

USE THE COMPLETE BLOOD COUNTS AND ESR AS BIOMARKERS PROGNOSTIC TOOL IN BREAST CANCER PATIENTS ATTENDING SURGERY DEPARTMENT AT ESUT TEACHING HOSPITAL, PARKLANE ENUGU NIGERIA.

ABSTRACT

Background: Full blood count is a prerequisite investigation requested from all breast cancer patients before and during treatment. Poor parameters adversely influence the outcome of cancers.

Objective: This study investigated the use of Complete Blood Count (CBC) and Erythrocyte Sedimentation Rate (ESR) as biomarker prognostic tool in breast cancer subjects.

Method: The sample size comprised of 110 female subjects and controls with ages between 21-70 years. A longitudinal study method was used. The samples were collected from apparently healthy individuals as control, pre-treatment at diagnosis and the treatment samples at different stages of the treatment. Questionnaire used obtained other demographic information. The data was analyzed with IBM SPSS PC. Version 20.0; SPSS Inc., Chicago, III., USA.

Result: Results showed that increased Neutrophil/Lymphocyte Ratio (NLR) and decreased Lymphocyte / Monocyte Ratio (LMR) were significantly associated with increased hazard ratio (HR) and decreased OS at $p < 0.05$ while Platelet/Lymphocyte Ratio (PLR) had no significant difference at $P > 0.05$ in Breast Cancer. In CBC and ESR, control, pre-treatment and treatment period, red blood cell (RBC) parameters and total white blood cell (TWBC) parameters decreased significantly at $p < 0.05$ in treatment results compared to the pre-treatment and control results while others showed no significant increase at $p < 0.05$ in treatment results compared to pre-treatment results. Treatment red cell distribution width (RDW) and mean platelet volume (MPV) observed a significant increase ($p < 0.05$) compared to the control and pre-treatment results. Age group 21-30 years showed more susceptibility than other age groups with lowest mean \pm SD in CBC and ESR but with no significant difference at $p > 0.05$.

Conclusion: This study supports the concept that biomarkers such as CBC and ESR can be used as a prognostic tool in early detection, treatment and monitoring of the disease progression in these subjects

Keywords: *Breast cancer, inflammation, Complete Blood Count, Hospital, Nigeria.*

1.1: INTRODUCTION

“The relation between cancer and the immune system has been increasingly recognized over the past three decades”². “While immune-surveillance is a strong line of defense by which transformed cells are cleared by cells like lymphocytes and natural killer cells, chronic inflammation is an established risk factor for developing several types of cancer including colon cancer, hepatocellular carcinoma and gastric cancer”¹. “In addition, the tumor microenvironment is infiltrated by a heterogeneous population of immune cells, each playing a different role in the

cross-talk between cancer cells and the host, either favoring or suppressing tumor progression. For example, a subset of myeloid cells which is expanded in cancer patients are myeloid-derived suppressor cells (MDSCs). These are immature myeloid cells of granulocytic or monocytic lineages that are elevated in cancer. MDSCs are capable of suppressing anti-tumor T cell activity and promoting tumor angiogenesis”². “In fact, higher numbers of circulating MDSCs is a poor prognostic indicator in esophageal, gastric and pancreatic cancers. On the other hand, higher lymphocyte infiltration in the tumor (tumor-infiltrating lymphocytes, TILs) is a good prognostic indicator in HNSCC”³. “In turn, cancer cells modify the behavior of neutrophils by inducing the release of cytokines and metalloproteinases, increasing their chemotactic potential and inhibiting apoptosis, which perpetuates cancer-associated inflammation. This suggests that different subsets of the inflammatory arsenal play opposing roles in shaping cancer behavior”⁴. “It is evident that components of the CBC can provide valuable prognostic information in solid tumors and hematologic malignancies that are not only limited to survival predictions or assessment of disease progression, but also are important tools when evaluating response to treatment. Thus, true assessment of the utility of the CBC as an inexpensive, established, and globally accessible prognostic factor in many malignancies requires careful studies of the sample results obtained. It is likely that future prospective studies examining the biology behind the prognostic value of the different components of the CBC count would later yield significant therapeutic progress and a thorough understanding of disease pathogenesis. The breast cancer incidence rate is much lower in Asian countries as compare to western countries. The cancer incidence is increasing in all regions of the world with majority of rise seen in developing countries”⁵. “Contrary to the recent drop in the breast cancer incidence in Western countries, the incidence in Korea has been gradually rising for more than a decade. Breast cancer has by far the highest incidence of all cancer types among women around the world”⁶.

“In Nigeria, breast cancer is the most common cancer in females accounting for 49.9%, followed by cervical cancer in 22.4%, and others are ovarian 21.9% and colorectal 2.3%. Liver cancer was the least common cancer 1.4%. The peak incidence of breast cancer in Korea is among women 45–49 years of age, whereas that in the USA and Canada is among women 75–79 years of age. Although estrogen exposure, unfavorable lifestyles, and genetic factors are known to be major risk factors for breast cancer, the unique epidemiological features of breast cancer among Korean women are not properly understood”⁷. “In Nigeria, all the breast cancer deaths were female and the majority of these deaths occurs in young adult and the middle aged group, and these groups accounts for 294 (79.9%) deaths. 100 (27.3%), 93 (25.4%), 76 (20.7%), and 1 (0.2%) of the cases are seen in the age groups 41–50, 31–40, 21–30, and 11–20 years, respectively. Others are seen above the age of 50 years but no death was recorded above the age of 80 years. The male to female ratio was 1:3. A study done from 1981-1990 comprising of 1842 breast cancer women with 17.2% presented with stage I and II; 73.8% presented with stage III. The peak age was between 36-45 years”⁸. “The peak age of breast cancer in Nigeria was reported to be between 35-39 years in 1999”⁹. “Age is an important risk factor for breast cancer, as women over 50 years of age accounted for approximately 78% of new breast cancer cases and 87% of breast cancer-related deaths in 2011 in the United States. However, the worldwide incidence of breast cancer among younger women has increased such that breast cancer is the most frequently diagnosed form of cancer among women aged <40 years. Therefore, it is very important to understand the association between age at diagnosis and breast cancer survival”¹⁰. “It has been suggested that age at diagnosis is related to breast cancer survival, but the data regarding this issue were conflicting”¹¹. “Most of the currently available data indicate that young age is associated with a poor prognosis due to the presence of more invasive disease among this population which is supported by other studies”^{12, 13}. “Although some studies have

noted that elderly women experience poorer outcomes than younger patients, however, the relationship between age and breast cancer prognosis remains unclear and controversial”¹⁴.

The majority of previous studies have reported that young age is associated with a poor prognosis among breast cancer patients, but this issue remains controversial, as the results of studies performed in Iran¹⁵, Nigeria¹⁶, Egypt¹⁷, and even the United States¹⁸ do not support this conclusion. However, most of these studies allocated patients into two groups and used age cut-offs of 35 or 40 years. As a result, the age ranges of the older groups in these studies were extremely large and included middle-aged and senile patients, which may have affected the results of their studies. A few studies performed comprehensive analyses involving patients of all ages. “A retrospective cohort study of 767 breast cancer patients in Brazil, the results of which indicated that women aged ≥ 70 and ≤ 35 exhibited shorter cancer-specific survival than patients aged between 36 and 69 years”.¹⁹ A study analyzed 493 breast cancer patients diagnosed from 1998–2005 in Australia and found that women under 40 years and over 70 years exhibited poorer overall survival than women between 40 and 69 years”²⁰. Similarly, “study done on 4,453 women who were diagnosed with breast cancer between 1961 and 1991 at a single institution in Sweden and were followed up for 10 years regarding breast cancer-specific mortality. They found that women fewer than 40 and above 80 years of age had poorer prognoses than women in other age groups. Younger patients were more likely to be diagnosed with a higher grade and a more advanced stage of disease. This may be due to poor breast cancer screening in young women, as the incidence of the disease in this population is low, which results in patients having larger masses and more advanced disease when they are diagnosed”²¹. “Additionally, younger patients were more likely to be hormone receptor-negative, and elderly patients were more likely to be hormone receptor-positive”²².

BREAST CANCER IN CBC AND ESR

“Full blood count is a prerequisite investigation requested from all cancer patients before surgery, use of chemotherapy and/or radiotherapy. Poor parameters adversely influence the outcome of cancers”.²³ “Hematological parameters and markers of the systemic inflammatory response have been correlated with prognosis in several solid cancers”²⁴. “Anemia is a common morbidity encountered in most solid cancer patients and, as a consequence, cancer patients suffer from shortness of breath, fatigue, and decreased energy, among other symptoms. Anemia of cancer may also be evident at initial diagnosis. Activation of the immune system appears to be the driving force for a global diminution of erythropoiesis, analogous to chronic inflammatory conditions observed in anemia of chronic disease”²⁵. “It is postulated that the immune system may be mobilized to stimulate production of inflammatory cytokines that can impede erythropoiesis. Consequently, there is insufficient differentiation and proliferation of erythroid precursors, leading to anemia”²⁶. “Inflammatory cytokines can also impair iron metabolism which can result in reduced serum iron levels and iron retention within the reticuloendothelial system. Tumors can also produce cytokines, which induce iron sequestration, thereby decreasing RBC production. Shortened RBC survival may also result from over expression of inflammatory cytokines”²⁶. “Anemia can result from bone marrow invasion by solid tumors. Myelophthisis, resulting from bone marrow replacement by solid tumors or hematologic malignancies, may manifest as anemia or pancytopenia. Breast cancer is among the most common tumors associated with bone marrow replacement”²⁷. “While anemia in patients with this cancer is often produced by the cancer itself; the addition of chemotherapy significantly increases the proportion of patients with anemia”²⁸. “The myelosuppressive effects of cytotoxic chemotherapy agents on erythropoiesis are generally cumulative in nature and up to 50% of patients with cancer may develop chemotherapy-induced anemia over the course of chemotherapy”²⁷. “A steady increase

in the rate of anemia occurs with additional cycles of chemotherapy as evidenced by data from the European Cancer Anaemia Survey (ECAS). This study showed that the rate of anemia (hemoglobin [Hb] <12 g/dL) increased from 19.5% in cycle 1 to 46.7% by cycle 5. The percentage of patients with more severe anemia (grades 2 and 3) also increased with greater numbers of chemotherapy cycles”²⁹ . “Patients can also become anemic within the first 2 cycles of chemotherapy as evidenced by data from a separate analysis of ECAS data in patients who were not anemic (Hb>12 g/dL) prior to initiating chemotherapy. In this analysis, 62% of patients experienced an Hb decline by 1.5 g/dL within a median time of 6.1 to 7.2 weeks and 51% experienced an Hb decline by 2 g/dL within a median time of 7.3 to 8.9 weeks”³⁰ . “Depending on the chemotherapeutic agent or regimen, anemia may be mild in degree (grade 1 or 2) in about 10%–85% of patients. Moderate or severe anemia will develop in about 2%–55% of patients and require intervention”³¹ .

“Chemotherapy may cause anemia in multiple ways. First, some chemotherapeutic agents will affect the production of new RBCs by damaging normal bone marrow precursor hematopoietic cells. When these cells are damaged, the ability of the bone marrow to produce new RBCs is impaired. Some drugs, such as platinum-containing agents, are nephrotoxic, and also affect the development of new RBCs by interfering with erythropoietin production by the kidneys”³² .

