

# Serum C-reactive protein as potential independent prognostic factor for breast cancer

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## ABSTRACT

**BACKGROUND:** The possible involvement of inflammation in breast carcinogenesis has potential prognostic and therapeutic consequences. We investigated whether C-reactive protein is expressed in breast cancer and evaluated its correlation with clinicopathological findings.

**METHODS:** We analyzed CRP in plasma samples of 219 cases of metastatic and nonmetastatic invasive cancer and carcinoma in situ, selected among participants in a retrospective study. Circulating plasma CRP measurements were performed at the time of primary diagnosis.

**RESULTS:** We observed higher levels of CRP in patients with cancer compared with patients with non-invasive tumors ( $p=0.0037$ ) and a significant association between elevated CRP levels and advanced tumor stage only in patients with non-metastatic breast cancer. The CRP level significantly increases with the size of the breast cancer in patients with non-metastatic disease: Tis 1.58 mg/L, T1 3.14 mg/L, T2 6.19 mg/L, T3 5.85 mg/L, T4 20.97 mg/L;  $p=0.0020$ .

**CONCLUSION:** Our data suggest that elevated plasma CRP levels are associated with advanced stages of breast cancer and bad prognosis. CRP levels might be an independent and potential long-term prognostic factor for breast cancer and could be probable used as tumor marker in patients without inflammatory diseases. Non-steroidal anti-inflammatory drugs could be used in the treatment of malignancy. Several trials are being conducted to study the use of COX (enzyme cyclo-oxygenase) inhibitors in the treatment of breast cancer.

**Keywords:** Breast cancer, prognosis, inflammation, tumor maker.

## INTRODUCTION

Chronic inflammation has been hypothesized for many years to be associated with cancer development and progression and has been studied for a long time<sup>(1-3)</sup>.

Serum C-reactive protein (CRP), an acute phase protein and a sensitive nonspecific marker of systemic inflammation, has been reported to be associated with a number of cancers, including breast cancer. Its expression is induced by pro-inflammatory cytokines and is produced mainly in hepatocytes<sup>(17)</sup>. However, results in the literature

## SOMMARIO

**BACKGROUND:** Il possibile coinvolgimento dell'infiammazione nella carcinogenesi ha un potenziale prognostico e delle conseguenze terapeutiche. Abbiamo investigato sull'eventualità che la proteina C-reattiva sia coinvolta nel cancro al seno e abbiamo valutato la sua correlazione con i riscontri clinicopatologici.

**METODOLOGIA:** abbiamo analizzato il CRP nei campioni di sangue di 219 casi di cancro invasivo metastatico e non, e nel carcinoma in situ, selezionati tra i partecipanti agli studi retrospettivi. I valori di CRP nel plasma sono stati misurati al momento della diagnosi primaria.

**RISULTATI:** Abbiamo osservato un maggiore livello di CRP in pazienti con il cancro rispetto a pazienti con tumori non invasivi ( $p = 0.0037$ ) e una significativa correlazione tra i livelli di CRP elevati e il tumore in uno stadio avanzato presente nei pazienti con cancro al seno non metastatico.

Il livello di CRP aumenta significativamente con l'ingrandirsi del cancro al seno non metastatico: Tis 1.58 mg/L, T1 3.14 mg/L, T2 6.19 mg/L, T3 5.85 mg/L, T4 20.97 mg/L;  $p=0.0020$ .

**CONCLUSIONI:** I nostri dati suggeriscono che elevati livelli di CRP nel sangue sono associabili con stadi avanzati di cancro al seno e prognosi maligne. I livelli di CRP potrebbero essere un potenziale fattore di prognosi a lungo termine del cancro al seno e probabilmente potrebbe venir usato come marcatore tumorale nei pazienti senza malattie infiammatorie.

