PRE-TREATMENT NEUTROPHIL TO LYMPHOCYTE RATIO MAY BE AN USEFUL TOOL IN PREDICTING SURVIVAL IN EARLY TRIPLE NEGATIVE BREAST CANCER PATIENTS.

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ABSTRACT

Background. There is a growing body of evidence that immune response plays a large role in cancer outcome. The neutrophil to lymphocyte ratio (NLR) has been used as a simple parameter of systemic inflammation in several tumors. The purpose was to investigate the association between pre-treatment NLR, disease-free survival and overall survival in patients with early triple negative breast cancer (TNBC).

Patients and methods: We reviewed the records of patients with stage I-III TNBC at our Institution from 2006 to 2012. The association between pre-treatment NLR and survival was analyzed. The difference among variables was calculated by chi-square test. DFS and OS were estimated using Kaplan-Meier method. Cox analysis was performed to analyze clinical parameters for their prognostic relevance.

Results: A total of 90 patients were eligible. There was no significant correlation among pre-treatment NLR and various clinical pathological factors. Patients with NLR higher than 3 showed significantly lower DFS (p=0.002) and OS (p=0.009) than patients with NLR equal or lower than 3. The Cox proportional multivariate hazard model revealed that higher pre-treatment NLR was independently correlated with poor DFS and OS, with hazard ratio 5.15 (95% confidence interval [CI] 1.11-23.88, p=0.03) and 6.16 (95% CI 1.54-24.66, p=0.01) respectively.

Conclusion: Our study suggests that pre-treatment NLR may be associated with DFS and OS patients with early TNBC. Further validation and a feasibility study are required before it can be considered for clinical use.

Keywords: Neutrophil, lymphocyte, ratio, prognosis, survival, triple negative, breast cancer.

INTRODUCTION

Triple negative breast cancer (TNBC) represents approximately 10–20% of breast cancers and is associated with an unfavourable outcome with a frequent occurrence of visceral metastases [1,2]. Although, several molecular markers have been evaluated in TNBC, in order to define TNBC with a more aggressive behaviour and poor prognosis, traditional histological parameters, such as tumor size and lymph vascular invasion are still consistent in providing prognostic informations in the group of TNBC [3 - 14]. Nevertheless, new clinical and laboratory factors are required that could be accurate and reproducible, but also easily performed. Increasing evidence supports the involvement of inflammation in cancer development, progression, metastasis and relapse [15,16]. The combined index, using neutrophil and lymphocyte counts in the form of neutrophil to lymphocyte ratio (NLR), has been used as simple parameter to assess the systemic inflammation. It is correlated with prognosis in several tumors, such as colorectal, gastric, pancreatic, non-small-cell lung, hepatocellular, ovarian, cervical and renal cancers [17 - 29]. Previous studies have investigated the role of NLR in predicting survival and mortality even in early breast cancer patients [30 - 32]. Based on the lack of any clinical prognostic features predicting prognosis in the subgroup of TNBC, the purpose of this study was to investigate the association between pre-treatment NLR, disease-free survival (DFS) and overall survival (OS) in patients with early TNBC.

PATIENTS AND METHODS

Patients

We retrospectively identified patients who were diagnosed and completed the treatment of invasive breast cancer at our Istitution from January 2006 to December 2012. Medical record were reviewed to find data on patient's medical history, age, sex, pathologic results such as tumour size, lymph node status, hormonal status, human epidermal growth factor receptor 2 (HER-2), receptor status and laboratory data. Patients with ductal carcinoma in situ with or without micro-invasion and

patients with lack of information on pathologic or laboratory results were excluded. We also excluded patients with stage IV breast cancer or inflammatory breast cancer, patients who were diagnosed preoperatively with systemic inflammatory or chronic disease such as Systemic lupus Erythematosus (SLE), any haematological disorders, liver cirrhosis, end-stage renal disease, pregnancy-related breast cancer, recent treatment with steroids or cytokines or granulocyte stimulating factor (G-CSF).

