Cytogenetic surveys in patients undergoing therapeutic irradiation were carried out mainly for validating the chromosomal biodosimetry after inhomogeneous exposure [4–6]. Rarely the cytogenetic analysis has also been applied in clinical radiobiology, presumably for comparing a chromosomal toxicity from various methods of radiation therapy [7–9].

Despite the sufficient number of publications with the results of the chromosomal surveys in uterine cancer patients undergoing radiation therapy [10–13], no data have been published yet concerning the peculiarities of radiation–induced cytogenetic effects in persons after pre–irradiation tumor cryodestruction. In our previous study the absence of genotoxic influence of the cryosurgical procedure on the chromosomal damage yield in human lymphocytes has been shown in patients sampled before the radiation treatment [14]. The objectives of the present survey were to find out whether the pre–irradiation tumor cryodestruction is able to cause the enhancement of the local radiation reactions in normal tissues in persons with pre–irradiation tumor cryodestruction.

Key Words: chromosome aberrations, uterine cancer, external gamma-therapy, tumor cryodestruction.
mean age 63 years). Malignancy stages were verified hystologically and distributed as follows: T1A — 17.1% cases (7 persons), T1B — 39.0% cases (16 persons), T1C — 12.2% cases (5 persons), T2 — 22.0% cases (9 persons), T3—4 — 9.8% cases (4 persons).

All patients received the radiation therapy as a part of complex scheme of uterine cancer treatment. Patients were irradiated externally from therapeutic gamma—irradiation device (ROCUS—AM with 60Co source of 1.25 MeV) in classic dose fractionation regimen. Daily fractions of 2 Gy were delivered 5 times per week to the pelvic AP and PA portals with irradiated field size 14 x 16 cm or 16 x 18 cm, until total dose of 40—45 Gy was accumulated at the end of the treatment. In several cases the radiation therapy was designed as the combination of the external and intracavitary pelvic irradiation. The external irradiation followed the standard scheme as described above. The intracavitary irradiation started after accumulation of 14—20 Gy dose from the distant exposure and was performed in the intervals between external irradiations using the AGAT—B brachytherapy device with single doses 5 Gy and 1.25 Gy to the points A and B, respectively. In these cases the total doses at the end of the radiotherapy were 50—55 Gy at the point A and 12—14 Gy at the point B from brachytherapy and about 40 Gy at both points from the external irradiation. Equal percentage of persons who received the intracavitary irradiation in addition to the external gamma therapy was present in the groups of cryosurgically treated and non—treated persons. In patients underwent tumor cryodestruction the cryogenic treatment of uterine endometrium was carried out using N2O3 at —70 °C on the therapeutic device AGC—01, twice for 5 min, under ultrasound control, 24—48 h before the start of radiation therapy course [1, 2].

Blood samples for cytogenetic analysis were collected 1—3 days before the start of radiation therapy, at the middle and at the end of irradiation course. Blood lymphocytes were cultivated according the standard protocol [3] in the Eagle’s MEM supplemented with 20% foetal calf serum and 2% PHA (Gibco, UK) at the temperature 37.5 °C during 48—50 h. Colcemid blocked metaphases were hypotonically treated and fixed in methanol/glacial acetic acid mixture. Cell suspensions were dropped onto slides and stained by Giemsa. Metaphases contained 46 chromosomes were scored and non—treated persons. In irradiated persons with tumor cryodestruction (groups I²²a and I²²b), the total dose was delivered according to the following scheme: 14 cm or 16 cm at the end of the radiotherapy were 50—55 Gy at the point A and 12—14 Gy at the point B from brachytherapy and about 40 Gy at both points from the external irradiation. Equal percentage of persons who received the intracavitary irradiation in addition to the external gamma therapy was present in the groups of cryosurgically treated and non—treated persons.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells scored</th>
<th>Aberration type</th>
<th>Y ± SE (per 100 cells)</th>
<th>Aberration per cell distribution</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>Aberrations</td>
<td>Aneuploids</td>
</tr>
<tr>
<td>I</td>
<td>4743</td>
<td>Dic + CR</td>
<td>0.32 ± 0.08</td>
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</tr>
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</table>

