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Immunological and quality-of-life profiles in women with breast cancer: Complementary versus conventional care

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Short title Immunological and QoL profiles in breast cancer

Summary

Background: Previous studies showed that women with breast cancer treated in anthroposophic clinic versus conventional care increased quality-of-life (QoL)-parameters fighting spirit and anxiety coping. We have now analyzed immune and QoL-factors in these two groups for possible differences during the first 6 months after admission, prompted by anthroposophic studies, including mistletoe extracts, showing beneficial immune system effects. **Material and Methods:** Fourteen immunological variables, including leukocyte-count, lymphocyte-count, activated T-cells (CD4⁺ and CD8⁺), NK-cells, B-cells, IL1 β , IL6, IL10, and oxytocin were longitudinally analyzed in both groups (n=2x26). A panel of QoL-parameters were analyzed using three different instruments. Statistical evaluation included that each patient was its own control. **Results:** Cytotoxic CD8⁺ T-cell frequency (% of lymphocytes analyzed by flow-cytometry) significantly decreased over time in the anthroposophic group versus the conventional group (repeated measures ANOVA, p=0.05). No major differences were observed in other immunological parameters, whereas QoL-variables, anxiety decreased and physical symptoms increased/improved significantly in the anthroposophic group (p=0.04 and p=0.05). **Conclusion:** Overall, women with breast cancer in anthroposophic or conventional therapy did not differ in their immune profiles over time, with exception of decreased cytotoxic T-cells in the anthroposophic group. Improvement in physical symptoms along with less anxiety in this group may have influenced brain-immune axis resulting in lower frequency of CD8⁺ T-cells, a feature associated with less aggressive cancer stages. To evaluate whether this observation is associated with good or bad prognosis, further detailed analyses of memory and naïve CD8⁺ T-cells at tumor site and in blood circulation are essential.

Introduction

Breast cancer is the second most common cancer in the world after lung cancer, and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers) [1]. Aspects of quality-of-life (QoL) and overall life situation for women with breast cancer and their families has developed as an important area of research [2]. Many patients with cancer and other serious diseases often choose complementary medicine including anthroposophic medicine. The principles underlying anthroposophy were developed by Rudolf Steiner (1861–1925) and Ita Wegman (1876–1943). The word is derived from Greek, *anthropos* (human) and *sophia* (wisdom). The idea is to develop spiritual wisdom through human self-knowledge [3, 4]. In anthroposophic medicine, natural scientific methods used in conventional medicine are not contraindicated. The effectiveness of anthroposophic therapies are important to evaluate also from a patient preference point-of-view. As a step towards better evaluation of these therapies practiced in Sweden, we performed several QoL studies on women with breast cancer, who received anthroposophical care (A-group) in addition to conventional care and compared these with patients receiving conventional care only (C-group) [5-8]. Three instruments were used for evaluation of perceived quality-of-life: 1) Life Satisfaction questionnaire (LSQ) [6, 9, 10], 2) the European Organization for Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire (QLQ)-C30, and 3) a coping scale named Mental Adjustment of Cancer (MAC scale) [11]. It was previously shown that women receiving anthroposophic medicine increased their perceived QoL over a period of 1 year [7]. A 5-year follow-up study on the same cohort of women (n= 60 + 60) showed that the mortality rates did not differ significantly in the two groups (11 died in the A-group during the first year and 9 in the C-group, respectively), and the 1st year increase in QoL found in the A-group was not retained at 5 years [8]. QoL parameters during the first 6 months have not been presented previously.

Psychological and physiological stressors such as cancer, acute hypoglycemia, starvation, shift work, cardiac infarction, trauma, and pain affect the immune system via multiple neuroendocrine mediators (brain-immune axis) [12]. Conversely, alterations in the immune system, such as infections and inflammatory conditions, might have strong effects on brain and behavior (sleep pattern, fatigue, libido, social withdrawal etc.) via cytokines and neuropeptides, released by immune cells and binding to hypothalamic cytokine receptors [13]. Salutary interventions that influence and dampen the hypothalamus-pituitary-adrenal gland (HPA)-axis include good nursing care, social support and social interactions. Women

with breast cancer frequently show compromised immune functions with elevated immunological activation and pro-inflammatory cytokines release [14]. A history of childhood maltreatment has been found associated with increased profiles of pro-inflammatory signaling [15], as well as cancer-related psychological distress [16].

