Ratio of concentrations of estrogen receptors to progesterone receptors (ER/PR) in the cytosol of breast cancers (stratification by forming of groups differing in PR)

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The ratio of cytosol concentrations of estrogen receptors to progesterone receptors (ER/PR) can help at the diagnosis of the excessive production of estrogens or (on the contrary) of the lowered function of ER or of the too small expression of the PR gene. We divided the statistical set into the groups with the approximately same concentrations of PR for stronger judgement of this ratio because PR is nearly not changing due to the age (in contrast to the age unstable ER). We used this stratification into the PR-limited groups at the radio-receptor analysis of 147 patients.

1) The ER/PR quotient was higher in the older patients but predominantly it was approximately 10-times lower in case of the high PR than in case of the low PR. This is why the more than 10-fould error can arise at uncorrected judgement whether ER of some patient is inadequately high or low in the comparison with her PR. It implies that e.g. in case of any one patient it is possible to infer the excessive production of ER from the comparison of her ER/PR – best only in the range of her PR-limited group (and in the addition – taking account of the age). It can be important for therapy and prognosis.

2) The interpersonal differences of ER and ER/PR were approximately 10-times smaller in PR-limited groups than in the whole statistical set. This is why e.g. the correlation coefficients of the age increase of ER and ER/PR in the PR-limited groups were more favourable than in the whole non-stratified statistical set.

In case if PR decreases in the higher age in case of some authors, it is necessary to create the PR-limited groups by another manner. For instance, 20 % of the tumors with the highest PR from each age group will be in the same PR-limited group (despite the fact that they differ in PR). The impact of the age will be then more marked in case of ER/PR than in case of ER only because the numerator elevates and denominator decreases. The impact of the ovarian cycle might be detected more sensitively on the same principle.

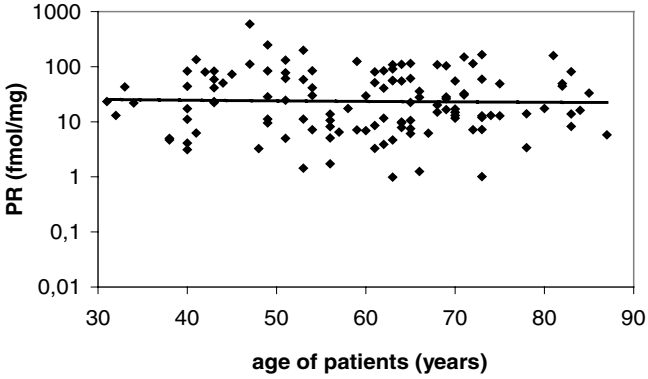
It is possible to analyse by this manner the possibility to transform the receptor results to the average age or to the optimal phase of the ovulation cycle to prevent e.g. the false negativity of ER. The principles of this mathematical approach might be exploited even for a judgement of the prognosis and therapy on the basis of the mutual ratio of different isotypes of receptors for one hormone only (ERα / ERβ or PRA / PRB). It concerns not only the breast cancers but also the cancers of the uterus.

Key words: Breast cancer, estrogen receptors, progesterone receptors, ratio ER to PR, isotypes (ERα / ERβ or PRA / PRB), estrogens, age, ovarian cycle, hormonal therapy.

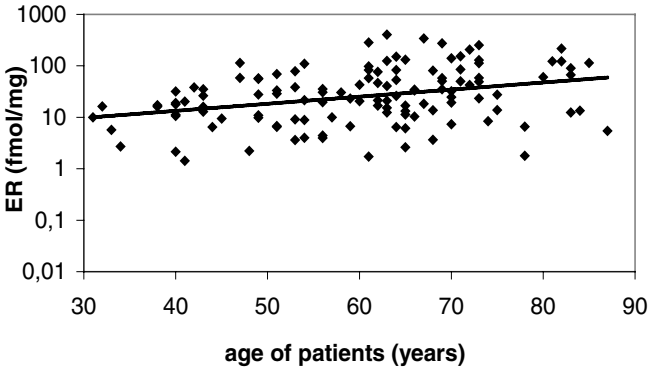
The result of quantification of hormonal receptors (fmol) expressed as their concentration in the cytosol of the breast cancer is influenced in different extent by the percentage of the stroma (e.g. blood vessels) because the stroma differs for each patient. It can be described as the variability of the amount of the cancerous and non-cancerous part of proteins (mg) in the denominator of the ratio fmol/mg. In case of ER and PR absence in the stroma – the impact of the stroma's percentage on the concentration of receptors in the tumor is indirectly proportional.