Pathological characteristics

Based on pathology reports, we identified tumors lacking immunohistochemical expression of ER, PR and HER2. ER and PR were considered positive if there were at least 1% positive invasive tumor nuclei in the sample. HER-2 status was evaluated by immunohistochemistry (IHC) using a semiquantitative score (0–3+). Tumor staining was compared to the staining of normal breast epithelium from the same patient as a negative control. For clinical purposes, no staining or weak (1+) and incomplete membrans' staining was considered a negative result. Patients with 2+ IHC staining for HER2 underwent fluorescence in-situ hybridization to confirm HER2 negativity. Triple-negative status (ER negative, PR negative and HER-2 negative) was finally diagnosed and re-reviewed by the single study pathologist of our Institution. Rare histological types of TNBC (apocrine, medullary, adenoid cystic and metaplastic carcinomas) were excluded from this analysis.

Laboratory Data

The NLR was defined as the absolute neutrophil count divided by absolute lymphocyte count. The NLR was calculated from the full blood count routinely performed immediately after breast cancer diagnosis and before the initiation of any treatment modality, including surgery (pre-treatment NLR). The cut-off value of 3 was decided as the maximum (sensitivity+specificity) point according to receiver operating characteristics curves (figures 1 and 2). Patients were further divided into two groups, A (NLR$\leq$ 3) and B (NLR>3).

Statistical analysis

Patients who were not reported as died at the time of the analysis were censored at the date they were last known to be alive. Disease-free survival (DFS) was defined as the interval between the date of diagnosis of TNBC to the date of relapse or progression of disease, or the date of death from any cause, while overall survival (OS) was defined as the interval between histological diagnosis to death or last follow-up visit. Survival distribution was estimated by the Kaplan—Meyer method. The association between categorical variables was estimated by Chi square test. The Cox multivariate proportional hazard regression model was used to evaluate the effects of the prognostic factors on survival. Significant differences in probability of surviving between the strata were evaluated by log-rank test. Hazard ratios and 95% confidence intervals (CIs) were estimated from regression coefficients. A significance level of 0.05 was chosen to assess the statistical significance. Statistical analysis was performed with MedCalc package (MedCalc® v9.4.2.0).

RESULTS

A total of 90 patients were eligible for analysis. The median value of NLR was 2.93 (range 1.62-13.47). The distribution of the baseline NLR of the 90 patients is shown in Figure 3. 17 patients (18.9%) showed higher pre-treatment NLR (group B). Median age at diagnosis was 53 years (range 28-79). The median follow-up time was 53.8 months (13.1-195.2). Pathological T stage was T1 in 52 and T2-T4 in 38 patients. Lympho-nodes were disease-positive in 42.3% of cases. Ductal tumors (91.1%), a grading of 3 (90%) and a high proliferative index (Ki-67>20%) (83.4%) were the most commonly observed categories. Vascular invasion and necrosis were found in 15.5% and 16.6% of patients, respectively. Patient characteristics are summarized in Table 1.

There was no significant correlation among pre-treatment NLR and various clinical pathological factors, including age, menopausal status, tumour size, lymph nodes status, grading, Ki-67, necrosis and lympho-vascular invasion (Table 2). Patients with NLR higher than 3 showed significantly

lower DFS (p=0.002) and OS (p=0.009) (Figures 4 and 5), than patients with NLR equal or lower than 3. A better OS was also correlated to the absence of necrosis (p=0.003) (Figure 6). The Cox proportional multivariate hazard model revealed that higher pre-treatment NLR was independently correlated with poor DFS and OS, with hazard ratio 5.15 (95% confidence interval [CI] 1.11-23.88, p=0.03) and 6.16 (95% CI 1.54-24.66, p=0.01) respectively. Multivariate statistical analysis also confirmed necrosis as an independent prognostic variable influencing OS (p=0.01; HR=6.92, 95% 1.48-32.35) (Tables 3 and 4).