Social support in breast cancer survivors is linked to lower inflammation and reduced amygdala reactivity (threat related brain regions) [14]. Oxytocin is a neuropeptide with psychosocial and psychophysiological functions, and a mediator of anti-stress, well-being, social interaction [17]. Immunological and genetic surveillance of several types of cancers including breast cancer have been reported [18].

The aim of this study was to evaluate immune and quality-of-life parameters in women receiving conventional versus anthroposophic integrated with conventional care for detection of possible differences in these parameters within the first 6 months after admission. The immune parameters were selected to reflect possible stress-related alterations in distribution of lymphocyte subsets, as well as inflammatory and anti-inflammatory conditions.

Materials and Methods

Study Design and Patients

The subjects were women with breast cancer who themselves chose the anthroposophic hospital for treatment. In the previously reported main study, 60 women who met the inclusion criteria, i.e. 75 years old or younger, residing in Stockholm, being able to communicate in Swedish, and expected to live and stay in Sweden for at least 6 months, were enrolled in the study from November 1995 to January 1999. Each woman received conventional medical cancer treatment in addition, and was individually matched with a woman (from an oncology out-patient department in the southern Stockholm area) who received conventional treatment only, as previously described [7]. All women in the A-group had anthroposophic care in addition to conventional care. The first 26 consecutive women from each group (n=60) that entered the study were selected for immunological analysis, thus the present study consists of 26 women in the A-group and 26 women in the C-group group, respectively. The mean age in the A-group group was 49.2 years (range 28 - 69 years) and the mean age in the C-group group was 49.9 years (range 36 – 68 years). The groups were similar also with regards to staging of breast cancer, and proportion of women that within the last 3 months had received cytostatic drugs, irradiation therapy, or no treatment (table 1). Neither age, staging nor treatment differed significantly between the groups (t-test and Chi-square test >0.1).

Ethical approval

All procedures involving human participants were approved after ethical investigation by the Regional Research Ethics Committee at the Karolinska Institute, Stockholm, Sweden: Dnr 95-293 and Dnr 01-015, E. Hamrin, in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards [19]. Informed consent was obtained from all individual participants included in the study.

Quality-of-Life Instruments

Three different instruments to evaluate QoL variables were used in this study. **1.** The mini-MAC is a revised version of the widely used MAC scale, developed for measuring mental adjustment to cancer in a general cancer population [11]. The five parameters are: a.

Helpless-Hopeless, e.g. “I feel completely at a loss about what to do” (8 items). b. Cognitive Avoidance, e.g. “I distract myself when thoughts about my illness come into my head” (4 items) c. Fighting Spirit, e.g. “I try to fight the illness” (4 items) d. Anxious Preoccupation, e.g. “I worry about the cancer returning or getting worse” (8 items) f. Fatalism, e.g. “I’ve had a good life; what’s left is a bonus” (5 items). **2.** LSQ consists of 34 items on 7-point scales [9]. The 34 items are allocated to six different factors as follows: physical symptoms (ps), sickness impact (si), quality of daily activities (qda), socioeconomic situation (ses), quality of family relation (qfa) and quality of close friend relationship (qfr). **3.** EORTC Study Group on Quality of Life has developed a modular measurement system for evaluating quality of life in cancer patients [20]. This consists of 30 items expressed in six functional scales and nine symptom scales. In order to avoid redundancy with the other scales and to reduce the number of parameters in the significance testing we decided to use only the “ql” (global quality of life) parameter from the EORTC scale.

Interventions

Both groups had been treated according to a special regional care program for all patients belonging to the Stockholm/Gotland region. The women who chose anthroposophic care were additionally treated with a special care program during their stay at the anthroposophic hospital (mean value 14 days, range 5–29 days). The individually composed anthroposophic therapies consisted of holistic care including natural products, Iscador (a cancer adjuvant medicament consisting of mistletoe extract), diets, art therapy, rhythmic therapy, therapeutic massage and hydrotherapy as previously described [8]. Most of the patients in the A-group continued to use Iscador after the first 2 weeks of anthroposophic hospital visit.

Procedure

In the previously reported main study, the women were followed for 5 years and were assessed by the questionnaires on six occasions as follows: on admission, after 1 month, after 3 months, after 6 months, after 1 year and after 5 years [8]. In the immunological study we used the questionnaire assessments on admission, after 3 months and after 6 months, which corresponds to the time points when the blood samples were obtained for measurements of immunological parameters.