The impact of the mentioned indirect proportion on both ER and PR must be quantitatively identical despite the fact that (at investigation of the receptor increase with the tumor-stroma ratio) the interpersonal differences are greater and therefore the statistical significance smaller in case of PR than in case of ER [1]. In case of such tumors that differ mutually only in the percentage of the stroma, the ER/PR quotients must be the same because the amount of proteins is shortened in these quotients.

In addition to this impact of the stroma, also the impact of some cancerous – non-stroma cells (such of them that are nega-



Graph 1. Dependence of PR on the age



Graph 2. Dependence of ER on the age

tive in both receptors) on the fmol/mg ratio is consistent with the influence of the "non-cancerous" proteins of the stroma (analogical indirect proportion). Again, this component of the tumor has the quantitatively identical influence on both ER and PR.

The ER/PR quotient eliminates both of these differences among the patients (in the percentage of the stroma proteins and in the percentage of the hormonally independent cancer cells). Using this quotient we may study more precisely e.g. the influence of estrogens on the level of ER and PR – but only with the help of some additional corrections. To explain these corrections we connected our considerations with the analysis of the age dependence of the ER/PR quotient within the statistical set of our patients.

The age increase of cytosol ER in the breast cancers was proven in many works (that we cited earlier – [2]). On the contrary – in case of PR – some authors observed the age decrease of PR – most frequently as the decrease of the number of PR(+) patients [3, 4, 5, 6, 7, 8, 9,]. It might be explained by the decrease of the blood concentration of estrogens [10]. Nevertheless, this decrease of PR is probably only small because in other works the age change of PR was insignificant or undetectable by either of the methods: qualitatively – by the number of PR(+) patients and quantitatively – by comparing of the differences among PR concentrations [11, 12, 13, 14, 15, 16]. Or even on the contrary, the age increase of PR was observed in a smaller number of works – evaluated both – qualitatively – by the number of PR(+) patients [17], and quantitatively – by comparing PR concentrations [18].

It implies that (in contrast to the age unstable ER) the cytosol concentration of PR can serve as a constant for sorting of the statistical set because PR is only minimally changed by the hormonal influences of the age. This is why we investigated the age changes of the ER/PR quotient in the PR-limited groups.

Material and methods

We examined the excisions from the mammacarcinomas of 147 patients (predominantly) from the hospital in Pardubice. If it was possible, the operations were performed in the first phase of the menstrual cycle. No patient had been healed with the Tamoxifen before surgical treatment. This statistical set was not sorted on basis of e.g. the histological properties of tumors.

In this work the results of the same tumors are newly statistically evaluated – the results of which we published in the works about the influence of the age on ER [2] and about the influence of the age on the PR/ER quotient [19]. However, the analysis of the influence of age on ER and on ER/PR was only simple in these cited works – without the creation of PR-limited groups.

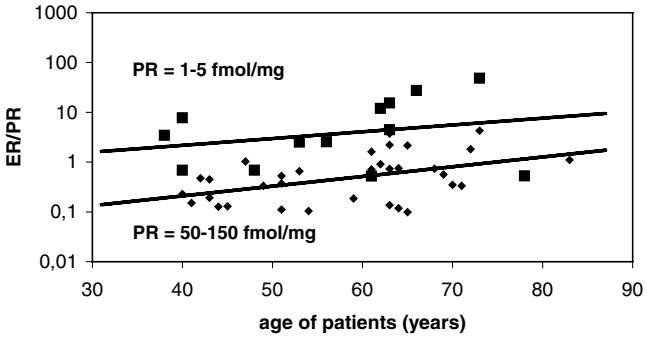
The examinations of excisions were performed in the Research Institute for Organic Syntheses Pardubice – Rybitvi in the years 1993 – 1994. After homogenisation of the tissue in the apparatus Ultraturax (IKA Werke) and after ultracentrifugation in the evacuated and cooled centrifuge VAC 600 (Janetzki) the radio-receptor analysis was performed using the ^{3}H estradiol, progesterone analog ^{3}H ORG.2058 (Amersham) and coated coal.