DISCUSSION

Inflammation is involved in breast cancer development, tumor angiogenesis and progression. The pro-tumorigenic activity mediated by immune system cells and associated inflammatory mediators, is countered by antitumor immunity [33 – 35]. Moreover, recent studies suggested that inflammation could be also responsible for treatment resistance during therapy [36 – 38] and even involved in relapse and metastasis process in breast cancer, promoting the angiogenic switch [39 - 43]. Furthermore, it has been shown that the presence of a lymphocytic infiltrate in several tumor types could be considered a predictor of a favourable outcome. In breast cancer tumor-infiltrating lymphocytes is associated with a better survival, a better response to anthracycline-based chemotherapy; as well as better response to neoadjuvant chemotherapy [44 – 46]. Recent studies have identified different immune response signature, based on the combination of high levels of tumor-associated macrophages, robust Th2 responses, and low CTL/NK cell infiltration, in breast cancer correspondent to the molecular profiles, that could provide useful information on patient prognosis [47,48]. Several studies have also investigated the relation between systemic inflammation and breast cancer survival, reporting a significant association between shorter survival and elevated concentration of circulating inflammatory biomarkers, such as serum amyloid A (SSA) and Systemic C-reactive protein (CRP) and serum interleukin-6 [49,50].

NLR has been already evaluated in breast cancer. In a large cohort of 442 patients observed that only in luminal A patients NLR (>2.5) was able to identify a poor prognosis [30]. Similar results were reported by Azab et al in 316 BC patients. In the highest NLR quartile (NLR >3.3) showed a significant increase in all-cause mortality rate at 1-,2- and 5-year follow-up compared with the lowest three NLR quartiles, suggesting that NLR is an independent, significant predictor of short- and long-term mortality in BC patients [31]. In a recent retrospective analysis, NLR continued to be statistically significant predictor of 5-year mortality in all lymphocyte count subsets, better than PLR (platelet to lymphocyte ratio) [32].

We investigated the prognostic role of pre-treatment NLR in TNBC subtype and our study suggests that increased pre-treatment NLR may be associated with worse DFS and OS in patients with early TNBC. The role of the neutrophils/lymphocyte ratio could represent a new accurate and reproducible laboratory index to identify TNBC patients with poorer prognosis. Circulating granulocyte neutrophil cells count, at the numerator, were been shown to contain and secrete the majority of cytokines, such as vascular endothelial growth factor (VEGF), interleukin-18 (IL-18) and matrix metalloproteinases (MMM), that create the optimal environment for tumor growth, progression and metastasis [51 – 54]. Neutrophilia is already considered as adverse outcome predictor in several tumors [31, 55 – 57]. On the other hand, cytotoxic T Lymphocytes (CTL) are known to induce apoptosis of cancer cells and inhibit tumor growth while CD8+ T lymphocyte infiltration is associated with better overall patient outcomes. However, the lymphocyte count and the neutrophil absolute count, that represent the denominator and the numerator respectively, are greatly influenced by various physiological, pathological and physical factors; NLR superiority is due to the stability of the ratio compared with the absolute cellular counts [31].

Furthermore, our data showed a correlation between OS and necrosis in the histological sample; in particular the absence of necrosis was shown to be associated with a better outcome in our patients. Actually, necrosis is usually considered to be immunologically harmful because of the sudden release of proinflammatory mediators. Necrotic cell death causes the release of proinflammatory

cytokines, such as IL-8, IL-10, TNF-alpha or of terminal mediators of inflammation, that are known to promote recruitment of inflammatory cells and inducing the cytokines and chemokines cascade. Therefore, necrosis could represent a link between inflammation and stromagenesis, angiogenesis, and suppression of the adaptive immune response, mechanisms involved in tumor growth, and could be charge also in cell resistance to therapy.

We are aware of some limitations in our study. It is a retrospective analysis in a single institution, on a small number of patients. However, to our knowledge, it is the first analysis showing that pre-treatment NLR could predict DFS and OS in TNBC patients. Because of the lack of any other clinical prognostic features, further validation work and feasibility study are required before the results of this study can be considered for clinical use.

Other interesting evidence are emerging about the role of the tumor- infiltration immunophenotype in TNBC in predict clinical outcome [45, 58-60], which should be interestingly integrated with our data. Prospective studies are needed to determine the immunogenic mechanisms underlying NLR variations and to adequately assess the potential role of NLR in guiding patient selection and treatment decisions. Groups defining staging for neoplasms are strongly encouraged to assess and incorporate measures of the presence of apoptosis, autophagy, and necrosis as well as the nature and quality of the immune infiltrate.

