Estradiol measurement in translational studies of breast cancer

Per Eystein Lønning*

Institute of Clinical Medicine, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway
Department of Oncology, Haukeland University Hospital, Bergen, Norway

Abstract

Plasma estrogen measurement with use of radioimmunoassays has been instrumental in the development of aromatase inhibitors for endocrine therapy of postmenopausal breast cancer. However, due to low plasma estrogen concentrations in postmenopausal women, direct radioimmunoassays lack the sensitivity required. While certain laboratories have developed highly sensitive assays for research purposes revealing plasma estrogen suppression consistent with results from tracer studies, such assays are time and labor-consuming due to need for pre-analytical chromatographic purification, sample concentration and sometimes conversion of precursors to products. While novel chromatographic methods involving mass spectrometry analysis are likely to replace such radioimmunoassays in the future, so far a limited number of laboratories have developed suitable assays with a detection limit (around 1 pM) that is required for analyzing plasma estrogen levels in patients during treatment with potent aromatase inhibitors.

© 2014 The Author. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

Contents

1. Introduction .......................................................... 26
2. Tracer studies .......................................................... 27
3. Plasma estrogen measurements in relation to treatment with aromatase inhibitors ........................................ 27
4. Tissue estrogen levels .................................................. 28
5. Discussion .............................................................. 29
6. Acknowledgments ...................................................... 30
7. References ............................................................ 30

1. Introduction

While the history of endocrine therapy in breast cancer started more than a century ago, the scientific rationale arose with the discovery of [1] and confirmation of the predictive value of [2] the estrogen receptor (today known as ERα). In parallel, introduction of what was known at that time as an adrenotoxic antiepileptic, aminoglutethimide, in an attempt to achieve a "medical adrenalectomy" [3], indirectly led to introduction of aromatase inhibition as a successful endocrine therapy for postmenopausal women. While clinically efficient [4], subsequent studies by the Hershey group revealed sustained androgen production, despite estrogen suppression [5], a question solved when tracer studies [6] revealed that aminoglutethimide directly inhibited peripheral aromatization of androstenedione into estrone (E1). The discovery that aminoglutethimide executed its anti-tumor efficacy through aromatase inhibition had profound influence on subsequent development of endocrine therapy for breast cancer. While effective, aminoglutethimide treatment was associated with substantial side effects [7], for which reason much efforts were placed on developing novel, less toxic agents (see Ref. [8]). Importantly, the successful implementation of aromatase inhibition as a major endocrine for breast cancer, from which more than a million breast cancer women around the world currently are benefitting, had not been possible without these careful translational studies applying sensitive radioimmunoassays, in concert with tracer studies, to define the
pharmacological mechanisms of action of aminoglutethimide and, aromatase inhibition as a useful endocrine treatment option in breast cancer.

2. Tracer studies

While tracer studies and plasma estrogen assessment in concert confirmed aromatase inhibition to act as a potent aromatase inhibitor in vivo [9], plasma estrogen radioimmunoassays lacked the sensitivity to detect estrogen suppression >90%, which was what to expect based on the tracer results. Thus, direct assessment of in vivo aromatase inhibition by tracer studies were considered to be the “gold standard”. With new aromatase inhibitor compounds in development, in collaboration with Professor Mitch Dowsett and his team, we initiated a program for in vivo assessment of aromatase inhibition. Using an HPLC method to separate estrogen metabolites [10], we developed an assay allowing assessment of in vivo aromatase inhibition with an average detection limit >99.1% [11–18]. The results are depicted in Table 1; in brief, while most compounds (the so-called 1st and 2nd generation compounds) caused in vivo aromatase inhibition <90%, the three 3rd generation compounds; the steroidal inhibitor exemestane, as well as the non-steroidal compounds anastrozole and letrozole, each caused on average >98% aromatase inhibition. Most importantly; these endocrine results were paralleled by clinical findings; while the 1st and 2nd generation compounds in general revealed clinical efficacy similar to tamoxifen [8], the three 3rd generation compounds revealed superiority, also with respect to clinical efficacy, and are today used as routine endocrine therapy for postmenopausal women in the adjuvant setting [19].