Farmaci anti-infiammatori non steroidei possono essere usati nel trattamento delle neoplasie maligne. Sono stati condotti numerosi trials sullo studio dell'utilizzo dei COX (enzima ciclo-ossigenasi) inibitori nel trattamento del cancro al seno.

are inconsistent and remain controversial. There are two hypotheses that might explain the correlation between CRP and cancer: 1) high CRP levels are a result of cancer or a premalignant state; 2) chronic inflammation with elevated CRP concentrations promotes cancer growth<sup>(6)</sup>. The production of cytokines and growth factors, the induction of cyclooxygenase-2 in macrophages and epithelial cells, the generation of mutagenic reactive oxygen, and nitrogen species are possible mechanisms for carcinogenesis<sup>(18)</sup>. Cytokines (interleukin-1, interleukin-6, tumour necrosis factor) regulate the synthesis of CRP. The role of elevated CRP levels in patients with carcinoma is not yet clear.

Nozoe et al. first described an association between CRP and carcinogenesis in patients with oesophageal

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squamous carcinoma<sup>(19)</sup>. They demonstrated an increased incidence of peritoneal, lymph node and liver metastases, intravascular invasion and poor prognosis in patients with preoperatively increased CRP levels.

It has been reported that serum CRP expression has a prognostic value for gastric, colorectal, esophageal carcinoma, multiple myeloma and malignant fibrous histiocytoma<sup>(19-23)</sup>. It has not been clarified, however, whether the serum CRP expression is a prognostic indicator in patients with breast cancer.

Some previous studies have reported that breast cancer patients have elevated levels of CRP before surgery, especially in women with advanced disease<sup>(16)</sup>. The results showed that the higher the CRP level at diagnosis, the worse the prognosis was. Elevated levels have been associated with poor prognosis in patients with breast cancer<sup>(6,12)</sup>.

Therefore, the aim of our retrospective study was to investigate the association between serum CRP levels before treatment and the pathological stages of breast cancer.

## PATIENTS AND METHODS

We analyzed CRP in plasma samples of 532 cases of invasive cancer and 49 cases of carcinoma in situ, selected among participants in a retrospective study.

The study population included participants who were diagnosed and treated at the Department of Gynaecology at the Saarland University Hospital between January 2010 and December 2012. Patients with documented bacterial infections and high CRP levels were excluded from the study. Information concerning age, menopausal status, diagnosis, and clinical pathology were collected for each patient and are summarized in Table I.

The breast cancer patients were staged according to TNM-UICC classification. 532 patients were diagnosed histologically with ductal infiltrating carcinoma, along with 49 of carcinoma in situ. Tumor size was classified as T1 (less than or equal to 2 cm) in 297 (50.77 %), T2 (tumor size between 2 and 5 cm) in 157 (26.84 %), T3 (tumor size more than 5 cm) in 27 (4.62 %) and T4 (tumor extends to chest wall) in 29 (4.96 %) of the cases.

**Table I:** Main clinical-pathological tumor characteristics of 586 breast cancer patients

Characteristic	Patients	Percent	Characteristic	Patients	Percent
<i>Age</i>			<i>Menopausal status</i>		
< 50 years	122	20.82 %	Premenopausal	117	19.97 %
50-70 years	296	50.52 %	Perimenopausal	35	5.97 %
> 70 years	168	28.66 %	Postmenopausal	433	73.89 %
<i>Histological diagnosis</i>			<i>Grading</i>		
Infiltrating carcinoma	532	91.57 %	G1	61	11.80 %
In situ carcinoma	49	8.43 %	G2	339	65.57 %
			G3	117	22.63 %
<i>Tumor size</i>			<i>Nodal involvement</i>		
T1	297	50.77 %	N0	400	68.61 %
T2	157	26.84 %	N1	123	21.10 %
T3	27	4.62 %	N2	20	3.43 %
T4	29	4.96 %	N3	13	3.26 %
<i>Metastatic site</i>			<i>HER2/neu status</i>		
M0	538	95.05 %	Negative	436	74.40 %
M1	28	4.95 %	Positive	89	15.19 %
<i>ER status</i>			<i>PgR status</i>		
Negative	109	18.60 %	Negative	213	36.35 %
Positive	477	81.40 %	Positive	370	63.14 %
<i>Local relapse</i>			<i>Death</i>		
No	571	97.44 %	No	574	97.95 %
Yes	15	2.56 %	Yes	12	2.05 %

There were 400 patients with negative lymph nodes and 162 patients with positive lymph nodes. Main tumor characteristics are shown in Table I.