Patients with cancer may develop anemia secondary to poor nutrition in general or due to reduced function in the gastrointestinal (GI) tracts to absorb nutrients²⁶. Folate deficiency may develop in anorexic patients with cancer, while vitamin B₁₂ deficiency can arise in patients who have undergone gastric or small bowel resection or bypass or have atrophy of stomach parietal cells, which produce intrinsic factor necessary for vitamin B₁₂ absorption ³³. Iron deficiency

anemia due to blood loss or the inability to absorb iron in the GI tract often occurs in patients with malignancies of the GI tract, including colorectal cancers²⁸. Nutrient deficiencies in folate, vitamin B₁₂ or iron may lead to anemia because all of these nutrients are essential to red blood cell (RBC) production and development³⁴.

Therefore, the hemoglobin concentration in peripheral blood was also studied as a prognostic factor in malignant disorders. The role of hemoglobin levels in clinical outcomes has been extensively examined in solid cancers, such as cervical, ovarian, breast, prostate, renal, liver, and endometrial cancer. Study established an association between hemoglobin levels and survival in solid carcinoma. Because of these studies done, close attention should be paid to anemia before and during treatment, with the goal of maintaining adequate hemoglobin levels and, as a consequence, ideally improving cancer outcomes and quality of life³⁵.

The white blood cell count (total and differentials) and packed cell volume predict disease severity and mortality risk³⁶. For example, elevated WBC counts predict a worse prognosis in patients with cancer and anemia predicts increased risk of death of solid cancer patients³⁷. Solid cancer patients with an absolute granulocyte count of 6000/mm³ or more were observed to have a shorter survival than the ones with less than 6000/mm³. A similar phenomenon was observed independently in patients with advanced carcinoma of the colon³⁸. A significantly worse 5-year cancer-related survival for patients with peripheral blood monocyte count >300/mm³ than for patients with a count <300/mm³ was observed in Japan³⁹. The prognostic significance of neutrophils, lymphocyte, platelet, mean platelet volume, platelet-lymphocyte ratio and neutrophils-lymphocyte ratio in patients with locally and advanced gastric cancer were assessed in Turkey and found to influence overall survival⁴⁰. Total leucocyte count (TLC), if elevated, predicts poorer prognosis⁴¹. The white blood cell count (total and differentials) and platelet count predicts disease severity and mortality risk. White blood cell (WBC) count, an

inflammatory biomarker, has become a useful predictor of certain diseases as well as a marker of infection ⁴². An elevated WBC count, even within the normal range, has been associated with breast cancer incidence and mortality rate ⁴³. The role of WBC count as a surrogate for inflammation has not been examined in the context of well-known effect modifiers for breast cancer development. Several studies have attempted to identify the association between WBC counts and solid cancer risk especially in breast cancer, but no consistent evidence has been found ⁴⁴.

The role of neutrophils in human cancers is relatively small. From an initial interest in the 1980s, the number of publications on neutrophils in cancer-related studies has been steadily going down ⁴⁵. However, this trend is now beginning to change with the realization that neutrophils are indeed important players in cancer development, as reflected by several recent reviews ⁴⁶. Also the role of neutrophils in cancer is multifactorial and not fully understood. Neutrophils reflect a state of host inflammation, which is a hallmark of cancer ⁴⁷. They can participate in different stages of the oncogenic process including tumor initiation, growth, proliferation or metastatic spreading ⁴⁸. The various roles of neutrophils in cancer development and progression, by several groups have recently explored the role of neutrophils and other markers of host inflammation on clinical outcomes. Thus, an elevated neutrophil count is an adverse prognostic factor incorporated in a contemporary prognostic score for metastatic Renal Cell Carcinoma (mRCC) treated with targeted therapy ⁴⁹. Furthermore, most data are available for the ratio of neutrophils to lymphocytes measured in the peripheral blood, the so-called Neutrophil-To-Lymphocyte Ratio (NLR). An elevated NLR is associated with worse outcomes in many solid tumors, both in early and advanced stage of cancer ⁵⁰. Moreover, an elevated NLR is associated with lower response rates in castration-resistant prostate cancer treated with abiraterone or docetaxel⁵¹. Also, an early decrease of NLR in response to targeted treatment

appears to be associated with more favorable outcomes and higher response rates in patients with RCC, even after adjustment for known prognostic factors including NLR at baseline⁵⁰. In contrast a rising NLR during the first weeks of treatment had the opposite effect⁵². These findings make NLR a biomarker easy to evaluate, and that have potential for the identification of early responders.

Peripheral blood neutrophil counts are increased in patients with cancer. Tumours produce granulocyte colony-stimulating factor (G-CSF) which skews the neutrophil retention/release balance in bone marrow, leading to this increase in blood neutrophils⁵³. In direct contrast, neutropenia in patients undergoing chemotherapy has been shown (by meta-analysis) to be beneficial to survival⁵⁴. This may of course just be a reflection of adequate toxicity of the drug being achieved to kill tumour cells. It must also be remembered that blood neutrophil levels increase under other conditions, such as infection. Within the same patient, neutrophils may display varying roles at different sites. Furthermore, appropriate inflammatory responses are dependent upon a functioning balance of neutrophil production, release from bone marrow, recruitment to the site of injury and clearance. Dysregulation of this homeostatic process, for example, by tumour-derived G-CSF, could perpetuate malignancy. In many patients with advanced cancer, elevated counts of neutrophils in blood are found. How cancers induce neutrophilia is uncertain, but production of granulocyte-macrophage colony-stimulating factor (GM-CSF) is a possible mechanism in several types of cancer⁵⁵. In addition, other cytokines such as granulocyte colony-stimulating factor (G-CSF), interleukin- (IL-) 1, and IL-6 produced by tumors seem to contribute to elevated neutrophil numbers in blood⁵⁶. This neutrophilia is associated with poor prognosis in several types of cancers, such as lung, melanoma, and renal carcinomas⁵⁷. In agreement with this; the presence of neutrophils within certain tumors seems also to be an indicator of poor prognosis.

Although the diagnosis and treatment of breast cancer (BC) has improved in the past few years, it is still the most common female malignancy worldwide ⁵⁸. In 2012, >1.7 million women were diagnosed with BC, and >521,000 women died of the disease ⁵⁹. A very steep increase in incidence and mortality was observed in 2012 compared to 2008 (21.2% and 13.6%, respectively). Developing countries have a lower incidence of BC than developed countries, but the mortality is high due to the lack of diagnosis and treatment ⁶⁰. Due to its complex nature, the progression and prognosis of BC are not yet well understood. Some of the proven molecular prognostic assays are expensive and inaccessible to most patients ⁶¹. Therefore, predictive factors with economical and practical advantages are desirable. Previous studies found that the neutrophil-to-lymphocyte ratio (NLR) could provide a prompt representation of the state of inflammation, which might play key roles in tumor growth, progression, invasion, and metastasis ⁶². Not only elevated numbers of neutrophils in peripheral blood as reflected by NLR are of prognostic relevance, but also their presence in the tumor can be associated with clinical outcome ⁶³. In this context, it should be noted that what mainly impact the worse outcome is the presence of inflammation within the tumor, and the assessment of neutrophils is an indirect measure of this and can vary among tumor types.

Lymphocytes represent an important component of the inflammatory microenvironment favoring the initiation and progression of malignancies. On the other hand, lymphocytes are key effectors of antitumor immune responses, for instance as they sense senescent cells (CD4⁺ T lymphocytes), as they release cytokines and stimulate other immune cells (NKT cells) or as they exert direct cytolytic activities against transformed cells (NK and CD8⁺ T lymphocytes). Elimination and equilibrium are achieved via lymphocytes, mainly the T cell subpopulation ⁶⁴.

In cancer patients the “healthy” response against the tumor is counteracted by a suppressive, tumor-driven effect. This hypothesis is strengthened by recent studies showing that the absence or presence of T cells in colorectal cancer specimens more accurately predicted the outcome than using standard prognostic factors⁶⁵. Other studies in different types of cancer, mainly cervical and breast cancer have also shown similar results⁶⁶. These studies further confirmed the importance of the immune response in prognosis alongside other more established factors⁶⁷. Recent studies also support the case of immunoediting by observing that tumor infiltration by lymphocytes is linked to tumor-associated immune response, mainly showing that the presence of tumor infiltrating lymphocytes may be associated with improved prognosis and clinical outcome in cancer patients⁶⁸. The composition of lymphocytic populations in blood, ascites and tumors is regulated by various cytokines and chemokines produced by the tumors or the components of the immune system⁶⁹.

The higher the lymphocyte count, the better the overall survival, the lower the platelet-lymphocyte ratio, the better the overall survival. Some researchers did a work on the lymphocytes in the peripheral blood of patients with breast cancer. They found out that the peripheral blood lymphocyte counts were found to be significantly lower in the short-survivors when compared with the long survivors. They concluded that lymphocyte count may be a host factor that influences survival in breast cancer⁷⁰. Another work analyzed the correlation between curability by conventional treatment of the 589 cases of the different types of solid cancer with reasonable possibilities of cure, and the total number of leukocytes in peripheral blood. A positive significant correlation was found between cancer curability and the total number of peripheral lymphocytes, a negative correlation was found between the total number of peripheral neutrophils (segmented and none segmented) and cancer curability. No correlation was found between curability of cancer and monocytes, eosinophil, or basophils. The molecular

mechanisms by which cytotoxic drugs induce depletion of lymphocytes have not been defined and may involve proliferative arrest in lymphocyte precursor compartments or, alternatively, direct induction of apoptosis in mature cells. Their findings indicate that the immunologic activity of peripheral lymphocytes may be a favorable factor in the cure of cancer by conventional treatment⁷¹.

It has been postulated that monocytes promote tumor progression and support host antitumor immunity. Moreover, an increased monocyte count in the peripheral blood is considered a predictive factor of poor prognosis in solid cancer patients⁷². Study was done on the pre-treatment monocyte count (MC) in patients who had had liver resection due to hepatocellular carcinoma, as well as in patients who underwent hepatic surgery due to colorectal metastasis. This work reported that a pre-treatment MC of $\geq 300/\text{mm}^3$ was an independent prognostic indicator of tumor recurrence in liver cancer patients with hepatocellular carcinoma. In the study of 97 patients with colorectal live metastasis, univariate analysis showed that patients with a pre-treatment monocyte count $>300/\text{mm}^3$ had a worse five-year cancer related survival when compared with patients with a monocyte count $\leq 300/\text{mm}^3$ ($p=0.04$), but disease-free survival rates did not differ between both groups⁷³. Elevated pre-treatment monocyte counts in a multivariate analysis were found to be an independent prognostic factor for cancer-related survival. A high peripheral MC (alone or combined with neutrophil count) has also been associated with adverse outcomes in cervical and ovarian cancer⁷⁴.

An increased pre-operative platelet count has been identified as an adverse prognostic indicator in bronchial cancer, gastric and gynecological malignancies⁷⁵. A high platelet count is associated with tumor progression and poor survival in patients with solid cancers especially in esophageal carcinoma⁷⁶. The relationship between cancer and thrombosis was established in the

late 19th century by Armand Trousseau⁷⁷. Since then, thrombocytosis has been associated with cancer prognosis. Clinical studies have investigated the frequency of high platelet count in patients with cancer and the role of high platelet count in patient outcomes. The overall survival (OS) of patients with ovary cancer, lung cancer, and breast cancer has been related to thrombocytosis at the time of diagnosis⁷⁸. Except diagnosis, poor prognosis of colorectal cancer and renal cancer are related to high platelet counts at presurgery⁷⁹. Another work reported that platelet count is a predictor of metastasis and venous thromboembolism in patients with cancer⁸⁰. Thrombocytopenia, is a common hematological complication of liver cancer caused by decreased production of hormone thrombopoietin (TPO) in the damaged liver and increased destruction of platelets through phagocytosis in the enlarged spleen, as well as the loss of hematopoietic function in bone marrow due to alcohol abuse or viral infection⁸¹.

