutions and a State system of cancer registration. Practical realization and implementation of the information system in oncology at the regional level began in the mid-'90-s receiving governmental support according to the Order of the Ukraine Ministry of Public Health "On the establishment of a National Cancer Registry of Ukraine" dated December 30, 1996 [14]. In 2001, the creation of the system was completed by including all regions of Ukraine in the Registry [13,14].

A close cooperation between the CMR and NCR began in 2005. Today, the CMR is in some way a part of NCR [19-21]. Note however, that cross-check of the registries reveals some discordances. The CMR is a specialized Clinico-morphological Registry that includes particular age group (at high risk for developing thyroid cancer) and only operated cases with pathologically confirmed diagnosis. In contrast, besides the CMR records, National Cancer Registry also includes cases that had not been operated with only preoperative cytological conclusion about the possibility of thyroid cancer available. Moreover, the calculation of cases at the regional level in the CMR is based on the patient's place of residence at the time of Chernobyl accident, while the NCR system is based on the patient's place of residence at surgery. In other words, these differences should always be taken into account when comparing the two data Registries or analyzing the incidence according to data of one or another registry.

Pathological verification of diagnosis is essential for the case to be included in the CMR. The detailed pathological report including tumor type, subtype, invasive properties and

tumor size is mandatory for cases operated at the Department of Surgery of Endocrine Glands of the IEM, and for the material (paraffin blocks, histological specimens) from patients operated elsewhere in Ukraine yet receiving radioactive iodine therapy at the Department of Clinical Radiology of the IEM (see Chapter 4).

Sources of information for the CMR are currently the reports of regional endocrinology services, data of regional oncological institutions (via the NCR of Ukraine), and clinical medical information system (MIS) of the IEM (www.therdep.com.ua). The principles of inclusion and recording of personal data coming from different sources correspond to the classical rules and procedures of cancer registries [22-24]. Automated procedures and control of completeness and quality of personal data play an important role [25].

Software realization of Clinico-morphological Registry

Entering and storage of Registry data is implemented using MS SQL Server software. This

- ensures data integrity;
- allows the use of a standard relational model for designing data structures and typical SQL for queries;
- provides a reliable import/export of data from most databases;
- allows performing application tasks using typical software tools (MS Access, Delphi, C + +, *. net , etc.).

The upgraded structure of registry databases provides some additional features:

- supports both Ukrainian and Russian versions of patient's surname and first name;
- implements a mechanism of saving changes or multiple patient's attributes' values (e.g. name, location, diagnosis);
- formalizes the place of residence according to the standard administrative territorial codifier "KOATO".

In addition, links have been established with databases with the geographical component at the regional, district and settlement level (MapInfo format), which extend the capabilities of data analysis and allow building various thematic maps. Today, the logical structure of the Registry includes three functional components: CaseRegist, CaseManager, and CaseReport.

- CaseRegist is a module of primary registration of data reported by the regional endocrinologists and IEM patients.
- CaseManager is a module of analysis, linkage, and verification of the Registry information. With this component, a comparative analysis of information from multiple sources is conducted including the search for and removal of duplicate records. A verified analytical version of the Registry record is generated that remains active for the year. A list of problematic cases to be verified by an expert morphologist and/or refined through queries to regional services could be created. The module also stores archives of the versions of analytical tables of the Registry from previous years; information about changes or refining of certain attributes of patients to be included in the next version of the Registry is accumulated.

- CaseReport component contains the current version of analytical tables of the Registry and a set of typical queries to generate standard numerical tables, charts and graphs that describe the available data for particular time and can be used for the preparation of reports, presentations, and publications.

The demographic component of the Clinico-morphological Registry

The demographic component of the Registry is based on a series of periodic publications of the State Statistics Committee of Ukraine; publications of regional statistics departments may be also involved detailing certain parameters for a specific region.

Census data represent the most reliable and detailed source of information on Ukrainian population. Our study period (from 1986 to 2010) includes two censuses: Ukrainian SSR census of 1989 and the All-Ukrainian census of 2001 [26, 27].

Primary sources of demographic data also include publications on the distribution of population by age and sex [28-33], the number of current and permanent population of Ukraine at the regional level, and annual demographic data records.

General statistical collections and the official website of the State Statistics Committee (www.ukrstat.gov.ua) [34] may also be used as sources of current demographic information.

The demographic component of the Registry includes data on the population of Ukraine for the period 1986 to 2010, and the following age groups are selected for monitoring:

- children under 14 years old (born before 1987);
- children under 14 years old (born in 1987 or later);
- adolescents aged 15 to 18 years (born before 1987);
- adolescents aged 15 to 18 years (born in 1987 or later);
- adults aged over 19 years (born before 1987, beginning from 1968);
- adults aged over 19 years (born in 1987 or later);
- *in utero* cohort (born from April 27 to December 31, 1986).

Population in the Registry (i.e., population size for which cases are registered) is growing from year to year: while for the first year of study (1986) it was slightly less than 14 million people, in 2010 it includes over 26 million subjects.

The demographic base of the Registry by attained age and ratio between exposed and unexposed subjects varies from year to year. The proportion of children and adolescents is monotonically decreasing (only that of born after Chernobyl remain practically unchanged), while the proportion of adults in the Registry is increasing (including those born both before and after Chernobyl).

The demographic base of the Registry allows assessing:

- annual dynamics of the age group from 0 to 14 years (born before and after Chernobyl separately);
- annual dynamics of the age group from 15 to 18 years (born before and after Chernobyl separately);
- annual dynamics of the age group over 19 years who were aged from 0 to 18 years at the time of the accident or born after Chernobyl;
- annual dynamics in the *in utero* cohort.

Since the size of groups in each region and in the whole of Ukraine is known, it is possible to make arbitrary combinations of regions and to compare the indices for the regions with the Ukraine as a whole.

To calculate the incidence, the most adequate determination of the number of person-years in a given cohort for a particular year is to use the average population size. It should be taken into account that we are not dealing with an exact number but with estimates characterized by some uncertainty. Some worsening of the quality of demographic estimates by the end of the study period (1986-2010) could be expected due to postponement of the next census of Ukraine to 2014 or later. A very informative overview of the problems of quality of demographic data, uncertainties in population parameters, and possible problems of their interpretation was recently published [35].

Methodology of descriptive analysis of the incidence

Current analysis of thyroid cancer incidence in younger age groups of the population of Ukraine represents methodologically, in general, a continuation of a series of previous publications [36-41]. By age at the time of accident and conditions of exposure, the study subjects are divided into the following three groups:

- exposed as children, aged 0-14 years at the time of accident (0-14 AE);
- exposed as adolescents, aged 15-18 years at the time of accident (15-18 AE);
- exposed *in utero*, born in May-December 1986 (EIU).

The first group (0-14 AE) is the largest and includes about 11.2 million subjects; the second group (15-18 AE) about 2.2 million persons, and the size of the cohort exposed prenatally is estimated to be about 0.52 million. It should be noted that, according to the conditions of CMR establishment, registered population includes those born beginning from December 1, 1968, i.e. actually the second group includes the subjects aged from 15 to <18.32 years at exposure (the population aged 18.32-18.99 belongs to the age cohort born in 1967).

Subdivision of population into these three groups, besides general medical considerations, has dosimetric rationale as well. Radiation doses to the thyroid have strong age dependency [42-46]. Under similar radioecological conditions, the maximal radiation dose is received by the population of younger age. For regional estimates of mean radiation dose [43], the relative exposure dose (normalized to the dose in adults) for children aged one year is 4.5-6; for children aged 5 years – 3-4; for children aged 10 years – 1.7-2; and for children aged 14 – about 1.5.

Similar age relationships are also inherent to risk coefficients (ERRD, EARD) of developing radiogenic cancer per exposure unit [4,47]. These patterns, taken together, indicate the highest expected radiogenic effects in the younger age groups of 0-4 years old and 5-9 years old compared with those aged 10 years and older.

Effects of thyroid exposure *in utero* are markedly different from those of postnatal irradiation, and information on risks is very limited [4,48,49].

The group born from 1987 was also used in later analyses as a control because it was not exposed to Chernobyl fallout and therefore does not include Chernobyl radiogenic cancers.

For the analysis by age at diagnosis, cases are subdivided into those diagnosed in children (aged 0-14 years at diagnosis), adolescents (aged 15-18 years at diagnosis), and adults (aged ≥19 years at diagnosis). Such subdivision is traditional for oncoepidemiology that recons childhood cancers a particular category [50,51]. A relatively new trend is to consider the group of adolescents and young adults (AYA) as patients requiring specific, tailored diagnostic procedures and streamlined procedures for cancer treatment [51-53].

Current analysis used a partition of 28 regions of Ukraine into two groups according to the level of thyroid exposure in children and adolescents [44,45, Chapter 2]:

- 6 most contaminated regions (Zhytomyr, Kyiv, Rivne, Chernihiv, Cherkasy regions and Kyiv City; average thyroid exposure dose in the group 0-18 years old at the time of the accident >35 mGy);
- 21 regions with relatively lower levels of contamination (average thyroid exposure dose in the group 0-18 years old <35 mGy).

Childhood and adolescent population of the 6 most contaminated regions accounted for 19-20% of the total population of corresponding age of Ukraine (Tables 3.1 and 3.2). It should be stressed that Zhytomyr, Kyiv, and Chernihiv regions are located at a distance up to 200 km from Chernobyl Nuclear Power Plant; the residents of northern areas of these regions received the highest thyroid doses [3,54].

Table 3.1
Thyroid cancer cases in selected groups of Ukrainian population in 1986-2010 (by periods of diagnosis)

	Population [a] million	1986-1989	1990-1994	1995-1999	2000-2004	2005-2010	1986-2010
0-14 years at exposure	11.1	59	383	827	1221	2554	**5044**
6 regions	2.2	15	191	384	574	1019	**2183**
21 regions	8.9	44	192	443	647	1535	**2861**
15-18 years at exposure	2.2	45	162	291	411	733	**1642**
6 regions	0.4	9	41	89	156	269	**564**
21 regions	1.8	36	121	202	255	464	**1078**
Subjects exposed *in utero*	0.5	0	3	5	26	78	**112**
6 regions	0.1	0	1	2	9	32	**44**
21 regions	0.4	0	2	3	17	46	**68**
Subjects born in 1987 and later	12.0 [b]	0	0	11	103	392	**506**
6 regions	2.5 [b]	0	0	3	32	123	**158**
21 regions	9.5 [b]	0	0	8	71	269	**348**
Total cases		**104**	**548**	**1134**	**1761**	**3757**	**7304**

[a] - exposed population on 1 January 1987; [b] - estimation of unexposed population on 1 January 2011

Table 3.2
Thyroid cancer cases in selected groups of Ukrainian population in 1986-2010 (by age at surgery)

	Population [a] million	Aged 0-14 years at surgery	Aged 15-18 years at surgery	Aged 19+ years at surgery	Total
0-14 years at exposure	11.1	453	496	4095	**5044**
6 regions	2.2	275	244	1664	**2183**
21 regions	8.9	178	252	2431	**2861**
15-18 years at exposure	2.2	-	31	1611	**1642**
6 regions	0.4	-	5	559	**564**
21 regions	1.8	-	26	1052	**1078**
Subjects exposed *in utero*	0.5	13	27	72	**112**
6 regions	0.1	5	11	28	**44**
21 regions	0.4	8	16	44	**68**
Subjects born in 1987 and later	12.0 [b]	177	183	146	**506**
6 regions	2.5 [b]	57	58	43	**158**
21 regions	9.5 [b]	120	125	103	**348**
Total cases		**643**	**737**	**5924**	**7304**

[a] - exposed population on 1 January 1987; [b] - estimation of unexposed population on 1 January 2011

In this work, the period of study is 1986-2010. The annual dynamics of cases and disease incidence are shown in Tables 3.3 and 3.4, respectively. In the analysis of time trends, the two-year intervals are used. Also, periods under comparison are subdivided into five intervals: 1986-1989 – the period shorter than the minimal duration of latency of radiation-induced thyroid cancer [4,47] when only sporadic thyroid cancers were diagnosed, and four subsequent intervals (1990-1994, 1995-1999, 2000-2004, 2005-2010) when both sporadic and radiogenic thyroid cancers are diagnosed in the population.

Analysis of thyroid cancer incidence in 1986-2010 according to the data of Clinico-morphological Registry

Tables 3.1 and 3.2 summarize data on thyroid cancer in the defined groups, and show population size in the 6 and 21 regions. Table 3.1 shows the number of cases for the five study periods. Table 3.2 shows the distribution of cases by age at diagnosis, depicting cancers diagnosed in childhood, adolescence, and adulthood.

Thus, the current analysis involves 6,798 cases in the exposed population, including

5,044 exposed as children, 1,642 cases in irradiated adolescents, and 112 cases of prenatal exposure. In subjects born after Chernobyl, i.e. in the unexposed population, 506 cases were diagnosed.

Detailed data on the number of cases, overall incidence for the whole of Ukraine, and for the 6 contaminated regions and 21 control regions are presented in Tables 3.3-3.7.

Data analysis for the groups by age at exposure takes into account that in every calendar year we are dealing with a virtual cohort with fixed characteristics of exposure that lived certain period after exposure. Thus, for a selected population group, over time, from year to year, the parameters of attained age change (increase) (minimum, maximum, median), as well as does the time after exposure. Attained age significantly affects the sporadic and radiogenic risks; time elapsed after exposure can also be an important parameter for calculating radiogenic risk. The attained age and time after exposure uniquely determine the age at exposure.

Detailed data on thyroid cancer cases and trends in the incidence among those exposed as children are shown in Table 3.3 and Figure 3.1. During the study period (1986-2010) in this group, the age varied from 0 to 14 years old in 1986 and from 24 to 38 years old in 2010. Table 3.3 shows the cases for the whole of Ukraine, as well as for 6 and 21 regions. Population size of this group was estimated to be 11.1 million persons at the beginning of 1987. This group showed the maximal number of cases (5,044) with about 43% of these (2,183 cases) registered in the 6 most contaminated regions. A significant increase in the incidence was observed in the first postlatent period (1990-1994) and continued until the end of the study (2010). Ascending trends are common to all four groups represented in Fig. 3.1 (males in 6 regions, females in 6 regions, males in 21 regions, females in 21 regions), although the growth rate and incidences are different for each group. For the low-dose regions, the increasing incidence is largely determined by the attained age; in the high-dose regions, there is an additional influence of radiogenic component and of the factor of intensive screening. The maximal incidence is expectedly observed in females from 6 contaminated areas (about 15 cases per 100,000 person-years). Incidence rates in the most contaminated areas are significantly higher than those in low-contaminated regions, although their ratio tends to be lower.

Rates in individuals exposed in adolescence are presented in Table 3.4 and Figure 3.2. The population size of this group was 2.2 million. In 2010, the age of this group reached 39-42 years old. The cumulative number of cases is 1,642, of which 564 (34%) were diagnosed in 6 regions with maximal thyroid doses. The main trends of incidence for males and females, in general, are similar to those in exposed during childhood. Due to differences in median attained age (about 9 years), the absolute values of the incidence for all four categories (males in 6 regions, females in 6 regions, males in 21 regions, and females in 21 regions) are somewhat higher than the corresponding values in exposed at childhood age. The incidence in females from 6 most contaminated regions reached 16-18 cases per 100,000 person-years at the end of the study period.

Figure 3.3 demonstrates incidence trends for those exposed at the age from 0 to 18 years old for different periods of study. Areas with the highest incidence (Kyiv, Chernihiv, Zhytomyr, and Kyiv-City) are located around the Chernobyl Nuclear Power Plant. Of note,

there was a pronounced increase in the incidence in this group in low-contaminated regions during the last time period (2005-2010). Figure 3.4 presents ranked values of cumulative incidence [55] for those exposed at the age 0-18 years in postlatent period (1990-2010). High-dose regions, except for Rivne, demonstrate a value above the average for Ukraine. Also, an increased incidence was noted in Kherson and Vinnitsa regions. It should be mentioned that a number of studies [56-59] found Polisya (Rivne, Volyn, Zhytomyr regions) to be an area with low incidence of sporadic thyroid cancer. The contribution of radiogenic cancers led to the increase in incidence in this region, but the values were found to be slightly below the average for Ukraine (Rivne region) or below the extreme values for Kyiv and Chernihiv regions (Zhytomyr region). The increased incidence in the industrial regions of Ukraine (Kherson, Dnipropetrovsk, and Zaporizhya regions) was mostly observed in 2000-2010, and does not seem to be associated with radiation factor.

An estimate of the proportion of cases due to radiation among the total number of cases in the study population is of particular interest. Since thyroid cancers induced by radiation do not have definite markers and cannot be isolated from the whole group, the estimate can only be obtained within a particular statistical model describing the dynamics of the disease depending on exposure doses (risk assessment model). So, the proportion of radiogenic cancers for the Ukrainian population aged from 0 to 18 years at the time of accident and for the study period 1990-1997 was estimated to be 30% [56]. A comparative analysis at the level of regions of the country for the period of 1990-2001 [59] resulted in an estimate of the proportion of thyroid cancers attributable to Chernobyl radiation of 30% for the whole population of Ukraine and about 50% for the residents of the 3 most contaminated areas (Kyiv, Zhytomyr, and Chernihiv regions). Similar analysis performed in the individual cohort study for the residents (at the time of accident) of the 8 radiation-contaminated areas adjacent to the Chernobyl Nuclear Power Plant, yielded an estimate of excess cancer cases diagnosed during 1998-2000 of about 80% [60], and for the cases operated in 2001-2007 from 50 to 60% [61].

For individuals exposed *in utero*, data are presented in Tables 3.1, 3.2, and Figure 3.5. The population size of this group was estimated to be 0.52 million people. The number of diagnosed cases is 112. For the purpose of easier interpretation of data, Figure 3.5 also shows incidence trends for the cohort exposed at the age of 0-4 years old, and for the cohort of unexposed subjects born in 1987-1989 which could be considered a rough estimate of sporadic thyroid cancer incidence. The *in utero* group displayed an extremely low incidence for the period 1990-1999. During 2000-2010, however, the incidence increased sharply and reached values characteristic to the exposed subjects aged 0 to 4 years. If the increase in incidence in the *in utero* group was of radiogenic nature, it should be noted that the period of latency was substantially longer. Excess incidence for the *in utero* group was largely observed in the 6 contaminated regions where 44 cases were diagnosed (39%). Recently started, a study of radiation effects from *in utero* exposure is now actively carried out [48,49] in the framework of prospective observations. Since the number of cases of thyroid cancer registered in these studies so far is very low (<10), it would be promising to analyze the risks for this group in well-designed analytical ecologic or case-control studies using cases from the 6 contaminated areas.

Table 3.3
Thyroid cancer cases and incidence per 100,000 childhood population in 1986 (0-14 years old at exposure)

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cases (F)	4	7	8	22	29	31	64	59	82	92	108	108	143	170	145	214	213	180	228	241	308	331	386	407	446
Cases (M)	4	3	4	7	17	16	27	31	27	33	43	33	41	56	39	55	51	52	44	60	65	69	74	72	95
Cases (Both)	8	10	12	29	46	47	91	90	109	125	151	141	184	226	184	269	264	232	272	301	373	400	460	479	541
Incidence	0.1	0.1	0.1	0.3	0.4	0.4	0.8	0.8	1.0	1.1	1.4	1.3	1.7	2.0	1.7	2.4	2.4	2.1	2.5	2.7	3.4	3.6	4.1	4.3	4.9
6 regions, Cases (Both)	3	0	3	9	18	24	49	45	55	61	72	58	85	108	98	131	129	103	113	121	157	165	173	198	205
21 regions, Cases (Both)	5	10	9	20	28	23	42	45	54	64	79	83	99	118	86	138	135	129	159	180	216	235	287	281	336
6 regions, Incidence	0.1	0.0	0.1	0.4	0.9	1.1	2.3	2.1	2.6	2.9	3.4	2.8	4.0	5.1	4.7	6.2	6.1	4.9	5.4	5.8	7.5	7.9	8.2	9.4	9.8
21 regions, Incidence	0.1	0.1	0.1	0.2	0.3	0.3	0.5	0.5	0.6	0.7	0.9	0.9	1.1	1.3	1.0	1.5	1.5	1.4	1.8	2.0	2.4	2.6	3.2	3.1	3.7

Table 3.4
Thyroid cancer cases and incidence per 100,000 adolescent population in 1986 (15-18 years old at exposure)

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cases (F)	8	9	8	6	18	17	23	37	36	54	37	48	59	54	57	70	64	72	79	95	86	95	114	111	106
Cases (M)	3	5	2	4	2	5	8	7	9	6	8	8	9	8	11	14	24	13	7	13	18	21	19	23	32
Cases (Both)	11	14	10	10	20	22	31	44	45	60	45	56	68	62	68	84	88	85	86	108	104	116	133	134	138
Incidence	0.5	0.6	0.4	0.4	0.9	1.0	1.4	2.0	2.0	2.7	2.0	2.5	3.0	2.8	3.0	3.8	3.9	3.8	3.9	4.8	4.7	5.2	6.0	6.0	6.2
6 regions. Cases (Both)	1	5	0	3	5	8	7	10	11	20	13	15	26	15	26	31	34	35	30	40	45	46	50	44	44
21 regions, Cases (Both)	10	9	10	7	15	14	24	34	34	40	32	41	42	47	42	53	54	50	56	68	59	70	83	90	94
6 regions, Incidence	0.2	1.1	0.0	0.7	1.1	1.8	1.6	2.3	2.5	4.5	2.9	3.4	5.9	3.4	5.9	7.0	7.7	7.9	6.8	9.1	10.2	10.4	11.3	10.0	10.0
21 regions, Incidence	0.6	0.5	0.6	0.4	0.8	0.8	1.3	1.9	1.9	2.2	1.8	2.3	2.3	2.6	2.3	3.0	3.0	2.8	3.1	3.8	3.3	3.9	4.6	5.0	5.2

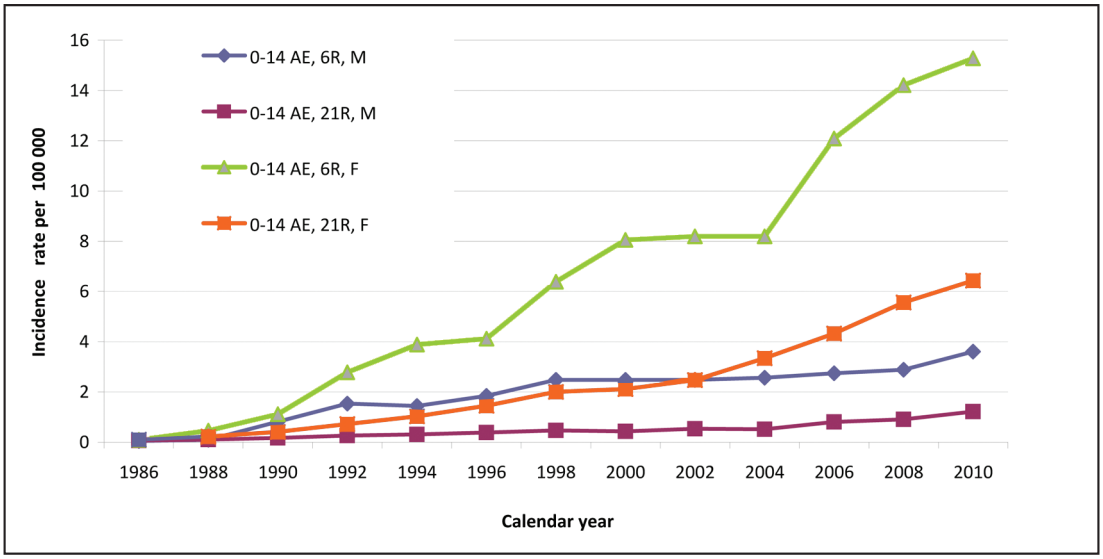

Figure 3.1. Time trends of thyroid cancer incidence in 6 and 21 regions of Ukraine for cohort aged 0-14 years at exposure. AE – age at exposure; 6R – 6 northern regions; 21R – other 21 regions; F – female; M – male.

Importantly, the interpretation of the number of cases and incidence in the groups defined by age at diagnosis (at surgery) should take into account that the composition of such groups changes from year to year as the cohorts become older. Both the size of the population and the proportion of exposed and unexposed subjects in the group are changing.

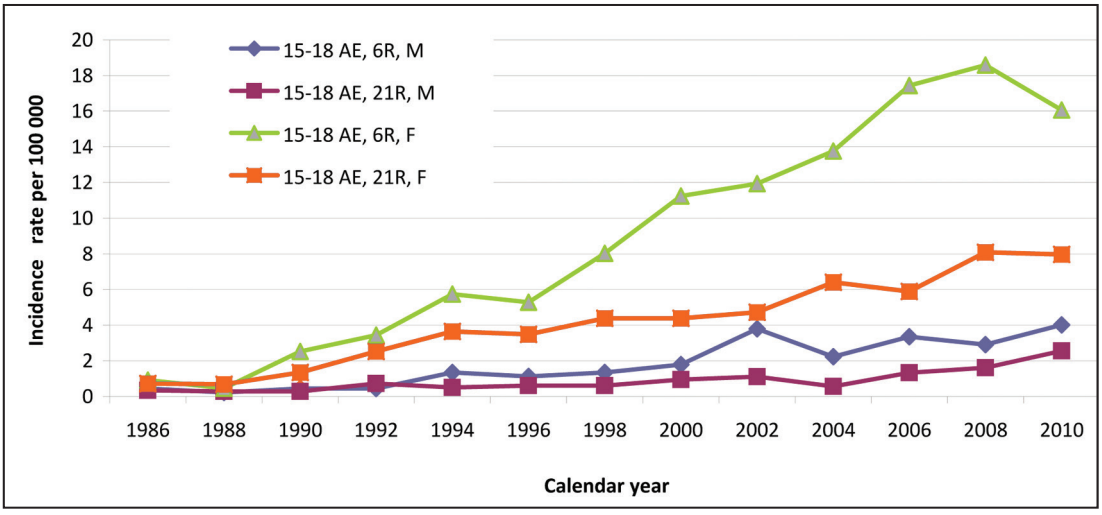

Figure 3.2. Time trends of thyroid cancer incidence in 6 and 21 regions of Ukraine for cohort aged 15-18 years at exposure. AE – age at exposure; 6R – 6 northern regions; 21R – other 21 regions; F – female; M – male.

Table 3.5
Thyroid cancer cases and incidence per 100,000 childhood population (0-14 years old at surgery: born before 1987, and in 1987 and later)

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Born before 1987																									
Cases (F)	4	7	7	7	17	15	35	28	30	35	35	29	31	15	10	0	-	-	-	-	-	-	-	-	-
Cases (M)	4	2	3	4	13	8	19	20	20	14	20	7	11	8	6	2	-	-	-	-	-	-	-	-	-
Cases (Both)	8	9	10	11	30	23	54	48	50	49	55	36	42	23	16	2	-	-	-	-	-	-	-	-	-
Incidence	0.1	0.1	0.1	0.1	0.4	0.3	0.8	0.8	1.0	1.1	1.4	1.2	1.8	1.5	2.1	0.7	-	-	-	-	-	-	-	-	-
6 regions, cases (Both)	3	0	3	4	12	15	37	31	31	34	37	25	26	14	7	1									
21 regions, cases (Both)	5	9	7	7	18	8	17	17	19	15	18	11	16	9	9	1									
6 regions, incidence	0.1	0.0	0.2	0.2	0.7	1.0	2.8	2.6	3.0	3.8	4.9	4.1	5.7	4.6	4.6	2.0	-	-	-	-	-	-	-	-	-
21 regions, Incidence	0.1	0.1	0.1	0.1	0.3	0.1	0.3	0.3	0.4	0.4	0.6	0.4	0.9	0.7	1.5	0.5	-	-	-	-	-	-	-	-	-
Born in 1987 and in subsequent years																									
Cases (F)	-	-	-	-	-	-	-	-	-	1	0	1	1	2	10	8	19	7	6	18	7	14	8	11	18
Cases (M)	-	-	-	-	-	-	-	-	-	0	2	0	1	3	3	7	6	2	3	3	1	3	2	5	5
Cases (Both)	-	-	-	-	-	-	-	-	-	1	2	1	2	5	13	15	25	9	9	21	8	17	10	16	23
Incidence	-	-	-	-	-	-	-	-	-	0.02	0.03	0.02	0.03	0.1	0.2	0.2	0.3	0.1	0.1	0.3	0.1	0.3	0.2	0.2	0.4
6 regions, cases (Both)	-	-	-	-	-	-	-	-	-	-	-	1	-	2	2	6	11	3	2	4	3	5	2	7	9
21 regions, cases (Both)	-	-	-	-	-	-	-	-	-	1	2	-	2	3	11	9	14	6	7	17	5	12	8	9	14
6 regions, Incidence	-	-	-	-	-	-	-	-	-	-	-	0.08	-	0.1	0.1	0.4	0.7	0.2	0.1	0.3	0.2	0.4	0.2	0.5	0.7
21 regions, Incidence	-	-	-	-	-	-	-	-	-	0.02	0.04	-	0.04	0.1	0.2	0.1	0.2	0.1	0.1	0.3	0.1	0.2	0.2	0.2	0.3

Table 3.6
Thyroid cancer cases and incidence per 100,000 adolescent population (15-18 years old at surgery: born before 1987, and in 1987 and later)

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Born before 1987																									
Cases (F)	1	4	0	5	6	7	7	9	19	8	15	12	18	29	30	32	29	20	5	4	-	-	-	-	-
Cases (M)	10	9	8	15	9	13	17	13	11	20	23	11	24	31	10	21	19	14	13	3	-	-	-	-	-
Cases (Both)	11	13	8	20	15	20	24	22	30	28	38	23	42	60	40	53	48	34	18	7	-	-	-	-	-
Incidence	0.4	0.5	0.3	0.7	0.5	0.7	0.8	0.8	1.0	1.0	1.3	0.8	1.4	2.0	1.3	1.7	2.1	2.2	2.3	2.8	-	-	-	-	-
6 regions, cases (Both)	1	4	0	5	6	7	7	9	19	8	15	12	18	29	30	32	29	20	5	4	-	-	-	-	-
21 regions, cases (Both)	10	9	8	15	9	13	17	13	11	20	23	11	24	31	10	21	19	14	13	3	-	-	-	-	-
6 regions, incidence	0.2	0.7	0.0	0.9	1.0	1.2	1.2	1.6	3.3	1.4	2.6	2.1	3.1	4.8	4.9	5.2	6.29	6.5	3.3	8.2	-	-	-	-	-
21 regions, incidence	0.5	0.4	0.3	0.6	0.4	0.6	0.7	0.6	0.5	0.9	1.0	0.5	1.0	1.3	0.4	0.9	0.8	1.1	2.1	1.4	-	-	-	-	-
Born in 1987 and in subsequent years																									
Cases (F)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	3	9	9	7	5	13	7
Cases (M)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	7	14	22	19	14	16	15	15
Cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	11	17	31	28	21	21	28	22
Incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.7	1.0	1.4	1.1	0.8	0.9	1.3	1.1
6 regions, cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	4	3	9	9	7	5	13	7
21 regions. cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	7	14	22	19	14	16	15	15
6 regions, incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	1.3	0.7	1.7	1.7	1.4	1.0	2.9	1.6
21 regions, incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.6	0.8	1.1	0.9	0.7	0.8	0.8	0.9

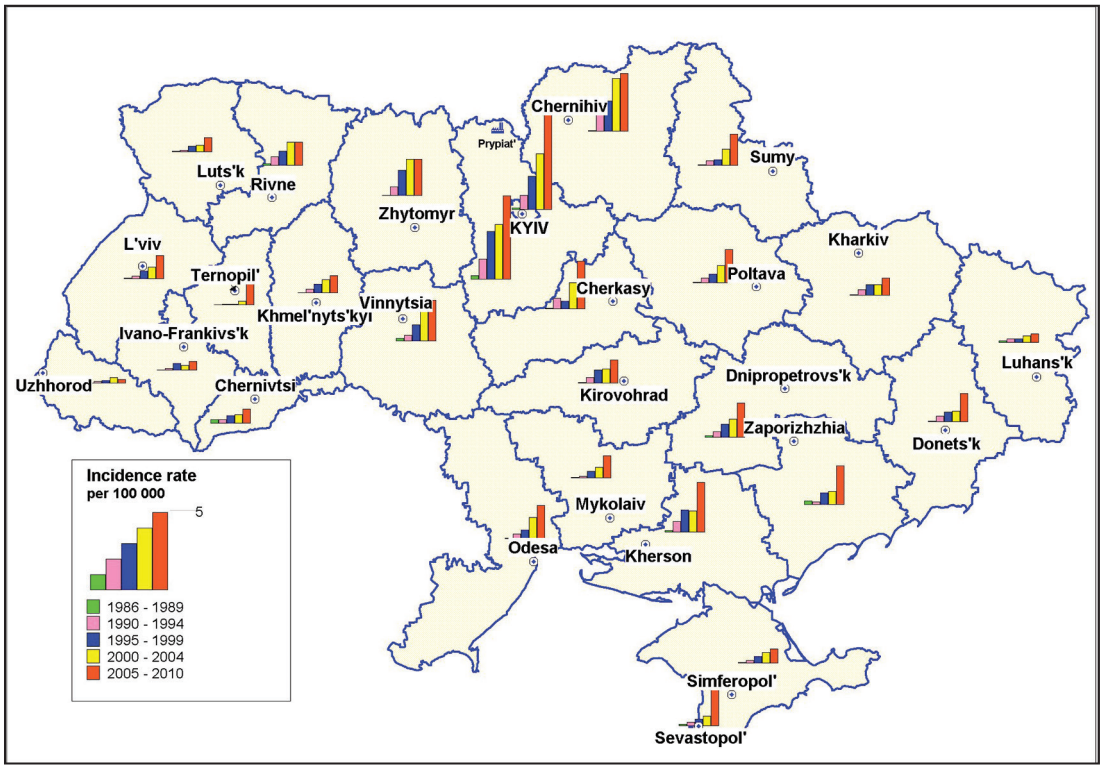

Figure 3.3. Region (oblast) specific time trends of thyroid cancer incidence in the cohort aged 0-18 years at exposure, both sexes.

Table 3.5 and Figure 3.6 demonstrate the statistics of cases and incidence trends for the age group 0-14 years at diagnosis. The incidence in this group was approximately 0.1 case per 100,000 person-years of childhood population during the period of latency (1986-1989), and then displayed a sharp increase already noticeable in the early postlatent period (1990-1992). Incidence in females from the 6 most contaminated regions increased in 1992-1998 more than 20-fold as compared to the period of latency. The increased incidence in females and males from the 6 regions started to decline synchronously after 1998, with transition of the most exposed group (aged 0-4 years at the time of the accident) to older age categories.

Since 2001, this group does not include exposed subjects (even those potentially irradiated *in utero*) any more. Incidences in males from the 6 and 21 regions are not significantly different, while that in females from the 6 most contaminated regions is approximately 2-fold higher than in less contaminated regions. Incidence rate for the group aged 0-14 years is widely used in cancer statistics, and the observed values (0.2-0.4 cases per 100,000 person-years) for unexposed Ukrainian population can be compared with data from other countries and other registries. Such a comparison with the ranked data from 28 European countries [11] suggests that the incidence in Ukraine for the group 0-14 years in the period 2003-2010 was about the European average.

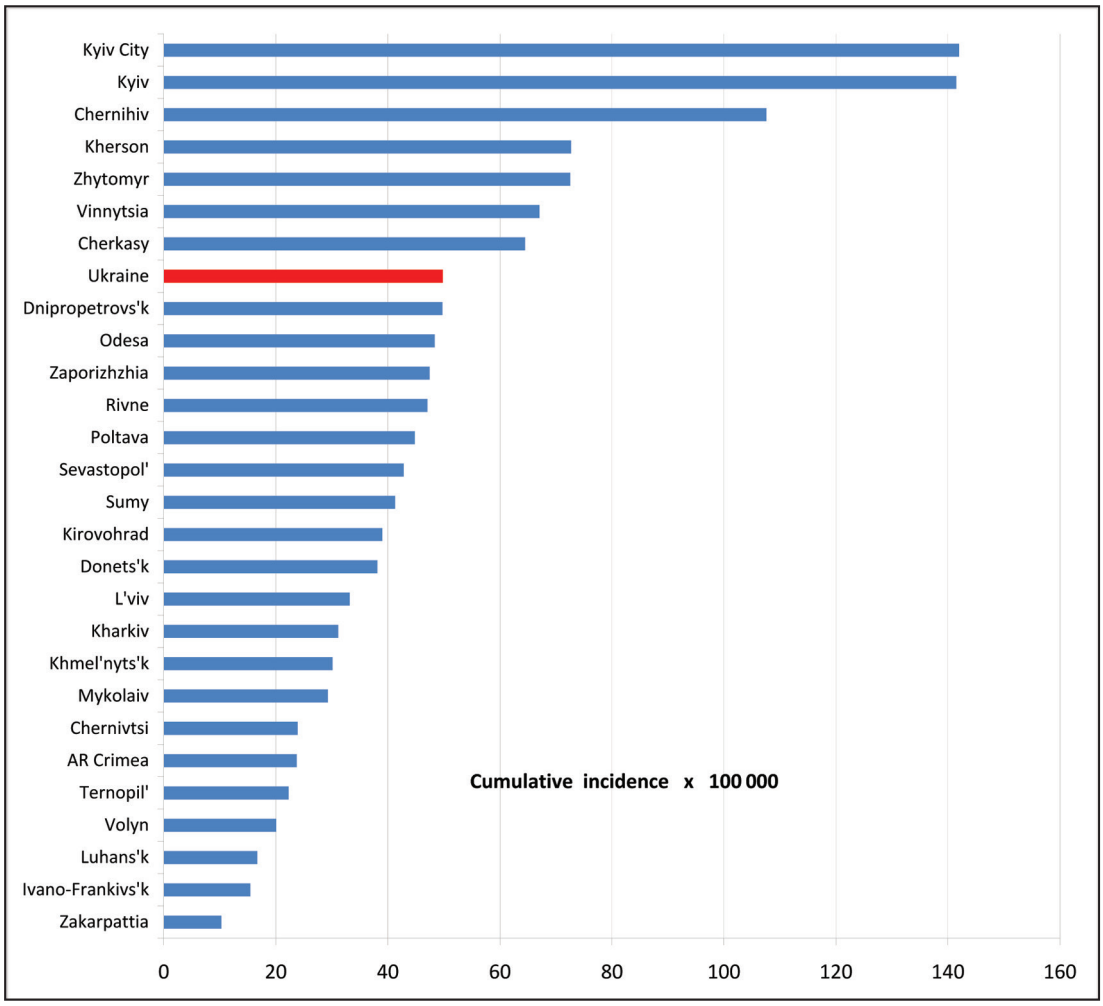

Figure 3.4. Ranking plot of region (oblast) specific cumulative incidence x 100 000 in 1990-2010 for the cohort aged 0-18 years at exposure, both sexes.

In the age group of 15-18 years old at diagnosis, the increasing incidence in the 6 high-dose regions was more gradual compared to the childhood group and reached maximal values both in females and males in 1998-2002 (Table 3.6 and Figure 3.7). Exposed population was not in the group since 2006, after which the incidence in males and females in the 6 and 21 regions became closer.

In the age group of 19+ years old at diagnosis, incidence trends were markedly different from the younger groups (Figure 3.8). This group has no upper age limit, and its size, median age, and age structure change every year. Incidence rates in the four categories (males in 6 regions, females in 6 regions, males in 21 regions, females in 21 regions) show increasing trends. The maximal rates were observed in females from the 6 most contaminated regions, followed by that in females from 21 regions, then in males from the 6 regions and the minimal was in males from 21 regions. It should be noted that in the "adult cohort", the exposed and unexposed populations are "mixed" beginning from 2006 (Table 3.7).

Table 3.7
Thyroid cancer cases and incidence per 100,000 in subjects aged 19+ years at surgery (born before and after Chernobyl)

	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Born before 1987																									
Cases (F)	0	2	3	4	19	19	33	54	65	91	83	113	148	166	165	253	246	233	300	338	400	431	511	531	570
Cases (M)	0	0	1	4	2	7	12	12	9	17	21	26	22	40	34	53	59	54	50	73	85	92	96	100	131
Cases (Both)	0	2	4	8	21	26	45	66	74	108	104	139	170	206	199	306	305	287	350	411	485	523	607	631	701
Incidence	-	0.6	0.4	0.5	0.9	0.8	1.2	1.4	1.5	1.8	1.5	1.9	2.1	2.3	2.1	2.9	2.7	2.4	2.8	3.0	3.7	3.9	4.5	4.7	5.2
6 regions, cases (Both)	-	1	0	3	5	10	13	15	16	39	33	37	68	80	88	133	134	119	141	161	208	213	229	249	256
21 regions, cases (Both)	-	1	4	5	16	16	32	51	58	69	71	102	102	126	111	173	171	168	209	250	277	310	378	382	445
6 regions, incidence	-	1.5	-	0.9	1.1	1.6	1.7	1.6	1.5	3.2	2.4	2.5	4.1	4.5	4.5	6.4	6.0	5.0	5.5	6.0	7.8	8.0	8.5	9.2	9.5
21 regions, incidence	-	0.4	0.5	0.4	0.9	0.7	1.0	1.4	1.4	1.4	1.3	1.7	1.6	1.8	1.4	2.1	1.9	1.8	2.1	2.4	2.6	2.9	3.5	3.6	4.1
Born in 1987 and in subsequent years																									
Cases (F)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	7	20	38	49
Cases (M)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	5	8	10
Cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	9	25	46	59
Incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	0.5	1.1	1.6	1.7
6 regions, cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	2	9	14	15
21 regions, cases (Both)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	7	16	32	44
6 regions, incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5	0.6	1.9	2.4	2.1
21 regions, incidence	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	0.5	0.9	1.4	1.6

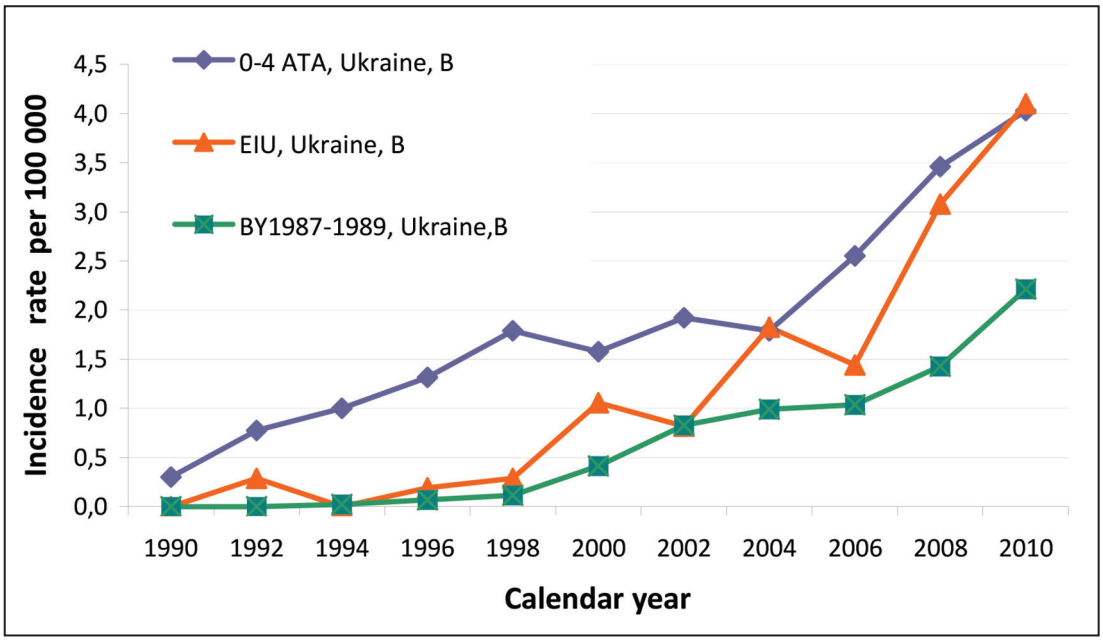

Figure 3.5. Thyroid cancer incidence in 3 birth cohorts, both sexes: aged 0-4 years at exposure (ATA – age at exposure; B – both sexes); exposed *in utero* (EIU – exposed *in utero*, Ukraine – whole Ukraine, B – both sexes); born in 1987-1989 (BY – year of birthday; Ukraine – whole Ukraine, B – both sexes).

