IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 7 Ser.4 (July. 2020), PP 12-18
www.iosrjournals.org

Expressions of CD 31 and VEGF as Predictors Anthracycline-Based Neoadjuvant Chemotherapy Response in Local Breast Cancer Patients

Dr.HeruPurwanto, Sp.B(K)Onk,M.Epid (Oncology Consultant Surgery, Master of Epidemiology) [1] Dr. dr.MochamadBachtiarBudianto,Sp.B(K)Onk,FINACS-FICS (Oncology Consultant Surgery)[2]
Dr.AlfiyannulAkhsan,Sp.B (General Surgery) [3].

[1]*Division of Oncology Surgery,Department of Surgery, Faculty of Medicine, University of Airlangga,Dr. Soetomo Hospital Surabaya, Indonesia*
[2]*Division of Oncology Surgery,Department of Surgery, Faculty of Medicine, University of Brawijaya, Dr. Saiful Anwar Hospital Malang, Indonesia*
[3] *Division of Oncology Surgery,Department of Surgery, Faculty of Medicine, University of Brawijaya, Dr. Saiful Anwar Hospital Malang, Indonesia*

Abstract:
CD31 expression is correlated with tumor cells that spread in the duct system. VEGF is one of the most prominent growth factors for physiological and pathological control in the process of angiogenesis. VEGF has been studied as an important key in the process of angiogenesis of several tumors, including breast cancer. This study design use observational analytic cohort method. The study population was female patients with locally advanced breast cancer who underwent anthracycline-based neo-advance chemotherapy at Dr. Saiful Anwar Malang from June 2019 to January 2019. The relationship between CD31 expression with NAC response has a correlation coefficient of 0.601 with p value of 0,000, because the value of p = 0,000 <0.05 (α = 5%), so it can be concluded that there is a significant relationship between CD31 expression with NAC response. The relationship between VEGF expression and NAC response has a correlation coefficient of 0.600 with a p value of 0,000, because the value of p = 0,000 <0.05 (α = 5%), so it can be concluded that there is a significant relationship between VEGF expression and NAC response .

Keywords*: CD 31, VEGF expression, anthracycline-based neoadjuvant chemotherapy, breast cancer*

Date of Submission: 23-06-2020 Date of Acceptance: 11-07-2020

I. Background

Breast cancer is the most common malignancy in women throughout the world. The GLOBOCAN 2018 data released by the WHO's International Agency for Research on Cancer (IARC) shows that breast cancer incidence is relatively high at 24.2% of all malignancies in women with a mortality rate of 15% and an estimated 2.1 million newly diagnosed cases in 2018 or 25% of all cancer cases (IARC, 2018). Based on a report from the Ministry of Health of the Republic of Indonesia in 2012, the incidence of breast cancer was 26 per 100,000 women, followed by cervical cancer with 16 over 100,000 women. According to Hospital Information System data (SIRS) in 2013, breast cancer ranked first inpatients in all hospitals in Indonesia (28.7%). It is recorded that breast cancer patients first come when in an advanced stage (stage III / IV) by 60-70% (MOH, 2016).

Clinical and pathological parameters such as staging, grading angiogenesis and some molecular biology criteria are significant prognostic factors in breast cancer (Rekha, 2018). Angiogenesis is defined as the growth of new blood vessels from existing blood vessels and consists of several processes, including: extracellular matrix remodeling, endothelial cell migration, proliferation and differentiation of microvessels and anastomosis (Sarcevic B, 2001). In breast cancer, Immunohistochemical examination of VEGF in tumor mass is associated with a cytometric CD31 microvessel density picture. (Agouza, 2010). Some studies describe the factors associated with microvessel density (MVD) testing to determine the quantity of angiogenesis that can be a predictor of tumor properties and the effects of antiangiogenesis treatment. The amount of this MVD can be determined through its morphology by examining Haematoxylin and Eosin (H&E) and immunohistochemical staining including Cluster of Differentiation 31 (CD 31) and Vascular Endothelial Growth Factor (VEGF) (Longatto, 2010). The use of CD31 antibodies to determine the effect of oseltamivirmonotherapy has been

studied in TNBC (Triple Negative Breast Cancer) patients and found reduced tumor vascularization after treatment is characterized by decreased expression of CD31 from immunohistochemical examinations before and after treatment interventions (Haudokis, 2001).

