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RET/PTC GENE REARRANGEMENTS FREQUENCY IN PAPILLARY THYROID CARCINOMA WORLDWIDE DEPENDING ON TIME AFTER CHERNOBYL NUCLEAR POWER PLANT ACCIDENT (POOLED-ANALYSIS). POSSIBLE CONTRIBUTION OF FACTORS OF DIAGNOSIS, 'AGGRESSIVE SURGERY', RADIATION, AND AGE

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Частота генных перестроек *RET/PTC* в папиллярных карциномах щитовидной железы в странах мира в зависимости от времени после аварии на Чернобыльской атомной электростанции (pooled-анализ). Возможный вклад факторов диагностики, «агрессивной хирургии», облучения и возраста

ABSTRACT

Results of pooled-analysis of primary data in the database formed from molecular epidemiological sources on *RET/PTC* gene rearrangements frequency in papillary thyroid carcinoma developed spontaneously and after Chernobyl nuclear power plant accident showed declined chronological trends for *RET/PTC1*, *RET/PTC3* and *RET/PTC* after the incident separately for cohorts from Europe, USA + Canada and the Asia-Pacific region have been demonstrated. The above trend for *RET/PTC3* and *RET/PTC* in total was similar to trends observed in carcinomas of Chernobyl etiology (Belarus, Russia and Ukraine), although there was no apparent time dependence for *RET/PTC1* level in this case. Observed trends could not be connected to any chronological changes in the degree of carcinoma differentiation or age factor for tumor.

As chronological changes of *RET/PTC* frequency in carcinomas in different continents and regions can not be explained by the radiation factor of the Chernobyl accident, it was concluded about the predominant contribution of the 'human factor' associated with 'overestimation' and 'overdiagnosis' of early forms of thyroid tumors in connection with increased vigilance after the Chernobyl accident. Apparently, these factors, along with improvement of instrumental methods at that time, coupled with the 'aggressive surgery', took place worldwide resulting in detection of early forms of the occult carcinomas and microcarcinomas. The frequency of *RET/PTC* in such tumors is higher than in conventional tumors. The frequence of these subjective factors is likely to decrease with time from the date of the Chernobyl accident.

Key words: *pooled-analysis, RET/PTC gene rearrangements, papillary thyroid carcinoma, the time after the Chernobyl accident, overestimation*

РЕФЕРАТ

На основе объединенного анализа (pooled-анализа) первичных данных из сформированной базы молекулярно-эпидемиологических источников по частоте генных перестроек *RET/PTC* в папиллярных карциномах щитовидной железы, развившихся спонтанно и после аварии на Чернобыльской атомной электростанции (ЧАЭС), были продемонстрированы спадающие хронологические тренды для показателей *RET/PTC1*, *RET/PTC3* и *RET/PTC* суммарно для когорт из Европы, США + Канады и стран Азиатско-Тихоокеанского региона. Указанный тренд для *RET/PTC3* и *RET/PTC* суммарно был аналогичен обнаруженному и для карцином чернобыльской этиологии (Белоруссия, Россия и Украина), хотя для уровня *RET/PTC1* в этом случае видимая временная зависимость отсутствовала. Выявленный тренд не мог быть обусловлен ни хронологическими изменениями в степени дифференцировки карцином, ни фактором различного возраста опухоленосителей.

В связи с невозможностью объяснить радиационным фактором аварии на ЧАЭС обнаруженные хронологические изменения частоты *RET/PTC* в карциномах разных континентов и регионов, сделан вывод о преобладающем вкладе «человеческого фактора», связанного со «сверхоценкой» и «сверхдиагностикой» ранних форм опухолей щитовидной железы в связи с тревогой после чернобыльского инцидента. По-видимому, эти факторы, плюс инструментальное улучшение на тот период, вкупе с «агрессивной хирургией», имели место по всему миру. В результате всюду выявлялись более ранние формы оккультных карцином и микрокарцином, частота *RET/PTC* в которых выше, чем в обычных опухолях. С отдалением времени обследования от года аварии на ЧАЭС частоты выявления названных субъективных факторов уменьшалась.

Ключевые слова: *объединенный (pooled) анализ, генные перестройки RET/PTC, щитовидная железа, папиллярная карцинома, завышенные оценки, время после чернобыльской аварии*

In memory of Professor
Samuel Yarmonenko
(1920–2011)

Introduction

Earlier, in 1990–1991, finding in Belarus of first child thyroid cancer cases after the accident at the Chernobyl Nuclear Power Plant [1] led initially to the assertions of the epidemic nature of such tumors after the incident [1][1], to the absolutisation of screening effect, and to denial of association between radiation and thyroid cancer in general (see Ref. [3]). Until that time the duration of the latent period, according to basic studies, was considered to be much longer (in the pooled-analysis [4] maximum

[1] "…it was postulated that 'these thyroid cancers might represent the beginning of an epidemic' " [2].

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frequency of thyroid cancer was 15–19 years after the radiation exposure). But the further diagnosis of new cases of thyroid cancer in children who were residents of contaminated areas (Belarus, and then Ukraine and Russia [2, 3]), removed any doubts about the association (connection) with radiation. This conclusion is generally acknowledged by international authorities [2, 5–7]. But the question remains about the magnitude of this association, because thyroid cancer is associated not only with the irradiation exposure but also with other causes. Uncertainties in the risk assessment of radiogenic cancers [8], particularly for thyroid cancer after the accident may be due to the following factors:

(a) In addition to the low basal frequency of childhood thyroid cancer as a whole [5, 9], in the republics of the Soviet Union before the Chernobyl accident there were difficulties with the assessment of the relevant values. Uncertainties were explained by the fact that graph of thyroid cancer statistical reporting was included in a section "other solid tumors" [5, 10]). Thus, the frequency of thyroid cancer before the accident remained unknown, but the relevance of data for other countries was limited by the influence of the ethnic factor, as evidenced by the special comparative studies [2, 11, 12].

(b) When trying to identify the dose–effect relationship there were uncertainties in the dosimetry data of both internal (due to radioactive iodine) and external exposure in contaminated regions [2, 5, 10, 13–15]. The effect of "recall bias" was also possible at the interview regarding the frequency of use of radioiodine-contaminated milk after the accident, use of local products, etc. [5].

(c) Application of new diagnostic methods of thyroid cancer coincided with the period after the Chernobyl accident. With the help of ultrasound and fine-needle aspiration biopsy it was possible to detect small tumors, i.e. microcarcinomas and occult carcinomas, which could not be detected earlier [2, 3, 5, 16–18]. This allowed revealing thyroid cancer at the early stages than before the incident were recorded [2, 3, 6]. This rule applies not only to the Chernobyl accident. For example, in recent decades, an increasing frequency of thyroid cancer in Switzerland [19] and other countries [4, 20] is also thought to be attributed to this factor. For the most contaminated regions of Belarus and Ukraine, the introduction of new diagnostic methods led to an increase in the registration rate of thyroid cancer in the 1988–1999 with a factor of 3, and for the other regions of Ukraine with the factor 2 [5, 16, 21].