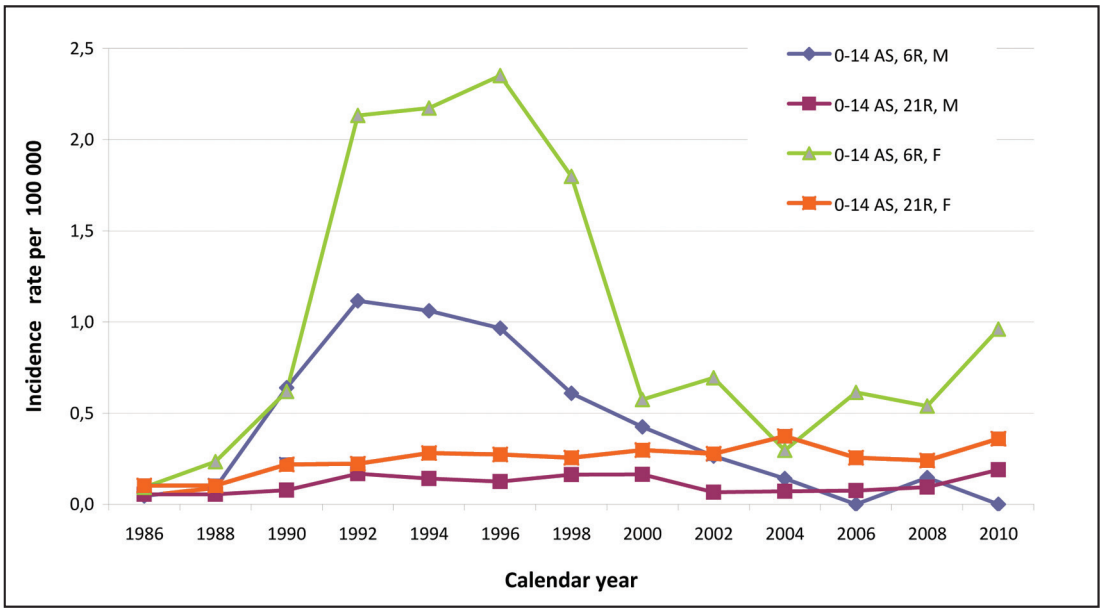

Figure 3.6. Time trends of thyroid cancer incidence among children aged 0-14 years at surgery. AS – age at surgery; 6R – 6 northern regions of Ukraine; 21R – other 21 regions of Ukraine; F – female; M – male.

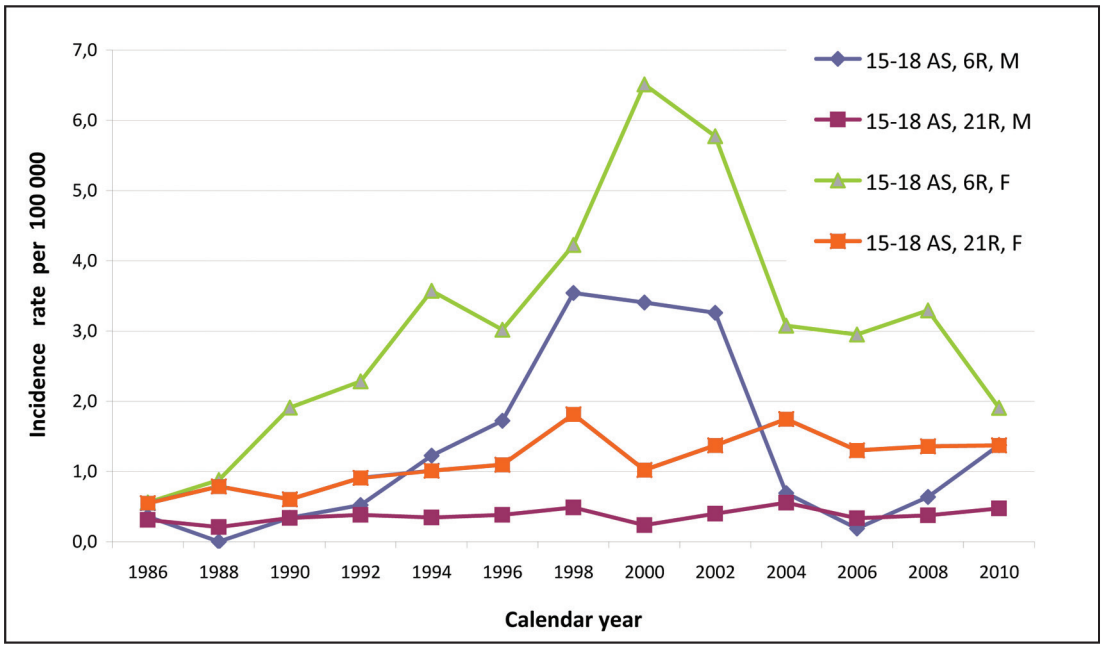

Figure 3.7. Time trends of thyroid cancer incidence among adolescents aged 15-18 years at surgery. AS – age at surgery; 6R – 6 northern regions of Ukraine; 21R – other 21 regions of Ukraine; F – female; M – male.

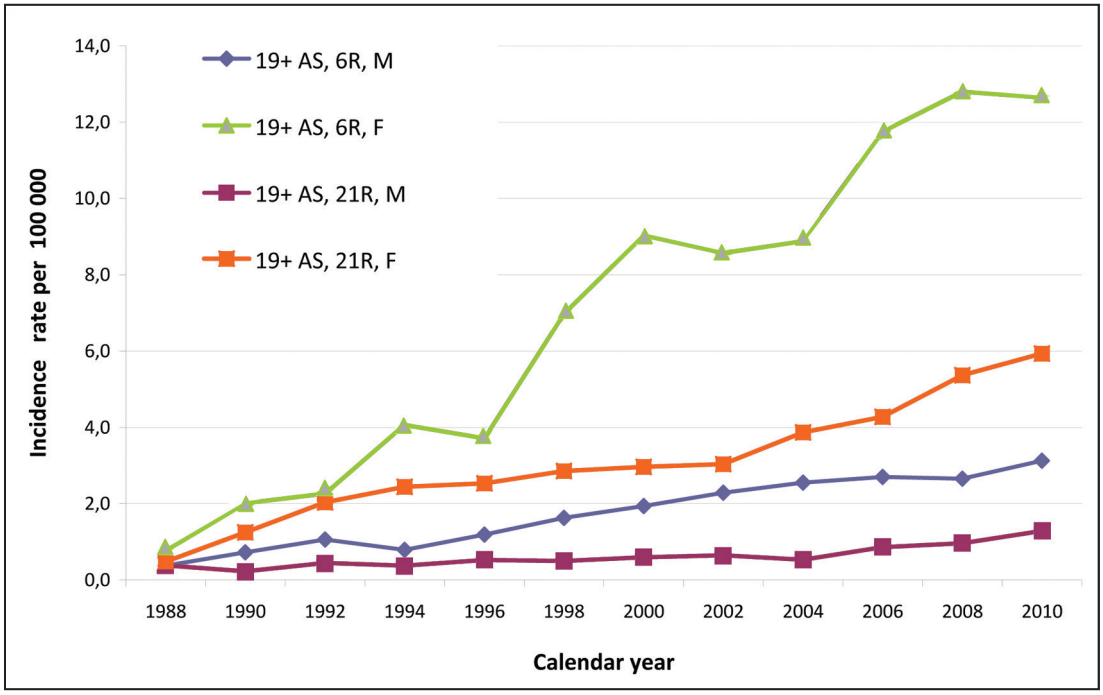

Figure 3.8. Time trends of thyroid cancer incidence among adults aged 19+ years at surgery. AS – age at surgery; 6R – 6 northern regions of Ukraine; 21R – other 21 regions of Ukraine; F – female; M – male.

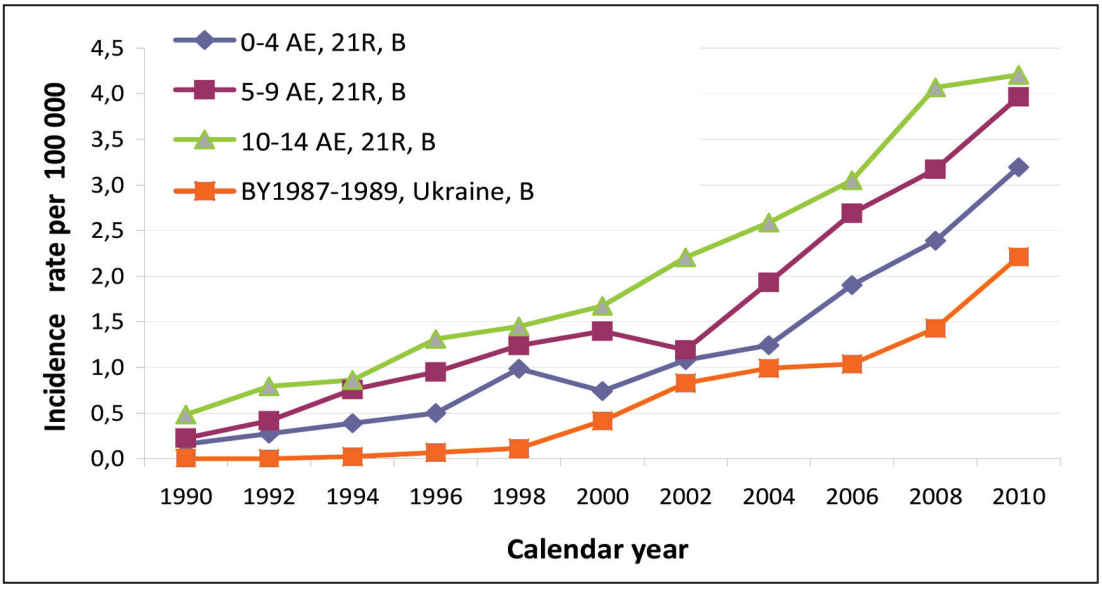

Figure 3.9. Time trends of thyroid cancer incidence in 21 less contaminated regions of Ukraine among 3 exposed and 1 unexposed birth cohorts. AE – age at exposure; 6R - 6 northern regions of Ukraine; B – both sexes; BY – year of birthday; Ukraine – whole Ukraine.

As the next step, we consider in a descriptive analysis the birth cohort effect for exposed and unexposed populations by comparing the incidence trends in the three exposed cohorts (0-4, 5-9, and 10-14 years old at the time of exposure), and in the unexposed cohort (nearest by age to the exposed cohorts) born in 1987-1989. Time trends are considered separately for the 6 regions and 21 regions without subdivision by sex. The size of cohorts (0-4, 5-9, and 10-14 years old at exposure) were 0.75, 0.71 and 0.73 million for high-dose regions, respectively, and about 3 million in each age group for the 21 low-dose regions. The size of the unexposed cohort born in 1987-1989 for the whole of Ukraine was about 2.2 million.

Figure 3.9 presents incidence trends in the postlatent period for these four cohorts in the 21 low-dose regions. At the beginning of the study period (1990-1992), the incidence in each cohort did not exceed 0.5 case per 100,000 person-years. In subsequent years, the cohorts showed a gradual growth of incidence, and at the end of the study incidence rates ranged from 2.2 to 4.1 cases per 100,000 person-years. It is noteworthy that during the analyzed period of time the incidences displayed a strictly ordered character according to attained age. For each fixed period, the cohort born in 1987-1989 showed the lowest incidence; the next higher value of incidence was observed in the cohort aged 0-4 years at exposure (the difference in the attained age with the previous cohort was about 4 years); then in the cohort aged 5-9 years at exposure, and the maximal value was in the cohort of 10-14 years at exposure. The shapes of trends for all cohorts are similar and close to the exponential curves.

Figure 3.10 presents incidence trends in postlatent period for similar four cohorts in the 6 high-dose regions. The trend in the cohort of subjects born in 1987-1989 is the same as in Fig. 3.9, and exponentially increases from 0 to 2.2 cases per 100,000 person-years.

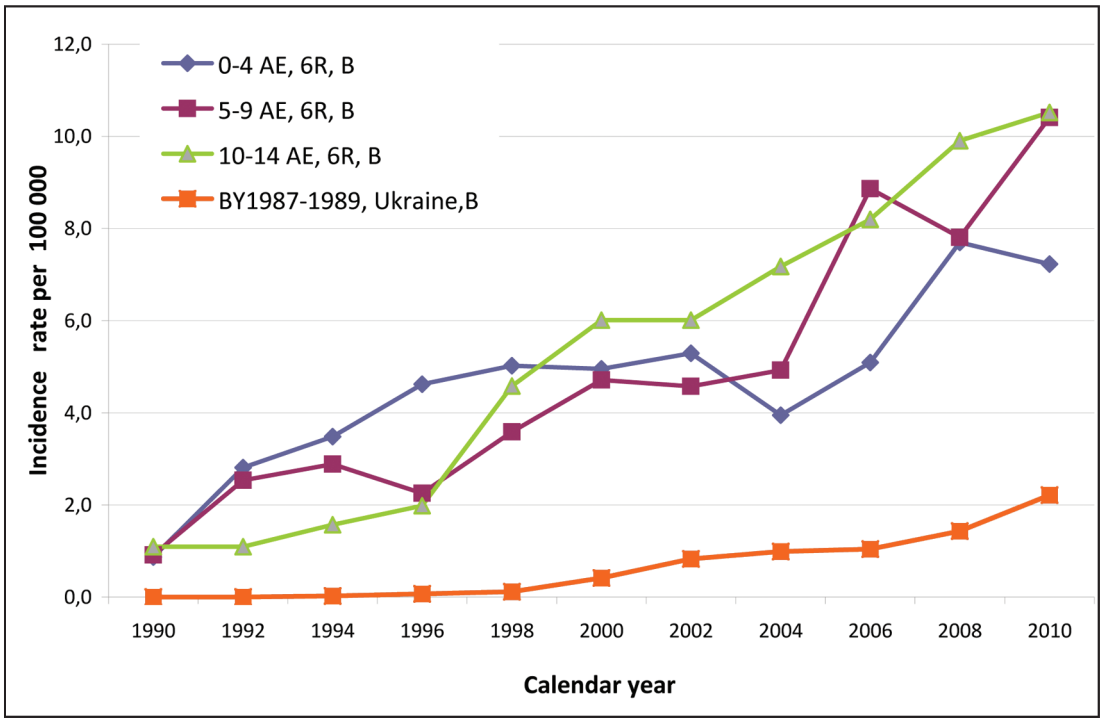

Figure 3.10. Time trends of thyroid cancer incidence in 6 northern regions of Ukraine among 3 exposed, and 1 unexposed birth cohorts. AE – age at exposure; 6R - 6 northern regions of Ukraine; B – both sexes; BY – birtnday year; Ukraine – whole Ukraine.

In the exposed cohorts, the sporadic incidence is supplemented by the radiogenic component and possibly by the factor of screening [58,62]. The trends in the exposed cohort are less "smooth", the values vary within the wide range (from 1 to 10.5 cases per 100,000 person-years) and do not show the "natural" ordering by attained age. Moreover, in the period of 1990-1998, the highest incidence was observed in the youngest cohort of 0-4 years old at exposure, which had accumulated maximal thyroid doses. From 1998 to 2006, the incidence in this cohort was almost stable: about 5 cases per 100,000 person-years, after which it continued to increase.

In the cohort aged 5-9 years old at exposure the incidence began to grow as rapidly as in the younger cohort in 1990, but until 2004 it was lower. The incidence in the older age group (10-14 years old at exposure) was slowly increasing until 1996, after which the growth accelerated and, beginning from 2000, it occupied a "natural" position with the highest incidence rate.

At the end of the study period (2010), the rate ratios in the groups of 0-4, 5-9, and 10-14 years old at exposure for 21 regions to the cohort born in 1987-1989 were 1.4, 1.7, and 1.9 (Fig. 3.9), respectively. The ratios for the 6 high-dose regions are remarkably higher: 3.3, 4.7, and 4.8, respectively.

Thyroid cancer incidence at the level of the whole population according to the data of National Cancer Registry

Finally, we will briefly discuss the incidence and mortality rates for the whole population of Ukraine in the period 1999-2010 according to the National Cancer Registry data [13]. During this period of time, the number of newly reported cases of thyroid cancer increased steadily from 2,093 (360 males and 1,733 females) cases in 2001 to 2,869 (508 males and 2,361females) in 2010. The annual increase in the incidence was 77.6 cases; it was faster in females (62.8 cases) compared to 14.8 cases in males. The sex ratio (the ratio of the number of cases in females to the number of cases in males) was 4.7 on average for the period 2001-2010 ranging from 4.2 to 5.3.

Figures 3.11 and 3.12 represent the trends of thyroid cancer incidence and mortality, respectively, due to thyroid cancer for the whole population of Ukraine for the period 1999-2010 according to the NCR [13]. During this period, the incidence showed an ascending trend both in females (from 6.5 to about 9 cases per 100,000) and males (from 1.9 to 2.5 cases per 100,000). At the same time, mortality rates vary within the ranges 1-1.2 per 100,000 in females and 0.5-0.6 in 100,000 in males with a tendency to a slow decrease. A similar relationship between the incidence and mortality is typical for the registries of other countries [1,8,9]. Such a ratio (declining trend of mortality and increasing incidence) reflects the fact that the increase in incidence is largely due to the growing number of curable well-differentiated tumors, and also due to improvements in the quality of diagnosis over time.

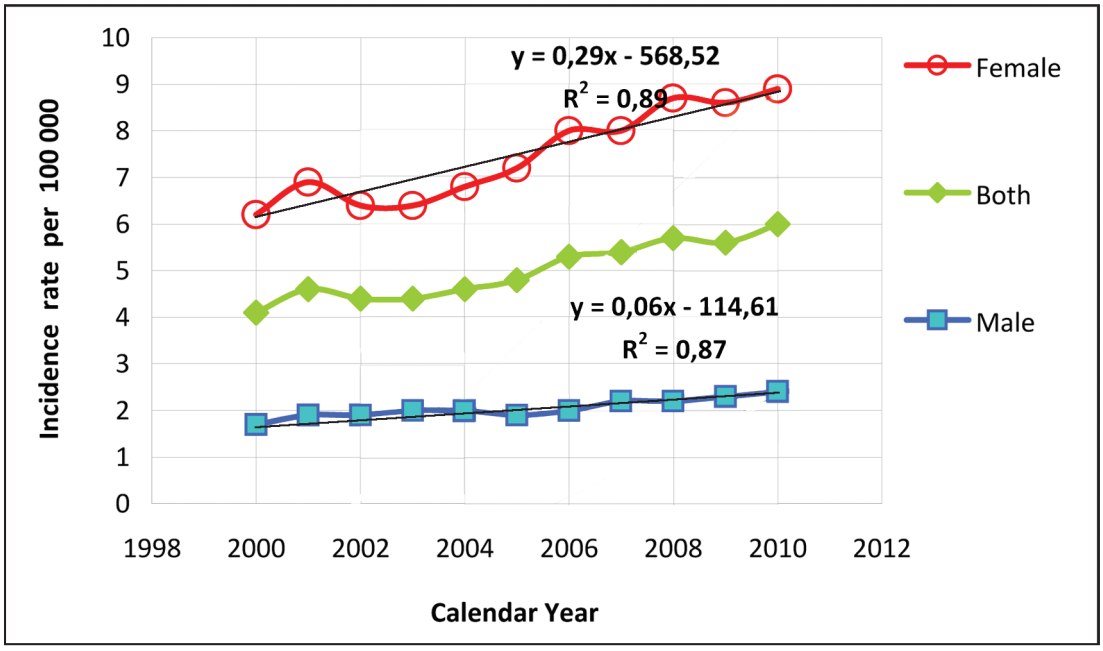

Figure 3.11. Age-adjusted incidence rates of thyroid cancer per 100,000 general population of Ukraine in 2000-2010. Data from ref. [13].

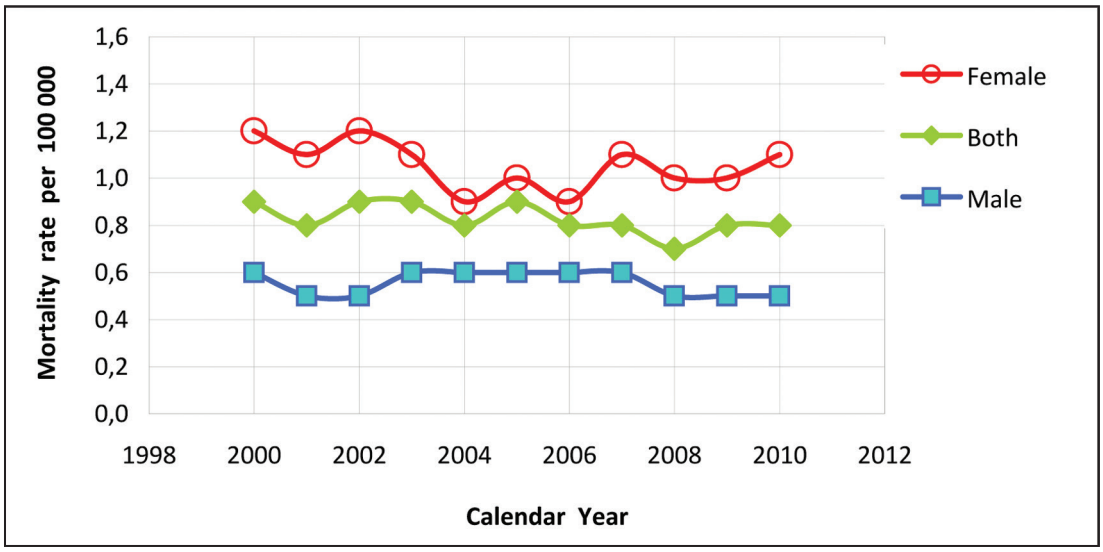

Figure 3.12. Age-adjusted mortality rates of thyroid cancer per 100,000 general population of Ukraine in 2000-2010. Data from ref. [13].

In summary, this chapter demonstrates that among the exposed population of children and adolescents (aged 0-18 years at the time of accident), there has been a significant increase in thyroid cancer incidence after the minimal period of latency; this tendency persists for the period of 20 years (1990-2010). At the same time, incidence rate in the 6 most contaminated regions exceeded that in 21 low-contaminated regions for all postlatent periods of study. However, in both high- and low-contaminated regions, incidence rate displays a descending tendency, perhaps indicating a gradual decrease in the contribution of radiation factor in the increased thyroid cancer incidence in the cohort aged 0-18 years at the time of Chernobyl accident. Among those exposed *in utero*, a significant growth of incidence started after 15 years since accident and continued to grow during the further period.

By age at diagnosis, peak incidences in childhood and adolescent groups were observed in 1995-1997 and 2000-2002, respectively. Since 2002 there are no exposed subjects (including the *in utero* cohort) in the childhood group and since 2006 in the adolescent group. Thus, in these age categories all childhood and adolescent cases of radiogenic thyroid cancer have been realized. In the corresponding groups of unexposed subjects aged 0-14 and 15-18 years at the time of diagnosis, thyroid cancer incidence is comparable to the average rate in European countries.

Incidence of sporadic thyroid cancer markedly increases from the age of 25-30 years old; it may, therefore, be expected that the proportion of radiogenic cancers in Ukraine will decline, even in the regions with relatively high levels of radiation exposure. On the other hand, as shown in a recent study [63], a statistically significant contribution of radioinduced thyroid cancers developing after acute external irradiation can be observed even 50-60 years after exposure. This fact justifies the need for continuous monitoring and analysis of the incidence of thyroid cancer in the population groups exposed as a result of Chernobyl catastrophe.

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Chapter 4

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Thyroid cancer pathology in Ukraine after Chernobyl

Two main types of thyroid carcinomas may arise from the cells of follicular epithelium: papillary and follicular. These two types differ by their structure (though follicles are often present in papillary carcinomas), molecular-biological characteristics, and clinical behaviour. Besides, medullary thyroid carcinomas (derived from parafollicular neuroendocrine C-cells), as well as poorly differentiated and anaplastic carcinomas may occur in the gland. The two latter types of tumors may develop from preexisting well-differentiated tumors. Nonepithelial tumors developing from lymphoid cells (thyroid lymphoma) or from mesenchymal tissue components (thyroid sarcomas) are also known [1-4].

The following are definitions of the main types of thyroid carcinomas based on the WHO Histological classification [5]:

- papillary thyroid carcinoma (PTC) is a malignant epithelial tumor derived from follicular cells, with characteristic changes in the nuclei (increased size, "ground glass" clearing, pseudo-intranuclear inclusions and grooves);
- follicular thyroid carcinoma (FTC) is an encapsulated or partly encapsulated malignant epithelial tumor, derived from follicular cells, with signs of marked invasion into tumor capsule and/or tumor capsule vessels, without changes in tumor cell nuclei characteristic for PTC;
- medullary thyroid carcinoma (MTC) is a malignant tumor derived from C-cells;
- poorly differentiated thyroid carcinoma (PDTC) is a malignant tumor derived from follicular cells, with signs of decreased differentiation and being an intermediary between well-differentiated (PTC and FTC) and undifferentiated (anaplastic) thyroid carcinoma, both by histological structure, aggressiveness and clinical behaviour;
- anaplastic (undifferentiated) thyroid carcinoma (ATC) is the most malignant epithelial thyroid tumor derived from follicular cells, partly or completely consisting of undifferentiated cells.

According to the above-mentioned classification, the present chapter describes and analyses the main types of thyroid carcinomas which were detected in the group

at increased risk for development of radiation-induced thyroid cancer in the period of a significant rise in its incidence after Chernobyl: 1990-2010 (see Chapter 3). A total of 2,960 cases diagnosed in children and adolescents of Ukraine (aged 0 to 18 years at the time of the Chernobyl accident) as well as in those who were born after the accident are reviewed (Table 4.1).

Morphological characteristics of 2,658 thyroid carcinomas in individuals born before Chernobyl are considered for three age groups: children operated on at the age from 4 to 14 years old, adolescents operated on at the age from 15 to 18 years old, and adults operated on at the age from 19 to 42 years old. Also, a comparative analysis of morphological changes is carried out for four time periods: 1990-1994, 1995-1999, 2000-2004, and 2005-2010 (Table 4.2). Overall, 287 thyroid cancers in children (out of 453 detected in Ukraine, 63.4%), 244 carcinomas in adolescents (out of 527 detected in Ukraine, 46.3%), and 2,127 carcinomas in adults (out of 5,706 detected in Ukraine, 37.3%) were studied for the period 1990-2010. Practically all cancers in children and adolescents included in the analysis have been additionally verified by the international experts, Professors VA LiVolsi and ED Williams, in the framework of joint international projects. Furthermore, 1,512 thyroid carcinomas in children, adolescents and adults operated in 1998-2010 included in the international Chernobyl Tissue Bank have been additionally verified by a Panel of experts-pathologists of the Project (see Chapter 6). It should be noted that since 2001, children born before Chernobyl were no longer registered because they naturally moved over to the age group of "adolescents" who, in turn, moved to the group of "adults" beginning from 2005.

Table 4.1
Total number of thyroid cancer cases under study

	Born before Chernobyl		**Born after Chernobyl**	
Type	number	%	number	%
PTC	2478	93.2	264	87.4
FTC	137	5.1	32	10.6
MTC	39	1.5	6	2.0
PDTC	4	0.2	-	-
Total	**2658**	100	**302**	100
		2960		

PTC – papillary thyroid carcinoma; FTC – follicular thyroid carcinoma; MTC – medullary thyroid carcinoma; PDTC – poorly differentiated thyroid carcinoma

As shown in Table 4.2, among all cancers, ***papillary thyroid carcinoma*** was most prevalent, and accounted for more than 90% of cases in all age groups and for all time periods. This fully corresponds to the previously obtained numerous data published by scientists from Ukraine, Belarus, and Russian Federation [6-15], and to the findings of joint scientific projects carried out in cooperation between the affected countries and leading research centres of the world [16-25].

It has been established that PTC was the most common malignant thyroid tumor not only after internal radiation exposure, but also after external exposure of head and neck

area, especially in childhood [1-4,26-30]. Thyroid cancers that had been detected after Hiroshima and Nagasaki A-bombings [31-35] or the hydrogen bomb test in the Marshall Islands were also mainly PTCs [36].

Table 4.2
Number of thyroid cancer cases in patients born before Chernobyl

	Children aged up to 14 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Type	number	%	number	%	number	%	number	%	number	%
PTC	127	97.0	135	93.1	10	90.1	-	-	272	94.8
FTC	2	1.5	6	4.1	-	-	-	-	8	2.8
MTC	2	1.5	4	2.8	1	0.9	-	-	7	2.4
PDTC	-	-	-	-	-		-	-	-	-
Total	**131**	100	**145**	100	**11**	100	-	-	**287**	100
	Adolescents aged from 15 to 18 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	27	96.4	81	92.0	117	91.4	-	-	225	92.2
FTC	1	3.6	7	8.0	10	7.8	-	-	18	7.4
MTC	-	-	-	-	1	0.8	-	-	1	0.4
PDTC	-	-	-	-	-	-	-	-	-	-
Total	**28**	100	**88**	100	**128**	100	-	-	**244**	100
	Adults aged from 19 to 42 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	13	92.9	149	96.8	605	91.5	1214	93.5	1981	93.1
FTC	1	7.1	4	2.6	45	6.8	61	4.7	111	5.2
MTC	-	-	1	0.6	10	1.5	20	1.6	31	1.5
PDTC	-	-	-	-	1	0.2	3	0.2	4	0.2
Total	**14**	100	**154**	100	**661**	100	**1298**	100	**2127**	100
	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	167	96.5	365	94.3	732	91.5	1214	93.5	2478	93.2
FTC	4	2.3	17	4.4	55	6.9	61	4.7	137	5.1
MTC	2	1.2	5	1.3	12	1.5	20	1.6	39	1.5
PDTC	-	-	-	-	1	0.1	3	0.2	4	0.2
Total	**173**	100	**387**	100	**800**	100	**1298**	100	**2658**	100

PTCs in Ukrainian patients varied in size from 0.3 to 75 mm. The analysis shows that the prevalence of tumors sized up to 10 mm (Table 4.3) - when combining all time periods - was increasing significantly and succesively in the age series: children (10/272, 3.7%) – adolescents (27/225, 12.0%) – adults (458/1981, 24.2%), p=0.0001 (here and hereafter, the Chi-square test for trend or Fisher's Exact text are used for comparison of subgroups).

A significant ascending linear trend (p=0.0001) in the frequency of "small" PTCs was also noted for the combined age groups in time elapsed after Chernobyl, i.e. by time periods: 1990-1994 (5/167, 3.0%) – 1995-1999 – (21/365, 5.7%) – 2000-2004 (95/732, 13.0%) – 2005 -2010 (374/1214, 30.8%). An inverse linear relationship was observed in the analysis of the frequency of carcinomas sized more than 40 mm (Table 4.3). The frequency was decreasing gradually and significantly (p=0.0001) both in age series: children (57 out of 272 cases, 20.9%) – adolescents (23 out of 225 cases, 10.2%) – adults (181 out of 1981 cases, 9.1%), and by time periods: 1990-1994 (33/167, 19.8%) – 1995-1999 (60/365, 16.5%) – 2000-2004 (70/732, 9.5%) – 2005-2010 (98/1214, 8.1%).

Analysis of small encapsulated tumors versus non-encapsulated or partly encapsulated did not reveal significant differences in age or time series. By contrast, significant ascending linear age and time trends (p=0.0001) were found for fully encapsulated large tumors (sized more than 40 mm): children (3 out of 57 cases, 5.3%) – adolescents (5 out of 23 cases, 21.7%) – adults (84 out of 181 cases, 46.4%); 1990-1994 (1/33, 3.0%) – 1995-1999 (8/60, 13.3%) – 2000-2004 (26/70, 37.1%) – 2005-2010 (56/98, 57.1%).

For the combined encapsulated tumors of any size, significant ascending linear trends were also noted (p=0.0001): children (21 out of 272 cases, 7.7%) – adolescents (35 out of 225 cases, 15.6%) – adults (582 out of 1981 cases, 29.4%); 1990-1994 (9/149, 6.0%) – 1995-1999 (91/365, 24.9%) – 2000-2004 (172/732, 23.5%) – 2005-2010 (363/1214, 29.9%).

PTC is generally known to display varying histological structures and therefore it is further subdivided into subtypes or variants. According to the WHO Histological classification, these variants include classic papillary, follicular, macrofollicular, solid, oxyphilic-cell, clear-cell, diffuse-sclerosing, tall-cell, columnar-cell, cribriform-morular, and Warthin-like variants. Papillary microcarcinoma is also considered to be a separate variant [5].

With regard to the classic papillary variant, at least 80% of tumors featured typical papillary formations with characteristic fibrovascular core and optically clear ("ground-glass") nuclei (Fig. 4.1 A) containing intranuclear grooves and pseudo-cytoplasmic inclusions. Most of the tumor cells showed positive immunohistochemical staining with antithyroglobulin antibodies (Fig. 4.1 B).

In the *follicular variant of PTC,* typical papillary structures are scarce or absent (Fig. 4.2 A). Cleared nuclei with chromatin localized at the periphery is the main distinctive feature of this subtype. Positive immunohistochemical reaction with antithyroglobulin antibodies, similarly to the classic papillary variant, was observed in most tumor cells (Fig. 4.2 B).

In the *solid variant of papillary thyroid carcinoma*, tumors with alveolar-solid growth pattern were most prevalent (Fig. 4.3 A). Areas with solid-trabecular structures were occasionally observed (Fig. 4.3 B). Papillary structures were generally absent, but small follicular areas could occur. The fact that tumors of this subtype are PTCs is substantiated by the structure of tumor cell nuclei. Intranuclear grooves and nuclear pseudoinclusions were best seen on electron microscopy (Fig. 4.3 D, E). Thyroglobulin in tumor cells, unlike in the classic papillary and follicular variants, was detected only focally (Fig. 4.3 C).

Table 4.3
Size of papillary thyroid carcinomas in patients born before Chernobyl

Size, mm	Children aged up to 14 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	1	0.8	-	-	-	-	-	-	1	0.4
6-10	2	1.6	7	5.2	-	-	-	-	9	3.3
11-20	41	32.3	71	52.6	3	30.0	-	-	115	42.3
21-30	41	32.3	17	12.6	5	50.0	-	-	63	23.2
31-40	15	11.8	10	7.4	2	20.0	-	-	27	9.9
41-50	15	11.8	18	13.4	-	-	-	-	33	12.1
51-60	9	7.0	6	4.4	-	-	-	-	15	5.5
> 60	3	2.4	6	4.4	-	-	-	-	9	3.3
Total	**127**	100	**135**	100	**10**	100	-	-	**272**	100
	Adolescents aged from 15 to 18 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	6	5.1	-	-	6	2.7
6-10	1	3.7	7	8.6	13	11.1	-	-	21	9.3
11-20	10	37.0	38	46.9	51	43.6	-	-	99	44.0
21-30	7	25.9	19	23.5	28	23.9	-	-	54	24.0
31-40	5	18.6	7	8.7	10	8.6	-	-	22	9.8
41-50	1	3.7	6	7.4	8	6.8	-	-	15	6.7
51-60	-	-	3	3.7	-	-	-	-	3	1.3
>60	3	11.1	1	1.2	1	0.9	-	-	5	2.2
Total	**27**	100	**81**	100	**117**	100	-	-	**225**	100
	Adults aged from 19 to 42 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	20	3.3	98	8.1	118	6.0
6-10	1	7.7	7	4.7	56	9.3	276	22.7	340	17.2
11-20	3	23.0	67	43.0	289	47.8	439	36.2	798	40.3
21-30	5	38.5	45	30.2	130	21.5	215	17.7	395	19.9
31-40	2	15.4	10	6.7	49	8.1	88	7.2	149	7.5
41-50	1	7.7	12	8.0	33	5.4	53	4.4	99	5.0
51-60	1	7.7	3	2.0	20	3.3	28	2.3	52	2.6
>60	-	-	5	3.4	8	1.3	17	1.4	30	1.5
Total	**13**	100	**149**	100	**605**	100	**1214**	100	**1981**	100

Continuation of Table 4.3

	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	1	0.6	-	-	26	3.6	98	8.1	125	5.0
6-10	4	2.4	21	5.7	69	9.4	276	22.7	370	14.9
11-20	54	32.3	176	48.2	343	46.9	439	36.2	1012	40.8
21-30	53	31.7	81	22.2	163	22.3	215	17.7	512	20.6
31-40	22	13.2	27	7.4	61	8.3	88	7.2	198	8.0
41-50	17	10.2	36	9.9	41	5.6	53	4.4	147	5.9
51-60	10	6.0	12	3.3	20	2.7	28	2.3	70	2.8
>60	6	3.6	12	3.3	9	1.2	17	1.4	44	1.8
Total	**167**	100	**365**	100	**732**	100	**1214**	100	**2478**	100

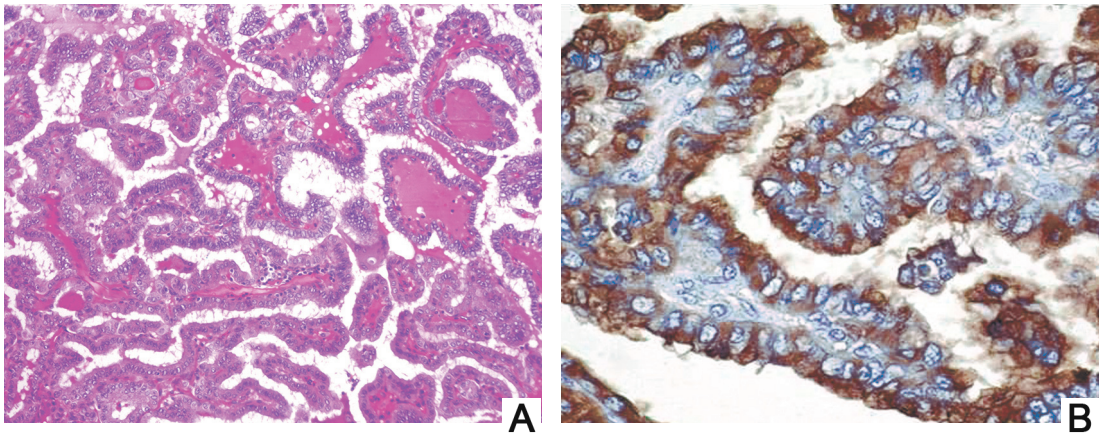

Figure 4.1. Classic papillary carcinoma. (A) Typical papillary structures with well-developed fibrovascular core and cleared tumor cell nuclei. Haematoxylin and eosin, original magnification x100. (B) Strong diffuse cytoplasmic immunostaining for thyroglobulin, original magnification x200.

These three subtypes accounted for more than 50% of all PTCs under study for all age groups and all periods of time (Table 4.4).

Of note, PTCs were not always monomorphic histologically which is a difficulty when ascribing tumors to one of the three main variants. In many cases tumors had a mixed growth pattern (herein referred to as "mixed variant") (Fig. 4.4), comprising a combination of papillary, follicular or solid components (Table 4.5).

Diffuse sclerosing variant was rather rare, 8.7% cases in children in the first period of time (Table 4.4). The frequency of this variant was significantly decreasing (p=0.0001) both in age and time series (Table. 4.4). Tumors with this structure were characterized by:

- diffuse extension of tumoral foci throughout the thyroid
- fibrous-sclerotic changes
- marked thyroiditis
- abundance of psammoma bodies
- foci of squamous-cell metaplasia.

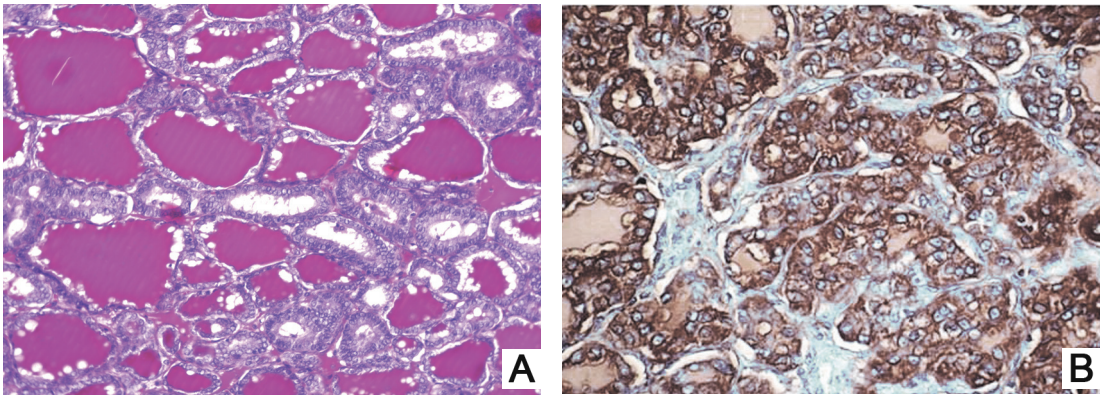

Figure 4.2. Follicular variant of papillary thyroid carcinoma. (A) Diffuse nuclear features of papillary carcinoma. Haematoxylin and eosin, original magnification x100. (B) Strong diffuse cytoplasmic immunostaining for thyroglobulin, original magnification x100.

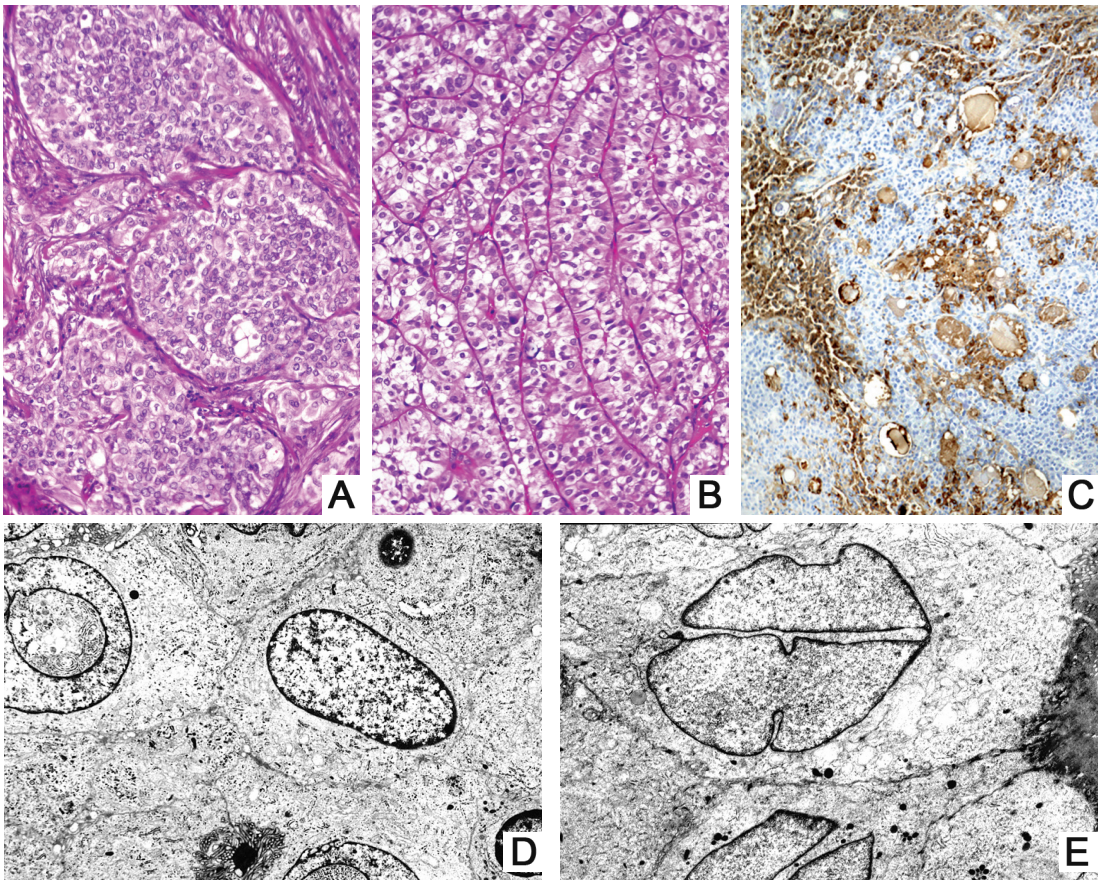

Figure 4.3. Solid variant of papillary thyroid carcinoma. (A) Alveolar-solid growth pattern. Haematoxylin and eosin, original magnification x100. (B) Trabecular growth pattern. Haematoxylin and eosin, original magnification x100. (C) Focal immunostaining for thyroglobulin, original magnification x50. (D) Intranuclear inclusions on electron microscopy, original magnification x5,000. (E) Nuclear grooves on electron microscopy, original magnification x7,000.