Medical Investigations

These included laboratory tests, x-ray, scintigraphy, ultrasound of liver etc., but no differences between the groups were reported.

Immunological Analysis

An immunological test package including 14 variables was performed at admission, after 3 and 6 months, at the same time point when questionnaires were answered. Immune phenotyping by flow cytometry was done as part of a clinical laboratory facility at Karolinska Hospital Stockholm/Huddinge under the guidance of Dr Birger Christensson. Blood samples (2 x 5 ml EDTA-tubes for flow cytometry, and 1 x 5 ml in serum-tubes) were obtained in the morning (non-fasting) and processed within 24 hrs. Fresh cells were used to enumerate circulating leukocytes and lymphocytes using a cell counter, and proportions of T cells (CD3⁺), T-helper cells (CD3⁺CD4⁺), T-cytotoxic cells (CD3⁺CD8⁺), natural killer (NK) cells (CD3-CD56⁺), B cells (CD19⁺) were measured by FACScan flow cytometer equipped with Cell Quest software program (Becton, Dickinson and company (BD), San Diego, CA) using antibodies from BD. Immune cells were stained according to manufacturer's protocol and reported as proportion (%) of lymphocytes. The instrument gating was set to exclude dead cells as characterized by forward and side-way scatter. In addition, flow cytometry was used to assess activation status of T cell populations by expression of the proportion (%) of CD4⁺ or CD8⁺ T cells expressing the IL2-receptor (CD25), CD38 or HLA-DR (see table 1). In addition, levels of cytokines IL1 β , IL6 (pro-inflammatory) and IL10 (anti-inflammatory) were analyzed in batch by multiple bead array (Milliplex MAP human cytokine panel from Millipore, Billerica, MA), according to the manufacturer's instructions using frozen sera stored at -70° C. The lowest detection level was 3.2, 0.13 and 0.32 pg/mL, respectively. The neuropeptide oxytocin was analyzed by an enzyme-linked immunosorbent assay (Correlate-EIA oxytocin kit, Assay Designs, Ann Arbor, MI), according to the manufacturer's instructions. The lowest detection level was 4.7 pg/mL.

Statistical Analysis

The main question was to analyze whether A and C groups differed over time. A strict procedure, in order to avoid mass significance due to multiple testing, was therefore applied involving analysis of variance (ANOVA) for repeated measures (adjusted for sample size and age) to identify any significant differences between the two groups over time (0, 3 and 6 months). Only when a significant difference between the groups was found ($p \leq 0.05$), we subsequently used t-test to investigate any differences between the groups at each time point. These p-values were derived from one-sided testing, since it was at this point already noted which group had the higher mean value.

Results

Immunological Differences between Anthroposophic and Conventional Care Groups

We found a significant decrease over time in the proportion of cytotoxic CD8⁺ T-cells in the anthroposophic group compared with the conventional care group ($p=0.05$ by repeated measures ANOVA, corrected for age) (figure 1). At admission (time point “0”) there was no difference in the cytotoxic T cell fraction between the groups (t-test, $p=0.9$), while the decrease over time in the anthroposophic group led to a significantly lower proportion at 6 months (t-test, $p=0.01$ between-group comparison at one time point). Among the other immunological variables, including serum levels of IL1 β , IL6, IL10 or oxytocin, we found no significant differences between the groups over the course (table 2).

Quality of Life Differences between Anthroposophic and Conventional Care Groups

Regarding QoL measurements, the A-group showed a high level of anxiety (a MAC scale item) at inclusion ($p=0.01$, between-group comparison at one time point compared with the C-group, figure 2). However, the level decreased over time, which was in contrast to the C-group that had a low anxiety level at inclusion and remained low over time (repeated measures ANOVA, $p=0.04$ for comparison between groups over time). There was also a difference over time (repeated measures ANOVA, $p=0.05$) for physical symptoms (ps), one of the items in the life satisfaction questionnaire. The A-group started at a low level (= high physical symptoms = “bad condition”), $p=0.007$ (t-test at time point 0) compared with the C-group (figure 3) but increased (*i.e.* less physical symptoms) to 3 months and then remained the same, while the C-group had a high level at all points. Among the other variables, no significant differences over time were noted between the groups (table 3).