We used the average association constant from the high concentrations of ER and PR to improve the tangent of the Scatchard line for calculation of the concentration of receptors in the region of their low values where it is not possible to calculate this tangent correctly by the classical mode. In this way we lowered the possibility of false zero results.

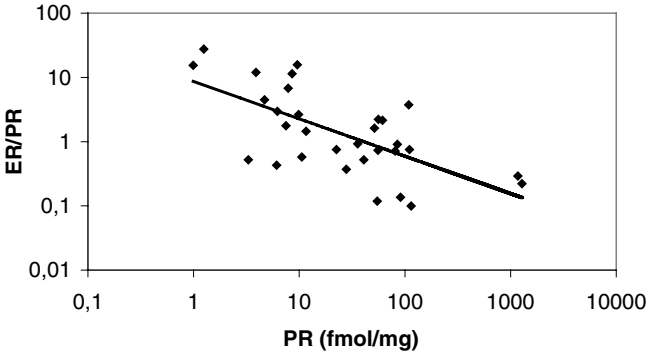
Now we sorted the statistical set into different groups that differ in the limited interval of PR values (PR = "zero", 1-7, 7-15, 15-50, 50-150, more than 150 fmol/mg). The age changes of the ER values and ER/PR quotients were investigated within these groups. On the contrary, the dependence of the ER/PR quotient on PR was analysed within the groups of the approximately same age. If some PR or ER value was smaller than 1 fmol/mg, such tumor was not used for calculation of the ER/PR quotient.

Results

The relative number of patients with PR 10 and more fmol/mg suggests the mild age decrease of PR (younger than 50



Graph 3: Dependence of ER/PR on the age (PR 5-50 fmol/mg omited)



Graph 4: Dependence of ER/PR on PR (61 – 67 years)

years = 68.6 % and older = 58.9 %). It is concordant with the first group of citations about this problem in the Introduction – with the works in which this mode of qualitative evaluation of each patient as PR(+) or PR(-) prevailed.

But (despite the success of this simplified clinical method of evaluation) at quantitative evaluation of each patient (instead of (+) and (-)), no age decrease of the line inserted through PR values of the whole statistical set was found (graph 1). It is in the concordance with the second group of these citations.

Nevertheless, there was found approximately the 10-fould age-increase of the values ER and ER/PR quotients (graphs No. 2 and 3).

Maximal differences of ER and ER/PR among the patients of the same age are more than 100-fould. This is why the correlation coefficients of the straight lines inserted after logarithmic calculation of the values of axis y was small in these graphs (0.35 for the age increase of ER/PR of all patients – without sorting into PR-limited groups).

If we evaluate only the patients with the nearly identical PR values, the age increase of ER and ER/PR is much better observable in these PR-limited groups than in the whole non-sorted statistical set – thanks to the smaller distances of ER and ER/PR from the inserted line (graph 3). The correlation coefficient of the age increase of ER/PR for the PR-limited group of PR = 15 – 50 fmol/mg is 0.61. And it increases even more at "thinning" of this PR-limited group (PR = 30 – 50 fmol/mg) despite the smaller number of patients in this last cited group. Even the most favourable correlation coefficient for the theoretical PR-limited group with zero differences among PR may be estimated from this correlation increase.