Tumoral foci had generally solid or papillary-solid structure. A marked invasion of tumor tissue and psammoma bodies to lymphatic vessels was characteristic (Fig. 4.5).

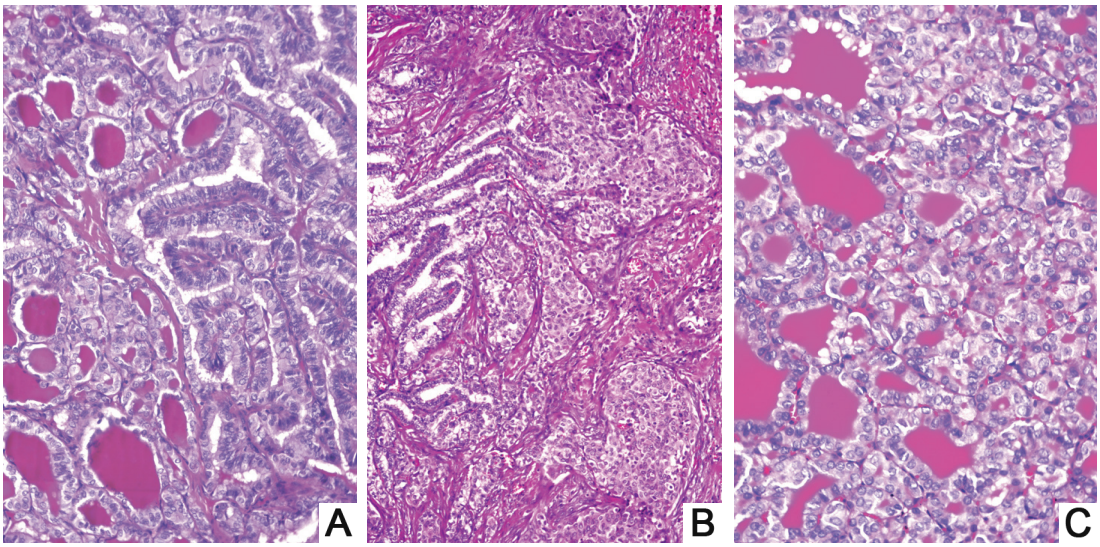

Figure 4.4. Mixed variant of papillary thyroid carcinoma. (A) Papillary-follicular growth pattern. Haematoxylin and eosin, original magnification x100. (B) Papillary-solid growth pattern. Haematoxylin and eosin, original magnification x50. (C) Solid-follicular growth pattern. Haematoxylin and eosin, original magnification x100.

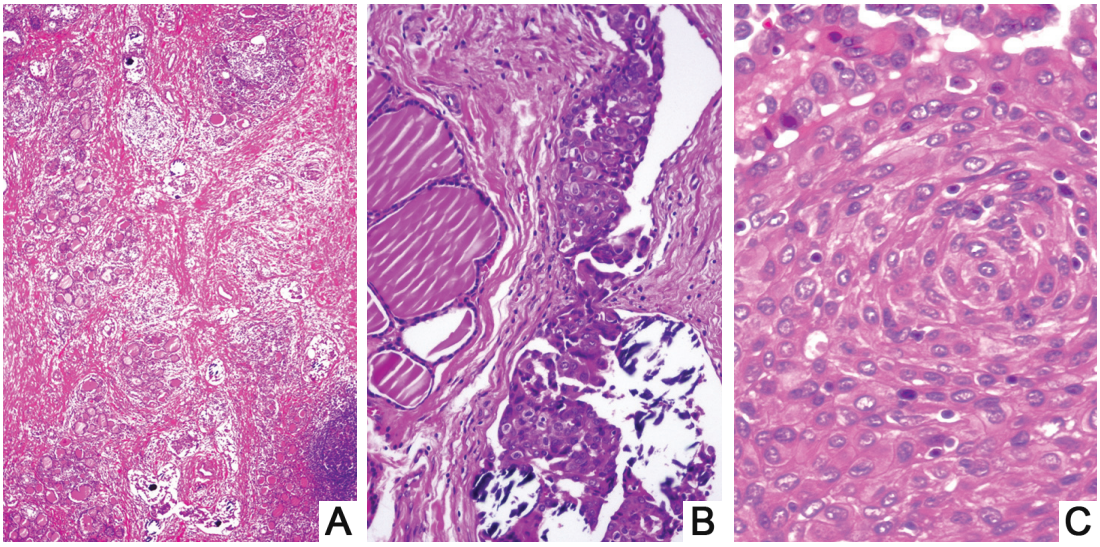

Figure 4.5. Diffuse sclerosing variant of papillary thyroid carcinoma. (A) Diffuse tumor growth, numerous psammoma bodies, marked fibrosis, lymphocytic infiltration. Haematoxylin and eosin, original magnification x20. (B) Tumor aggregates inside lymphatic vessels. Haematoxylin and eosin, original magnification x100. (C) Squamous-cell metaplasia. Haematoxylin and eosin, original magnification x200.

Table 4.4
Subtypes of papillary thyroid carcinomas in patients born before Chernobyl

	Children aged up to 14 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Subtype	Number	%	number	%	number	%	number	%	number	%
PV	9	7.1	19	14.1	2	20.0	-	-	30	11.0
FV	46	36.2	17	12.6	2	20.0	-	-	65	23.9
SV	38	29.9	17	12.6	2	20.0	-	-	57	21.0
Mixed V	23	18.1	76	56.3	4	40.0	-	-	103	37.9
DSV	11	8.7	6	4.4	-	-	-	-	17	6.2
Warthin	-	-	-	-	-	-	-	-	-	-
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	**127**	100	**135**	100	**10**	100	**-**	-	**272**	
	Adolescents aged from 15 to 18 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	4	14.8	21	25.9	23	19.6	-	-	48	21.3
FV	10	37.0	13	16.0	24	20.5	-	-	47	20.9
SV	5	18.5	8	10.0	11	9.4	-	-	24	10.7
Mixed V	8	29.7	35	43.2	58	49.6	-	-	101	44.9
DSV	-	-	4	4.9	-	-	-	-	4	1.8
Warthin	-	-	-	-	1	0.9	-	-	1	0.4
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	**27**	100	**81**	100	**117**	100	**-**	-	**225**	
	Adults aged from 19 to 42 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	2	15.4	54	36.2	223	36.9	388	32.0	667	33.7
FV	6	46.1	30	20.1	102	16.9	194	16.0	332	16.8
SV	2	15.4	12	8.1	25	4.1	79	6.5	118	5.9
Mixed V	3	23.1	51	34.3	242	40.0	541	44.6	837	42.3
DSV	-	-	2	1.3	2	0.3	2	0.1	6	0.3
Warthin	-	-	-	-	11	1.8	8	0.7	19	0.9
Cribriform	-	-	-	-	-	-	2	0.1	2	0.1
Total	**13**	100	**149**	100	**605**	100	**1214**	100	**1981**	100
	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	15	9.0	94	25.8	248	33.9	388	32.0	745	30.0
FV	62	37.1	60	16.4	128	17.5	194	16.0	444	17.9
SV	45	26.9	37	10.1	38	5.2	79	6.5	199	8.1
Mixed V	34	20.4	162	44.4	304	41.5	541	44.6	1041	42.0
DSV	11	6.6	12	3.3	2	0.3	2	0.1	27	1.1
Warthin	-	-	-	-	12	1.6	8	0.7	20	0.8
Cribriform	-	-	-	-	-	-	2	0.1	2	0.1
Total	**167**	100	**365**	100	**732**	100	**1214**	100	**2478**	100

PV – classic papillary variant; FV – follicular variant; SV – solid variant; Mixed V – mixed variant; DSV – diffuse sclerosing variant; Warthin – Warthin-like variant; Cribriform – cribriform-morular variant

Table 4.5
Structural components of mixed variant of papillary thyroid carcinoma in patients born before Chernobyl

	Children aged up to 14 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Structure	Number	%	number	%	number	%	number	%	number	%
PF	3	13.0	12	15.8	1	25.0	-	-	16	15.5
PS	4	17.4	7	9.2	-	-	-	-	11	10.7
PFS	2	8.7	1	1.3	-	-	-	-	3	2.9
SF	14	60.9	56	73.7	3	75.0	-	-	73	70.9
Total	**23**		**76**		**4**	100	**-**	**-**	**103**	100
	Adolescents aged from 15 to 18 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	Number	%	number	%	number	%	number	%	number	%
PF	1	12.5	12	34.9	25	43.1	-	-	38	37.6
PS	-	-	7	20.0	11	19.0	-	-	18	17.8
PFS	-	-	-	-	5	8.6	-	-	5	5.0
SF	7	87.5	16	45.7	17	29.3	-	-	40	39.6
Total	**8**		**35**	100	**58**	100	**-**	**-**	**101**	100
	Adults aged from 19 to 42 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	Number	%	number	%	number	%	number	%	number	%
PF	2	75.0	20	39.2	148	61.2	252	46.6	422	50.4
PS	-	-	11	21.6	32	13.2	100	18.5	143	17.1
PFS	-	-	5	9.8	10	4.1	34	6.2	49	5.9
SF	1	25.0	15	24.4	52	21.5	155	28.7	223	26.6
Total	**3**	100	**51**	100	**242**	100	**541**	100	**837**	100
	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	Number	%	number	%	number	%	number	%	number	%
PF	6	17.6	44	27.2	174	57.2	252	46.6	476	45.7
PS	4	11.8	25	15.4	43	14.1	100	18.5	172	16.5
PFS	2	5.9	6	3.7	15	5.0	34	6.2	57	5.5
SF	22	64.7	87	53.7	72	23.7	155	28.7	336	32.3
Total	**34**	100	**162**	100	**304**	100	**541**	100	**1041**	

PF – papillary-follicular variant; PS – papillary-solid variant; PFS – papillary-follicular-solid variant; SF – solid-follicular variant

According to the literature, the development of PTC with diffuse-sclerosing structure has been associated with previous radiation exposure [1,2,37,38]. However, studies of «post-Chernobyl» carcinomas did not confirm this notion as such tumors were observed in not more than in 7.0-9.0% cases and mostly in children [10,11,16].

In less than 1% of cases, the *Warthin-like variant* (Table 4.4) was found in later periods (2000-2004 and 2005-2010, mostly in adults). Its distinctive feature is a profound intratumoral thyroiditis (Fig. 4.6). Tumors were represented by oxyphilic cells and generally had papillary or papillary-solid structure; they were also characterized by the very strong diffuse immunohistochemical reaction for thyroglobulin (Fig. 4.6 D) and TTF-1 (Fig. 4.6 E). Referring the Warthin-like variant to PTC is justified by enlarged and cleared nuclei (Fig. 4.6 C). The proliferative activity of tumor cells (immunohistochemical reaction with anti-Ki67 antibodies) was not high, less than 5% (Fig. 4.6 F), which is, again, characteristic of PTC in general [3,4]. The lesions ranged in size from 5 to 42 mm, all of them were non-encapsulated. Lymph node metastases were identified in 7 out of 19 cases (36.8%),

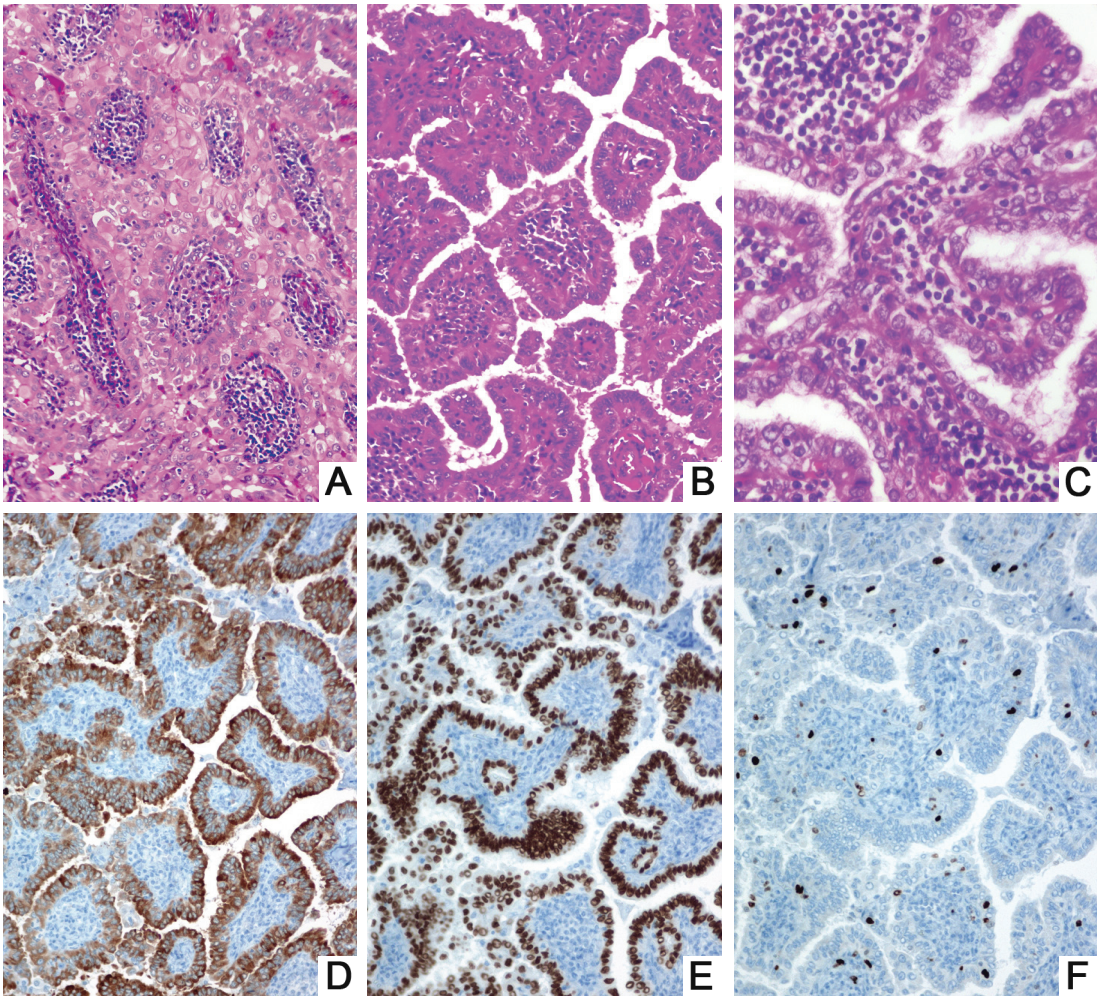

Figure 4.6. Warthin-like variant of papillary thyroid carcinoma. (A, B) Papillary-solid and papillary growth pattern, profound intratumoral thyroiditis. Tumors are represented by oxyphilic cells. Haematoxylin and eosin, original magnification x100. (C) Nuclear features of Warthin-like variant. Haematoxylin and eosin, original magnification x200. (D) Strong diffuse cytoplasmic staining for thyroglobulin, original magnification x100. (E) Strong nuclear reactivity for TTF-1, original magnification x100. (F) Focal nuclear reactivity for Ki67, original magnification x100.

but none showed distant metastases (Table 4.9). Our data are in agreement with the opinion of other authors [39] that these tumors behave similarly to conventional PTCs. The impact of radiation on the development of this subtype is not established yet.

In two cases (female patients aged 26 and 27 years), an even more rare variant of PTC, the *Cribriform-morular*, was identified (0.1%). Tumors were represented by the nodule-like lesions localized between markedly sclerosed stroma (Fig. 4.7 A, 4.7 B). An essential difference between this and other subtypes was the presence of numerous morulas (Fig. 4.7 C, 4.7 D). Tumor areas had solid or follicular structure with the colloid being practically absent in all follicles. In the solid areas, cells were of polygonal shape; nuclei were more dense than in other subtypes and contained a small number of intranuclear inclusions. Immunohistochemical reaction with antithyroglobulin antibodies was practically negative, while the reaction with anti-TTF-1 antibodies was highly intensive and diffuse in tumor cell nuclei but virtually absent in the nuclei of the cells within morulas (Fig. 4.7 E). A highly intensive reaction to ß-catenin was revealed in the nuclei and cytoplasm of tumor cells; staining in morulas was rather weak and had a diffuse pattern (Fig. 4.7 F). Nuclei positive for Ki67 were rare both in tumor and in morular cells (Fig. 4.7 G); weakly positive for TP53 (p53) nuclei were detected in 3-5% of tumor and morular cells (Fig. 4.7 H). The described tumors fully correspond to the data available in the literature [3,4] not only by morphological characteristics, age and gender of patients, but also by the diagnosis of familial adenomatous polyposis (FAP) in these individuals as stated in their medical records. No impact of radiation on the development of this subtype of PTC has been found.

The most aggressive variants according to the literature [1,3,4,37,40], the *Tall-cell* and *Columnar-cell variants*, were not observed among PTCs under study. In three cases in adults (0.3%), only isolated tall-cell areas were noted in the tumors with papillary-trabecular architecture. Columnar-cell areas were detected in an adult patient only in one tumor (0.1%) which was, again, of papillary-trabecular structure. Such areas demonstrated pseudostratified columnar cells with subnuclear and supranuclear cytoplasmic vacuoles (Fig. 4.8 A). Thyroglobulin in such areas was expressed at the apical part of cells and in the narrowed fine intrapapillary space (Fig. 4.8 B); practically all nuclei of tumor cells expressed TTF-1 (Fig. 4.8 C), and only few (2-3%) expressed Ki67 (Fig. 4.8 D). The reaction with anti-TP53 antibodies was negative.

Further, we analyse three main subtypes of PTC (classic papillary, follicular, and solid variants) and tumors with mixed growth pattern for age and time related changes.

In the first five years of a significant rise in thyroid cancer incidence (1990-1994), PTCs in children operated at under 15 years of age (127 out of 167 cases, 76.0%) were the most prevalent. 66.1% of these tumors had follicular and solid structure (Table 4.4). Of note, the follicular variant differed from that described in adults by more roundish nuclei and the presence of solid component (up to 20.0%) in all cases, commonly in the areas of intrathyroidal or extrathyroidal extension. Besides, in the tumors with mixed growth pattern ("mixed variant") in this age group (Table 4.5), the prevalence of the solid-follicular architecture was significantly higher than those of other structural combinations ($p<0.0018$ vs papillary-follicular; $p<0.0058$ vs papillary-solid, and $p<0.0001$ vs papillary-follicular-solid).

Such architectural particularities of tumors in children prompted the introduction of the special solid-follicular "childhood variant" of PTC [38], which combines tumors of solid, follicular, and solid-follicular variants.

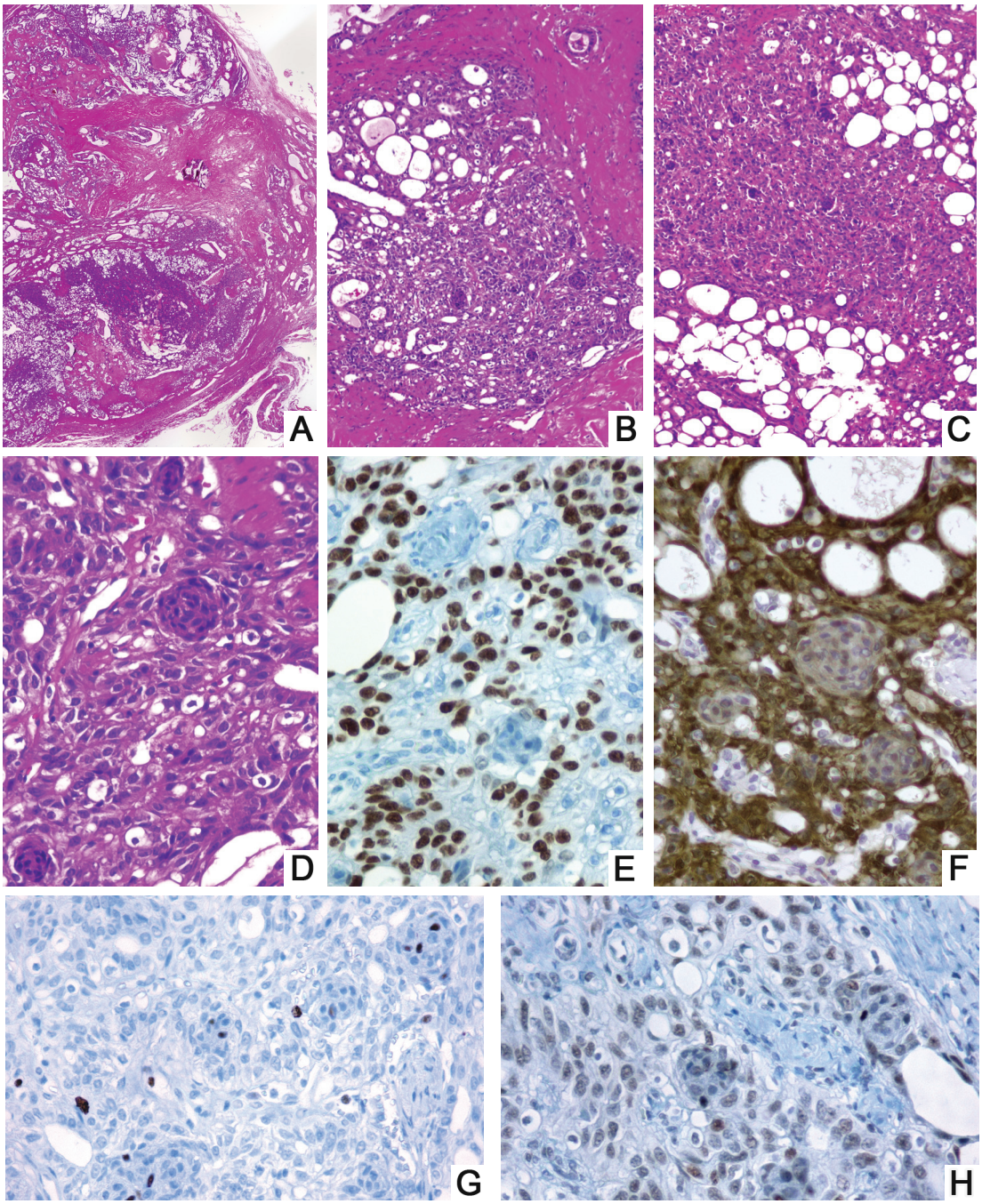

Figure 4.7. Cribriform-morular variant of papillary thyroid carcinoma. (A, B) Nodule-like lesions, marked stromal sclerosis. Haematoxylin and eosin, original magnification x10-panoramic; x50. (C, D) Numerous morulas and empty follicles. Haematoxylin and eosin, original magnification x50; x200. (E) Diffuse strong nuclear reactivity for TTF-1, original magnification x200. (F) Diffuse strong cytoplasmic and nuclear staining for ß-catenin in tumor cells; diffuse and weakly positive staining in morulas. Original magnification x200. (G) Focal nuclear reactivity for Ki67 in tumor and morular cells, original magnification x200. (H) Focal weak nuclear reactivity for TP53 in tumor and morular cells, original magnification x200.

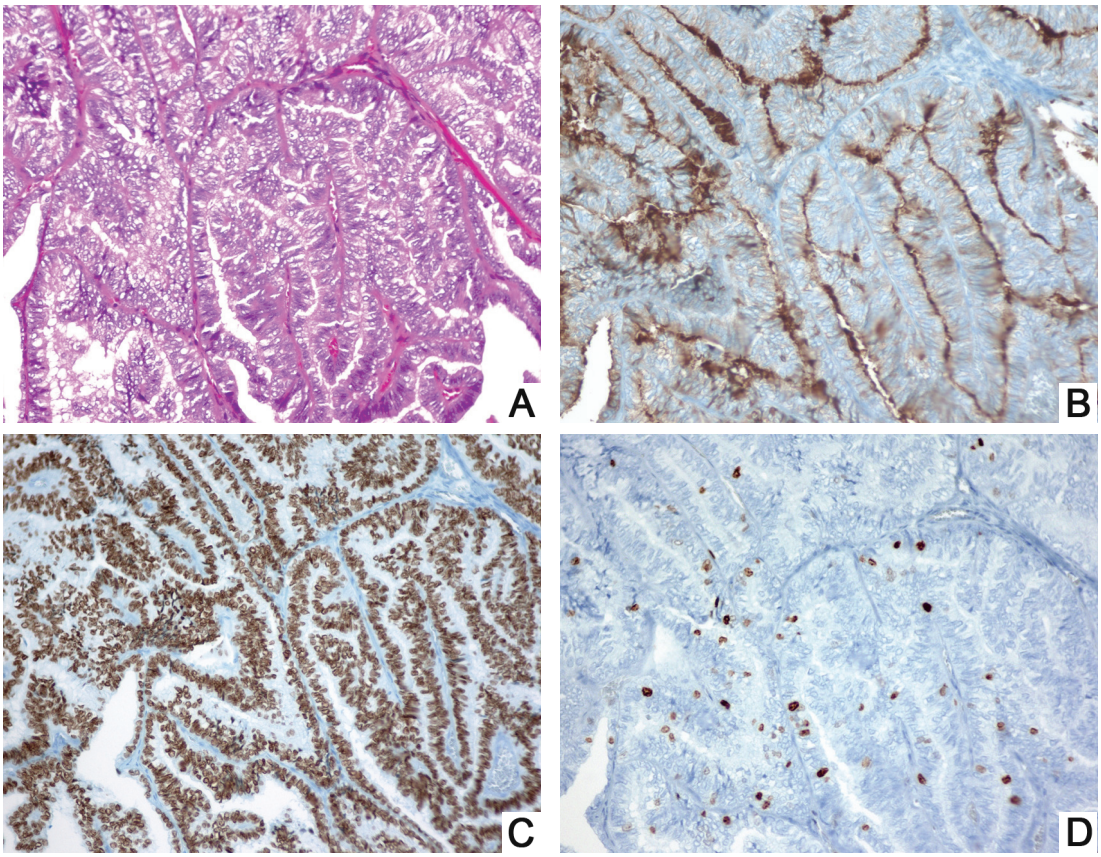

Figure 4.8. Columnar-cell areas of papillary thyroid carcinoma. (A) Papillary-trabecular growth pattern, prominent subnuclear vacuoles, and moderate pseudostratification. Haematoxylin and eosin, original magnification x100. (B) Strong apical cytoplasmic thyroglobulin staining, original magnification x100. (C) Diffuse strong nuclear reactivity for TTF-1, original magnification x100. (D) Focal nuclear reactivity for Ki67, original magnification x100.

During the first decade after Chernobyl, tumors of "childhood variant" accounted for up to 80% in patients operated at the age under 15 years in Ukraine and Belarus. These PTCs were characterized by marked intrathyroidal and extrathyroidal extension, invasion of lymphatic and blood vessels, and very frequent regional metastases [11,17,18,20,41,42]. It should be noted that such a "combined" solid-follicular variant of PTC was much more prevalent among children of Ukraine and Belarus affected by the Chernobyl accident compared to nonexposed children of England and Wales [11,17].

In addition, it has been established in Ukraine that the relative risk of development of "childhood variant" PTC was increasing with thyroid exposure dose: 2.2-fold at the dose 0.05 to 0.2 Gy, 5.2-fold at 0.2 to 1.0 Gy, and 6.7-fold at >1.0 Gy. Overall, the relative risk of development of such tumors at thyroid exposure dose exceeding 0.05 Gy was 3.7 [43].

In children of Russia, the follicular variant of PTC [44] was most common in post-Chernobyl years. No correlation between morphological structure and thyroid exposure dose was found [14].

It has been claimed that the presence of a marked solid component determined the aggressiveness of biological behaviour of PTC in children [16,17,18,20,38,45,46]. In this context, of interest are data obtained later by a group of experts-pathologists (which also included representatives of Ukraine, Belarus and Russia) in the framework of an international project for establishment of the Chernobyl Tissue Bank. The group performed an analysis of the histological structure of PTCs and their latency [47]. Three groups of children have been selected differing by age at exposure, age at surgery, and by the period of latency (defined as time interval between the Chernobyl accident and date of surgery). A significant difference was found in the prevalence of less differentiated (solid) and more differentiated (papillary and follicular) structural components depending on latency. PTCs developing after a short latency were characterized by the more prominent solid component and by more pronounced intrathyroidal and extrathyroidal extension as compared to those occurring after a longer latency. The latter tumors generally displayed papillary-follicular growth pattern and peritumoral fibrosis, which are usually considered to be less aggressive pathological features.

It needs to be emphasized that PTCs with architecturally less differentiated solid areas should not be erroneously assimilated into the poorly differentiated thyroid tumors group [1,3,4,25,30,37,48] as these are different in both histological structure and prognosis.

The same group of pathologists proposed later that the abundance of the solid component in "post-Chernobyl" childhood PTCcould also be influenced by a moderate iodine deficiency observed in the affected countries as compared to England and Wales, and especially to Japan [49].

Since 25 years have already passed after the Chernobyl accident, children who had experienced the strongest impact of radioactive iodine from fallout have already moved over to the age category of "adults" for a long time. Therefore, it appears inappropriate to continue using the term "childhood variant" today; for this reason, our analysis was based on generally accepted PTC subtypes. The highest percentage of tumors with a solid structure, which, as mentioned above, are architecturally less differentiated, was observed in children (21.0%). This is in line with the results of other studes of radiation-induced or sporadic PTC in children [3,4,25,29,46]. The frequency of tumors with classic papillary structure was, on the contrary, the highest in adults (33.7%), with a highly significant age trend (p=0.0001).

The ratio of PTC subtypes significantly changed with time after Chernobyl, i.e. with increasing period of latency. In all age groups the percentage of tumors with solid structure was gradually decreasing while that of classic papillary and, especially, mixed structure was increasing (Table 4.4). Similarly to the age-related changes, time-related linear trends were also highly significant (p=0.0001). Besides, in all age groups combined, the frequency of the solid variant decreased from 26.6% in the first period of observation (1990-1994) to 6.5% in the last period (2005-2010) while the frequency of the classic papillary variant increased from 9.0 to 32.0%. Structural combinations of the mixed variant also markedly changed with time (Table 4.5). The frequency of tumors with solid-follicular structures gradually decreased (from 64.7% in 1990-1994 to 28.7% in 2005-2010), and the percentage of tumors with papillary-follicular structure increased (from 17.6% в 1990-1994 to 46.6% in 2005-2010).

Invasive features of PTCs under study (extrathyroidal extension to soft tissues adjacent to the thyroid, regional and distant metastases), and their relationship to tumor size and multifocality as assessed according to the seventh edition of TNM Classification [50], also changed significantly with time after Chernobyl (Table 4.6).

Table 4.6
Size of papillary thyroid carcinomas and the prevalence of lymph node and distant metastases in patients born before Chernobyl

1st period: surgery in 1990-1994

	All age groups						
	N0	N1a	N1b	Total pT		M0	M1
pT/tumor size	number	number	number	number	%	number	number
pT1a (up to 5 mm)	1			1	0.5	1	
pT1a (6-10 mm)	3			3	1.8	3	
pT1am (1-5 mm)							
pT1am (6-10 mm)							
pT1b (11-20 mm)	17	6		23	13.8	23	
pT1bm (11-20 mm)							1
pT2 (21-40 mm)	23	4	2	29	17.4	28	1
pT2m (21-40 mm)							
pT3 (>40 mm)*	8	2	2	12	7.2	11	
pT3m (>40 mm)*							30
pT3 (any size)**	11	13	61	85	50.9	55	13
pT3m (any size)**			14	14	8.4	1	
Total	63 (37.7%)	25 (15.0%)	79 (47.3%)	**167**	**100**	122 (73.1%)	45 (26.9%)

2nd period: surgery in 1995-1999

	All age groups						
	N0	N1a	N1b	Total pT		M0	M1
pT/tumor size	number	number	number	number	%	number	number
pT1a (up to 5 mm)							
pT1a (6-10 mm)	16	1		17	4.7	17	
pT1am (1-5 mm)							
pT1am (6-10 mm)							
pT1b (11-20 mm)	70	22	4	96	26.3	95	1
pT1bm (11-20 mm)	3			3	0.8	3	
pT2 (21-40 mm)	28	14	3	45	12.3	45	
pT2m (21-40 mm)	2			2	0.5	2	
pT3 (>40 mm)*	10	2	1	13	3.6	13	
pT3m (>40 mm)*							
pT3 (any size)**	39	34	92	165	45.2	115	50
pT3m (any size)**			24	24	6.6	3	21
Total	168 (46.0%)	73 (20.0%)	124 (34.0%)	**365**	**100**	293 (80.3%)	72 (19.7%)

Continuation of Table 4.6

3rd period: surgery in 2000-2004

	All age groups						
	N0	N1a	N1b	Total pT		M0	M1
pT/tumor size	number	number	number	number	%	number	number
pT1a (up to 5 mm)	24			24	3.3	24	
pT1a (6-10 mm)	40	6	2	48	6.6	48	
pT1am (1-5 mm)	1	1		2	0.3	2	
pT1am (6-10 mm)	6	2	1	9	1.2	9	
pT1b (11-20 mm)	181	51	17	249	34.0	247	2
pT1bm (11-20 mm)			2	2	0.3	1	1
pT2 (21-40 mm)	75	24	13	112	15.3	110	2
pT2m (21-40 mm)	7	1		8	1.0	8	
pT3 (>40 mm)*	20	1	1	21	2.9	21	
pT3m (>40 mm)*	1			2	0.3	2	
pT3 (any size)**	56	42	128	226	30.9	196	30
pT3m (any size)**	5	6	18	29	3.9	23	6
Total	416 (56.8%)	134 (18.3%)	182 (24.9%)	**732**	**100**	691 (94.4%)	41 (5.6)

4th period: surgery in 2005-2010

	All age groups						
	N0	N1a	N1b	Total pT		M0	M1
pT/tumor size	number	number	number	number	%	number	number
pT1a (up to 5 mm)	79	1	3	83	6.8	83	
pT1a (6-10 mm)	173	24	9	206	17.0	206	
pT1am (1-5 mm)	11	1	1	13	1.1	13	
pT1am (6-10 mm)	30	6	2	38	3.1	38	
pT1b (11-20 mm)	228	62	20	310	25.5	310	
pT1bm (11-20 mm)	32	8	3	43	3.6	43	
pT2 (21-40 mm)	150	25	16	191	15.7	189	2
pT2m (21-40 mm)	16	3	6	25	2.1	25	
pT3 (>40 mm)*	44	11		55	4.5	55	
pT3m (>40 mm)*	9	2		11	0.9	11	
pT3 (any size)**	70	34	88	192	19.8	176	16
pT3m (any size)**	13	8	26	47	3.9	43	4
Total	855 (70.4%)	185 (15.3%)	174 (14.3%)	**1214**	**100**	1192 (98.2%)	22 (1.8%)

*- no extrathyroidal extension; ** - extrathyroidal extension

While during the first period of observation (1990-1994), the signs of extrathyroidal extension were identified in 59.3% cases, their frequency gradually decreased with increasing latency to 23.7% in 2005-2010. Such a tendency was also noted in the analysis of the frequency of regional and distant metastases. The percentage of cases with metastases to lymph nodes decreased from 62.3 to 29.6%, and that of distant metastases to the lung (detected during postoperative treatment of patients with radioiodine), decreased from 26.9 to 1.8%. All the above changes were characterized by significantly descending linear trends (p=0.0001).

The frequency of tumor foci detected in the form of separate lesions in contralateral lobe or sometimes in the affected lobe as well (Tm) in the first three time periods, i.e. during 1990-2004, practically did not differ (Table 4.7): 8.4% (14/167), 7.9% (29/365), and 7.1% (52 cases out of 732). Only in 2005-2010, the frequency of multifocal tumors significantly increased as compared with the first period of time (p=0.0309).

Also, it should be noted that before 2000, the "additional" tumoral foci which did not differ structurally from the main tumor were mostly seen among cases of "aggressive" PTCs sized more than 10 mm with signs of extrathyroidal extension and regional as well as distant metastases (Table 4.6, 4.7 and Fig. 4.9).

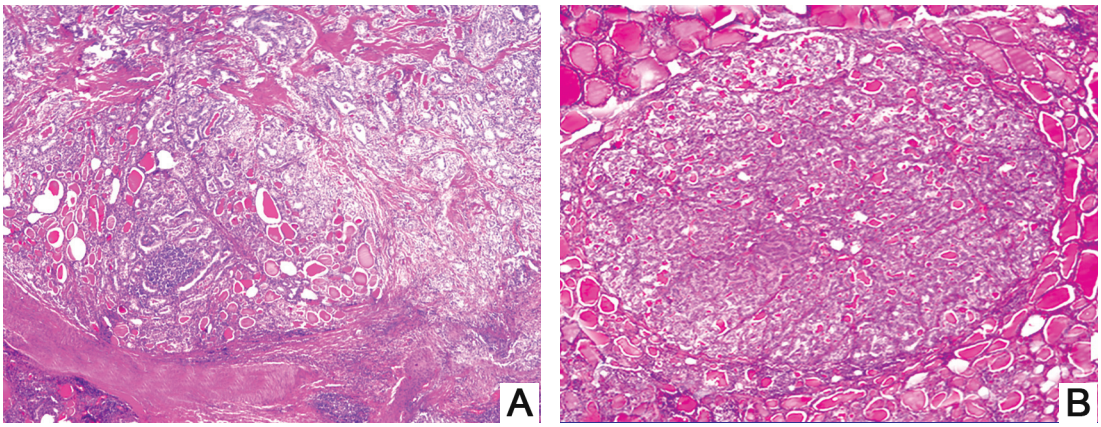

Figure 4.9. Multifocal papillary thyroid carcinoma. (A) Nonencapsulated main tumor sized 15 mm in the left lobe, follicular variant with the solid growth pattern. Haematoxylin and eosin, original magnification x10. (B) Multifocal lesion in the right lobe sized 5 mm with the solid-follicular structure. Haematoxylin and eosin, original magnification x20.

In 2000-2004, and especially in 2005-2010, different foci of multifocal tumors were characterized by variations in size, including microtumors (Table 4.7), by the degree of encapsulation, and by structural and invasive features. For an instance, in the same patient, there might be an encapsulated tumor of mixed structure sized more than 10 mm, and a separate nonencapsulated microtumor of mixed structure in one lobe (Fig. 4.10 A-E). In the contralateral lobe there might be, again, an encapsulated tumor of mixed structure sized more than 10 mm, and a nonencapsulated oxyphilic-cell tumor of the solid structure with signs of marked intratumoral and peritumoral thyroiditis (Fig. 4.10 F-H). In this example tumors are likely to be multiple PTCs developing independently.

Invasive features of PTCs also depended on patients' age. The highest frequency of tumors displaying intrathyroidal and extrathyroidal extension, vascular invasion, regional metastases to cervical lymph nodes and distant metastases to the lung was found in children (Table 4.8). For all these features, significantly descending linear age trends were found (p=0.0001).

Morphological signs of aggressiveness (extrathyroidal extension, vascular invasion, regional metastases) were revealed in all histological subtypes, mostly in nonencapsulated tumors (Table 4.9). In encapsulated PTCs (Fig. 4.11), tumor capsule invasion was observed in most cases, intrathyroidal extension in not more than 40.0%, and was limited only to isolated tumoral foci outside the tumor in adjacent thyroid tissue (Fig. 4.11 D).

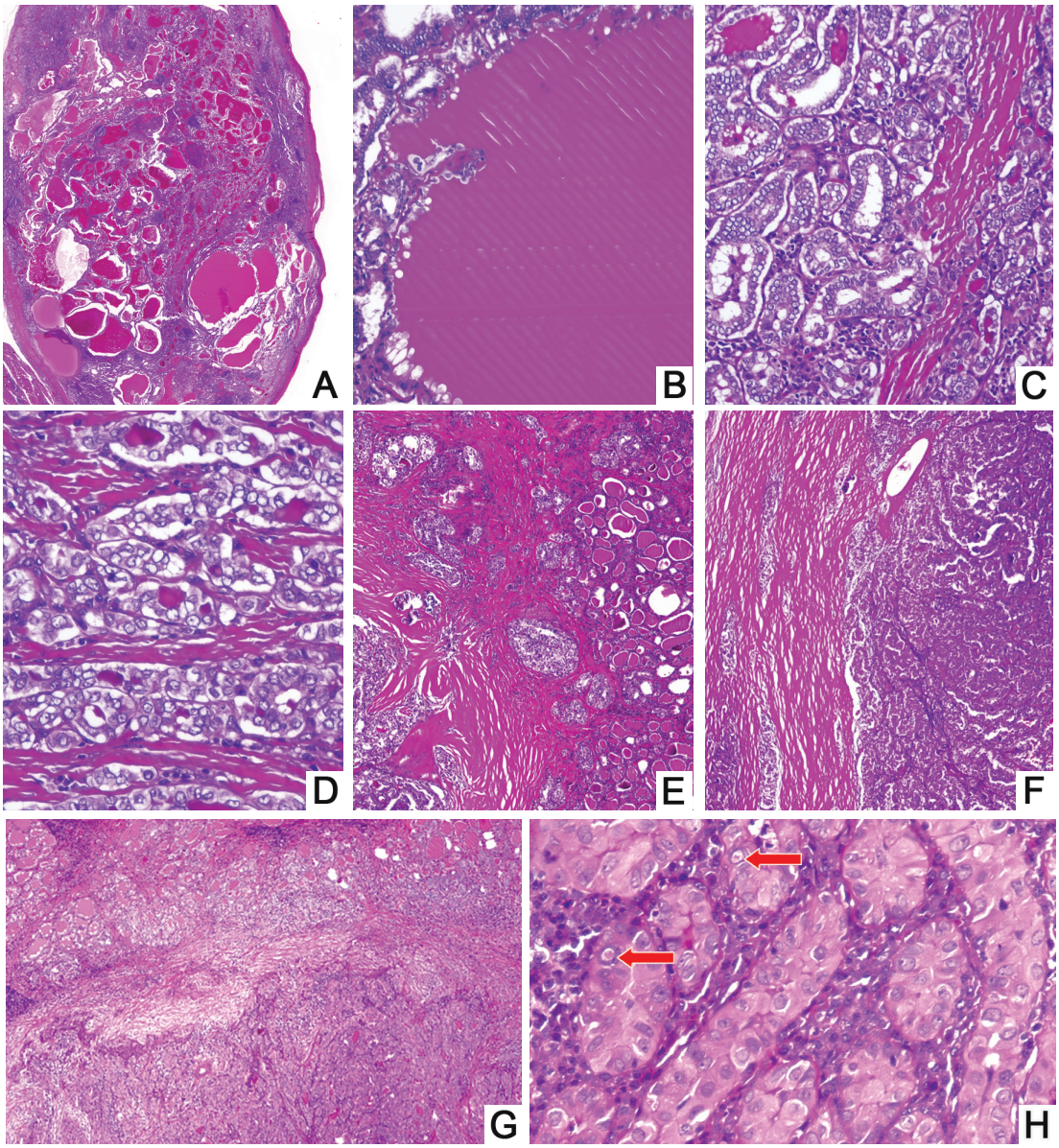

Figure 4.10. Multiple papillary thyroid carcinomas revealed in the same patient. (A) Encapsulated tumor in the right lobe sized 22 mm with the mixed growth pattern. Haematoxylin and eosin, original magnification x10-panoramic. (B) High-power image of the same tumor. Macrofollicular growth pattern. Haematoxylin and eosin, original magnification x100. (C) High-power image of the same tumor. Microfollicular growth pattern. Haematoxylin and eosin, original magnification x100. (D) High-power image of the same tumor, different area. Solid-follicular component between fibrotic stroma. Haematoxylin and eosin, original magnification x200. (E) Nonencapsulated tumor in the right lobe sized 6 mm with the solid-follicular growth pattern and small papillary loci. Marked stromal fibrosis. Haematoxylin and eosin, original magnification x20-panoramic. (F) Encapsulated tumor in the left lobe sized 11 mm with the papillary-solid growth pattern. Evident tumor capsule invasion. Haematoxylin and eosin, original magnification x20. (G) Nonencapsulated oxyphilic-cells tumor in the left lobe sized 9 mm with the solid and trabecular growth pattern. Evident intratumoral and peritumoral thyroiditis. Haematoxylin and eosin, original magnification x20. (H) High-power of the same tumor. Intranuclear pseudoinclusions in oxyphilic tumor cells. Haematoxylin and eosin, original magnification x200.