RESULTS

Chromosome aberrations. The analysis of chromosomal rearrangement level changes during the radiation therapy was concentrated on the chromosome type aberration and dicentric plus centric ring yields, depending on the cryosurgical treatment status of patients. After pooling the individual results within each group a distribution of aberrations among cells was obtained and the mean aberration frequencies with standard errors were calculated (Table). Papworth’s u—test showed the significant overdispersion of total aberrations and chromosome exchanges per—cell distributions comparing with the Poisson statistics in the middle and at the end of radiation treatment (σ²/Y > 1, u > 1.96) irrespectively to the patients’ cryosurgical treatment status. The mean frequencies of cells containing dicentrics and centric rings in groups IIa and IIb were identical; the dispersion to aberration yield ratio; u — Papworth’s test values; * statistically significant difference between groups IIIa and IIIb.

Table. Aberration level and aberration—per—cell distribution in patients during radiation therapy

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yields of chromosome exchanges have shown no difference as well (t = 0.59; p > 0.05). The absence of statistical difference between these groups was also found for the yields of chromosome type aberrations and the levels of aberrant cells (respectively, t = 1.22 and t = 1.90; p > 0.05). The distributions of dicentrics and centric rings among cells were not statistically different between groups IIa and IIb (χ² = 2.383; p = 0.123), but for total aberration distribution the difference appeared to be significant (χ² = 5.248; p = 0.022). That was caused by the 1.7–times higher proportion of cells containing ≥3 aberrations within the aberrant fraction in persons who were not cryosurgically treated in comparison with the group IIb.

At the end of radiation therapy 2-fold increase of the total aberration yield and 1.7-fold increase of the dicentrics and centric rings yield comparing with the values in the middle of the course were present in both IIIa and IIb groups. Group IIIa displayed somewhat lower meanings of these two indices than the group IIIb, but the magnitude of difference was non-significant for both parameters (t = 1.43 for total aberrations and t = 1.76 for dicentrics and rings; p > 0.05). In contrast to closeness of aberration yields, the levels of aberrant cells and metaphases containing dicentrics and centric rings appeared to be statistically higher in the group IIIa compared with the group IIIb (t = 2.97 and 2.59, respectively); p < 0.01).

The distributions of chromosome exchanges and total chromosome aberrations among cells didn’t show the sufficient difference between groups IIIa and IIIb (χ² = 7.306, p = 0.121 and χ² = 7.791, p = 0.254 respectively). But the proportion of mostly damaged cells (containing ≥4 aberrations) within the aberrant lymphocyte fraction appeared to be 1.3 times higher in the group IIIa, and the same effect was found for the percentage of cells with ≥3 dicentrics and centric rings among the cells with chromosome exchanges.

The dicentrics and centric rings yield in cells containing chromosome exchanges in persons with and without pre-irradiation cryosurgical treatment was plotted against the mean number of external irradiation dose fractions at the time of sampling (Fig. 1). At the middle of the radiation therapy this parameter was statistically indistinguishable for patients with or without tumor cryodestruction (t = 1.30; p > 0.05). Between the middle and the end of radiation treatment the increase of dicentrics and centric rings yield in cells containing chromosome exchanges was more pronounced in persons without cryosurgical treatment: t = 4.39 for comparing groups IIIa and IIa; t = 3.17 for comparing groups IIIb and IIb (p < 0.001 in both cases). At the final point this index in patients with tumor cryodestruction appeared to be significantly lower than the value in the group IIIa (t = 2.04; p < 0.05).

**Genomic abnormalities.** The changes of genomic abnormalities levels measured in patients during radiation treatment are shown on the Fig. 2. Before irradiation course the spectrum of genomic abnormalities was restricted to polyploids only. At the middle and the end of radiation therapy this parameter was statistically indistinguishable for patients with or without tumor cryodestruction (t = 1.30; p > 0.05). Between the middle and the end of radiation treatment the increase of dicentrics and centric rings yield in cells containing chromosome exchanges was more pronounced in persons without cryosurgical treatment: t = 4.39 for comparing groups IIIa and IIa; t = 3.17 for comparing groups IIIb and IIb (p < 0.001 in both cases). At the final point this index in patients with tumor cryodestruction appeared to be significantly lower than the value in the group IIIa (t = 2.04; p < 0.05).