3. Plasma estrogen measurements in relation to treatment with aromatase inhibitors

The results from in vivo tracer studies, in concert with the results from large randomized studies, underline the imperative of maximal aromatase inhibition for optimal clinical efficacy. Thus, while randomized studies revealed superiority for third-generation aromatase inhibitors as compared to tamoxifen [19], in contrast first- and second-generation compounds were found of similar efficacy, but not superior, as compared to conventional therapy [8,20]. Tracer studies however are laborious and expensive to conduct and may be applied to small patient groups only. Thus, there is a need for simpler methods, like plasma estrogen measurement.

A key problem relates to low plasma estrogen levels in postmenopausal women, in particular when on aromatase inhibitor therapy. Taking into account plasma levels of estradiol (E2), E1 and estrone sulfate (E1S) to be in the 15–20 pM, 70–80 pM and 4–500 pM ranges, respectively [21], the assays need sensitivity limits of a few pM to detect potential suppression >98%. Developing highly sensitive radioimmunoassays with a detection limit of about 1 pg/ml (3.7 pM), the Herhsey group revealed significant differences with respect to plasma estrogen suppression between the second-generation aromatase inhibitor CGS16949A [22] and letrozole [23]. Notably, as for both studies the difference in plasma estrogen suppression was corroborated by similar findings with respect to suppression of urinary estrogen secretion.

A problem related to use of 3H-labelled standards in radioimmunoassays relates to the limited specific activity of these standards (in the 50–160 mCi/mmol range). A higher specific activity may be achieved with use of 125I-labelled compounds (specific activity in the 2000 Ci/mmol range). The first sensitive 125I-based RIA for E2 measurement in patients treated with aromatase inhibitors was developed by Professor Mitch Dowsett at the Royal Marsden Hospital [24] and subsequently used to measure plasma E2 suppression with different aromatase inhibitors [25–30]. Some years later, learning this assay for E2 measurement, we used the same 125I-E2 standard and E2 antibody developing a highly sensitive assay for plasma E1S measurement. The procedure involved taking the samples through multiple purification steps (Fig. 1), hydrolysis and finally conversion of unconjugated E1 into E2 [31].

Taking this approach further, we improved our assay, allowing E1 as well as E1S to be converted into E2, each steroid to be measured with the same 125I-E2 assay (Table 2). The approach involved adding minor amounts of 3H-E2 as well as 3H-E1 and 3H-E1S for recovery standard including correction of the final results. As for this assay, we achieved a detection limit of 0.67 pM for E2, 1.14 pM for E1S, and 0.55 pM for E1S [32], a significant improvement as compared to our earlier 3H-based methods [33]. Applying this assay to patients on treatment with letrozole versus exemestane [18], we recorded a mean suppression of plasma E2 of 92.8% versus 95.2%, for E1 96.3% versus 98.8%, and for E1S 95.3% versus 98.8%, respectively [34]. It should be noted however that, even with this sensitive assay, 5 out of 12 patients had plasma levels of E2 below detection limit during anastrozole treatment; corresponding figures for letrozole was as high as 11 out of 12 [34].

Another interesting approach was taken by Dr. Klein and her team who used an ultrasensitive recombinant cell bioassay to measure estrogen levels in patients on treatment with letrozole [35]; this approach is discussed in detail in another paper in this issue. A particular problem relates to plasma estrogen measurement for patients on steroidal aromatase inhibitors, such as exemestane.

Table 1

| Drug                  | Dose                                    | Mean inhibition | References |
|-----------------------|-----------------------------------------|-----------------|------------|
| **First/second generation compounds** |                                         |                 |            |
| Aminoglutethimide (AG) | 1000 mg daily                           | 90.6%           | [13]       |
| Rogletamide           | 400/800/1600 mg daily                    | 50.6%/63.5%/73.8% | [13]       |
| Fadrozole             | 2 mg/4 mg daily                         | 82.4/92.6%      | [11]       |
| Formestane            | 125 mg/250 mg daily                     | 62.3%/70.0%/57.3% | [14]       |
| Formestane            | 250 mg/500 mg i.m./2 w                  | 84.8%/91.9%     | [12]       |
| Formestane            | 500 mg i.m./w                           | 91.3%           | [15]       |
| Formestane + AG       | 500 mg i.m./w 1000 mg daily             | 94.2%           | [15]       |
| **Third-generation compounds** |                                         |                 |            |
| Exemestane            | 25 mg daily                             | 97.9%           | [17]       |
| Anastrozole           | 1 mg daily                              | 96.7%           | [16]       |
| Anastrozole           | 10 mg daily                             | 98.1%           | [16]       |
| Anastrozole           | 1 mg daily                              | 97.3%           | [18]       |
| Letrozole             | 2.5 mg daily                            | >99.1%          | [18]       |

