in vivo *24*: 227-230 (2010)

Impact of Adjuvant Chemo- and Radiotherapy on the Cellular Immune System of Breast Cancer Patients

THERESA MELLIOS, HONG LIOE KO and JOSEF BEUTH

Institute of Naturopathy, University of Cologne, 50931 Köln, Germany

Abstract. *The relevance of an uncompromized immune system for the development and progress of breast cancer still is a matter of intensive research. The impact of chemo- and/or radiotherapy on peripheral blood immune cell counts and activity of breast cancer patients (n=660) was investigated by flow cytometry. Not only the absolute counts, but also the comparison of those counts with standard values were evaluated. Most studies do not consider this comparison. As compared to pre-treatment values, leukocytes, lymphocytes, B-lymphocytes, T-lymphocytes, helper T-cells and CD25$^+$ T-cells (activated T-cells) were significantly reduced after chemo- and/or radiotherapy. However, mean cell counts remained within the normal range. Statistically non-significant down-regulation was detected for cytotoxic T-cells, suppressor T-cells and natural killer cells, which are of prime importance as far as tumor development and defence are concerned. The impact of the therapy is not predictable for individual cases. However, only less than 20% of the breast cancer patients were immunocompromized after chemo- and/or radiotherapy.*

Breast cancer is the most frequent malignancy in women in Germany and worldwide with rising incidence and falling mortality since 1980 (1). The multimodal treatment comprises secondary prevention, operation, chemo- and/or radiotherapy as well as hormone and immune therapy with antibodies against Her2/neu.

Beyond these evidence-based therapies, most patients want to improve the outcome and their quality of life with complementary medicine. The American Cancer Society defines complementary medicine or methods as those that are used along with regular medical care. If these treatments are carefully chosen and managed, they may enhance comfort and well being (2). Some complementary treatments have been tested, *e.g.* nutrition, sports, psycho-oncology, while others have not (3).

Treatment with immunomodulating mistletoe extract is the most frequently used complementary therapy in Germany. Its efficacy in terms of improving quality of life and stimulating the immune system has been shown (4-6), however, it still is controversially discussed.

Nothing is mentioned about complementary treatments and their indication in the recent S3 guideline for breast cancer. To make a scientifically based recommendation concerning complementary immunotherapy for patients, their cellular immune system should be measured six to eight weeks after chemo- and/or radiotherapy. During this time interval the cellular immune system usually recovers from therapy-induced suppression.

This investigation was performed to analyse the cellular immune system of breast cancer patients before and after guideline based chemo- and/or radiotherapy.

Patients and Methods

This investigation analyses the cellular immune system of 660 breast cancer patients who attended consultation concerning complementary treatment at the Institute of Naturopathy at the University of Cologne. The mean age of the patients varied statistically significantly among the four groups. However, the values were all within the sixth decade and therefore differences were negligible (7-9). Also negligible was the TNM status of the patients which was not considered important for this analysis of the impact of chemo- and/or radiotherapy on the cellular immune system. Data were collected before and 6-8 weeks after the patients had finished adjuvant treatment in accredited breast cancer centres in Cologne. Therefore the patients were divided into four groups: without adjuvant treatment (n=391); after chemotherapy (n=55); after radiotherapy (n=52); after chemo- and radiotherapy (n=162). Peripheral blood cells were measured by flow cytometry (FACSCalibur™; BD Biosciences, San Jose, USA) and analysed with the statistic software programme SPSS. The standard values used in the Institute of Naturopathy are presented in Table I (7).

The comparison of the individual cell counts with the standard values was a strictly descriptive data analysis.

Correspondence to: Professor Josef Beuth, Institute for Naturopathy University of Cologne, Joseph-Stelzmann-Str. 9, 50931 Cologne, Germany. Tel: +49 2214786414, Fax: +49 2214787017, e-mail: Hans.Beuth@uk-koeln.de

Key Words: Breast cancer, guideline-based adjuvant treatment, immunosuppression, complementary treatment.