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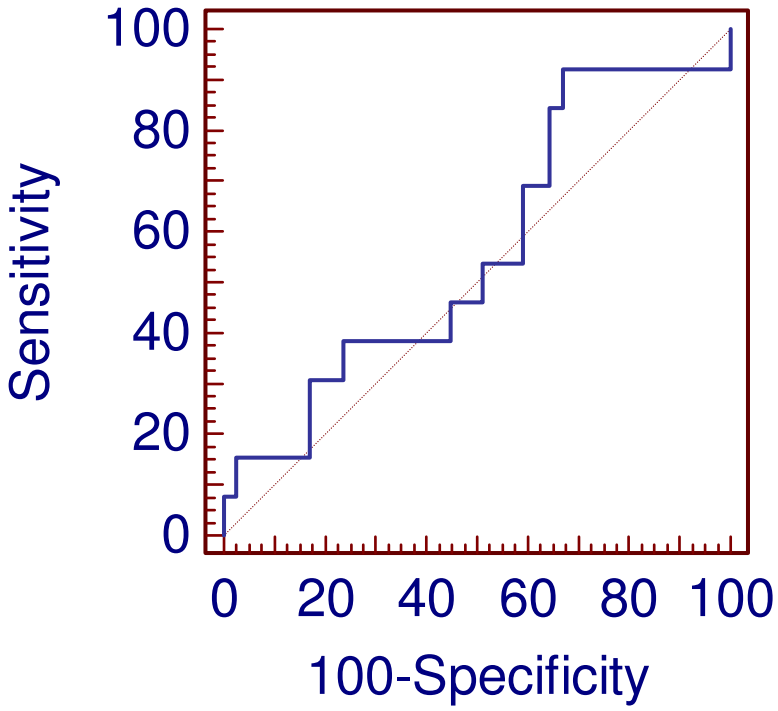

Figure 1: ROC curve for DFS.

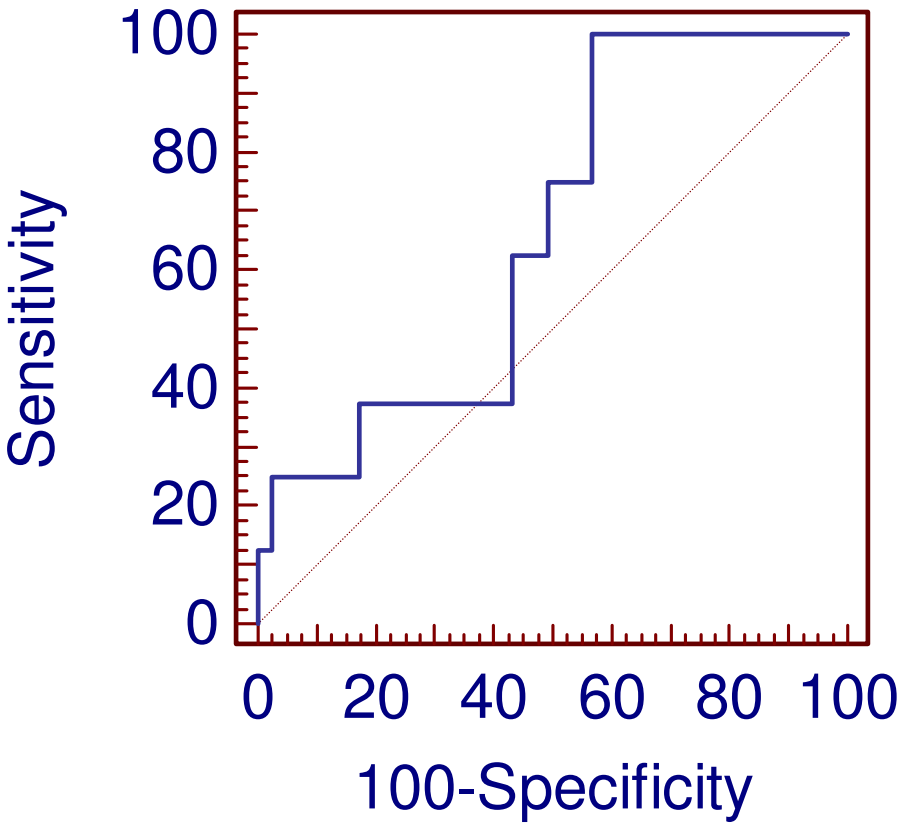

Figure 2: ROC curve for OS.