(d) There are also uncertainties in the accurate diagnosis of thyroid cancer. During a period of 1974–1988 WHO revised histological criteria for thyroid cancer, resulting in tumor previously considered follicular become considered as papillary (for a review see [19]). A lack of qualified pediatric oncologists should also be noted, as it was mentioned in [18] with reference to leading Russia pediatric oncologist. As noted in [3], "almost all the thyroid nodules in children, regardless of their sizes were considered as potentially malignant neoplasms" (this is the "ascertainment bias" [16]).

(e) Investigation bias was also possible due to more frequent and thorough examination of individuals from the contaminated regions and/or with suspected on radiation exposure [16]. This bias is applied for the residents of areas affected after the Chernobyl accident [5, 18, 22, 23], and for the liquidators [2, 5, 22]. Similarly, when examining persons from the affected regions to non-thyroid reasons, health workers and physicians could simultaneously on their own initiative examine the thyroid in these persons ("diagnostic suspicion bias") [5].

(f) The screening effect is widely known, and it clearly noticeable in cases of thyroid cancer. For example, in [4, 24] a sevenfold increase of thyroid cancer incidence in the exposed patient cohort from the USA was explained by this factor. There are other such examples [25]. Screening allows to detect asymptomatic tumors, and as a consequence causes a sharp increase in thyroid cancer statistics, leading to a reduction of latent period. Indirectly, this is reflected by the low mortality among surgically treated patients (0.3–0.6 %) [2]. On the other hand, in [22, 26] authors have demonstrated that the screening effect appeared only in relatively short-term studies of thyroid cancer incidence (3–5 years; the factor 1.4–1.9), while in long-term studies this phenomenon disappears. Depending on the possible scenario model the value of screening effect for the victims after the accident territories of Ukraine is estimated at 1.0 and 2.5 [27]. According to UNSCEAR 2000 [2], international screening programs in the contaminated after the Chernobyl accident areas did not contribute significantly themselves to the increase in the incidence of thyroid cancer, but accompanying factors (more advanced diagnostics and a variety of subjective biases) could have an impact.

(g) Overestimation effect because of overdiagnosis could be a result of all the mentioned factors. This phenomenon was characteristic not only for the Chernobyl etiology tumors [18, 23] but also, for example, for a permanent increase in the frequency of thyroid cancer in the United States, which was not accompanied by increased mortality [28]. The essence of this phenomenon lies in the identification of more sub-clinical forms of the occult carcinomas [28].

(h) Overestimation could lead to "aggressive surgical approach" in the 1990s, resulting in that almost all thyroid nodules in children from the affected regions were considered as potentially malignant and removed surgically [3, 18]. As noted in [3], "Appearance of 'aggressive surgery' term has reason behind it. Aggressive surgery also contributed to reduction of minimal latency period."

(i) Particularly for the countries from the former Soviet Union we can not forget also the factor of iodine deficiency, which could lead to an increased risk of thyroid cancer due to relatively high doses on this body organ [2, 5, 6, 29, 30]. There are facts of iodine deficiency and endemic goiter in the affected regions of Belarus [31], Ukraine [32], and Russia [33].

The search for new facts, even indirect, that may shed light on the extent of radiation attribution for childhood thyroid cancer after the accident, continues to be relevant in the present because the dose for the induction of these cancers are likely to be the lowest among other solid cancers [2, 4, 35–38]. The aim of the present study is to find these facts in the field of molecular epidemiology of thyroid cancer after the Chernobyl incident. Our pooled-analysis is devoted to assessing the frequency of *RET/PTC* gene rearrangements[2] in papillary thyroid carcinomas sporadic and radiogenic etiology for contingents from different continents of the world depending on the time after the Chernobyl accident.

Different studies with similar design, revealing a trend of reducing of *RET/PTC* frequency with increasing time after the Chernobyl accident were performed early by other authors [45–47] (attempt to analyze corresponding published data are also known [48–51]). But these studies were local in nature, and in some cases interpretation of the results is questionable [45, 46][3]. However, decrease of *RET/PTC* frequency in carcinomas depending on the time after irradiation has been shown for the victims of the atomic bombings [52, 53].

But similar trends for *RET/PTC* frequency in thyroid tumors in the past decade have been demonstrated for Italy [54, 55] and the USA [56], that is for countries to a lesser degree affected by the Chernobyl emissions [57]. While the phenomenon in first source was attributed to the Chernobyl accident [54], in the second source the effect was attributed to a decrease in the level of US medical radiation doses from decade to decade [56][4]. These explanations can not be considered satisfactory (see also below).

As a result, the reasons for the fall of *RET/PTC* frequency in the aftermath of the Chernobyl accident (with a parallel increase of *BRAF* gene mutations frequency [43, 54–56]) in papillary thyroid carcinomas are not clear, and, most importantly, it is unclear whether this trend is associated with objective, rather than subjective factors. It is known that in the occult carcinoma and microcarcinoma *RET/PTC* frequency higher than in mature tumors [59–62], in connection with which there may be mentioned the possibilities discussed above "aggressive surgery" leads to the removal of the earlier forms of carcinomas [3, 18]. Since the phenomenon first was to take place in the most contaminated after the Chernobyl accident regions, it seems appropriate to compare the chronological dynamics of changes in the *RET/PTC* frequency in carcinomas in different regions of the world to varying degrees affected by the incident.

Material and Methods

"Database" on the molecular epidemiology of RET/PTC and the studied parameters

We published characteristics of completed base of sources ("Database") on the molecular epidemiology of *RET/PTC* in sporadic and radiogenic thyroid carcinoma previously in [63]. At the end of 2014 the base contained, apparently, about 100 % of all possible reports on the topic (197 works[5]), while more than 90 % is represented by the original papers. Data collection was carried out during a period of more than one year.

For previous pooled-analysis [63] and in present paper only studies of papillary thyroid carcinomas, i.e., the form of thyroid cancer with the strongest association with irradiation [40–43, 63, 64], and works with the detection of *RET/PTC* mainly by PCR at various modifications [63] are included.

As in our previous work [63], were used the frequencies of the two main types of rearrangements (*RET/PTC1* and *RET/PTC3*), as well as *RET/PTC* frequency in total (i.e., the sum of all types of rearrangements studied in a particular work) as the main parameters. When analyzing carcinomas with multiple

[2] *RET/PTC* gene rearrangements are generated as a result of formation, due to chromosomal translocations and inversions, chimeric constructs between the tyrosine kinase domain of *RET* gene and fragments of different donor genes. The result is a structurally modified form of proto-oncogene *RET*, the expression of which leads to overproduction of *RET/PTC*-oncoproteins having constant tyrosine phosphorylation activity. The latter is believed to play a role in initiation/promotion of the papillary thyroid carcinoma. Previously, *RET/PTC* assumed the role of a molecular marker of radiogenic thyroid tumors, but much of the data showed the ambiguity of the situation [5, 39–44]. *RET/PTC* are probably the most studied gene and/or chromosomal changes in thyroid tumors (more than 200 publications on the molecular epidemiology of mid-2015 [44]).

