

Study on IL-2 and CA 15-3 level as combined biomarkers in monitoring chemotherapeutic response among invasive breast cancer patients

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Abstract. In Malaysia, breast cancer is the most frequent type of disease among women. This study was designed to determine the clinical usefulness of carbohydrate antigen (CA 15-3) and interleukin 2 (IL-2) levels as combined biomarkers in monitoring breast cancer patient's response to chemotherapy. Ethical approval was obtained to recruit patients with histologically confirmed invasive ductal carcinoma (IDC) attending Oncology Clinic at Advanced Medical and Dental Institute. Whole blood was collected from 10 IDC breast cancer patients' pre and post primary chemotherapy. Plasma was separated from the whole blood to determine the CA 15-3 level and IL-2 level using enzyme-linked immunosorbent assay (ELISA) pre and post-treatment. In addition, the histological findings, tumour stage and other patients' data were obtained from the medical record. Findings showed that IL-2 had borderline significant changes between pre- and post-chemotherapy ($p = 0.074$) whereas for CA 15-3, there was insignificant differences of CA 15-3 level between pre and post-chemotherapy ($p > 0.05$). It was noted that only CA 15-3 level had significant correlation with tumour size. This study demonstrates that IL-2 level requires further investigation in a larger sample size to correlate its potential use as combined biomarker with CA 15-3 in monitoring response to chemotherapy.

1. Introduction

The *MUC1* gene was first recognized in human milk, where it is shed from the milk secreting epithelial cells [1]. Further, it is now known to be expressed on different types of hematopoietic cells as well [2, 3]. However, this polymorphic transmembrane glycoprotein is frequently overexpressed on malignant glandular cell surfaces, resulting in increased levels shed into the blood of some patients with carcinomas [4, 5]. Numerous circulating mucinous markers, including carbohydrate antigen 15-3 and 27.29 (CA 15-3 and CA 27.29) are secreted products of the polymorphic *MUC1* gene, and can be used as biomarkers in patients with breast cancer [2]. Although high levels of the protein CA 15-3 is produced by tumour tissue, it is not usually elevated in serum until the cancer has reached an advanced stage. A high CA15-3 level of about >32 U/mL typically indicates advanced breast cancer and a larger tumour burden [6]. Both CA 15-3 and CA 27.29 antigen are approved by the FDA as a biomarker for breast cancer in 1997 for the detection of recurrent breast cancer, and also in the monitoring of the response to therapy for metastatic breast cancer [6, 7]. However, they have to be used in conjunction with diagnostic imaging, clinical history, and physical examination [8, 9].

Interleukin-2 (IL-2) is a multi-potential cytokine secreted by T helper 1 cells (Th1) which is involved in the growth and differentiation of a wide range of immunologic functions, particularly in T



and B cells. Additionally, it enhances the cytolytic functions of natural killer (NK) cells [10-13]. It is also known to have some function in the proliferation of several non-lymphoid cells [11]. Interleukin-2 gene expression in hepatocarcinoma patient with interleukin-2 positive tumours was reported to show favourable prognosis [14]. A previous study also suggested that reduced plasma IL-2 level concentration in patients with breast cancer was a possible risk factor for relapse [15]. In fact, this study is the first to assess IL-2 level as a potential biomarker in monitoring breast cancer patient's response to chemotherapy. Early detection of breast cancer is a challenge due to its heterogeneity and complexity. Thus, a profile test with multiple biomarkers should be conducted to characterize the full spectrum of this heterogeneous disease rather than a single analyte [16]. In this study, CA 15-3 and IL-2 levels were measured pre and post-chemotherapy treatment to assess their usefulness in monitoring patient's response to chemotherapy.

2. Materials and Method

The aim of this study was to monitor response to chemotherapy based on both CA-153 and IL-2 level instead of a single biomarker. This was achieved by measuring the difference in both biomarkers (CA-153 and IL-2) level pre- and post-chemotherapy among invasive breast cancer patients.