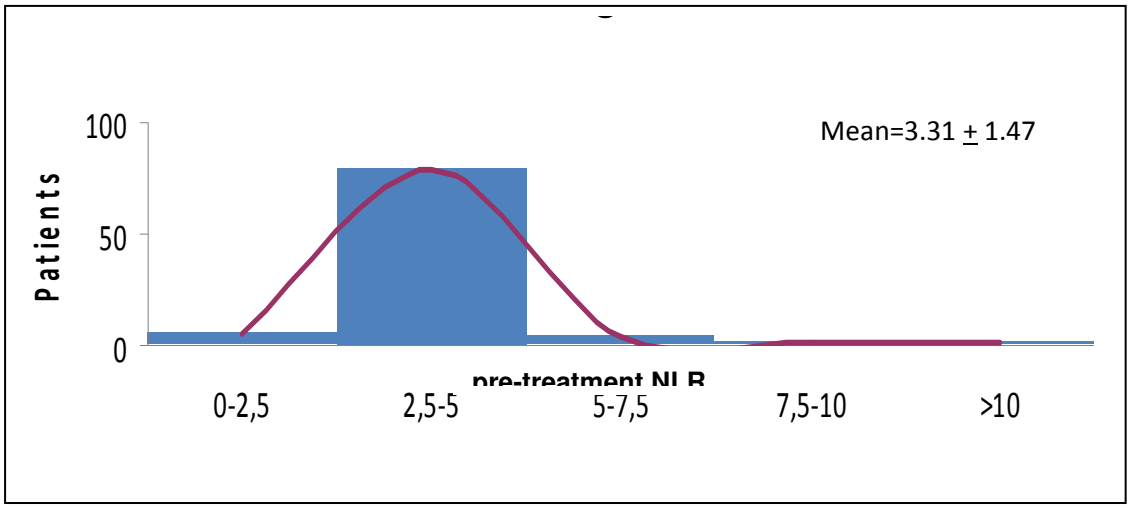

Figure 3. Histogram of the baseline NLR in the peripheral blood of 90 patients with TNBC.