The whole extent of the interpersonal variability of the ER/PR quotient in the non-sorted statistic set (graph 3) is not greater than the extent of the variability of ER or PR (graph 1 and 2). The relative error of ER/PR is therefore not the summation of the relative error of ER with relative error of PR. It speaks about the good potency of the ER/PR quotient to make the corrections of the interpersonal variability of the percentage of the receptor negative tissue (such as the variability of the stroma).

Nevertheless, this band of all ER/PR values (non-sorted into the PR-limited groups) in the graph 3 is not thinner than the bands of the points in the graphs 1 and 2. It can be explained by the fact that the ER/PR quotient makes the corrections of the variability – only in the percentage of receptor's positive cancerous cells (in the connection to the percentage of the negative cancerous cells and stroma).

On the contrary, in case of the intracellular concentrations of both receptors in the receptor positive cells – the making of corrections of the interpersonal variability has not been performed yet (e.g. by transformation to the median intracellular concentrations). These two different causes of the interpersonal variability (the uncorrected variability of the intracellular concentrations – and the corrected (by ER/PR ratio) variability of the amount of positive and negative cells and stroma) might be approximately of the similar extent.

When we lowered the variability of PR by creation of the PR-limited groups the variability of ER and ER/PR also decreased. In addition to the above cited elevation of the correlation coefficients, it is possible to quantify that each PR-limited group in the graph 3 is then more than 10 times thinner than the whole unsorted statistical set. Furthermore, the "residual" interpersonal variability of ER and ER/PR in the PR-limited groups might be also quantified.

Nevertheless, we cannot say that this residuum is the variability of only e.g. the intracellular concentrations of ER. This part of the variability of ER is smaller. It is explainable as follows: The tumors with the small percentage of PR(+) cells and high intracellular concentration of PR can be in the same cytosol PR-limited group as the tumors with the high percentage of PR(+) cells and low intracellular concentration of PR. The same mathematical principle concerns also ER.

From the graph 3 is also evident that ER/PR is more than 10 times higher in case of the lowest PR than in the highest PR (statistically significantly). The high PR-limited group is below the low PR-limited group because PR is in the denominator.

Therefore, when we evaluated the groups formed (on the contrary) on the basis of the same age – the ER/PR quotient

decreased with the increase of PR (graph 4). It is important from the statistical point of view that similar statistically significant decrease was observed in six different age groups despite the fact that their graphs were not so representative as in the average age group with the highest number of patients.

The graph 3 shows also that there is more than one type of dependence between ER and PR. The other type of dependence prevails over the (in the Introduction explained) direct proportion because the inserted lines are not identical. Also the graph 4 proves that the direct proportion does not prevail between ER and PR because the inserted line of this graph is not horizontal.

It is the proof of simultaneous influence of the other principles than the amount of e.g. the stroma. There are the saturation dependences of regulation mechanisms of the intracellular concentrations of receptors. These dependences (other than proportion) summate with the direct proportion between ER and PR caused e.g. by the influence of the stroma that is explained in the Introduction.

Discussion

1. Necessity of performing of corrections of the ER/PR quotient

It would be advantageous for some investigations when the median of the (receptor negative tissue eliminating) ER/PR quotient would be the same in all PR-limited groups and independent on the age. It would be a strong simplification, similarly to the identical boundary of ER positivity (10 fmol/mg) used for all patients with no respect to the age and amount of the stroma. It would be advantageous if e.g. tumors with the genetically lowered ability to form PR would be over the median of all ER/PR values, and the patients with the elevated level of estrogens (that can depress the production of ER) below this median.

Such simplified understanding of these problems can arise from the assumption that these genetic influences are quantitatively much more significant than the complicating factors (as e.g. the influence of age). The 10-fould age changes of ER/PR (observable in our graph 3) are than the first cause of the non-usability of this quotient without performing of its corrections.

These age changes are explained predominantly as the changes of the intracellular concentration of ER [20, 21, 22]. The cause of these changes might be found in the age changes of the concentration of hormones because it was proven not only in vivo – as the low levels of ER in the breast cancers of the young women in comparison with the old ones [e.g. 23] but also in the cell cultures where the exposition to the estradiol caused the reduction of the ER amount [24]. The percentage of ER(+) epithelial and stromal cells in the mammary gland of the ovarectomised mice decreased significantly 24 hours after the estrogen injection and the 2.5-fould increase of the progesterone receptors (PR) in the cytosol was observed [10]. This explains the suitability of performing of mathematical corrections of the receptors' age changes (the main principle).