[3] The authors did not study *RET/PTC* frequency in carcinomas of residents of various regions affected after the Chernobyl accident, but, in fact, they estimated the frequency of themselves carcinomas in the regions.

[4] This assumption is questionable, since the well-known fact the steady increase in the intensity of medical exposure in all developed countries [58] (including accumulated annual doses: UNSCEAR 2008 [58]; figures VIII, IX, table 7, etc.).

[5] By mid-2015 it was added only single relevant sources.

rearrangements as a parameter of *RET/PTC* frequency in total, we accounted a total number of tumors with rearrangements on the entire pool of carcinomas in the cohort, but not the frequency of rearrangements on the entire pool of carcinomas (see rationale in [63][6]).

For the analysis from the database following parameters included in works were extracted: the output of the paper with the year of its publication, geographic region of investigated population, the mean/median age of cohort (or its age range)[7], the number of carcinomas studied and the number of identified *RET/PTC1*, *RET/PTC3*, and *RET/PTC* in total for calculating the frequency of these parameters.

Characterization of pooled-analysis of data

The type of analysis, conducted in this study, combining data from a sum of sources, is a pooled-analysis that differs from meta-analysis type. Meta-analysis, as a rule, is a summation with the specific approaches which included the sources weighing, and then statistical analysis of the final results of individual studies, while the pooled-analysis operates with a set of raw primary data of each work (see in the guidelines [65–67]). In both cases determination of heterogeneity degree for number of variations is performed before to choose a statistical model for the summation. Depending on the latter parameter received it is possible to combine the data in two models: "Fixed effect model" and "Random effect model" [65–67]. Sometimes the model of "simple pooling data", i.e., the calculation of the frequency index in simple proportion was used [68].

In some cases, pooled-analysis, like meta-analysis, provides an initial "weighing" of sources before combining. For example, in pooled-analysis [4] authors used weighing in accordance with the reciprocal of the variances. In other cases, this step is not carried out (for example, a large-scale worldwide study of the chromosomal aberrations frequency by pooled-analysis [69]).

The program we used for meta-analysis (see below.) weighing of variants according to specified criteria can be carried where necessary out automatically.

Statistical analysis and presentation of results

Our approach involved combining of the raw primary data from individual publications, which were grouped chronologically according to five-year periods after the Chernobyl accident, based on the year of publication of the paper[8]. Each group of data[9] for a particular five-years period was checked for heterogeneity. In the case of non-homogeneity for calculation the Random effect model was used, and in the case of homogeneity the Fixed effect model[10] was used. For some time-points there was only one work (noted below). In these cases, the calculation was carried out on the model of a simple proportion [63, 68].

Calculations of proportions, i.e., of *RET/PTC* frequency according to the above statistical models, Odds ratios, 95 % CI, the comparison of Odds ratio for groups by using of two-tailed Fisher's exact test and Pearson's χ^2 test, and the study of strata heterogeneity by χ^2 test based on the *H* index and I^2 criterion [70] was performed using the program WINPEPI (J.H. Abramson, version 11.39).

Cochran–Armitage test for *p*-trend was applied using XLSTAT (version 2015.3.01.19349).

Analysis of the data by regression and the calculation of correlation coefficients and their statistical significance were performed using the software Statistica (version 10). Plotting was carried out also using this software.

Conflict of interest and the possibility of subjective biases

Conflict of interest and subjective preconditions were absent. The work carried out within the broader budget theme and was not supported by any other funding sources. The purpose of the work is only a passing and therefore premeditated biased are unlikely. The time frame when performing work was absent.

Results and Discussion

1. Dynamics of changes in RET/PTC frequency in papillary thyroid carcinoma of Chernobyl cohorts depending on the period after the Chernobyl accident

Information for related analyzed sources can be found in our study [63]. Database at the beginning of 2015 included 30 studies, most devoted to the Chernobyl children cohorts, although in 6 papers adult cohorts, including liquidators [71] have also been studied. The time period is 20 years (from 1994 to 2014).

Fig. 1 shows *RET/PTC* frequencies in carcinomas of the named population depending on the five-year periods after the Chernobyl accident.

[6] Briefly, it is likely that multiple rearrangements within a single tumor may be linked due to external or internal causes: a lack of antioxidants, genomic instability, genetically determined defect in DNA repair and so forth [63].

[7] In the presence of such data in the publication or the ability to obtain them from the primary data.

[8] Dividing of carcinomas according to five-year periods hereinafter partly conditional as in some works the authors could study of stored frozen tumor earlier periods. But in most publications this information was not indicated, so we based only on the year of the publication.

[9] It consisted of *RET/PTC* frequency index and related to its 95 % Confidence Intervals (CI) for each work; calculation method see below.

[10] The homogeneity was found only for very small samples.

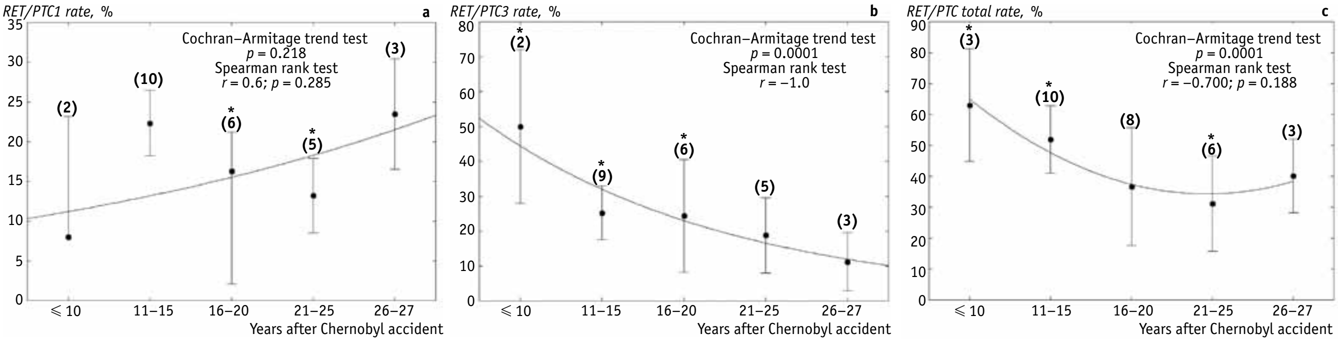

Fig. 1. The frequency *RET/PTC* in thyroid carcinomas in population affected by the Chernobyl accident according to the period after the incident. The abscissa submitted five year periods after the Chernobyl accident; the ordinate shows the frequencies of *RET/PTC1* (*a*), *RET/PTC3* (*b*) and *RET/PTC* in total (*c*), in %. Presented the value of the results of pooled-analysis and 95 % CI (if homogeneity of the sample per time point was obtained, the calculations of proportions were carried out using Fixed effect model, if sample was heterogeneous the calculation was carried out by Random effect model; see Section "Material and methods"). In the parentheses a number of studies on the time-point is presented; an asterisk reflects that the differences for Odds ratios are significant compared with the value for the last period (p varies from 7.9×10^{-6} to 0.018)