2.1. Sample size, patients selection and ethical approval

Sample size was calculated using PS software version 3.0.43 (Dupont & Plummer, 1997) based on comparing 2 means. To detect the difference of 7.5 pg/ μ l in IL-2 with 80% power and $\alpha = 0.05$, 9 subjects are required (SD was estimated 7.19 pg/ μ l). By anticipating a 10% attrition rate, a total of 10 subjects were recruited in this study. A total of 10 patients attending Oncology Clinic at Clinical Trial Complex (CTC), Advanced Medical and Dental Institute (AMDI) were recruited in this study after obtaining the ethical approval. Inclusion criteria were based on histologically confirmed invasive breast cancer patients of ductal origin and women who were post pubertal. Meanwhile, exclusion criteria include tumours of non-ductal origin, male breast carcinoma, and women who were pre pubertal.

2.2. Biochemical analysis

From each patient, (1-2 ml) venous whole blood sample was collected pre and post primary chemotherapy in EDTA anticoagulant tubes. The samples were centrifuged at 4000 rpm for 5 minutes to separate the plasma for clinical chemistry analysis. The plasma was transferred into Eppendorf tubes and stored at -80°C until analysis. Clinical chemistry analyses were done to determine the levels of the tumour markers i.e. CA 15-3 and IL-2 in the patients' blood by ELISA method (enzyme-linked immunosorbent assay). CanAg CA15-3 EIA kit (Fujirebio Diagnostics, Japan) was used for measuring the level of CA 15-3 in the patient's plasma as recommended by the manufacturer's instructions.

2.3. Statistical analysis

Data was analysed using IBM SPSS Statistics v.20 software (SPSS Inc., Chicago, IL, USA). Distribution normality tests were performed using histogram (SPSS). The quantitative variables were summarized as median and Interquartile Range (IQR) with 95% Confidence interval (CI) as they were not normally distributed. Accordingly, Wilcoxon Signed Ranks non-parametric two-tailed test was used to compare the median of the tumour marker levels before and after chemotherapy. The correlation between the levels of each tumour marker and tumour size and lymph node metastasis was determined by using Spearman's Rank Correlation Coefficient test and p -values less than 0.05 were regarded as statistically significant.

3. Results

3.1. Patients' demographic characteristics and histological findings

All of the 10 patients were females from Malaysian population with histologically confirmed IDC (invasive ductal carcinoma), and who were post pubertal receiving primary chemotherapy. The histological findings, tumour stage and other patients' data were obtained from the patients' medical

record in Oncology Clinic at AMDI. The mean age of the patients was 48.4 years (range 40-67 years). Tumour size was classified as T2 (tumour size between 2 and 5 cm in greatest dimension) in 7/10 patients (70%) and T4 (tumour extends to chest wall) in 3/10 (30%) of cases according to the TNM classification. The percentage of patients with negative lymph nodes and positive nodes was 50 % for each. Whole blood samples were collected pre and post different cycles of primary chemotherapy. The number of samples collected in cycle 1 was 3/10 (30%) and in cycle 2 was 6/10 (60%) whereas in cycle 4 was 1/10 (10%).

3.2. Biochemical findings

The level of CA 15-3 for each sample before and after treatment is shown in Figure 1. There was no significant difference of CA 15-3 levels in the samples of the patients pre and post-chemotherapy as shown in Table 1. Correlation between the levels of CA 15-3, tumour size and lymph node status was determined by using Spearman's Rank Correlation Coefficient test. There was a significant moderate positive correlation between the CA 15-3 level and the tumour size $r = 67\%$ ($p < 0.05$) (Figure 2). Meanwhile, the correlation between CA 15-3 level and lymph node status was moderate positive $r = 56\%$ ($p > 0.05$) even though the p -value did not show any significant results. The level of IL-2 for each sample before and after treatment is illustrated in Figure 3. No significant difference of IL-2 levels was found in the samples of the patient's pre and post-chemotherapy as shown in Table 2. Correlation between the IL-2 level, tumor size and lymph node status was determined by using Spearman's Rank Correlation Coefficient test. No significant correlation was observed between IL-2 level and the tumour size $r = 25\%$ ($p > 0.05$) or between IL-2 level and lymph node status in patients $r = 22\%$ ($p > 0.05$) as well.

