Effect of Aromatase Inhibitors on Lipid Metabolism, Inflammatory Response and Antioxidant Balance in Patients with Breast Carcinoma

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Abstract. *Background: Aromatase inhibitors may affect lipid metabolism, inflammatory response and antioxidant balance. Patients and Methods: One hundred and eighty-six post-menopausal patients with breast carcinoma underwent evaluation of parameters of lipid metabolism, inflammatory response and antioxidant balance immediately before as well as 2 and 4 months after the start of therapy with aromatase inhibitors. Results: A significant increase in total, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol, lipoprotein (a), retinol, C-reactive protein and fibrinogen was observed. The changes of serum lipid concentrations were restricted mostly to the patients pre-treated with tamoxifen who had significantly lower baseline levels of these parameters. Conclusion: An increase of serum cholesterol, lipoprotein (a), C-reactive protein and fibrinogen in patients treated with aromatase inhibitors is the result of tamoxifen withdrawal rather than a direct effect of therapy. No significant changes in serum lipids were observed in patients treated with aromatase inhibitors in the first-line setting.*

Breast carcinoma is the most common malignancy in women (1). The progress accomplished in the therapy of breast carcinoma has led to improved survival. In addition to early diagnosis, the improvement of survival of patients diagnosed with breast carcinoma patients is also the result of systemic treatment, including cytotoxic chemotherapy and hormonal therapy (2). Hormonal therapy has been shown to be effective in patients with estrogen and/or progesterone receptor-positive breast carcinoma in the setting of early breast carcinoma, as well as advanced or metastatic disease. Hormonal therapy represents the principal systemic treatment for many patients with breast carcinoma. For several decades, tamoxifen was the mainstay of hormonal therapy of breast carcinoma. More recently, data have accumulated demonstrating the efficacy of third-generation aromatase inhibitors in patients who failed to respond to tamoxifen, as well as benefit compared to tamoxifen in the first-line therapy of advanced disease or adjuvant therapy of early breast carcinoma (3-7).

The incidence of breast carcinoma increases with age. Partly because of improved survival, associated diseases emerge as important causes of death in patients with breast carcinoma. Prominent among comorbidities in patients with breast carcinoma are cardiovascular disorders (8). Cardiovascular disorders are the principal cause of death in the Western world, and, similarly to breast carcinoma, increase in incidence with age. Most cardiovascular disorders are caused by atherosclerosis. Some of the risk factors of atherosclerosis, *e.g.* elevated serum lipids or blood pressure, may be affected by anticancer therapy, including endocrine treatments. Menopause also accelerates atherosclerosis (9). In earlier studies, it was demonstrated that tamoxifen favorably affected parameters of cholesterol metabolism (10-15). On the other hand, the administration of aromatase inhibitors may have an adverse effect on serum lipids (16).

Administration of anticancer agents is associated with oxidative stress. Disorders of antioxidant balance are among the factors involved in the pathogenesis of the toxicity associated with radiotherapy or chemotherapy. Oxidative

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Key Words: Aromatase inhibitors, breast carcinoma, C-reactive protein, neopterin.

stress also plays an important role in atherosclerosis (17). The atherogenic potential of serum lipids is affected by liposoluble antioxidants (18). Antioxidant supplementation, *e.g.* administration of vitamin E, may retard the progression of atherosclerosis (19). Vitamin E represents the major antioxidant in the serum (20). The term vitamin E denotes several naturally occurring tocopherols and tocotrienols, but alpha-tocopherol is responsible for most vitamin E activity in animal tissues. Disorders of antioxidant balance involving vitamin E are also thought to be involved in the toxicity associated with radiotherapy (21), or chemotherapy (22). In earlier studies, a decrease in serum alpha-tocopherol was observed during systemic chemotherapy (23-26). Retinol is a major circulating form of vitamin A that also has antioxidant activity and plays an essential role in many physiological functions, including vision, growth, development, differentiation, and immune response (20).