On the basis of the data presented in Fig. 1 we can say that the conclusions reached earlier by several authors for the local Chernobyl samples [45–47] (including reviews and review section of the studies [48–51]) in our pooled-analysis appears to be confirmed, that is, the level of *RET/PTC1* has some tendency to increase from the five-year period after the Chernobyl accident (for Cochran–Armitage trend test and Spearman rank correlation test, although in both cases not statistically significant, see. Fig. 1, *a*). For *RET/PTC3* and *RET/PTC* in total frequency index decreases, for Cochran–Armitage trend test it was obtained an inverse relationship from time with a very high significance in both cases (p <0,0001; Fig. 1, *b*, *c*). Spearman rank test also showed a high level for a significant inverse correlation with time for *RET/PTC3* frequency ($r = -1.0$; Fig. 1, *b*), and a clear tendency to that for *RET/PTC* in total ($r = -0.7$; $p = 0.188$; Fig. 1, *c*).

It should be recalled that according to *in vitro*, *ex vivo* [5, 40, 41, 44, 51] and *in vivo* [63], namely *RET/PTC1* is most radiogenic rearrangements, and therefore it is expected to see a reduction of its frequency as temporary separation from the incident at Chernobyl. But this is clearly not observed (Fig. 1, *a*).

When analyzing dependencies shown in Fig. 1, the question may arise about the impact of confounding and subjective factors, such as more in-depth and extensive research frequency *RET/PTC* in the early periods of determination of more frequent thyroid carcinomas after the Chernobyl accident. This explanation, however, is not true. Fig. 2 shows the number of carcinomas per one work according to the five-year period after the incident, and

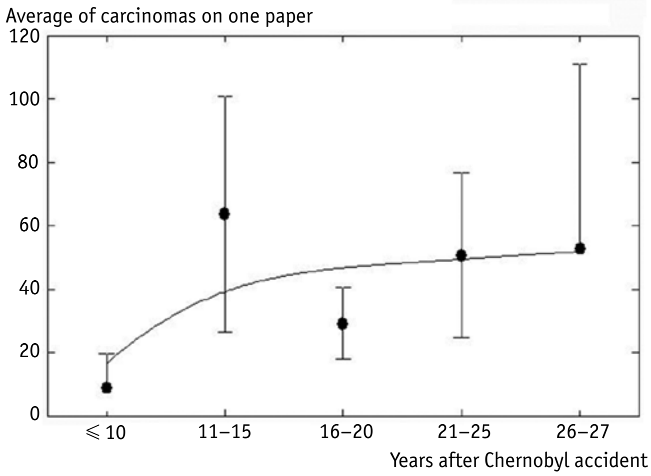

Fig. 2. Comparative scope of studies on *RET/PTC* frequency in Chernobyl cohorts thyroid carcinomas depending on the time after the accident.
On the abscissa a five-year period after the incident is represented; ordinate displays the average number of carcinomas studied for one work and 95 % CI

we can see that the scope of the study did not decrease significantly over time.

There raises a question about of the possible mechanisms for obtained chronological changes in indexes. A similar relationship was found for *RET/PTC* frequency in total in carcinomas of individuals affected by the atomic bombing, and a peak of about 20 years was marked (median was 22 years [52]) followed by a monotonic decrease [52, 53]. But also question arises about the specificity of these recent decades' trends for irradiated cohorts?

2. Dynamics of changes in RET/PTC frequency in sporadic papillary thyroid carcinoma from different continents and regions of the world depending on the period after the Chernobyl accident

Our "database" (i.e., base of published sources) is characterized, as mentioned above, by apparently 100 % completeness of sources, allowed to separate publications by continents (list of works characteristics presented in [63]). The closest to the affected region of the Chernobyl accident, in addition to the territories of Belarus, Ukraine and Russia, is Europe. The European sample for sporadic carcinoma was largest among all analyzed groups in our study (63 works). A substantial amount of data has been accumulated also for the United States and Canada (25 works), and for the Asia-Pacific region (China, Taiwan, Japan, Korea, Hawaii, Australia, Tasmania, New Caledonia; a total of 24 publications). It is understood that the latter two groups, especially the Asia-Pacific, were the least affected by the Chernobyl fallout: thyroid doses estimated in UNSCEAR 1988 for such countries were small [57].

Fig. 3 shows the combined data for chronological trends of *RET/PTC* frequency indexes in different continents and regions depending on the five-year period after the Chernobyl accident, and Fig. 4 presents data which show that the extent of relevant studies on all continents, as in the case of the residents of Chernobyl (see above Fig. 2) does not decrease with time.

Fig. 3 shows that the time dependence for frequencies of *RET/PTC3* and *RET/PTC* in total in carcinomas of the European population decreases, similarly to the curve for Chernobyl cohorts (Cf. Fig. 1, *b*, and Fig. 3, *b*, *c*). The high statistical significance on the Cochran–Armitage trend test (p <0.0001) and the obvious tendency to inverse correlation on Spearman rank test (for *RET/PTC3* and *RET/PTC* in total, respectively, $r = -0.8$; $p = 0.104$ and $r = -0.7$; $p = 0.188$).

However, for *RET/PTC1* unlike to the Chernobyl cohorts, the declining trend from the five-year period after the accident was detected (Fig. 3, *a*). This trend has, once again, a high statistical significance on the Cochran–Armitage test (p <0.0001) and a tendency to inverse correlation on Spearman rank test ($r = -0.5$; $p = 0.391$). Given that *RET/PTC1* frequency in carcinomas of victims after the accident statistically significant relationship was not identified (only the tendency, see above Fig. 1, *a*), in general we can say that the chronological trend indicators for the European contingent reflect relationships similar to those shown for the Chernobyl cohorts from Belarus, Ukraine and Russia.

Geographically Europe is close to the affected by the accident regions, and doses on thyroid from the Chernobyl fallout are already calculated for its population [2, 5][11], along with assessment of possible risks of thyroid cancer (for example, [72, 73]; see also paragraphs D189 and D250 in [5]). Whatever the epidemiological reality of these risks, it can be argued that among all continents Europe is theoretically the most affected after the Chernobyl accident. Therefore, the chronological coincidence of trends for some indicators considered as radiogenic [40–56] for the European contingent, on the one hand, and for the residents of Belarus, Ukraine and Russia on the other hand, can not be represented awesome in theory. While the assessed risks of thyroid cancer in these two cases, of course, incommensurable [2, 5, 6].