Extrathyroidal extension was registered only in 1.2% cases in adults among fully encapsulated tumors with subcapsular localization; regional metastases were found in isolated cases in each age group. Regional lymph node metastases in adults were more frequently seen in the classic papillary variant than in follicular or solid variants (Table 4.10). A higher rate of lymph node metastases in encapsulated classical papillary thyroid carcinomas was also reported in an independent study [51]. No distant metastases to the lung in patients with encapsulated PTC were found during the whole period of observation (Table 4.10). This is in excellent agreement with the opinion of J. Rosai: "Papillary thyroid carcinoma totally surrounded by a capsule may still be associated with nodal metastases, but the incidence of distant metastases or tumor death is nearly zero" [4].

Table 4.7
Size of multifocal papillary thyroid carcinomas in patients born before Chernobyl

Biggest lesion, mm	**Children aged up to 14 years at surgery**									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	-	-	-	-	-	-
6-10	-	-	-	-	-	-	-	-	-	-
11-20	3	23.1	1	11.1	-	-	-	-	4	18.2
21-30	4	30.7	-	-	-	-	-	-	4	18.2
31-40	1	7.7	-	-	-	-	-	-	1	4.5
41-50	3	23.1	1	11.1	-	-	-	-	4	18.2
51-60	1	7.7	4	44.5	-	-	-	-	5	22.7
>60	1	7.7	3	33.3	-	-	-	-	4	18.2
Total	**13**	100	**9**	100		**0**	-	-	**22**	100
	Adolescents aged from 15 to 18 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	-	-	-	-	-	-
6-10	-	-	-	-	1	10.0	-	-	1	5.2
11-20	-	-	4	50.0	4	40.0	-	-	8	42.1
21-30	-	-	1	12.5	3	30.0	-	-	4	21.1
31-40	1	100	2	25.0	1	10.0	-	-	4	21.1
41-50	-	-	1	12.5	1	10.0	-	-	2	10.5
51-60	-	-	-	-	-	-	-	-	-	-
>60	-	-	-	-	-	-	-	-	-	-
Total	**1**	100	**8**	100	**10**	100	-	-	**19**	100

Continuation of Table 4.7

	Adults aged from 19 to 42 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	2	4.8	15	8.5	17	7.4
6-10	-	-	-	-	11	26.2	44	24.8	55	23.8
11-20	-	-	5	41.7	10	23.8	62	35.0	77	33.3
21-30	-	-	2	16.7	8	19.0	21	11.9	31	13.4
31-40	-	-	-	-	2	4.8	17	9.6	19	8.2
41-50	-	-	-	-	5	11.9	10	5.6	15	6.5
51-60	-	-	1	8.3	4	9.5	4	2.3	9	3.9
>60	-	-	4	33.3	-	-	4	2.3	8	3.5
Total	**0**	-	**12**	100	**42**	100	**177**	100	**231**	100
	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
up to 5	-	-	-	-	2	3.8	15	8.5	17	6.3
6-10	-	-	-	-	12	23.1	44	24.8	56	20.6
11-20	3	21.4	10	34.5	14	26.9	62	35.0	89	32.7
21-30	4	28.7	3	10.3	11	21.2	21	11.9	39	14.3
31-40	2	14.3	2	6.9	3	5.8	17	9.6	24	8.8
41-50	3	21.4	2	6.9	6	11.5	10	5.6	21	7.7
51-60	1	7.1	5	17.3	4	7.7	4	2.3	14	5.1
>60	1	7.1	7	24.1	-	-	4	2.3	12	4.5
Total	**14**	100	**29**	100	**52**	100	**177**	100	**272**	100

In nonencapsulated PTCs, the most pronounced intrathyroidal extension was noted in the diffuse-sclerosing variant (Fig. 4.5A) apparently stemming from its definition. For patients of all ages with diffuse sclerosing variant of PTC, a high frequency of extrathyroidal extension and metastases to lymph nodes was registered as well (Table 4.9). Metastases most commonly had papillary-solid structure with marked signs of oxyphilic-cell and squamous-cell metaplasia (Fig. 4.12). In children and adolescents with diffuse sclerosing variant of PTC, distant metastases to the lung were also found in 23.5 and 25.0% cases, respectively. These data additionally attest to the aggressive behaviour of this variant of PTC [1,3,4,29], but, as already mentioned above, the small number of cases does not allow establishing a link between such a tumor structure and exposure to Chernobyl fallout.

Our review of the three main subtypes of PTC (classic papillary, follicular and solid) and of the mixed-type PTC shows that the identification of the (formerly) "childhood variant" [17,38] which combines - as already pointed out - tumors with solid, follicular, and solid-follicular structures, was pathologically justified. All signs of aggressiveness: vascular invasion, multifocality, extrathyroidal extension, regional lymph node and distant metastases were most pronounced namely in children who had nonencapsulated tumors with such structure (Table 4.9).

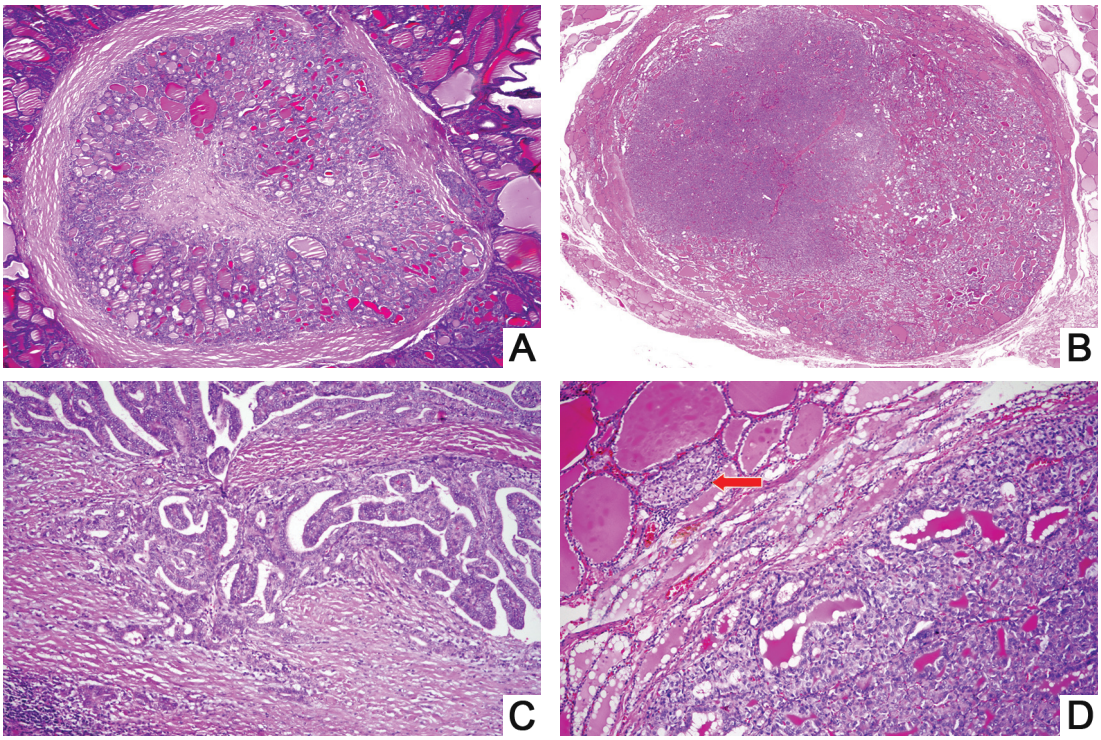

Figure 4.11. Fully encapsulated papillary thyroid carcinomas. (A) Tumor with the follicular growth pattern. Evident tumor capsule invasion and central fibrosis. Haematoxylin and eosin, original magnification x20-panoramic. (B) Tumor with the solid-follicular growth pattern. Haematoxylin and eosin, original magnification x20-panoramic. (C) Tumor with the papillary growth pattern. Tumor capsule invasion. Haematoxylin and eosin, original magnification x50. (D) Tumor with solid-follicular growth pattern. Intrathyroidal extension of the tumor. Haematoxylin and eosin, original magnification x50.

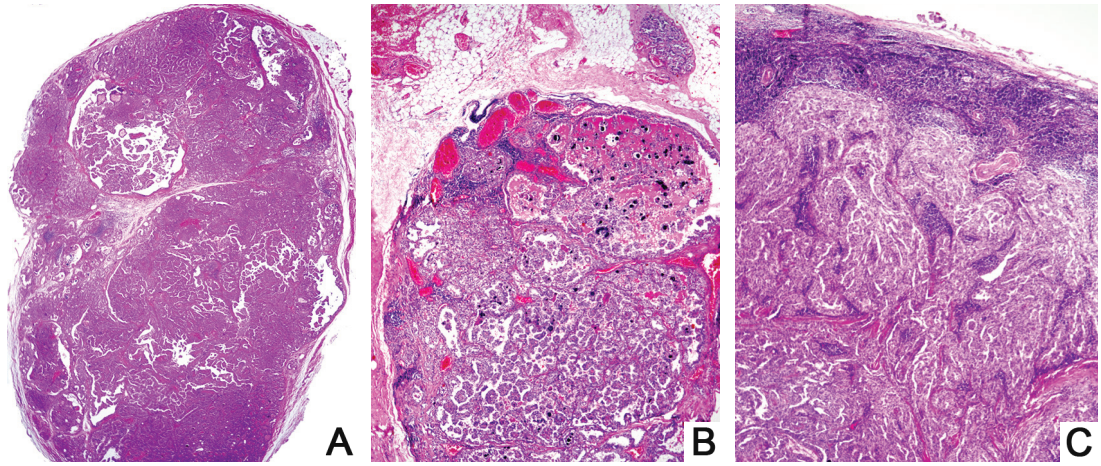

Figure 4.12. Lymph node metastases of the diffuse-sclerosing variant of papillary thyroid carcinomas. (A) Papillary-solid growth pattern. Haematoxylin and eosin, original magnification x10-panoramic. (B) Papillary growth pattern. Cystic changes, numerous psammoma bodies, extra-lymph node extension. Haematoxylin and eosin, original magnification x20. (C) Papillary-solid growth pattern. Evident oxyphilic-cell metaplasia. Haematoxylin and eosin, original magnification x20.

Table 4.8
Invasive properties of papillary thyroid carcinoma in patients born before Chernobyl

Subtype (number)	Intrathyroidal extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
Children aged up to 14 years at surgery														
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (30)	21	70.0	20	66.7	1	3.3	-	-	14	46.7	16	53.3	4	13.3
FV (65)	32	49.2	40	61.5	25	38.5	4	6.2	43	66.2	38	58.5	21	32.3
SV (57)	33	57.9	43	75.4	23	40.4	8	12.3	50	87.7	45	78.9	19	33.3
PFV (16)	8	56.0	9	56.3	1	6.3	1	6.3	8	50.0	11	68.8	4	25.0
PSV (11)	5	45.5	5	45.5	2	18.2	1	9.1	4	36.4	9	81.8	1	9.1
PSFV (3)	2	66.7	1	33.3	-	-	-	-	2	66.7	3	100	-	-
SFV (73)	50	68.5	54	74.0	31	42.5	8	11.0	58	79.5	53	72.6	23	31.5
DSV (17)	17	100	17	100	-	-	-	-	7	41.2	9	52.9	4	23.5
Warthin (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cribriform (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (272)	168	**61.8**	189	**69.5**	83	**30.5**	22	**8.1**	186	**68.4**	184	**67.6**	76	**27.9**
Adolescents aged from 15 to 18 years at surgery														
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (48)	26	54.2	22	45.8	3	6.3	3	6.3	18	37.5	22	45.8	5	10.4
FV (47)	24	51.1	21	44.7	11	23.4	1	2.1	19	40.4	22	46.8	6	12.8
SV (24)	17	70.8	11	45.8	11	45.8	3	12.5	15	62.5	10	41.7	4	16.7
PFV (38)	17	44.7	13	34.2	5	13.2	4	10.5	16	42.1	20	52.6	5	13.2
PSV (18)	12	66.7	6	33.3	2	11.1	3	16.7	9	50.0	13	72.2	4	22.2
PSFV (5)	3	60.0	3	60.0	1	20.0	-	-	3	60.0	5	100	2	40.0
SFV (40)	26	65.0	20	76.9	9	22.5	5	12.5	21	52.5	22	55.0	12	30.0
DSV (4)	4	100	4	100	-	-	-	-	4	100	4	100	1	25.0
Warthin (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cribriform (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (225)	129	**57.3**	100	**44.4**	42	**18.7**	19	**8.4**	105	**46.7**	118	**52.4**	39	**17.3**

Continuation of Table 4.8

Subtype (number)	Adults aged from 19 to 42 years at surgery													
	Intrathyroidal extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (667)	200	30.0	203	30.4	29	4.3	69	10.3	133	19.9	225	33.7	10	1.5
FV (332)	100	30.1	58	17.5	49	14.8	38	11.4	63	19.0	79	23.6	8	2.4
SV (118)	40	33.9	39	33.1	37	31.4	17	14.4	41	34.7	35	29.7	6	5.1
PFV (422)	170	40.3	129	30.6	47	11.1	45	10.7	136	32.2	192	45.5	19	4.5
PSV (143)	61	42.7	65	45.5	21	14.7	20	14.0	47	32.9	59	41.3	9	6.3
PSFV (49)	25	51.0	20	41.7	16	32.7	7	14.3	14	28.6	17	34.7	4	8.2
SFV (223)	65	29.1	50	22.4	49	22.0	32	14.3	48	21.5	63	23.8	9	4.0
DSV (6)	6	100	6	100	-	-	-	-	5	83.3	6	100	-	-
Warthin (19)	5	26.3	6	31.6	3	15.8	3	15.8	4	21.1	7	36.8	-	-
Cribriform (2)	2	100	1	50.0	1	50.0	-	-	-	-	1	50.0	-	-
Total (1981)	674	**34.0**	577	**29.1**	252	**12.7**	231	**11.7**	491	**24.8**	674	**34.0**	65	**3.3**

Table 4.9

Invasive properties of nonencapsulated and partly encapsulated papillary thyroid carcinoma in patients born before Chernobyl

Subtype (number)	Children aged up to 14 years at surgery													
	Intrathyroidal extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (26)	18	69.2	17	65.4	1	3.8	-	-	14	53.8	15	57.7	4	15.4
FV (57)	31	54.4	38	66.7	21	36.8	4	7.0	43	75.4	37	64.9	21	36.8
SV (54)	31	57.4	42	77.8	22	40.7	8	14.8	50	92.6	44	81.5	19	35.2
PFV (14)	7	50.0	9	64.3	1	7.1	1	7.1	8	57.1	10	71.4	4	28.6
PSV (11)	5	45.5	5	45.5	2	18.2	1	9.1	4	36.4	9	81.8	1	9.1
PSFV (3)	2	66.7	1	33.3	-	-	-	-	2	66.7	3	100	-	-
SFV (69)	49	71.0	53	71.0	30	43.5	8	11.6	58	84.1	53	76.8	23	33.3
DSV (17)	17	100	17	100	-	-	-	-	7	41.2	9	52.9	4	23.5
Warthin (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cribriform (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (251)	160	**63.7**	182	**72.5**	77	**30.7**	22	**8.8**	186	**74.1**	180	**71.7**	76	**30.3**

Continuation of Table 4.9

Adolescents aged from 15 to 18 years at surgery

Subtype (num)	Intrathyroidal extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (40)	22	55.0	21	52.5	3	7.5	3	7.5	18	45.0	20	50.0	5	12.5
FV (40)	21	52.5	19	47.5	11	27.5	1	2.5	19	47.5	21	52.5	6	15.0
SV (23)	16	69.5	10	43.5	11	47.8	3	13.0	15	65.2	10	43.5	4	17.4
PFV (31)	15	48.4	12	38.7	5	16.1	3	9.7	16	51.6	20	64.5	5	16.1
PSV (15)	11	73.3	6	40.0	2	13.3	3	20.0	9	60.0	13	86.7	4	26.7
PSFV (5)	3	60.0	3	60.0	1	20.0	-	-	3	60.0	5	100	2	40.0
SFV (31)	23	74.2	18	58.1	7	22.6	5	16.1	21	67.7	22	71.0	12	38.7
DSV (4)	4	100	4	100	-	-	-	-	4	100	4	100	1	25.0
Warthin (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cribriform (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total (190)	115	**60.5**	93	**48.9**	40	**21.1**	18	**9.5**	105	**55.3**	115	**60.5**	39	**20.5**

Adults aged from 19 to 42 years at surgery

	Intrathyroidal extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (499)	145	29.1	170	34.1	21	4.2	57	11.4	129	25.9	210	42.1	10	2.0
FV (178)	73	41.0	48	27.0	24	13.4	16	9.0	63	35.4	72	40.4	8	4.5
SV (74)	33	44.6	36	48.6	24	32.4	13	32.4	40	54.1	33	44.6	6	8.1
PFV (336)	134	39.9	113	33.6	37	11.0	36	10.7	136	40.5	185	55.0	19	5.7
PSV (128)	53	41.4	61	47.7	16	12.5	20	15.6	45	35.2	58	45.3	9	7.0
PSFV (33)	18	54.5	17	51.5	13	39.4	4	12.1	14	42.4	17	51.5	4	12.1
SFV (124)	45	36.3	46	37.1	24	19.4	24	19.4	48	38.7	52	41.9	9	7.3
DSV (6)	6	100	6	100	-	-	-	-	5	83.3	6	100	-	-
Warthin (19)	5	26.3	6	31.6	3	15.8	3	15.8	4	21.1	7	36.8	-	-
Cribriform (2)	2	100	1	50.0	1	50.0	-	-	-	-	1	50.0	-	-
Total (1399)	514	**36.7**	504	**36.0**	163	**11.7**	173	**12.4**	484	**34.6**	641	**45.8**	65	**4.6**

Table 4.10
Invasive properties of fully encapsulated papillary thyroid carcinoma in patients born before Chernobyl

Children aged up to 14 years at surgery

Subtype (number)	Tum capsule invasion		Intrathyroid extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroid extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (4)	4	100	3	75.0	3	75.0	-	-	-	-	-	-	1	25.0	-	-
FV (8)	7	87.5	1	12.5	2	25.0	4	50.0	-	-	-	-	1	12.5	-	-
SV (3)	3	100	2	66.7	1	33.8	1	33.3	-	-	-	-	1	33.3	-	-
PFV (2)	2	100	1	50.0	-	-	-	-	-	-	-	-	1	50.0	-	-
PSV (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PSFV (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SFV (4)	1	25.0	1	25.0	1	25.0	1	25.0	-	-	-	-	-	-	-	-
Total (21)	17	**81.0**	8	**38.1**	7	**33.3**	6	**28.6**	0	**-**	0	**-**	4	**19.0**	0	**-**

Adolescents aged from 15 to 18 years at surgery

Subtype (number)	Tum capsule invasion		Intrathyroid extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroid extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (8)	8	100	4	50.0	1	12.5	-	-	-	-	-	-	2	25.0	-	-
FV (7)	7	100	3	42.9	2	28.6	-	-	-	-	-	-	1	14.8	-	-
SV (1)	1	100	1	100	1	100	-	-	-	-	-	-	-	-	-	-
PFV (7)	7	100	2	28.6	1	14.3	-	-	1	14.3	-	-	-	-	-	-
PSV (3)	3	100	1	33.3	-	-	-	-	-	-	-	-	-	-	-	-
PSFV (0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SFV (9)	9	100	3	33.3	2	22.2	2	22.2	-	-	-	-	-	-	-	-
Total (35)	35	**100**	14	**40.0**	7	**20.0**	2	**5.7**	1	**2.9**	0	**-**	3	**8.6**	0	**-**

Adults aged from 19 to 42 years at surgery

Subtype (number)	Tum capsule invasion		Intrathyroid extension		Lymphatic invasion		Vascular invasion		Multifocality		Extrathyroidal extension		Regional mts		Distant mts	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%	number	%
PV (168)	167	99.4	55	32.7	33	19.6	8	4.8	12	7.1	4	2.4	15	8.9	-	-
FV (154)	129	83.8	27	17.5	10	6.5	25	16.2	22	14.3	-	-	7	4.5	-	-
SV (44)	39	88.6	7	15.9	3	6.8	13	29.5	4	9.1	1	2.3	2	4.5	-	-
PFV (86)	82	95.3	36	41.9	16	18.6	10	11.6	9	10.5	-	-	7	8.1	-	-
PSV (15)	13	86.7	8	53.3	4	26.7	5	33.3	-	-	2	13.3	1	6.7	-	-
PSFV (16)	14	87.5	7	43.8	3	18.8	3	18.8	3	18.8	-	-	-	-	-	-
SFV (99)	82	82.8	20	20.2	4	4.0	25	25.3	8	8.1	-	-	1	1.0	-	-
Total (582)	531	**91.2**	160	**27.5**	73	**12.5**	89	**15.3**	58	**10.0**	7	**1.2**	33	**5.7**	0	**-**

By contrast, in adolescents and adults with tumors of such structural "combination", no signs of the higher aggressiveness were found. This may probably be due to the fact that the architecture of the follicular variant in adolescents and adults differs from that in children. In older patients, the solid component is absent or confined to very small foci in the areas of invasive growth. On the other hand, when combining tumors with the solid and solid-follicular structure in adolescents and adults, the frequency of extrathyroidal extension was significantly higher ($p<0.05$) as compared to other subtypes: in adolescents 66.7% (36/54 cases of the solid and solid-follicular subtypes) vs 50.7% (69/136 cases of other subtypes), and 44.4% (88/198 cases of the solid and solid-follicular subtypes) vs 33.0% (396/1201 cases of other subtypes) in adults. In addition, in the adult group, such a pooling showed a significantly higher frequency, as compared to other subtypes, of vascular invasion ($p<0.001$), multifocality ($p<0.01$) and distant metastases (Table 4.9). Thus, despite the finding that the prevalence of PTCs with the solid and solid-follicular structure was significantly declining with increasing patients' age and latency, morphological signs of aggressiveness in such tumors were still preserved.

According to the WHO classification [5], *papillary microcarcinoma* represented by the tumors sized up to 10 mm is considered to be an independent subtype. We already noted that the prevalence of microcarcinomas in the groups under study was significantly rising with increasing age of patients and latency (Table 4.3), i.e. time elapsed after Chernobyl. The presence of the "full" capsule (Fig. 4.13 A) was found in 20.8% tumors sized up to 5 mm (26/125) and in 22.4% (83/170) sized from 6 to 10 mm, i.e. most "small" tumors were nonencapsulated (Fig. 4.13 B-C).

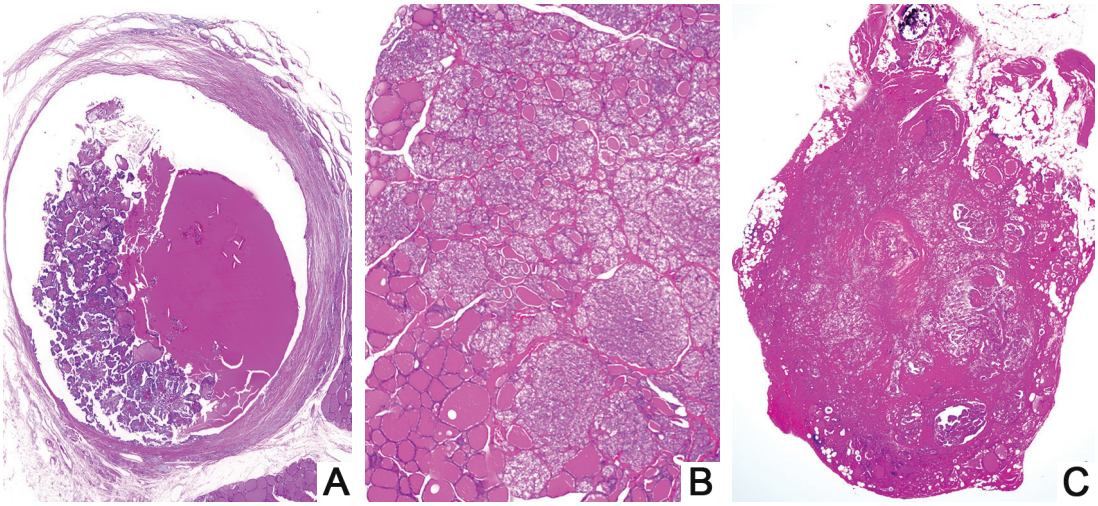

Figure 4.13. Papillary microcarcinomas. (A) Fully encapsulated tumor sized 7 mm with the papillary growth pattern. Haematoxylin and eosin, original magnification x20-panoramic. (B) Nonencapsulated tumor sized 7 mm with the solid growth pattern. Tumoral extension to the thyroid capsule and adjacent thyroid tissue. Haematoxylin and eosin, original magnification x20. (C) Tumor sized 5 mm with the papillary-solid growth pattern. Extrathyroidal extension, marked intratumoral fibrosis. Haematoxylin and eosin, original magnification x10.

Microcarcinomas, similarly to large size PTCs, may have different structure: classic papillary (Fig. 4.13 A), follicular, solid (Fig. 4.13 B) or mixed (Fig. 4.13 C). No statistically significant predominance of any histological variant – among both encapsulated and nonencapsulated tumors – was found in microcarcinomas (Table 4.11, 4.12).

The invasive features of microcarcinoma depended on tumor size and encapsulation. Encapsulated tumors sized up to 5 mm, regardless of architecture, were minimally invasive in all 26 cases. Although tumor cells might display signs of invasion into the tumor capsule, not a single case showed spread outside its limits; there were no signs of vascular invasion or metastases to lymph nodes. In three cases (11.5%) diagnosed during the last period of observation microtumors were multiple, as was mentioned above.

Table 4.11
Subtypes of fully encapsulated micro-PTC in patients born before Chernobyl

sized from 1 to 5 mm

All age groups

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Subtype	number	%	number	%	number	%	number	%	number	%
PV	-	-	-	-	2	40.0	7	33.3	9	34.7
FV	-	-	-	-	1	20.0	7	33.3	8	30.8
SV	-	-	-	-	-	-	1	4.8	1	3.8
PFV	-	-	-	-	-	-	2	9.5	2	7.7
PSV	-	-	-	-	1	20.0	1	4.8	2	7.7
PSFV	-	-	-	-	-	-	1	4.8	1	3.8
SFV	-	-	-	-	1	20.0	2	9.5	3	11.5
Warthin	-	-	-	-	-	-	-	-	-	-
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	**5**	100	**21**	100	**26**	100

sized from 6 to 10 mm

All age groups

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	-	-	3	30.0	5	35.7	15	25.0	23	27.4
FV	-	-	2	20.0	4	28.6	12	20.0	18	21.4
SV	-	-	1	10.0	-	-	9	15.0	10	11.9
PFV	-	-	2	20.0	3	21.5	10	16.7	15	17.8
PSV	-	-	2	20.0	1	7.1	2	3.3	5	6.0
PSFV	-	-	-	-	-	-	5	8.3	5	6.0
SFV	-	-	-	-	1	7.1	7	11.7	8	9.5
Warthin	-	-	-	-	-	-	-	-	-	-
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	-	-	**10**	100	**14**	100	**60**	100	**84**	100

Encapsulated microcarcinomas sized from 6 to 10 mm were also minimally invasive in general. However, in two cases out of 84 (2.4%), in the presence of follicular or papillary-follicular structures, there were metastases to lymph nodes (N1a); in 7 cases (8.3%) signs of lymphatic invasion, and in 7 cases (8.3%) multiple microtumors were seen.

Nonencapsulated microcarcinomas, similarly to the tumors of larger size, were characterized by the more pronounced invasive features as compared to encapsulated ones. Even very small tumors (up to 5 mm) of the follicular, solid, and mixed structure displayed signs of moderate intrathyroidal spread in 11.1% cases (11/99), lymphatic invasion in 8.1% (8/99), vascular invasion in 1.0% (1/99), extrathyroidal extension in 2.0% (2/99, Fig. 4.13C), and metastases to lymph nodes (N1a) in 6.1% of cases (6/99).

Table 4.12
Subtypes of nonencapsulated and partly encapsulated micro-PTC in patients born before Chernobyl

sized from 1 to 5 mm

Subtype	**All age groups**									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	1	100	-	-	7	33.3	10	13.0	18	18.2
FV	-	-	-	-	6	28.5	17	22.1	23	23.2
SV	-	-	-	-	1	4.8	9	11.7	10	10.1
PFV	-	-	-	-	3	14.3	13	16.9	16	16.2
PSV	-	-	-	-	1	4.8	9	11.7	10	10.1
PSFV	-	-	-	-	1	4.8	-	-	1	1.0
SFV	-	-	-	-	2	9.5	18	23.3	20	20.2
Warthin	-	-	-	-	-	-	1	1.3	1	1.0
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	**1**	100	-	-	**21**	100	**77**	100	**99**	100

sized from 6 to 10 mm

	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PV	-	-	2	18.2	21	38.2	75	34.7	98	34.3
FV	1	25.0	1	9.1	8	14.5	28	13.0	38	13.3
SV	2	50.0	1	9.1	1	1.8	10	4.6	14	4.9
PFV	-	-	2	18.2	15	27.3	47	21.8	64	22.4
PSV	-	-	2	18.2	5	9.1	26	12.0	33	11.5
PSFV	-	-	-	-	-	-	9	4.2	9	3.1
SFV	1	25.0	3	27.2	5	9.1	20	9.2	29	10.1
Warthin	-	-	-	-	-	-	1	0.5	1	0.4
Cribriform	-	-	-	-	-	-	-	-	-	-
Total	**4**	100	**11**	100	**55**	100	**216**	100	**286**	100

Invasiveness of the tumors sized from 6 to 10 mm was significantly higher compared to that of microcarcinomas sized up to 5 mm: intrathyroidal extension was observed in 22.0% (63 out of 286 cases, p=0.0178); lymphatic invasion in 24.8% (71/286, p=0.0003); extrathyroidal extension in 16.1% (46/286, p=0.0001); lymph node metastases in 23.3% (67/286, p=0.0001). However, all these were significantly lower than in nonencapsulated

carcinomas sized more than 10 mm for all age groups (Table 4.9). It should be emphasized that not in a single case of PTC sized up to 10 mm distant metastases to the lung were detected in the course of postoperative follow-up of patients.

In Belarus, by contrast with Ukraine, the prevalence of post-Chernobyl microcarcinomas was much higher: 39.2% just in children operated on at the age under 15 years [52,53]; in some cases distant metastases to the lung were identified [54]. Thus, tumor size alone cannot be a determinant of the choice of treatment tactics.

Table 4.13
Concomitant thyroid diseases in patients with micro-PTC born before Chernobyl

sized from 1 to 5 mm

All age groups

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Pathology	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	-	-	-	-	-	-
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	5/26	19.2	19/98	19.4	24/125	19.2
Nodule	-	-	-	-	6/26	23.1	15/98	15.4	21/125	16.8
MNG	1/1	-	-	-	4/26	15.4	18/98	18.4	23/125	18.4
Graves'	-	-	-	-	4/26	15.4	22/98	22.4	26/125	20.8
Chronic thyroiditis	-	-	-	-	4/26	15.4	6/98	6.1	10/125	8.0
Total	1/1	-	0	-	23/26	**88.5**	80/98	**81.6**	104/125	**83.2**

sized from 6 to 10 mm

All age groups

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	-	-	-	-	-	-
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	8/69	11.6	16/276	5.8	24/370	6.5
Nodule	-	-	3/21	14.3	7/69	10.1	29/276	10.5	39/370	10.5
MNG	-	-	2/21	9.5	3/69	4.3	20/276	7.2	25/370	6.8
Graves'	-	-	-	-	-	-	2/276	0.7	2/370	0.5
Chronic thyroiditis	-	-	3/21	14.3	3/69	4.3	62/276	22.4	68/370	18.4
Total	0/4	-	8/21	**38.1**	21/69	**30.4**	129/276	**46.6**	158/370	**42.7**

Modern diagnostic methods allow detection of tumors even of minimal size. Our analysis of the presented material shows that thyroid nodule, multiple nodules, and Graves' disease were the reason for surgical intervention in 75.2% of cases of microcarcinoma sized up to 5 mm, and only in 24.3% cases sized from 6 to 10 mm. In the rest of cases, the papillary microcarcinoma was the main thyroid disease, and the main reason for surgery (Table 4.13). Concomitant chronic thyroiditis (presented in Table 4.13) was not the reason for surgery. Undoubtedly, tumors sized up to 1-2 mm could be detected incidentally only on microscopic examination of histological specimens from operated patients. If surgery was performed

for benign thyroid disease, e.g. multinodular goiter or Graves' disease (Fig. 4.14 A-B), such microcarcinomas are considered to be "occult". The presence of microcarcinomas sized 2 mm and larger may be suspected at a thorough examination of surgical material and during sample selection for subsequent microscopic analysis as demonstrated in the case of follicular adenoma (Fig. 4.14 C).

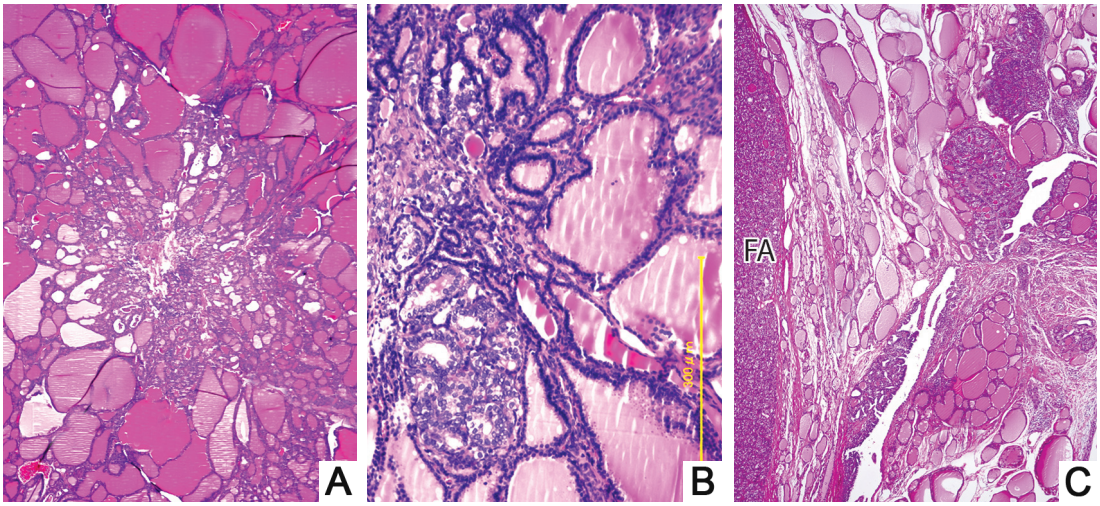

Figure 4.14. Occult papillary microcarcinomas. (A, B) Nonencapsulated micro-PTCs sized 2 mm and 0.3 mm with the follicular growth pattern revealed in patients with Graves' disease. Haematoxylin and eosin, original magnification x20, x100. (C) Nonencapsulated micro-PTCs sized 3 mm with the papillary-follicular growth pattern revealed in patients with follicular adenoma (FA). Haematoxylin and eosin, original magnification x20.

As a whole, our analysis of 2,478 cases of PTC showed the presence of concomitant thyroid disease in children in 11.0%, in adolescents in 16.9%, and in adults in 33.1% cases (Table 4.14). There was a significant ascending age-related linear trend (p=0.0001). The frequency of concomitant thyroid disease was also increasing in time from the first (1990-1994) to the last (2005-2010) period of observation (p=0.0001 for trend).

Besides the concomitant benign thyroid disease, additional coexisting thyroid cancers of smaller size than the PTC were noted in several cases; for this reason such tumors were referred to as "concomitant". In 2 cases these were FTCs and in 2 cases MTCs (Table 4.14). One MTC in a boy who underwent hemithyroidectomy at the age of 7 years for a PTC with the solid structure, was diagnosed at repeated surgery (completion thyroidectomy, neck dissection at level VI) two years later [11,42]. The second MTC, sized 7 mm, was identified in the left lobe of a female patient aged 25 years with a PTC sized 11 mm in the right lobe (Fig. 15 A-C).

Follicular adenomas were concomitant to PTC only in adults, beginning from the period 2000-2004 (2.8%), and their frequency somewhat increased, though insignificantly, in the last period of observation (4.4%).

Solitary and multiple (MNG) hyperplastic nodules were identified in all groups; their frequency significantly increased in a pairwise comparison: from 1.2% in children to 10.2% in adults (p=0.0001), and from 0.6% in 1990-1994 to 12.7% in 2005-2010 (p=0.0001); the ascending linear age and time trends were also significant (p=0.0001).

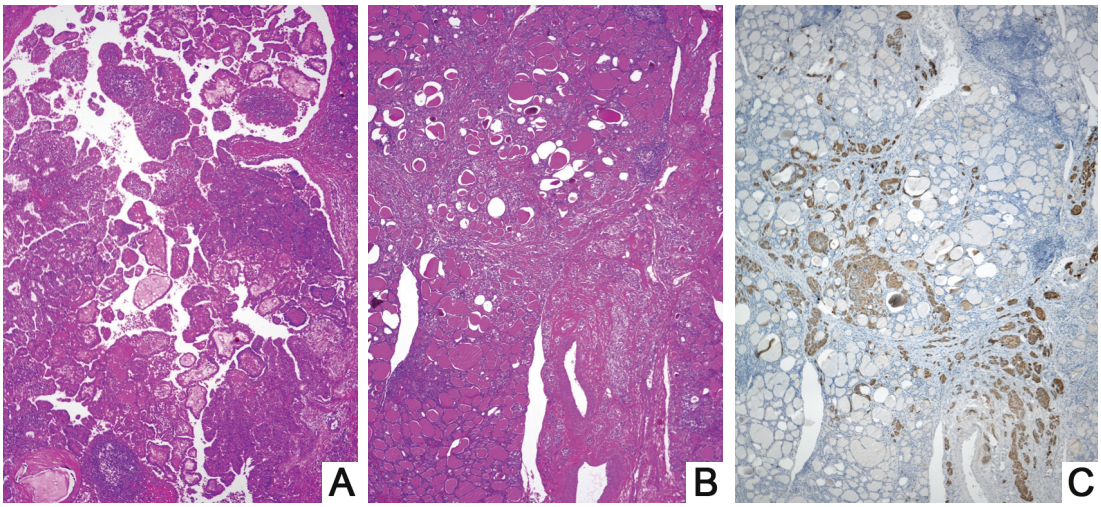

Figure 4.15. Two types of thyroid cancer coexisting in the same patient. (A) Nonencapsulated PTC with the papillary-solid growth pattern. Pronounced oxyphilic-cell metaplasia, Warthin-like loci. Haematoxylin and eosin, original magnification x20. (B) Nonencapsulated micro-medullary thyroid carcinoma with the solid growth pattern. Haematoxylin and eosin, original magnification x20. (C) Immunostaining of same area for calcitonin, original magnification x20.

PTCs in patients with Graves' disease were detected in a small number of cases (1.6%), only in adults and only beginning from 2000, i.e. from the age of 32 years old. These were mainly "occult" cancers or those detected at visual examination of surgical material. PTCs sized more than 10 mm in patients with Graves' disease were identified only in two cases.

The frequency of concomitant marked chronic thyroiditis of grade 3+/4+ (focal thyroiditis of grade 1+/2+ was not taken into account) practically did not differ between children and adolescents (9.6% and 10.7%, respectively) but was significantly increased in adults in a pairwise comparison with both children (p=0.0011) and adolescents (p=0.0105). A significant increase over time was observed between the first and last periods of observation (p=0.0007); the corresponding linear trend was also significant (p=0.0001).

Thus, most morphological characteristics of PTC in children, adolescents and adults who were aged up to 18 years during Chernobyl accident, have two main patterns: age-related and time-related, which displayed significantly descending or ascending linear trends.

Considering other types of thyroid cancer, it should be noted that ***follicular thyroid carcinoma*** (FTC) was detected in Ukraine in a small percentage of cases: 2.8% in children, 7.3% in adolescents, and 5.2% in adults (Table 4.2), similarly to other countries affected by the Chernobyl catastrophe [7,10,14].

The tumors under study represented encapsulated solitary nodules exceeding 2 cm in more than 70% of cases (in all age groups). Tumors sized less than 1 cm were detected in 1 out of 18 cases in adolescents (5.6%) and in 7 out of 111 cases in adults (6.3%). Most FTCs had microfollicular-solid structure. The oxyphilic-cell variant of FTC was not found in children, but was identified in 2 cases in adolescents (11.1%) and in 11 cases (9.9%) in adults. The diagnosis of "follicular thyroid carcinoma" was based on generally accepted criteria: marked "hook-like" or "mushroom-like" expansion through the tumor capsule and/or in blood vessels of the tumor capsule (Fig. 4.16).

Table 4.14
Concomitant thyroid diseases in patients with PTC born before Chernobyl

Children aged up to 14 years at surgery

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Pathology	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	-	-	-	-	-	-
MTC	1/127*	0.8	-	-	-	-	-	-	1/272	0.4
FA	-	-	-	-	-	-	-	-	-	-
Nodule	-	-	1/135	7.4	-	-	-	-	1/272	0.4
MNG	1/127	0.8	1/135	7.4	-	-	-	-	2/272	0.8
Graves'	-	-	-	-	-	-	-	-	-	-
Chronic thyroiditis	8/127	6.3	16/135	11.9	2/10	20.0	-	-	26/272	9.6
Total	10/127	**7.9**	18/135	**13.3**	2/10	**20.0**	**-**	**-**	30/272	**11.0**

* - MTC, 12 mm, n/encaps, sol,T3N0M0

Adolescents aged from 15 to 18 years at surgery

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	1/117*	0.9	-	-	1/225	0.4
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	2/81	2.5	4/117	3.4	-	-	6/225	2.7
Nodule	-	-	2/81	2.5	3/117	2.6	-	-	5/225	2.2
MNG	-	-	1/81	1.8	1/117	0.9	-	-	2/225	0.9
Graves'	-	-	-	-	-	-	-	-	-	-
Chronic thyroiditis	1/27	3.7	9/81	11.1	14/117	12.0	-	-	24/225	10.7
Total	1/27	**3.7**	14/81	**17.3**	23/117	**19.7**	**-**	**-**	38/225	**16.9**

* - FTC, 12 mm, microfol, T1bN0M0

Adults aged from 19 to 42 years at surgery

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	-	-	1/1214*	0.1	1/1981	0.05
MTC	-	-	-	-	-	-	1/1214**	0.1	1/1981	0.05
FA	-	-	-	-	17/605	2.8	53/1214	4.4	70/1981	3.5
Nodule	-	-	3/149	2.0	30/605	5.0	89/1214	7.3	122/1981	6.2
MNG	-	-	2/149	1.3	12/605	2.0	66/1214	5.4	80/1981	4.0
Graves'	-	-	-	-	6/605	1.0	26/1214	2.1	32/1981	1.6
Chronic thyroiditis	1/13	7.7	21/149	14.1	104/605	17.2	224/1214	18.5	350/1981	17.7
Total	1/13	**7.7**	26/149	**17.4**	169/605	**27.9**	460/1214	**37.9**	656/1981	**33.1**

* - FTC, 10 mm, T1aN0M0; ** - MTC, 7 mm, encaps, sol, T1aN0M0

Continuation of Table 4.14

	All age groups									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
FTC	-	-	-	-	1/732	0.1	1/1214	01	2/2478	0.1
MTC	1/167	0.6	-	-	-	-	1/1214	0.1	2/2478	0.1
FA	-	-	2/365	0.5	21/732	2.9	53/1214	4.4	76/2478	3.0
Nodule	-	-	6/365	1.6	33/732	4.5	89/1214	7.3	128/2478	5.2
MNG	1/167	0.6	4/365	1.1	13/732	1.8	66/1214	5.4	84/2478	3.4
Graves'	-	-	-	-	6/732	0.8	26/1214	2.1	32/2478	1.3
Chronic thyroiditis	10/167	6.0	46/365	12.6	120/732	16.4	224/1214	18.5	400/2478	16.1
Total	12/167	**7.2**	58/365	**15.9**	194/732	**26.5**	460/1214	**37.9**	724/2478	**29.2**

Invasion of two and more capsular vessels was found in 108 out of 137 cases (78.8%); therefore, 21.2% of FTCs in children and adolescents born before Chernobyl were minimally invasive. An aggressive widely invasive FTC with extension to extrathyroidal connective tissue and the trachea, with regional metastases to lymph nodes and distant metastases to the lung (pT4aN1aM1) was observed only in one case in an adolescent female patient aged 15 years whom we described earlier [42].