Table 1: The patients' demographic data

Study participant No.	Age (year)	Height (cm)	Weight (kg)	Cycle of collection	Chemotherapy x no. of cycles	Tumor stage (TNM) system	Tumor size (cm)	Size (cm) in max. dimen.
1	50	138	35.0	C4	TC x4	T4NxMx	15x15x5	15
2	40	154	56.5	C2	AC-T x4	T4N2Mx	12x12	12
3	43	156	63.6	C1	AC x4	T2N0Mx	4.5x3.5x3.4	4.5
4	41	160	62.0	C2	AC-T x4	T2N2M0	4x3x2.5	4
5	45	162	73.8	C2	AC-T x4	T2N1M0	2.8x2.5x3.8	3.8
6	42	155	56.0	C2	FEC x6	T2N0Mx	3.5x2.5x2.5	3.5
7	60	142	79.0	C2	AC-T x4	T2N2Mx	3.5x2.5x2	3.5
8	49	158	53.2	C2	AC x4	T2N0Mx	1x1x3	3
9	67	149	67.4	C1	AC-T x4	T4NxM1	17x13	17
10	47	142	51.7	C1	AC-T x4	T2N1Mx	5x3x2	5

A, Adriamycin; C, Cyclophosphamide; T, Docetaxel; F, Fluorescent; E, Epirubicin

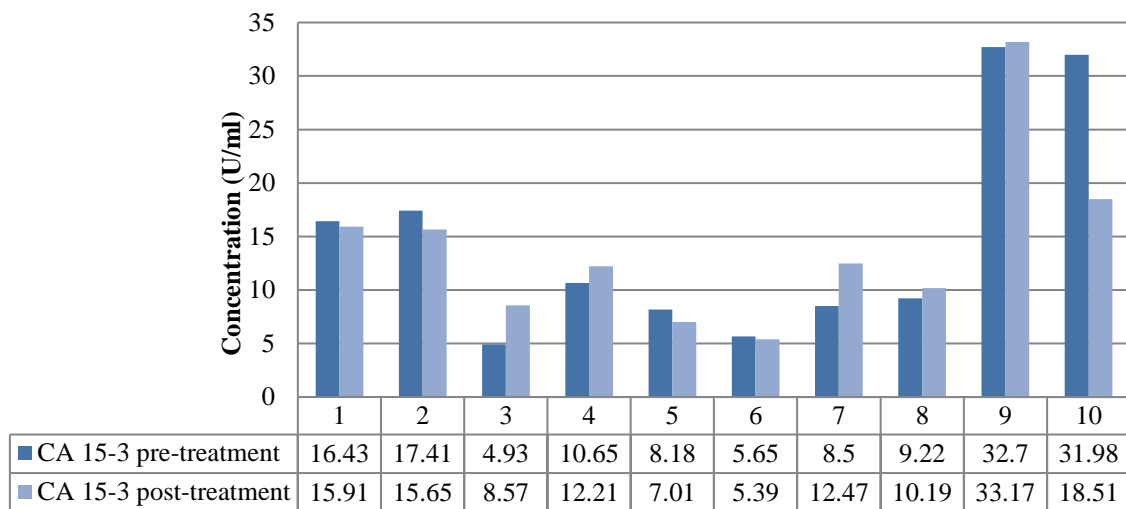
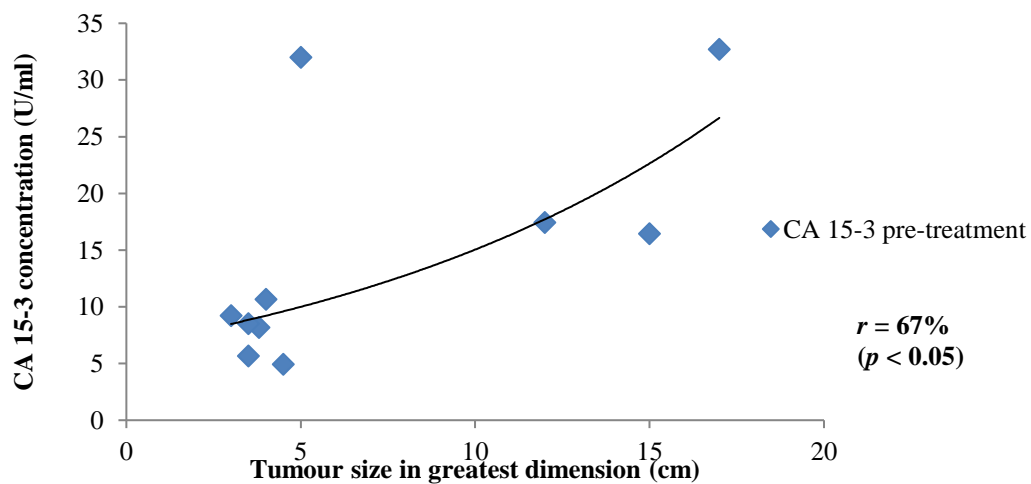