Infiltration of the bone marrow by metastatic tumour cells can result in bone marrow failure and resultant hematologic abnormalities. As cancer cells invade the healthy marrow, they replace hematopoietic stem cells, leading to the depletion of multiple cell lines. A study done describe a typical pattern of anemia, thrombocytopenia, and increased mortality⁸². Another work described 3 cases in which patients presented with both epithelial malignancies and presumed ITP. The mechanism of carcinoma-associated ITP has not yet been elucidated. Age might be a contributing factor, given that many of the individuals described in the literature with both ITP and carcinoma are older than the average patient with isolated ITP. With advanced age comes immune system dampening and modulation that could potentially predispose to both malignancy and thrombocytopenia⁸³. Other theories include an increase in anti-platelet antibody with carcinoma, as well as the presence of immune-modulating oncogenic viruses⁸⁴.

Recently, there has been intense interest in the prognostic value of peripheral blood biomarkers in colorectal cancer (CRC). Inflammation has been reported to be involved in

carcinogenesis and disease progression and local cancer-related inflammation can be reflected by a systemic inflammatory response (SIR) ⁸⁵. Nearly a third of cancer patients have thrombocytosis at diagnosis and aberrant activation of platelets has been shown to be associated with CRC⁷⁹. Lymphocytes are essential components of the tumor microenvironment, which contributes to carcinogenesis ⁸⁶. Monocytes have been reported to influence CRC progression and can be used to predict prognosis⁸⁷. Therefore, a comprehensive evaluation of the literature is warranted. Routine peripheral blood counts may be useful prognostic factor for evaluating the accuracy of risk stratification in patients with solid cancers. Since chemotherapy and other solid cancer treatment affect the full blood count, it is important to know the extent of these effects by comparing the full blood count results before and during treatment in these subjects.

Erythrocyte sedimentation rate (ESR) is the most widely used laboratory test for evaluating the inflammatory status in clinical practice, including infection, autoimmune and malignant diseases⁸⁸. Elevated ESR is frequently encountered in patients with cancer. The outcome in various malignancies depends on the type of the underlying disorder, the stage and duration of the disease, and the regimen and intensity of the antitumor treatment ⁸⁹. In addition, an elevated ESR level has also been identified as a prognostic factor adversely affecting survival in cancer patients ⁹⁰. A number of studies indicated that an increased ESR level is associated with worse survival; patients with higher ESR values in various malignancies, including colorectal cancer ⁸⁸, renal cell cancer ⁹¹head and neck cancer ⁹² soft tissue sarcoma⁸⁹, breast cancer ⁹³, and prostate cancer ⁹⁴, had a shorter survival compared with those with normal ESR levels.

The complete blood count is a prerequisite investigation for solid cancer patients before the use of any treatment ⁹⁵. Complete blood counts are routinely performed during chemotherapy and other breast cancer treatments to check the number of each type of blood cell circulating in

the body. The complete blood count also helps to check for different side effects of chemotherapy. Blood counts are monitored regularly before each cycle of treatment in breast cancer patients, since cancer treatments affect the bone marrow's ability to make blood cells. Chemotherapy medications and radiation exposure can significantly reduce the levels of blood cells. This reduction increases the risk of infection, fatigue and bleeding. Complete blood count especially lymphocytic count reflects the response of cellular immunity in a cancer patient. The alteration in hematological parameters influences the disease progression. Hemoglobin (Hb) and packed cell Volume (PCV) are indirectly associated with increased risk of cardiac failure in cancer patients⁹⁶.

It is evident that components of the CBC count can provide valuable prognostic information in solid tumors and hematologic malignancies that are not only limited to survival predictions or assessment of disease progression, but also are important tools when evaluating response to treatment. Thus, true assessment of the utility of the CBC count as an inexpensive, established, and globally accessible prognostic factor in many malignancies requires careful studies of the sample results obtained. It is likely that future prospective studies examining the biology behind the prognostic value of the different components of the CBC count would later yield significant therapeutic progress and a thorough understanding of disease pathogenesis.

In this present study, complete blood count was studied in order to determine and compare their pre-treatment and treatment CBC results for prognostic values during the courses of chemotherapy to prevent the risk of unpleasant and life threatening side effects such as anaemia, fatigue, infections and bleeding. Also to prevent disruption of delivery of the treatment, due to none efficient monitoring of the CBC which can result in change to the planned dose and time.

NLR, PLR, LMR IN BREAST CANCERS

Many inflammatory factors are associated with BC prognosis. However, the association between the preoperative NLR and prognostic value in BC patients remains controversial⁹⁷. An increasing number of studies had concentrated on the relationships between NLR and the prognosis of tumor, and the breast cancer were also included⁹⁸. In a retrospective, longitudinal, cohort study of 437 consecutive female breast cancer patients, conclusion was drawn that NLR, as an independent predictor of breast cancer mortality, was superior to platelet-to-lymphocyte ratio (PLR)⁹⁹. While another researcher, indicated that both increased NLR and PLR are associated with poor survival in breast cancer, but only NLR is independently correlated with OS and Disease-Free Survival (DFS)¹⁰⁰.

A work reported that NLR was shown to be better than derived Neutrophil/Leukocyte–Lymphocyte Ratio (dNLR) in terms of predicting prognosis in patients with breast cancer and a high pretreatment NLR (NLR > 4) was associated with poor survival (DFS and OS) in patients¹⁰¹. Another work found that preoperative high NLR was a significant diagnostic predictor of distinction of breast cancer from benign proliferative breast disease and elevated NLR was also an important prognostic marker for primary invasive breast cancer in a randomized controlled trial, and the optimal cutoff for NLR was 2.96¹⁰². In multivariate analysis, NLR is an independent predictor of short- and long-term mortality in breast cancer patients with NLR > 3.3 after adjusting for possible confounder¹⁰³. As for patients undergoing breast cancer surgery, NLR \geq 4 is associated with a higher risk of relapse and NLR \geq 3 is associated with a higher risk of relapse and higher mortality¹⁰⁴.

A work demonstrated that patients with NLR \geq 2.06 showed poorer response to neoadjuvant chemotherapy and a lower pathological Complete Response (pCR) rate than those with NLR < 2.06. High NLR was an independent prognostic factor for poor DFS and breast

cancer-specific survival (BCSS) in these patients with breast cancer undergoing preoperative chemotherapy¹⁰⁴. However, some researchers also discovered that cell death induced by some types of chemotherapy can improve CTL responses. Therefore, it is necessary to consider the stability of NLR in the process of drug therapy¹⁰⁵. Clinicians may think over what kinds of drugs the patients have taken prior to employing NLR as the prognostic factor of breast cancer.

Furthermore, when it comes to the diagnosis of breast cancer in the early stages, it should be noted that at this period the indicators of immune system appear not to be strong enough for the detection, due to a lack of systemic abnormalities in the body. When it comes to stages, the value of NLR ratio would rather likely play a predictive role. Japanese researchers also reported that preoperative NLR might be an independent prognostic factor for survival in Japanese patients with breast cancer, meanwhile they pointed out that NLR was significantly higher in patients with lower body mass index¹⁰⁶. As for Chinese patients with breast cancer, researchers found that patients with high NLR >2.57 showed a significantly lower OS than those with lower NLR¹⁰⁷. Another work reported that NLR is independently correlated with OS and DFS; the cutoff value of NLR (3.0) was consistent with that of most of previous studies¹⁰⁰.

It should be noted that some studies indicate patients with a lower NLR had a better prognosis, whereas other studies have failed to show an association (Jia *et al.*, 2015). Thus, a meta-analysis of the association between the pretreatment NLR and the prognosis of BC patients is warranted¹⁰⁹. Another study evaluated pretreatment blood PLR, NLR and LMR for its prognostic values in patients with breast cancer. This work comprised of 436 breast cancer patients with median age of 52.5 years (25-78 years). The cut-off value NLR was ≥ 2.65 , LMR ≥ 0.28 and PLR ≥ 190.9 . This work concluded that elevated pretreatment NLR (≥ 2.65) and PLR (≥ 190.9) were associated with lower overall survival (OS) in breast cancer (BC) while

LMR did not affect OS¹¹⁰. In another work done using 239 breast cancer patients, they evaluated also the pretreatment LMR in all the patients. A cut-off value was set at 6.00 using ROC. A total of 119 BC patients (49.8%) were classified in the high LMR group and 120 BC patients (50.2%) in the low LMR group. The low LMR group had significant worse disease-free survivals (DFS) in these patients while high LMR had significant response to therapy¹¹¹.

AIM

To use the peripheral blood cells as an assessment of inflammatory biomarkers in breast cancer patients attending Surgery Out Department at ESUT Teaching Hospital, Parklane Enugu.

SPECIFIC OBJECTIVE

1. To determine the complete blood count and erythrocyte sedimentation rate of the subjects at their pre-treatment and treatment period.
2. To calculate the neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) in the subjects as prognostic biomarkers.
3. To determine age group susceptibility.

JUSTIFICATION OF THE STUDY

This work will be used to show that a simple, lower costs and less complicated procedure complete blood count can compare favourably with the more extensive, expensive and complex molecular markers for the diagnosis and prognosis in these subjects using longitudinal analysis.

STATEMENT OF THE PROBLEM

The encumbrances and delay in the early detection, diagnosis and treatment on cancer subjects has led to early deaths, so the development of simple, cost effective, fast and non-invasive method will go a long way in alleviating the delays in treatment of these subjects

ensuring chances of long time survival. Determination of the specific age group that is more susceptible to these solid cancers will increase awareness and screening in that age group.

LIMITATION OF STUDY

The economic hardship on these subjects affected their availability for treatment hence decreased the sample size. More subjects would have been included if this study follow-up was done for more than two years period.

SCOPE OF THE STUDY

This work is a longitudinal study that involves the use of questionnaire in obtaining demographic and clinical information needed in this research. It involved also the use of fully automated machines that helped produce results with precision, accuracy and quality assurance.

MATERIALS AND METHODS

STUDY SITE

This study was conducted in ESUTH Teaching Hospital Parklane GRA Enugu, Enugu State. Enugu was created on 27th August 1991. Enugu State is one of the five states in the South Eastern geopolitical zone of Nigeria and was the administrative capital of the former East Central State. It has an area of 8727.1 square kilometers. It is bounded by Anambra State on the west, Imo and Abia States on the south, Kogi state on the north and Ebonyi and Benue States on the east. The state has a projected population of over 3.5 million people. The major municipal cities are the capital, Enugu and Nsukka. Within Enugu State, there are six (6) district hospitals, thirty-six (36) cottage hospitals and three hundred and sixty-six (366) primary health care centers, health centers and health posts. ESUT Teaching Hospital is one of the hospitals offering comprehensive surgical health services in Enugu. The people of Enugu are mainly Igbo by tribe, though other tribes like Hausa, Yoruba etc, are well represented as well. The inhabitants of

Enugu state are mainly civil servants, artisans, students, farmers and traders. The prevalent religion in the area is mainly Christianity of several denominations ranging from Roman Catholic, Anglican to Protestants. Though there are few inhabitants who practice Islam and Traditional religion.

STUDY DESIGN

This was a longitudinal study. The pre-treatment samples were collected at diagnosis and the treatment samples collected at different stages of the treatment. The base line samples collected from the subjects were used as control and compared with other subsequent samples collected from same subjects at various stages of the treatment and the changes noted were reported.