On the contrary, the North American continent, even in theory, should have been affected by the Chernobyl fallout much less, not to mention the Asia-Pacific region. The corresponding estimates in UNSCEAR 1988 to indicate this fact [57] (Figure XXII, XXIII, Table 11–13, etc.). However, as can be seen from Fig. 3, all three indices of *RET/PTC* frequency in sporadic carcinomas from these regions is also reduced by the five-year periods after the Chernobyl accident. Unlike Chernobyl and European cohorts, a downtrend starts with a "second Five-Year", that is, after 11–15 years after the accident (Fig. 3, *d–i*). Cochran–Armitage trend test showed, however, in almost all cases highly statistically significant decreasing trend for the whole period of observation (i.e., from the first term to ≤10 years after the incident). The exception was only observed in two cases: for *RET/PTC3* frequency for the North American continent and *RET/PTC1* frequency for Asia-Pacific region[12]. However, for the total index of *RET/PTC* frequency in sporadic carcinomas in both regions declining trends was statistically highly significant (p <0.0001 and $p = 0.003$, respectively; Fig. 3, *f*, *i*). Significant trends in Spearman correlations were also observed in almost all cases (for the period from the second time point). Moreover, for total *RET/PTC* frequency in carcinomas for North American contingent the inverse correlation was absolute ($r = -1.0$).

Thus, the declining trends for the frequency of the main indicators of *RET/PTC* are clearly identified not

[11] "The average thyroid dose to residents of the other European countries was about 1.3 mGy". "In the other European countries [except Belarus, Ukraine and Russia], the average thyroid doses to pre-school children are estimated to be less than 20 mGy" [5] (paragraphs 33 and B75; see also Tables B17 and B18).

[12] The calculation in these cases of the trend parameters according to the Cochran–Armitage test after elimination of the first time point did not led to statistically significant results.

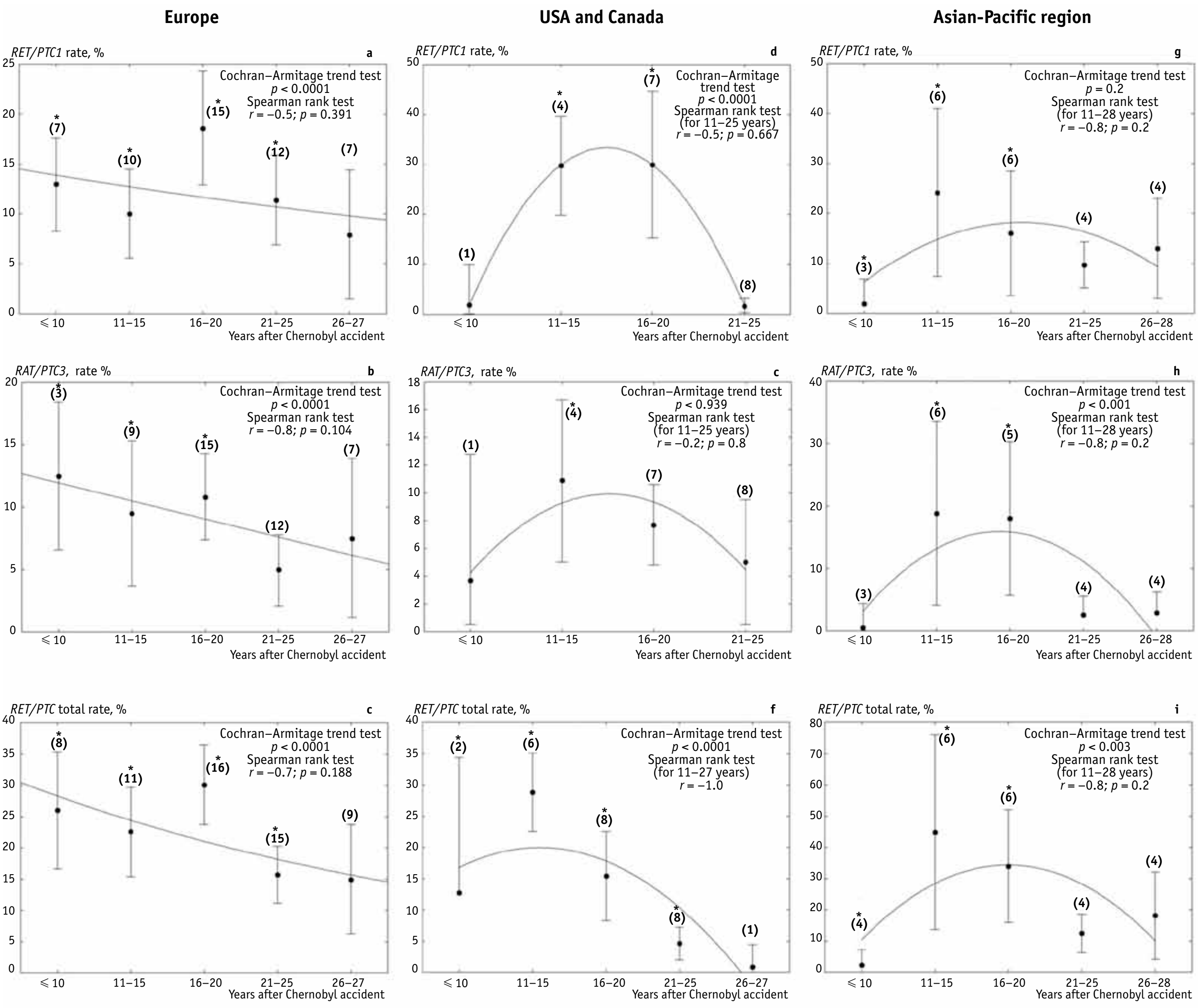

Fig. 3. The *RET/PTC* frequency indexes in sporadic thyroid carcinomas in different continents and regions depending on the period after the Chernobyl accident.

The abscissa submitted five years after the incident; the ordinates shows the frequencies of *RET/PTC1* (*a*, *d*, *g*), *RET/PTC3* (*b*, *e*, *h*) and *RET/PTC* in total (*c*, *f*, *i*), in %. Europe – *a*, *b*, *c*; USA + Canada – *d*, *e*, *f*; Asia-Pacific region – *g*, *h*, *i*. Values from pooled-analysis and 95 % CI (if homogeneity of the sample per time point was obtained, the calculations of proportions was carried out by Fixed effect model, if sample was heterogeneity the calculation was carried out by Random effect model; see Section "Material and methods") are presented. In the parentheses a number of studies on the time-point is presented; an asterisk reflects that the differences for Odds ratios are significant compared with the value for the last period (p varies from $7.5*10^{-23}$ to 0.046, the main part of the values is less than 0.001)

only in presumably suffered from the Chernobyl fallout Europe, but even in very distant geographical regions. It should be noted that a statistically significant relationship could only be obtained in the case of an ordinal time scale which corresponded of five-year periods after the accident. In the study of Pearson linear correlation for *RET/PTC* frequency depending on the year of publication in a continuous scale, only a tendency to significance and low correlation coefficients were mainly observed (Table).