Another molecule associated with oxidative stress is neopterin, an unconjugated pteridine synthesized from guanosine triphosphate (27). Human monocytes/macrophages produce significant quantities of neopterin when stimulated with interferon-γ, and increased concentrations of neopterin in serum or urine are considered to be an indicator of systemic immune activation (28). Increased urinary and serum neopterin concentrations have been reported in patients with different primary tumors, including metastatic breast carcinoma (27, 29, 30). Elevated neopterin concentrations were limited to patients with active disease (27). Increased urinary neopterin is also associated with poor prognosis in breast carcinoma (31). Increased neopterin concentrations were also described in patients with atherosclerosis (32-34). Systemic immune activation is closely associated with systemic inflammatory response. C-reactive protein is the parameter most widely used to assess systemic inflammatory response. Similarly to neopterin, C-reactive protein concentrations are increased in the serum of patients with atherosclerosis. Moreover, increased serum C-reactive protein levels predict the risk of future cardiovascular events (35-39).

The aim of the present study was to prospectively evaluate serum lipids, parameters of antioxidant balance and systemic immune and inflammatory activation in patients with breast carcinoma treated with third-generation aromatase inhibitors.

Patients and Methods

Patients. One hundred and eighty-six post-menopausal patients, aged (mean±standard deviation) 62±10 years, with histologically verified breast carcinoma were included in the present study. The patients were evaluated immediately before, 2 months and 4 months after the start of therapy with third-generation aromatase inhibitors. One hundred and thirty-two patients were treated with letrozol, 41 with anastrozol and 13 with exemestane. One hundred and thirteen patients were treated with aromatase inhibitors in the first-line setting, including 71 patients treated with systemic chemotherapy within the previous 6 months, 42 patients who had had no prior systemic treatment, and 73 patients who had had aromatase inhibitors after prior hormonal therapy with tamoxifen. The study was approved by the institutional Ethical Committee, and the patients provided their informed consent.

Measurement of serum lipids, parameters of glucose metabolism and proteins. Blood samples were drawn from a peripheral vein after an overnight fast. The samples were transferred immediately to the laboratory, centrifuged (1,600 × g, 10 minutes, 16˚C) and the serum was separated and analyzed immediately or frozen at –20˚C until analysis. Serum lipoproteins were separated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions using gradient ultracentrifugation in sodium chloride solutions (OPTIMA MAX-XP ultracentrifuge; Beckman Coulter, Fullerton, USA), and cholesterol, triglyceride and alpha-tocopherol contents of the fractions were analyzed using the same methods as described below for serum samples. Serum cholesterol, triglyceride, C-reactive protein, lipoprotein (a), glucose, total protein and albumin were determined on a MODULAR analyzer (Hoffmann-La Roche, Basel, Switzerland) using commercially available kits. Homocysteine concentration was determined immunochemically (Immulite 2000; Siemens Healthcare Diagnostics, Deerfield, USA). Glycosylated hemoglobin was determined by high-performance liquid chromatography using a Variant II Turbo System (Bio-Rad, Hercules, CA, USA) according the instruction of the manufacturer and expressed according the IFCC reference system (40).

Measurement of serum alpha-tocopherol and retinol. Serum alpha-tocopherol and retinol were determined before and during the therapy by high-performance liquid chromatography as described elsewhere (41). In the liquid-liquid extraction procedure, 500 μl of serum was deproteinized by cool ethanol denatured with 5% methanol (500 μl, 5 minutes, 4˚C). Subsequently, 2.5 ml of *n*-hexane were added to this mixture and extracted for 5 minutes by a vortex apparatus. After centrifugation (1,600 × g, 10 minutes, 0˚C), an aliquot (2 ml) of the clean extract was separated and evaporated under nitrogen (60˚C). The residue was dissolved in 400 μl methanol and analyzed by reversed-phase high-performance liquid chromatography using external standard calibration. The analyses were performed using a Perkin Elmer high-performance liquid chromatography setup (Norwalk, USA) comprising an LC 200 pump, LC 200 autosampler, LC column oven 101 thermostat and LC 235C diode array detector attached to a Perkin Elmer Turbochrom Chromatography Workstation version 4.1. Separation of alpha-tocopherol and retinol was performed using Chromolith Performance RP-18e, 100×4.6 mm monolithic columns (Merck, Darmstadt, Germany). As the mobile phase 100% methanol was used at a flow rate of 2.5 ml min^{-1} and column pressure of 3.3 MPa. A block heater LC oven 101 (Perkin Elmer, Norwalk, USA) was utilized to keep the analytical column temperature at 25˚C. The injection volume was 50 μl. The detection of alpha-tocopherol and retinol was carried out at 295 nm and at 325 nm, respectively.