Nevertheless, even the elimination of the age changes of the ER/PR quotient by its transformation onto the average age (or by plotting of our graph 3 and insertion of the line – without sorting to PR-limited groups) is insufficient. It is impossible to state one boundary value – e.g. the median of the age-transformed ER/PR values (or only one "common" inserted boundary line in the graph 3) that could divide the whole statistical set to the patients with their age-corrected ER/PR quotient either great, or small.

Such mode of searching of e.g. the patients with the small binding capacity of ER (that is compensated by the elevated production of ER without elevation of production of PR) would give the incorrect results. In case of the small amount of the stroma or a great percentage of receptor positive cells – such patients – with such ER mutation can be also in the highest PR-specific group but it is impossible to find them by this "global" manner. Nearly all tumors with minimal PR expression will be above them – including those that have not the elevated intracellular ER concentration (searched by such screening).

For explaining of this suggestion, it is necessary to stress that the group of tumors with the high PR is 10 times lower than the group with the low PR in the graph 3 (instead of being scattered within the same – or less differing level). The tumors with the high ER/PR from the group PR 50 – 150 fmol/mg are not in the upper edge of the whole non-sorted statistical set but in its centre (because the majority of tumors of this group is in the lower half of the graph 3).

Therefore, it is necessary to take into account the dissimilarity of the values of ER/PR within different PR-limited groups. It is necessary at the use of this quotient for evaluation of not only the above cited genetic defect of ER but also of e.g. the influence of some factors e.g. on ER (acting on ER similarly to the age changes).

Another lines (between the lines of the graph 3) might also be constructed for the age dependence of the most probable (= adequate) ER/PR values – for any individual value PR (for the median of any other – newly created PR-limited group). Therefore, each tumor can acquire its own line in this manner (derived from PR of this tumor) in order to provide information, about the distance of this tumor in the vertical direction from this line of the ER/PR values (that are most normal in different age for the judged PR value).

Only when the ER/PR of the judged tumor is compared with this "its own" PR-age line (for "its own" PR) it might be determined whether ER/PR of this tumor is higher than adequate (= "above-adequate") from the point of PR's view (above this theoretical line) or whether it is quite the contrary – i.e. "below-adequate".

It is also possible to derive the scatter for each such PR-age line of ER/PR "of one evaluated patient" from the scatter of the values in the graph 3 (and the boundaries for the extreme values). Thus, we can answer questions such as "whether the excess of ER over PR is too excessive in case of some

tumor". Analogically, it is possible to search the excessive or insufficient PR in the (on the contrary) ER-limited groups – but only in the range of the relevant age group (as in the graph 4) or after age transformation of ER.

2. Analysis of the influence of hormones on the concentration of ER and PR

The differences of ER/PR among the patients within the PR-limited groups are approximately 10 times smaller than in the non-sorted statistic set (graph 3). Therefore, we can say that the use of PR-limited groups is an advantageous tool for analysis of the age's influence on ER/PR because we have proven the improvement of the correlation coefficients in comparison with the non-sorted statistical set.

Theoretically (in case of the greater statistical sets), it is possible to elevate the correlation coefficients more and more by the additional thinning of PR-limited groups. It is even possible to create the theoretical (ideal) PR-limited groups with the zero differences among PR of their tumors. The relative differences of ER/PR among the patients must be the same as the relative differences of only ER in such theoretical group because PR is constant. It concerns the intra-group variability as well as age changes where the relative differences of ER are again the same as in case of ER/PR. This is why it is sometimes more advantageous to investigate the age changes of ER instead of ER/PR in any such ideal group because it is simpler.