Discussion

This study on women with breast cancer, who were treated in two different regimens, included the first 6 months after admission to conventional or anthroposophic care and focused on immunological and QoL profiles and compared the patients in the two groups. Anthroposophic medicine today is very much used especially in many European countries [4] and several studies indicate both preclinical and clinical effects of cancer breast therapy adjuvants such as Iscadore, a mistletoe extract [21]. However, evidenced-based complementary medicine studies are scarce.

The major and most important finding of this study is that the proportion of cytotoxic CD8⁺ T cells decreased in A-group patients over the 6 months period, which was not found in the C-group. What is the function of CD8⁺ cells in breast cancer and what does the change in CD8⁺ cells imply? As a background, CD8⁺ T cells participate in the defense against viruses and certain cancers. Most tumor cells express antigens that can mediate an apparently specific and functional immune response including recognition by host CD8⁺ T cells. However, most tumors that are detected clinically must have evaded antitumor immune responses to grow progressively, pointing at strong tolerance inducing conditions [22]. Recent therapies are therefore focused on breaking this tolerance using therapeutic monoclonal antibodies against molecules (called check-points of immunity) that decide activation versus tolerance of immune responses, for example PD-1, PD-L1 or CTLA-4 [23]. Humanized antibodies against these targets are used for treatment of several tumor types and now evaluated in clinical trials including breast cancer [24]. In breast cancer, significantly higher blood CD8⁺ T cell frequencies, both total CD8⁺ cells and especially memory CD8⁺ cells, were observed in patients with metastatic (more aggressive) versus non-metastatic (less aggressive) disease [25]. The authors observed an increase in CD8⁺ frequencies both in the tumor microenvironment, and in the circulation [25]. Based on these observations, we suggest the following interpretation of our findings: The statistically significant reduction of CD8⁺ cells over time (repeated measures ANOVA) found in the A-group, but not in the C-group, may indicate that complementary treatment generates a less aggressive disease, at least during the observation time of 6 months. Sixteen of the 21 women in the A-group who took part in the 5-year follow up had continued the contact with the anthroposophic hospital in different ways, but it is unknown as to what extent [8]. However, in a 5 year follow-up study, there was no difference in mortality between the two groups [8]. An important aspect of further studies is to characterize the different CD8⁺ subtypes such as naïve and memory CD8⁺ T cells

and their differentiation state, particularly in view of the fact that tumor cells potentially induce immunosenescence reminiscent of cytomegalovirus-induced immunosenescence [25].

The anthroposophic A-group showed a high level of anxiety at inclusion in contrast to the C-group. This fact points at some limitations of the study, which must be considered. First, a randomized clinical study would have been preferable, but it was not possible considering the resources available and the regulations of the Swedish health care system. Thus, the present design with individually matched pairs was an option. But since the women took initiatives themselves to be referred to anthroposophic care, there could be differences between the women in A-group and the women in the C-group that were not controlled for by the matching procedure. The women were individually matched regarding stage of disease, age, treatment during the 3 months before entering the study and prognosis. There were no differences between the groups regarding children or marital status, but there was a difference regarding the professions of the women on admission of the study [26]. In the A-group 23% compared with none in the C-group were working as artists, journalists or other cultural workers. This could be one explanation to why some of the women choose the anthroposophic hospital and it could also mean that the two groups differed psychologically ie. anxiety level differed at admission. Noteworthy, personality traits (extraversion vs. introversion) have been identified to show different gene expression profiles related to pro-inflammatory signaling [27]. It is also known that stress-release programs, ethical caring, choir singing, and psychotherapy have beneficial effects for women with breast cancer [28-31], and an important stress-reducing aspect of anthroposophic care is the individually composed therapy consisting of holistic care including natural products, Iscador, diets, art therapy, rhythmic therapy, therapeutic massage and hydrotherapy [3, 4]. A deeper psychoneuroimmunological understanding of these effects induced by intervention programs are needed [32].

Fourteen immunological parameters were longitudinally investigated in the present study. In the evaluation process over time, from time of admittance, to 3 months and finally at 6 months, it is important to recognize that each patient is its own control, which is a strength of this study. There is a large inter-individual variation in immune parameters such as lymphocyte populations. However, the intra-individual variation over time is limited, hence the inter-individual variation is overcome by the design of the study [33]. A potential limitation was that several immunological and QoL parameters were investigated. However, the issue of mass-significance was overcome by using a strict and stringent statistical approach taking the multi-parameter design into account.