STUDY POPULATION

The sample size comprised of 110 female subjects and controls with ages between 21-70 years. There was no ethnicity differentiation. Questionnaires were used to obtain other demographic characteristics, clinical/provisional diagnosis, their life styles, and the staging of these solid cancers. Follow up of the subjects began at entry of this study. Subjects were followed monthly for a period of six months depending on the scheduled clinical appointments of the subjects by their clinician

CRITERIA

Exclusion criteria:

1. Subjects suffering from other types of health problems like liver cirrhosis, active bleeding, intestinal obstructions, diabetes, hyper blood pressure, non-solid cancers examples leukemia, lymphomas, myelomas, mixed cancers like adenosquamous carcinomas, mixed mesodermal tumors, and other types of solid cancers.

2. Subjects with the presence of any diagnosed haematological system diseases.

Inclusion criteria:

1. All subjects suffering from all forms of breast cancer, as diagnosed by their clinician at the different stages of the illness.
2. Subjects with life expectancy of more than three years.

DATA COLLECTION

Subjects' data including demographics (example ages, sexes, level of education occupation and soon on) and clinicopathological features (cancer location, and stages) were all obtained using questionnaires. The cancer staging was performed according to the 7th edition of the Union for International Cancer Control- American Joint Committee on Cancer Association on cancer classifications. Blood sampling were performed to measure erythrocyte sedimentation rate (ESR), total and differential leucocytes counts, platelet counts for the calculation of neutrophil/lymphocyte ratio (NLR), platelet / lymphocyte ratio (PLR), lymphocyte / monocyte ratio (LMR). These ratios are defined as the total number of neutrophils, platelets, monocytes divided by the total number of lymphocytes.

SAMPLE PROCESSING

Sequestrated sample of a total five milliliters of blood were collected by venipuncture at the antecubital vein from all the subjects at different stages (pre-treatment and treatment). The blood samples were collected in dipotassium ethylenediaminetetraacetic acid (K₂EDTA) containers commercially prepared and processed immediately. Stored blood samples were not used in this research work. The complete blood count (CBC) and ESR were done as soon as possible or at least within thirty minutes to one hour from the time of collection. The sample

collections and processing were done at all the different stages of cancer in this research work and results analyzed.

DETERMINATION OF HAEMATOLOGICAL PARAMETERS

Haematological parameters such as; haematocrit (HCT), haemoglobin concentration (Hb), total white cell count (TWCC), differential white cell count, total platelets count, MCHC, MCV, MCH were immediately analyzed on samples collected in EDTA tubes by a haematological analyzer “Be-5300 – Mindray” Japan. Determination of erythrocyte sedimentation rate was done using Westegren method.

Statistical Analysis

Sample size was calculated using Graphpad Prism of Statmate Software version 2.0. A sample size of 110 has 90% power to detect a difference between means of 0.33 with significant difference level (alpha) of 0.05 (two-tailed). The mean and standard deviation (mean value \pm SD) of the data were tabulated for each group. The data was analyzed with IBM Statistical Package for Social Sciences (SPSS PC. version 20.0; SPSS Inc., Chicago, III., USA). The ROC curve was used to determine the different cut-offs in the ratios (NLR, LMR and PLR). Cox proportional-hazards regression analysis was used to evaluate the prognostic factors (ages, duration, ratios and their cancer diagnosis). Overall survival (OS) was defined as the duration from diagnosis to death or last follow-up. The ANOVA and Tukey HSD post- hoc test were used to compare the results obtained within controls, pre-treatment and treatment result; age groups and the age group susceptibility.

RESULTS

Table 1: Demographic table of the Breast cancers.

A sample size of 110 female subjects with age range mean±SD of 44.±11.3 were studied. Educational qualifications were: primary, 0(0%); secondary, 62(56%) and tertiary, 48(44%). There occupations were: civil servants, 38(35%); business, 62(56%) and students, 10(9%). The duration was calculated from the onset of diagnosis to the end of this research and it was calculated in months. The duration was grouped into three categories in this work. The duration on each of the cancer were reported in mean±SD. The total number and percentage was reported for Breast Ca. Average treatment intervals in weeks (mean±SD) were: breast cancer (37.0±19.0). The controls of 110 subjects used in this work were apparently healthy individuals. A total number of ten breast cancer subjects were lost to death.

TABLE 1: SOCIODEMOGRAPHIC CHARACTERISTICS OF POPULATION

	Total number		Percentage	
	Test Subjects	Control	Test Subjects	Control
Gender (Females)	110	110	100	100
Level of Education				
Primary	0	0	0	0
Secondary	62	0	56	0
Tertiary	48	110	44	100
Occupation				
Civil Servants	38	55	35	55
Business	62	0	56	0
Students	10	65	9	65
Age(years)Mean±SD	44.0±11.3			
Age Groups				
21-30	14	40	12.7	36.4
31-40	33	25	30.0	22.7
41-50	29	28	26.4	25.5
51-60	28	12	25.5	10.9
61-70	6	5	5.5	4.5
Duration (months)mean±SD	29.5 ±12.7			
11-30	64	0	58.0	0
31-50	41	0	41.0	0
51-70	5	0	5.0	0

Table 2: High and Low optimal cut-off values in the Breast cancers with their total number and percentages respectively.

Receiver Operating Characteristic (ROC) curve calculated using Youden index for AUC (area under the curve) were constructed between death events and censors. The optimal cut-off values of pretreatment NLR, LMR, and PLR were calculated using ROC curve. According to these optimal cut-off values, the 110 subjects were classified into two groups: high and low NLR, LMR, and PLR with their respectively percentage.

TABLE 2: HIGH AND LOW OPTIMAL CUT-OFF VALUES IN BREAST CANCERS WITH THEIR TOTAL NUMBER AND PERCENTAGES RESPECTIVELY.

	NLR	LMR	PLR
Optimal cut-off	2.45	2.60	9950.0
Sensitivity	0.89	0.97	1.000
specificity	0.045	0.450	0.000
AUC	0.990	0.983	0.980
p-value	0.00001	0.00001	0.000
High (N %)	25(23%)	56(51%)	73(66%)
Low (N %)	85(77%)	54(49%)	37(34%)

Table 3: The prognostic purposes of NLR, LMR and PLR in breast cancer

A total of 85(77%) of breast Ca subjects had low NLR (<2.45) while 25(23%) had high NLR (>2.45). In LMR, 54(49%) had low ratio (<2.60) and 56(51%) had high ratio (>2.60). 37(34%) had low PLR (<9950) while 73(66%) had high PLR (>9950). Positive signs were observed in these ratios' constant coefficient (B) NLR (0.21) and LMR (0.60). Hazard ratio(HR) for NLR is 1.23 (95%CI:1.12-1.35; p=0.0000) and LMR is 0.60 (95%CI:0.42-0.80; p=0.0000) meaning that high NLR and lower LMR ratios are associated with increased HR and decrease or shortened overall survival (OS) time in the subjects. A unit increase in NLR and decrease LMR by 1.0 increase HR of the ratio values by 1.23(NLR) and 0.60(LMR) folds. Also a unit increase

in NLR and decrease LMR decreases the OS time in the subjects by 1.35 and 0.80 months respectively. But in PLR, HR equals 1.0, meaning that there is no effect between high PLR and low PLR in these subjects even with the high significant p-value. The graphs summarize the result (figure 1 to 3), increase NLR and decrease LMR, decreases the survival time.

**TABLE 3: THE PROGNOSTIC PURPOSES OF NLR, LMR AND PLR IN BREAST
CANCER**

Covariates (mean/N)	Coefficient (β)	Standard error	P-value	Exp(B) (Hazard ratio)	95% CI for Exp(B) Lower Upper	
NLR(2.32) [:0 <2.45(85); 1>2.45(25)]	0.21	0.05	0.0001*	1.23	1.12	1.35
LMR (6.41) [0:<2.60(54);1>2.60(56)]	0.60	0.20	0.0001*	0.60	0.42	0.80
PLR (145.0) (0:<9950.0(37);1>9950.0(73))	0.03	0.01	0.01*	1.00	1.00	1.01

P<0.05*-signifies a significant difference.