Nevertheless, it is necessary to take into account also the results of those authors that found the age decrease of PR (not only the age increase of ER). They might have used different timing of the operation with respect to the menstruation cycle. For instance, based on the conclusions of Pujol et al [6] we may deduce that if (in case of some author) the premenopausal patients are operated on the day ovulation and slightly before (when PR is the highest) the age decrease of PR can be the more significant than in case of authors that performed the surgery one week before the ovulation.

In such case the judgement of the ER/PR quotient is more sensitive than investigation of ER only (for investigation of the age influence of ovarian estrogens on these steroid receptors). It is caused by the fact that the numerator changes with the age in the opposite direction than the denominator.

Nevertheless, in such case, the sorting of the statistical set into groups would have to be performed in the other manner. For instance the highest PR-limited group must not include the tumors with the same PR (from the whole statistical set) but the tumors that have the highest PR in their age group (e.g. 20 % patients with the highest PR from each age group) – because the highest PR is lower in the old patients than the highest PR in the young ones in case of such works.

The ER/PR quotient together with creation of such flexible PR-limited groups could probably be a convenient and very sensitive tool also for the analysis of the ovarian cycle's influence on the steroid receptors in the breast tumors. Not only ER but also PR changes according to the time – even during the first half of the menstrual cycle [e.g. 6] when e.g. the operations of our patients were performed.

In such case (analogically as at the age decrease of PR) e.g. the tumors with the highest PR from the early postmenstruation phase should be included in the same PR-limited group as the highest PR tumors from ovulation phase. Creation of the rigid statistical subgroups with the same PR (with no regard to the influence of estrogens and progesterone on PR) would be incorrect because it would artificially connect the statistical groups of tumors of the non-identical types of the hormonal dependence.

In this manner we might therefore contribute to the solution of the question whether and how it is suitable to transform the receptor results to the average age or to the optimal phase of the ovulation cycle. The prognostic studies based on these transformations could modify the decision about the antiestrogen therapy.

3. Other works on the ER/PR ratio

Majority of authors that published the information about the mutual ratio of ER and PR did not use the quantitative evaluation but the qualitative one. Therefore, it concerned e.g. the prognosis of ER(+), PR(+) patients in comparison with the ER(+), PR(-) or with ER(-), PR(+) or with the ER(-), PR(-) patients. For instance Hurlimann et al [3] found that in case of PR(-) the prognosis is bad not only in case of ER(-) but also ER(+). (Most frequently the value 10 fmol/mg is used as the boundary between (+) and (-) for both ER and PR.)

Nevertheless, it is not possible to use these publications for solving the question – whether the prognosis of e.g. the patient with ER = 11, PR = 9 fmol/mg differs from ER 100, PR 5 fmol/mg. In addition to this difference within the ER(+), PR(-) group there is a question of a prognostic similarity of ER(+), PR(-) with ER(++), PR(+) (in case when the difference between such two tumors is caused only e.g. by the difference in the amount of the stroma).

That is why not only we [19] but also other authors [25, 14, 26] have documented the basic information for the study of the possibility to exploit the numerical ratio PR and ER in the breast tumors. In addition to the fact that ER/PR quotient increases (PR/ER decreases) with the age (due to the increase of ER) it was documented in some of these works that the band of ER/PR values is approximately of the same width (in the graph of the dependence on the age) as the band of ER values only or as the band of PR values only [19, 14]. Nevertheless, the sorting to the PR-limited groups was neither used in these two works nor in the works of Strnad et al [26] and Ashba a Traish [25], which are described in the following paragraphs.

Ashba and Traish [25] used the qualitative comparison (+) and (-) as well as the quantitative one – numerical values in fmol/mg. Nevertheless, they report the medians of the ER/PR (PR/ER) quotient in different age without the evaluation of the whole-group scatter. They explain the importance of the ER/PR quotient in their Discussion on the examples ER(+) or

(-) and PR(+) or (-). As an example of the probable ER(+), PR(-) cause they use the ER mutation that is able to bind the hormone but not to transfer the signal onto DNA. Nevertheless, we suppose that apart from this qualitative approach might be suitable to respect also the quantitative tendencies – not only (+) and (-). However, in concordance with these authors we presume that the same ER/PR quotient values are often of different prognostic importance in PR(+) tumors and in PR(-) tumors. This is why we suppose that the boundaries of the PR-limited groups for stratification should be stated on basis of these old experiences.