Determination of urinary neopterin. Neopterin was determined by high-performance liquid chromatography (42). Briefly, after centrifugation (5 minutes, 1,300 ×g) and diluting 100 μl of urine specimens with 1.0 ml of mobile phase containing 2 g of disodium-EDTA per liter, samples were injected onto a column and high-performance liquid chromatography was performed using an

Table I. *Changes in the investigated parameters in the whole group (n=186).*

Parameter	Visit 1 (baseline)	Visit 2	*p*-Value compared to visit 1	Visit 3	*p*-Value compared to visit 1
Time (days)	0	64±12	-	126±15	-
Cholesterol					
Total (mmol/l)	5.83±1.16	5.98±1.15	0.005	6.08±1.16	0.0002
VLDL (mmol/l)	1.32±0.42	1.38±0.41	0.05	1.39±0.43	0.007
LDL (mmol/l)	2.97±0.80	3.12±0.72	0.02	3.18±0.79	0.001
HDL (mmol/l)	1.27±0.39	1.24±0.33	0.49	1.25±0.30	0.63
Triglycerides					
Total (mmol/l)	1.59±0.80	1.54±0.75	0.14	1.57±0.71	0.51
VLDL (mmol/l)	0.97±0.54	1.00±0.57	0.88	0.99±0.52	0.49
LDL (mmol/l)	0.45±0.13	0.42±0.13	0.0007	0.42±0.12	0.0004
HDL (mmol/l)	0.19±0.13	0.16±0.06	0.0002	0.17±0.13	0.003
Lipoprotein (a) (g/l)	0.42±0.46	0.44±0.49	0.002	0.44±0.44	0.001
Homocysteine (μmol/l)	12.4±4.2	12.7±4.9	0.21	12.2±4.7	0.81
Glucose (mmol/l)	5.6±1.7	5.6±1.6	0.09	5.6±1.7	0.19
Glycosylated hemoglobin (%)	3.9±1.0	3.9±0.9	0.47	4.0±1.0	0.11
Retinol (μmol/l)	1.64±0.41	1.71±0.42	0.0006	1.73±0.37	0.0001
Alpha-tocopherol					
Total (μmol/)	25.09±6.32	25.19±6.31	0.84	26.03±6.49	0.02
VLDL (μmol/l)	7.65±3.42	7.78±3.32	0.57	8.28±3.50	0.0009
LDL (μmol/l)	10.51±3.37	10.89±3.20	0.03	11.34±3.48	0.0003
HDL (μmol/l)	6.31±2.67	6.24±2.56	0.84	6.68±2.38	0.02
Alpha-tocopherol/ cholesterol ratio (10^{-3})	4.43±1.25	4.29±1.12	0.08	4.34±1.01	0.52
Urinary neopterin (μmol/mol creatinine)	190±80	193±78	0.89	199±90	0.74
C-reactive protein (mg/l)	4.5±7.6	4.5±5.6	0.14	4.9±6.7	0.004
Total protein (g/l)	73.2±4.9	74.2±5.0	0.0003	74.9±4.8	0.00001
Albumin (g/l)	44.2±2.5	44.3±2.8	0.29	44.7±2.8	0.10
Fibrinogen (g/l)	3.65±0.80	3.88±0.80	0.0001	3.92±0.65	0.00001
Hemoglobin (g/l)	137±12	139±12	0.00004	139±11	0.000002
Platelets (10^9/l)	234±55	239±84	0.76	240±80	0.57
Leukocytes (10^9/l)	5.2±1.6	5.1±1.7	0.03	5.4±1.8	0.11

Values are the mean±standard deviation of the respective parameters.

LC1084A with HP 79850 A integrator (Hewlett-Packard, Palo Alto, CA, USA) or a Prominence LC20 (Shimadzu, Kyoto, Japan) as described elsewhere (42). Neopterin was identified by its native fluorescence (353 nm excitation, 438 nm emission) and quantified by external standard method. Creatinine was determined by Jaffé reaction after dilution of the sample 1:50 on a MODULAR analyzer (Hoffmann-La Roche, Basel, Switzerland) using a commercial kit according the manufacturer's instructions, and neopterin concentrations were expressed as neopterin/creatinine ratio (μmol/mol creatinine).