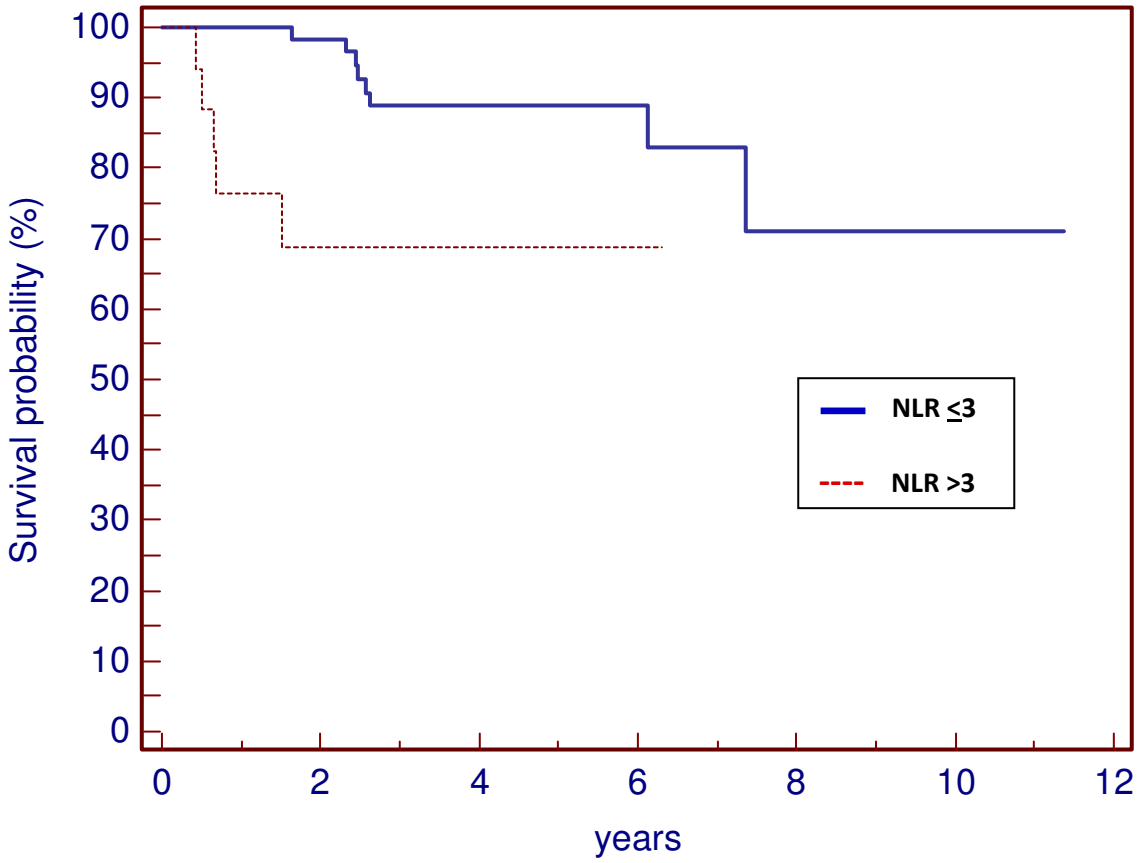

Figure 4. DFS of patients with early TNBC based on NLR (p=0.002).

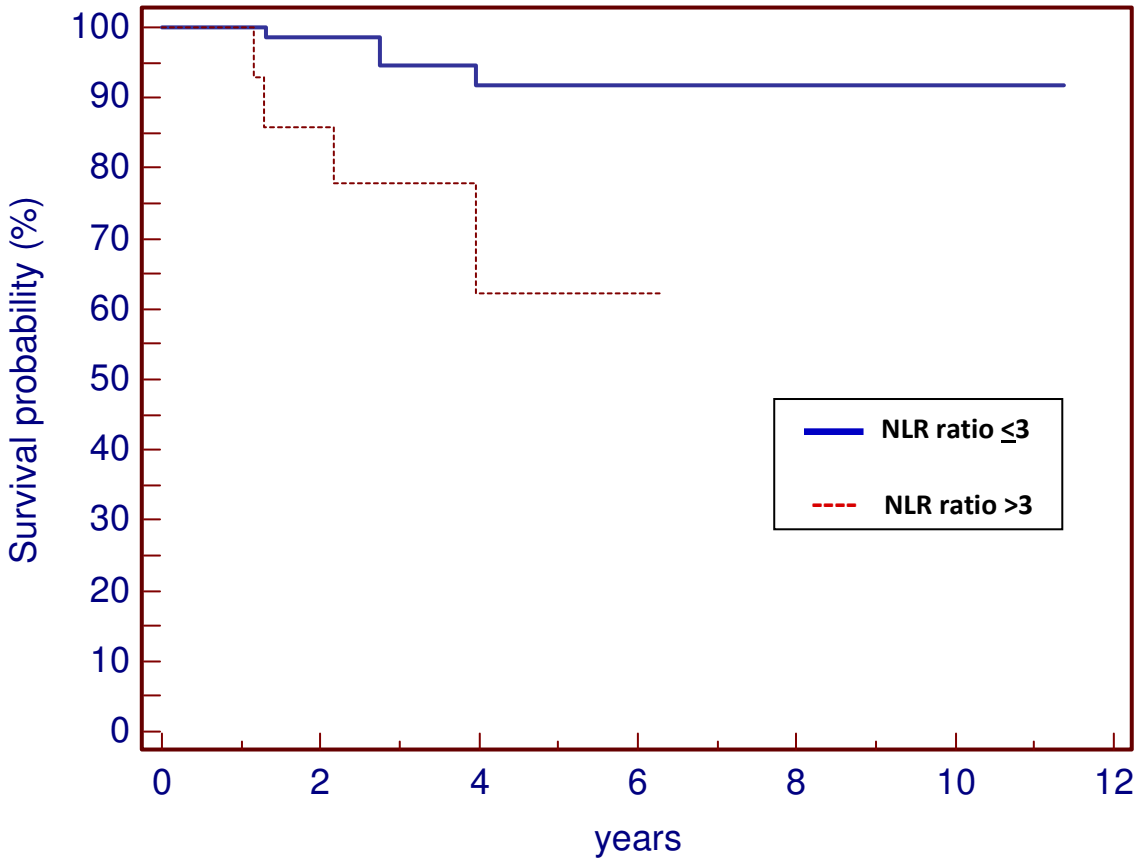

Figure 5. OS of patients with early TNBC based on NLR (p=0.009).

