INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

POTENTIAL PROGNOSTIC ROLES FOR IL-6 AND CRP IN IRAQI WOMEN WITH BREAST CANCER

[a]Janan Majeed Al-Akeedi, [b]Aqeel Shakir Mahmod & [c]Maha Abed Ali
[a]Department of Medical Analysis, College of Pharmacy / University of Baghdad/Iraq
[b]Oncologist/Baghdad Hospital/Baghdad/Iraq
[c]Cancer Central Hospital/Baghdad/Iraq

ABSTRACT
Breast cancer is a disease that continues to plague females during their entire lifetime. IL-6 and CRP are found to be elevated in various inflammatory and malignant diseases and their levels are found to correlate with the extent of the disease. A total of 80 Iraqi female patients attending the National Cancer Institute in Baghdad Teaching Hospital were subjected to this study The primary objective of this study was to determine the preoperative serum levels of IL-6 and CRP in breast carcinoma and to compare the two parameters in regards to their reliability in the prognosis of breast cancer in Iraqi women. ROC analysis shows that using IL-6 with cutoff point ≥11.3 has a sensitivity of 50% and specificity of 100% and area under curve (UAC) 0.79, 95% CI (0.687-0.875), P=0.0001, whereas the corresponding values of sensitivity and specificity and for CRP were 61.7, 71.2 respectively and the AUC was 0.65, 95% CI(0.54-0.76), P=0.009. Results show that the comparison between two ROC curves was not significant (P=0.056), but in such diseases it was well known that the specificity is very important to prevent treating normal people with cancer drugs, hence applying the two tests could be more safe to detect the prognosis of breast cancer in Iraqi women. The primary test is CRP (higher sensitivity) and IL-6 as a completing test to confirm the absent of disease because of its high specificity.

KEYWORDS: IL-6 CRP breast cancer ROC curve.

INTRODUCTION

Cytokines are intracellular signaling polypeptide produced by activated cells that regulate immune responses, the acute phase reaction and hematopoiesis and play a central role in host defense (Hirano et al.1990).IL-6 is a glycoprotien of molecular weight of 26KDa ,composed of 184 amino acids (Kashimoto, 2006). IL-6 have multiple activities ,it has been suggested that IL-6 is the main factor involved in host response to a foreign pathogen, also plays a role in immunological response and inflammation (Culig *et al*., 2005). Its produced by monocytes and macrophages and in a smaller percentage of fibroblasts ,endothelial cells, lymphocytes T and B, chondrocytes and amnion cells. The production of IL-6 and interferon, tumor necrosis factor, DNA viruses and RNA viruses (Gobal *et al*., 2002).In addition IL-6 activates the synthesis of glucocorticoids by secreting corticotropin and adenocorticotropin (Scheller *et al*., 2006). CRP is a protein produced in the liver, predominantly under transcriptional control by the cytokine IL-6 originating from the site of pathology. CRP is a classical acute phase protein displaying rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection and tissue damage (Johnson, 2006). Previous epidemiological studies have reported that elevated CRP levels may be associated with poor prognosis of several types of solid cancers (Allins *et al*., 2009) including endometrial, cervical, colorectal, pancreatic, hepatocellular, esophageal, renal cells, bladder, prostate, ovarian and non-small-cell lung cancer (Dowd *et al*., 2010).

MATERIALS & METHODS

Patients

A total of 80 Iraqi female patients who were attending the National Cancer Institute in Baghdad Teaching Hospital. The patients were divided into two clinical subgroups:
Group 1: 60 patients with breast cancer
Group 2: 20 women have no cancer (control)
Basic blood investigations, chest x-ray, ECG and CT scan were done for all the patients and the diagnosis of cancer were confirmed.

Samples

Blood samples were drawn for IL-6 and CRP levels on admission. The blood samples were collected using standard tubes and stored at 20-25 C.
The samples for IL-6 were analyzed using ELISA, while the samples for CRP were measured by immune agglutination test.

Statistical analysis

Data of IL-6 were tested for normality by using frequency distribution plot and the D Agonstino-Pearson test was also used to confirm the result. As the data was not normally distributed, the Mann-Whitney test was used to compare the significant differences between control and patients. ROC curve was applied to evaluate the effective of the two parameters in detection of breast cancer in Iraqi women. Youden Index was submitted to identify the cutoff

point of IL-6. The analysis of data was submitted by MedCalc software.