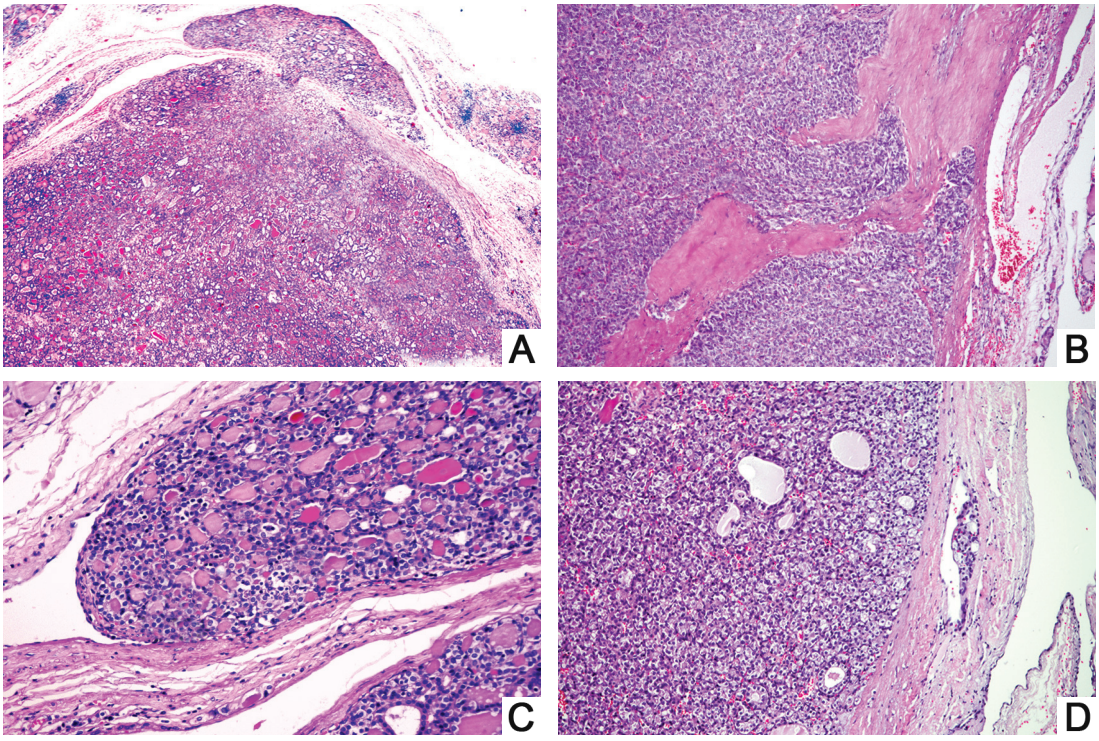

Figure 4.16. Follicular thyroid carcinomas. (A) Mushroom-like expansion through the capsule. Haematoxylin and eosin, original magnification x10. (B) Capsular invasion in a hook-like fashion. Haematoxylin and eosin, original magnification x50. (C, D) Vascular invasion. Aggregates of tumor cells are seen within the vessel lumen attached to the wall and covered by endothelium. Haematoxylin and eosin, original magnification x100, x50.

Among thyroid diseases concomitant to FTC, PTC (represented mostly by microcarcinomas sized up to 10 mm) was detected in 5 adults (Table 4.15). In 1 case, an encapsulated PTC sized 21 mm was smaller than the coexisting FTC nodule (35 mm), and therefore it was referred to the category of "concomitant disease". Solitary and multiple hyperplastic nodules were identified in 5.1%, chronic thyroiditis in the extratumoral tissue in 10.2% of cases.

There are different opinions regarding the impact of radiation exposure on the development of FTC. Some authors suggest the existence of such [3,26,55] while other [56] have not revealed significant link between FTC and previous exposure.

Our analysis of the frequency of FTC in Ukraine after Chernobyl in the group at high risk for radiation-induced thyroid cancer showed no significant differences between different age groups. However, a comparison of the frequency of FTC for different time periods after the accident revealed a significantly increasing linear trend (p=0.0334). Undoubtedly, 137 FTCs comprise too small number as compared to 2,478 PTCs, and further special investigations are needed to draw valid conclusions regarding association with thyroid dose.

Medullary thyroid carcinoma (MTC) in the cohort under study was diagnosed even less frequently: 2.4% in children, 0.4% in adolescents, and 1.5% in adults (Table 4.2). Tumor size varied from 4 to 75 mm; in 84.6% cases (33 out of 39) tumors were nonencapsulated, 87.2% carcinomas (34 out of 39) had the solid structure (Fig. 4.17), and only 12.8% displayed "spindle cell-solid" growth pattern. In all cases there was a positive immunohistochemical reaction with anti-calcitonin antibodies which confirmed the diagnosis of MTC. Seven out of 8 MTCs in children and adolescents (87.5%) were characterized by extrathyroidal extension, metastases to lymph nodes (T3N1bM0); 2 of these patients had distant metastases to the liver and brain (T3N1bM1). In adults, tumor aggressiveness was less pronounced: extrathyroidal extension and regional metastases were found in 12 out of 31 (38.7%) cases, distant metastases to the liver were detected in 1 female patient (3.2%). In the latter patient with MEN 2A syndrome (she had been operated for pheochromocytoma before), a papillary microcarcinoma sized 0.2 cm was identified (Fig. 4.18).

Here we do not analyze MTC in greater detail since many studies of post-Chernobyl thyroid cancer have not revealed any effect of radiation on the risk for development of tumors of this type [7,10,11,14].

Poorly differentiated thyroid carcinoma (PDTC) was diagnosed in 4 cases, all in adult female patients, accounting for 0.2% of all cancers under study (Table 4.2). Two of them were encapsulated, sized 26 and 35 mm, with trabecular-solid structure and invasion into tumor capsule and capsular vessels (T2N0M0). Both carcinomas developed in the presence of PTC as certain tumor areas displayed characteristic nuclear features. Two PDTCs were nonencapsulated, sized 50 and 52 mm, were of the insular and solid structure (T3N1bM0). In case of PDTC with the solid structure, the tumor was widely invasive (Fig. 4.19) with marked intrathyroidal and extrathyroidal extension, and necrotic areas. Tumor cells had signs of oxyphilic-cell metaplasia, were sometimes multinuclear, with marked nucleoli (Fig. 4.19 B). In some tumor areas, a focal positive reaction with anti-thyroglobulin antibodies was revealed (Fig. 4.19 C); the reaction with anti-TTF-1 antibodies was diffuse and strong (Fig. 4.19 D). A high proliferative activity of the tumor was confirmed by the reaction with anti-Ki67 antibodies. In many areas there were more than 50.0% of positively stained nuclei (Fig. 4.19 E). In such areas up to 20.0% of nuclei were also positive for TP53 (Fig. 4.19 F).

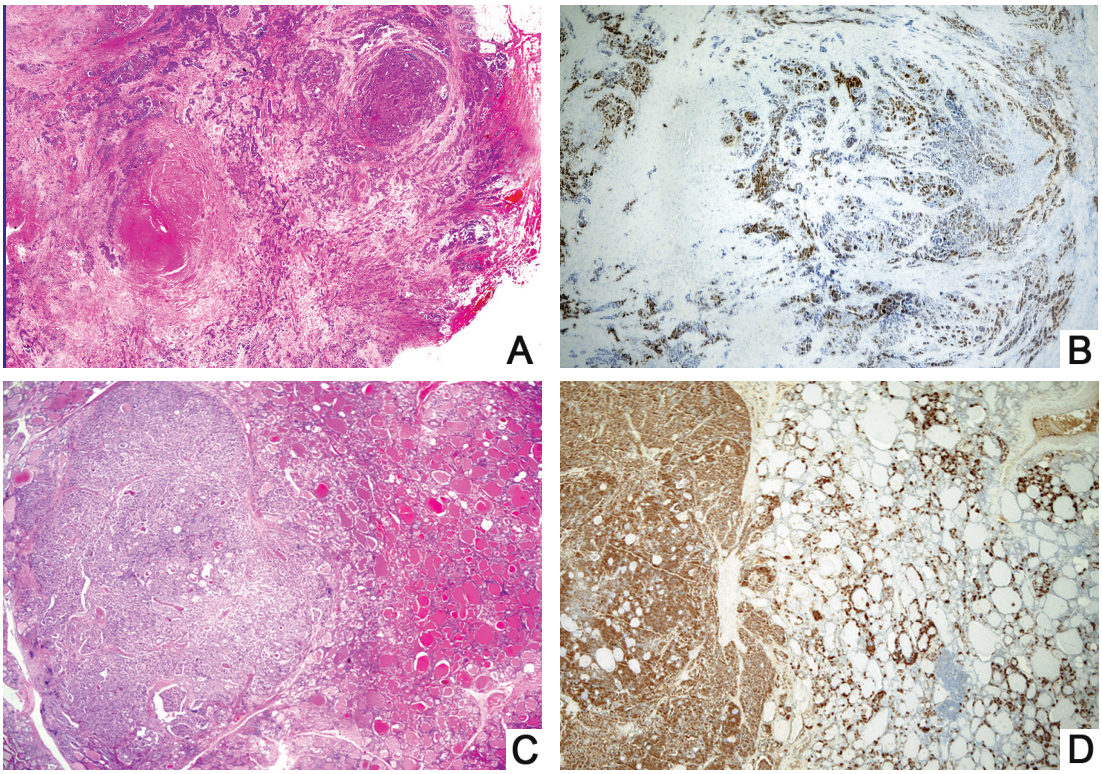

Figure 4.17. Medullary thyroid carcinoma. (A) Tumor with the solid growth pattern, marked stromal fibrosis, extrathyroidal extension and central necrosis. Haematoxylin and eosin, original magnification x10-panoramic. (B) Immunostaining for calcitonin of the same area, original magnification x20. (C) Tumor with the solid growth pattern and vascular invasion. Haematoxylin and eosin, original magnification x10. (D) Immunostaining for calcitonin of the same area. Marked C-cell hyperplasia in peritumoral areas, original magnification x10.

Table 4.15
Concomitant thyroid diseases in patients with FTC born before Chernobyl

	Children aged up to 14 years at surgery									
	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
Pathology	number	%	number	%	number	%	number	%	number	%
PTC	-	-	-	-	-	-	-	-	-	-
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	-	-	-	-	-	-
Nodule	-	-	-	-	-	-	-	-	-	-
MNG	-	-	-	-	-	-	-	-	-	-
Graves'	-	-	-	-	-	-	-	-	-	-
Total	0/2	-	0/6	-	-	-	-	-	0/8	-

Continuation of Table 4.15

Adolescents aged from 15 to 18 years at surgery

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	-	-	-	-	-	-	-	-	-	-
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	2	20.0	-	-	2	11.1
Nodule	-	-	-	-	1	10.0	-	-	1	5.6
MNG	-	-	1	14.3	-	-	-	-	1	5.6
Graves'	-	-	-	-	-	-	-	-	-	-
Chronic thyroiditis	1	-	-	-	1	10.0	-	-	2	11.1
Total	1/1	**-**	1/7	**14.3**	4/10	**40.0**	**-**	**-**	6/18	**33.3**

Adults aged from 19 to 40 years at surgery

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	-	-	-	-	2*	4.4	3*	4.9	5*	4.5
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	1	2.2	-	-	1	0.9
Nodule	-	-	-	-	1	2.2	5	8.2	6	5.4
MNG	-	-	-	-	2	4.4	4	6.6	6	5.4
Graves'	-	-	-	-	-	-	1	1.6	1	0.9
Chronic thyroiditis	-	-	-	-	4	8.9	8	13.1	12	10.8
Total	0/1	**-**	0/4	**-**	10/45	**22.2**	21/61	**34.4**	31/111	**27.9**

* - PTCs: 21 mm, encaps, FV, T2N0M0; 8 mm, partly encaps, FV, T1aN0M0; 7, 6, mm, n/encaps, FV, T1aN0M0; 5 mm, n/encaps, PFV, T1aN0M0

All age groups

	1990-1994		1995-1999		2000-2004		2005-2010		1990-2010	
	number	%	number	%	number	%	number	%	number	%
PTC	-	-	-	-	2	3.6	3	4.9	5	3.6
MTC	-	-	-	-	-	-	-	-	-	-
FA	-	-	-	-	3	5.5	-	-	3	2.2
Nodule	-	-	-	-	2	3.6	5	8.2	7	5.1
MNG	-	-	-	-	2	3.6	4	6.6	7	5.1
Graves'	-	-	-	-	-	-	1	1.6	1	0.7
Chronic thyroiditis	1	25.0	1	5.9	5	9.1	8	13.1	14	10.2
Total	1/4	**25.0**	1/17	**5.9**	14/55	**25.5**	21/61	**34.4**	37/137	**27.0**

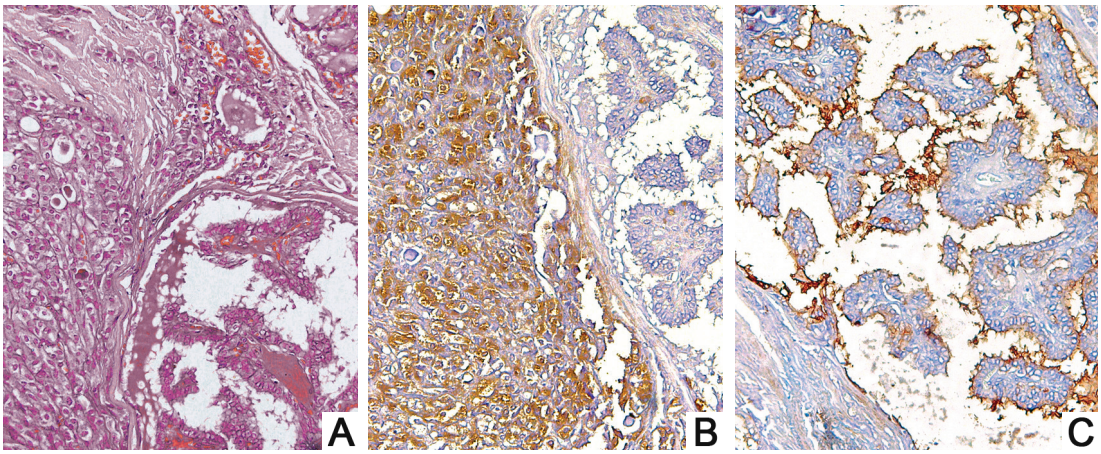

Figure 4.18. Coexistence of two different types of thyroid carcinomas in one patient. (A) Encapsulated papillary microcarcinoma sized 2 mm with the classic growth pattern and cystic changes seen in vicinity to the nonencapsulated medullary thyroid carcinoma with the solid growth pattern. Haematoxylin and eosin, original magnification x100. (B) Immunostaining for calcitonin of the same area, original magnification x100. (C) Immunostaining for thyroglobulin of the same area, original magnification x100.

In the most aggressive among all 4 PDTCs case, microfoci of PTC were also identified (Fig. 4.20). They were characterized by a strong reaction with antibodies to thyroglobulin (Fig. 4.20 D) and TTF-1 (Fig. 4.20 E), but unlike PDTC areas there were only isolated Ki67 positive nuclei (Fig. 4.20 F). TP53 expression was not detected in the PTC areas.

Tumor metastases completely replaced lymphatic nodes, and their structure and immunohistochemical characteristics did not differ from those of the main tumor (Fig. 4.21). The only difference was the lower frequency of nuclei positive for TP53: 5-10% (Fig. 4.21 F).

Modern handbooks of thyroid pathology do not consider the role of radiation in the development of PDTC [3,4]. However, at least some tumors may develop from preexisting well-differentiated PTC or FTC. It could not be ruled out that radiation may promote dedifferentiation of PTC cells. In our series, patients' age was 21 and 24 (encapsulated tumors) and 35 and 38 years old (nonencapsulated tumors), while typical mean age of patients with PDTC specified in handbooks is 55-60 years old. This issue, from our point of view, deserves particular attention in future, because 3 out of 4 cases of PDTC were detected in the last period of follow-up, namely, in 2008-2010.

Summarizing our data on pathology of thyroid cancer in Ukraine in the cohort aged from 0 to 18 years at the time of the Chernobyl accident, it should be noted that for the whole study period (1990-2010) the relative frequency of PTC exceeded 90.0% despite patients' age at surgery increased over this period from 4 (the youngest children operated in 1990) to 42 years old (the oldest adults operated in 2010). Thus, PTC is undoubtedly the type of radiogenic «Chernobyl» cancer; this may be considered a proven fact.

At the same time, a unique "structural portrait" of radiogenic Chernobyl PTC could not been established either by our group in Ukraine, or other authors [14,23,53,57]. Most likely, the development of PTC in patients of the group at risk (0 to 18 years at Chernobyl) followed the common mechanisms of thyroid carcinogenesis (yet displaying a higher or lower frequency of certain genetic abnormalities which correlate with structural changes in the tumor) that presumably underlie sporadic thyroid carcinogenesis.

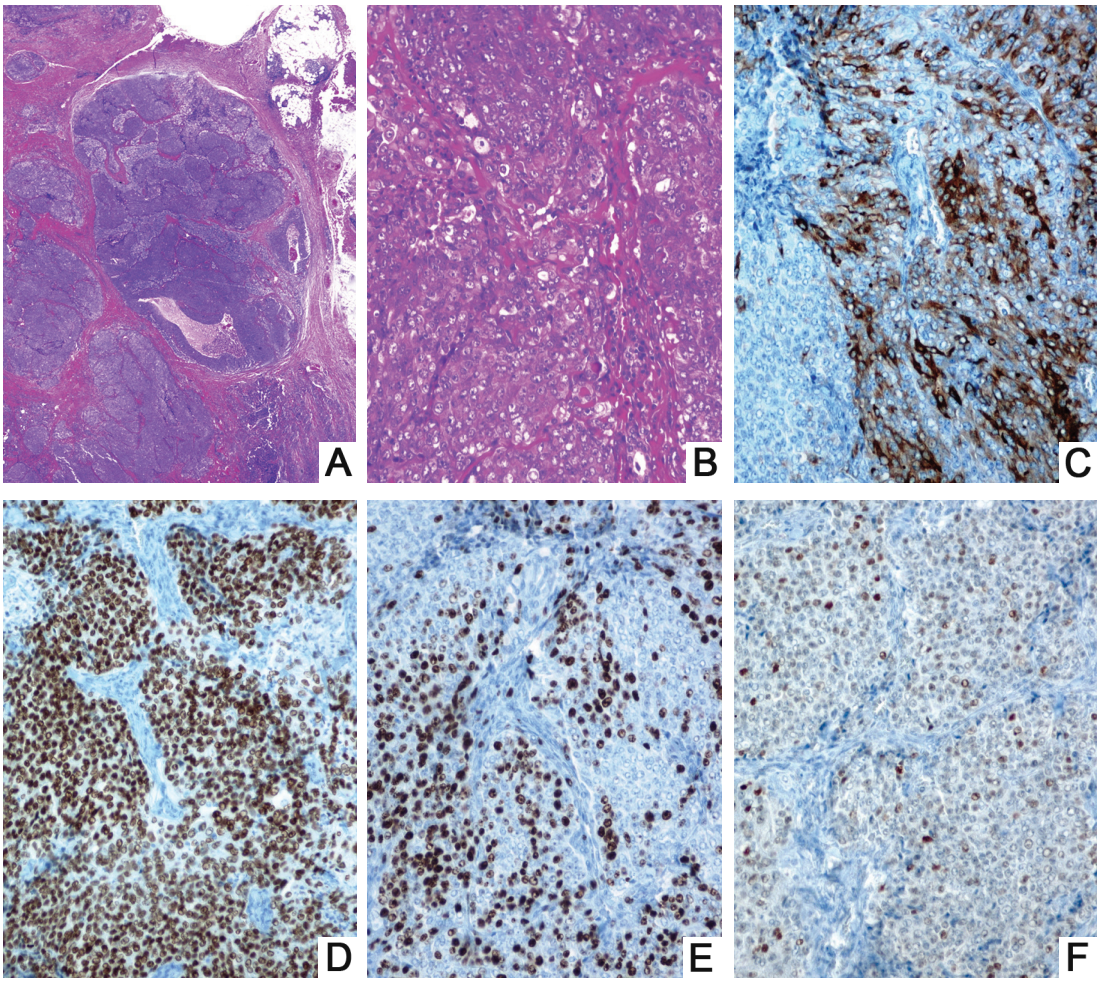

Figure 4.19. Poorly differentiated thyroid carcinoma. (A) Widely invasive nonencapsulated tumor with the solid growth pattern. Marked fibrosis, necrotic foci, extrathyroidal extension. Haematoxylin and eosin, original magnification x10-panoramic. (B) High-power image of the same tumor. Numerous nuclei with prominent nucleoli. Haematoxylin and eosin, original magnification x100. (C) Focal immunostaining for thyroglobulin, original magnification x100. (D) Diffuse strong nuclear reactivity for TTF-1, original magnification x100. (E) Diffuse strong nuclear reactivity for Ki67. Original magnification x100. (F) Moderate nuclear reactivity for TP53, original magnification x100.

Morphological characteristics of PTC are significantly changing with patients' age at surgery and over time after Chernobyl, i.e. with latency. Our analysis provides the evidence for two major patterns (age-related and time-related) seen for most pathological characteristics under study: "ascending" in some cases or "descending" in other linear trends. Significantly descending age and time trends are found for the frequency of architecturally less differentiated solid and solid-follicular subtypes of PTC, and invasive characteristics: intrathyroidal and extrathyroidal extension, vascular invasion, regional metastases to lymph nodes and distant metastases to the lung, which are important and favorable for the postoperative prognosis and patients' quality of life.

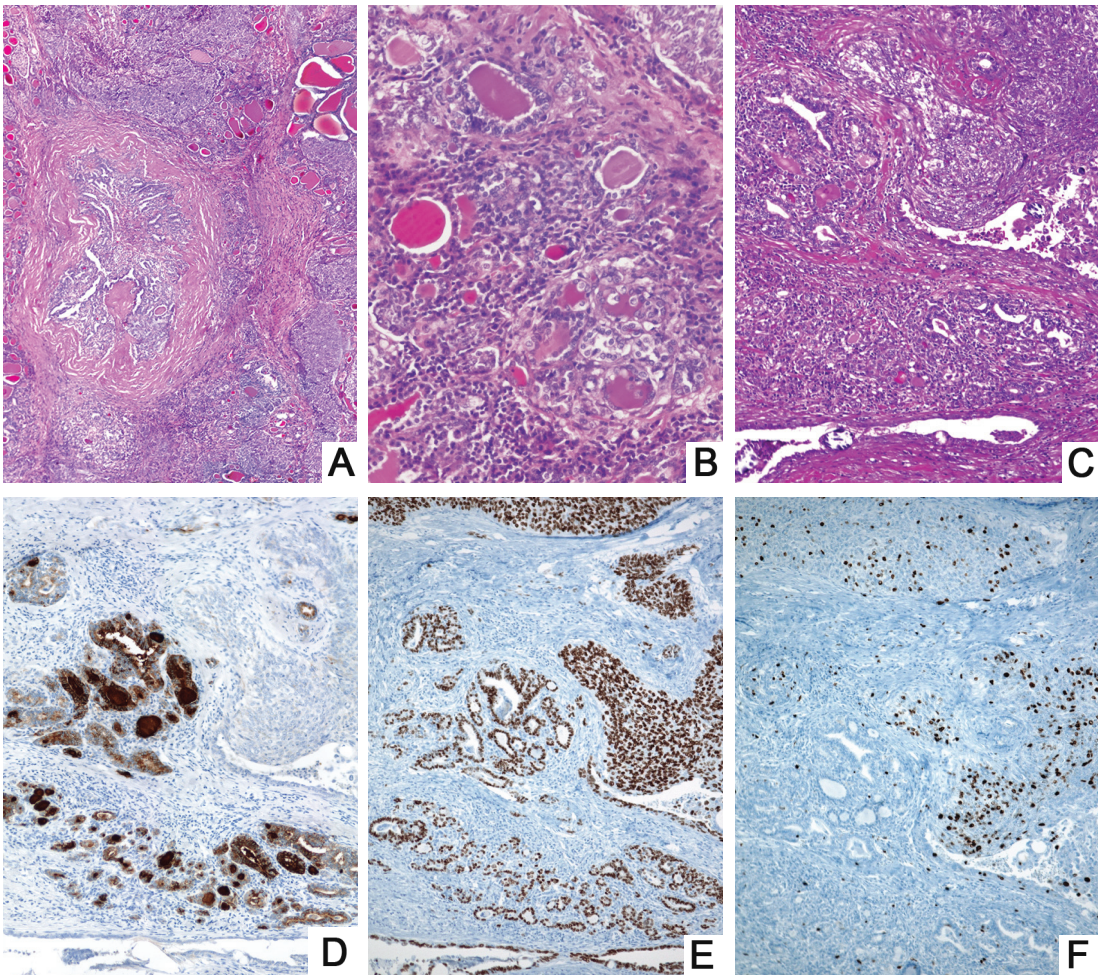

Figure 4.20. Microfoci of papillary thyroid carcinoma in poorly differentiated thyroid carcinoma. (A) Fibrotic area with the papillary-follicular growth pattern. Haematoxylin and eosin, original magnification x50-panoramic. (B) Microfoci with the follicular growth pattern. Haematoxylin and eosin, original magnification x100. (C) Microfoci with the follicular growth pattern and numerous psammoma bodies in lymphatic vessel. Haematoxylin and eosin, original magnification x50. (D) Strong thyroglobulin immunostaining of PTC foci, original magnification x100. (E) Diffuse strong nuclear reactivity for TTF-1 in both PTC and PDTC areas, original magnification x50. (F) Isolated Ki-67 positive nuclei in PTC foci. Original magnification x50.

Ascending linear trends are established for the frequency of structurally more differentiated papillary and papillary-follicular subtypes of PTC, for the prevalence of tumors sized up to 10 mm, and encapsulated tumors. These, again, may be considered as favorable, since "small" and encapsulated PTCs are characterized by significantly weaker invasive features compared with tumors of larger size and nonencapsulated ones. Thus, with increasing time after Chernobyl, PTC, based on its morphological characteristics, clearly becomes less aggressive. It would be difficult, however, to unambiguously determine if this is due only to the increasing patients' age or also to the increasing with age frequency of sporadic PTC.

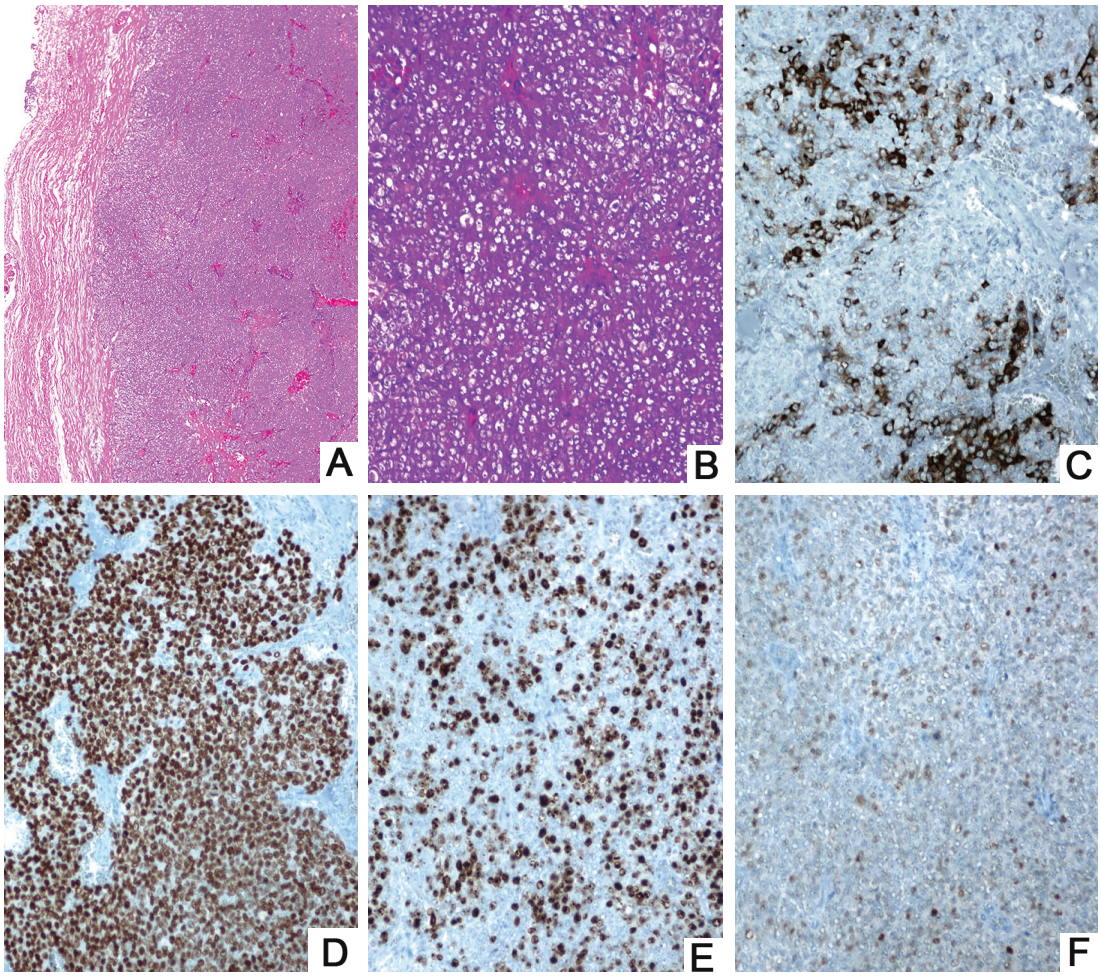

Figure 4.21. Lymph node metastasis of poorly differentiated thyroid carcinoma. (A) Metastasis of PDTC with the solid growth pattern completely replacing lymph node. Haematoxylin and eosin, original magnification x10-panoramic. (B) High-power image of the same section. Numerous nuclei with prominent nucleoli similar to those observed in the primary tumor. Haematoxylin and eosin, original magnification x100. (C) Focal immunostaining for thyroglobulin, original magnification x100. (D) Diffuse strong nuclear reactivity for TTF-1, original magnification x100. (E) Diffuse strong nuclear reactivity for Ki67. Original magnification x100. (F) Focal nuclear reactivity for TP53 showing labeling of 5% of the nuclei, original magnification x100.

As an attempt to at least partly answer this question, a comparative analysis of morphological characteristics of PTCs detected in age-matched groups of patients born before Chernobyl (radiogenic PTCs) and after Chernobyl (sporadic, non-radiogenic PTCs) will be performed. The next chapter describes the results of such analysis.

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Chapter 5

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Comparative pathological analysis of papillary thyroid carcinoma in age-matched groups of patients born before and after Chernobyl

The aim of this chapter is to compare structural characteristics and invasive features of papillary thyroid carcinomas (PTCs) between the groups of patients who were children at the time of the Chernobyl accident and thus were exposed to radioactive iodine in fallout (the radiogenic cancers) and those who were born since 1987 and were not irradiated (so-called sporadic cancers). We recognize that the radiogenic group will inevitably include some cases of non-radiation aetiology, and attempted to minimize these through the process of selection described below.

In Chapter 3 and our previous papers [1-5] it has been shown that thyroid cancer incidence was significantly higher in the six northern Ukraine regions with high levels of contamination by radioactive iodine. Among these, three areas are the most affected: Kiev, Zhytomir, and Chernigov regions. A simple calculation of the number of cases per 1,000 residents in relevant populations for 25 years (from 1986 to 2010) demonstrates evident difference: it is about 3 times higher in those exposed as children and 2 times higher in those exposed as adolescents as compared with less contaminated regions (Table 5.1).

Table 5.1
Number of cases and prevalence per 1,000 of population of thyroid cancer cases in Ukraine in subjects who were children and adolescents at the time of Chernobyl accident

	Children	Adolescents	Total
Whole of Ukraine	5044/11100000=**0.5/1000**	1642/2200000=**0.8/1000**	6686/13300000=**0.5/1000**
21 less or not contaminated regions	2861/8900000=**0.3/1000**	1078/1760000=**0.6/1000**	3939/10660000=**0.4/1000**
3 out of 6 most contaminated regions	1098/1025000=**1.1/1000**	254/195000=**1.3/1000**	1352/1220000=**1.1/1000**

Also, as was reported earlier [6-9], the highest dose-dependent additional incidence was observed in children whose age in 1986 did not exceed 4 years. It therefore is reasonable to assume that the fraction of papillary carcinomas attributable to radiation would be the largest among those whose age at exposure ranged from 0 to 4 years and who at the time of accident were residents of the three most affected regions of Ukraine (Kiev, Chernigov, and Zhytomir regions).

For this reason, for the present investigation we selected pathological data for the period from 1990 to 2010 (see Chapter 4), and included in the analysis only cases diagnosed in residents of the three most contaminated regions who were aged up to 4 years at Chernobyl. These are: 114 out of 272 PTCs in children aged up to 14 years at surgery, 66 out of 225 in adolescents aged from 15 to 18 years at surgery, and 59 out of 361 PTCs in adults aged 19-23 years. Age at surgery in adults was limited from 19 to 23 years since among patients born after Chernobyl no one was older than 23 years in 2010. All 264 PTCs detected in subjects born after the accident were included in the analysis regardless of their place of residence because all these cases are sporadic. Characterstics of the age-matched groups are specified in Table 5.2.

Table 5.2
Age-matched groups of radiogenic and sporadic PTC in the study

	Number of cases		Mean age		F:M	
	Radiogenic	Sporadic	Radiogenic	Sporadic	Radiogenic	Sporadic
Children	114	111	11.6	12.0	68:46=1.5:1	83:28=3.0:1
Adolescents	66	97	16.8	16.8	44:22=2.0:1	72:25=2.9:1
Adults	59	56	21.2	20.8	44:15=2.9:1	46:10=4.6:1

In all groups, both in radiogenic and sporadic PTCs, female patients were prevailing, but in the group of children with radiogenic tumors the proportion of male patients was greater as compared with the group of children with sporadic carcinomas (Table 5.2), with a substantial difference in F:M ratio (1.5:1 vs 3:1, respectively).

There were no significant differences in mean size of tumor when comparing either different age groups or radiogenic and sporadic carcinomas in each age group. In children with radiogenic PTC the frequency of microcarcinomas measuring up to 10 mm was 2.2 times lower as compared to children with sporadic PTC (Table 5.3) suggesting that screening had not shifted tumor size to the lower values in the exposed regions.

Table 5.3
Mean size of radiogenic and sporadic PTC in the study

	Children		Adolescents		Adults	
	Radiogenic	Sporadic	Radiogenic	Sporadic	Radiogenic	Sporadic
Mean size (mm)	23.6	24.0	19.1	23.3	20.6	21.3
≤10 mm: n (%)	7/114(6.1)	5/111(13.5)	14/66(21.2)	16/97(16.5)	13/59(22.0)	13/56(23.2)

It should be emphasized that "architecturally" all PTCs under study, regardless of patients' date of birth or age at diagnosis, were almost all represented by three common histological variants: classical papillary, follicular, solid, and by the mixed-type tumors (herein reffered to as "mixed variant") which comprise combinations of the mentioned growth patterns. The diffuse-sclerosing variant was identified in a small number of cases (mainly among children), and there were isolated cases of Warthin-like and Cribriform-morular variants (Table 5.4). Among the mixed variants of PTC, the papillary-follicular and solid-follicular combinations were most frequent (Table 5.5).

Comparison of radiogenic PTCs selected for the present analysis with all morphologically studied cases in patients born before the Chernobyl accident (described in the previous chapter) shows that such a selection did not result in changes in the distribution of main PTC subtypes, in the ratio of structural components of mixed variant, or frequency of the combined (solid+solid-follicular and papillary+papillary-follicular) variants in all age groups (see Tables 4.4, 4.5 of Chapter 4 and Tables 5.4, 5.5, 5. 6 of this Chapter). Despite the maximum age of adult patients has considerably decreased after selection (from 42 years in the total group to 23 years in the selected group), all significant linear age trends (assessed by the Chi-square test for trend) identified in the previous chapter, were preserved. In the selected groups of PTCs, linear decreasing trends of frequency of solid, solid-follicular and combined (solid+solid-follicular) variants ($p<0.0115$; $p<0.0009$; $p<0.0001$, respectively), and increasing linear trends of frequency of papillary, papillary-follicular and combined (papillary+papillary-follicular) variants in the age series of children-adolescents-adults remained unchanged ($p<0.0001$; $p<0.0013$; $p<0.0001$, respectively). A significant increasing linear age trend was also noted for the frequency of fully encapsulated PTCs ($p<0.0001$, Table 5.7).

The selection had no effect on the invasive properties of radiogenic PTCs either (Table 4.8 of Chapter 4 and Table 5.7 of this Chapter). In the selected cases, significant decreasing linear trends of frequency of intrathyroidal spread and extrathyroidal extension, vascular invasion, regional and distant metastases in the age series of children-adolescents-adults were also confirmed (all trends, $p<0.0001$).

Thus, our selection of cases by patients' age and place of residence at the time of the Chernobyl accident was not associated with significant changes in morphological characteristics of PTCs determined in corresponding age groups for the whole of Ukraine, i.e. there was no detectable selection bias.

Considering the frequency of histological subtypes in sporadic PTCs (in patients born after the Chernobyl accident), it should be noted that the linear trends similar to those in radiogenic cancers were not found in age series (Tables 5.4, 5.5, 5.6) except for a decreasing trend in the frequency of solid variant ($p<0.0502$, marginally). Analysis of encapsulated PTCs also showed no age relationship (Table 5.7).

By contrast, invasive properties of sporadic (Table 5.7) as well as of radiogenic PTCs were significantly decreasing in the age series of children-adolescents-adults (intrathyroidal extension, $p<0.0470$; extrathyroidal extension, $p<0.0001$; vascular invasion, $p<0.0008$; regional metastases, $p<0.0004$, distant metastases to the lung, $p<0.0106$).

Conceivably, radiogenic and sporadic papillary carcinomas, when comparison is performed in the age series, are characterized by the same trends for invasive features, but display different character of distribution of main histological subtypes.

Table 5.4
Subtypes of PTC in patients born before (radiogenic) and after Chernobyl (sporadic cancers)

	Children up to 14 y.o. at surgery				Adolescents 15-18 y.o. at surgery				Adults 19-23 y.o. at surgery			
	Radiogenic		Sporadic		Radiogenic		Sporadic		Radiogenic		Sporadic	
	n	%	n	%	n	%	n	%	n	%	n	%
Classic papillary	11	9.6	18	16.2	14	21.2	28	28.9	20	33.3	12	21.4
Follicular	25	21.9	17	15.3	14	21.2	9	9.3	13	22.0	8	14.3
Solid	24	21.1	17	15.3	5	7.6	10	10.3	5	7.5	3	5.3
Mixed	47	41.3	54	48.7	31	47.0	48	49.5	21	35.6	29	51.8
Diffuse-sclerosing	7	6.1	5	4.5	1	1.5	1	1.0	-	-	1	1.8
Warthin-like	-	-	-	-	1	1.5	1	1.0	-	-	2	3.6
Cribriform	-	-	-	-	-	-	-	-	-	-	1	1.8
Total	114	100	111	100	66	100	97	100	59	100	56	100

Table 5.5
Structural combinations of mixed subtypes of PTC in patients born before (radiogenic) and after Chernobyl (sporadic cancers)

	Children up to 14 y.o. at surgery				Adolescents 15-18 y.o. at surgery				Adults 19-23 y.o. at surgery			
	Radiogenic		Sporadic		Radiogenic		Sporadic		Radiogenic		Sporadic	
	n	%	n	%	n	%	n	%	n	%	n	%
Papillary-follicular	9	19.1	20	37.0	13	41.9	19	39.6	12	57.1	7	24.1
Papillary-solid	5	10.6	10	18.5	8	25.8	8	16.7	3	14.3	6	20.7
Papillary-follicular-solid	2	4.3	7	13.0	1	3.2	6	12.5	-	-	4	13.8
Solid-follicular	31	66.0	17	31.5	9	29.0	15	31.2	6	28.6	12	41.4
Total	47	100	54	100	31	100	48	100	21	100	29	100

Table 5.6
PTCs with most prominent solid patterns (SV+SFV) and most prominent papillary patterns (PV+PFV) in patients born before (radiogenic) and after Chernobyl (sporadic cancers)

	Children up to 14 y.o at surgery				Adolescents 15-18 y.o at surgery				Adults 19-23 y.o at surgery			
	Radiogenic		Sporadic		Radiogenic		Sporadic		Radiogenic		Sporadic	
	n	%	n	%	n	%	n	%	n	%	n	%
Solid+Solid-Follicular variants	55/114	48.2	34/111	30.6	14/66	21.2	25/97	25.8	11/59	18.6	15/56	26.8
Papillary+Papillary-Follicular variants	20/114	17.5	38/111	34.2	27/66	40.9	47/97	48.5	32/59	54.2	19/56	33.9

Table 5.7
Invasive proprties and tumor encapsulation in patients born before (radiogenic) and after Chernobyl (sporadic cancers)

	Children up to 14 y.o at surgery				Adolescents 15-18 y.o at surgery				Adults 19-23 y.o at surgery			
	Radiogenic		Sporadic		Radiogenic		Sporadic		Radiogenic		Sporadic	
	n	%	n	%	n	%	n	%	n	%	n	%
Intrathyroidal extension	97	85.1	74	66.7	35	53.0	61	62.9	35	59.3	28	50.0
Extrathyroidal extension	76	66.7	60	54.1	30	45.0	40	41.2	19	32.2	12	21.4
Multifocality	7	6.1	11	9.9	7	10.6	11	11.3	5	8.5	9	16.1
Vascular invasion	97	85.1	79	71.2	44	66.7	64	66.0	27	45.8	24	42.9
Regional metastases	77	67.5	71	64.0	35	53.0	54	55.7	16	27.1	19	33.9
Distant metastases	27	23.8	14	12.6	9	13.6	6	6.2	-	-	1	1.8
Fully encapsulated	6	5.3	22	19.8	10	15.2	15	15.5	19	32.2	15	26.8

Taking into account that earlier comparative analyses of PTC in children and adolescents of Ukraine, Belarus, and Russia born before and after Chernobyl have not revealed substantial morphological differences [10-14], in the present study we used deepened univariate and multivariate statistical approaches to each of the three age-matched groups.

To compare pathological parameters between radiation-induced and sporadic PTC by univariate analysis, Fisher exact test (FT) was used for categorical data, and two-tailed Mann-Whitney test for quantitative measurements.

Univariate statistical analysis of main subtypes of PTC in children (Table 5.8) showed that among radiogenic PTCs the frequency of tumors with solid-follicular strucuture (solid + solid-follicular variants) was significantly higher than that in sporadic PTCs ($p<0.009$). Conversely, the frequency of sporadic PTCs with papillary-follicular structure (papillary + papillary-follicular variants, $p<0.006$) was significantly higher than in radiogenic tumors.

Comparison of invasive features also revealed the most pronounced differences in childhood groups: radiogenic PTCs were characterized by a significantly higher frequency of intrathyroidal extension ($p<0.002$), vascular invasion ($p<0.015$) and distant metastases ($p<0.033$).

In addition, in the group of children with radiogenic PTC, a significantly lower frequency was observed for the fully encapsulated tumors as compared with sporadic PTCs ($p<0.001$), and for the frequency of microcarcinomas sized up to 10 mm, but the difference is not quite statistically significant ($p<0.074$). The groups of children with radiogenic and sporadic PTCs also differed significantly in F:M ratio ($p<0.026$) as mentioned above.

Among adolescents and adults no significant differences between any of the above parameters were observed (Table 5.8).

Multivariate analysis in each age group of patients was performed using standard logistic regression modeling. The following variables were tested:

age at surgery (continuous; years);
sex (categorical, M or F);
tumor size ≤10mm or >10 mm (categorical; yes or no);
complete tumor capsule (categorical; yes or no);
histological subtype (categorical; solid+solid follicular or other);
lymph node metastases (categorical; yes or no);
distant metastasis to the lung (categorical; yes or no);
intrathyroidal spread (categorical; yes or no);
extrathyroidal extension (categorical; yes or no);
multifocality (categorical; yes or no);
vascular invasion (categorical; yes or no).

Non-automatic backward elimination was applied to the full model that initially included all the variables listed above. Once the most appropriate model was determined, the maximum likelihood estimates of the respective parameters and their 95% confidence intervals were calculated.