In conclusion, the present results indicate that complementary therapy did not differ significantly in their immune profiles over time compared with conventional therapy, with one significant exception of decreased cytotoxic CD8⁺ T - cells in the anthroposophic group. Significantly improved physical symptoms and less anxiety over time in this group may have influenced this reduced frequency of CD8⁺ T cells, a feature reported to be associated with less aggressive cancer stages. This finding is a basis for further studies on memory and naïve CD8⁺ T function in breast cancer and the relation to good or bad prognosis, particularly in view of recent findings that CD8⁺ memory cytotoxic T cells have shorter telomere length and are associated with stress related hormones and lifestyle changes [34] [35].

Disclosure statement

The authors declare that there is no conflict of interests.

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Table 1. Matching of patients at admission

| Matching Parameter ¹ | Anthroposophic group n=26 | Conventional group n=26 |
|---|------------------------------|----------------------------|
| Stage of disease | | |
| Advanced | 9 | 10 |
| Limited | 17 | 16 |
| Age | | |
| Mean/SD | 49.2/9.4 | 49.9/7.8 |
| >50 years | 10 | 9 |
| ≤50 years | 16 | 17 |
| Prognosis | | |
| Favorable | 10 | 11 |
| Unfavorable | 16 | 15 |
| Treatment (cytostatics and/or x-ray) 3 months before admission | 19 | 19 |
| Supportive anthroposophic care also including Iscador | 26 | 0 |

Matching was tested with chi-square for categorical variables and t-test for continuous data, all p-values were >0.1.

¹The matching criteria were based on:

a) disease stage at entry—advanced inoperable tumor, distant metastases, or operable tumor with or without node metastases;

(b) age—over or under 50 years;

(c) treatment during the 3 months before entering the study—radiotherapy, chemotherapy, or no treatment; and (d) prognosis—favorable or unfavorable.

The prognosis was related to the frequency and location of metastases. The criteria for a favorable prognosis included inoperable disease confined to the breast, or limited to regional or distant lymph nodes, lung, or bone. Patients with liver or other visceral metastases, or several metastatic sites, were defined as having an unfavorable prognosis. Prognosis was only applied as a matching variable in cases with metastatic disease.

Table 2. Immunological markers, comparison between anthroposophic (A) and conventional (C) groups over time (repeated measures ANOVA at admission (0 month), 3 and 6 months)*.

| Immunological parameters | Description | p-value |
|--------------------------|---|---------|
| LPK | Leukocyte count | 0.32 |
| Ly | Lymphocyte count | 0.91 |
| CD3 | Pan T cell marker | 0.32 |
| CD4 | T helper cells | 0.16 |
| CD8 | T cytotoxic cells | 0.05* |
| CD19 | B cells | 0.82 |
| CD56 | Natural killer cells | 0.70 |
| CD25 on CD4 cells | IL2 receptor, activation marker on CD4 ⁺ cells | 0.07 |
| CD38 on CD8 cells | CD38, activation marker on CD8 ⁺ cells | 0.88 |
| HLA-DR on CD8 cells | HLA-DR, activation marker on CD8 ⁺ cells | 0.71 |
| IL-1 β | Interleukin 1-beta, proinflammatory cytokine | 0.87 |
| IL-6 | Interleukin 6, proinflammatory cytokine | 0.96 |
| IL-10 | Interleukin 10, anti-inflammatory cytokine | 0.17 |
| oxytocin | Neuropeptide | 0.30 |

*Adjusted for age and sample size

Major lymphocyte populations (T, B, NK, CD4, CD8) were expressed as proportion (%) of lymphocytes. Subpopulations were expressed as proportion (%) of their mother population (CD4 or CD8). The CD4 and CD8 populations were also positive for CD3, while NK cells were negative for CD3.

Cytokine and oxytocin levels in serum were analyzed by multiplex bead technology and ELISA, respectively.