RESULTS

The data of IL-6 were not normally distributed (Figure 1) which was also confirmed by D'Agostino-Pearson test (P <0.0001). Mann-Whitney test was used to compare the differences in IL-6 between control and patients and the differences were significant (P=0.0001) (Median for control= 7.15, patients=11.4). ROC curve for IL-6 shows that the cutoff point according to youden index was ≥11.3 with a sensitivity of 50% and specificity of 100% (Figure 2) and area under curve (UAC) 0.79, 95% CI (0.687-0.875),P=0.0001.Criterion values and coordinates of the ROC curve for IL-6 were shown in table (1).

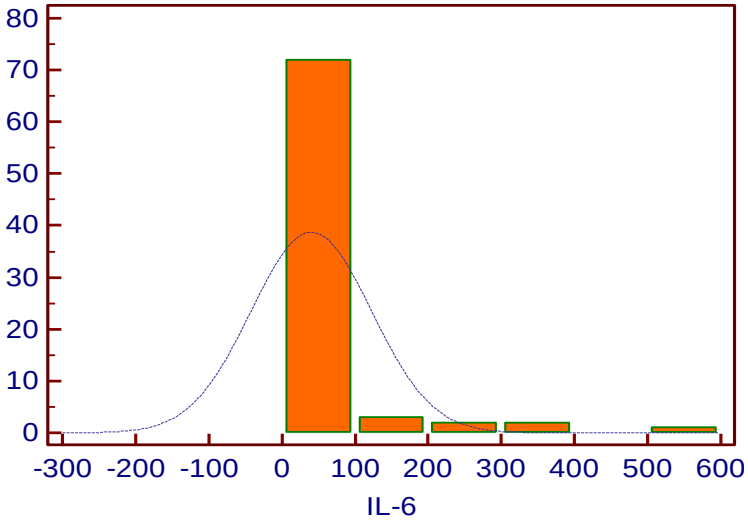

FIGURE 1: Frequency distribution of IL-6

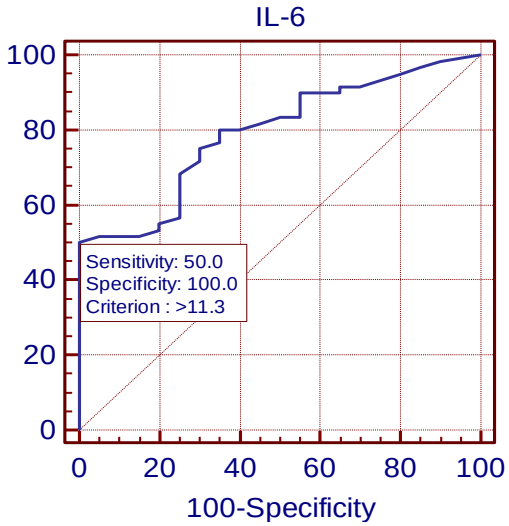

FIGURE 2: ROC curve for IL-6

TABLE 1: Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI
≥5.2	100.00	94.0 - 100.0	0.00	0.0 - 16.8	1.00	1.0 - 1.0		
>5.2	98.33	91.1 - 100.0	10.00	1.2 - 31.7	1.09	0.9 - 1.3	0.17	0.02 - 1.7
>5.5	96.67	88.5 - 99.6	15.00	3.2 - 37.9	1.14	0.9 - 1.4	0.22	0.04 - 1.2
>5.8	95.00	86.1 - 99.0	20.00	5.7 - 43.7	1.19	0.9 - 1.5	0.25	0.06 - 1.0
>6	91.67	81.6 - 97.2	30.00	11.9 - 54.3	1.31	1.0 - 1.8	0.28	0.09 - 0.8
>6.1	91.67	81.6 - 97.2	35.00	15.4 - 59.2	1.41	1.0 - 2.0	0.24	0.08 - 0.7
>6.3	90.00	79.5 - 96.2	35.00	15.4 - 59.2	1.38	1.0 - 1.9	0.29	0.1 - 0.8
>6.4	90.00	79.5 - 96.2	40.00	19.1 - 63.9	1.50	1.0 - 2.2	0.25	0.10 - 0.6
>6.5	90.00	79.5 - 96.2	45.00	23.1 - 68.5	1.64	1.1 - 2.5	0.22	0.09 - 0.5
>6.7	86.67	75.4 - 94.1	45.00	23.1 - 68.5	1.58	1.0 - 2.4	0.30	0.1 - 0.7
>6.8	83.33	71.5 - 91.7	45.00	23.1 - 68.5	1.52	1.0 - 2.3	0.37	0.2 - 0.8
>7.1	83.33	71.5 - 91.7	50.00	27.2 - 72.8	1.67	1.1 - 2.6	0.33	0.2 - 0.7
>7.2	81.67	69.6 - 90.5	55.00	31.5 - 76.9	1.81	1.1 - 3.0	0.33	0.2 - 0.6
>7.3	80.00	67.7 - 89.2	60.00	36.1 - 80.9	2.00	1.2 - 3.5	0.33	0.2 - 0.6
>7.4	80.00	67.7 - 89.2	65.00	40.8 - 84.6	2.29	1.2 - 4.2	0.31	0.2 - 0.6
>7.5	78.33	65.8 - 87.9	65.00	40.8 - 84.6	2.24	1.2 - 4.1	0.33	0.2 - 0.6
>7.6	76.67	64.0 - 86.6	65.00	40.8 - 84.6	2.19	1.2 - 4.0	0.36	0.2 - 0.6
>7.8	75.00	62.1 - 85.3	70.00	45.7 - 88.1	2.50	1.3 - 5.0	0.36	0.2 - 0.6