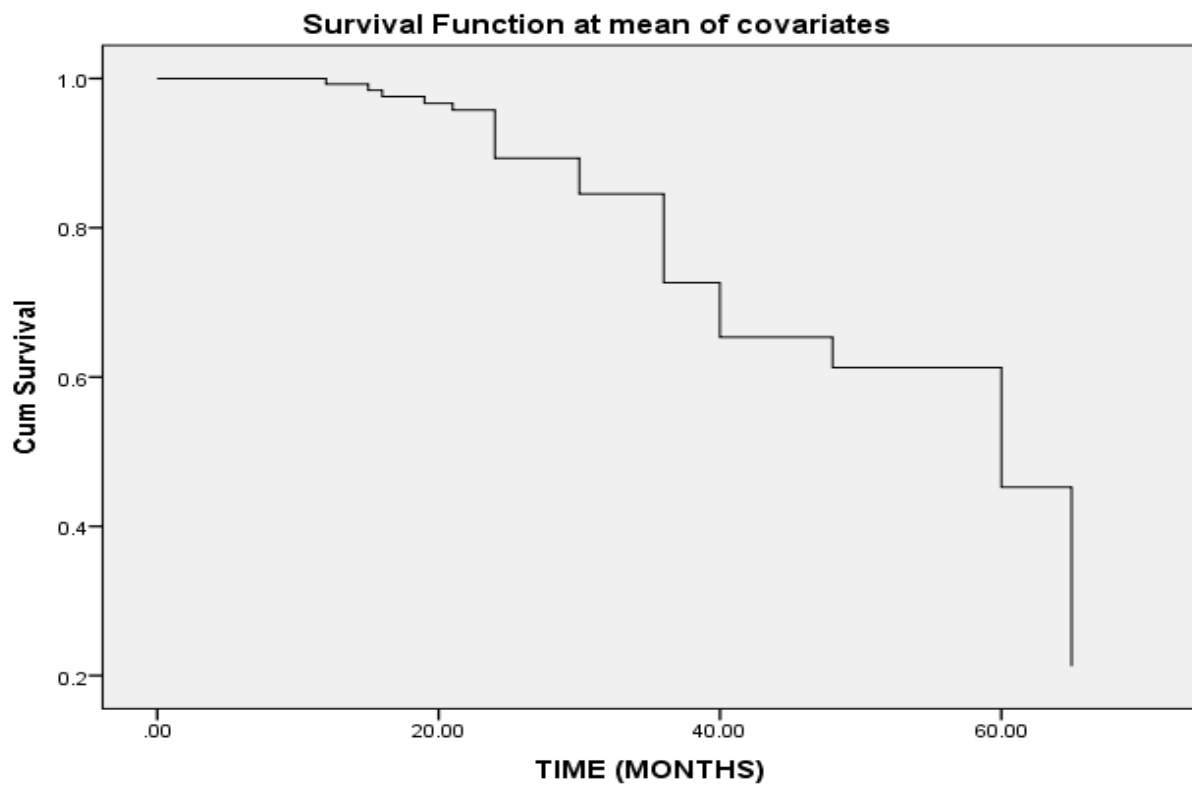


FIGURE 1: NLR SURVIVAL FUNCTION

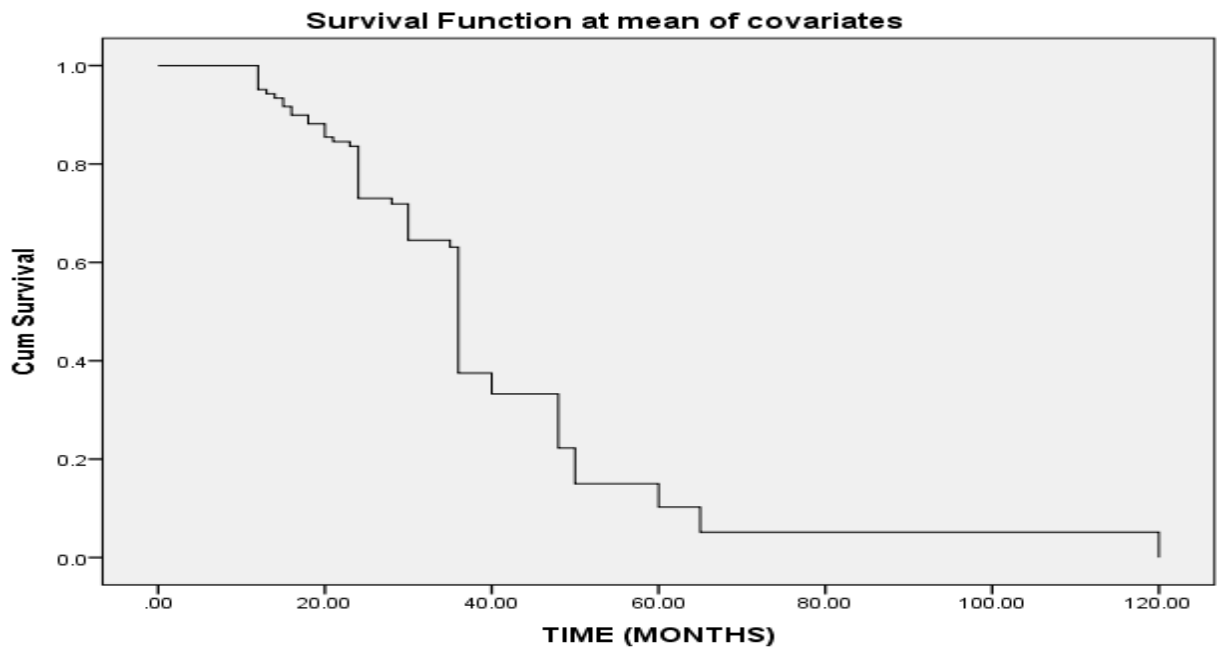


FIGURE 2: LMR SURVIVAL FUNCTION

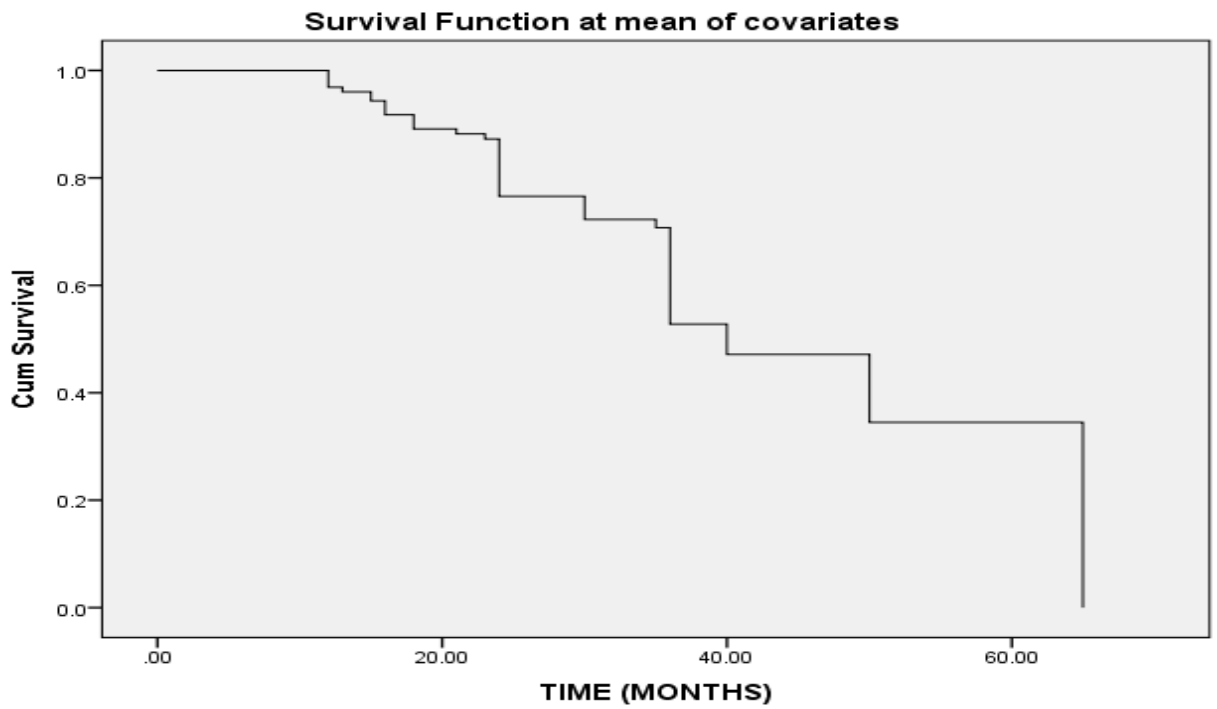


FIGURE 3: PLR SURVIVAL FUNCTION

Table 4: Comparing the CBC and ESR results in controls, pre-chemotherapy and chemotherapy in Breast cancer(BCa) subjects using ANOVA with Turkey HSD Post-Hoc

Analysis of Variance (ANOVA) was used to calculate the difference between and within the controls (N=110), pre-treatment (N=110) and treatment (N=90) CBC and ESR result in BCa subject. A significant difference at $P \leq 0.05$ between and within all the RBC parameters, TPLT and WBC parameters were observed. A Turkey post-hoc of the mean \pm SD was carried out on the RBC parameters, ESR TPLT, MPV, TWBC, ANC, ALC and AMC within and between the control, pre-treatment and treatment results. In TRBC, a significant decreased in treatment (3.75 \pm 0.6) at $p=0.0001$ with control ((4.1 \pm 0.1) and pre-treatment (4.14 \pm 0.7). In HB, a significant decreased in treatment (9.95 \pm 1.4) at $p=0.0001$ with control (12.5 \pm 0.1) and pre-treatment (11.20 \pm 1.8). In HCT, a significant decreased treatment (29.90 \pm 1.4) at $p=0.0001$ with control (36.9 \pm 3.4) and per-treatment (33.93 \pm 5.4). In MCHC, a significant decreased treatment (30.83 \pm 2.6) at $p=0.0001$ with control (32.6 \pm 2.2) and per-treatment (33.93 \pm 2.3). In MCV, a significant decreased treatment (85.0 \pm 7.5) at $p=0.0004$ with control (82.0 \pm 1.8) and also pre-treatment (81.7 \pm 6.4). In MCH, a significant decreased treatment (26.22 \pm 2.8) at $p=0.0001$ with control (30.0 \pm 1.9) and per-treatment (26.14 \pm 3.0). In RDW, a significant increased treatment (14.0 \pm 2.0) at $p=0.0001$ with control (11 \pm 1.0) and per-treatment (11 \pm 1.9). In TPLT, a significant increased treatment (280372 \pm 98058.9) at $p=0.0003$ with control (224.855 \pm 39.198) and per-treatment (272.481 \pm 116.262). In MPV, a significant decreased treatment (10.0 \pm 1.9) at $p=0.01$ with control (10.5 \pm 0.9) and pre-treatment (11 \pm 3.3). In TWBC, a significant decreased treatment (4.96 \pm 4.5) at $p=0.0001$ with control (4.0 \pm 0.7) and pre-treatment (6.20 \pm 4.3) was observed. In ANC, a significant decreased treatment (3.5 \pm 2.1) at $p=0.01$ was observed between control (4.3 \pm 1.0) and per-treatment (5.1 \pm 2.7); also control (4.3 \pm 1.0) and treatment (3.5 \pm 2.1) at $p=0.02$; pre-treatment (5.1 \pm 2.7) and treatment (3.5 \pm 2.1) at $p=0.0001$. In ALC, a significant decreased

treatment (1.7±0.7) at p=0.001 with control (2.9±0.5) and pre-treatment (2.5±1.5) at p=0.01; control (2.9±0.5) and chemotherapy (1.7±0.7) at p=0.0001, pre-treatment (2.5±1.5) and treatment (1.7±0.7). In AMC, significant decreased treatment (0.02±0.3) at p=0.0001 with control (0.5±1.0) and pre- treatment (0.2±0.3). In ESR, significant increased treatment (66.40±30.3) at p=0.0001 with the control (14.63±6.3) and pre- treatment (44.70±29.8) was observed.

TABLE 4: COMPARING THE CBC AND ESR RESULTS IN CONTROLS, PRE- TREATMENT AND TREATMENT IN BREAST CANCER SUBJECTS USING ANOVA WITH TURKEY HSD POST-HOC

Parameter	Control (mean±SD) (N=110)	Pre- treatment (mean±SD) (N=110)	Treatment (mean±SD) (N=90)	f- value	p-value	A vs B	A vs C	B vs C
TRBC(×10¹²l)	4.1±0.1	4.14±0.7	3.75±0.6	11.5	0.0001*	0.9	0.0001*	0.0001*
HB(g/dl)	12.5±1.0	11.20±1.8	9.95±1.4	83.2	0.0001*	0.0001*	0.0001*	0.0001*
HCT (%)	36.9±3.4	33.93±5.4	29.90±4.3	68.7	0.0001*	0.0001*	0.0001*	0.0001*
MCHC(g/dl)	32.6±2.2	31.35±2.3	30.83±2.6	16.9	0.0001*	0.001*	0.0001*	0.14
MCV(fl)	82.0±1.8	81.7±6.4	85.00±7.5	10.9	0.0001*	0.9	0.0004*	0.0001*
MCH(pg/cell)	30.0±1.9	26.14±3.0	26.22±2.8	19.8	0.0001*	0.0001*	0.0001*	1.0
RDW (%)	11±1.0	11±1.9	14±2.0	81.2	0.0001*	0.0001*	0.0001*	0.0001*
PLT(×10⁹l)	224.855 ±39.198	272.481 ±116.262	280.373 ±98.060	12.1	0.0001*	0.0003*	0.0001*	0.8
MPV (%)	10.5±0.9	11±3.3	10±1.9	10.9	0.0001*	0.2	0.0001*	0.01*
TWBC(×10⁹l)	4.0±0.7	6.2±4.3	4.96±4.5	10.5	0.0001*	0.0001*	0.2	0.02*
ANC(×10³l)	4.3±1.0	5.1±2.7	3.5±2.1	15.1	0.0001*	0.01*	0.02*	0.0001*
ALC((×10³l)	2.9±0.5	2.5±1.5	1.7±0.7	35.3	0.0001*	0.01*	0.0001*	0.0001*
AMC((×10³l)	0.5±1.0	0.2±0.3	0.2±0.3	51.1	0.0001*	0.0001*	0.0001*	1.0
AEC((×10³l)	0.2±0.4	0.1±0.3	0.1±0.3	3.20	0.06	0.07	0.09	0.9
ABC((×10³l)	-	-	-	-	-	-	-	-
ESR(mm/hr)	14.3±6.3	44.70±29.8	66.40±30.3	122.5	0.0001*	0.0001*	0.0001*	0.0001*

P<0.05*-signifies a significant difference. A (control), B (pre- treatment), C (treatment)

Table 5: CBC and ESR results at different age groups in breast cancer

There were no significant differences at $P > 0.05$ observed in all the CBC and ESR parameters measured within and between the different age groups using ANOVA.