Analysis of hemoglobin, peripheral blood cell count and fibrinogen. Peripheral blood cell count was performed as described elsewhere (43). Hemoglobin was measured by a photometric method using sodium lauryl sulfate; leukocytes and platelets were determined by impedance method using a Sysmex XE-2100 blood analyzer (Sysmex, Kobe, Japan). Fibrinogen was determined by Clauss clotting time method (STA Compact; Diagnostica STAGO, Paris, France).

Statistics. The parameters before and during the treatment were compared by Wilcoxon signed rank test. Differences between different groups were analyzed by Mann-Whitney *U*-test. The decision on statistical significance was based on $p=0.05$ level. The analyses were performed using NCSS software (Number Cruncher Statistical Systems, Kaysville, UT, USA).

Results

As shown in Table I, the concentrations of total, VLDL and LDL cholesterol, lipoprotein (a), retinol, alpha-tocopherol, LDL alpha-tocopherol, total protein, C-reactive protein, fibrinogen, hemoglobin and leukocytes increased significantly compared to baseline during the first months of therapy with aromatase inhibitors. LDL and HDL triglycerides decreased significantly.

Significant differences were observed in baseline parameters of lipid metabolism and inflammatory response between 73 patients with prior hormonal therapy (in all cases tamoxifen) and 113 patients with no prior hormonal therapy (Table II). The patients treated with first-line aromatase inhibitors were significantly older and had significantly

Table II. *Comparison between patients treated with first-line aromatase inhibitors and tamoxifen-pretreated patients.*

Parameter	No prior hormonal therapy (n=113)	Prior hormonal therapy (tamoxifen) (n=73)	*p*-Value
Age (years)	64±11	60±9	0.04
Cholesterol			
Total (mmol/l)	6.22±1.18	5.22±0.82	<0.000001
VLDL (mmol/l)	1.39±0.40	1.20±0.42	0.0002
LDL (mmol/l)	3.20±0.84	2.60±0.57	<0.000001
HDL (mmol/l)	1.33±0.46	1.18±0.24	0.02
Triglycerides			
Total (mmol/l)	1.61±0.90	1.56±0.63	0.71
VLDL (mmol/l)	0.99±0.58	0.93±0.47	0.56
LDL (mmol/l)	0.44±0.13	0.46±0.13	0.29
HDL (mmol/l)	0.18±0.09	0.21±0.18	0.02
Lipoprotein (a) (g/l)	0.42±0.48	0.39±0.43	0.81
Homocysteine (μmol/l)	13.2±4.3	11.0±3.6	0.0001
Glucose (mmol/l)	5.6±1.5	5.6±2.0	0.50
Glycosylated hemoglobin (%)	3.9±0.9	4.1±1.1	0.15
Retinol (μmol/l)	1.67±0.40	1.61±0.44	0.23
Alpha-tocopherol			
Total (μmol/)	26.47±6.54	23.01±5.37	0.0001
VLDL (μmol/l)	8.11±3.90	6.91±2.33	0.06
LDL (μmol/l)	11.19±3.44	9.44±2.97	0.00005
HDL (μmol/l)	6.58±2.72	5.89±2.55	0.12
Alpha-tocopherol/cholesterol ratio (10^{-3})	4.31±1.00	4.61±1.53	0.27
Urinary neopterin (μmol/mol creatinine)	202±88	172±62	0.02
C-reactive protein (mg/l)	4.6±8.3	4.3±6.1	0.41
Total protein (g/l)	73.0±5.3	73.5±4.2	0.50
Albumin (g/l)	44.3±2.6	44.0±2.4	0.39
Fibrinogen (g/l)	3.97±0.69	3.12±0.68	<0.000001
Hemoglobin (g/l)	136±13	137±10	0.61
Platelets (10^9/l)	241±55	221±53	0.005
Leukocytes (10^9/l)	5.2±1.7	5.3±1.6	0.51

Values are the mean±standard deviation of the respective parameters.

higher baseline total, VLDL, LDL and HDL cholesterol, HDL triglycerides, homocysteine, alpha-tocopherol, LDL alpha-tocopherol, urinary neopterin, fibrinogen and platelets compared to patients pre-treated with tamoxifen.