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**DISCUSSION**

The chromosome type aberrations and dicentrics plus centric rings yields were chosen as the main end-
entered cells, so the yield of aberrations per aberrant
entered there from an intact body part. Each next dose
that takes place within the time intervals between the
one is lymphocyte circulation over the patients' body
dose-effect relationship for this end-point. The second
by undamaged stem cells, that results in a decrease of
fraction. The first one is a dilution of aberrant cell frac-
tion with non-aberrant lymphocytes newly produced
in patients' body, affecting the simply additive accu-
ration assumed. Thus, a concurrent dynamics of the aber-
level and aberration–per–aberrant cell yield dur-
ring the radiotherapy course reflect the radiation dose
accumulation and the changes of the volume of irri-
ated cell fraction, but also provide the semiquantitative
assessment of the lymphocyte precursors proliferation
activity and the rate of lymphocyte circulation between
irradiated and non–exposed body sections in thera-
papeutically exposed persons [18]. This model was ap-
plied in present study for explaining the peculiarities of
cytogenetic effects found in persons treated and non–
treated with pre–irradiation tumor cryodestruction.

In present study a dramatic increase of chromosome
type aberration frequency in patients during radiothera-
py course was found, and that was accompanied by
the significant overdispersion of aberrations–among–cells
distribution comparing with Poisson statistics. Both ef-
effects are the most commonly reported cytogenetic
changes seen in persons undergoing external partial
body therapeutic irradiation [4–8, 10–13]. The extend of
dicentrics and total chromosome aberrations yields ri-
sing in our survey seem to be in a good consistence
with the reports concerning the cytogenetic effects in ex-
ternally irradiated uterine or cervical cancer patients [10–
13]. A non–random aberration distribution in human lym-
phocytes in case of low–LET radiation exposure reflects
the inhomogeneity of dose distribution in patients' body,
when the total lymphocyte population is presented by
the superposition of irradiated and non–irradiated cell
fractions [3, 15].

In cases of the inhomogeneous exposure the chro-
mosome aberration frequency calculated for the total
number of cells scored (i.e. the direct cytogenetic in-
dex) represents the average radiation dose accumu-
lated in the total human body and thus depends on the
relative volume of irradiated lymphocyte fraction. Un-
like that, the yield of chromosome aberrations in aber-
rant cells is a function of radiation dose accumulated in
the irradiated part of the body only, irrespectively to the
irradiated fraction volume. So, a combination of these
direct and per–unit cytogenetic parameters allows to
carry out the biological dosimetry of the non–uniform
irradiation, e.g. after the first dose fraction of external
partial body therapeutic exposure [3–6].

However, we recognised that during in vivo frac-
tionated therapeutic irradiation two processes take place
in patients' body, affecting the simply additive accu-
mulation of the aberration level and the aberration–per–
aberrant cell yield produced by each subsequent dose
fraction. The first one is a dilution of aberrant cell frac-
tion with non–aberrant lymphocytes newly produced
by undamaged stem cells, that results in a decrease of the
aberration frequency comparing with the expected
dose–effect relationship for this end–point. The second
one is lymphocyte circulation over the patients' body
that takes place within the time intervals between the
dose fractions and leads to diluting the aberrant cells
inside the irradiated zone by non–aberrant cells, which
entered there from an intact body part. Each next dose
induces aberrations in both already irradiated and newly
entered cells, so the yield of aberrations per aberrant
cell becomes lower than that of expected if no circula-
tion assumed. Thus, a concurrent dynamics of the aber-
ration level and aberration–per–aberrant cell yield dur-
ing the radiotherapy course reflect the radiation dose
accumulation and the changes of the volume of irri-
ated cell fraction, but also provide the semiquantitative
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plied in present study for explaining the peculiarities of
cytogenetic effects found in persons treated and non–
treated with pre–irradiation tumor cryodestruction.