The data in table shows that although the patterns in terms of the trend sign were almost the same, nonetheless for indexes of carcinomas from the Asia-Pacific region even a tendency to linear correlation is practically absent. On the other hand, for almost as little affected

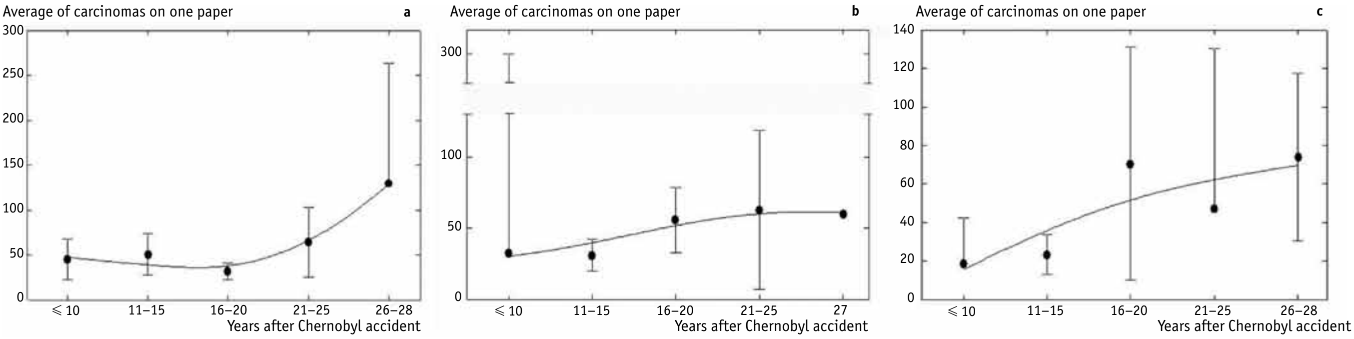

Fig. 4. Comparative scope of studies on *RET/PTC* frequency in sporadic thyroid carcinomas for groups from different continents and regions of the world depending on the time after the accident.
Europe – *a*, USA + Canada – *b*, the Asia-Pacific region – *c*. On the abscissa a five-year period after the incident is represented; ordinate displays the average number of carcinomas studied for one work and 95 % CI

Table

Pearson linear correlation between the indexes of *RET/PTC* frequency in carcinomas of analyzed contingents depending on the year of study/publication (continuous scale)

Cohort	*RET/PTC1*		*RET/PTC3*		*RET/PTC* суммарно	
	r	*p*	*r*	*p*	*r*	*p*
Chernobyl residents	–0,020	0,922	–0,404	0,045	–0,354	0,055
Sporadic Europe	–0,099	0,488	–0,198	0,187	–0,259	0,040
Sporadic USA + Canada	–0,391	0,088	–0,380	0,099	–0,615	0,001
Sporadic Asia-Pacific region	–0,032	0,884	–0,183	0,427	–0,067	0,754

the North American continent and in Europe there was a statistically significant linear trends for the main index, i.e. the frequency of *RET/PTC* in total.

It can be concluded that the calculation of *RET/PTC* frequency depending on the five-year period after the accident in the ordinal scale according to the Cochran–Armitage trend test was the most accurate (Fig. 1 and 3). Using this approach, a high probability of significant trends of decreasing as the time-distance from the accident, at least for *RET/PTC* frequency in total, are certain in all cases.

Revealed phenomena is very similar in different world regions, including virtually unaffected by the accident at the Chernobyl nuclear power plant in terms of its radiation factor, it is difficult to explain by the effects of radiation. Yet it is unlikely, as alleged by the authors from Italy after studying of national cohort of sporadic carcinomas [54, 55] that the reduction in the intensity of the of Chernobyl fallout was the cause of the declining trend of *RET/PTC* frequency also in this country.

Similarly, the decline of indexes for carcinomas of the United States for more than 30-year period [56] hardly can be uniquely caused by radiation factor, although, again, with it tied the authors of this study revealed their chronological changes during 1974–1985 years[13]. But the fact of the steady reverse growth of rate of thyroid carcinomas themselves for the whole time period, both in the US and around the world (see, for example, in [19, 20]), led the authors of [56] to opposite conclusion: the predominantly non-radiation conditionality of these tumors.

The molecular mechanism of *RET/PTC* induction associated with the generation of DNA double-strand breaks by reactive oxygen species; it is proved [74–76]. However, in serious doubt the assumption that over the past decade in Europe, the US and the former Soviet Union the levels of oxidative stress and DNA damage permanently reduced, the more so because, as we said, the intensity of medical exposure only increases [58].

In preparing of the material for pooled-analysis for sporadic carcinomas we examined the primary data of virtually every study to identify cohorts of individuals with possible radiation exposure in the past. Indexes for these individuals were isolated from sporadic pools, and included to group of radiogenic carcinomas. In the

[13] Tumor 1974–1985 period had a history of medical exposure in childhood in 19 % of cases, and in 2009 only 2 % of cases [56]. If it had therapeutic effects, or diagnostic in essential doses (it is not clear from the context of the paper), then the union in a single cohort of radiogenic and sporadic carcinomas by authors [56] does not seem valid, and the findings of their study do not seem reasonable.

absence of the individual data for these mixed cohorts, the corresponding information in the pooled-analysis was not introduced (for details see ref. [63]). Therefore, unlike the US study [56], we studied groups homogeneous for radiation factor; in any case, at the level of the primary published data.

It can be seen that a satisfactory explanation of downtrend of *RET/PTC* frequency in sporadic carcinomas worldwide so far is not proposed. The attempt to connect the identified phenomenon with the chronological changes in the degree of tumor differentiation (that is able to affect the level of genetic changes [41, 51]) does not lead to success. Indeed, it was shown, for example, in the United States [77] and Denmark [78] for a period of 1970–2000-ies the increase of thyroid cancer differentiation level[14]. But the level of *RET/PTC* depends on the differentiation level of carcinomas in direct proportion. Despite some contradictory data in the earliest studies [79], this rearrangement has a low probability of progression in poorly differentiated and anaplastic carcinomas [41, 51, 80–83][15].

However, since the fact of dependence of the differentiation degree of thyroid cancer by the age of tumor-bearer is known ("...age is a key prognostic indicator for well-differentiated thyroid cancer" [84]), it is important to find out how *RET/PTC* frequency in thyroid carcinoma is connected with age factor in all its range.