Multivariate logistic regression analysis (Table 5.9) confirmed significant differences between radiogenic and sporadic PTCs in children. Four parameters (male gender, absence of tumor capsule, solid+solid-follicular tumor architecture, and a higher frequency of intrathyroidal spread) were found to be independently associated with radiogenic PTC. The fifth parameter, the higher frequency of lymph node metastases, showed just marginal association with radiogenic PTCs.

Table 5.8
Univariate statistical analysis of PTCs in patients born before (radiogenic) and after Chernobyl (sporadic cancers) in age-matched groups

Children

Parameters	OR	95% CI	*p*-value
Sex (M vs F)	1.89	1.08-3.35	0.026
Age at operation (years)	0.93	0.83-1.04	0.190
Tumor size (≤10 mm vs >10 mm)	0.42	0.16-1.07	0.074
Tumor capsule (yes vs no)	0.22	0.09-0.58	0.001
Subtype (solid+solid-follicular vs other)	2.11	1.22-3.64	0.009
pN (pN1 vs pN0)	1.14	0.66-1.99	> 0.5
M (M1 vs M0)	2.13	1.06-4.42	0.033
Multifocality (yes vs no)	0.59	0.21-1.56	0.287
Intrathyroidal extension (yes vs no)	2.78	1.47-5.43	0.002
Extrathyroidal extension (yes vs no)	1.67	0.97-2.88	0.063
Vascular invasion (yes vs no)	2.31	1.97-4.47	0.015

Adolescents

Parameters	OR	95% CI	*p*-value
Sex (M vs F)	1.44	0.73-2.86	0.379
Age at operation (years)	1.01	0.76-1.33	> 0.5
Tumor size (≤10 mm vs >10 mm)	0.69	0.29-1.64	0.394
Tumor capsule (yes vs no)	0.92	0.38-2.14	> 0.5
Subtype (solid+solid-follicular vs other)	0.78	0.37-1.63	> 0.5
pN (pN1 vs pN0)	0.92	0.49-1.72	> 0.5
M (M1 vs M0)	2.42	0.83-7.56	0.106
Multifocality (yes vs no)	0.94	0.33-2.52	> 0.5
Intrathyroidal extension (yes vs no)	0.69	0.36-1.29	0.241
Extrathyroidal extension (yes vs no)	1.21	0.64-2.27	> 0.5
Vascular invasion (yes vs no)	1.03	0.53-2.00	> 0.5

Adults

Parameters	OR	95% CI	*p*-value
Sex (M vs F)	1.57	0.64-3.96	0.324
Age at operation (years)	1.28	0.96-1.73	0.097
Tumor size (≤10 mm vs >10 mm)	0.94	0.34-2.53	> 0.5
Tumor capsule (yes vs no)	1.30	0.58-2.94	> 0.5
Subtype (solid+solid-follicular vs other)	0.63	0.26-1.51	0.374
pN (pN1 vs pN0)	0.72	0.32-1.61	0.427
Multifocality (yes vs no)	0.48	0.14-1.50	0.211
Intrathyroidal extension (yes vs no)	1.46	0.70-3.07	0.315
Extrathyroidal extension (yes vs no)	1.74	0.76-4.12	0.191
Vascular invasion (yes vs no)	1.12	0.54-2.35	> 0.5

Table 5.9
Results of multivariate statistical analysis of PTC in patients born before (radiogenic) and after Chernobyl (sporadic cancers) in age-matched groups

Children

Parameters	OR	95% CI	*p*-value
Sex (M vs F)	1.86	1.02-3.43	0.042
Tumor capsule (yes vs no)	0.27	0.09-0.73	0.009
Subtype (solid+solid-follicular vs other)	1.89	1.05-3.33	0.031
pN (yes vs no)	1.89	0.96-3.70	0.064
Intrathyroidal spread (yes vs no)	2.80	1.36-5.98	0.005

Adolescents

Parameters	OR	95% CI	*p*-value
Extrathyroidal extension (yes vs no)	3.00	1.07-9.91	0.037

Adults

Parameters	OR	95% CI	*p*-value
Age at operation (older vs younger)	1.49	1.08-2.12	0.014
pN (yes vs no)	0.34	0.11-0.93	0.034
Multifocality (yes vs no)	0.32	0.08-1.05	0.060
Extrathyroidal extension (yes vs no)	3.56	1.25-1.51	0.017

In adolescents, only a higher chance of extrathyroidal extension in radiogenic PTCs was noted. In adults, the logistic regression revealed a significant association for four parameters: age at surgery, lymph node metastases, and extrathyroidal extension. A higher frequency of extrathyroidal extension was associated with radiogenic PTCs. On the contrary, a higher frequency of lymph node metastases, younger age and higher frequency of multifocality (marginally) were associated with sporadic PTCs (Table 5.9). Of note, univariate statistical analysis showed no significant differences for any of these parameters in adolescents and adults.

Thus, radiogenic papillary carcinomas in each of the age-matched groups had certain differences from sporadic ones. The most pronounced differences were observed in age-matched groups of children; these were confirmed on both univariate and multivariate statistical analyses.

The data obtained suggest that after radiation exposure at the age 0-4 years old, the most rapidly progressing towards clinical manifestations PTCs (latency in subjects operated on in childhood ranged from 3.8 to 13.8 years, mean 9.4 years, Table 5.10) were independently associated with the solid and/or solid-follicular growth pattern, absence of tumor capsule, and such an aggressive morphological feature as intrathyroidal spread as compared to sporadic PTCs in the age-matched group. It should be noted that the higher aggressiveness of radiogenic Chernobyl papillary carcinomas in children of Ukraine and Belarus with a shorter latency was associated with the presence of a marked solid component, as reported in our previous study [15].

Apparently, the slower development of radiogenic tumors in adolescents and adults (mean latency 14.4 and 18.8 years, respectively, Table 5.10) levels off the differences in histological architectonics seen in children. On multivariate analysis, associations were found only for the greater chance of extrathyroidal extension in adolescents and adults and also for the lower chance of nodal disease in adults, but no correlation was found with tumor morphology in these age groups.

It is worth noting that such a "convergence" of morphological characteristics between radiogenic and sporadic PTCs with increasing patients' age and, naturally, the latency of radiogenic tumors, could perhaps be explained, at least in part, by different thyroid doses received during the Chernobyl accident by children at surgery on one hand, and adolescents and/or adults at surgery on the other. This hypothesis is based on the results of our cohort studies obtained within the Ukrainian-American Project [16]. It was shown that after the 1st screening during the period 1998 to 2000 (mean latency 14.6 years), the number of thyroid cancer cases per 1000 cohort members was the highest (10.2/1000) in the high-dose group in which thyroid doses were more than 1 Gy as compared with the low-dose group with thyroid doses less than 0.3 Gy (1.3/1000, $p<0.0001$). By contrast, after the 4th screening during 2006-08 (mean latency 20.9 years), such tendency was no longer observed (1.2/1000 and 1.5/1000 respectively, $p<0.753$). It therefore seems possible that the rapid progression of radiogenic PTCs in children at surgery might be associated with higher exposure doses as compared with radiogenic tumors in adolescents and adults which are characterized by the longer latency.

To verify this supposition, we analyzed *individual reconstructed ^{131}I thyroid doses* in all cases of radiogenic PTCs which have been calculated by a method described in a recent article [17] and in Chapter 2 of this monograph for thyroid tumors included in the Chernobyl Tissue Bank (CTB). Mean ^{131}I thyroid dose in our cohort which included 239 children aged 0 to 4 years at exposure was 1.016 Gy (95% CI 0.701-1.332; median 0.336 Gy; minimum 0.040 Gy; maximum 24.110 Gy).

In the comparison of three age groups with different latency by the Kruskal-Wallis nonparametric ANOVA, no significant differences between exposure doses were found. Although the median of doses was somewhat decreasing in the series children-adolescents-adults, no significant linear trend was found (Table 5.10). A plausible explanation of the absence of significant differences between thyroid doses would be that all Ukrainian-American cohort members had direct measurements of thyroid activity (see Chapter 2) unlike patients in the present study, who had direct measurements of thyroid activity only in 16.3% cases (39 out of 239). The accuracy of dose estimates in the Ukrainian-American cohort was obviously higher with narrower CI range. In addition, patients distributions by age at the time of Chernobyl (0 to 4 years) and at surgery (4 to 23 years) differed significantly from those included in the Ukrainian-American project [16,18,19] in which the age at exposure exceeded 4 years in most cases, there were practically no patients operated in childhood, and the age of operated adults reached 34 years.

A more detailed analysis of mean thyroid doses for those pathological parameters that distinguished radiogenic PTCs from sporadic ones showed that doses were significantly higher in children/Females comparing with adults/Females. When comparing the doses of females and males, those were higher for males in all age groups, especially in adolescents and adults, but without significant difference (Table 5.11).

Analysis of dose relationship with PTC size (microcarcinoma vs other) demonstrated different trends in children, adolescents and adults. While in children the mean dose for microcarcinomas was insignificantly lower than in PTCs of larger size, in adolescents and adults microcarcinoma was associated with higher doses, and in adolescents such a difference was significant (Table 5.12).

In patients of all age groups with non-encapsulated PTC mean doses were higher than in patients with fully encapsulated tumor (Table 5.13), but significant differences were not found either in children in whom this parameter was strongly associated with radiogenic cancer, or in older patients in whom this association was absent.

For the combined solid + solid-follicular subtypes of PTC, which were associated with radiogenic cancer in children, mean doses did not display statistically significant differences either in the series children-adolescents-adults or when the doses were compared to those for all other subtypes of PTC (Table 5.14).

Morphological features of tumor agressiveness (intrathyroidal spread, extrathyroidal extension, nodal disease), were not associated with significant differences in mean doses in all age groups either. Also, no dose differences were found in patients with or without morphological manifestations of agressiveness (Tables 5.15-5.17). A significantly higher exposure dose was noted only in adolescents without intrathyroidal spread as compared to patients with such or with corresponding adults (Table 5.15).

Tumor multifocality, which was marginally associated with sporadic PTC in adults, was associated in radiogenic carcinomas of this age group with a higher (compared with children and adolescents) exposure dose (Table 5.18) but no significance could be expected taking into account the range of dose estimates from 0.155 to 12.279 Gy in 5 observations.

Table 5.10
Latency and thyroid radiation doses in patients with radiogenic PTC (born before Chernobyl)

	Latency (years)			Doses (Gy)		
Parameters	Children (n=114)	Adolescents (n=66)	Adults (n=59)	Children (n=114)	Adolescents (n=66)	Adults (n=59)
Mean	9.4	14.8	18.9	0.995	1.333	0.704
95 % CI	9.0-9.9	14.3-15.2	18.3-19.4	0.591-1.398	0.487-2.179	0.269-1.139
Minimum	3.8	11.7	14.8	0.048	0.040	0.044
Median	9.4	14.4	18.8	0.406	0.341	0.261
Maximum	13.8	17.9	22.6	16.584	24.110	12.280
p-value			<0.0001			0.226
p-trend			<0.0001			0.467
p-value	<0.0001 [a]			0.801 [a]		
p-value	<0.0001 [b]	<0.0001 [c]		0.098 [b]	0.182 [c]	

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults

Table 5.11
Thyroid radiation doses in females and males with radiogenic PTC (born before Chernobyl)

	Females, doses, Gy			Males, doses, Gy		
Parameters	Children (n=68)	Adolescents (n=44)	Adults (n=44)	Children (n=46)	Adolescents (n=22)	Adults (n=15)
Mean	1.023	0.962	0.459	0.953	2.075	1.317
95 % CI	0.970-1.476	0.320-1.607	0.275-0.714	0.187-1.721	0.208-4.359	0.379-3.014
Minimum	0.048	0.040	0.044	0.057	0.068	0.101
Median	0.459	0.327	0.240	0.329	0.366	0.434
Maximum	13.216	13.286	3.175	16.584	24.110	12.279
p-value			0.046			0.551
p-trend			0.113			0.472
p-value	0.235 [a]			0.234 [d]	0.295 [e]	0.139 [f]
p-value	0.015 [b]	0.217 [c]				

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: female vs male; [e] - adolescents: female vs male; [f] - adults: female vs male;

Table 5.12
Thyroid radiation doses and tumor size in patients with radiogenic PTC (born before Chernobyl)

	Size ≤10 mm, doses, Gy			Size ≥11 mm, doses, Gy		
Parameters	Children (n=7)	Adolescents (n=14)	Adults (n=13)	Children (n=107)	Adolescents (n=52)	Adults (n=46)
Mean	0.396	4.323	1.380	1.034	0.528	0.513
95 % CI	0.179-0.612	0.473-8.174	0.620-3.380	0.605-1.464	0.310-0.746	0.304-0.722
Minimum	0.152	0.203	0.087	0.048	0.070	0.044
Median	0.342	2.136	0.274	0.416	0.299	0.259
Maximum	0.715	24.110	12.279	16.584	4.508	3.175
p-value			0.022			0.115
p-trend			0.664			0.084
p-value	0.025 [a]			0.102 [a]		
p-value	0.757 [b]	0.017 [c]		0.086 [b]	0.798 [c]	
p-value				0.645 [d]	0.0006 [e]	0.905 [f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: ≤10 mm vs ≥11 mm; [e] – adolescents: ≤10 mm vs ≥11 mm; [f] - adults: ≤10 mm vs ≥11 mm

Table 5.13
Thyroid radiation doses and full tumor encapsulation in patients with radiogenic PTC (born before Chernobyl)

Parameters	Fully encapsulated, doses, Gy			Non- and partly encapsulated, doses, Gy		
	Children (n=6)	Adolescents (n=10)	Adults (n=19)	Children (n=108)	Adolescents (n=56)	Adults (n=40)
Mean	0.327	0.612	0.362	1.032	1.462	0.866
95 % CI	0.126-0.527	0.092-1.315	0.161-0.563	0.607-1.457	0471-2.454	0.229-1.504
Minimum	0.083	0.087	0.044	0.048	0.040	0.087
Median	0.402	0.327	0.202	0.406	0.359	0.285
Maximum	0.540	3.400	1.626	16.584	24.110	12.279
p-value			0.424			0.732
p-trend			0.534			0.737
p-value	0.635 [a]			0.842 [a]		
p-value	0.849 [b]	0.344 [c]		0.412 [b]	0.642 [c]	
p-value				0.348 [d]	0.838 [e]	0.089 [f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: full encapsulation vs other; [e] – adolescents: full encapsulation vs other; [f] - adults: full encapsulation vs other

Table 5.14
Thyroid radiation doses and combined Solid+Solid-Follicular subtype of PTC in patients born before Chernobyl

Parameters	Solid+Solid-Follicular subtype, doses, Gy			Other subtypes, doses, Gy		
	Children (n=55)	Adolescents (n=14)	Adults (n=11)	Children (n=59)	Adolescents (n=52)	Adults (n=48)
Mean	0.752	0.730	0.716	1.222	1.496	0.701
95 % CI	0.433-1.071	0.315-1.144	0.107-1.325	0.494-1.951	0.424-2.558	0.177-1.226
Minimum	0.056	0.089	0.045	0.048	0.040	0.044
Median	0.369	0.352	0.285	0.428	0.327	0.254
Maximum	6.880	2.160	3.015	16.584	24.110	12.279
p-value			0.855			0.134
p-trend			0.920			0.366
p-value	0.591 [a]			0.417 [a]		
p-value	0.911 [b]	0.966 [c]		0.052 [b]	0.212 [c]	
p-value				0.387 [d]	0.814 [e]	0.654 [f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: SV+SFV vs other; [e] – adolescents: SV+SFV vs other; [f] - adults: SV+SFV vs other

Table 5.15
Thyroid radiation doses and intrathyroidal spread in patients with radiogenic PTC (born before Chernobyl)

Parameters	Intrathyroidal spread, doses, Gy			No intrathyroidal spread, doses, Gy		
	Children (n=97)	Adolescents (n=35)	Adults (n=35)	Children (n=17)	Adolescents (n=31)	Adults (n=21)
Mean	0.973	0.644	0.828	1.121	2.111	0.523
95 % CI	0.573-1.373	0.268-1.021	0.116-1.540	0.486-2.729	0.349-3.873	0.213-0.959
Minimum	0.048	0.040	0.045	0.096	0.072	0.044
Median	0.453	0.292	0.285	0.316	0.428	0.235
Maximum	16.584	4.595	12.279	13.216	24.110	3.175
p-value			0.238			0.067
p-trend			0.692			
p-value	0.120[a]			0.196[a]		
p-value	0.289[b]	0.725[c]		0.308[b]	0.028[c]	
p-value				0.436[d]	0.039[e]	0.436[f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: intrathyroidal spread, yes vs no; [e] – adolescents: intrathyroidal spread, yes vs no; [f] - adults: intrathyroidal spread, yes vs no

Table 5.16
Thyroid radiation doses and extrathyroidal extension in patients with radiogenic PTC (born before Chernobyl)

Parameters	Extrathyroidal extension, doses, Gy			No extrathyroidal extension, doses, Gy		
	Children (n=76)	Adolescents (n=30)	Adults (n=19)	Children (n=38)	Adolescents (n=36)	Adults (n=40)
Mean	0.786	0.726	0.601	1.413	1.839	0.753
95 % CI	0.508-1.063	0.281-1.171	0.204-0.997	0.314-2.513	0.318-3.361	0.128-1.378
Minimum	0.048	0.046	0.045	0.065	0.040	0.044
Median	0.410	0.330	0.285	0.396	0.341	0.259
Maximum	6.880	5.167	3.015	16.584	24.110	12.279
p-value			0.456			0.262
p-trend			0.534			0.391
p-value	0.646[a]			0.987[a]		
p-value	0.372[b]	0.594[c]		0.150[b]	0.175[c]	
p-value				0.648[d]	0.541[e]	0.928[f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: extrathyroidal extension, yes vs no; [e] – adolescents: extrathyroidal extension, yes vs no; [f] - adults: extrathyroidal extension, yes vs no

Table 5.17
Thyroid radiation doses and lymph node metastases in patients with radiogenic PTC (born before Chernobyl)

Parameters	Lymph node metastases, doses, Gy			No lymph node metastases, doses, Gy		
	Children (n=77)	Adolescents (n=35)	Adults (n=16)	Children (n=37)	Adolescents (n=31)	Adults (n=43)
Mean	0.983	0.692	0.642	1.020	2.057	0.727
95 % CI	0.560-1.407	0.294-1.090	0.149-1.135	0.106-1.934	0.296-3.819	0.148-1.306
Minimum	0.048	0.040	0.045	0.065	0.072	0.044
Median	0.416	0.251	0.247	0.397	0.390	0.261
Maximum	13.216	5.167	3.175	16.584	24.110	12.279
p-value			0.176			0.107
p-trend			0.570			0.200
p-value	0.117[a]			0.245[a]		
p-value	0.186[b]	0.855[c]		0.463[b]	0.028[c]	
p-value				0.616[d]	0.021[e]	0.658[f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: lymph node metastases, yes vs no; [e] – adolescents: lymph node metastases, yes vs no; [f] - adults: lymph node metastases, yes vs no

Table 5.18
Thyroid radiation doses and multifocality in patients with radiogenic PTC (born before Chernobyl)

Parameters	Multifocality, doses, Gy			No multifocality, doses, Gy		
	Children (n=7)	Adolescents (n=7)	Adults (n=5)	Children (n=107)	Adolescents (n=59)	Adults (n=54)
Mean	0.496	0.888	2.736	1.027	1.386	0.515
95 % CI	0.172-0.821	-0.632-2.409	-3.889-9.361	0.598-1.459	0.448-2.324	0.324-0.703
Minimum	0.150	0.089	0.155	0.048	0.040	0/044
Median	0.457	0.251	0.403	0.397	0.350	0.254
Maximum	1.056	4.595	12.279	16.584	24.110	3.175
p-value			0.642			0.145
p-trend			0.200			0.213
p-value	0.535[a]			0.937[a]		
p-value	>0.999[b]	0.432[c]		0.070[b]	0.093[c]	
p-value				0.944[d]	0.499[e]	0.289[f]

[a] - children vs adolescents; [b] - children vs adults; [c] - adolescents vs adults; [d] - children: multifocality, yes vs no; [e] – adolescents: multifocality, yes vs no; [f] - adults: multifocality, yes vs no

Given mean thyroid doses in patients of three age groups with radiogenic papillary carcinomas did not differ significantly for virtually all morphological characteristics under study, we performed an additional deepened analysis of the dose-response relationship.

Those pathological parameters that appeared to be significant on the multivariate analysis of radiation-induced vs sporadic PTCs were examined for their association with thyroid dose using *univariate and multivariate standard logistic regression modeling*:

$$P(a \mid z) = \frac{1}{1+e^{-z}}$$

where *a* – a pathological parameter and *z* is:

for the univariate analysis

$$z = Const + OR(D_j)$$

where *Const* is a constant, $OR(D_j)$ is the Odds Ratio for the dose group D_i (i=0, .. , 4), relative to the dose group D_0 with the OR=1 (referent group). All exposed patients were subdivided in quartiles thereby determining the dose range for each quartile;

for the multivariate analysis:

$$z = Const + bP_k + OR(D_j)$$

where *Const* is a constant, P_k – pathological parameters, $OR(D_j)$ is the Odds Ratio for the dose group D_j relative to the dose group D_0 with the OR=1 (referent group);

Two types of logistic regression models were applied:

- patients were stratified by eight dose intervals (i=0, .. ,8);
- a threshold dose model (two dose intervals) was used for which the reference dose interval was determined by optimizing the model according to binomial maximum likelihood.

The following pathological parameters (P_k) were tested for the multivariate analysis: age at surgery (continuous; years); sex (categorical, M or F); tumor size ≤10 mm or >10 mm (categorical; yes or no); complete tumor capsule (categorical; no or yes); histological subtype (categorical; solid+solid follicular or other); lymph node metastases (categorical; yes or no); distant metastasis to the lung (categorical; yes or no); intrathyroidal spread (categorical; yes or no); extrathyroidal extension (categorical; yes or no); multifocality (categorical; yes or no).

The linear or linear-quadratic terms for the dose were forcedly introduced as independent variable(s) in addition to the parameters used for the multivariate analysis of radiation-induced vs. sporadic PTCs.

EPICURE software package was used for the analysis. Non-automatic backward elimination was applied to the full model that initially included all the variables listed above. The binomial maximum likelihood estimates and the likelihood-based 95% CI were calculated for parameters of all the above-introduced models. Likelihood ratio statistics were used to calculate *p*-values for the studied effects and to test the statistical significance of the respective parameters. The results of these analyses are presented in Tables 5.19-5.21.

In *children*, the following pathological parameters were significantly different between radiation-induced and sporadic PTCs: absence of full tumor capsule, histological subtype (solid + solid-follicular vs other), intrathyroidal spread (yes vs no), and lymph node metastases (yes vs no), marginally (Table 5.19).

Table 5.19
Univariate and multivariate statistical analyses of association of different pathological parameters in exposed children

Tumor capsule, children

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.1885	1.00	referent	-
0.1885 - < 0.387	0.46	0.02-5.12	> 0.5
0. 387 - < 0.7923	1.50	0.23-12.1	> 0.5
0.7923 – 16.584	NA [a]	NA	NA
p-trend linear regression			> 0.5 [b]
	multivariate, stratified		
Extrathyroidal extension	0.03	0.00-0.26	< 0.001 [c]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
0.110 - < 0.185	NA	NA	
0.185 - < 0.274	NA	NA	
0.274 - < 0.406	0.26	0.01-4.32	0.37
0.406 - < 0.547	1.48	0.12-20.9	> 0.5
0.547 - < 0.851	NA	NA	
0.851 - < 1.907	NA	NA	
1.907 –	NA	NA	
linear dose model *p*-trend			0.1 [b]
	multivariate, dose threshold		
Extrathyroidal extension	0.06	0.00-0.43	0.004 [c]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
≥ 0.110	0.13	0.13-1.21	0.07 [b]

[a] there were no or too few cases in the given dose range to fit the model; [b] indicates non-significant association of full tumor encapsulation with thyroid dose; [c] indicates significantly decreased chance of fully encapsulated tumors among cases with extrathyroidal extension

Continuation of Table 5.19

Histological subtype, children

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.1885	1.00	referent	-
0.1885 - < 0.387	1.64	0.58-4.76	0.35
0.387 - < 0.7923	1.24	0.44-3.58	> 0.5
0.7923 – 16.584	2.06	0.72-6.12	0.18
p-trend linear regression			> 0.5 [a]
	multivariate, stratified		
Extrathyroidal extension	0.23	0.09-0.54	< 0.001 [b]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
0.110 - < 0.185	1.22	0.26-5.94	> 0.5
0.185 - < 0.274	0.97	0.20-4.80	> 0.5
0.274 - < 0.406	1.88	0.40-9.22	0.42
0.406 - < 0.547	1.37	0.29-6.60	> 0.5
0.547 - < 0.851	0.80	0.16-3.81	> 0.5
0.851 - < 1.907	5.07	1.00-31.89	0.05
1.907 –	1.22	0.25-5.94	> 0.5
linear dose model *p*-trend			0.1 [a]
	multivariate, dose threshold		
Extrathyroidal extension	0.24	0.10-0.56	0.002 [b]
Thyroid dose (Gy)			
< 0.851	1.00	referent	-
≥ 0.851	2.03	0.81-5.23	0.31 [a]

[a] indicates non-significant association of histological subtype (solid+solid-follicular) with thyroid dose; [b] indicates significantly decreased chance of tumors with growth pattern other than solid or solid-follicular among cases with extrathyroidal extension

Continuation of Table 5.19

Intrathyroidal spread, children

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.1885	1.00	referent	-
0.1885 - < 0.387	0.38	0.07-1.54	0.18
0.387 - < 0.7923	0.58	0.11-2.61	0.48
0.7923 – 16.584	1.56	0.24-12.61	> 0.5
p-trend linear regression			0.43 [a]
	multivariate, stratified		
pT	8.48	2.05-43.28	0.003 [b]
pN	7.96	1.90-41.07	0.004 [c]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
0.110 - < 0.185	0.96	0.03-20.62	
0.185 - < 0.274	0.78	0.03-13.42	
0.274 - < 0.406	0.21	0.01-3.97	0.37
0.406 - < 0.547	0.68	0.02-11.27	> 0.5
0.547 - < 0.851	1.56	0.05-31.79	
0.851 - < 1.907	3.33	0.08-160.1	
1.907 –	1.52	0.04-54.32	
linear dose model *p*-trend			0.1 [a]
	multivariate, dose threshold		
pT	8.21	2.27-34.17	0.001 [b]
pN	6.54	1.73-28.86	0.001 [c]
Thyroid dose (Gy)			
< 0.547	1.00	referent	-
≥ 0.547	2.80	0.71-13.57	0.147 [a]

[a] indicates non-significant association of intrathyroidal spread with thyroid dose; [b] indicates significantly increased chance of intrathyroidal spread among tumors with advanced pT category; [c] indicates significantly increased chance of intrathyroidal spread among cases featuring lymph node metastases

Continuation of Table 5.19

Lymph node metastases, children

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.1885	1.00	referent	-
0.1885 - < 0.387	0.76	0.24-2.33	> 0.5
0.387 - < 0.7923	0.76	0.24-2.33	> 0.5
0.7923 – 16.584	0.84	0.27-2.65	> 0.5
p-trend linear regression			> 0.5 [a]
	multivariate, stratified		
Sex	2.95	1.03-9.55	0.042 [b]
Tumor capsule	0.11	0.01-0.84	0.034 [c]
M	4.36	1.00-31.23	0.049 [d]
Vascular invasion	0.05	0.01-0.21	0.004 [e]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
0.110 - < 0.185	1.48	0.20-10.8	> 0.5
0.185 - < 0.274	0.89	0.15-5.42	> 0.5
0.274 - < 0.406	2.21	0.26-18.37	0.45
0.406 - < 0.547	2.15	0.29-16.09	0.46
0.547 - < 0.851	0.81	0.15-4.44	> 0.5
0.851 - < 1.907	0.58	0.10-3.49	> 0.5
1.907 –	2.26	0.31-16.42	0.42
linear dose model *p*-trend			> 0.5 [a]
	multivariate, dose threshold		
Sex	2.89	1.05-9.33	0.041 [b]
Tumor capsule	0.15	0.01-0.93	0.036 [c]
M	4.41	1.00-28.41	0.048 [d]
Vascular invasion	0.05	0.01-0.20	< 0.001 [e]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
≥ 0.110	1.56	0.44-4.27	0.72 [a]

[a] indicates non-significant association of lymph node metastases with thyroid dose; [b] indicates significantly higher chance of lymph node metastasis in males; [c] indicates significantly decreased chance of lymph node metastases among cases with fully encapsulated tumor; [d] indicates significantly increased chance of lymph node metastases among cases with distant metastases; [e] indicates significantly decreased chance of lymph node metastases among cases without vascular invasion

In *adolescents*, only one pathological parameter was significantly different between radiation-induced and sporadic PTCs: extrathyroidal extension (yes vs no) (Table 5.20).

Table 5.20
Univariate and multivariate statistical analyses of association of extrathyroidal extension in exposed adolescents

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.1938	1.00	referent	-
0. 1938 - < 0.341	0.70	0.17-2.77	> 0.5
0.341 - < 0.802	1.43	0.36-5.81	> 0.5
0.802 – 24.11	0.45	0.10-1.88	0.28
p-trend linear regression			0.19 [a]
	multivariate, stratified		
pN	7.18	1.76-39.45	0.005 [b]
Vascular invasion	0.11	0.02-0.47	0.002 [c]
Thyroid dose (Gy)			
< 0.095	1.00	referent	-
0.095 - < 0.194	0.61	0.05-6.20	> 0.5
0.194 - < 0.264	1.54	0.13-20.72	> 0.5
0.264 - < 0.341	0.74	0.05-8.96	0.49
0.341 - < 0.425	2.19	0.17-32.21	> 0.5
0.425 - < 0.802	3.42	0.28-49.84	> 0.5
0.802 - < 2.449	0.47	0.04-5.06	0.42
2.449 –	0.67	0.04-10.43	0.37
linear dose model *p*-trend			> 0.5 [a]
	multivariate, dose threshold		
pN	7.18 [b]	1.76-39.45	0.005 [b]
Vascular invasion	0.11 [c]	0.02-0.47	0.002 [c]
Thyroid dose (Gy)			
< 0.194	1.00	referent	-
≥ 0.194	1.88	0.08-23.17	0.52 [a]

[a] indicates non-significant association of extrathyroidal extension with thyroid dose; [b] indicates significantly increased chance of extrathyroidal extension among cases with lymph node metastases; [c] indicates significantly decreased chance of extrathyroidal extension among cases without vascular invasion

In *adults*, the following pathological parameters were significantly different between radiation-induced and sporadic PTCs: extrathyroidal extension (yes vs no), lymph node metastases (yes vs no), and multifocality (yes vs no, marginally) (Table 5.21).

Table 5.21
Univariate and multivariate statistical analyses of association of different pathological parameters in exposed adults

Lymph node metastases, adults

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.160	1.00	referent	-
0. 160 - < 0.261	0.55	0.09-2.82	0.47
0.261 - < 0.560	0.73	0.14-3.51	> 0.5
0.560 - 12.28	0.74	0.15-3.52	> 0.5
p-trend linear regression			> 0.5 [a]
	multivariate, stratified		
Sex	0.05	0.02-0.53	0.044 [b]
Tumor capsule	0.01	0.00-0.22	0.034 [c]
Vascular invasion	0.02	0.00-0.17	< 0.001 [d]
Thyroid dose (Gy)			
< 0.102	1.00	referent	-
0.102 - < 0.155	0.03	0.00-1.79	0.13
0.155 - < 0.202	0.20	0.00-5.35	0.35
0.202 - < 0.261	0.01	0.00-0.20	0.012
0.261 - < 0.396	0.13	0.00-2.44	0.19
0.396 - < 0.562	0.04	0.00-1.54	0.13
0.562 - < 1.260	0.01	0.00-0.21	0.013
1.260 –	0.12	0.00-3.04	0.23
linear dose model *p*-trend			> 0.5 [a]
	multivariate, dose threshold		
Sex	0.06	0.03-0.51	0.042 [b]
Tumor capsule	0.01	0.00-0.21	0.036 [c]
Vascular invasion	0.02	0.00-0.18	< 0.001 [d]
Thyroid dose (Gy)			
< 0.102	1.00	referent	-
≥ 0.102	0.08	0.00-1.88	0.14 [a]

[a] indicates non-significant association of lymph node metastases with thyroid dose; [b] indicates significantly decreased chance of lymph node metastasis in males; [c] indicates significantly decreased chance of lymph node metastases in cases of fully encapsulated tumors; [d] indicates significantly decreased chance of lymph node metastases among cases without vascular invasion

Continuation of Table 5.21

Multifocality, adults

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.160	1.00	referent	-
0. 160 - < 0.261	NA [a]	NA	NA
0.261 - < 0.560	3.50	0.34-75.98	0.27
0.560 – 12.28	1.00	0.04-26.97	> 0.5
p-trend linear regression			0.12 [b]
	multivariate, stratified		
Tumor size	0.10	0.01-0.69	0.021 [c]
Thyroid dose (Gy)			
< 0.110	1.00	referent	-
0.110 - < 0.185	NA	NA	> 0.5
0.185 - < 0.274	NA	NA	> 0.5
0.274 - < 0.406	NA	NA	> 0.5
0.406 - < 0.547	NA	NA	> 0.5
0.547 - < 0.851	NA	NA	> 0.5
0.851 - < 1.907	NA	NA	> 0.5
1.907 –	NA	NA	> 0.5
Linear/quadratic dose model *p*-trend			0.11 [b]
	multivariate, dose threshold		
Tumor size	0.10	0.01-0.69	0.026 [c]
Thyroid dose (Gy)			
< 0.406	1.00	referent	-
≥ 0.406	4.05	0.49-87.00	0.20 [b]

[a] there were no or too few cases in the given dose range to fit the model; [b] indicates non-significant association of multifocality with thyroid dose; [c] indicates significantly decreased chance of multifocality among small tumors

Continuation of Table 5.21

Extrathyroidal extension, adults

Parameters	OR	95% CI	*p*-value
	univariate		
Thyroid dose (Gy)			
< 0.160	1.00	referent	-
0. 160 - < 0.261	0.80	0.16-3.91	> 0.5
0.261 - < 0.560	1.00	0.21-4.68	> 0.5
0.560 – 12.28	1.00	0.20-4.66	> 0.5
p-trend linear regression			> 0.5 [a]
	multivariate, stratified		
Tumor capsule	0.05	0.00-0.37	0.002 [b]
Vascular invasion	0.08	0.01-0.36	< 0.001 [c]
Thyroid dose (Gy)			
< 0.102	1.00	referent	-
0.102 - < 0.155	0.78	0.02-24.50	> 0.5
0.155 - < 0.202	0.57	0.03-11.74	> 0.5
0.202 - < 0.261	0.23	0.01-4.56	0.32
0.261 - < 0.396	0.51	0.02-9.09	> 0.5
0.396 - < 0.562	0.56	0.03-10.18	> 0.5
0.562 - < 1.260	0.15	0.00-3.24	0.23
1.260 –	0.32	0.02-6.00	0.44
linear dose model *p*-trend			> 0.5 [a]
	multivariate, dose threshold		
Tumor capsule	0.03 [b]	0.00-0.36	0.002 [b]
Vascular invasion	0.07	0.00-0.32	< 0.001 [c]
Thyroid dose (Gy)			
< 0.102	1.00	referent	-
≥ 0.102	0.42	0.04-4.45	0.45 [a]

[a] indicates non-significant association of extrathyroid extension with thyroid dose; [b] indicates significantly decreased chance of extrathyroid extension in encapsulated tumors; [c] indicates significantly decreased chance of extrathyroid extension among cases without vascular invasion.

Thus, as shown by the results of either univariate or any type of multivariate analysis (Tables 5.19-5.21), no evidence of significant association with thyroid dose was found for any pathological parameter tested in all age groups.

The only exception was a marginally significant association of the combined solid + solid-follicular subtype of PTC in children with the dose range from 0.851 to 1.907 Gy (Table 5.19), whose lower limit almost coincides with mean thyroid exposure dose (0.752 Gy) in children with these growth patterns (Table 5.14). In view of recently published results of molecular genetic study of PTCs identified in the Ukrainian-American cohort, which has established a positive association between *RET/PTC* rearrangements and ^{131}I thyroid dose with an inflection point at 1.6 Gy [20], this fact draws attention. Notably, namely *RET/PTC* rearrangements, *RET/PTC3* in particular, are most common among children and are associated with the solid and solid-follicular tumor structure [21, 22].

As a whole, radiogenic papillary carcinomas in children exposed after the Chernobyl accident at the age under 4 years (mean ^{131}I thyroid dose 1.016 Gy) and subdivided by their age at surgery into three groups (children, adolescents and adults) significantly differed from sporadic carcinomas in age-matched groups for a number of morphological parameters. Univariate and multivariate statistical analyses revealed the most substantial differences in both histoarchitectonics and invasive features of radiogenic PTCs, demonstrating their more aggressive behaviour especially well seen in age-matched groups of children. In adolescents and adults, morphological differences between radiogenic and sporadic tumors were found only for isolated parameters and those were revealed only by multivariate analysis.

Such a "convergence" of morphological differences between radiogenic and sporadic PTCs with increasing patients' age (hence the latency of radiogenic tumors), was not associated with the lower thyroid exposure dose or a certain dose range for most morphological manifestations of tumor agressiveness. A significant association was noted only between solid + solid-follicular subtype and a dose range from 0.851 to 1.907 Gy in children, for which an association with *RET/PTC* rearrangements was recently reported [20].

It is quite possible that future molecular genetic investigations employing advanced technologies, e.g next generation sequencing, will discover new genetic/epigenetic alterations unknown at present that are associated with radiation thyroid carcinogenesis, which, in turn, will allow better understanding of the mechanisms underlying more pronounced morphological signs of aggressiveness of radiogenic PTCs with short latency.

Continuous ongoing verifications of thyroid doses in children and adolescents of Ukraine after Chernobyl aimed at the reduction of uncertainties in dose estimates [23, 24] are highly important for the subsequent analyses of dose-response relationship. These would possibly allow establishing more confident associations between morphological parameters and exposure doses.

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Chapter 6

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Ukrainian contribution to the international Chernobyl Tissue Bank (CTB)

Cancer is an extremely complex disease that involves the interaction of biological pathways on a number of levels. Activation of the individual pathways does not necessarily occur in an independent fashion through parallel linear routes, but operates through large and complex networks of interacting pathways. Interactions between pathways can occur at a number of different levels and can interact directly, e.g. via phosphorylation events or indirectly, e.g. via regulation of gene expression.

In addition to understanding the interaction of pathways within a cell, it is clear that cancer cells do not exist within a vacuum. They respond to signals from outside of the individual cell, between different types of cell within a tissue (e.g. epithelial cells responding to signals generated from endothelial or stromal cells), and with stimuli external to the tissue, e.g. hormones, etc. Therefore cancer can be considered to be an extremely complicated system, one in which when one key node is blocked by use of an antineoplastic agent, for example, has the opportunity to re-route the signaling to overcome the block.

Most cancer researchers use rather reductionist approaches and focus their studies either on a particular gene of interest, or a particular pathway. The ability that we now have through the generation of "omics" data to provide data on multiple pathways simultaneously means that we need a paradigm shift in cancer research. The ability to generate several types of "omics" data from each individual cancer specimen will provide much more information about the system in general. This necessitates the ability to provide analytes of different types to be used in individual "omics" platforms to generate data on genome sequence, DNA copy number, epigenomic, transcriptomic, proteomic, and metabolomic data. The data then needs to be integrated to identify the key genes and pathways driving an associated phenotype, such as drug response or clinical outcome.

Tissue banks may play a key role in this shift in our approach to the characterization of cancer by not only supplying human biosamples to researchers using complimentary technologies, but also providing a platform for the data on an individual patient to be collated and correlated with clinical information.

The Chernobyl Tissue Bank was established in 1998 and designed with a systems biology approach to radiation-induced thyroid cancer in mind. This paper sets out the strategy for the development of the CTB, and, in particular, the substantial contribution made by Ukraine to its success.

The Chernobyl Tissue Bank – the paradigm for a cancer resource designed for systems biology

The CTB has been funded by four sponsors (the National Cancer Institute of the US, the European Commission (EC), the Sasakawa Memorial Health Foundation of Japan (SMHF) and the World Health Organization (WHO)) and is supported by two of the countries most affected by fallout from the reactor accident, Ukraine and the Russian Federation.

In the early 1990s, a number of projects studying the effect of the Chernobyl accident were funded by the four sponsors listed above. The increase in thyroid cancers in children and adolescents in Belarus and Ukraine had been confirmed in a number of publications [1,2]. By 1995, it was becoming apparent that several European research groups were unknowingly receiving material from the same patients for research, and that there were discrepancies in the pathological diagnoses being applied to the same tumor. Subsequently, a report to the EC confirmed that there had indeed been considerable overlap since 1995 among a number of EC-funded molecular biology projects [3]. It was then recognized that a cooperative tissue bank would reduce the duplication of research effort and provide better scientific data on the health effects of the Chernobyl accident. Following agreement on the various protocols, the Chernobyl Tissue Bank officially started collecting material on October 1, 1998. The full history and detail on the ethics and governance of the project have already been published [4].

Study cohort

The CTB study cohort comprises all patients with thyroid carcinomas and cellular follicular adenomas from the contaminated oblasts (the Russian and Ukrainian equivalent of a US state) of the Russian Federation (Bryansk, Kaluga, Tula, and Oryol) and Ukraine (Kiev, Kiev City, Cherkassy, Chernigov, Rovno, Zhitomyr) who were born on or after April 26, 1967 (i.e., aged under 19 years at the time of the Chernobyl accident) and operated on or after the October 1, 1998. In addition, a number of cases have been collected from other areas of Ukraine, relatively less contaminated by radioactive fallout. The collection currently comprises 4,288 cases of thyroid cancer and cellular follicular adenoma from patients who were under 19 years at the time of the Chernobyl accident; 2,267 of these are from Ukraine. Frozen material is available on 1,727 out of the 2,267 cases from Ukraine, and DNA and RNA has already been extracted from a quarter of these cases. Collection of blood samples began in late 1999 and samples of serum and whole blood have been collected from around 950 Ukrainian patients. One important feature of the project is that it also collects biosamples from patients resident in the areas of Ukraine and Russia exposed to

radioactive fallout, but who were not exposed to radioiodine, as they were born more than 9 months after the accident. These cases form an age- and residency-matched cohort of patients who develop spontaneous thyroid neoplasia. This is the ideal cohort for comparison with those who were exposed to radioiodine in 1986. The current number of cases in this valuable cohort is 379 (245 with a diagnosis of cancer, of which 124 are from Ukraine). This number is much lower than those exposed to radioiodine – the incidence of thyroid cancer being approximately the same as the background spontaneous rate from uncontaminated regions – of the order of 1 per million per year.

The current project therefore consists of two banks of biological material and information comprising:

- Snap frozen and formalin fixed, paraffin embedded samples from tumor, normal tissue and, where possible, metastatic tissue from postoperative specimens,
- nucleic acids extracted from these specimens,
- vials of serum from patients whose thyroid tissue is held in the bank,
- samples of whole blood,
- DNA extracted from blood,
- Results from research projects supplied with samples from the CTB.

The Ukrainian contribution to the project represents 64% of the total.

Data management within the CTB

The data management infrastructure for the CTB was designed to facilitate a systems biology approach to thyroid cancer. It comprises two separate databases, plus an integrated database that serves as a portal for researchers to access information on samples and data held, and to apply for access to both data and samples (Fig. 6.1).

One, centralized web-accessible database, held on secure servers at Imperial College London holds anonymised information on donors to the CTB and the biological samples donated by them. Regular, automated transfer of patient data back to secure servers in Ukraine and Russia ensures that each center has a local mirror copy.

Detailed standard operating procedures for the collection and documentation of specimens and blood samples have been agreed upon with professional staff involved in the collection of material, and ethical standards agreed upon with the relevant authorities, conforming to the requirements of each country involved and those of the funding organisations. Each donor is identified by a unique alphanumeric code. Samples from each donor are identified by suffixes to this code enabling the specimens and any derivatives from them to be linked to the tissue block they were derived from and the individual donor. Tissue and blood samples, and extracted materials are recorded within tables in the CTB database. The database schema allows easy transfer of data between different database systems such as IBM DB2, Oracle, Microsoft SQL Server, MySQL, and PostgreSQL.