* Formestane = 4-hydroxyandrostenedione.
** Administered as 125 mg b.i.d.
*** Administered as 250 mg once daily.
levels a magnitude higher as compared to plasma and E1
and E1 gradients; as averaged and E1 levels in estro-
levels and tumor tissue and E2 levels correlated even stronger to their corresponding
by as well as E1 and E1 are converted into E1 as well as E1
overweight as compared to normal-weight individuals [41].
high BMI despite a similar degree of aromatase inhibition among
[40,41] revealed slightly higher plasma estrogen levels related to
plasma estrogen levels with these sensitive radioimmunoassay’s
inhibition for overweight/obese patients [37–39]; analyzing
index (BMI). Conflicting data have challenged efficacy of aromatase
the same studies have been analyzed with respect to body mass
[32].

Mean levels (ML), detection limits and isotope used for the radioimmuno-assays (125I or 3H) with respect to different methods used at different time periods for measurement of postmenopausal estrogen levels in relation to aromatase inhibitor therapies in our laboratory.

| ML  | E2  | 15 pM | 70 pM | 400 pM | References |
|-----|-----|-------|-------|--------|------------|
| 125I: 1.3 pM 8.7% 3H: 5.3 pM 7.6% 125I: 36.1 pM 9.0% | 31 |
| 125I: 2.6 pM 0.7% | 30 |
| 125I: 0.67 pM 4.4% 125I: 1.14 pM 1.6% 125I: 0.55 pM 0.14% | 32 |

Mean levels (ML) = consistent with expect based on steroid disposition [54,55]. The 125I radioimmunoassay was modified from original version by Dowsett and colleagues [24].

Due to potential interacting metabolites, samples collected from patients on treatment with such compounds need pre-purification with use of HPLC before radio immunoassaying [36].

Apart from differentiating between first/second generation aromatase inhibitors on the one hand as compared to the highly potent third-generation compounds on the other side, plasma estrogen measurements have been able to discriminate also between highly potent third-generation compounds like anastrozole and letrozole. Thus, two independent studies, both applying a cross-over design, have confirmed letrozole to be a more portent estrogen measurements have been able to discriminate also
plasma estrogen concentrations [21,48]. These findings are in agreement with the results from Professor Millers group revealing the bulk of tumor tissue estrogens to have a plasma origin [49] and the findings of Dunbier et al., reporting a strong correlation between postmenopausal plasma E2 levels and tumor tissue expression of estrogen-regulated genes [50].

Based on these findings, we proposed a new hypothesis, explaining tissue to plasma hormone gradients based on physical-chemical properties for each individual compound [51]. Considering unconjugated E2 and E1, these compounds are highly lipophilic, explaining a high tissue to plasma concentration gradient. In contrast, E1S is a water-soluble conjugate. While the concentration of plasma E1S exceeds the concentration of circulating E1 and E2 by an average factor of 8 and 40, respectively [21], this is due to the fact that most unconjugated E2 and E1 are converted into E1S which, on the other hand, has a plasma clearance rate of only about 10% the clearance rate of the unconjugated estrogens [52,53]. Our hypothesis does not exclude local estrogen production; nor is it inconsistent