Figure 1: The levels of CA 15-3 in the samples pre and post-chemotherapy

Table 2: Statistical analysis on difference of CA 15-3 concentration pre and post-treatment

Variable	No. of subjects	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	Z statistic ^a	P value ^a
CA15-3 (all C)	n =10	9.9 (13.51)	12.3 (8.38)	-0.153	0.878
CA 15-3 (C1)	n =3	31.9 (-)	18.5 (-)	0.0	1.0
CA 15-3 (C2)	n =6	8.8 (4.79)	11.2 (6.66)	-0.314	0.73

^a Wilcoxon Signed Ranks Test, IQR = Interquartile Range, C = Cycle of chemotherapy



*r: correlation coefficient

Figure 2: The correlation between the level of CA 15-3 in the samples of the patients (x-axis) and tumour size in the greatest dimension (y-axis)

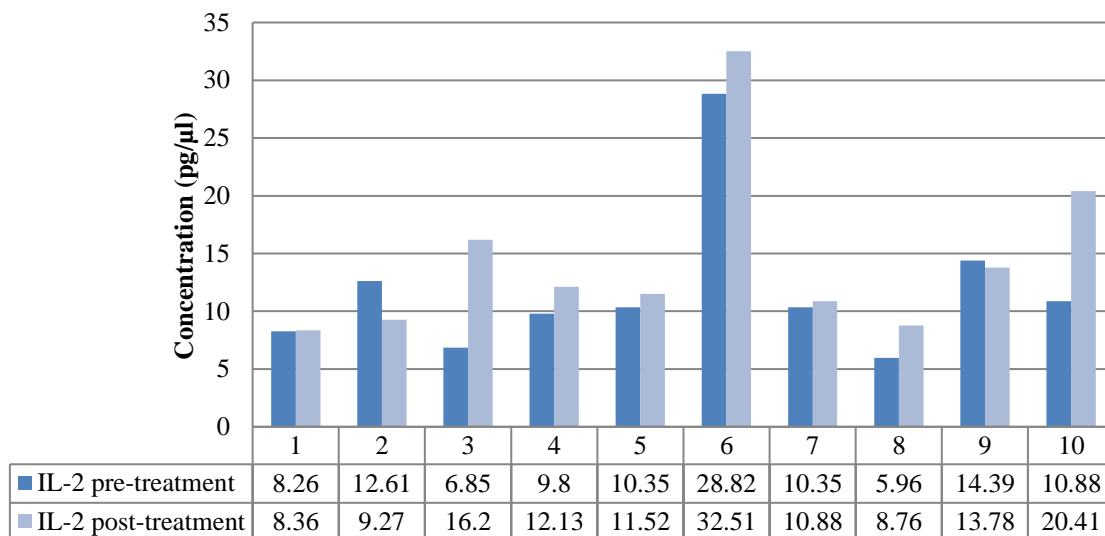


Figure 3: The levels of IL-2 in the samples of the patients' pre and post-chemotherapy