Table I. *Normal range of blood cell counts and activity as described (7).*

Cell type	Count/µl (normal range)
Leukocytes $\times 10^3$	4,0-10,0
Lymphocytes	1100-3400
Monocytes	300-600
Granulocytes	1400-6500
T-lymphocytes	800-2000
B-lymphocytes	80-600
Helper T-cells	500-1100
Cytotoxic T-cells	40-400
Suppressor T-cells	200-900
NK cells	180-400
$CD25^+$ T-cells	180-410

Results

Peripheral blood cells of 660 breast cancer patients were analysed before and after (6-8 weeks) adjuvant chemo- and/or radiotherapy. For each cell type descriptive statistics, characterization in form of box plots and bar charts for the leukocytes were performed. As shown in Figures 1 and 2, chemo- and/or radiotherapy reduced leukocyte counts, however, mean values remained within the normal range.

As shown in Table II, counts of leukocytes, lymphocytes, B-lymphocytes, T-lymphocytes, helper T-cells and $CD25^+$ T-cells (activated T-cells) were significantly reduced after chemo- and/or radiotherapy as compared to pre-treatment values. Most suppressed were helper T-cells and B-cells, with values about 30% below normal range. Cytotoxic T-cells, suppressor T-cells and NK cells, which are important for tumour development and progress were non-significantly reduced after chemo- and/or radiotherapy. Table II shows mean and standard deviation for all cell populations investigated.

Besides these observations concerning immune cell counts and activity in patients with or without chemo- and/or radiotherapy (n=660), a separate investigation was performed in 33 patients who had their cellular immune system intraindividually checked before and after adjuvant therapies. Results were comparable to those of the whole (interindividual) group. An observation in this patient group was that although absolute counts and arithmetic mean were lower after adjuvant treatment, some patients presented increased cell counts after treatment. This observation confirms that in the individual case results are not predictable.

Decreased counts of one cell type does not automatically mean a compromized immune system. Synergistic acting cells such as NK cells and cytotoxic T-cells can compensate the function of each other.

Table II. *Mean and standard deviation for all cell populations investigated.*

	no T	CT+RT	CT	RT
Leukocytes $\times 10^3$				
Arithmetic mean	7.27	4.92*	5.05*	5.11*
Standard deviation	2.22	1.33	1.82	1.37
Lymphocytes				
Arithmetic mean	1833.22	1379.98*	1479.67*	1448.85*
Standard deviation	638.85	432.62	602.58	467.36
Monocytes				
Arithmetic mean	498.25	403.49*	465.56	402.81*
Standard deviation	234.74	248.47	269.99	158.88
Granulocytes				
Arithmetic mean	4951.71	3201.19*	3102.05*	3223.29*
Standard deviation	1903.61	1162.69	1395.88	1105.68
T-lymphocytes				
Arithmetic mean	1400.03	1025.95*	1088.05*	1054.94*
Standard deviation	517.64	346.67	439.63	375.95
B-lymphocytes				
Arithmetic mean	259.31	172.04*	154.60*	165.98*
Standard deviation	159.36	117.07	118.28	101.48
Helper T-cells				
Arithmetic mean	989.46	613.90*	665.42*	694.85*
Standard deviation	399.77	237.16	300.90	272.82
Cytotoxic T-cells				
Arithmetic mean	109.54	92.71	105.82	92.50
Standard deviation	90.82	81.74	119.68	79.89
Suppressor T-cells				
Arithmetic mean	328.21	329.51	324.18	274.58
Standard deviation	155.82	166.48	196.26	148.67
NK cells				
Arithmetic mean	233.15	234.10	267.18	248.15
Standard deviation	133.06	121.11	166.62	109.99
$CD25^+$ T-cells				
Arithmetic mean	449.47	338.85*	342.38*	334.63*
Standard deviation	214.70	114.21	178.75	166.89

No T: no adjuvant therapy; CT: adjuvant chemotherapy; RT: adjuvant radiotherapy. *statistically significantly different from no T.

Discussion

The evaluation of counts and activity of peripheral blood immune cells is an important contribution to the aftercare of breast cancer patients who ask for immunostimulating complementtary treatments. Most patients take complementary medicine during and after the recommended standard therapies (3). The indication, however, has to be analysed to avoid useless or even harmful treatment, especially immunostimulating therapies. Therefore, analysis of the cellular immune system six to eight weeks after finishing tumordestructive treatments may be regarded a helpful instrument.