**4. Tissue estrogen levels**

Much interest has focused on issue estrogen levels since van Landeghem [42] and others three decades ago reported breast cancer tissue E2 levels a magnitude higher as compared to plasma levels in postmenopausal women. Thus, issues have been raised with respect to local estrogen synthesis by aromatization [43] as well as de-conjugation of E1S [44]. Using our sensitive radioimmunoassay’s on tissue samples following HPLC purification (Fig. 2), we were able to detect tissue levels of E2 as well as E1 and E1S with high degree of sensitivity [45]. Studying tumor tissue samples collected before and during treatment with anastrozole or letrozole [34,46], we confirmed effective tissue estrogen suppression with no evidence of “escape” for any single tumor. Further, studying tissue estrogens across benign and malignant breast tissue [21], we confirmed elevated tissue to plasma E2 as well as E1 gradients; as for benign tissue, the tissue to plasma ratio for E2 and E1 averaged about 2 and 5, respectively. As for E1S, however, we found a tissue to plasma gradient averaging 0.1 only, contrasting previous findings obtained by others with use of a direct radioimmunoassay [47]. Interestingly, we confirmed elevated tumor E2 levels in estrogen receptor positive but not in estrogen receptor negative tumors; these elevated levels were found positively correlated to transcriptional levels of the estrogen receptor as well as the reductive 17 hydroxy steroid dehydrogenase B7, but negatively correlated to the oxidative B2 and B12 dehydrogenases [48]. However, tissue E2 and E1 levels correlated even stronger to their corresponding plasma estrogen concentrations [21,48]. These findings are in agreement with the results from Professor Millers group revealing the bulk of tumor tissue estrogens to have a plasma origin [49] and the findings of Dunbier et al., reporting a strong correlation between postmenopausal plasma E2 levels and tumor tissue expression of estrogen-regulated genes [50].
with the finding that estrogen receptor expression as well as dehydrogenase activity to some degree may influence the ratio between E2 and E1 in breast cancer tissue. However, the finding of similar tissue to plasma estrogen gradients in pre- and postmenopausal women despite substantial differences with respect to plasma estrogen levels between these groups indicate tissue to plasma equilibrium to be a rapid event [51]. Thus, local synthesis would be of minor importance to local estrogen concentration, in as much as estrogens synthesized locally would be quickly buffered by the circulating pool of hormones.

5. Discussion

Radioimmunoassay of plasma and tissue estrogen levels, in concert with tracer studies, has contributed significantly to our understanding of estrogen disposition. This has been mandatory to development of aromatase inhibition, currently the major endocrine treatment option for postmenopausal breast cancer patients. In contrast, there have been many publications reporting plasma estrogen levels much higher than should be assumed based on theoretical calculations [54,55] and the need for caution with respect to critically interpreting the results in the literature cannot be overemphasized [55]. Further; recent studies applying radioimmunoassays have contributed to our understanding of estrogen disposition in general, including important topics like explaining elevated tissue to plasma estrogen levels.

Measurement of plasma estrogens in postmenopausal women, due to their low levels, are time- and labor-dependent methods, involving multiple steps such as chromatographic separation and conversion [55]. The topic becomes complicated in particular when measuring estrogen levels in patients on treatment with aromatase inhibitors. For example, patients on treatment with letrozole, the most potent aromatase inhibitor currently in clinical use, will frequently have estradiol levels below the level of detectability. In contrast, as estrone–sulfate circulates at much higher levels, this steroid is usually in the detectable range in women receiving aromatase inhibitor therapy. Due to its high plasma level, E1S has been considered a potential source for tissue estrogens through uptake and hydrolysis [56]. While E1S may easily be hydrolyzed into unconjugated hormones, its potential contribution to tissue estrogen levels, based on arguments raised above, may be questioned. Whether plasma E1S may be a significant contributor to tissue E2 or not, plasma E1S may be a good proxy parameter for estrogen suppression with potent aromatase inhibitors as it exist at equilibrium with unconjugated E1 and E2 [21]. When considering tissue estrogen measurements, this becomes even more demanding, in general and requires pre-purification of samples by use of HPLC.

Taken together, while reliable radioimmunoassays are available and have played an important role in translational research, such methods are too time- and labor-demanding to be functional for routine purposes. While different liquid and gas chromatographic methods over the years gradually have improved and should be expected to replace use of radioimmunoassays for research and,
in particular, routine analysis in the future, it should be emphasized that, at this stage, there are only a few laboratories around the world with methods documented to have a detection limit allowing plasma estrogen assessment in patients on treatment with aromatase inhibitors. While such methods are expected to continuously improve, notably, they should be subject to the same strict criteria documenting sensitivity and specificity in the low concentration range with reproducibility similar to what has been shown with respect to the most sensitive radioimmunoassay’s [55].

Acknowledgments

The author wish to thank key members of my team who over many years participated in our studies cited in this paper; Mr. DagfinnEkse, Dr. Junger Geisler, Mr. Nhat Duong and Mrs. Hildegunn Helle who, sadly, passed away by December 2013, at an age of 42, due to incurable cancer.