3. The RET/PTC frequency in thyroid carcinomas according to age in a continuous scale

According to our source database, the vast majority of Chernobyl cohorts tumors matched to children's and young age (see the relevant data in [63]).

On the other hand, we know in total only 24 studies of *RET/PTC* frequency in pediatric sporadic carcinomas[16]. Three works present the contingent from Ukraine, 14 works present the groups from Europe, three papers present the cohorts from the United States and three investigations present populations from China and Japan; another group presents of Saudi Arabia (this work is not included in the pooled-analysis). Thus, the vast majority of samples of sporadic carcinomas from Europe, US + Canada, and countries in Asia, Australia and Oceania which included in the pooled-analysis corresponded to adulthood tumor.

One of the key provisions set out for *RET/PTC* frequency in papillary thyroid carcinoma as a result of a more than 20-year global research, was the thesis of the predominance of this indicator for childhood tumors [44, 63]. Although studies similar dependence on age was not confirmed in a number of works [47, 60, 85–91] (there are other examples), the lack of association in these cases was due, perhaps, only to a weak statistical power of some local researches. Since there is evidence that this dependence pattern exists – either statistically significant [81, 92] or as a trend, sometimes obvious [93–96].

It should be noted that almost in none of the cited publications, to our knowledge, the authors attempted to determine the correlation between *RET/PTC* frequency and mean age of cohort in a continuous scale. Probably the work [90], in which the connection was not found, is a somewhat exception.

Previously we have statistically confirmed with our pooled-analysis that childhood carcinomas had higher frequencies of *RET/PTC1*, *RET/PTC3*, and *RET/PTC* in total, and the phenomenon was detected for both sporadic and, in general, for radiogenic cancer [63]. But, like most other authors, our study was based only on the binary principle ("scale"): children – adults. And the question of how the cohort age in the "analog" scale connected with *RET/PTC* frequency in tumors of their representatives went unanswered.

The existing database of sources made it possible to conduct an appropriate analysis. Not all publications with primary data allowed to determine the mean/median age of cohorts. But for the 87 studies on *RET/PTC* frequency in sporadic thyroid carcinomas relevant information could be either extracted or calculated.

There was information in the following categories:

- Published by the authors of the mean and/or median cohort ages.
- Presented in the primary material individual data on *RET/PTC* in parallel to the tumor-bearer age. In such cases, we calculated the mean and the median age of the cohort.
- Age ranges presented by authors for investigated groups ("from" and "to"). There we used conventional approach when in analysis we took the middle of such analyzed ranges. In cases where it was stated in the publication simply, for example, "less than 20 years old", the cohort age was taken as "20 years". In some cases calculation were made on the scattergrams of age, was taken to a weighted average of the published values for several groups, etc.

[14] Improved diagnostic is not the only explanation for this, as an increasing number of incidents with tumors of all sizes [77], and reduction depending on the time the number of anaplastic carcinomas on the order of less than increasing the number of differentiated tumors [78].

[15] Poorly differentiated and anaplastic thyroid carcinoma may arise as *de novo*, and come from a pre-existing well-differentiated tumors [41, 51].

[16] The list includes all publications of primary data that could isolate indexes for childhood tumors. Often, the entire group in a some work included only 1–2 child carcinoma, for which data include by us in the stratum at an earlier pooled-analysis [63].

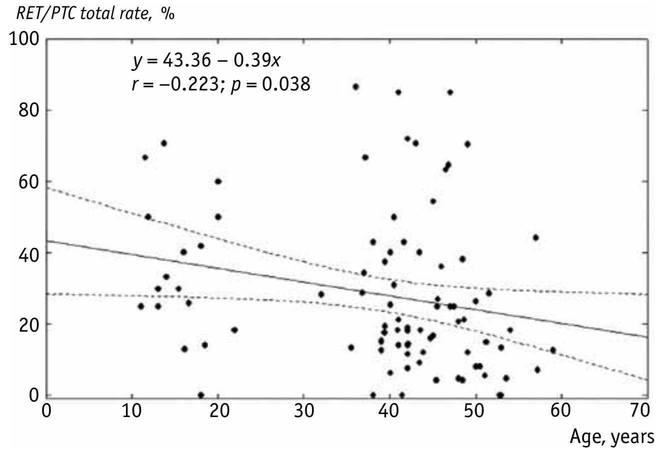

Fig. 5. Dependence of *RET/PTC* frequency in total in thyroid carcinomas on the cohort age.
The abscissa submitted the cohort age (median, mean, or otherwise obtained by the estimation; details in the text) years; the ordinates shows *RET/PTC* frequency in total, %. Dotted lines reflect the 95 % CI

The mean and/or median age of groups were available for 78 % of the sample (mean were for 59 works (68 %), and median for 45 works (52 %)).

The basis of the total analyzed indicators initially undertook of median cohort age, as the most suitable central tendency for the abnormal distribution of values. In cases where the median values were not available in the analysis included the mean age of the cohort or the result of the above estimation approaches.

The calculation of the parameters of Pearson linear correlation on age did not reveal the slightest statistically significant trends for frequencies of *RET/PTC1* and *RET/PTC3* separately (respectively: $n = 78$; $r = –0.067$; $p = 0.559$ and $n = 77$; $r = –0.093$; $p = 0.419$). Nevertheless, for *RET/PTC* frequency in total though weak, but significant association was revealed ($n = 87$; $r = –0.223$; $p = 0.038$; Fig. 5).

When replacing as the base parameter the median age with mean age the association did not acquire greater significance. In this case, the correlation index for *RET/PTC* frequency in total shifted over the limit of statistical significance, albeit to a small extent ($n = 87$; $r = –0.206$; $p = 0.055$).

It may be thought that we used a inadequate conditional approach to the introduction of the sample including the estimated value of the average of the authors' age range, weighted average values, etc. (see above) covered yet of 22 % variants. Therefore, such values of samples were eliminated, and the correlation statistics calculated strictly accurate for median and/or mean values of cohort age. Nothing encouraging was received, however. Moreover, even the only significant linear correlation between *RET/PTC* frequency in total and cohort age disappeared ($n = 68$; $r = –0.146$; $p = 0.238$).

Thus, based on these data, it can be concluded that the dependence of *RET/PTC* frequency on the age for predominantly adult group (see the age distribution in Fig. 5[17]) if it exists, is very weak. Although it may be reversed. It follows that the observed differences in the chronological trends for the different continents and regions of the world (see Fig. 4 above) are unlikely due to age as a factor, and the associated change in the degree of differentiation of carcinomas.

However, there is an explanation of this phenomenology, and it reflects in many ways the "human factor".