No difference for total chromosome aberrations and
dicentrics plus centric rings frequencies was observed
between groups either IIa and IIb or IIIa and IIIb. Taking
into account that irradiated portals were equally loca-
ted and had the same size, a conclusion could be made
that the similar doses were accumulated in similar ir-
radiated body volume in both cryosurgically treated and
non–treated patient groups. From this a strong evidence
arises that the cryodestruction didn't impact on the bi-
ologically equivalent radiation load accumulation, but also
didn't negatively influence the lymphocyte precursors
proliferation rate or enhance the patients' chromosome
radiosensitivity in normal tissue cells.

In contrast to the coincidence of the direct cytoge-
etic parameters, patients groups with and without pre-
irradiation cryoprocedure displayed a difference for the
per–unit index dynamics during the second half of the
radiotherapy course. That was expressed as a 3–times
more sufficient rising of the level of cells containing
chromosome exchanges but accompanied by a dis-
tinctively lower proportion of heavily damaged cells
within aberrant lymphocyte fraction in cryosurgically

treated patients comparing with the positive control.

Taking into account the equality of accumulated ra-
diation dose and the similarity of leucopenia restora-
tion, the lymphocyte circulating rate should be consi-
dered as the main factor causing the changes of aber-
ration–per–cell distribution and dicentric plus centric ring
yield in cells containing chromosome exchanges found
in cryosurgically treated patients. Possibly, in these
persons a relatively higher proportion of “fresh” non–
aberrant lymphocytes entered the irradiated body zone
within the intervals between subsequent irradiation frac-
tions, partially replacing the already exposed cells. So,
the relatively lower number of lymphocytes inside the
irradiated part were able to “collect” the aberrations in-
duced by several dose fractions, that results in lower
frequency of heavily damaged cells and lower yield of
dicentrics and rings per aberrant cell in patients un-
derwent the pre–irradiation cryodestruction. The en-
hancement of lymphocyte circulation rate was suffi-
cient enough to provide the 13% decrease of dican-
trics and rings per aberrant cell yield in compare with
non–cryosurgically treated group at the end of radia-
tion therapy.

In addition to chromosomal rearrangement frequen-
cy increasing, a quantitative change for the polyploids
and endoreplications level was detected in patients’ lymphocytes during radiation treatment. The similar moderate rising rate of the genomic abnormalities yield was observed in our previous investigations and also was noted in 12 other cytogenetic surveys of radiation therapy patients [12, 19]. The absence of chromosome aberrations in polyploids indicates that this type of cytogenetic damage arises in cells beyond the irradiated body part, so polyploidisation is not caused by anaphase dicentric bridging. The alternative mechanism may involve the total genome amplification if a specific reply to the action of clastogenic factors, which are known to be induced in human blood plasma by radiation exposure [20]. Thus, the genomic abnormalities could not be used as a quantitative radiation-specific markers, but may indicate the genome instability development in somatic cells of cancer patients during radiation treatment.

The difference found between groups for genomic abnormalities dynamics supports the assumption concerning more rapid lymphocirculation in cryosurgically treated persons. Between the middle and the end of irradiation course the total level of genomic abnormalities demonstrated the low declining trend in cryosurgically treated patients, that was in contrast to 1.5-fold rising in patients without tumor cryodestruction. In line with our hypothesis, the relative decline in producing the genomic abnormalities corresponds to the respectively lower clastogenic factors output, which could be afforded by lower dose accumulation in irradiated fraction of plasma in case of increased lymphocirculation rate. The discrepancy for genomic abnormality level between cryosurgically treated and non-treated groups occurred concurrently with the difference observed for dicentrics and rings per aberrant cell yields, i.e. within the second half of radiation treatment. Thus, one can assume that both effects possibly could have the same reason, and the result of more rapid lymphocirculation between irradiated and non-exposed body zones became quantitatively pronounced at the end of irradiation course.