Differential response in the parameters of lipid metabolism, oxidative balance and inflammatory response was observed during the first four months of therapy with aromatase inhibitors based on prior tamoxifen treatment. As shown in Table III, concentrations of total, VLDL and LDL cholesterol, lipoprotein (a), retinol, alpha-tocopherol, C-reactive protein, total protein, fibrinogen, hemoglobin and platelets significantly increased compared to baseline, while the concentrations of total, LDL and HDL triglycerides decreased. An increase in alpha-tocopherol was evident only after 4 months, and alpha-tocopherol/cholesterol ratio actually decreased. In contrast, in patients treated with first-line aromatase inhibitors (*i.e.* without prior tamoxifen therapy) less extensive changes were observed (Table IV), including a rise in retinol, total protein, hemoglobin and platelets.

Discussion

In the present study, a significant increase in total, VLDL and LDL cholesterol and lipoprotein (a) accompanied by changes in serum concentrations of alpha-tocopherol and retinol was observed. However, cholesterol increased only in patients pre-treated with tamoxifen, and no changes in cholesterol concentrations were observed in patients treated with aromatase inhibitors in the first-line setting. Thus, the present data indicate that the increase in serum cholesterol and other changes of laboratory parameters are caused by the withdrawal of tamoxifen rather than by the suppression of endogenous estrogen production induced by aromatase inhibitors. In contrast to cholesterol, triglyceride concentrations decreased after tamoxifen withdrawal. Tamoxifen withdrawal also led to a significant increase in C-reactive protein and fibrinogen concentrations. Serum total protein concentrations also increased significantly during the therapy. No effect was observed on serum homocysteine

Table III. *Parameters of lipid metabolism and systemic inflammatory response in patients pre-treated with tamoxifen (n = 73).*

Parameter	Visit 1 (baseline)	Visit 2	*p*-Value compared to visit 1	Visit 3	*p*-Value compared to visit 1
Time (days)	0	64±12	-	126±15	-
Cholesterol					
Total (mmol/l)	5.22±0.82	5.71±0.92	<0.000001	5.92±0.99	<0.000001
VLDL (mmol/l)	1.20±0.42	1.32±0.40	0.00001	1.32±0.38	0.003
LDL (mmol/l)	2.60±0.57	3.00±0.62	0.000003	3.15±0.68	<0.000001
HDL (mmol/l)	1.18±0.24	1.21±0.22	0.28	1.22±0.26	0.046
Triglycerides					
Total (mmol/l)	1.56±0.63	1.47±0.77	0.03	1.45±0.64	0.049
VLDL (mmol/l)	0.93±0.47	0.98±0.62	0.92	0.93±0.46	0.69
LDL (mmol/l)	0.46±0.13	0.42±0.13	0.003	0.41±0.11	0.0001
HDL (mmol/l)	0.21±0.18	0.16±0.07	0.001	0.16±0.05	0.004
Lipoprotein (a) (g/l)	0.39±0.43	0.46±0.48	0.000003	0.49±0.48	0.0001
Homocysteine (μmol/l)	11.0±3.6	11.2±3.9	0.37	11.2±3.4	0.19
Glucose (mmol/l)	5.6±2.0	5.5±1.7	0.72	5.7 ±2.0	0.77
Glycosylated hemoglobin (%)	4.1±1.1	4.0±0.9	0.92	4.1±1.1	0.28
Retinol (μmol/l)	1.61±0.44	1.71±0.42	0.02	1.68±0.38	0.001
Alpha-tocopherol					
Total (μmol/l)	23.01±5.37	23.89±6.46	0.20	24.89±5.71	0.004
VLDL (μmol/l)	6.91±2.33	7.19±3.08	0.62	7.68±2.51	0.01
LDL (μmol/l)	9.44±2.97	10.40±3.08	0.001	11.08±3.56	0.000006
HDL (μmol/l)	5.89±2.55	5.77±2.79	0.89	6.39±2.41	0.09
Alpha-tocopherol/cholesterol ratio (10^{-3})	4.61±1.53	4.20±0.94	0.006	4.26±0.94	0.03
Urinary neopterin (μmol/mol creatinine)	172±62	189±81	0.21	190±83	0.49
C-reactive protein (mg/l)	4.3±6.1	4.7±5.1	0.01	5.1±5.7	0.0004
Total protein (g/l)	73.5±3.7	74.9±3.7	0.001	75.6±4.0	0.00001
Albumin (g/l)	44.0±2.4	44.5±2.3	0.19	45.2±2.5	0.001
Fibrinogen (g/l)	3.12±0.68	3.68±0.76	<0.000001	3.85±0.71	<0.000001
Hemoglobin (g/l)	137±10	139±8	0.01	141±8	0.00001
Platelets (10^9/l)	221±53	234±55	0.004	241±55	0.0001
Leukocytes (10^9/l)	5.3±1.6	5.3±1.5	0.24	5.7±1.7	0.21