Probably discussed in section "Introduction" diagnostic bias in connection with the accident at the Chernobyl nuclear power plant, plus the instrumental improvement, coupled with the "aggressive surgery" in the aftermath of the Chernobyl accident [3, 18], there have been all over the world: from the Ukraine and Belarus to Europe, North America and the Asia-Pacific region. A similar phenomenon was characteristic not only for Chernobyl tumor etiology, but, for example, for a permanent increase in the incidence of thyroid cancer in the United States (though not accompanied by an increase in mortality) (*28*). Perhaps, due to this worldwide detected earlier forms of occult carcinoma and microcarcinomas which characterized by higher *RET/PTC* frequency than in conventional tumors [59–62] (up to 77 % compared with 47 % in clinical forms of carcinomas [61]).

Over time, the medical caution, of course, gradually subsided.

In Europe, such caution seems to have originated in the first period of detection of increased frequency of carcinomas in Belarus (the beginning of the 1990s (*1–3*), which was realized in the immediate activation partially subjective diagnosis and surgery included occult and microcarcinomas. As a result, the level of Europe's parameters of *RET/PTC* proved the largest in the earliest period (see Fig. 3 *a–c* above). But for the more distant from Chernobyl continents "alarm" probably sounded later, bringing to North America and Asia-Pacific countries the maximum values of the indicators have moved five years (Fig. 4, *d–i*). This explanation seems to be the most likely.

[17] Which presents, of course, not all relevant studies. In some works the primary information about the age had only qualitative in nature (children or adult) and use it for the above correlation analysis was impossible.

Conclusion

Presented pooled-analysis of the gene rearrangements *RET/PTC* frequency in thyroid papillary carcinoma was the further development of the studies of radiation attribution for thyroid cancers after the Chernobyl accident. Noted earlier [45–51] the fact of reducing of *RET/PTC* frequency in total and *RET/PTC3* frequency time after the Chernobyl incident in our case was confirmed by the analysis of data from all the relevant global researches. From one to other five-year period after the accident, the levels of these indexes declined steadily. But for *RET/PTC1*, which is considered to be the most radiogenic type of rearrangement [5, 40, 41, 44, 51, 63] similar changes with regard to tumors of the Chernobyl etiology has not been found either by us in the present study (significant changes were absent, see Fig. 1 *a*) or by other authors (there is uptrend) [45, 46]. In principle, *a priori*, can still be explained by these chronological dependences for *RET/PTC* in total and *RET/PTC3* by less influence of accident at the Chernobyl nuclear power plant factor, if they were not known data about similar chronological dynamics and for sporadic carcinomas from Italy [54, 55] and the USA [56].

In our study, the completeness of sources and accessibility published in their primary information allowed to conduct a pooled-analysis to identify chronological trends after the accident for index of *RET/PTC* frequency in sporadic thyroid carcinomas for almost all major continents and regions. Almost similar to the detected for the Chernobyl cohorts chronological dynamics of changes in the frequency of *RET/PTC3* and *RET/PTC* in total for the European contingents was found, although in this case the level of *RET/PTC1* also decreased monotonically according to the five-year periods after the Chernobyl accident[18]. As for the combined cohorts USA + Canada and the Asia-Pacific region (China, Taiwan, Japan, Korea, Hawaii, Australia, Tasmania, and New Caledonia) the dependence in nearly all cases (and for the *RET/PTC* frequency in total in all cases) characterized statistically significant declining chronological trends.

Thus, even for regions, slightly affected by Chernobyl fallout (according to UNSCEAR [2, 5, 57]), the similar to that shown for the contingent from Belarus, Russia and Ukraine chronological trend in relation to *RET/PTC* frequency in thyroid carcinomas is obtained. It is unlikely that it can be entirely attributed to the radiation factor, whether the Chernobyl accident or higher doses of medical exposure in previous years. In the latter case it is useful to pay attention to the abovementioned maximum values of indexes for Europe, North America and the Asia-Pacific region, which generally occurred at the time of the most active research and diagnosis of thyroid tumors in the post-Chernobyl period (Fig. 1 and 3). In this regard, not understandable why the alleged for USA [56] more intensively (based on dose levels) medical exposure distant past decades was realized precisely in this period, especially since real dose of medical exposure in all developed countries to permanently grow to date [58].

Explanation of the identified chronological changes trend by the degree of differentiation of thyroid carcinomas was not correct, because although in recent decades the degree of differentiation of thyroid cancer actually increases [77, 78], the dependence on *RET/PTC* frequency on the degree of carcinoma differentiation was no inversely, but directly proportional [41, 51, 80–83]. In our pooled-analysis no well significant association between *RET/PTC* frequency and median/mean cohort age of tumor in a continuous scale (Fig. 5, age was from 11.5 to 57.2 years) was found, while the degree of differentiation of cancer thyroid tumor on the age known enough [84].

As an explanation of our results the most probable hypothesis is related to overestimation and "overdiagnosis" of early forms of thyroid tumors [97], including in connection with the accident at Chernobyl, expressed earlier by several authors for the territories of countries of the former Soviet Union [3, 18, 22, 23]. Apparently, these factors, coupled with the instrumental improvement in the period of the 1990s, and with the "aggressive surgery" after the accident took place around the world: from the Ukraine and Belarus to Europe, North America and the Asia-Pacific region. As a result, everywhere the earlier forms of the occult carcinomas and microcarcinomas in which *RET/PTC* frequency was higher than in conventional tumors were revealed [59–62]. Perhaps and all types of tumors that were invisible even in the future were obtained too. Is it possible that at times and all not malignant tumors, as it was in its time for the Soviet republics too [3].

From remote survey of the Chernobyl accident, the intensity of these subjective factors, of course, declined.

Meanwhile, in the period before the Chernobyl accident [98], it was known that the irradiation of an adult thyroid does not have a high radiation sensitivity in relation to carcinogenesis [36, 99], and with medical exposures of ^{131}I in childhood dose induction of thyroid cancer is not are low [100, 101] (see also ref. [102, 103]). But even in Europe, not to mention the North American continent and the countries of the Asia-Pacific region, the dose of ^{131}I on thyroid rarely reached the level of very low

[18] Formally *RET/PTC1* frequency in Europe carcinomas was more similar on the parameter induced by radiation than for residents of the Chernobyl accident in the three countries of the former USSR. This, of course, absurd.

doses (up to 10 mGy [104, 105]), and much less the level of low doses (up to 100 mGy [6, 7, 104, 105]).

If the identified chronological trends of reducing of *RET/PTC* frequency in carcinomas worldwide are actually due to subjective reasons and "aggressive surgery", this phenomenon is deplorable. Since unjustified even while incomplete knowledge and exaggeration of Chernobyl effects from the 1990s to early 2000s years it could affect the fate of many people worldwide. This was repeatedly noted by various researchers, from the former Chairman of the UNSCEAR Z. Jaworowski [106], Academician L.A. Il'yin [107] and Russia's leading expert on Radiation Biology recent years S.P. Yarmonenko [108, 109] to a Russian analyst generalist S.V. Yargin [18, 23].

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