The samples database holds relevant information on the patient (date of birth, date of operation, sex, oblast of residence at the time of the accident and operation) together with pathological information and location coordinates for each sample of tissue, DNA or RNA

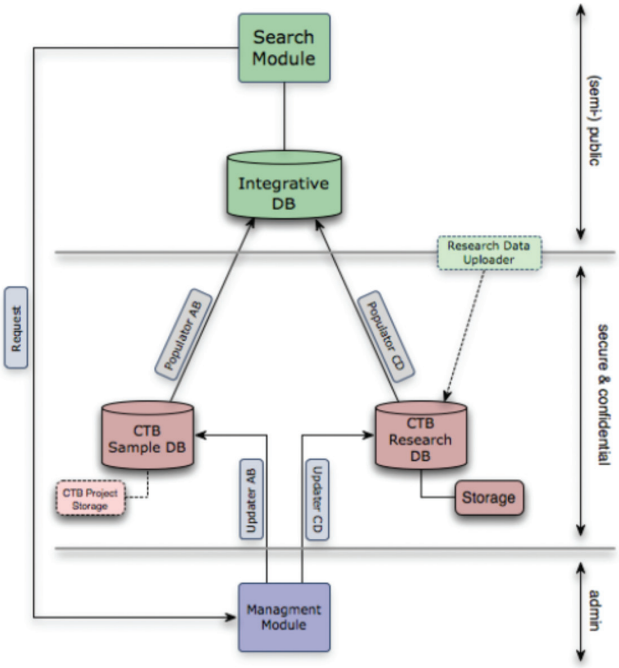

Figure 6.1. The CTB data warehouse combines the Samples, Research and Integrative Databases, Management and Search Modules with corresponding front-ends/interfaces. The system was developed with assistance from the Bioinformatics Support Services (BSS) at Imperial College, and continued close cooperation is essential for the smooth and secure integration of all the components of the overall system. Access to further samples and to information stored in the research database from the use of previous samples in research from the same patient is provided by the CTB portal.

extracted from tissue, and blood, serum and DNA extracted from blood, and information on the quality assurance of these derivatives is also recorded within the samples database. Dosimetry information for each patient has been provided in collaboration with Professor Ilya Likhtarov (see Chapter 2).

Security and integrity of the data in the CTB Data Warehouse is of paramount importance. With regards to the Samples database and corresponding front-end, the access to data sets is appropriately restricted according to a country (e.g. Ukraine, Russia) and a role (e.g. pathologist, lab technician, administrator) to which a user belongs. Integration of the Samples database with the rest of the system and transfer of data between different elements of the overall CTB Warehouse are submitted to the same strict security requirements and are designed to minimize the risks of data loss or theft. Regular, time and place restricted updates of the Integrative database from the Samples and Research databases provide the up-to-date link between the research data and the key clinical-data elements required by a researcher.

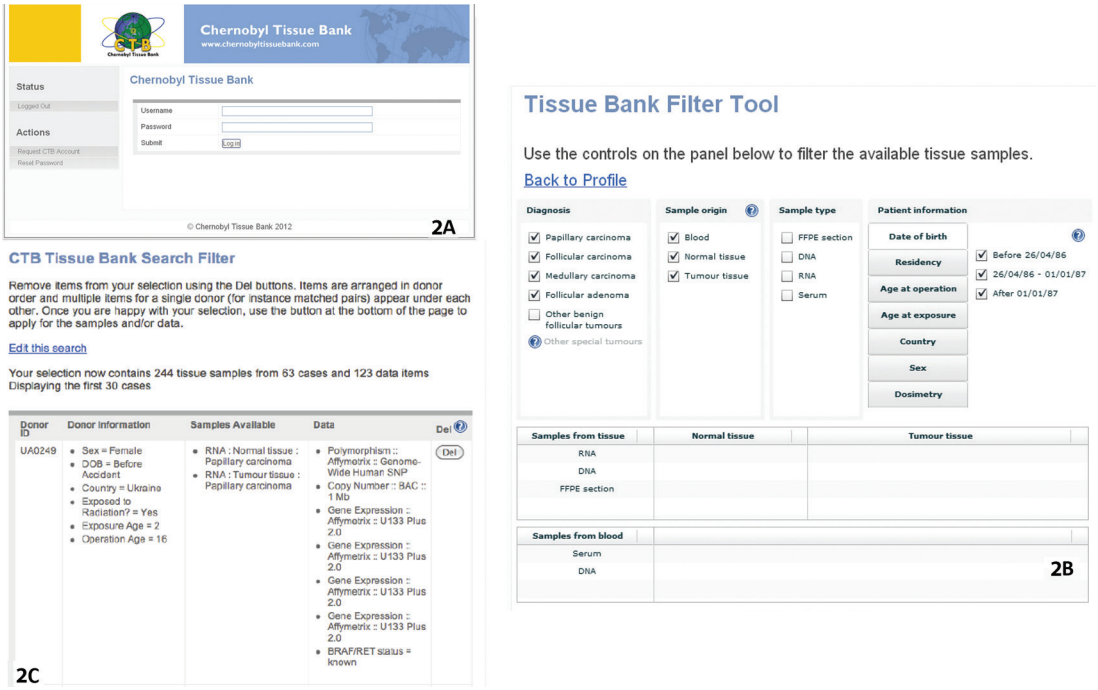

Figure 6.2. Representative screenshots of the CTB portal. (2A) Login entry page. (2B) User searches samples by selecting criteria of interest. As search filters are selected, numbers of samples matching criteria are shown. (2C) Representative search results page showing available samples, search criteria and additional data available for sharing.

*The CTB Portal (*https://cisbic.bioinformatics.ic.ac.uk/ctb/html_ctb_public/*)*

The CTB Portal provides on-line access to the resources of the CTB. Accessible either directly or from the CTB web site, the Portal gives access to a simple, but powerful, search facility that allows a researcher to search the database and check if samples are available that match the requirements for their study. Biomaterials are defined by: the type of thyroid tumor (from the consensus diagnosis of the Pathology Panel); the origin of the sample – blood, tumor tissue or normal tissue; the type of sample – FFPE section; extracted DNA or RNA; and key patient information such as age at exposure, residency, etc. The results of the search show the number of cases that match the criteria and the number of samples that are available. The system went live on the 25th anniversary of the Chernobyl accident. Representative screenshots of the Portal and the search filter are shown in Figure 6.2.

The PI is guided through the on-line application process to request samples. Management tools embedded within the Portal, and accessible only by the secretariat, facilitate the processing of applications and tracking progress through the review and approvals process. Once the applicant has submitted their application on-line, the secretariat checks that the application is complete. The status of the application is altered as

it progresses through each stage of the process and an e-mail is automatically sent to the PI acknowledging each change of status: (submitted for review, more information required, etc.). The software automatically compiles the various sections of the application into a PDF. The secretariat then forwards this to the External Review Panel for assessment of the scientific quality of the project requesting access.

The integrative database produces a comprehensive list of all the cases identified that match the search criteria entered by the applicant. This listing is available only to the secretariat and is a significant step in the automation of the process of selecting appropriate samples for a project. The process can never be totally automated and expert oversight of the pathological information will always be required. However, the initial screening facilitates this procedure.

The CTB Portal also provides access to the Data Warehouse both for PIs to upload data from their approved CTB projects and for other researchers to be able to see if data is available linked to the cases they have selected.

Use of CTB samples in research

Biospecimens from the CTB have been provided to the major research groups involved in the studies of the consequences of the Chernobyl accident. Information on the projects receiving biosamples can be found at http://www.chernobyltissuebank.com/research.html. Thirty projects have been approved for access to date; 11,901 samples have already been released to these projects. Of these, 11,498 were provided from the Ukrainian section of the CTB. Scientific evaluation of each project is provided by the CTB's External Review Panel (ERP) and the outcome of the review and, where appropriate, any feedback from the ERP is provided to the applicant.

This approach provides a basis that fosters international collaboration and reduces the chance of competition and even friction between groups in their requests for this material. Researchers who obtain material from the resource agree to provide the results of their investigation on a case-by-case basis to enable combined analysis to be carried out at a later date. The provision of extracted nucleic acid from thyroid tissue, rather than each researcher being provided with a small piece of tissue, maximises the amount of data that can, potentially, be obtained from a single operative specimen and enables multiple molecular biological studies to be carried out for each case. The median number of projects supplied by material from a single case at present is 4 with some being used in more than 9 projects. Details of the publications resulting from material supplied by the project are listed on the project website (www.chernobyltissuebank.com/papers.html). Data on Copy Number Alteration, mRNA expression, SNP and mutation/translocation of thyroid cancer key oncogenes *RET* and *BRAF*, is already available for over a quarter of the cases, with miRNA array and methylation DNA array data being made available through the EU funded EpiRadBio project. Similar data will also be available soon from patients enrolled in the Ukrainian-American Cohort.

Results of Research studies

The pathology of all cases submitted to the CTB is reviewed by an International Pathology Panel. Their review of the cases has suggested a new classification for thyroid cancer [5] and has shown that the latency affects the morphological subtype and aggressiveness of papillary cancers [6] with the frequency of the solid subtype falling from 24 to 6% over the first two decades after the accident, and a similar decline in the frequency of extrathyroid extension and lymph node metastases [7]. A more detailed explanation of the pathological features can be found in Chapters 4 and 5 in this book.

The results of molecular biological studies that have used material from the Chernobyl Tissue Bank are included in Chapter 7 in this book. An abstract of all projects authorized to use material from the CTB, and links to papers that have resulted from these projects, are available on the project website (www.chernobyltissuebank.com).

Thus, we can conclude that understanding the major drivers in tumour growth will depend increasingly on being able to take a systems biology approach, rather than an individual gene or analytical platform approach. It is already evident from the literature that a change in copy number at the DNA level does not always result in an increase in RNA expression of all of the genes coded for by the amplified region, and that identifying critical nodes within networks of converging signalling pathways will be necessary to understand the functional biology of cancer [8]. Collating all of the research data generated from the samples donated by those affected by the Chernobyl accident is a challenge. By collating the data in a central resource, the CTB will facilitate not only projects that require access to samples alone, but also projects that wish to link their results with data already available. Tissue banks are likely to be key players in this type of cancer research in future and should be designed from the outset to facilitate this aim.

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Chapter 7

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Molecular biology studies of Ukrainian thyroid cancer after Chernobyl

Cancer is known to be an extremely complex disease that is caused by a variety of different agents, including radiation. An understanding of the molecular mechanisms that generate cancer, and the link between molecular features and aetiology, prognosis and response to treatment is pivotal in the successful treatment of the patient. The unprecedented increase in thyroid cancer following the accident at the Chernobyl power plant provided the opportunity to understand the molecular biology of this type of malignancy, and to link molecular features with radiation aetiology. Thyroid cancer in young people is a rare disease, with an incidence rate of around 0.5–1.5 cases per million per year [1]. Initial reports suggested that thyroid cancers that were observed in young people following the Chernobyl accident were different from those from an unexposed population. However, more careful analysis showed that this rare variant, a solid type of papillary cancer that showed extensive invasion, was common in young patients, whether exposed to radiation or not [2]. This contrasts with the classic and follicular variants of papillary carcinoma found more commonly in adults. Since the pathological appearance of a tumor is related to the integration of the molecular events that are involved in its generation, a number of studies have concentrated on defining the various genetic and epigenetic changes involved.

Cases from Ukraine, provided via the Chernobyl Tissue Bank (CTB) have been used extensively in these analyses. The early studies concentrated on the frequency of activation of oncogenes already known to be pathogenic in papillary thyroid carcinoma (PTC). However, more complex molecular techniques have developed in the last decade, and the later studies concentrate on the identification of multiple molecular changes in these tumors.

Changes in the Genome

Somatic DNA – single oncogene studies

Genetic alterations that induce the Mitogen activated protein kinase (MAPK) signalling pathway are common in sporadic PTC. The MAPK pathway is involved in regulation of differentiation, survival and cell growth, and its malfunction is well known to be tumor

promoting [3]. Common alterations include both gene rearrangements and point mutations. Inversions and translocations of a given chromosomal region can lead to an activation of an oncogene that is inactive in the normal thyroid cell. The commonest rearrangements in PTC are those that involve paracentric inversion of part of chromosome 10, *RET/PTC1* and *RET/PTC3*, resulting in fusion with the *H4* gene (*CCDC6*, *D10S170*) and *NCOA4* gene (*RFG*, *ELE1*), respectively. The *BRAF* oncogene which is frequently mutated (point mutation) in adult PTC has also been found to be activated in a small number of radiation-induced PTC following inversion on chromosome 7q [4]. Alterations in the *BRAF* and *RET* oncogenes are usually mutually exclusive [5,6].

Other studies have identified chromosomal breakpoints on 1p32-36, 1p11-13, 1q22, 3p25-26 and 7q32-36 [7-9] and a deletion on 11q [10], but their importance in radiation-induced childhood PTC remains unclear. One reason why some rearrangements occur more frequently than others may be the close proximity of the two gene loci participating in the rearrangement within the nucleus. This theory is supported in a study by Nikiforova *et al*. on the PTC specific translocation of the *RET* and *H4* genes thereby forming *RET/PTC1* [11]. In vitro irradiation studies show increased DNA end-joining enzymatic activity which argues in favour of a radiation-specific response related to gene rearrangement in thyroid cells [12].

RET/PTC gene rearrangement in Ukrainian cases

The *RET* gene is located on chromosome 10q11.2. Its breakpoints for rearrangement are all located within intron 11. The encoded receptor tyrosine kinase has an extracellular domain, a transmembrane domain and an intracellular domain [13]. Fusion of *RET* with other genes provides the tyrosine kinase domain with a new promoter and 5' coding region which leads to constitutive expression of the protein and transmembrane domain is substituted by dimerization domains for ligand-independent activation [14]. There are 17 different *RET/PTC* rearrangements that have been identified in PTC, but inversions of chromosome 10 resulting in *RET/PTC1* and *RET/PTC3* are the most frequent. The *RET* proto-oncogene was already known to be involved in papillary thyroid carcinogenesis in studies of sporadic cases where rearrangements have been found in up to 40% of cases. Rearrangement has been shown to be restricted to the papillary type of thyroid cancer [15,16] and occur frequently in small papillary carcinomas suggesting that it is likely to be a driving event in early tumorigenesis. The breakpoint in the DNA occurs within an intron, which permits the use of reverse transcriptase PCR (RT-PCR) using RNA extracted from the tumors as a useful tool for identifying PTCs that harbour *RET/PTC* rearrangements. However, studies using this technique need to be interpreted with caution, as it has been shown that the results are heavily influenced by the PCR protocol used and tumor heterogeneity [17].

Initial studies post Chernobyl were carried out on Belarussian cohorts [18-20] and suggested an increased frequency of *RET/PTC* rearrangement in radiation induced PTCs in children. In a study on Ukrainian cases, reported by Santoro *et al*. [21], of the 106 papillary carcinomas which produced amplifiable RNA and were analysed for *RET* rearrangement using rearrangement specific RT-PCR, 20 were identified as positive for *RET/PTC1* and 15 for *RET/PTC3*; one other tumor was positive for both *RET/PTC1* and *RET/PTC3*. Analysis of the expression

of the extracellular and tyrosine kinase domains was also performed independently in a different institute in 45 of the 106 cases. Twenty-two of these 45 tumors were positive for expression of *RET* tyrosine kinase. No tumor sample showed positivity for the expression of the *RET* extracellular domain only, suggesting that the *RET* tyrosine kinase expression was due to the presence in these tumors of a *RET* rearrangement fusing the tyrosine kinase domain of the *RET* gene with an active promoter. Among these 45 cases investigated for *RET* tyrosine kinase expression, there were 20 with an identified *PTC1* or *-3* rearrangement, including one tumor which showed both *PTC1* and *-3*; 18 showed *RET* tyrosine kinase expression. The unexpected negative *RET* tyrosine kinase in two cases may have been the result of a lack of sensitivity. Twenty-five cases lacked either a *PTC1* or *-3* rearrangement, 21 were negative for *RET* tyrosine kinase expression, while four were positive. The positive *RET* tyrosine kinase in these four cases could have resulted from the presence of a *RET* rearrangement other than *PTC1* or *-3*. Overall agreement between the two techniques was therefore present in 39 out of 45 cases (87%). The type of *RET* rearrangement correlated with tumor morphology; *RET/PTC3* dominated in the predominantly solid subtype and *RET/PTC1* was more common in the classical subtype of PTC. This is consistent with previous studies on other Chernobyl-related tumor cohorts [20,22,19] and with transgenic models of thyroid cancer in animals [23,24]. A cohort of 28 PTCs from Ukraine and 39 from Belarus was examined by Thomas *et al*. [25]. Patients received surgery 9-11 years after exposure to radiation and their age range at operation was between 6-18 years. 60.7% of the Ukrainian and 51.3% of the Belarussian cohort were *RET/PTC* positive. 23 PTCs harboured the *RET/PTC3* rearrangement and 14 patients the *RET/PTC1*. *RET/PTC3* was found to be associated with the dominantly solid subtype of PTC, whereas *RET/PTC1* was associated with the classic subtype of PTC. Matched normal thyroid tissue samples were negative for *RET* rearrangements. In addition, lymph node metastases of 8 Ukrainian patients were examined. *RET* rearrangement was detected in all cases where the rearrangement was already present in the primary tumor. One metastasis was found to be *RET/PTC* positive although the primary tumor was negative. It had been shown that both ionizing and external radiation [26] were able to induce *RET* rearrangements *in vitro* [27], suggesting that *RET/PTC* rearrangement in these tumors was a potential marker for radiation exposure. *RET/PTC3* rearrangement is more prevalent than *RET/PTC1* and linked to the solid subtype, which was, at the time believed to be more frequent in radiation induced tumors. In the rare cases where it occurs in adult onset sporadic PTC, it was linked to a more aggressive, invasive phenotype with a higher potential to metastasize [28]. Since the early pathological studies suggested that the PTCs found in children post Chernobyl were more aggressive than their adult counterparts, it seemed logical to suggest that this rearrangement was important in the solid subtype, aggressive PTCs, that were frequent in the short latency PTCs, which were being examined at that time.

However, in all the studies reported above there was an inherent problem. Frequencies of *RET* rearrangement in young onset, post Chernobyl PTCs were compared with the frequencies of this oncogene in adult PTCs. Later studies [29], some using age-matched controls [30], have shown that the frequency of *RET* rearrangement in early onset PTC, regardless of prior radiation exposure of the patient, is much higher than that found in adult PTC, suggesting that the initial conclusions on *RET* rearrangement being a biomarker for radiation exposure were incorrect.

Tumor heterogeneity

RT-PCR is a useful tool, but does not allow identification of the proportion of cells that carry the *RET/PTC* rearrangement. It was noted that the strength of RT-PCR signal for *RET/PTC* varied considerably between cases, and that this might suggest that this may represent intratumoral heterogeneity. Unger *et al*. [31] therefore examined 32 patients from the Ukrainian cohort with a tumor latency of 9-12 years for the intratumoral distribution of the *RET/PTC* rearrangement using FISH. FISH is a DNA hybridization method targeting metaphase chromosomes or interphase nuclei on tissue sections. FISH is capable of detecting *RET* rearrangements without further knowledge of the fusion partners. It allows for examination at the single cell level and can contribute to a better understanding of the tumor heterogeneity. Interphase FISH was chosen for the detection of the chromosomal alterations involving the *RET* gene, [31,32]. Two YAC probes including regions within the *RET* target locus and distal to the *RET* sequence were chosen and labelling was performed using two different fluorescent dyes (Fig. 7.1). A confocal laser scanning microscope was used for visualisation. Cell nuclei were scored for the presence of a separated red and green FISH signal in addition to an overlapping signal. This helps to avoid misclassification, which is dependent on the location of the interphase chromosome 10 within the nucleus and the spatial proximity of *RET* to putative rearrangement partners. Overlapping signals occur only in normal nuclei, and split signals indicate an aberration of the *RET* gene. This approach has been further validated by using careful selection of controls, including *RET/PTC* positive and negative cell lines as well as wild type *RET* expressing controls (Fig. 7.2).

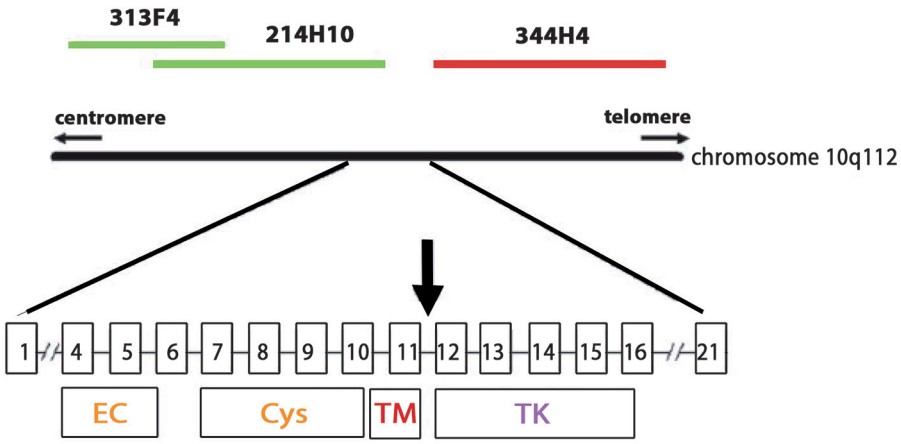

Figure 7.1. Mapping of YAC probes 313F4, 210H10 and 344H4 on chromosome 10q11.2. YAC clones 313F4 and 214H10 (FITC-labelled, green) map proximal to and include the *RET* locus, 344H4 (Cy3-labelled, red) maps distal to *RET*. Exons and different parts of the RET gene are indicated below (EC=extracellular domain, Cys=cystein-rich domain, TM=transmembrane domain, TK=tyrosine kinase domain).

The study reported by Unger *et al*. [31], comprised 29 PTCs, two follicular adenomas, and a follicular cancer. Detection of the *RET* rearrangement with conventional qRT-PCR revealed that 13 (40.6%) of the patients expressed the TK domain of *RET*, and in only

two and three patients, respectively, was a clear *RET/PTC1* and *RET/PTC3* signal detected. Investigation of the cases with FISH was more sensitive and 23 patients (72%) were positive for the *RET* rearrangement. A threshold for positivity was set as evidence of rearrangement in a minimum of 7% of cells within a tumor. The highest observed frequency of cells bearing the *RET* rearrangement positive FISH signal was 46%. In none of the tumors was a FISH positive signal observed in 100% of tumor cells. Further analysis showed that the distribution of FISH positive cells was inhomogeneous.

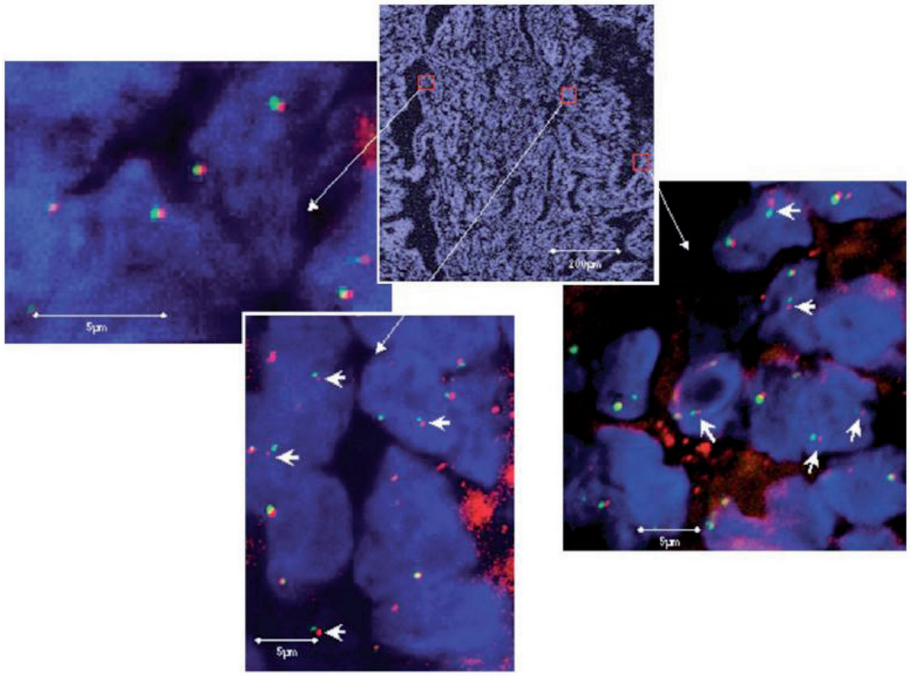

Figure 7.2. Example of FISH analysis with *RET*-specific YAC probes using confocal laser scanning microscopy (CLSM). Three different areas were randomly selected from the tumor as indicated at the 40x magnification image (TOPRO image only, top center). One viewing area (left side) shows only normal cells exhibiting overlapping FISH signals. Two other viewing areas (middle and right side) show a cluster of aberrant cells identified by split FISH signals (arrows). All images are superimposed from approximately ten different slices throughout the thickness of the tissue section. For a more precise evaluation a stepwise scoring every 0.5 µm is applied.

The same group repeated the *RET* rearrangement analysis on a Ukrainian cohort of 13 patients presenting with PTC after a shorter latency of 4-8 years post Chernobyl [32]. They compared this short latency group to the long latency group described previously for the distribution of the *RET* rearrangement within a given tumor sample. Examination with interphase FISH showed 10 of the patients (77%) were positive for a *RET* rearrangement with a frequency of rearranged cells between 11-54%. In 9 cases, the *RET* rearrangement was homogenously distributed all over the tumor tissue, whereas one case showed distinct clustering in certain areas of the tumor. Both studies identified a similar frequency of intertumoral rearrangement of *RET*, but the intratumoral distribution of the

RET rearrangement between the groups differed significantly. The longer latency group included a higher frequency of tumors with a heterogenous distribution and clustering of the rearrangement. At the same time, others had noted that tumor morphology was changing from a predominantly solid subtype in the shorter latency to a solid-follicular subtype in the longer latency group [33]. The reason for this may be the outgrowth of distinct subclones in longer latency tumors or the presentation of *RET/PTC* as a second event in thyroid carcinogenesis.

In a later study, reported by Hieber *et al*. [34], interphase FISH analysis was used to assess *RET* rearrangements of the cases. Aside from five other chromosomal rearrangements, 16 of the cases (72%) were positive for *RET* rearrangements. This is a rate similar to that observed in other studies [31,32]. With a threshold for *RET* positive of 7.1%, the number of rearranged cells per case varied from 10.6% to 41.5%. There was no obvious correlation with age or gender of the patients, but the median age at operation of the patients carrying rearrangements was higher (22 years) compared to the group with no rearrangement (18 years). Within the group carrying rearrangements, older patients showed greater tumor heterogeneity with respect to *RET/PTC* rearrangements, consistent with the earlier study by Unger *et al*. [31].

The BRAF proto-oncogene

BRAF is a RAF kinase which plays an important role in the MAPK pathway. It is member of the family of RAF proteins, and due to its serine/threonine kinase activity it phosphorylates the MAPK/ERK kinase upon stimulation by RAS [35]. It is frequently mutated in thyroid cancer, where the point mutation at nucleotide position 1799 is the most prominent one. A transversion from thymine to adenine (*T1799A*) changes the amino acid sequence in codon 600 from valine to glutamic acid (*V600E*); thus the abbreviations used to designate this mutation are $BRAF^{T1799A}$ or $BRAF^{V600E}$. This results in constituent activation of the gene, thus activating the MAPK pathway. The frequency of mutation varies in a number of studies from 36-69% in adult papillary thyroid carcinoma [36,37], including one study on Ukrainian tumors [30]. In adult cases, *BRAF* mutation significantly correlates with clinical parameters in PTC such as distant metastases, advanced clinical stage and disease specific mortality [38]. In children, the involvement of *BRAF* has been found to be somewhat different. Kumagai *et al*. [39] performed a study on childhood PTC from Japan and Ukrainian patients with PTC following the Chernobyl accident, and compared the frequency of *BRAF* mutation with that found in adult PTCs. Sequence analysis of the $BRAF^{T1799A}$ mutation in exon 15 found only one positive case in the young Japanese cohort. No mutations in *K-, H- or N-RAS* were detected. The same result was obtained for the childhood group (aged under 15 at diagnosis) within the Ukrainian cohort. However, among the cases of young adults, 8 out of 33 carried the $BRAF^{T1799A}$ mutation and two other cases carried *RAS* mutations, one in *NRAS* codon 61 and one in *HRAS* codon 61. The Ukrainian group was also examined for *RET* rearrangements. 5 out of 15 in the childhood group exhibited the rearrangement, and 12 out of 33 in the group of young adults. Mutation of *BRAF* and rearrangement of the *RET* oncogene were mutually exclusive. Interestingly, in this study, the presence of the mutation was not

associated with large tumor size, regional lymph node invasion, extrathyroidal invasion, or distant metastases.

Lima *et al*. [40] examined a cohort of 34 post-Chernobyl paediatric PTC from Ukraine and found that four were positive for the *BRAF*^{V600E} mutation. 14 of the patients had a *RET/PTC* rearrangement, but *BRAF* mutation and *RET* rearrangement were, again, mutually exclusive. The *BRAF* mutation occurs predominantly in the papillary and follicular variants, whereas the *RET* rearrangements were more common in the solid subtype of the tumor. The control group of sporadic pediatric PTCs contained no solid variants, and the frequency of *RET* rearrangement was not determined. Powell *et al*. [30] used 67 cases of post-Chernobyl PTC from Ukraine to examine whether the *BRAF* mutation was related to the age of the patients or the radiation exposure. 32 patients were aged above 30 at time of clinical presentation, and 35 patients were under 16. All patients, exposed or unexposed to radiation, were residents in the Ukraine and hence an ethnic difference as confounder can be excluded. Paired tumor – normal samples were analysed and 18 out of the 32 adult cases were found to harbour the *BRAF*^{V600E} mutation in the tumor but not in the normal tissue. The tumor morphology was of dominantly classic or dominantly follicular subtype. Only one child in the group of 35 exposed cases was positive for the mutation and none of the sporadic PTCs. Interestingly, the one case bearing the *BRAF* mutation was of a dominant papillary subtype.

In summary, *BRAF* mutations are more commonly seen in PTCs with a dominantly papillary morphology, and are more common in PTCs diagnosed in adulthood rather than childhood. The low frequency of *BRAF* mutation in PTCs from children exposed or not exposed to radiation suggests that, as with *RET* oncogene rearrangements, the age of the patient at diagnosis is a significant factor. It is therefore important to ensure that cases are appropriately age matched when seeking to identify radiation related changes in molecular biology of tumors. One study has also shown that in post-Chernobyl PTCs from Belarus, *BRAF* can be activated by gene rearrangement. In this case, the C-terminal region of the *BRAF* gene is fused to the first eight exons of the *AKAP9* gene building the *AKAP9/BRAF* fusion gene [4]. However, as is the case with the early studies of *RET* rearrangement in post Chernobyl PTCs, this study lacked age matched controls and it is therefore unclear whether this finding is related to radiation or to the age of the patient at operation.

Other proto-oncogenes

Although *RET/PTC* and *BRAF* alterations are the commonest oncogene alterations seen in PTC, rearrangements and mutations of other genes are also observed, but at a much lower frequency. *NTRK1* encodes the high affinity nerve growth factor (NGF) receptor, and is occasionally rearranged in PTC. *NTRK1* fusion partners are the genes *TMP3*, *TPR* and *TFG* [41,42]. The *NTRK1* rearrangement, is only present in about 11% of sporadic PTCs [43], and is rare (3.3%) in post-Chernobyl thyroid tumors [44-46].

Other types of thyroid cancer are known to involve much different oncogenes, such as the *RAS* oncogene [47] in follicular carcinoma or *TP53* in anaplastic carcinoma [48]. Mutations of the thyroid stimulating hormone receptor (*TSH*R) gene occur in follicular carcinoma and benign thyroid tumors [49,50]. Santoro *et al*. [21] examined whether one

of these molecular changes had an influence on the development of PTC after exposure to radiation. The group studied 128 PTC of post-Chernobyl patients who received surgery before the age of 15, all of whom were from Ukraine. In addition to *RET* rearrangement, reviewed in the section above, mutations in the *RAS, TP53* genes, and exon 10 of the *TSHR* were also analysed and correlated with the morphological status of the tumor samples.

Alterations in the other genes of interest were not found to be present in the post-Chernobyl PTC cohort. Other studies could also not demonstrate correlation of *TSHR* or *TP53* mutations in thyroid carcinogenesis after radiation exposure [51-54]. *RAS* mutations are not commonly found in sporadic PTCs, although they are identified in follicular thyroid tumors, and detection in radiation-induced childhood PTC is similarly rare [39,53,55].

These results show that mutations in the *TP53* and *H-, N-*, and *K-RAS* genes play no important role in the carcinogenesis of radiation-induced PTC.

Studies on genomic profiling

Array-based comparative genomic hybridisation (aCGH) is a technique that allows an objective and quantitative examination of copy number changes of the entire genome at a high resolution. BAC clones covering the whole genome in 1MB distances are spotted on glass slides and used for hybridisation with probe sequences of a tumor sample. Genomic alterations such as deletions or amplifications are detected with a greater sensitivity and improved resolution (1MB) than by karyotyping, but chromosomal rearrangements such as translocations and inversions cannot be identified using this technique. aCGH is also capable of detecting copy number variations which occur regularly in normal tissue and need to be distinguished from their pathogenic counterparts.

Unger *et al*. [56] analysed 33 patients who developed PTC following the Chernobyl accident for chromosomal aberrations using aCGH and validated the results by FISH. The cohort was a mix of 13 children and 20 adults with known *RET/PTC* rearrangement status. *RET/PTC* positive cases and *RET/PTC* negative cases were compared, as well as children versus adults. In all cases, deletions occurred with a higher frequency than amplifications. This might have been predicted, as radiation is thought to introduce chromosomal breaks which may lead to deletions, translocations or inversions rather than a chromosomal gain. Losses were predominantly on chromosomes 1, 6, 7, 9, 10, 11, 12, 13, 16, 19, 20, 22 and gains on chromosomes 10, 12, 19, 20, 21. Hierarchical clustering distinguished the three groups; childhood *RET/PTC* positive, adult *RET/PTC* positive and adult *RET/PTC* negative. *RET/PTC* positive cases differed significantly for a region on chromosome 1p which was deleted in adults, but not in children. All *RET/PTC* positive cases differed significantly from the negative cases in losses on 1p, 7p, 9, 13q and gains on 3q, 4p, 12q, 21q. Deletions of regions on 9q, 10p, 10q and 22q had also been observed in other studies on thyroid tumors [57-61]. Hence, these aberrations seem to be more specific for carcinogenesis in thyroid than for the exposure to radiation and hence cannot be considered as radiation specific biomarkers. Gene detection in the altered regions revealed 31 candidate genes which are known to be involved in tumor progression and 21 tumor suppressor genes which specifically map to deleted chromosomal regions. It is of note that the majority of these genes is known to be involved

in thyroid carcinogenesis, and a further study giving a comparison with sporadic PTCs would be necessary to define a specific role in radiation induced tumor development. The genes identified are involved in the molecular pathways of apoptosis, interleukin-27, angiopoietin receptor and the PI3K/MAP kinase. The data reflects a large heterogeneity in post-Chernobyl PTC which suggests the existence of various routes of tumor development. In terms of biomarker screening this may imply that a whole panel of markers will be necessary for an exact detection of a radiation signature.

Stein *et al*. [62] used tumor and matching normal tissue of 10 Ukrainian patients with post Chernobyl PTC to examine copy number changes and gene expression. The latency for these tumor patients was 13-14 years, and age at exposure varied from 3 months to 18 years whereas age at operation was from 14-31 years.

In studies on sporadic PTCs, copy number alterations (CNAs) are detected with a low frequency. Depending on the study, the occurrence of gains and losses ranges between 2-50% [7,59,63]. Detection is strongly dependent on the method used and the conventional CGH and cytogenetic approaches used in these studies have not the same high resolution as the currently used array-based CGH (aCGH). Stein *et al*. used high resolution SNP array CGH technology for CNA examination. Several amplifications on chromosome 22 were detected in all 10 samples. Also, chromosomes 1 and 12q show amplifications with high frequency. Further, less frequent amplifications on 5p, 9q, 16p, and 21q were also detected. Deletions were identified in chromosomes 21q and 14q, and several deletions on 1q, 6p, 9p, 10p, 13q, and 22 were restricted to 2 tumors specifically. In general, deletions were less common in PTC than amplifications independently from radiation exposure or sporadic occurrence. Although radiation is thought to generate an increased amount of deletions, translocations and inversions due to chromosomal damage, there is no direct evidence that the number of chromosomal aberrations in radiation-induced PTC is higher than in sporadic PTC. However, some aberrations seem to occur preferably after radiation exposure. Chromosome 22 was reported in several studies to be affected in post Chernobyl patients [59,64,65] and was associated with tumor aggressiveness in younger patients. Gene expression of genes in these regions was determined and compared with gene expression in sporadic tumors. 41 genes were specifically upregulated in post Chernobyl pediatric PTC but not in sporadic PTC, and the ones with the strongest overexpression were *TESC*, *PDZRN4*, *TRAA/TRDA*, *GABBR2*, *CA12*, *MPZL2*, *SCG5*, *PDZK1IPI*, *AMIG02*, *NOVA2*, and *TNIK*. The genes with the largest downregulation of expression from a total of 24 genes were *PAPSS2*, *PDLIM3*, *BEXI*, *ANK2*, *SORBS2*, *PPARGCIA*, *MT1M*, *CTGRF*, *LYVE1*, and *OGDHL*. Only one gene on chromosome 22, *FBLN1*, was reported to be specifically downregulated. It would be of interest to examine these genes further for their potential usefulness as biomarkers of radiation exposure.

Another study on chromosomal aberrations was carried out by Hieber *et al*. [34]. A cohort of 23 patients from the Ukraine who developed PTC after the Chernobyl accident were examined for chromosomal aberrations and *RET/PTC* rearrangements. The median age of the patients at operation was 21 years old. SKY (spectral karyotyping) was performed and revealed chromosomal aberrations in 14 of the cases, most frequent on chromosomes 7, 10, 11, 21, and 22. It is unknown whether these aberrations are related to radiation exposure as no group of sporadic PTC was included in this study [6,13,14]. As discussed earlier, studies on sporadic thyroid cancer had already described aberrations of chromosome 22 as a cytogenetic event in subtypes of PTC.

None of the above mentioned studies was able to define a biomarker specifically targeted to radiation exposure. This may be partially due to the design of the studies as confounders such as age at operation, ethnic origin and histology of the tumor play a role in the molecular phenotype of tumors, and the cohorts studied often lack matched controls.

Hess *et al*. [66] designed a study to specifically address the question whether there are genomic alterations that correlate with exposure to radiation. A cohort of 56 patients with PTC was matched by age at operation and residency. The patients were known to be exposed to radiation (33 patients born before April 1986) or not exposed to radiation (19 patients born after January 1987). A second cohort of 28 PTCs (16 exposed and 12 unexposed) matched on the same criteria was selected to validate the results. aCGH and FISH were performed as reported in a previous study [56] to evaluate chromosomal aberrations that differed between exposed and unexposed cases. Amplifications were found to be more frequent than deletions which are in agreement with the findings of Stein *et al*. [62]. In general, DNA gains (n=81) were more frequent than DNA losses (n=63). Five regions on chromosomes 1, 3, 4, and 12 were lost, and six regions on chromosomes 12, 19, 20, and 22 were gained in all cases investigated. Hierarchical cluster analysis separated the array CGH profiles into two main clusters, 1(n=23) and 2 (n=29). Cluster 2 was subdivided into two subclusters, 2-1 (n=14) and 2-2 (n=15). Cluster 1 contained significantly more *RET/PTC* positive cases compared with cluster 2 (p=0.0096). Large tumors (pT2 and pT3) were associated with cluster 1 (p=0.00087). Tumors that had metastases to regional lymph nodes (N1) were predominant in cluster 1 and cluster 2-2 (p=0.04). Univariate supervised analysis showed associations [false discovery rate (FDR) <0.05] of CNAs with tumor size (gain of 1q21.1–23.3, 7q22.1, 9p24.3, 10p15.3–15.1, 10q26.13–26.3, 11p11.12-cen, 12q24.11–24.23, and 16q22.1–23.3) and sex of patients (loss of 5q23.3–31.3). A DNA gain on chromosome 7 (7p14.1-q11.23, 32.1Mb in size) was exclusively associated with a subgroup of patients exposed to radiation after Chernobyl (univariate supervised analysis; FDR<0.05). The alteration was present in 13 out of 33 cases from the exposed group and in none of the cases from the unexposed group (p=0.0015, FDR=0.035). This finding was verified by array CGH analysis of an independent validation set consisting of 28 PTC cases (16 exposed and 12 unexposed). FISH analysis of individual cases revealed that up to 24% of tumor cells exhibit the amplification of 7q11. As the cohort was matched on the confounding parameters age, residency and *RET/PTC* status, the gain of 7q11 could be described as the first potential marker identified for exposure to radiation. Nine genes identified within this region were associated with tumor development according to gene ontology analysis. The five genes *CLDN3, CLDN4, LIMK1, PMS2L2*, and *RFC2* showed no significant change in expression, whereas the three genes *PMS2L3*, *PMS2L11*, and *STAG3L3* were significantly overexpressed in tumors with gain compared to those with normal copy number. *CLIP2* was found to be overexpressed in exposed tumors in general, independently from the presence of the gain of 7q11. *CLIP2* (Cap-Gly domain main containing linker protein 2) is a relatively unknown gene with putative functions in microtubule formation, chromosome segregation and cell division. As these are mechanisms which become perturbed in cancer development, there might be a cancer-related function of *CLIP2*, but its involvement in tumorigenesis, and especially radiation-induced tumor development, is currently unknown.

Interestingly, the gain on chromosome 7q11 was already reported in a previous study of Richter *et al*. [61], who used conventional CGH to analyse chromosomal aberrations in post-Chernobyl PTC. A cohort of 60 children presenting with PTC after the Chernobyl accident was used for the study. The 48 female and 12 male patients were matched on age at exposure, age at operation and residency. 30% (18 cases) had an altered chromosomal profile. The most frequent deletions were observed on chromosomes 16p, 16q, 20q and 22q, and amplifications of chromosomal regions on chromosomes 4, 7q11.2-21, 13q21-22, and 21. The region 7q11.2-21 overlaps with the region described in Hess *et al*., but none of the other CNAs identified in this study correspond with those found by Hess *et al*. The other CNAs idenified may be more related to the age of the patients at operation or the behavioural characteristics of the tumor. Deletions on chromosome 22 occur in PTC of patients of younger age and thyroid carcinoma with an increased invasive and metastatic potential [67,68,59]. Imbalances on chromosome 16 were reported earlier to be involved in various forms of thyroid cancer [69,57].

The results of these studies are summarised in Table 7.1.

Table 7.1
Overview of genomic abberrations found in the Ukrainian cohort

Author [ref.]	chromosomal alterations gains	chromosomal alterations losses	Observed in sporadic cases	genes/pathways
Unger *et al*., 2008 [56]	chromosomes 10, 12, 19, 20, 21	chromosomes 1, 6, 7, 9, 10, 11, 12, 13, 16, 19, 20, 22	chromosomes 9q, 10p, 10q and 22q	apoptosis, interleukin-27, angiopoietin receptor and the PI3K/MAP kinase pathways
Stein *et al*., 2011 [62]	chromosomes 22, 1, 12q, 5p, 9q, 16p, 21q	chromosomes 21q, 14q, 1q, 6p, 9p, 10p, 13q, 22		Upregulated: *TESC*, *PDZRN4*, *TRAA/TRDA*, *GABBR2*, *CA12*, *MPZL2*, *SCG5*, *PDZK1IPI*, *AMIG02*, *NOVA2*, *TNIK*. Downregulated: *PAPSS2*, *PDLIM3*, *BEXI*, *ANK2*, *SORBS2*, *PPARGCIA*, *MT1M*, *CTGRF*, *LYVE1*, *OGDHL*, *FBLN1*
Hieber *et al*., 2011 [34]	chromosome 7, 10, 11, 21 and 22			
Hess *et al*., 2011 [66]	chromosomes 12, 19, 20, 22, 7q11	chromosomes 1, 3, 4, and 12	chromosomes 1, 3, 4, 12, 19, 20, 22	Upregulated: *PMS2L3*, *PMS2L11*, *STAG3L3*, *CLIP2*
Richter *et al*., 2004 [61]	chromosomes 4, 7q11.2-21, 13q21-22, 21	chromosomes 16p, 16q, 20q, 22q	chromosomes 16, 22	

Germline Genome

Genetic association studies using germline DNA variants

Genetic association studies are aimed at the exploration of the link between genotype and phenotype to facilitate the determination of genetic risk factors for the potential development of disease. These studies evaluate gene polymorphism, usually the single nucleotide polymorphism (SNP) in retrospective or prospective case-control studies. In general, there are two methodological approaches to selecting which and/or how many SNPs need to be analyzed. The first one, the so-called candidate gene approach, hypothesizes that genetic variations in one or in a limited number of genes may influence the risk for or the phenotype of a given disease. A more comprehensive way is hypothesis-free; it employs analysis of the whole genome, hence the genome-wide association study (GWAS). To date, a number of investigations have been done in the area of thyroid cancer, mostly in sporadic cases, with a few of those exploring the radiation-induced thyroid cases.