Values are the mean±standard deviation of the respective parameters.

concentrations and glucose metabolism evaluated by serum glucose or glycosylated hemoglobin.

These findings are consistent with previous reports. Serum lipids have been studied in patients with breast carcinoma treated with endocrine therapy, sometimes with conflicting results. Tamoxifen administration has been reported to result in lower serum cholesterol concentrations (10-15). On the other hand, administration of aromatase inhibitors has been reported to result in increased (16) or unchanged serum lipid levels (44-48). The conflicting results reported in these studies may be due to different third-generation aromatase inhibitors used, different patient populations, including differences in previous therapy, and also differences in study design, *e.g.* sample collection or timing of follow-up visits. In some studies, non-fasting lipid concentrations were investigated (49), while in other studies samples were collected after an overnight fast (16, 44, 45, 50). Investigation of fasting samples may be more sensitive in detecting an effect of therapy. An increase of serum cholesterol and LDL cholesterol was observed after letrozole administration in patients with advanced breast carcinoma pretreated with tamoxifen (16). In a randomized study, letrozole administered sequentially after tamoxifen as an extended adjuvant therapy resulted in increased serum cholesterol, LDL cholesterol and lipoprotein (a) concentrations, but the increase in cholesterol was mostly not different in patients after tamoxifen withdrawal treated with placebo (50). In a small cohort of patients without invasive breast carcinoma treated in a prevention trial, letrozole had no significant effect on serum total, LDL and HDL cholesterol (51). No significant changes of serum total or LDL cholesterol were reported in two studies in patients treated with anastrozole (44, 45). In a trial comparing anastrozole with tamoxifen and combination of these agents administered in neoadjuvant setting, only a non-significant increase of cholesterol was observed in patients treated with anastrozol (49). However, a significant difference

Table IV. *Parameters of lipid metabolism and systemic inflammatory response in patients treated with first-line aromatase inhibitors (n = 113).*