>7.9	73.33	60.3 - 83.9	70.00	45.7 - 88.1	2.44	1.2 - 4.9	0.38	0.2 - 0.6
>8	71.67	58.6 - 82.5	70.00	45.7 - 88.1	2.39	1.2 - 4.8	0.40	0.2 - 0.7
>8.1	68.33	55.0 - 79.7	75.00	50.9 - 91.3	2.73	1.3 - 6.0	0.42	0.3 - 0.7
>8.3	66.67	53.3 - 78.3	75.00	50.9 - 91.3	2.67	1.2 - 5.8	0.44	0.3 - 0.7
>8.4	63.33	49.9 - 75.4	75.00	50.9 - 91.3	2.53	1.2 - 5.5	0.49	0.3 - 0.7
>9	61.67	48.2 - 73.9	75.00	50.9 - 91.3	2.47	1.1 - 5.4	0.51	0.3 - 0.8
>9.2	56.67	43.2 - 69.4	75.00	50.9 - 91.3	2.27	1.0 - 5.0	0.58	0.4 - 0.8
>10	55.00	41.6 - 67.9	80.00	56.3 - 94.3	2.75	1.1 - 6.8	0.56	0.4 - 0.8
>10.2	53.33	40.0 - 66.3	80.00	56.3 - 94.3	2.67	1.1 - 6.6	0.58	0.4 - 0.8
>10.8	51.67	38.4 - 64.8	85.00	62.1 - 96.8	3.44	1.2 - 10.1	0.57	0.4 - 0.8
>11	51.67	38.4 - 64.8	90.00	68.3 - 98.8	5.17	1.4 - 19.7	0.54	0.4 - 0.7
>11.2	51.67	38.4 - 64.8	95.00	75.1 - 99.9	10.33	1.5 - 70.9	0.51	0.4 - 0.7
>11.3	50.00	36.8 - 63.2	100.00	83.2 - 100.0			0.50	0.4 - 0.6
>11.5	48.33	35.2 - 61.6	100.00	83.2 - 100.0			0.52	0.4 - 0.7
>12.2	46.67	33.7 - 60.0	100.00	83.2 - 100.0			0.53	0.4 - 0.7
>13.6	45.00	32.1 - 58.4	100.00	83.2 - 100.0			0.55	0.4 - 0.7
>15.5	43.33	30.6 - 56.8	100.00	83.2 - 100.0			0.57	0.5 - 0.7
>15.6	41.67	29.1 - 55.1	100.00	83.2 - 100.0			0.58	0.5 - 0.7
>16.7	40.00	27.6 - 53.5	100.00	83.2 - 100.0			0.60	0.5 - 0.7
>16.8	38.33	26.1 - 51.8	100.00	83.2 - 100.0			0.62	0.5 - 0.8
>21.6	36.67	24.6 - 50.1	100.00	83.2 - 100.0			0.63	0.5 - 0.8
>22.6	35.00	23.1 - 48.4	100.00	83.2 - 100.0			0.65	0.5 - 0.8
>27.1	33.33	21.7 - 46.7	100.00	83.2 - 100.0			0.67	0.6 - 0.8
>34.6	31.67	20.3 - 45.0	100.00	83.2 - 100.0			0.68	0.6 - 0.8
>34.7	30.00	18.8 - 43.2	100.00	83.2 - 100.0			0.70	0.6 - 0.8
>43.2	28.33	17.5 - 41.4	100.00	83.2 - 100.0			0.72	0.6 - 0.8
>43.5	26.67	16.1 - 39.7	100.00	83.2 - 100.0			0.73	0.6 - 0.9
>50.6	25.00	14.7 - 37.9	100.00	83.2 - 100.0			0.75	0.6 - 0.9
>56.2	23.33	13.4 - 36.0	100.00	83.2 - 100.0			0.77	0.7 - 0.9
>56.4	21.67	12.1 - 34.2	100.00	83.2 - 100.0			0.78	0.7 - 0.9
>56.5	20.00	10.8 - 32.3	100.00	83.2 - 100.0			0.80	0.7 - 0.9
>56.6	18.33	9.5 - 30.4	100.00	83.2 - 100.0			0.82	0.7 - 0.9
>70	16.67	8.3 - 28.5	100.00	83.2 - 100.0			0.83	0.7 - 0.9
>70.4	15.00	7.1 - 26.6	100.00	83.2 - 100.0			0.85	0.8 - 0.9
>78.2	13.33	5.9 - 24.6	100.00	83.2 - 100.0			0.87	0.8 - 1.0
>100	11.67	4.8 - 22.6	100.00	83.2 - 100.0			0.88	0.8 - 1.0
>120	10.00	3.8 - 20.5	100.00	83.2 - 100.0			0.90	0.8 - 1.0
>150	8.33	2.8 - 18.4	100.00	83.2 - 100.0			0.92	0.8 - 1.0
>250	6.67	1.8 - 16.2	100.00	83.2 - 100.0			0.93	0.9 - 1.0
>260	5.00	1.0 - 13.9	100.00	83.2 - 100.0			0.95	0.9 - 1.0
>302	3.33	0.4 - 11.5	100.00	83.2 - 100.0			0.97	0.9 - 1.0
>318	1.67	0.04 - 8.9	100.00	83.2 - 100.0			0.98	1.0 - 1.0
>504	0.00	0.0 - 6.0	100.00	83.2 - 100.0			1.00	1.0 - 1.0