TABLE 5: RED BLOOD CELL PARAMETERS RESULTS AT DIFFERENT AGE GROUPS IN BREAST CANCER(BCa) USING ANOVA

Age groups (years/N)	TRBC $\times 10^{12}/l$	HB g/dl	HCT %	MCHC g/dl	MCV fl	MCH pg	RDW %	ESR mm/hr
21-30 (n=14)	3.9 \pm 0.8	10.9 ± 2.3	32.1 \pm 7.7	32.0 \pm 2.7	79.7 ± 8.1	25.6 \pm 2.3	13.5 ± 2.6	32.2 \pm 24.6
31-40 (n=33)	4.1 \pm 0.7	10.9 \pm 2.0	33.2 \pm 6.1	31.0 \pm 2.1	80.9 ± 6.0	25.6 \pm 4.0	14.2 ± 3.2	51.4 \pm 31.6
41-50 (n=29)	4.3 \pm 0.8	11.3 \pm 1.7	34.8 \pm 4.6	31.7 \pm 1.6	83.1 ± 5.1	26.5 \pm 2.8	12.6 ± 1.8	47.6 \pm 31.8
51-60 (n=28)	4.1 \pm 0.7	11.3 \pm 1.6	34.5 \pm 4.3	32.0 \pm 2.3	83.0 ± 5.5	26.5 \pm 2.0	13.2 ± 2.0	42.7 \pm 27.1
61-70 (n=6)	4.2 \pm 0.4	11.8 \pm 0.9	35.3 \pm 2.7	29.0 \pm 3.8	84.0 ± 2.6	27.0 \pm 0.9	12.9 ± 2.1	32.3 \pm 28.8
F (p) value	0.78 (0.5)	0.54 (0.7)	0.91 (0.5)	2.41 (0.06)	1.54 (0.2)	0.80 (0.6)	1.8 (0.1)	1.4 (0.2)

Not significant at $P > 0.05$

**TABLE 5: PLATELET AND WHITE BLOOD CELL PARAMETERS RESULTS AT
DIFFERENT AGE GROUPS IN BREAST CANCER(BCa) USING ANOVA**

Age groups (years/N)	PLT×10⁹l	MPV%	TWBC ×10⁹l	ANC× 10⁹l	ALC×1 0⁹l	AMC ×10⁹l	AEC ×10⁹l
21-30 (n=14)	263.500± 113.236	10.8 ±2.1	6.8± 2.9	4.2 ±2.1	2.5 ±0.8	0.09 ±0.1	0.03 ±0.06
31-40 (n=33)	264.697± 120.133	10.8 ±2.7	6.6± 6.9	5.0 ±4.3	3.2 ±4.7	0.2 ±0.5	0.1 ±0.2
41-50 (n=29)	295.414± 124.236	10.8 ±2.6	6.2± 2.2	5.2 ±2.1	2.3 ±0.8	0.2 ±0.2	0.06 ±0.1
51-60 (n=28)	265.679± 107.355	11.7 ±3.7	5.7± 2.3	4.5 ±2.6	2.2 ±1.0	0.3 ±0.5	0.08 ±0.1
61-70 (n=6)	251.167± 12.680	10.6 ±1.5	5.2± 1.6	4.0 ±1.5	2.1 ±0.5	0.1 ±0.3	0.02 ±0.04
F (p) value	0.40 (0.8)	0.5 (0.7)	0.33 (0.9)	0.5 (0.8)	0.7 (0.6)	0.8 (0.5)	1.0 (0.4)

Not significant at P >0.05

DISCUSSION

The relationship between NLR, LMR and PLR and prognostic significance in patients with breast cancers have been reported by many studies, however inconsistent results have been presented so far. No reference values have been established in Nigeria and Africa to the best of my knowledge. We made an attempt to determine different cut-off values for all the breast cancers using the pre-treatment CBC results. And in addition, determine the NLR, LMR and PLR ratio with their respective percentage in each group using longitudinal approach. Previous studies determined these ratios in retrospective studies. The duration of the disease was used to determine the overall survival using the different ratio cut-off values.

BREAST CANCER RATIOS

Although the diagnosis and treatment of breast cancer (BCa) has improved in the past few years, it is still the most common female malignancy worldwide⁵⁸. Developing countries have a lower incidence of BCa than developed countries, but the mortality is high due to the lack of diagnosis and treatment⁶⁰. Due to its complex nature, the progression and prognosis of BCa are not yet well understood. Some of the proven molecular prognostic assays are expensive and inaccessible to most patients. Therefore, predictive factors with economical and practical advantages are desirable. Studies found that the neutrophil-to-lymphocyte ratio (NLR) could provide a prompt representation of the state of inflammation, which might play key roles in tumour growth, progression, invasion, and metastasis BCa^{62,97,98}. In the prognostic determination of NLR, LMR, PLR in BC, so many researchers did a single ratio determination (NLR) with different NLR cut-off values. A cut-off value of >2.96 ¹⁰²; >4.0 ¹⁰¹ and >3.3 ⁹⁹ were used, only one researcher, used the three ratios (NLR, LMR, PLR) in prognostic determination in BCa¹¹⁰. It is increasingly recognized that the host inflammatory response play a critical role in the development and progression of BCa. LMR is a measure of the relative differences between lymphocytes and

monocytes counts and is an index of systemic inflammation. Lymphocyte and monocytes are two key components of the human immune system. The hosts' anticancer immune response relies mainly on lymphocytes which activates the anticancer immune response processes of the host system by releasing lymphatic factor to kill the cancer cells. A high LMR indicates an increase lymphocyte count and a decrease monocyte count while a low LMR has the opposite effect. A low LMR can thus be interpreted as not being favourable in the prognostic outcome in these subjects while a higher LMR indicated higher response and increased host defense against this cancer. High pre-treatment NLR is associated with higher risk of relapse for BCa patients being treated with surgery⁹⁸; poorer response to chemotherapy¹⁰⁵; lower overall survival (OS)^{107,110}.

In this present work, the three ratios: NLR of 2.45, LMR, 2.60 and PLR, 9950.0 were used the three ratios were used. This work confirmed that NLR, LMR and PLR had relationship with the duration of the cancer progression and overall survival. A lower pre-treatment NLR and higher LMR were independently associated with a favourable prognosis while a higher value NLR and lower LMR were associated with worse prognosis. The PLR in these subjects is not an independent prognostic marker in BCa. This can be explained that most of the subjects in this study present themselves at a later stage of this cancer when the cancer near metastased and the immune system are overwhelmed by this disease, hence the chances of lymphocytes performing their defense mechanism in no longer viable. The neutrophils are known to be able to aid the proliferation and survival of malignant cells, promote angiogenesis and metastasis while lymphocyte suppresses tumour growth and invasion through their cytolytic activities. So taken together, the subjects with high NLR will relatively have lymphocytopenia and as a result exhibit a poorer immune response to malignant advanced tumour, thereby worsening their prognosis. In **table 3 and figure 1-3** shows the shortened survival in these BCa subjects.

. The importance of these ratios cannot be over emphasized especially in monitoring cancer progression, therapy and response rate in BCa subjects. So in this work, the ratio results reported is consistent with other reported works done by previous researchers. No matter the predictors used in these ratios especially NLR in these subjects, NLR still remains an important prognostic biomarker in BCa.

BREAST CANCER: PRE-TREATMENT AND TREATMENT

Full blood count is a prerequisite investigation requested from all cancer patients before surgery, use of chemotherapy and/or radiotherapy. Poor parameters adversely influence the outcome of cancers²³. Hematological parameters and markers of the systemic inflammatory response have been correlated with prognosis in several solid cancers²⁴.

In this present study the treatment parameters (TRBC, Hb, PCV, ESR) sample results were significantly associated with decreased, values when compared with their pre-treatment sample results at $p < 0.05$. These results showed a classical case of anaemia in these subjects. This immune system once activated stimulates the production of inflammatory cytokines that impedes erythropoiesis hence leading to insufficient differentiation and proliferation of erythroid precursors leading to anaemia²⁷. Also these cytokines can be produced by the cancer cells themselves which then induces iron sequestration, thereby decreasing RBC production⁷⁶. Over expression inflammatory cytokines causes shortened RBC survival⁴². This work is consistence with works done by ^{27,76}. In this present work, there is significant decrease of the treatment TWBC compared to the pre-treatment TWBC even though the TWBC are within the normal range. An elevated WBC count, even within the normal range, has been associated with breast cancer incidence and mortality rate ⁴¹. The role of WBC count as a surrogate for inflammation has not been examined in the context of well-known effect modifiers for breast cancer

development. Several studies have attempted to identify the association between WBC counts and solid cancer risk especially in breast cancer, but no consistent evidence has been found⁴²..

This change could also be as a result of chemotherapy cytotoxic destruction effect on bone marrow resulting in these changes observed in this TWBC work²⁵ . Studies by⁴⁰ had attempted to identify the association between TWBC and other solid cancer risk, but no consistent evidence has been found most reports were done on neutrophils/ lymphocytes ratios. This present work observed a significant increase of treatment ESR to pre- treatment ESR test results in this cancer. The result coincides with the anaemia observed in these patients who may be caused by several factors including androgen deprivation, nutritional decline, bone marrow filtration, treatment – related toxicity and chronic inflammatory state. This work is in agreement with works done and reported by^{84,90}.

BREAST CANCER AND AGES

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer deaths in women worldwide, accounting for 23% of total cancer cases and 14% of all cancer related mortalities. In this study, age group susceptibility was determined using the CBC and ESR in breast cancers. In the breast cancer subjects, a total of 110 (40%) studied were. The age group 31 – 40 years had the highest incidence in this work (33(30.0%)), while the 61 – 70 (6(50%)) years had the smallest incidence of subjects enrolled in this work, 21 – 30 is the second smallest group of subjects enrolled (14(12.7%). There were no significant difference at $p>0.05$ within and between the different age groups observed in this work. However, from the CBC and ESR results obtained in this work, it showed that subjects 21 – 30 years age group had the lowest mean \pm SD values. This showed that these particular age groups were more susceptible to breast cancer than other age groups regardless of the number in this age group. Age is an important risk factor for breast cancer as women over fifty years of age accounted for approximately 78% new

breast cancer cases and 87% of breast cancer - related death in 2011, however the incidence of breast cancer among younger women has increased such that breast cancer is the most frequency diagnosed form of cancer among women aged <40 years (Fredholm *et al*, 2005).¹¹, had suggested that age at diagnosis is related to breast cancer survival, but the data regard these uses are conflicting. A poor prognosis in younger age group due to the presence of more invasive disease among this age group were reported^{12,?}. Also works by^{17, 18, 19} all reported a more susceptibility of age group <30 and >70 to breast cancer. However works done by¹⁴ in Nigeria; ^{15, 16} in USA reported a more susceptibility in mild age's groups.

The susceptibility of these younger age women tend to have more aggressive tumors and a higher recurrence rate are mostly caused by lack of screening for younger women, so this younger women tend to present with a larger palpable lumps and a more advanced stage. So in this present work the age group 21 – 30 years which were mostly affected, may be due to early section by mammography is not applicable in our environment due to cost and availability. Another reason is that even when mammography in used in younger women because they have a denser breast than postmenopausal, it is usually difficult to detect. So more aggressive awareness on self – examination and availability of this mammography should be made available by the government to hospitals. Routine screening should be done on monthly bases. This work in consistent to works done by¹², Fredholm *et al*, 2015, and Ibrahim *et al* ,2014.

CONCLUSION:

Components of CBC and ESR provides valuable prognostic information in breast cancer by predicting survival, assessment of diseases progression and response to treatment. Thus, these ratios may be considered for routine clinical use as reliable and low-cost biomarkers.

ETHICAL APPROVAL AND CONSENT

All subjects gave a written and informed consent while the study protocol was approved by the Research & Ethics Committee of Enugu State University of Science and Technology Teaching Hospital Park Lane G.R.A. Enugu-North Local Government Area.

RECOMMENDATIONS:

Pre-treatment ratios of NLR, LMR and PLR should be introduced in clinical practice as a routine laboratory for early detection, prognosis, easily reproducible and accessible

Identification of adequate cut-off values in these ratios over a pre-treatment and treatment period of time could add more accurate information in the type of therapy for use in these patients.