Parameter	Visit 1 (baseline)	Visit 2	*p*-Value compared to visit 1	Visit 3	*p*-Value compared to visit 1
Time (days)	0	64±12	-	126±15	-
Cholesterol					
Total (mmol/l)	6.22±1.18	6.16±1.24	0.41	6.19±1.24	0.72
VLDL (mmol/l)	1.39±0.40	1.41±0.42	0.76	1.43±0.46	0.33
LDL (mmol/l)	3.20±0.84	3.19±0.77	0.26	3.21±0.87	0.41
HDL (mmol/l)	1.33±0.46	1.26±0.37	0.09	1.27±0.33	0.38
Triglycerides					
Total (mmol/l)	1.61±0.90	1.59±0.73	0.96	1.64±0.75	0.44
VLDL (mmol/l)	0.99±0.58	1.00±0.54	0.88	1.02±0.56	0.62
LDL (mmol/l)	0.44±0.13	0.42±0.12	0.05	0.43±0.12	0.16
HDL (mmol/l)	0.18±0.09	0.16±0.06	0.03	0.18±0.16	0.68
Lipoprotein (a) (g/l)	0.42±0.48	0.41±0.48	0.92	0.37±0.44	0.77
Homocysteine (μmol/l)	13.2±4.3	13.6±5.2	0.36	12.9±5.3	0.23
Glucose (mmol/l)	5.6±1.5	5.6±1.5	0.07	5.6±1.5	0.18
Glycosylated hemoglobin (%)	3.9±0.9	3.9±0.9	0.37	3.9±1.0	0.23
Retinol (μmol/l)	1.67±0.40	1.71±0.42	0.01	1.76±0.36	0.02
Alpha-tocopherol					
Total (μmol/)	26.47±6.54	26.03±6.09	0.38	26.76±6.87	0.53
VLDL (μmol/l)	8.11±3.90	8.16±3.42	0.96	8.67± 3.98	0.62
LDL (μmol/l)	11.19±3.44	11.22±3.25	0.96	11.51±3.44	0.62
HDL (μmol/l)	6.58±2.72	6.54±2.37	0.71	6.87±2.35	0.13
Alpha-tocopherol/cholesterol ratio (10^{-3})	4.31±1.00	4.34±1.21	0.94	4.40±1.05	0.33
Urinary neopterin (μmol/mol creatinine)	202±88	195±77	0.39	205±94	0.91
C-reactive protein (mg/l)	4.6±8.3	4.4±5.9	0.97	4.7±7.3	0.35
Total protein (g/l)	73.0±5.3	73.7±5.7	0.04	74.4±5.2	0.0002
Albumin (g/l)	44.3±2.6	44.3±3.1	0.66	44.3±2.9	0.66
Fibrinogen (g/l)	3.97±0.69	4.00±0.80	0.76	3.96±0.61	0.89
Hemoglobin (g/l)	136±13	138±14	0.001	138±13	0.005
Platelets (10^9/l)	241±55	236±72	0.02	233±53	0.02
Leukocytes (10^9/l)	5.2±1.7	5.0±1.8	0.06	5.3±1.8	0.32

Values are the mean±standard deviation of the respective parameters.

in cholesterol concentrations was observed during therapy between patients treated with anastrozole and tamoxifen. No increase in cholesterol concentrations was evident after exemestane therapy in three studies (46-48). The effects of anastrozole, letrozole and exemestane on lipid metabolism were compared in healthy women (52). A statistically significant decrease of total cholesterol was observed after 12 weeks of therapy only for exemestane, but lowered total cholesterol in those treated with exemestane was associated with lower HDL cholesterol levels. Findings from the present cohort demonstrating that the increase in serum cholesterol in patients treated with aromatase inhibitors is the result of tamoxifen withdrawal rather than aromatase inhibition are consistent with these previous reports and may provide an explanation reconciling sometimes conflicting results of earlier reports.

An increase in cholesterol observed in the present study was associated with increased VLDL and LDL cholesterol as well as of lipoprotein (a) concentrations. Tamoxifen therapy has been reported to result in lower LDL and increased HDL cholesterol (14). In another study in breast cancer patients treated with tamoxifen a decrease in total and LDL cholesterol was accompanied by a decrease in lipoprotein (a) concentrations (11). The changes observed in VLDL and LDL cholesterol or lipoprotein (a) concentrations in the present cohort are therefore also linked to tamoxifen withdrawal.

Increased circulating homocysteine is associated with higher risk of atherosclerosis (53, 54). Tamoxifen has been reported to lower serum homocysteine concentrations (55, 56). In the present cohort, serum homocysteine was significantly lower in patients pre-treated with tamoxifen, but no effect of tamoxifen withdrawal was observed. This is in agreement with another report that found no change in plasma homocysteine after administration of aromatase inhibitors (57). A minor, but statistically significant increase of homocysteine was observed after two years of exemestane therapy (46). Thus, therapy of breast carcinoma with

aromatase inhibitors seems to have no effect on serum homocysteine, at least in the short term. Further confounding the analyses, malignant tumors may produce homocysteine, and tumor control may result in lowered circulating homocysteine concentrations (58, 59).

In contrast to the effect on serum cholesterol, tamoxifen therapy has been reported to result in increased serum triglyceride levels (14, 60, 61). Accordingly, a decrease in serum triglycerides was observed after tamoxifen withdrawal in the present cohort. In an earlier study, a decrease in serum triglyceride concentration was reported after therapy with anastrozol (44).