To evaluate peripheral blood leukocytes and their subpopulations it is important to be aware of possible confounders such as age, psychological constitution, additional medication and alimentation (7-13). Since all patients were treated in accordance with international guidelines optimized by recommendations for evidence-

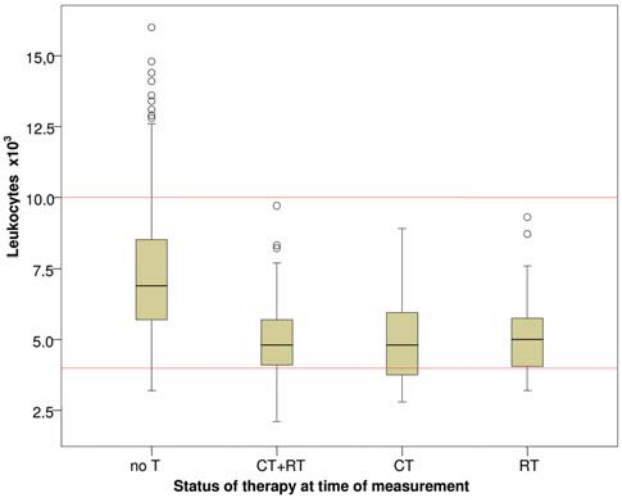
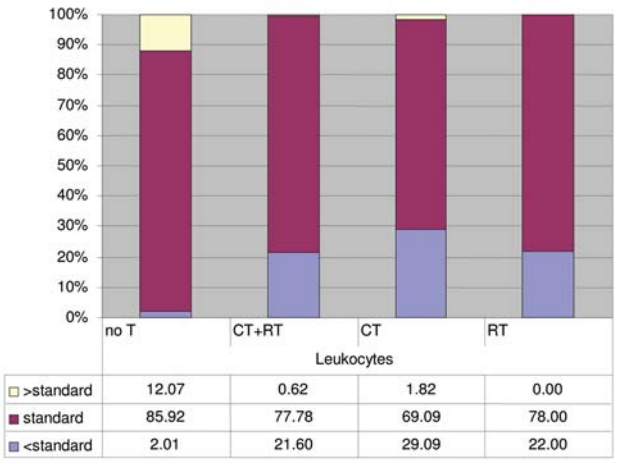


Figure 1. *Median, interquartile range and upper and lower whisker. Absolute counts decreased 6-8 weeks after termination of the adjuvant therapies. Approximately 75% stayed within the normal range. No T: no adjuvant therapy; CT: adjuvant chemotherapy; RT: adjuvant radiotherapy.*



Leukocytes	no T	CT+RT	CT	RT
>standard	12.07	0.62	1.82	0.00
standard	85.92	77.78	69.09	78.00
<standard	2.01	21.60	29.09	22.00

Figure 2. *Percentage of breast cancer patients with leukocyte counts within/below/above the normal range as shown in Table I. No T: no adjuvant therapy; CT: adjuvant chemotherapy; RT: adjuvant radiotherapy.*

based complementary therapies, *e.g.* nutrition, sports and psychooncology, confounders were considered.

Patients who are submitted to the "Disease Management Programme" for breast cancer receive an indication-based chemotherapy consisting of 5-fluorouracil, taxanes, anthracyclines and cyclophosphamide according to international guidelines. For these drugs depending on dose and application schedule, evidence was shown for immuno-suppressing and immunostimulating effects (14-19). Given as combination therapy after surgery of breast cancer, the suppressing qualities are dominant. It has been frequently shown that chemo- and/or radiotherapy down-regulate the immune systems. But most studies concerning this topic are lacking the comparison with standard values, which are very important for judging the indication for stimulating therapies.

In the present investigation, this comparison revealed that fewer than 20% of the patients have absolute immune cell counts below standard that require attention and treatment. Accordingly, it contributes to a better understanding of the immunosuppressing activities of adjuvant therapy. Since more than 60% of cancer patients in Germany receive immunomodulating therapies (3), this investigation confirms that the cellular immune system should be evaluated before starting complementary immunotherapy.

References

1 Krebs in Deutschland 2003-2004. Häufigkeiten und Trends. 6. überarbeitete Auflage. Robert Koch-Institut und Gesellschaft der epidemiologischen Krebsregister in Deutschland e. V. (Hrsg). Berlin, 2008.