CONFLICT OF INTEREST:

None declared.

FUNDING

This work was personally funded.

REFERENCES

1. Grivennikov S.I, Greten F.R, Karin M. "Immunity, inflammation, and cancer," Cell. 2010 ;(140)6:883–899.
2. Gabilovich D.I, Nagaraji S. Myeloid-derived suppressor cells as regulators of the immune system. Nature Review Immunology. 2013; 9(3):162–174.
3. Balampas, P., Michel, Y., Wagenblast, J .Tumour-infiltrating lymphocytes predict response to definitive chemoradiotherapy in head and neck cancer. British Journal of Cancer. 2014; 110(2):501–509.
4. Dumitru C.A, Fechner M.K, Hoffmann T.K, Lang S, Brandau S. A novel p38- MAPK signaling axis modulates neutrophil biology in head and neck cancer. Journal Leukocyte Biology. 2012; 91(4):591–598.
5. GLOBOCAN. Cancer Fact Sheet. Breast Cancer Incidence and Mortality Worldwide.2008; <http://globocan.iarc.fr/factsheets/cancers/breast.asp>.
6. Siegel, R.L, Miller, K.D, Jemal, A (2011) .Cancer statistics CA. Cancer Journal Clinical: 66: 7-30.

7. Agnoli, C. Metabolic syndrome and postmenopausal breast cancer in the ORDETcohort: a nested case-control study. *Nutrition Metabolism Cardiovascular Disease*. 2010; 20: 41–48.
8. Ihekwaba, F.N. Breast Cancer in Nigerian Women. *British Journal of Surgery*. 1992; 79 (8):771-775
9. Anyanwu S.N (1999). Breast cancer in eastern Nigeria: A ten year review. *West African Journal of Medicine*. 1999; 19:120-125.
10. DeSantis C., Siegel R., Bandi P., Jemal A. Breast cancer statistics. *CA Cancer Journal. Clinical*. 2011; 61:409–418.
11. Cvetanovic A, Popovic L, Filipovic S, Trifunovic J, Zivkovic N, Matovina-Brko G Young age and pathological features predict breast cancer outcome—report a dual Institution experience in Serbia. *Journal of the Balkan Union of Oncology*. 2016; 20(6):1407–1413.
12. Vostakolaei F.A, Broeders M.J, Rostami N, van Dijck J.A, Feuth T, Kiemeneij L.A,(2014). Age at diagnosis and breast cancer survival in Iran. *International journal of breast cancer*. 20(1):20-22.
13. Schonberg M.A, Marcantonio E.R, Li D, Silliman R.A, Ngo L, McCarthy E.P(2010). Breast cancer among the oldest old: tumor characteristics, treatment choices, and survival. *Journal of clinical oncology. Journal of the American Society of Clinical Oncology*; 28(12):2038–2045.
14. Ikpat O.F, Ndoma-Egba R, Collan Y. Influence of age and prognosis of breast cancer in Nigeria. *East African medical journal*. 2005;79(12):651–657.
15. Alieldin, N.H., Abo-Elazm, O.M., Bilal, D., Salem, S.E., Gouda, E., Elmongy, M., Ibrahim A.S. Age at diagnosis in women with non-metastatic breast cancer: Is it related to prognosis? *Journal of Egypt Natlande. Cancer Institute*. 2014; 26:23–30.
16. Crowe J.P Jr., Gordon N.H, Shenk R.R, Zollinger R.M Jr., Brumberg D.J, Shuck J.M. Age does not predict breast cancer outcome. *Achieves of Surgery*.1993; 129(5):483–487.
17. Balabram, D., Turra, C.M., Gobbi, H. Association between age and survival in a cohort of Brazilian patients with operable breast cancer. *Cadernos de saude publica*. 2015; 31(8):1732–1742.
18. Roder D.M, de Silva P, Zorbas H.M, Kollias J, Malycha P.L, Pyke .CM (2012). Age effects on survival from early breast cancer in clinical settings in Australia. *ANZ journal of surgery*; 82(7–8):524–528.
19. Brandt J, Garne J.P, Tengrup I, Manjer J. Age at diagnosis in relation to survival breast cancer: a cohort study. *World journal of surgical oncology*.2015; 13:33-37.
20. Kataoka A, Tokunaga E, Masuda N, Shien T, Kawabata K, Miyashita M (2014). Clinicopathological features of young patients (<35 years of age) with breast cancer in a Japanese Breast Cancer Society supported study. *Breast Cancer*; 21(6):643–650.
21. Chang R, Wong G.Y. Prognostic significance of marked leukocytosis in hospitalized. *Journal of General Internal Medicine*. 2011; 6:199–203.
22. Knaus W.A, Wagner D.P, Draper E.A, (2010). The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. *Chest*; 100:1619–1636.
23. Spivak J.L (2005). The anaemia of cancer: death by a thousand cuts. *National Review on Cancer*; 5:543-555.
24. Birgegård G, Aapro M.S, Bokemeyer C. Cancer-related anemia: pathogenesis, prevalence and treatment. *Oncology*. 2005; 68(suppl 1):3-11.
25. Marks P.W, Rosenthal, D.S (2009). Hematologic manifestations of systemic disease: infection, Chronicinflammation, and cancer. In: Hoffman R, Benz EJ, Shattil SJ, et al,

- eds. Hematology: Basic Principles and Practice. 5th ed. Philadelphia, PA: Churchill Livingstone Elsevier; 2309-2319
26. Bridges K.R, Pearson H.A. Cancer and anemia. In: Bridges KR, Pearson HA, (Ed). Anemias and Other Red Cell Disorders. New York, NY: McGraw-Hill Medical Publishing Division. 2008; PP. 58-80.
 27. Ludwig H, Van Belle S, Barrett-Lee P (2004). The European Cancer Anaemia Survey (ECAS): A large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patient. *European Journal of Cancer*; 40:2293-2306.
 28. Barrett-Lee, P.J., Ludwig, H., Birgegård, G. For European Cancer Anaemia Survey Advisory Board and Participating Centers. Independent risk factors for anemia in patients receiving chemotherapy: results from the European Cancer Anaemia Survey. *Oncology*.2006; 70:34-48.
 29. Groopman J.E, Itri L.M. Chemotherapy-induced anemia in adults: incidence and treatment. *Journal of Netherlands Cancer Institute*. 1999; 91:1616-1634.
 30. NCCN (2011). Clinical Practice Guidelines in Oncology. Cancer- and Chemotherapy-Induced Anemia. Version 2. National Comprehensive Cancer Network
 31. Kaushansky K, Kipps T.J (2005). Hematopoietic agents: growth factors, minerals, and vitamins. In: Brunton LL, Lazo JS, Parker KL, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th Ed. New York, NY: The McGraw Hill Companies, PP. 11-15.
 32. Guyton A.C, Hall J.E. Red blood cells, anemia, and polycythemia. In: Guyton AC, Hall JE, eds. Textbook of Medical Physiology. 11th ed. Philadelphia, PA: Elsevier Saunders: 2006; 419-428.
 33. Fuso L, Mazzola S, Marocco F. Pretreatment serum hemoglobin level as a predictive factor of response to neoadjuvant chemotherapy in patients with locally advanced squamous cervical carcinoma: a preliminary report, *Gynecology Oncology*.2005; 99(3, Suppl. 1):S187–S191.
 34. Serkies K, Badzio A, Jassem J.(2006) Clinical relevance of hemoglobin level in cervical cancer patients administered definitive radiotherapy, *Acta Oncology*, ;45(6):695–701.
 35. Grimm R.H.J.R, Neaton J.D, Ludwig W. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. *Journal of American Medical Association*.2015; 254:1932–1937.
 36. Ruckner H.W, Lavin T, Plaxe S.C, Stroch J.A, Livstone E.M,(2012). Absolute Granulocyte, lymphocyte, and monocyte counts. Useful determinants of prognosis for patients with metastatic Cancer of the stomach. *Journal of American Medical Association*; 24(7):1004–1006.
 37. Sasaki A, Kai S, Endo Y, (2017). Prognostic value of preoperative peripheral blood Monocyte count in patients with colorectal liver metastasis after liver resection. *Journal of Gastrointestinal Surgery*; 11:596–602.
 38. Aliustaoglu, M., Bilici, A., Ustaalioglu, B.B., Konya, V., Gucun, M., Seker, M. The effect of peripheral blood values on prognosis of patients with locally advanced gastric cancer before treatment. *Medical Oncology*. 2010; 27:1060–1065.
 39. Grimm R.H.J.R, Neaton J.D, Ludwig W. Prognostic importance of the white blood cell count for coronary, cancer, and all-cause mortality. *Journal of American Medical Association*.2002; 254:1932–1937.
 40. Erlinger, T. P., Muntner, P. & Helzlsouer, K. J. WBC count and the risk of cancer mortality in a national sample of U.S. adults: results from the Second National Health and Nutrition

- Examination Survey mortality study. *Cancer Epidemiology Biomarkers Previous*. 2014;13, 1052–1056.
41. Margolis, K. L., Rodabough, R. J., Thomson, C. A., Lopez, A. M., McTiernan, A. (2017). Prospective study of leukocyte count as a predictor of incident breast, colorectal, endometrial, and lung cancer and mortality in postmenopausal women. *Archives Internal Medicine*: 167:1837–1844.
 42. Allin, K. H., Bojesen, S. E. and Nordestgaard, B. G (2016). Inflammatory biomarkers and risk of cancer in 84,000 individuals from the general population. *International Journal of Cancer* ; 139: 1493–1500.
 43. Jamieson T, Clarke M, Steele CW, et al .Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis. *Journal Clinical Invest*. 2012; 122:3127–144.
 44. Fridlender Z.G, Sun J, Kim S (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. *Cancer Cell* ; 16:183–194.
 45. Hanahan D, Weinberg R.A (2011). "Hallmarks of cancer: the next generation". *Cell*; 144 (5): 646–674.
 46. Heng D.Y, Xie W, Regan M.M. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor- targeted agents: results from a large, multicenter study. *Journal Clinical Oncology*. 2009; 27:5794–5799.
 47. Templeton A.J, McNamara M.G, Šeruga B, Vera-Badillo F.E, Aneja P, Ocaña A, Leibowitz-Amit R (2014). Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *Journal of Natland Cancer Institute*. 106:124-126.
 48. Leibowitz-Amit R, Templeton A.J, Omlin A (2014). Clinical variables associated with PSA response to abiraterone acetate in patients with metastatic castration- resistant prostate cancer. *Annual Oncology*; 25:657–662.
 49. Wang L, Ma J, Liu, F (2014). “Expression of MUC1 in primary and metastatic human epithelial ovarian cancer and its therapeutic significance,” *Gynecologic Oncology*; 105; 3; 695–702.
 50. Jablonska J., Lang S., Sionov R.V. (2017). The regulation of pre-metastatic niche formation by neutrophils. *Oncotarget*; 8:112132–112144.
 51. Shitara K., Matsuo K., Oze I. (2011). Meta-analysis of neutropenia or leukopenia as a prognostic factor in patients with malignant disease undergoing chemotherapy. *Cancer Chemotherapy Pharmacology*; 68:301–307.
 52. McGary C. T., Miele M. E., Welch D. R. (2015). Highly metastatic 13762NF rat mammary adenocarcinoma cell clones stimulate bone marrow by secretion of granulocyte-macrophage colony-stimulating factor/interleukin-3 activity. *The American Journal of Pathology*; 147(6):1668–1681.
 53. Atzpodien, J., Reitz M (2008). Peripheral blood neutrophils as independent immunologic predictor of response and long-term survival upon immunotherapy in metastatic renal- cell carcinoma. *Cancer Biotherapy and Radiopharmaceuticals*;
 54. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V. (2012). "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010". *Lancet*; 380 (9859): 2095–2128.
 55. Torre, L.A, Bray, F, Siegel, R.L, Ferlay, J, Lortet-Tieulent, J, Jemal, A (2011). Global cancer statistics, *CA Cancer Journal of Clinical*. ; 65: 87- 108.