Strnad et al [26] studied the ER/PR quotient in benign lesions of the breast (instead of the cancers). Also they used the numerical comparison in fmol/mg as well as the qualitative one ((+) and (-)). On the basis of their results with the praecarcinoses they report that the prevalence or trend to the prevalence of ER over PR is an important marker of the imminent cancerogenesis. They also cite e.g. the work of Khan et al [27] in this context. They suggest that it is possible to consider the high amount of ER in the benign breast lesion as a sign of elevated sensitivity of this target tissue towards the circulating estrogens. Moreover, they also assume that suitable hormonal therapy used for the suppression of the breast lesion proliferation may be applied on the basis of the hormonal receptor's prevalence.

This is the approach – that enables to respect the quantitative differences e.g. among different ER(+), PR(+) patients. Furthermore, these authors [26] point out predominantly the influence of the ovulation cycle and the influence of the age on ER/PR similarly to Ashba and Traish [25].

Additionally, we currently suppose (in contrast to these two works [25 and 26]) that at the judgement of the ER/PR quotient the corrections (adjusting) for the diagnostic purposes should be performed with respect to the question – whether it concerns the tumor from the high or on the contrary middle or low PR-limited group. We have shown the statistical proofs for it predominantly in the graph 3.

Based on our preliminary studies we suppose that it will be suitable to use the additional stratification – e.g. according to the differences in TNM or in the therapy or in the hormonal concentrations, etc. for statistically significant proof of the influence of ER/PR ratio on the length of the patient's survival. The reason is that the survival is a result of many other factors that elevate the scatter of the statistical set.

On the contrary, we assume the greater statistical significance in case of the use of the ER/PR quotient for the analysis of the hormonal influences because it concerns the dependences that assert themselves "immediately" – during one day before biopsy [10]. The very similar possibility of elevation of the diagnosis accuracy was proven in the (similarly hormonally dependent) tumors of the other organ. In case of the adenocarcinoma of the uterine cervix the significant differences of PR level and of the PR/ER quotient were found if the patients were stratified according to the estradiol concentrations [28].

4. Ratios of ER isotypes α/β and PR isotypes A/B

In comparison with the literature concerning the ER/PR ratio, we have found more works describing the dependence of the prognosis and therapy on the mutual ratio of different ER isotypes (ERα / ERβ) or on the ratio of different PR isotypes (PRA / PRB). It concerns not only the breast cancer but also the endometrial cancer.

The expression of ERα correlated positively with all biological parameters of the good prognostic profile of the breast tumors. On the contrary, the expression of ERβ can cause the false positive interpretations if there are only results of the whole ER at disposal [29].

The over-expression of PRA in the cells of the breast tumor can be connected with the inhibition of the progestin effect and with the bad prognosis [30]. In case of the cancer of the endometrium – it is possible to cite that e.g. the decrease of PRB (= again the relative elevation of PRA influence) can predict that the endometrial tumors are badly differentiated and that they do not react to the progestin therapy [31].

We suppose that also in case of these ratios (of different isotypes of receptors for only one hormone) it is suitable to solve the possibility of performing of corrections that result from our mathematical approach. It means predominantly the sorting of tumors to the statistical groups on the basis of such isotype of the receptor that does not change according to the age or ovarian cycle.

For instance Cheng et al [32] found in the breast gland of the monkeys (macaques) that the excess of estradiol during the ovarian cycle and the cells' entrance into the cell cycle lowers only the expression of the ERα isoform and that the proliferating cells produce PR of only B isotype. It is therefore possible to deduce that in the time interval between menstruation and ovulation it would be suitable to create the ERβ-limited groups for investigation of the ERα / ERβ quotient (and on the contrary PRA-limited groups for investigation of the PRA / PRB quotient).

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