2 American Cancer Society: Guidelines for using complementary and alternative medicine. 2008. www.cancer.org.

3 Beuth J: Evidenzbasierte Komplementäronkologie – Aktuelle Studien und Ausblick. Der Onkologe *13*: 534-542, 2007.

4 Semiglazov VF, Stepula VV, Dudov A, Schnitker J and Mengs U: Quality of life is improved in breast cancer patients by standardised mistletoe extract PS76A2 during chemotherapy and follow-up: a randomised, placebo-controlled, double-blind, multicentre clinical trial. Anticancer Res *26*: 1519-1530, 2006.

5 Schumacher K, Schneider B, Reich G, Stiefel T, Stoll G, Bock PR, Hanisch J and Beuth J: Influence of postoperative complementary treatment with lectin-standardized mistletoe extract on breast cancer patients. A controlled epidemiological multicentric retrolective cohort study. Anticancer Res *23*: 5081-5088, 2003.

6 Beuth J, Schneider B and Schierholz JM: Impact of complementary treatment of breast cancer patients with standardized mistletoe extract during aftercare: A controlled multicenter comparative epidemiological cohort study. Anticancer Res *28*: 523-528, 2008.

7 Hannet I, Erkeller-Yuksel F, Peter Lydyard P, Deneys V, DeBruère M: Developmental and maturational changes in human blood lymphocyte subpopulations. Immunol Today *13*: 215-218, 1992.

8 Murta EF, de Andrade JM, Falcão RP and Bighetti S: Lymphocyte subpopulations in patients with advanced breast cancer submitted to neoadjuvant chemotherapy. Tumori *5*: 403-407, 2000.

9 Falcão RP, Ismael SJ and Donadi EA: Age-associated changes of T lymphocyte subsets. Diagn Clin Immunol *5*: 205-208, 1987.

10 Herbert TB and Cohen S: Stress and immunity in humans: a meta-analytic review. Psychosom Med *55*: 364-379, 1993.

11 Sabbioni MEE, Bernhard J, Siegrist HP, Schmitz SFH, Gertsch MC, Thuerlimann B, Bonnefoi H Perey L, Herrmann R, Goldhirsch A and Huerny C: Does subjective burden of early breast cancer and its treatment affect immune measures during adjuvant therapy? Breast Cancer Res Treatm *87*: 75-86, 2004.

12 Sabbioni MEE, Castiglione M, Huerny C, Siegrist HP, Bacchi M, Bernhard J, Thuerlimann B, Bonnefoi H, Perey L, Goldhirsch A and Senn HJ: Interaction of tamoxifen with concurrent cytotoxic adjuvant treatment affects lymphocytes and lymphocyte subsets counts in breast cancer patients. Supp Care Cancer *7*: 149-153, 1999.

13 Good RA and Fernandes G: Nutrition, immunity, and cancer-a review. Part I: Influence of protein or protein-calorie malnutrition and zinc deficiency on immunity. Clin Bull *8*: 3-12, 1979.

14 Ehrke MJ: Immunomodulation in cancer therapeutics. Int Immunopharmacol *3*: 1105-1119, 2003.

15 Danesi R, Conte PF and Del Tacca M: Pharmacokinetic optimisation of treatment schedules for anthracyclines and paclitaxel in patients with cancer. Clin Pharmacokin *37*: 195-211, 1999.

16 Arinaga S, Akiyoshi T and Tsuji H: Augmentation of the generation of cell-mediated cytotoxicity after a single dose of adriamycin in cancer patients. Cancer Res *46*: 4213-4216, 1986.

17 Kotsakis A, Sarra E, Peraki M, Koukourakis M, Apostolaki S, Souglakos J, Mavromanomakis E, Vlachonikolis J and Georgoulias V: Docetaxel-induced lymphopenia in patients with solid tumors: a prospective phenotypic analysis. Cancer *89*: 1380-1386, 2000.

18 Chan OTM and Yang LX: The immunological effects of taxanes. Cancer Immunol Immunother *49*: 181-185, 2000.

19 Grünberg E, Eckert K and Maurer HR: Docetaxel treatment of HT-29 colon carcinoma cells reinforces the adhesion and immunocytotoxicity of peripheral blood lymphocytes *in vitro*. Int J Oncol *12*: 957-963, 1998.

Received December 2, 2009
Revised February 4, 2010
Accepted February 9, 2010