Candidate gene approach

In a study investigating loss of heterozygosity (LOH) for three different SNPs of the *RET* gene (G691S, L769L, and S904S), no evidence of such was observed in 28 of 46 radiation-related papillary thyroid carcinomas (PTC) from Ukraine and Belarus that were heterozygous for at least one of the three SNPs under analysis [70]. A microarray investigation performed later and independently was consistent with these findings [71]. In the additional 68 cases, the rare S allele of G691S was significantly more frequent in patients older than 30 years old as compared to the younger subjects. Since excess radiation risk for PTC in the individuals exposed after they are 20 years old is low and it further declines with age at exposure, the study concluded that *RET* polymorphisms may rather influence sporadic but not radiation-induced thyroid carcinogenesis.

A study of the Arg72Pro polymorphism of the *TP53* gene (which encodes a tumor suppressor protein TP53 commonly reffered to as p53) in 48 pediatric/adolescent and 68 adult patients with PTC exposed to Chernobyl radiation from Ukraine and Russia, and 53 adult patients with sporadic PTC and 313 healthy controls from Russia found that the Arg/Arg homozygotes were significantly less frequent in adult patients than in children and adolescents [72]. No LOH or imbalanced *TP53* allele expression was observed in tumor tissues of heterozygous individuals. It was proposed that *TP53* genotype other than Arg/Arg may contribute to the risk of development of PTC in individuals exposed to radiation during their late childhood, adolescence or in young adulthood, particularly in females aged between 18 and 30. Interestingly enough, an elevated risk for thyroid cancer was reported in females exposed to Chernobyl fallout at the age below 30 years in a radiation epidemiological investigation [73].

A subsequent study extended to the analysis of 9 SNPs in 5 genes involved in DNA damage response (*ATM*, *XRCC1*, *TP53*, *XRCC3* and *MTF1*) performed in 255 PTC patients (123 from Chernobyl areas and 132 sporadic) and 596 healthy controls (198 residents of Chernobyl areas and 398 subjects without history of radiation exposure) demonstrated that

the *ATM* G5557A and *XRCC1* Arg399Gln polymorphisms, regardless of radiation exposure, were associated with a decreased risk of thyroid cancer [74]. Of note, the *ATM* IVS22-77 T>C and *TP53* Arg72Pro SNPs interacted with radiation exposure: the *ATM* IVS22-77 associated with the increased risk of sporadic PTC whereas *TP53* Arg72Pro correlated with the higher risk of radiation-induced PTC in adult patients, in support to the study which involved the Ukrainian patients described above [72]. An issue of gene-gene and gene-environment interactions was addressed in the analysis of *ATM*/*TP53* genotypes. Some of those strongly associated with either sporadic or radiation-induced cancer suggesting that polymorphism of these genes may modify the risk for PTC of different etiology.

Genome-wide association studies

The genome-wide association studies (GWAS), sometimes also referred to as molecular epidemiology investigations, are conducted using advanced technologies to rapidly and cost-effectively analyze genetic differences between individuals with specific diseases compared to healthy individuals. Usually, the studies are performed in two or more stages. First, genotyping of a large number of SNPs in a relatively limited sample set, which is called the training set, is performed on microarrays. The purpose of this step is to tentatively determine the potential candidate polymorphisms at liberal *p* value. Subsequently, genotyping of full or narrower set of SNPs in, again, a relatively small sample set is undertaken to achieve more stringent *p* value. Further on, a validation study is performed by genotyping a narrow set of pre-determined SNPs in an extended sample set to increase *p* value stringency or to disprove the association. This last step is commonly performed in the single-track assays such as e.g. TaqMan. Finally, a strict statistical meta-analysis is done, which combines the results obtained at different stages, with correction for multiple testing. Eventually, one or several SNPs might be determined as conferring the association with the risk for disease. A functional study might be further undertaken to clarify the mechanism by which this SNP or a gene with which this SNP is in strong linkage disequilibrium (LD) might be involved in the pathogenesis of a given disorder.

Before discussing the results obtained in Chernobyl PTCs, it is necessary to mention several appropriately powered studies of genetic predisposition to sporadic thyroid cancer published recently. The first one, employing a sample set of 962 cases of differentiated thyroid cancer (i.e., mostly PTC and limited number of FTC combined) and 38,923 controls, reported rs965513, a SNP located at chromosome 9q22.33, 59 Kb upstream (centromeric) to the *FOXE1* gene encoding a thyroid-specific transcription factor TTF2, as the strongest genetic marker (OR=1.75; 95% CI 1.59-1.94; $p=1.7\times10^{-27}$) associating with thyroid malignancy in individuals of Iceland, Spain and the USA of European descent [75]. This study also claimed another SNP, rs944289, on chromosome 14q13.3 in the area of the *NKX2-1* gene that encodes the TTF1 transcription factor, to be a marker for thyroid cancer (OR=1.37; 95% CI 1.24-1.52; $p=2.0\times10^{-9}$). A consequent study of the same group, based on a sample set of up to 1,150 cases and 41,448 controls, found 3 additional SNP associating with low TSH levels in population also to be the markers of risk for thyroid cancer. These are rs966423 on 2q35 (OR=1.34; 95% CI 1.22-1.47; $p=1.3\times10^{-9}$), rs2439302 on 8p12 (OR=1.36; 95% CI 1.23-1.50; $p=2.0\times10^{-9}$) and rs116909374 on 14q13.3 (OR=2.09;

95% CI 1.68-2.60; p=4.6x10^{-11}) [76]. With regard to genetic markers in the *FOXE1* proximity, it is important to take into account the results of an independent study of 768 tag-SNPs in 97 genes which were initially genotyped in 615 PTC cases and 525 controls from Spain and then in 482 patients with PTC and 532 controls from Italy for validation [77]. The target genes were selected based on their differential expression in primary thyroid tumors or the involvement in thyrocyte biology, metabolism and/or carcinogenesis such as the MAP kinase, JAK/STAT and TGF-beta pathways. An SNP, rs1867277, within the LD block spanning *FOXE1* and located at the 5'UTR of the gene was identified to associate with PTC (OR=1.49; 95% CI 1.30–1.70; p=5.9x10^{-9}). Functional study demonstrated that this SNP affects *FOXE1* expression by differentially recruiting the USF1/USF2 transcription factors.

At present, the results of only one investigation of Chernobyl PTCs from Belarus employing GWAS have been reported [78]. The developments of this project, however, required the involvement of additional PTC cases and controls from Ukraine. Therefore, it seems pertinent to describe this study here.

During the first phase of the molecular epidemiology study undertaken in the frame of Nagasaki University's Global COE Program supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan for 2007-12, a total of 667 patients from Belarus diagnosed for PTC in 1989–2009 and 1,275 controls from Belarus and Russia were studied, of which 408 cases and 627 controls were genotyped using the Illumina microarrays accommodating over 500,000 human SNPs; the rest of samples were subjected to the validation study. Statistical meta-analysis has discovered 3 SNPs at chromosome 9q22.33 significantly associating with Chernobyl PTC with a p-value order of 10^{-9} (Fig. 7.3). This value indicated a very significant association which surmounted the genome-wide threshold of 2x10^{-7}. For one of the detected SNPs, rs965513, which was used for validation, a p-value of 4.8x10^{-12} and an odds ratio of 1.65 (95% CI 1.43–1.91) was obtained after meta-analysis.

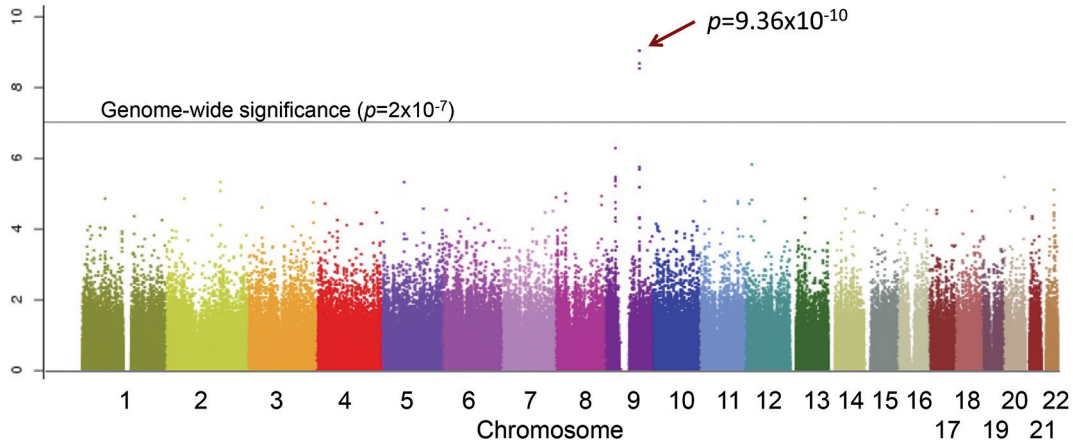

Figure 7.3. Manhattan plot of the GWAS results of the first stage of Chernobyl PTC study. The p values calculated by the Trend chi-square test for 506,840 autosomal SNPs are plotted in -log$_{10}$ scale with respect to their chromosomal positions. The horizontal line indicates the Bonferroni adjusted p value for genome-wide significance.

In the second phase of the study, the sample set was expanded to include additionally 286 PTC cases from Belarus and 145 from Ukraine, and 257 controls from Belarus, 157 from Ukraine and 620 from Poland. Thus, a total of 1,098 PTCs from Belarus and Ukraine, and 2,309 controls (both cases and controls that passed the quality control) from the three countries were included. The number of samples genotyped using Illumina microarrays was 847 PTC cases and 1,240 controls; validation study using single-track TaqMan assays included 274 cases and 1,023 controls.

Among all the targets tested, only 3 SNPs displayed significance in the training and validation sets while all other did not pass validation. Two SNPs were around or in the *FOXE1* gene locus on chromosome 9q22.33: rs965513 (OR=1.69; 95% CI 1.51–1.90; p=5.8x10^{-19}), and rs1867277 (OR=1.52; 95% CI 1.26–1.83; p=1.4x10^{-5}). The third SNP that displayed significant association was in the *NRG1* locus on chromosome 8p12, rs2439302 (OR=1.35; 95% CI 1.16–1.57; p=9.1x10^{-5}). Notably, all three SNPs detected in our Chernobyl series had effect size, in terms of OR, very similar to those reported in sporadic thyroid cancer studies. Based on these observations it was concluded that these SNPs are the markers of PTC of either radiation or sporadic etiology.

It is worth mentioning that besides the 3 SNPs that displayed significant association signals, the study also identified 11 potential candidate SNPs in or around 10 genes which were significant on microarray analysis with p values ranging 4.7x10^{-7} – 1.6x10^{-4}. However, none of them, when subjected to validation study, passed it displaying the p-values from 0.07 – 0.944. Therefore, all of them were conservatively judged as having no association with Chernobyl PTC even though their significance (a p-value) for association ranged 6.0x10^{-6}–5.0x10^{-3} on final meta-analysis. The SNPs in vicinity of *NKX2-1*, *MBIP* and *DIRC3* previously reported to be associating with sporadic thyroid cancer were insignificant in the study thus pointing on the specific association of these genes with sporadic thyroid cancer.

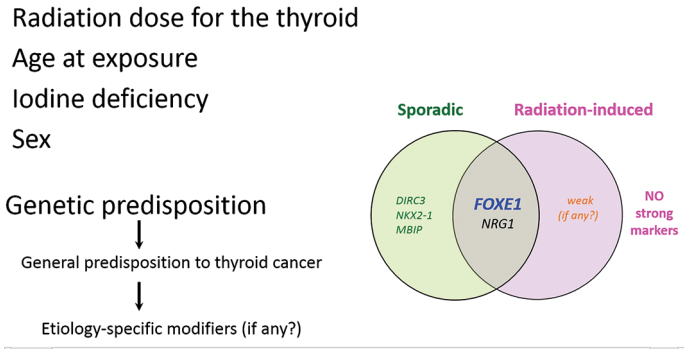

Figure 7.4. Risk factors for development of papillary thyroid carcinoma after internal exposure to radioiodine. Genetic predisposition is added to those previously established to affect the risk: exposure dose, younger age at exposure, iodine deficiency, male sex. Note that genetic markers associating with risk for Chernobyl PTC are the same found for sporadic PTC, around or in the *FOXE1* (the strongest) and *NRG1* gene loci. Several markers appear to be associating with the risk for sporadic thyroid cancer only: in or around the *DIRC3*, *NKX2-1* or *MBIP* genes.

As a whole, molecular epidemiology study of Belarussian and Ukrainian PTCs leads to an important corollary that among the genetic factors affecting risk for radiation-induced thyroid cancer, the strongest markers are the same that confer predisposition to the sporadic form of this type of malignancy. Genetic markers of radiation-related and sporadic partly overlap yet the absence PTC-associating markers on 14q13.3 (i.e., *NKX2-1* or *MBIP*), and 2q35 (*DIRC3*) from the Chernobyl series suggests they are specific to PTC developing without radiation history. As shown in Figure 7.4, the results obtained in the study of Chernobyl PTC permit the inclusion of genetic predisposition to the list of risk factors for radiation-induced thyroid carcinogenesis known from the earlier experience. The fact that GWAS has not revealed genetic markers specifically associating with radiation-related but not sporadic thyroid cancer does not rule out the possibility of their existence. Further studies with higher resolution, such as e.g. next-generation sequencing, may shed light on this problem and will probably refine our understanding of radiation-induced carcinogenesis by addressing issues of gene-gene and gene-environment interactions.

Possible implication of FOXE1 in pathogenesis of PTC

The three genetic studies, two of sporadic thyroid cancers and one of radiation-induced Belarussian and Ukrainian PTC, have concordantly identified the *FOXE1* (*TTF2*) locus as a marker of inherited susceptibility for PTC of different etiology [75,77,78]. The intronless *FOXE1* is a member of the forkhead/winged helix family of evolutionarily conserved transcription factors [79]. In humans, FOXE1 is a key player in thyroid organogenesis, thyrocyte precursors migration and differentiation with onset of expression in the thyroid primordium at Carnegie stage 15 [80,81]. FOXE1 is a transcription activator of thyroperoxidase (*TPO*) and thyroglobulin (*TG*) genes [82,83].

FOXE1 involvement in thyroid diseases remains scarcely addressed. Using RT-PCR or *in situ* hybridization, Sequeira *et al*. found *FOXE1* (*TTF-2*) expression in about 60% of human thyroids [84]. In benign thyroid lesions *FOXE1* expression was observed in 43–100% cases. In thyroid malignancies, *FOXE1* was expressed in 44% follicular carcinomas, 65% PTC and 0 (of 2) anaplastic carcinomas. Nonaka *et al*. reported strong diffuse immunohistochemical TTF-2 staining in 50 – 100% tumor cells in PTC, follicular adenoma, follicular carcinoma and poorly differentiated thyroid carcinoma [85]. Medullary thyroid carcinomas were weakly positive in 75% cases and anaplastic thyroid carcinomas were virtually all negative. In a study by Zhang *et al*., nuclear expression of TTF-2 was found to be gradually decreasing from follicular adenoma to anaplastic carcinoma in accordance with tumor dedifferentiation [86]. Abnormal TTF-2 expression in the cytoplasm displayed the opposite trend except for anaplastic carcinoma in which TTF-2 expression was generally low. Despite genetic studies strongly suggest FOXE1 implication in PTC, and there is an alteration of FOXE1 expression and localization in cancer cells, its role in tumor development remains unknown.

To clarify the role of FOXE1 in PTC, the patterns of FOXE1 immunohistochemical expression in 42 tumors and adjacent normal thyroid were analyzed, and then their relationship with morphological characteristics of the tumor and patients' genotypes was investigated [87].

FOXE1 exhibited nuclear and cytoplasmic staining in normal thyroid follicular cells. Nuclear immunoreactivity was strong or moderate, while cytoplasm showed weak-intensity

staining. Approximately one-third of all nuclei were not stained, whereas no cytoplasmic expression was noticed in about 25% of cells. More uniform pattern of FOXE1 expression was observed in thyroid cells at the vicinity of PTC border within 100-300 μm tissue layer in which most cells showed strong nuclear and moderate cytoplasmic immunoreactivity.

FOXE1 expression in cancer tissue displayed approximately the same extent as in the normal counterpart. Cells at the tumor border and those in close proximity to the border showed the highest intensity of cytoplasmic and nuclear expression. Neoplastic cells in the tumor center exhibited substantially lower intensity scores and an obvious loss of nuclear expression.

A significant increase of total FOXE1 score in cancer tissue compared to normal thyroid ($p<0.001$) which was contributed mainly by the cytoplasmic expression was found while the nuclear expression remained higher in non-neoplastic thyroid cells ($p<0.001$ and $p<0.05$, respectively). Thus, cytoplasmic FOXE1 expression was an essential feature of cancer cells whereas prominent nuclear expression was characteristic for normal cells.

Statistical analysis confirmed preliminary observations of the gradient FOXE1 expression from central to peripheral areas of tumor and normal counterparts. Both cancer and surrounding normal thyroid tissue demonstrated the strongest FOXE1 expression in the areas immediately adjacent to the tumor border comparing with the staining scores of distant regions ($p<0.001$). Of note, the highest nuclear scores were on the normal tissue side and the highest cytoplasmic scores on the cancer side. There were notable changes in FOXE1 immunoreactivity with distance from the tumor border, such as increasing number of negative nuclei in normal thyroid tissue and increasing number of negative nuclei along with decreasing cytoplasmic intensity in cancer tissue (Fig. 7.5).

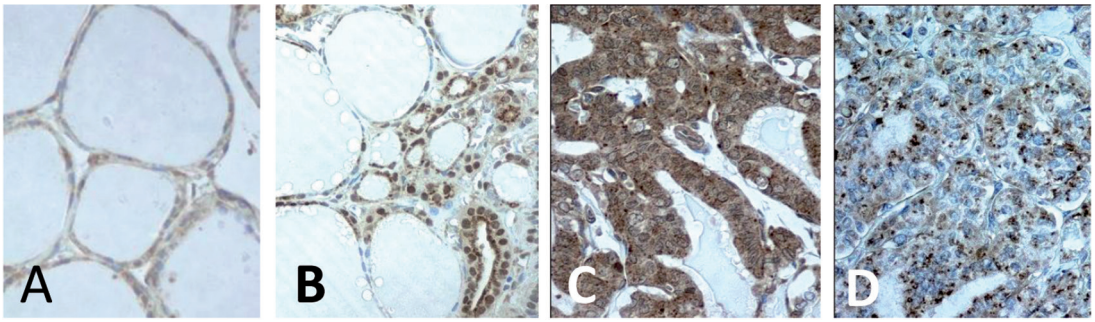

Figure 7.5. Four distinct patterns of immunohistochemical FOXE1 expression in different zones of normal thyroid (A and B) and tumor tissue (C and D), original magnification ×200. (A) In the normal thyroid tissue at the distance >300 μm from the tumor border there is a considerable variation of nuclear and cytoplasmic FOXE1 expression with 25 – 35% FOXE1 negative cells. (B) In the normal thyroid tissue immediately (≤300 μm) adjacent to invasive or encapsulated tumor border) most cells are FOXE1 positive with the highest nuclear and moderate cytoplasmic intensity. (C) In the tumor tissue at the invasive front or subcapsular region (<300 μm) regardless tumor border contacts adjacent thyroid parenchyma or extrathyroid tissues, virtually all cells demonstrate the strongest cytoplasmic and moderate nuclear FOXE1 expression. (D) Tumor tissue at the distance >300 μm from the tumor border shows mainly monomorphic low to moderate cytoplasmic expression with negative or weak nuclear staining.

On multivariate logistic regression analysis it was demonstrated that among all demographic and pathological variables entered in the model, only two, namely tumor multifocality (p=0.032) and rs1867277 polymorphism in the *FOXE1* 5′ UTR (p=0.037), significantly associated with nuclear FOXE1 expression in the zone of tumor cells confined within the 300 μm to the tumor border; capsular invasion displayed marginal significance (p=0.051). Both multifocality and variant genotype other than homozygous for the major allele (the dominant model of inheritance) associated with the higher FOXE1 expression.

Thus, FOXE1 displays an aberrant cytoplasmic expression in PTC suggestive of a possibility of a role other than transcription factor or of the existence of other factors causing translocation; perhaps such translocation may contribute to cancer cell biology. FOXE1 overexpression in the cytoplasm in tumor cells may also reflect activation of FOXE1 regulating pathways (Shh/Gli and Wnt) which are switched on during thyroid carcinogenesis [88]. The precise role of cytoplasmic FOXE1 requires functional studies to determine whether it is an active process involved in carcinogenesis or it is a consequence of malignant transformation.

The interface between normal and cancer tissues is a battlefield of invading cancer cells and the host. Many biological processes in cancer show highest intensity at the border, e.g. cell metabolism changes, proliferation, neovascularization, and invasion. The significant difference of FOXE1 expression between the periphery and the center of PTC is in line with these notions of particular processes at the tumor border. The gradient in non-neoplastic thyroid tissue adjacent to PTC was also found. Such kind of distribution is not described well for normal counterpart. Observations of the sharp reduction of FOXE1-positive cells with the distance from the tumor border imply FOXE1 involvement in communication at the host/tumor interface.

Transcriptomic studies

Gene expression analysis

The previous sections have concentrated on the molecular aberrations identified in the genome. Although many of these will result in changes at the RNA level, other factors can affect RNA expression. The next section therefore summarises data from transcriptomic studies using RNA expression techniques.

The power of the microarray technology in contrast to conventional analysis is in enabling an observation of the genome-wide gene expression profile for a given population within a single experiment. Gene-specific oligonucleotide sequences are spotted on glass slides and used for hybridisation of a preprocessed RNA sample. Technical issues such as non-specific binding of the fluorophore or hybridisation artefacts can influence the evaluation and need to be controlled for. The large amount of data created may be difficult to analyse and needs implementation of explorative statistical tools for normalisation and correction, as well as for detection of significant hits. Gene expression microarray studies are carried out to define diagnostic and prognostic markers and signatures. In Ukrainian post-Chernobyl PTCs, several studies were specifically designed to address the question whether there is a molecular signature for radiation-induced thyroid cancer. Table 7.2 gives an overview of the outcome. No significant findings were reported concerning the transcriptome of exposed and non exposed tumor tissue, but various studies identified subsets of genes involved in thyroid carcinogenesis and their related signalling pathway regulations.

Table 7.2
Overview of conducted gene expression studies in the Ukrainian cohort

Author [ref.]	Hypothesis	Transcriptome	Pathway	Genes
Abend *et al.*, 2012 [103]	dose-response relationship between ^{131}I dose and gene expression	2500 genes differentially expressed in relation to ^{131}I dose	Cell cycle & growth and differentiation, Cell adhesion, Microenvironment/metabolic changes, Transcription factor and DNA modification through methylation ; w/o significant enrichment of a certain pathway	*AJAP1*, *FAM38A*, *CA12*, *LMO3*, *ZNF493*, *MTA1*, *SLC19A1*, *CDK12*, *ACVR2A*, *GALNT7*, *SLC43A3*
Delys *et al.*, 2007 [94]	gene expression is differentially regulated in sporadic/radiation-induced PTC compared to normal thyroid	differetial expression of 44.5% of genes on the array	Immune response, Cytokine activity, Chemokine activity, Thyroid hormone generation, EGFR signaling pathway, Activation of JNK activity , MAP kinase phosphatase activity, Extracellular matrix, Peptidase activity, Protease inhibitor activity, Laminin complex, Collagen, Cell–cell adhesion, Integrin complex; *p*-values below 0.05	*ANXA1*, *CDH3*, *CLDN1*, *DUSP5*, *GPX1*, *HMGA2*, *NELL2*, *NRCAM*, *SLIT1*, *THBS2*, *TNC*, *BCL2*, *EGR1*, *EGR2*, *FLRT2*
Detours *et al.*, 2005 [89]	radiation-signature in post-Chernobyl childhood PTC	no separation of radiation-induced from sporadic PTC	n/a	radiation-induced and sporadic datasets show a high correlation
Detours *et al.*, 2007 [90]	radiation-signature in post-Chernobyl childhood PTC	similar between radiation-induced and sporadic;	n/a	two subsets of 256 and 118 genes separate radiation-induced from sporadic;
			homologous recombination	*XRCC2*, *SHFM1*, *RAD51C*, *MUS81*, *RAD51L1*, *RAD51*, *RAD50*, *RAD54B*, *RAD54L*, *NBS1*, *RAD52*, *XRCC3*, *BRCA1*
Dom *et al.*, 2012 [93]	radiation-signature in post-Chernobyl childhood PTC	separation of normal exposed from normal not exposed tissue	Chronic myeloid leukaemia, Neutrophin signalling pathway, MAPK signalling pathway, Insulin signalling pathway, Renal cell carcinoma, Pancreatic cancer, Regulation of actin cytoskeleton, Spliceosome, mTOR signalling pathway, Apoptosis, Focal adhesion	403 gene signature
Stein *et al.*, 2010 [62]	gene expression changes in radiation-induced childhood PTC in comparison to the matching normal tissue	n/a	Cancer, Cellular Growth and Proliferation, Reproductive System Disease, Cellular Movement, Cell-to-Cell Signaling, and Cell-Mediated Immune Response	*LRP4*, *IGSF1*, *ODZ1*, *CITED1*, *SLIT1*,*HMGA2*, *SERPINA1*, *KCNJ2*, *AGR2*, *TACSTD2*, *NELL2*, *CHI3L1*, *TPO*, *TFF3*, *CRABP1*, *COL9A3*, *MATN2*, *SLC4A4*, *DIO1*, *KIT*, *CCL21*, *FBLN1*, *FHL1*, *MPPED2*

Detours *et al*. [89] performed Micromax Microarray experiments to evaluate the gene expression profile of twelve post-Chernobyl PTCs. In addition, eight sporadic PTCs and thirteen autonomous adenomas from Belgium, France and the US were arrayed. The group was interested whether the gene expression pattern of the radiation exposed cases would reveal a radiation-related signature and applied several unsupervised and supervised analyses to the obtained microarray data. Unsupervised hierarchical clustering was able to distinguish between PTCs and adenomas, but could not separate the sporadic from the post-Chernobyl PTCs. Similarly, *RET/PTC* rearrangement was not a criterion which could separate the PTCs into two groups. Significance analysis of microarray (SAM) found 168 genes to be differentially expressed between adenomas and PTC with a false discovery rate (FDR) of 1%. Amongst these, a signature of six genes was capable of accurately differentiating between adenoma and PTC. No such genes were found when analysing sporadic PTC versus post-chernobyl PTC. Instead, a significant correlation of the two groups was found which could not be seen for the adenomas. This correlation was independent from the methods used and was validated *in silico* using PTC data from Affymetrix microarrays. Patients were not matched on age, residency, or sex within this study. Surprisingly, despite the high variation in the clinical parameters of the patients, the data correlated significantly and indicated a similarity between sporadic and post-Chernobyl cases. The data suggested that no molecular expression signature for radiation-induced thyroid cancerogenesis exists.

Two years later the same group [90] repeated the analysis on Agilent microarrays, which support a broader range of genes. The Ukrainian cohort of twelve patients exposed to radiation was compared to a French cohort of 14 patients without known radiation history. The distribution of histological subtypes was the same in the two groups. Frequencies of *RET/PTC* rearrangement and *BRAF* mutation were also comparable. Screening for global expression profiles revealed no difference between the French and the post-Chernobyl PTCs. However, application of a supervised classification algorithm identified a subset of genes which was capable of distinguishing French and post-Chernobyl tumors with an error rate of 12%. In further experiments *in vitro* the same authors showed that 200 mM H_2O_2 (peroxide) or 2.5 Gy γ-radiation cause a similar transcriptional response in a B lymphocyte cell line with a subset of 293 genes having a fold change greater than 1.5. 118 out of these genes were also able to separate the French and the post-Chernobyl cohort with an error of 15%. This implies that peroxide had a major influence in carcinogenesis in the French cohort. H_2O_2 is a byproduct of thyroid hormone synthesis and known for its high DNA damaging potential due to its oxidizing properties. H_2O_2 is considered as a highly reactive oxygen species, and free radicals were shown to cause cancers in the thyroid of Tg-a1BAR mice [91]. Similarly, ionizing radiation is creating reactive oxygen through the radiolysis of water, which is highly abundant in the cellular environment. The etiological agents peroxide and radiation both create reactive oxygen species as a cancer initiating event which supports the finding of similar global gene expression profiles. An additional signature of thirteen genes involved in homologous recombination was able to distinguish the two cohorts with an error rate of 15%. The authors matched the groups on clinical parameters such as morphology and known molecular aberrations. However, they did not

match on age and residency, two independent confounders known to affect data analysis. It would also have been interesting to see whether the magnitude of *RET/PTC* or *BRAF* expression had an influence on the gene expression profile as described in literature [92], but such data was not provided.

A more recent study again tried to address the question whether a radiation-specific signature is detectable in post-Chernobyl thyroid cancer. Dom *et al*. [93] chose a carefully selected cohort. 22 patients who were exposed to radiation and born before April 1986 were matched on age and residency in Ukraine to 23 patients born after March 1987 and hence not exposed to radiation. The cases were subjected to Affymetrix microarrays and expression of single genes was validated using qRT-PCR.

The authors searched for global gene expression variations using hierarchical clustering and principal components analysis which successfully separated tumor from normal thyroid. However, similar analyses were not able to reveal any differences between exposed and non exposed cases. A subset of eight genes was selected according to known involvement of their products in carcinogenesis: carbonic anhydrase 12, BH3-interacting domain death agonist, clusterin, cyclin D2, trefoil factor 3, low-density lipoprotein receptor-related protein 1B, dual specificity phosphatase 1 (DUSP1) and thrombospondin, type I, domain-containing 7A, and validated by qRT-PCR. All of them were found to be differentially regulated in tumor versus normal thyroid tissue.

Supervised analysis of the data could not distinguish exposed from non exposed tumors either, but the normal tissue seemed to have differential gene expression in both groups.

Potential confounding factors such as the clinical parameters sex, age at operation, patients' place of residence (region/oblast) at the time point of the Chernobyl accident, morphological subtype of PTC, TNM classification, presence of *BRAF* mutations or *RET/PTC* rearrangements, tumor size and methodological parameters such as batch effects of microarray hybridisation or RNA storage time were analysed. The age at operation and the time of storage of frozen tissue prior to RNA extraction were found to be associated with the radiation exposure, and data was adjusted accordingly. The corrected data was evaluated using SAM and a signature of 403 genes was found to distinguish exposed from non exposed normal thyroid tissue. All of them were upregulated in the exposed normal tissue. The results were validated *in silico* using an external data set. Gene ontology and KEGG analysis of the gene signature in DAVID found pathways with an impact on cancer development and proliferation, mainly MAPK, insulin and mTOR signalling pathways, and enrichment of cell adhesion-related genes.

Thus, the study was not able to show any differences between exposed and non exposed tumors, but the matching contralateral normal tissue in these patients reveals significant differences. This could be related to the character of the tumor which is known to evolve into diverse subpopulations, whereas the surrounding normal tissue does not evolve but may keep a tumor-initiating expression profile. However, such hypotheses remain speculative and require validation in an independent set of cases. The signature identified could be used to classify a PTC cohort in the two categories - exposed and non exposed, but was associated with a false positive rate of 30%.

Pathway regulation

Although scientists could not detect a clear radiation-related signature in post-Chernobyl childhood PTC, with the methods used, the collected data highlighted certain signalling pathways in exposed over unexposed tumor tissue. The point mutations and gene rearrangements observed in PTC stress out a major role for the MAPK pathway in thyroid tumorigenesis. *BRAF* mutations and *RET* as well as *NTRK* rearrangements lead to constitutive activation of these genes and are supposed to interfere with their downstream partners perturbing the pathway cascade. Expression status of other genes - especially in post-Chernobyl PTC - was rarely explored. The study of Delys *et al*. [94] aimed to identify certain signalling pathways and their regulation in relation to the physiopathology of the disease. 12 PTCs from Ukrainian patients which were exposed to radiation and 15 sporadic PTCs from French patients plus matching normal tissues were arrayed on Agilent cDNA microarrays. The statistical algorithm Significance Analysis of Microarray (SAM) was applied, and it was possible to significantly distinguish a characteristic gene expression profile for PTC from normal thyroid tissue. The differences in gene expression may relate to the observed differences in morphology between tumor and normal tissue. To identify individual differentially regulated genes, the data was correlated with two independent PTC data sets [95,96]. Only genes which show the same regulation in at least two of the data sets were selected for further pathway regulation studies. This resulted in a profile of 451 up- and 233 downregulated genes. Eleven upregulated genes (*ANXA1, CDH3, CLDN1, DUSP5, GPX1, HMGA2, NELL2, NRCAM, SLIT1, THBS2, TNC*) and four downregulated genes (*BCL2, EGR1, EGR2, FLRT2*) identified in the microarray data sets were validated by qRT-PCR. Additionally, 20 genes were already identified in the literature to correlate with the phenotype of sporadic thyroid cancer [97]. GO term analysis with DAVID revealed a participation of the genes in the gene list in eleven pathways with a p value <0.05. Many of the pathways are related to the immune response which may more reflect the infiltration of immune cells into the tumor rather than changes in the tumor cell itself. Alteration of cytokines and chemokines was observed in this study as well, which is consistent with other studies where PTC is known to evoke a chronic inflammation *in vivo* and *in vitro* [98]. This could have a direct influence on the recruitment of lymphatic cells into the tumor tissue.

Surprisingly, no deregulation of the MAPK pathway could be found, which would be expected for PTC because of the *BRAF* mutations and *RET/PTC* gene rearrangements which lead to a constitutive expression of those genes. On the other hand, EGF signalling, an activator of the MAPK pathway, was significantly altered, whereas ERK specific inhibitors of the DUSP family were highly overexpressed as well. c-jun N-terminal kinase (JNK) and p38 pathway was another gene cascade significantly altered. Its involvement in thyroid carcinogenesis was already suggested previously by immunohistochemical detection in tumor but not normal thyroid tissue [99]. Also, overexpression of proteases and protease inhibitors which are known for remodelling of the ECM was highly abundant in PTC versus normal tissue. This is a well known cancer initiating event and documented to have a participation in thyroid cancer [100,101].

Cell migration is another mechanism playing an important role in carcinogenesis. Its consequences for tumor development can be observed and are described histopathologically [102]. Delys *et al*. [94] identified several genes from the integrin, cadherin and claudin family being deregulated in the tumor tissue, which could have a direct influence on the invasive behaviour of the tumor. It would be interesting to apply a principal component analysis to these 26 cases of the study to see whether clinical parameters match with these findings, and tumors with a higher invasive behaviour consistently show a stronger upregulation of these pathways.

The study could identify a clear difference between PTC and normal tissue and found many pathways which are known to be involved in tumor development being differentially regulated. The study could not distinguish between radiation-induced and sporadic tumors, suggesting that the etiologic agent may be responsible for tumor initiation but has no influence on tumor progression.

Transcriptomic profiles and radiation dose

Previous studies showed that, especially in gene expression analysis, the use of differing methods and statistical evaluation algorithms, the sample size and/or selection of control populations lead to inconsistent findings between studies. These factors may have contributed to the conclusion that neither a radiation-specific signature nor a gene regulation mechanism is present in the Ukrainian post-Chernobyl PTC cases. Despite this, a more recent study performed by Abend *et al*. [103] aimed to address the question whether a dose-related response to ^{131}I is present and detectable. The patients included in this study were enrolled in the Ukraine-American Cohort Study. The patients were all below 18 years old at the time of accident and individual ^{131}I measurements were taken within two months after the accident [104]. 63 tumor/normal pairs were split in a screening set with 32 cases for Agilent oligo microarray gene expression analysis and a validation set with 31 cases for qRT-PCR validation of altered genes. Arraying found 2500 genes that were significantly differentially expressed between the tumor and the normal tissue, but none of the *p* values survived multiple testing using false discovery rate (FDR). 75 genes were selected based on analysis of a dose-dependent differential expression, and this list includes also genes with evidence from previous studies. qRT-PCR found 11 of those genes were differentially expressed in tumor but not in normal tissue in relation to the dose received. Nine of the eleven genes are involved in the pathways cell adhesion (*AJAP1, FAM38A*), energy metabolism (*CA12*), transcription or DNA methylation (*LMO3, ZNF493, MTA1, SLC19A1*), and growth/differentiation (*CDK12, ACVR2A*). These pathways are known for their participation in the cellular response to ionizing radiation [105, 106]. The importance of five genes (*CA12, GALNT7, LMO3, SLC43A3 and FAM38A*) was also confirmed by others in post-Chernobyl and other radiation-induced childhood thyroid tumors [62, 107]. The differential expression of the genes is dose-dependent and linear, however, the three genes *SLC19A1, SLC43A3* and *ZNF493* exhibit some non-linearity. The cellular response to radiation is known to be non-linear, and changes over time with an early response to DNA damage and a later survival with chromosomal rearrangements [108,109], and the non-linearity in differential expression may reflect this. Taken together, the eleven genes show

a clear difference in expression levels between exposed tumor and exposed normal tissue, and further studies on the genomic architecture of these genes will give more insight on their ability to serve as biomarkers for radiation exposure.

In the most recently reported study using material from the patients enrolled in the Ukrainian-American cohort, Leeman-Neill *et al*. [110] hypothesized that chromosomal aberrations such as *RET/PTC* and point mutations have different associations with ^{131}I dose. The case series included 26 males (42%) and 36 females (58%) who were resident in the areas surrounding Chernobyl, i.e., the Zhytomir (n= 17; 27.4%), Kiev (n=12; 19.4%), or Chernigov (n=33; 53.2%) regions (oblasts), and who were between 5 months and 17 years old (mean 8.0 years) at the time of the Chernobyl accident. The estimated ^{131}I dose for patients in the study ranged from 0.008 Gy to 8.6 Gy, with a mean dose of 1.3 Gy. Surgical removal of PTCs occurred between October 1998 and December 2007, with time between exposure and surgery ranging from 12.5 to 21.6 years (mean: 16.5 years).

RET/PTC rearrangement was the most common genetic alteration and was found in 22 (35%) cases, including 14 *RET/PTC1* and 8 *RET/PTC3* rearrangements. Point mutations in the *BRAF* and *RAS* genes were found in 9 (15%) and 5 (8%) of the tumors, respectively. All 9 *BRAF* mutations were *BRAF*^{V600E}. Four *RAS* mutations were detected in *NRAS* codon 61 and 1 in *HRAS* codon 61. No *KRAS* mutations were found in codons 12 and 13. In addition, these tumors were studied for *PAX8/PPAR*γ rearrangement, a prototypic genetic alteration found in follicular thyroid carcinoma that occurs with lower prevalence in the follicular variant of PTC. Two tumors were positive for *PAX8/PPAR*γ; both were of the follicular variant of PTC. In both cases, the fusions were between exon 9 of *PAX8* and exon 1 of *PPAR*γ, with several expected splice variants of the chimeric *PAX8/PPAR*γ transcripts detected. One tumor had more than 1 mutation, harbouring an *NRAS* point mutation in codon 61 and *PAX8/PPAR*γ rearrangement. Twenty five (40%) tumors revealed none of the studied mutations. Patients with *BRAF* or *RAS* mutations had the lowest dose (0.27 Gy and 0.20 Gy, respectively). *RET/PTC1* and *RET/PTC3* were associated with a higher dose of 1.04 Gy and 1.54 Gy. The highest dose of 1.97 Gy had tumors without any of the observed aberrations. There was also a significant correlation with the age of the patients, where *BRAF* and *RAS* mutations associated with older age and *RET/PTC* rearrangements with younger onset. Hence, it is difficult to differentiate between the known effects of age at diagnosis on the molecular phenotype of PTC, and the effects of dose, especially in such a small study. The cohort was not age matched or subdivided between children and adolescents (aged <19) and young adults (aged >19) at surgery. Children have been shown to receive a relatively higher dose of radiation due to the smaller size of their thyroid.

Interestingly, there was a positive correlation of the chromosomal rearrangements with residency in the Zhytomir oblast, where the individuals also received a higher radiation dose. This oblast is known for a moderate iodine deficiency. As such a deficiency was shown to be a risk factor for thyroid cancer in a Belarussian cohort of post-Chernobyl thyroids [111], the authors suggest a similar contribution for their findings.

Further whole genome studies on cohorts matched for the confounders in this study (age, sex, region of residence) would be required to convincingly link the findings in this paper and that by Abend *et al*. [103] to dose of radiation.

In summary, it is now accepted that the initial studies suggesting that post- Chernobyl thyroid cancers showed a higher frequency of involvement of the *RET/PTC* oncogene were misinterpreted due to the lack of appropriate age-matched controls. It had been assumed that the molecular pathology of thyroid cancer was not influenced by age, but as the studies reported above have shown, young onset PTC shows very different pathology and morphology when compared with the same disease in adults. Studies using the newer "omic" technologies have suggested that there may be subtle differences between PTCs of a radiation aetiology compared with age-matched controls, but all of these studies require validation in separate cohorts of patients. The studies so far performed on the effect of inherited, germline polymorphisms suggest, as with their somatic counterparts, that there is little to differentiate radiation associated PTC from sporadic PTC. Attention is now turning to studies on potential epigenetic differences (miRNA and DNA methylation), and to next generation sequencing technologies to tease out more subtle differences associated with a radiation aetiology. Regardless of the final conclusions of these studies regarding the relationship between radiation and molecular phenotype, the Ukrainian cases provided to the general scientific community through the generosity of Ukrainian patients and the staff at the Institute of Endocrinology and Metabolism in Kiev, have been instrumental in increasing our knowledge of molecular pathology of thyroid cancer in general, and PTC in particular. It remains to be seen whether any of the data so far generated by these studies, and those yet to come, will lead to the development of better prognostic markers for thyroid cancer. Unlike many cancers, PTC has a releatively good prognosis, with a predicted death rate over 30-50 years of 1%. Despite this, there is a recurrence rate of 30% [112]. Identification of prognostic biomarkers, combined with clinical features would therefore aid patient stratification for follow-up.

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Conclusion

The present publication summarizes the results of numerous and diverse studies of thyroid cancer in children and adolescents of Ukraine affected as a result of the Chernobyl accident. We specifically have been focusing on long-term studies on reconstruction of individual thyroid doses, the relationship of thyroid cancer incidence with the age and place of residence of children at the time of the accident, clarification of changes in the structure and invasive properties of "Chernobyl" papillary thyroid carcinoma (PTC) in different age groups and in different periods after the Chernobyl accident, analysis of differences in the structure and invasive properties of radiogenic and sporadic PTC, and initial steps of the search for an association of individual molecular-genetic abnormalities with thyroid exposure dose. It is anticipated that further progress in discovery of radiation exposure markers or signatures will involve new advanced technologies, in particular, the whole genome analysis by next generation sequencing aimed at identification of possible genetic alterations unknown today which may underlie the development of radiogenic thyroid cancers.

We express a hope that this book will be useful to the readers professionally working in different fields of knowledge including radiation medicine, dosimetry, epidemiology, endocrinology, oncology, pathology, and molecular biology. The book may also be of interest to our Japanese colleagues in the analysis of health consequences of the accident at Fukushima Dai-ichi Nuclear Plant where a large-scale ultrasound thyroid screening in young population of Fukushima prefecture was initiated shortly after the disaster in March 2011.

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