Conclusion. The present survey clearly demonstrates that the cytogenetic analysis of peripheral blood lymphocytes is a powerful tool for applied radiobiology, allowing to evaluate a modificative action of the additional treatment method (e.g. the pre-irradiation tumor cryodestruction) on the radiation-specific effects development in human normal tissue cells during radiation therapy. The similar intensity of rising the radiation-specific chromosome rearrangement yields during radiation therapy in cryosurgically treated and non-treated uterine cancer patients groups indicates that the cryo-procedure doesn’t impact on the biologically equivalent radiation load accumulation, but also doesn’t have a negative influence on the lymphocyte precursors proliferation rate and doesn’t enhance the chromosome radiosensitivity in normal tissue cells. However, between the middle and the end of radiotherapy course the yield of dicentrics and centric rings per aberrant cell increased much less steeply in patients with tumor cryodestruction in compare with the positive control group. Concurrently, the frequency of polyploids and endoreplications didn’t increase in cryosurgically treated persons, that contrasted with statistically significant accumulation of cells with numerically abnormal genome in patients without cryodestruction. Both phenomena could be explained by enhanced lymphocirculation between irradiated and non-exposed body sections, that results in lower proportion of lymphocytes and blood plasma which accumulate the dose from several thera-peutic fractions. Taking into account the functional role of lymphocytes, the enhanced lymphocyte circulation may lead to more effective removing of radiation-killed cells and the toxic products of their decay from the irradiated body zone. That could be one of the possible mechanisms of reducing the negative local radiation reactions in normal tissues in persons underwent pre-irradiation tumor cryodestruction.

REFERENCES

ЦИТОГЕНЕТИЧЕСКИЕ НАРУШЕНИЯ В ЛИМФОЦИТАХ ПЕРИФЕРИЧЕСКОЙ КРОВИ У БОЛЬНЫХ РАКОМ МАТКИ В ХОДЕ ЛУЧЕВОЙ ТЕРАПИИ: ЭФФЕКТЫ ПРЕДЛУЧЕВОЙ КРИОДЕСТРУКЦИИ ОПУХОЛИ

В.А. Винников, А.А. Михановский, И.А. Мазник

Цель: определение возможного модифицирующего влияния предлучевой криодеструкции опухоли на радиационно-индуктированные эффекты в клетках нормальных тканей. Методы: классический цитогенетический анализ 50-часовых культур лимфоцитов периферической крови в группах пациентов, подвергавшихся или не подвергавшихся криохирургическому лечению, осуществляли до начала лучевой терапии, в середине и в конце курса внешнего гамма-облучения, проводимого в режиме классического дробного фракционирования (2 Гр за один сеанс). Результаты: в сравниваемых группах наблюдались одинаковые темпы повышения частоты радиационно-специфических хромосомных перестроек на протяжении лучевой терапии, что свидетельствовало об отсутствии влияния криопроцедуры на ход накопления биологически-эквивалентной дозы облучения и на радиочувствительность хромосом в клетках нормальных тканей. Поклеточное распределение аберраций у пациенток после криодеструкции опухоли характеризовалось сниженной пропорцией метафаз с большим числом хромосомных перестроек по сравнению с группой положительного контроля, особенно в конце лучевого лечения. У больных после криохирургического вмешательства во второй половине курса лучевой терапии наблюдалась замедленная темпы повышения выхода дисциентеров и колен на аберрантную клетку, что коррелировало с эффектом снижения частоты полиплоидов и эндорепликаций, в отличие от накопления геномных нарушений у лиц без криодеструкции опухоли. Выводы: наиболее вероятным источником модифицирующего влияния предлучевой криопроцедуры на цитогенетические показатели при лучевой терапии является усилена циркуляция лимфоцитов между облучаемой и необлучаемой зонами тела, что может отражать один из механизмов снижения риска развития местных лучевых реакций у пациенток, которым проводили предлучевую криодеструкцию опухоли.

Ключевые слова: аберрации хромосом, рак матки, дистанционная гамма-терапия, криодеструкция опухоли.