Circulating plasma CRP measurements were performed at the time of primary diagnosis in 219 participants. The cut-off level for serum CRP was established at 5 mg/L. We determined the plasma CRP levels in fresh plasma samples in our laboratories at the Department of Clinical Biochemistry, Saarland University Hospital.

The statistical analyses were carried out using SAS version 9.2 statistics software. The Kruskal-Wallis test was used for relating CRP levels to clinicopathological parameters. A p-value of less than 0.05 was considered to be significant.

## RESULTS

High circulating plasma levels (> 5 mg/L) of CRP were associated with advanced-stage breast cancer and metastasis. Of the 219 patients with data, 43 (19.63%) showed an abnormal CRP level at the time of primary diagnosis.

Patients diagnosed with invasive cancers had significantly higher levels of CRP than patients with carcinoma in situ;  $p=0.0037$ . A mean CRP concentration of 5.91 mg/L was found, while patients with noninvasive cancers had a mean CRP level of 1.25 mg/L.

### Relationship between CRP and tumor characteristics in adjuvant patients

We noted that CRP levels did differ significantly with tumor size. The CRP level significantly increases with the size of the breast cancer in patients with non-metastatic disease: Tis 1.58 mg/L, T1 3.14 mg/L, T2 6.19 mg/L, T3 5.85 mg/L, T4 20.97 mg/L;  $p=0.0020$  (Figure 1).

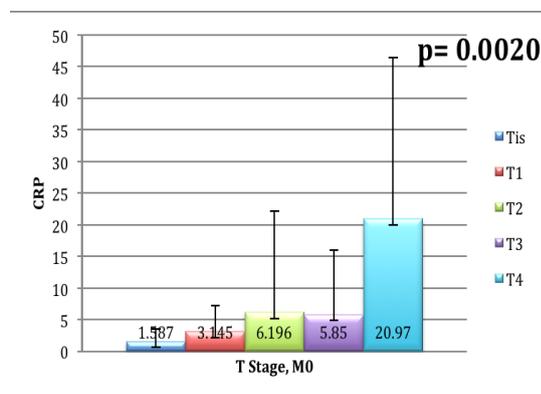


Figure 1: CRP and tumor stage

Serum levels of CRP in patients with non-metastatic and low-grade malignancy cancers (G1) did not differ significantly as compared with patients with medium (G2), respectively high-grade malignancy cancers (G3): 2.84 mg/L vs. 4.95 mg/L, respectively 4.84 mg/L,  $p>>0.05$  (Figure 2).

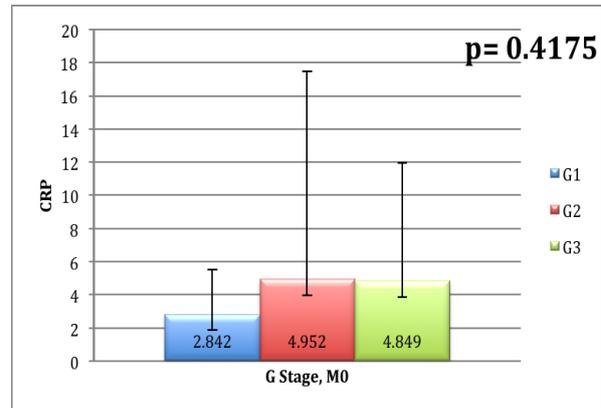


Figure 2: CRP and grading

We evaluated correlation of CRP level at the time of primary diagnosis with nodal burden. CRP concentrations were higher in patients with positive nodal status. Patients with no lymph node involvement (N0) had a mean CRP level of 4.07 mg/L, while patients with positive axillary lymph nodes as follows: N1 4.22 mg/L, N2 7.77 mg/L. There was only one patient with N3 breast cancer, who had a CRP level at the time of primary diagnosis of 1.80 mg/L. However, the observed differences of CRP levels are not significant (Figure 3).