As the data of CRP are dichotomous, no cutoff point was identified. The sensitivity and specificity of CRP was 61.7 and 71.2 respectively. UAC was 0.65, 95%CI (0.54-0.76), P=0.009 (Figure 3).

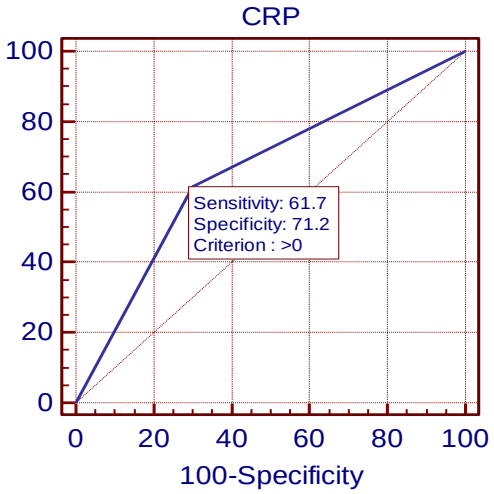

FIGURE 3**:** ROC curve for CRP

ROC curves of IL-6 and CRP were compared and, results revealed that the differences were not significant (P=0.056) (Figure 4). Although the sensitivity of CRP was higher than IL-6 (61.1% vs 50%), but the specificity was lower in CRP as compared with IL-6 (71.2 vs 100%). In such diseases it was well known that the specificity is very important to prevent treating normal people with cancer drugs, hence applying the two tests could be more safe to detect the prognosis of breast cancer in Iraqi women. The primary test is CRP (higher sensitivity) and IL-6 as a completing test to confirm the absent of disease because of its high specificity.