56. Jemal A, Bray F, Center M.M (2011). Global cancer statistics. *CA Cancer Journal of Clinical*; 61: 69-90.
57. Coates A.S, Winer E.P, Goldhirsch A (2015). Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer. *Annual of Oncology* ; 26(8):1533–1546.
58. Coussens L.M, Werb Z (2012). Inflammation and cancer. *Nature* ; 420(6917):860–867.
59. Jensen H.K, Donskov F, Marcussen N (2009). Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. *Journal Clinical Oncology*; 27:4709–4717.
60. Kohrt, H, Nouri N, Nowels K, Johnson D, Holmes S and Lee P.P (2005). “Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer,” *PLoS Medicine*; 2(9): 0904–0919.
61. Klemi, P.J; Pylkkänen,L; Kiilholma,P; Kurvinen,K and Joensuu,H (2005). “Protein detected by immunohistochemistry as a prognostic factor in patients with epithelial ovarian carcinoma,” *Cancer*; 76; 7: 1201–1208.
62. Wang J, Liu Y, Zhang N, Li X, Xin P, Bi J, Kong C (2017). Prognostic role of pre-treatment platelet to lymphocyte ratio in urologic cancer. *Oncotarget*; 8(41):70874–70882
63. Anderson, M.J., Shafer-Weaver, K., Greenberg, N.M and Hurwitz, M.A (2007). “Tolerization of tumor-specific T cells despite efficient initial priming in a primary murine model of prostate cancer,” *Journal of Immunology*; 178: 1268–1276.
64. Dougan, M and Dranoff, G (2009). “The immune response to tumors,” *Current Protocols in Immunology*; 85; 20.11.1–20.11.4.
65. Hashiguchi,Y; Tsuda,H; Inoue,T; Nishimura,S; Suzuki,T and Kawamura,N. “Alteration of cell cycle regulators correlates with survival in epithelial ovarian Cancer. 2008;
66. Bainbridge, E.T., Ford, C.H., Newman, C.E. Total lymphocyte counts in breast cancer. *Lancet*. 2018; 1:1203–1204.
67. Papatostas A.E, Lesnick G.J, Jenkins G, Aufses A.H Jr (2016). The prognostic significance of peripheral lymphocyte counts in patients with breast carcinoma. *Cancer*; 37:164–168.
68. Sasaki A, Iwashita Y, Shibata K (2006). Prognostic value of preoperative peripheral blood monocyte count in patients with hepatocellular carcinoma, *Surgery* 139(6):755–764.
69. Sasaki A, Kai S, Endo Y, (2007). Prognostic value of preoperative peripheral blood monocyte count in patients with colorectal liver metastasis after liver resection, *Journal of Gastrointestinal Surgery*; 11(5):596–602.
70. Bishara S, Griffin M, Cargill A. Pre-treatment white blood cell subtypes as prognostic indicators in ovarian cancer. *European Journal Obstetric Gynecology Reproduction Biology*. 2008; 138(1):71–75.
71. Pedersen L.M, Milan N (2016). Prognostic significance of thrombocytosis in patients with primary lung cancer. *European Respiratory Journal*; 9:1826–1830.
72. Shimada H,Oohira G, Okazumi S.I (2014). “Thrombocytosis associated with poor prognosis in patients with esophageal carcinoma,” *Journal of the American College of Surgeons*,(198) 5: 737–741.
73. Stone, R.L, Nick, A.M, McNeish, I.A (2012). “Paraneoplastic thrombocytosis in ovarian cancer,” *The New England Journal of Medicine*, (366), 7: 610–618.
74. Taucher, S., Salat, A., Gnant, M (2013) “Impact of pretreatment thrombocytosis on survival in primary breast cancer,” *Thrombosis and Haemostasis*, (89) 6: 1098–1106.
75. Ishizuka,M., Nagata,H., Takagi,K., Iwasaki,Y., and Kubota,K. “Preoperative thrombocytosis is associated with survival after surgery for colorectal cancer,” *Journal of Surgical Oncology*. 2012;(106)7: 887–891.

76. Sylman J.L; Mitrugno A; Tormoen G.W; Wagner T.H; Mallick P; McCarty O.J (2017), "Platelet count as a predictor of metastasis and venous thromboembolism in patients with cancer," *Convergent Science Physical Oncology*,3: 2-5.
77. Kajihara M, Okazaki Y, Kato S, Ishii H, Kawakami Y, Ikeda Y, Kuwana M (2007). Evaluation of platelet kinetics in patients with liver cirrhosis: similarity to Idiopathic thrombocytopenic purpura. *Journal of Gastroenterology Hepatology*; 22:112–118.
78. Nieder C, Haukland E, Pawinski A, Dalhaug A (2010). Anaemia and thrombocytopenia in patients with prostate cancer and bone metastases. *Biomedical Central Cancer*; 10:284-286.
79. Spivack M, Brenner S.M, Markham M.J, Snyder E.L, Berkowitz D (2009). Presumed immune thrombocytopenia and carcinoma: report of three cases and review of the literature. *American Journal of Medical Sciences*; 278:153–156.
80. Pedio G, Ruttner J.R, Odermatt B, Gut D (2014). Oncogenic viruses in the thrombocytopenic stage of experimental hipa–plasmacytoma. *Experientia*; 30:289–291.
81. Hanahan D, Weinberg R.A. "Hallmarks of cancer: the next generation". *Cell*.2011; 144 (5): 646–674.
82. Hoffmann T.K, Dworacki G, Tsukihiro T, Meidenbauer N, Gooding W, Johnson J.T, Whiteside T.L. Spontaneous apoptosis of circulating T lymphocytes in patients with head and neck cancer and its clinical importance. *Clinical Cancer Research*. 2012; 8:2553–2562.
83. Hamm A, Prenen H, Van Delm W, Di Matteo M, Wenes M, Delamarre E, Schmidt T, Weitz J, Sarmiento R, Dezi A, Gasparini G, Rothe F, Schmitz R. Tumour-educated circulating monocytes are powerful candidate biomarkers for diagnosis and disease follow-up of colorectal cancer. *Gut*. 2016; 65:990–1000.
84. Bochen K, Krasowska A, Milaniuk S, Kulczynska M, Prystupa A, Dzida G. Erythrocyte sedimentation rate-an old marker with new applications. *Journal of Pre-Clinical Clinical Research*.2011; 5:50–55.
85. Choi E.S, Kim H.S, Han I (2014). Elevated preoperative systemic inflammatory markers predict poor outcome in localized soft tissue sarcoma. *Annual Surgery Oncology*; 21:778–785.
86. Strojnik T, Smigoc T, Lah TT (2014). Prognostic value of erythrocyte sedimentation rate and C- reactive protein in the blood of patients with glioma. *Anticancer Research*; 34:339–347.
87. Sengupta S, Lohse C.M, Chevillie J.C, Leibovich B.C, Thompson R.H, Webster W.S, Frank I, Zincke H, Blute M.L, Kwon E.D.(2006). The preoperative erythrocyte sedimentation rate is an independent prognostic factor in renal cell carcinoma. *Cancer*; 106:304–312.
88. Chen, Y, Chen, K, Xiao, X. Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: a retrospective study. *Biomedical Central Cancer*. 2016; 16(1): 320-324.
89. Choi E.S, Kim H.S, Han I (2014). Elevated preoperative systemic inflammatory markers predict poor outcome in localized soft tissue sarcoma. *Annual Surgery Oncolog*; 21:778–785.
90. Johansson J.E, Sigurdsson T, Holmberg L, Bergström R (2012). Erythrocyte sedimentation rate as a tumor marker in human prostatic cancer. An analysis of prognostic factors in 300 populations-based consecutive cases. *Cancer*; 70:1556–1563.
91. Akinbami, A., Popoola, A., Adediran, A., Dosunmu, A., Oshinaike, O., Adebola, P. Full blood count pattern of pre-chemotherapy breast cancer. *Caspian Journal of Internal Medicine*. 2012; 4:574-579.
92. Mozaffarian D, Nye R, Levy WC (2013). Anemia predicts mortality in severe heart failure: the prospective randomized amlodipine survival evaluation (PRAISE). *Journal of American College Cardiology* 41:1933-1939.

93. Koh C.H, Bhoo-Pathy N, Ng K.L (2015). Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer. *British Journal of Cancer*. 2015; 113(1):150–158.
94. Forget P, Bentin C, Machiels J.P, Berliere M, Coulie P.G, De Kock M. Intraoperative use of ketorolac or diclofenac is associated with improved disease-free survival and overall survival in conservative breast cancer surgery. *British Journal of Anaesthetics*.2015; 113(Suppl 1):i82–i87.
95. Azab, B., Shah, N., Radbel, J., Tan, P., Bhatt, V., Vonfrolio, S., Habeshy, A. Pre-treatment neutrophil/lymphocyte ratio is superior to platelet/lymphocyte ratio as a predictor of long-term mortality in breast cancer patients. *Medical Oncology*. 2012; 30 (1):432-435.
96. Liu, C, Huang, Z, Wang, Q (2016). Usefulness of neutrophil-to-lymphocyte ratio and platelet- to-lymphocyte ratio in hormone-receptor-negative breast cancer. *Oncology Targets Therapy*; 9: 4653.
97. Dirican A, Kucukzeybek B.B, Alacacioglu A. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? *International Journal of Clinical Oncology*.2015; 20(1):70–81.
98. Ozyalvacli, G, Yesil, C, Kargi, E (2013). Diagnostic and prognostic importance of the neutrophil lymphocyte ratio in breast cancer. *Asian Pacific Journal of Cancer Preview*; 15(23): 10363–10366.
99. Chen, Y, Chen, K, Xiao, X. Pretreatment neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independent prognostic indicator in breast cancer patients: a retrospective study. *Biomedical Central Cancer*. 2016; 16(1): 320-324.
100. Casares, N, Pequignot, M.O, Tesniere, A. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *Journal of Experimental Medicine*. 2005; 202(12): 1691–1701.
101. Nakano, K, Hosoda, M, Yamamoto, M (2014). Prognostic significance of pre-treatment neutrophil:lymphocyte ratio in Japanese patients with breast cancer. *Anticancer Research*; 34(7): 3819–3824
102. Yao, M, Liu, Y, Jin, H (2014). Prognostic value of preoperative inflammatory markers in Chinese patients with breast cancer. *Oncology Targets Therapy*; 7: 1743–1752.
103. Jia W, Wu J, Jia H (2015). The peripheral blood neutrophil-to-lymphocyte ratio is superior to the lymphocyte-to-monocyte ratio for predicting the long-term survival of triple-negative breast cancer patients. *PLoS One*. ; 10(11):e0143061.
104. Pistelli M, De Lisa M, Ballatore Z (2015). Pre-treatment neutrophil to lymphocyte ratio may be a useful tool in predicting survival in early triple negative breast cancer patients. *Biomedical Central Cancer*; 15:195-199.
105. Huszno J, Kolosza Z., Mrochem-Kwarciak J, Rutkowski T, Skladowski K. The role of Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio, and Platelets in the Prognosis of Metastatic Renal Cell Carcinoma. *Oncology*. 2018; 97:7–17
106. Wataru G, Shinichiro K, Yuka A, Koji T, Katsuyuki T,(2018). Predictive value of lymphocyte-to- monocyte ratio in the preoperative setting for progression of patients with breast cancer. *Biomedical Central Cancer*: 18:1137-1140.