Table 3. Quality-of-Life variables, differences between anthroposophic (A) and conventional (C) group (ANOVA repeated measures over time 0, 3, and 6 months)

| QoL abbreviation | Description | Instrument | p-value |
|------------------|----------------------------------|------------|---------|
| fighting | fighting spirit | MAC scale | 0.85 |
| anxiety | anxiety | MAC scale | 0.04 |
| hope | helpless-hopeless | MAC scale | 0.69 |
| fatal | fatalism | MAC scale | 0.23 |
| avoid | avoidance | MAC scale | 0.44 |
| ps | physical symptoms | LSQ scale | 0.05 |
| si | sickness impact | LSQ scale | 0.18 |
| qda | quality of every day | LSQ scale | 0.96 |
| ses | socioeconomic situation | LSQ scale | 0.32 |
| qfa | quality of family relation | LSQ scale | 0.94 |
| qfr | quality of close friend relation | LSQ scale | 0.47 |
| ql | global quality of life | EORTC | 0.77 |

Figure legends

Fig. 1. The proportion of CD8⁺ cytotoxic T-cells over time. Comparison of conventional (C-group, blue line) and anthroposophic group (A-group, green line). At time of admission (0 months), and at 3 months there was no difference, but the proportion decreased in the A-group compared with the C-group (repeated measures over time ANOVA, $p=0.05$) and there was a significant difference at 6 months ($p=0.01$, t-test between groups at one time point). The mean values are corrected for sample size and age. SEM values are given for each mean.

Fig. 2. The anxiety level is higher in the A-group at inclusion ("0"), but decreases over time compared with the C-group (repeated measures over time ANOVA, $p=0.04$). The mean values are corrected for sample size and age. SEM values are given for each mean.

Fig. 3. Physical symptoms (ps) over time. The A-group scored low (=high load/suffering of physical symptoms) at inclusion compared with the C-group. During the course there is an increase (=improvement) in the A-group compared with the C-group (repeated measures over time ANOVA, $p=0.05$). The mean values are corrected for sample size and age. SEM values are given for each mean.

Figure 1

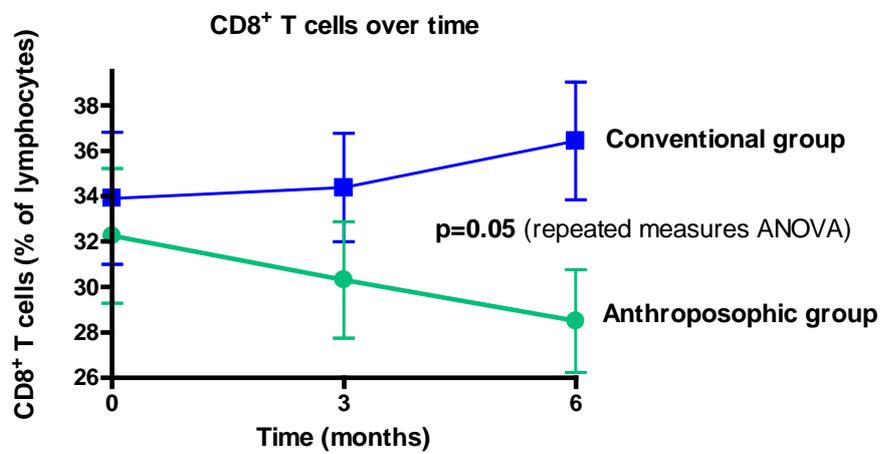


Figure 2

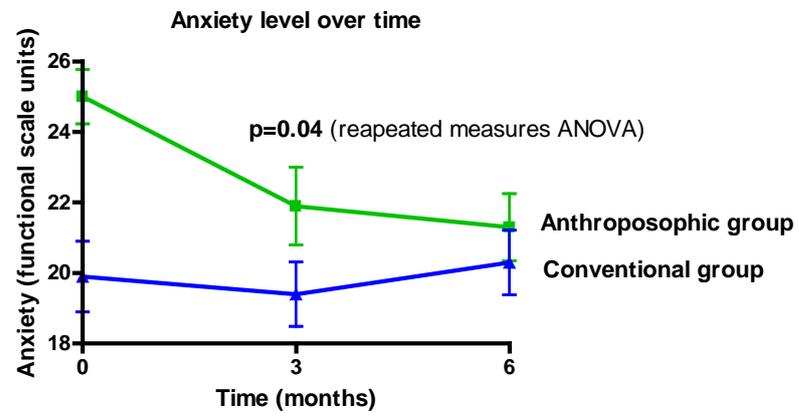


Figure 3