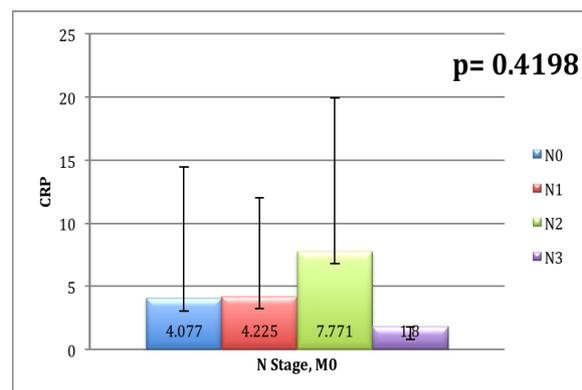
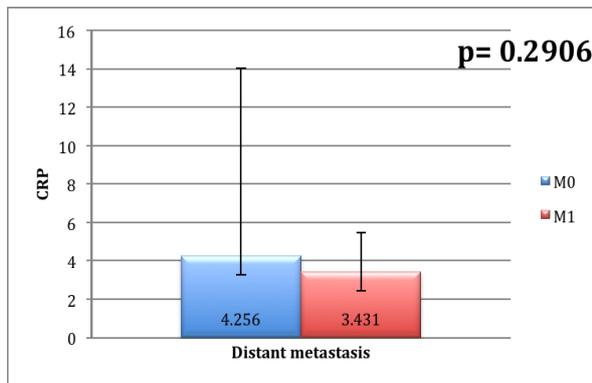


Figure 3: CRP and nodal status

### Relationship between CRP and tumor characteristics in patients with metastatic disease

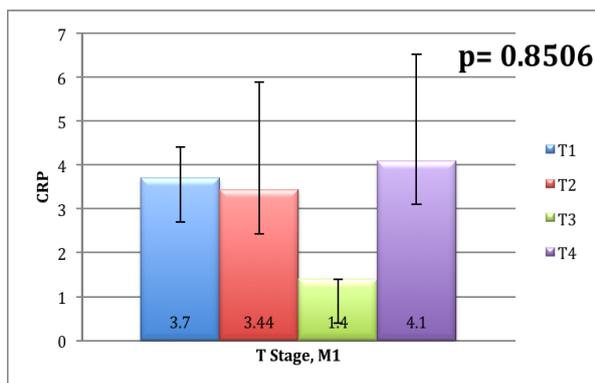
26 patients with data had distant metastasis at time of their initial breast cancer diagnosis as follows: 5 patients had lung metastases, 8 bone metastases, 2 liver metastases and the others had more than one

location of distant metastasis. Patients with metastatic disease had a mean CRP level of 3.43 mg/L, almost equal to patients with non-metastatic disease (Figure 4).



**Figure 4:** Relationship between CRP and distant metastases in patients with non-metastatic vs. metastatic disease

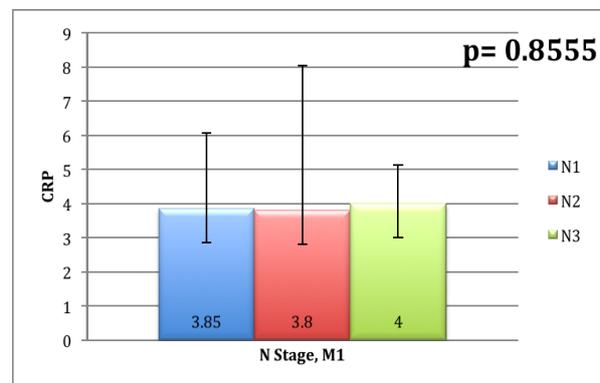
Non-significant elevations of CRP levels were observed in patients with T2, respectively T4 breast cancer and distant metastases (4.44 mg/L, respectively 4.10 mg/L). A CRP level of 3.70 mg/L was observed in patients with T1 breast cancer. There was only one patient with T3 breast cancer and distant metastases with a CRP level of 1.40 mg/L (Figure 5).