Table 3: Statistical analysis on difference of IL-2 concentration pre and post-treatment

Variable	No. of subjects	Pre-treatment Median (IQR)	Post-treatment Median (IQR)	Z statistic ^a	P value ^a
IL-2 (all C)	$n = 10$	10.3 (5.15)	11.8 (8.11)	- 1.784	0.074
IL-2 (C1)	$n = 3$	10.8 (-)	16.2 (-)	-1.069	0.285
IL-2 (C2)	$n = 6$	10.3 (7.28)	11.2 (8.08)	- 1.153	0.249

^a Wilcoxon Signed Ranks Test, IQR = Interquartile Range, C = Cycle of chemotherapy

4. Discussion

In this study, the results revealed that only 20 % of the patients had CA 15-3 level higher than the cutoff (> 30 U/ml) and there was insignificant differences of CA 15-3 level between pre and post-chemotherapy ($p > 0.05$). Additionally, no significant correlation ($p > 0.05$) was found between CA 15-3 and lymph node status, whilst there was a significant correlation ($p < 0.05$) between the level of this biomarker and the tumour size, suggesting that tumour tissue itself may be the source of serum CA 15-3 [16]. Previously higher CA 15-3 level was reported to correlate with larger tumour size and more advanced disease [17-19]. A study in which 818 patients with histologically verified diagnosis of invasive breast cancer had their CA 15-3 serum level determined pre-operatively. Finding showed that 15.2% of patients with clinically non-metastatic breast cancer had high serum CA 15-3 levels (> 30 U/ml) [20]. However, according to the present literature, CA 15-3 is not specific or sensitive enough to detect early breast cancer and the elevation of CA15-3 is not a reliable indicator in the diagnosis of breast cancer since normal serum levels can also be found in women with breast cancer [18].

Further analysis on IL-2 level was done to correlate its level with CA 15-3. We noted 80 % of the patients showed higher levels of IL-2 in post-chemotherapy than pre-chemotherapy and this result was in line with studies done by Fang et al., (2006) and Orditura et al., (2000). The IL-2 level showed borderline significant changes between pre- and post-chemotherapy ($p = 0.074$). Additionally, no significant correlation was observed between IL-2 level and the tumour size ($p > 0.05$) or between IL-2 level and lymph node status in patients ($p > 0.05$). IL-2 is a cytokine released from T helper lymphocytes. It promotes the generation, proliferation, differentiation of T lymphocytes, enhances the activity of natural killer cells, induces the generation of lymphokine-activated killer (LAK) cells, and promotes the production of antibodies by B lymphocytes. Through these mechanisms, it plays a vital role in antitumor immune responses [10, 21]. The increased IL-2 level in this study demonstrated that anti-tumour immune response of the patients was increased after receiving chemotherapy. Similar result was obtained from another study on IL-2 serum level among 60 advanced non-small-cell lung

(NSCL) cancer patients whereby a significant increase in IL-2 level was observed following chemotherapy [22].

5. Conclusion

This study revealed that IL-2 level was borderline significant when compared between pre- and post-chemotherapy, whereas CA15-3 serum level had significant correlation with the tumour size. It suggests the use of IL-2 in monitoring response to chemotherapy but requires further investigation in a larger sample size to correlate its potential as combined biomarker with CA 15-3.

6. References

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Acknowledgement

This study was funded by AMDI Student Research Fund, USM.