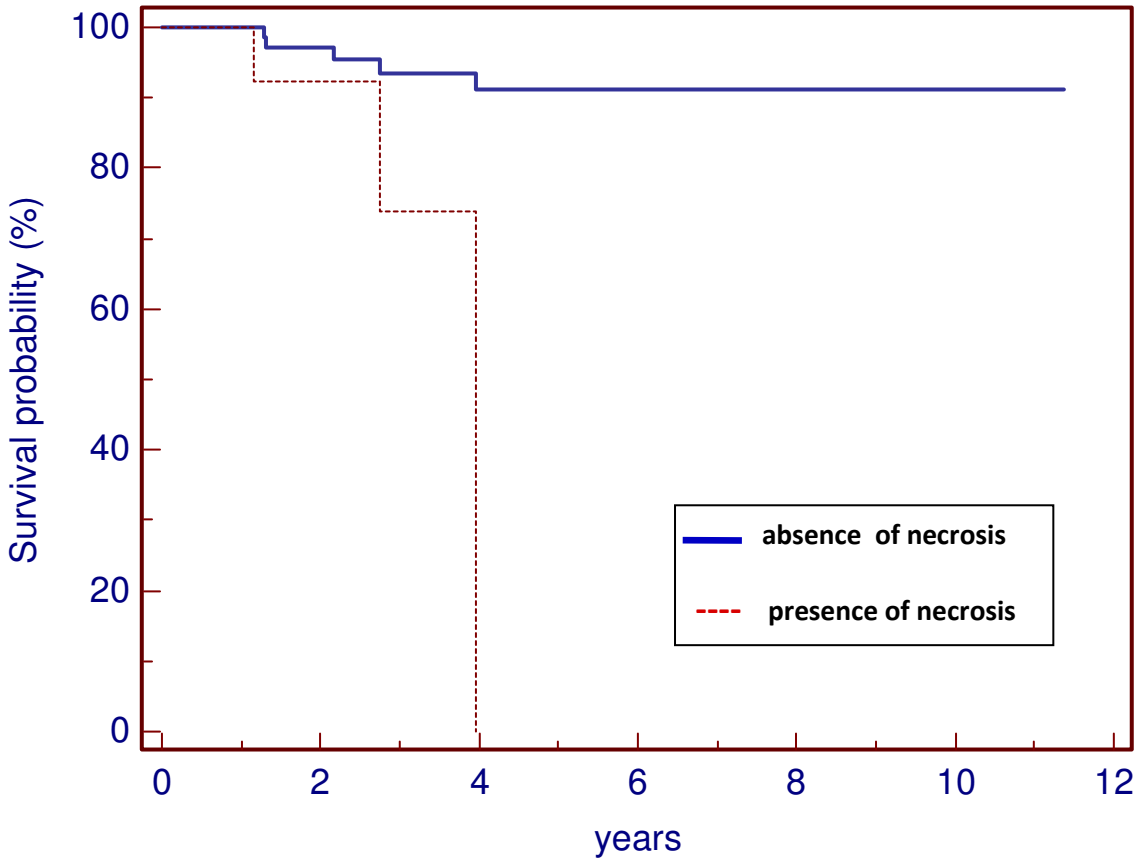

Figure 6. OS of patients with early TNBC based on presence of necrosis (p=0,003).

Additional files provided with this submission:

Additional file 1: tables.docx, 22K
http://breast-cancer-research.com/imedia/1657705965129417/supp1.docx
Additional file 2: cover letter.doc, 21K
http://breast-cancer-research.com/imedia/8553963291294197/supp2.doc