References

[1] Jensen EV, DeSombre ER, Jungblut PL. Estrogen receptors in hormone-responsive tissues and tumors. In: Wissler RW, Dao TL, Wood Jr S, editors. Endogenous factors influencing host-tumor balance. University of Chicago Press; 1967. p. 17-30.
[2] McGuire WL. Steroid receptors in human breast cancer. Cancer Res 1978;38:4289-91.
[3] Cash R, Brough AJ, Cohen MNP, Satoeh PS. Aminoglutethimide (Eleipten-Ciba) is an inhibitor of adrenal androgens; mechanism of action and therapeutic trial. J Endocrinol Metab 1967;27:1239-48.
[4] Santen RJ, Lipton A, Kendall J. Successful medical adrenalectomy with aminoglutethimide. JAMA 1974;230:1661-5.
[5] Samojobik E, Veldhuis JD, Weils SA, Santen RJ. Preservation of androgen secretion due to estrogen suppression with aminoglutethimide in the treatment of metastatic breast carcinoma. J Clin Invest 1980;65:602-12.
[6] Santen RJ, Santner S, Davis B, Veldhuis J, Samojobik E, Ruby E. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast cancer. J Clin Endocrinol Metab 1978;47:1257-65.
[7] Santen RJ, Worgul TJ, Lipton A, Harvey H, Boucher A. Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann Intern Med 1982;96:94-101.
[8] Lønning PE. Aromatase inhibitors in breast cancer. End-Rel Cancer Intern Med 1982;96:94-101.
[9] Santen RJ, Santner SJ, Tilson-Mallett N, Rosen HR, Samojlik E, Veldhuis JD. In: Santen RJ, Santner SJ, Tilson-Mallett N, Rosen HR, Samojlik E, Veldhuis JD, editors. In: Santen RJ, Santner SJ, Tilson-Mallett N, Rosen HR, Samojlik E, Veldhuis JD, editors. In estrogen suppression with aminoglutethimide in postmenopausal women with breast cancer. J Clin Endocrinol Metab 1989;64:887-94.
[10] Santen RJ, Mehta A, King N, Smith IE, Powles TJ, Stein RC, Coombes RC. An endocrine and pharmacokinetic study of four oral doses of formestane in postmenopausal breast cancer patients. Eur J Cancer 1992;28:1671-6.
[11] Lønning PE. Aromatase inhibitors in breast cancer patients on treatment with aromatase inhibitors. J Steroid Biochem Mol Biol 1995;55:409-12.
[12] Geisler J, Ekse D, Helle H, Duong N, Lønning P. An optimised, highly sensitive radioimmunoassay for the simultaneous measurement of estrogen, estradiol and estrone sulphate in the ultra-low range in human plasma samples. J Steroid Biochem Mol Biol 2008;109:90-5.
[13] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Cameron DA, Graham JD, Goldhirsch A, et al. Tissue estrogen is selectively elevated in receptor positive breast cancers while tumour estrogen is reduced independent of receptor status. J Steroid Biochem Mol Biol 2007;105:31-41.
[14] Santen RJ, Lønning PE, Iveson TJ, Smith IE, Ahern J, Smithers DA, Trunet PF, Dowsett M. Phase I study of the oral non-steroidal aromatase inhibitor C20267 in postmenopausal patients with advanced breast cancer. Cancer Res 1993;53:266-70.
[15] Dixon JM, Renshaw L, Young O, Murray J, Macaskill EJ, McHugh M, Folkerd E, Cameron DA, A’Hern RP, Dowsett M. Letrozole suppresses estrogen and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. J Clin Oncol 2008;26:1671-6.
[16] Lønning PE. Estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over-designed study. J Clin Oncol 2002;20:751-7.
[17] Iveson TJ, Smith IE, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[18] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Cameron DA, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[19] Dowsett M, MacNeil F, Jacobs S, Lønning PE, Powles TJ, Stein RC, Coombes RC. Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route. Cancer Res 1987;47:1957-61.
[20] Dowsett M, Cunningham DC, Stein RC, Evans S, Dehennin L, Hedley A, Coombes RC. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. Cancer Res 1989;49:1306-12.
[21] Dowsett M, Stein RC, Mehta A, Coombes RC. Potency and selectivity of the non-steroidal aromatase inhibitor C20494A in postmenopausal breast cancer patients. Clin Endocrinol 1990;32:623-34.
[22] Dowsett M, MacNeil F, Mehta A, Newton C, Haynes B, Jones A, Jarman M, Lønning PE, Powles TJ, Duong N, Lønning P. Endocrine and clinical effects of exemestane (Femara) and anastrozole (Arimidex) on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients. Lancet 2010;375:1493-9.
[23] Demers LM, Lipton A, Harvey HA, Kambic KB, Grossberg H, Brady C, Santen RJ. The efficacy of C20267 in suppressing estrogen biosynthesis in patients with advanced stage breast cancer. J Steroid Biochem Mol Biol 1993;44:867-91.
[24] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Cameron DA, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[25] Demers LM, Mehta A, King N, Smith IE, Powles TJ, Stein RC, Coombes RC. An endocrine and pharmacokinetic study of four oral doses of formestane in postmenopausal breast cancer patients. Eur J Cancer 1992;28:415-20.
[26] Iveson TJ, Smith IE, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[27] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Fotis T, Anker GB, Hall R. Relations between sex hormones, sex hormone binding globulins, insulin-like growth factors, fasting and insulin-like growth factor binding protein-1 in post-menopausal breast cancer patients. J Steroid Biochem Mol Biol 2005;95:409-12.
[28] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Fotis T, Anker GB, Hall R. Relations between sex hormones, sex hormone binding globulins, insulin-like growth factors, fasting and insulin-like growth factor binding protein-1 in post-menopausal breast cancer patients. J Steroid Biochem Mol Biol 2005;95:409-12.
[29] Lønning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Cameron DA, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[30] Iveson TJ, Smith IE, A’Hern RP, Dowsett M. Letrozole suppression of breast cancer hormone receptors in patients treated with aromatase inhibitor, letrozole. Clin Endocrinol 1995;45:2365-9.
[43] Miller WR. Aromatase activity in breast tissue. J Steroid Biochem Mol Biol 1991;39:783–90.