In the present study, increase in serum cholesterol was associated with increased retinol concentrations. An increase in serum alpha-tocopherol was less pronounced or absent, and alpha-tocopherol/cholesterol ratio actually decreased. Patients pretreated with tamoxifen had lower alpha-tocopherol concentrations, but also, although not significantly, a higher alpha-tocopherol/cholesterol ratio. The reduction in alpha-tocopherol/cholesterol ratio could be of significance for antioxidant balance and lipid oxidation. Moreover, the changes of alpha-tocopherol concentrations may be of importance in cancer patients. In previous studies, low vitamin E intake was associated with toxicity of chemotherapy in children with acute lymphoblastic leukemia (62). The administration of vitamin E has been shown to alleviate some side-effects of radiotherapy (21) and chemotherapy (22). Although serial monitoring of vitamin E levels may be a necessary prerequisite for any therapeutic use of this antioxidant vitamin, serum vitamin E is not routinely measured in cancer patients. In most reports published so far, alpha-tocopherol has been investigated in epidemiological studies in relation to cancer risk. For example, lower vitamin E concentrations have been reported in patients with breast cancer (63).

Previous therapy with tamoxifen may also explain other differences observed between patients treated with aromatase inhibitors in the first-line setting or after tamoxifen or changes during therapy. Tamoxifen is known to reduce fibrinogen concentrations (12, 13), and the increase in fibrinogen concentrations found here may thus be explained by tamoxifen withdrawal. An anti-inflammatory effect has also been reported for tamoxifen that manifested in lowered serum concentrations of C-reactive protein (12) or other acute-phase proteins (10). In the present cohort, the therapy with aromatase inhibitors had no effect on urinary neopterin, but the difference between urinary neopterin in patients pre-treated with tamoxifen compared to patients treated with first-line aromatase inhibitors may be explained by the anti-inflammatory effect of earlier tamoxifen therapy. Serum C-reactive protein increased significantly during the therapy with aromatase inhibitors in patients pre-treated with tamoxifen, but not in patients treated with first-line aromatase inhibitors, indicating a suppressive effect of tamoxifen on the systemic inflammatory response. Both fibrinogen and C-reactive protein are important laboratory markers of increased risk of cardiovascular events (38).

The decrease of total protein concentrations associated with tamoxifen therapy could be explained by hemodilution (10, 64). Tamoxifen therapy is known to induce hemodilution, resulting in lower concentrations of hemoglobin or albumin and lower peripheral blood cell counts (64). Significant changes observed during the first four months of therapy with aromatase inhibitors in total protein and hemoglobin concentrations and platelet counts could also be affected by changing hemodilution after tamoxifen withdrawal. However, an increase in total protein and hemoglobin was also observed in patients treated with first-line aromatase inhibitors, suggesting that tamoxifen withdrawal was not the only factor involved.

The clinical significance of laboratory changes associated with tamoxifen withdrawal and therapy with third-generation aromatase inhibitors is currently uncertain. Although the favorable effect of tamoxifen on cardiovascular risk factors is well proven, the effect on cardiovascular events is less certain, and in many epidemiological studies, no effect of tamoxifen on cardiovascular events was found (65, 66). For obvious reasons, the data on long-term cardiovascular safety of aromatase inhibitors is limited (67, 68). In agreement with the literature, data from the present cohort indicate that therapy with aromatase inhibitors *per se* does not have a significant adverse effect on serum cholesterol or other laboratory cardiovascular risk factors.

In conclusion, an increase of serum cholesterol, lipoprotein(a), C-reactive protein and fibrinogen in patients after the therapy with aromatase inhibitors seems to be associated with the withdrawal of tamoxifen rather than to reflect the direct effect of aromatase inhibitors. No significant changes in serum lipids were observed in patients treated with aromatase inhibitors in the first-line setting. Patients treated with tamoxifen had also lower serum C-reactive protein, urinary neopterin and fibrinogen levels.

Acknowledgements

Supported by a grant of the Internal Grant Agency of the Ministry of Health of the Czech Republic (NR9096-4).

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Received February 17, 2009
Revised May 11, 2009
Accepted May 21, 2009