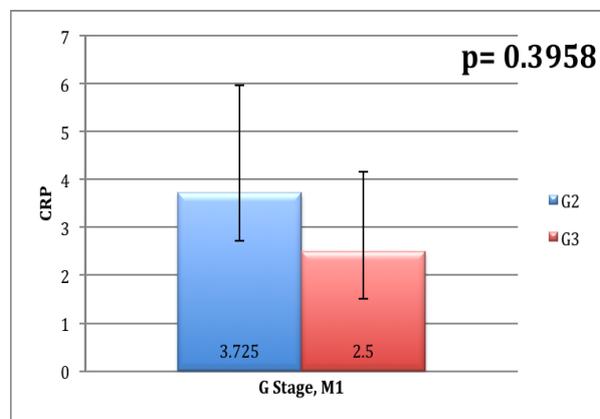
**Figure 5:** CRP and tumor stage

CRP serum levels increase with nodal status of breast cancer. This difference again did not reach statistical significance. A level of 3.15 mg/L was observed in nodal negative patients. Patients with N1 and N2 breast cancer had a CRP level of 3.85 mg/L and 3.80 mg/L, respectively, while a level of 4.00 mg/L was observed in patients with N3 breast cancer (Figure 6).

The CRP concentrations were independent of grading in patients with distant metastases (Figure 7).



**Figure 6:** CRP and nodal status

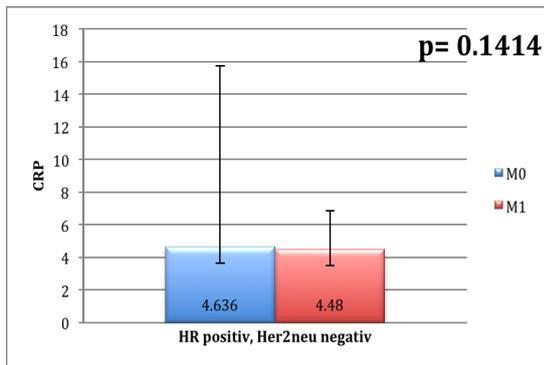


**Figure 7:** CRP and grading

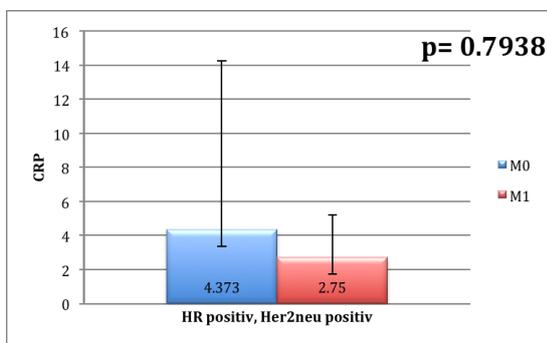
### Correlation of CRP level at the time of primary diagnosis with ER, PgR and HER2-expressions

We didn't notice any significant correlations of CRP levels with receptor status in our study. A detailed breakdown of distribution is shown in Figures 8-10.

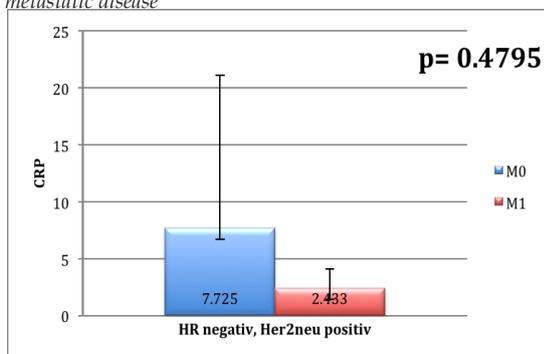
No difference was observed in patients with non-metastatic compared to metastatic hormone receptor positive, HER2-negative breast cancer (Figure 8). Patients with metastatic hormone receptor positive, HER2-positive breast cancer had non-significant lower CRP levels than patients with non-metastatic disease, 2.75 mg/L compared to 4.37 mg/L (Figure 9). Slightly higher CRP levels were observed in patients with non-metastatic triple negative breast cancer (3.52 mg/L) compared to patients with metastatic triple negative breast cancer (2.30 mg/L) (Figure 11). Similarly, patients with non-metastatic hormone receptor negative, HER2-positive disease had a non-significant higher CRP level than patients with metastases, 7.72 mg/L vs. 2.43 mg/L (Figure 10).