CD31 expression is correlated with tumor cells that spread in the duct system (Sapino, 2001). VEGF is one of the most prominent growth factors for physiological and pathological control in the process of angiogenesis. VEGF has been studied as an important key in the process of angiogenesis of several tumors, including breast cancer. Besides being influenced by VEGF, angiogenesis is also influenced by basic fibroblat growth factor (bFGF), angiopoietin, integrin activator and inhibitors such as thrombospondin and angiostatin and endostatin (Bruce, 2001). VEGF expression in tumor tissue is related to microvessel density (MVD) and poor prognosis in breast cancer (Rekha, 2018). Although some anti-angiogenic treatment strategies that target VEGF are promising, there is still a need for some research into this.

Anthracycline,daunorubicin,doxorubicin, and epirubicin antibiotics, which are widely used for the treatment of malignancies, have been evaluated for their effects on angiogenesis due to inhibition of type IV collagenase. In the chorioallantoic membrane (CAM) angiogenesis membrane system, anthracyclines inhibit vascular density at a dose of 5-20 micrograms / disk and biosynthesis of collagen proteins, which is an index of angiogenesis. The antiangiogenic effects of the antitumor agents mentioned above at therapeutically attainable concentrations can explain, at least in part, their antitumor properties because angiogenesis is an important process for tumor growth and metastasis. Antiangiogenic effects, not related to inhibition of metalloproteinases because higher concentrations are needed for that effect than for inhibition of angiogenesis.(Maragaudokis, 1994).Ng et al. In their study mentioned the effect of anthracycline-based chemotherapy treatment associated with a decrease in VEGF levels in breast cancer patients.

Objective assessment of neoadjuvant chemotherapy response from clinical parameters of tumor size is assessed based on RECIST and WHO criteria. Based on the RECIST (Response Evaluation Criteria in Solid Tumor) criteria, the response is good if it meets the CR (Complete Response) and Partial Response (PR) criteria, while the SD (Stable Disease) and PD (Progressive Disease) meet the criteria as a bad response to chemotherapy (2015). In his study, Wang J mentioned that there were 15% of patients who met the Complete Response criteria and 28% of patients experienced Partial Complete Response after undergoing anthracyclin-based neo-advanced in locally advanced breast cancer patients (Wang J et al, 2002). Several combinations of anthracycline-based chemotherapy are widely used as a choice of first-line neoadjuvant therapy in breast cancer. Data for 2015 in the Integrated Oncology Poly RSUD Dr. Saiful Anwar Malang The use of combination cyclophosphamide, doxorubicin and 5-FU (CAF) chemotherapy reached 62% of the total new chemotherapy of 434 patients. While the use of Epirubicin (CEF) 3%, Methotrexate (CMF) 6%, and other combinations 29% (Tabrani, 2017).

The explanation above motivates the authors to examine whether the expression of CD31 and VEGF can be used as a predictor of anthracycline-based neo-adjuvant chemotherapy response in local advanced breast cancer in Integrated Hospital Oncology Clinic of Dr. Saiful Anwar Malang.

II. ResearchMethods

This study design use observational analytic cohort method. The study population was female patients with locally advanced breast cancer who underwent anthracycline-based neo-advance chemotherapy at Dr. Saiful Anwar Malang from June 2019 to January 2019. The sample size was determined according to calculations based on the sample size formula using Lemeshow because of the known prevalence and unknown population. The method of sampling is done by consecutive sampling based on inclusion and exclusion criteria from June 2019 to January 2019, which is based on the order of the arrival of the patient to the Oncology Surgery clinic of RSSA Malang. All patients who meet the inclusion and exclusion criteria will be taken until the sample size is fulfilled.