[44] Santner SJ, Leszcynski D, Wright C, Manni A, Feil PD, Santen RJ. Estrone sulfate: a potential source of estradiol in human breast cancer tissue. Breast Cancer Res Treat 1986;7:35–44.

[45] Geisler J, Berntsen H, Lønning PE. A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol and estrone sulphate levels in breast cancer tissue. J Steroid Biochem Mol Biol 2000;72:259–64.

[46] Geisler J, Dette S, Berntsen H, Ottestad L, Lindtjorn B, Dowsett M, Lønning PE. Influence of neoadjuvant anastrozole (Arimidex) on intratumoral estrogen levels and proliferation markers in patients with locally advanced breast cancer. Clin Cancer Res 2001;7:1230–6.

[47] Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Mol Biol 2000;72:23–7.

[48] Haynes BP, Straume AH, Geisler J, A’Hern R, Helle H, Smith IE, Lønning PE, Dowsett M. Intratumoral estrogen disposition in breast cancer. Clin Cancer Res 2010;16:1790–801.

[49] Larionov AA, Berstein LM, Miller WR. Local uptake and synthesis of oestrone in normal and malignant postmenopausal breast tissues. J Steroid Biochem Mol Biol 2002;81:57–64.

[50] Dunbier AK, Anderson H, Ghazoui Z, Folkerd EJ, A’Hern R, Crowder RJ, Hoog J, Smith IE, Osin P, Nerurkar A, et al. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. J Clin Oncol 2010;28:1161–7.

[51] Lønning PE, Haynes BP, Straume AH, Dunbier A, Helle H, Knappskog S, Dowsett M. Exploring breast cancer estrogen disposition: the basis for endocrine manipulation. Clin Cancer Res 2011;17:4948–58.

[52] Lønning PE, Kvensland S, Thorsen T, Ueland PM. Alterations in the metabolism of oestrogens during treatment with aminoglutethimide in breast cancer patients: Preliminary findings. Clin Pharmacokinet 1987;13:393–406.

[53] Lønning PE, Johannessen DC, Thorsen T. Alterations in the production rate and the metabolism of oestrone and oestrone sulphate in breast cancer patients treated with aminoglutethimide. Br J Cancer 1989;60:107–11.

[54] Lønning PE, Geisler J, Johannessen DC, Elke D. Plasma estrogen suppression with aromatase inhibitors evaluated by a novel, sensitive assay for estrone sulphate. J Steroid Biochem Mol Biol 1997;61:255–60.

[55] Folkard E, Lønning PE, Dowsett M. Interpreting plasma estrogen levels in breast cancer: caution needed. J Clin Oncol 2014;32:1396–400.

[56] Santner SJ, Feil PD, Santen RJ. In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway. J Clin Endocrinol Metab 1984;59:29–33.