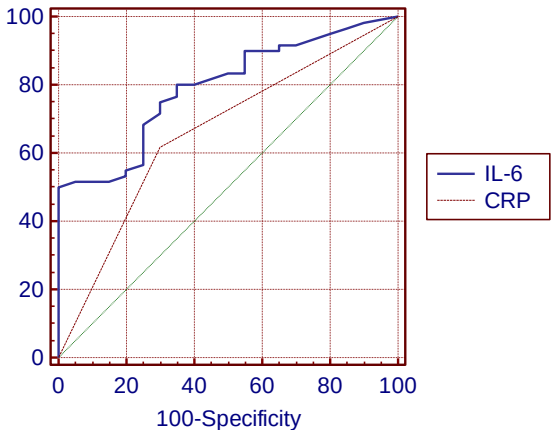

FIGURE 4: Roc curves comparison between IL-6 and CRP

DISCUSSION

The link between inflammation and cancer is an old concept that was first proposed by Virchow 1864,when he observed that inflammatory cells frequently infiltrate tumor stroma (Montovani *et al.*, 2008). Breast cancer is a very heterogenous disease. IL-6 and CRP have related roles in the inflammatory response. IL-6 induces CRP production in liver by activating Janus Kinases. However few studies have examined IL-6 and CRP together in the same population, which be important to determine whether measuring both markers provides better prognostic information or whether one marker alone is better than the other (Alexandrakis *et al.*, 2003). The study shows that the differences between IL-6 and CRP in UAC was not significant but the sensitivity and specificity were differed so using two parameters are very important to give the diagnosis more reliability. The study indicated That THE levels of IL-6 correlates with all THE aspects of Brest cancer like tumor size, age of THE patiënt and, before or after surgery (Praveen *et al*., 2011). IL-6 is à critical factor dring hematopoiesis and subsequent lymphocyte differentiation and activation.(Kishimoto 2006). IL-6 serum levels continue to increase with age rise until approximately age 70 years at which time Then gradually decline (Giuliane *et al.*, 2001). So detection of IL -6 and CRP level in serum for prognosis of Breast cancer Will be in use till THE. Age 70years. IL-6maydrive tumor progression, beside of enhance THE growth of THE fibroblasts of Breast cancer in an IL-6dependent manner(stublebaker et al 2008).many Breast tumor stromal celles provider à paracrine source ofIL-6 for THE Breast cancer cells within the Breast tumor microenvironment (Blick *et al*., 2008). Plasma CRP levels might sum up some prognostic information of well known tumor characteristics such as tumor stage and grade. Women with CRP levels had a 3.5fold increased risk of reduced overall survival suggesting that women with high CRP levels at the time of diagnosis have a particularly poor survival (Kristine *et al*., 2011).

REFERENCES

Hirano, T., Akira, S., Taga, T., and Kishimoto, T. (1990) Biological and clinical aspect of IL-6 Immunol Today, 170:443-454.

Kashimoto, T. (2006) IL-6 discovery of polio tropicytokines. Arthritis.Res.Ther. 8 (suppl2):s2.

Culig, Z., Stoner, H., Bartsch, G. (2005) IL-6 regulation of prostate cancer cell growth. J. cell. Biochem., 95:497-505.

Golab, J., Jakobisiak, M., Lasekm W. (2002) Cytokiny, Immunologia. Warsazawa,WydNauk PWN,15:198-205.

Scheller, J., Ohnesorge, N., Rose-John, S. (2006)IL-6 trans signaling in Chronic inflammation and cancer. Scand. J. Immunol. (2006):63:321-329.

Johnsons, A. M. (2005) Amino aids, peptiden and proteïnes. In Tietz textbook of Clinical chemistry and molecular diagnostics. 4th. edition. Edited by Burits CA, Ashwood ER, Bruns DE. St. Louis, MO: Elsevier Saunders:555-556.

Allin, K.H., Bojesen, S.E., Nordestgaard, B.G. (2009) Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer J. Clin. Oncol. : 27:2217-2224.

O Dowd, C., McRae, L.A., McMillan, D.C., Kirk, A. and Milroy, R. (2010) Elevated preoperative CRP predicts poor cancer specific survival in patients undergoing resection for non small cell lung cancer. J. Thorac. Oncol. 5:988-992.

Mantovani, A., Romerom P., Palucka, A.K., Marincola, F.M. (2008)Tumor immunity: effect or Response to tumor and role of the microinvironment. Lancet:371-(9614):771-783.

Alexandrakis, M.G., Passam, F.H., Ganotakis, E.S. (2003) :The significant of ESR, serum IL-6 and acute phase reactants protein levels in multiple myeloma. Clin. Lab. Haematol (2003):25:41-6.

Praveen, R. and Karuna Nithi, R. (2011) Clinical significance of preoperative drum IL-6and CRP level in breast cancer patients: World J. Of surgical oncology (2011):9:18.

Giuliani, N., Sansoni, P., Girasole, G.,Vescovini, R., Passeri, G., Passeri, M. (2001) Serum IL-6 ,soluble IL-6 receptor and soluble gp130 exhibit different Patterns of

age and menopause-related changes.Exp.Gerontol, 36:547-557.

Stublebaker, A.W., Storic, G., Werbeck, J.L., Sasser, A.K., Tavolari, S. (2008) Fibroblasts Isolated from common sites of breast cancer metastasis enhance cancer cell growth rates and invasiveness in an IL-6 dependent. Cancer Res., 68:9087-9095.

Blick,T., Widodo, E., Hugo, H., Waitham, M., Lenburg, M.E., Neve, R.M. (2008) Epithelial Mesenchymal transition traits in human breast cancer cell lines. Clin. Exp. Metastasis, 25:629-642.

Kristine, H., Borg, G., Nordestgaard, Henrik flyer and Stig E. Bojesen (2011) Elevated per Treatment levels of plasma CRP are associated with poor prognosis after breast cancer: a cohort study .Breast Cancer Research,13:R55.