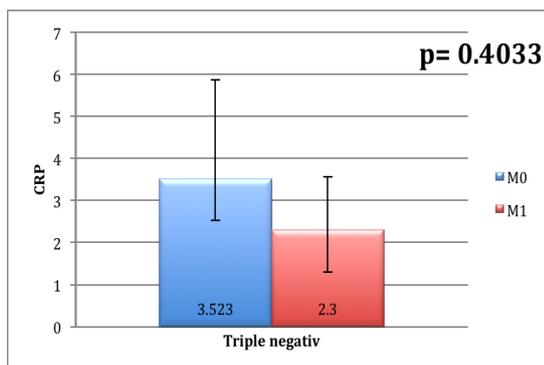
**Figure 8:** Relationship between CRP and hormone receptor positive, HER2/neu negative breast cancer in patients with non-metastatic vs. metastatic disease



**Figure 9:** Relationship between CRP and hormone receptor positive, HER2/neu positive breast cancer in patients with non-metastatic vs. metastatic disease



**Figure 10:** Relationship between CRP and hormone receptor positive, HER2/neu negative breast cancer in patients with non-metastatic vs. metastatic disease



**Figure 11:** Relationship between CRP and triple negative breast cancer in patients with non-metastatic vs. metastatic disease

## DISCUSSION

CRP is an acute-phase protein with a rapid level elevation in response to acute inflammation<sup>(4,5)</sup>. It is produced in the liver in response to elevated cytokine concentrations after inflammation<sup>(5)</sup>. Elevated CRP levels were also observed during chronic inflammatory diseases and cancer<sup>(6)</sup>.

Researchers found that there may be a correlation between elevated CRP levels and poor prognosis with a high risk of recurrence of many types of solid cancers, including breast cancer<sup>(7-10)</sup>. Some of previous publications reported that regardless of menopausal status, age, tumor size, lymph node status, presence of metastasis or receptor status, elevated levels of CRP resulted in poor prognosis<sup>(11)</sup>. In other studies, elevated CRP levels were associated with larger tumor size, lower tumor grade and presence of distant metastases<sup>(12)</sup>.

A meta-analysis of prospective cohort studies was performed to investigate the correlation between CRP levels and cancer risk<sup>(24)</sup>. The results showed that there is a significant evidence for concluding that elevated levels of CRP are associated with an increased risk of all-cancer.

We observed higher CRP levels in patients with invasive cancer compared to patients with non-invasive tumors and a significant association between elevated CRP levels and advanced tumor stage only in patients with non-metastatic breast cancer. Other studies suggest that a correlation between CRP and survival may be present only in patients with metastatic breast cancer<sup>(12-15)</sup>. The results are controversial.

Another large systematic review of the association between CRP levels and cancer was published<sup>(6)</sup>. Most of the studies evaluating CRP as a prognostic marker of cancer did not reach statistical significance. In most studies CRP levels were found to be higher in patients with cancer than in controls with benign diseases. Several studies showed contrasting findings and did not provide strong evidence to support the usefulness of high CRP levels in diagnosis of cancer<sup>(6)</sup>. We found no correlation between CRP levels and receptor status. Other studies, however, noticed a positive association between serum CRP levels and hormone positive breast cancer<sup>(25,26)</sup>.

Because of the short follow-up period of 17.5 months of the study, we could not establish a correlation between survival and serum CRP levels. A Danish study noticed that the higher the CRP level at diagnosis, the worse the prognosis was<sup>(11)</sup>. Elevated CRP levels at time of diagnosis were associated with increased risk of death from breast cancer. This is the largest study that has examined whether a correlation exists between CRP levels and prognosis. Other

previous studies have shown a similar association<sup>(27,28)</sup>.

Our data suggest that elevated plasma CRP levels are associated with advanced stages of breast cancer and bad prognosis and support the role of chronic inflammation in carcinogenesis. However, most of our results were not statistically significant and cannot

provide evidence for causality. CRP levels might be an independent and potential long-term prognostic factor for breast cancer. Further research effort with a longer follow-up period is needed to evaluate the role of elevated CRP levels in carcinogenesis.

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