Research Inclusion Criteria, as follows:

1. Women with locally advanced breast cancer who have never received chemotherapy
2. Agree to follow the study by signing informed consent.

Research Exclusion Criteria, as follows:

1. Patients with residif breast cancer. Patients with breast cancer whose disease reappears after a period of disease free interval or increased staging (progressive disease).
2. Sufferers with weak general condition; depicted in the performance status with a measurement scale Karnofsky value ≤70%.
3. Patients have received chemotherapy / radiotherapy before.
4. No paraffin blocks are available.
5. Patients who refuse to participate in this study.

So for this study required a sample of 64 samples, to anticipate the dropout used a sample of 67 samples.

In this study the research variables studied were:

1. Levels of CD31 and VEGF before getting neoadjuvant chemotherapy as an independent / independent variable.
2. Size of breast tumors before and after receiving anthracyclin neo-advance chemotherapy as an independent / independent variable.
3. Confounding variables are:Tumor subtype (there are 5 subtypes of breast cancer based on immunohistochemistry namely Luminal A, Luminal B (Her-2 negative), Luminal B (Her-2 positive), Her-2 overexpression and basal like).b. Number of cycles (based on NCCN there are 2 choices of cycles in chemotherapy namely 3 cycles and 6 cycles).

Data collection methods in this study were carried out by way of female patients with local advanced stages who met the inclusion and exclusion criteria of the study were given an explanation of the purpose and benefits of the examination, and requested approval to participate in the study by signing an informed consent and informed consent. Furthermore subjects' general data such as name, age, gender, address and telephone number are recorded. Other data is recorded according to the data collection form.

The study subjects then underwent CD 31 and VEGF 1 examination and tumor size a day before undergoing neodajuvant chemotherapy. Subjects will undergo chemotherapy according to the procedure for administering anthracycline-based regimens. Subsequent re-examinations of CD 31 and VEGF and tumor size will be performed after undergoing third neoajuvan chemotherapy.

Data management is carried out using the SPSS 23.0 program. Data is presented in the form of frequency distribution tables and cross tabulations. Data from the research variables are tested for normality data, if they meet the test using paired t test to see differences before and after treatment, while if it does not meet the assumption of test data normality using the Wilcoxon Test.

Research Results and Discussion

Research on the relationship between CD 31 expression and VEGF with neoajuvan chemotherapy responses in Local Advanced Stage Breast Cancer has been carried out on 81 patients who came to the Integrated Oncology Polyclinic at Saiful Anwar Hospital in Malang. Of the 81 patients included in the study sample as many as 4 people could not be followed by the development of therapy because they did not come back to undergo chemotherapy and as many as 6 patients were not found paraffin blocks from previous biopsy samples so that the number of research subjects that could be carried out by CD31 and VEGF immunohistokima examination was as much 71 paraffin blocks. All subjects who met the inclusion criteria were recorded characteristic data, measuring the diameter of the tumor with calipers or ultrasound and checking the expression of CD31 against the paraffin blocks biopsy. After being given neoadjuvan chemotherapy for 3 cycles, then the chemotherapy response was assessed with RECIST 1.1 criteria.

Characteristics of Research Subjects

Table 1: Characteristics of Research Subjects

	Frekuensi	Persentase
Usia 40 - 50 Tahun	29	40.85
61 - 70 tahun	25	35.21
51 - 60 Tahun	17	23.94
Hasil PA		
Grade I	6	8.45
Grade II	25	35.21
Grade III	40	56.34
ER		
-	10	14.08
+	56	78.87
Tidak Ada	5	7.04
PR		
-	26	36.62
+	32	45.07
Tidak Ada	13	18.31
Her2		
-	38	53.52
+	2	2.82
++	4	5.63

+++	10	14.08
Tidak Ada	17	23.94
Ki67		
-	18	25.35
+	28	39.44
Tidak Ada	25	35.21
Stadium		
IIIA	9	12.67
IIIB	55	77.46
IIIC	7	9.85
Ukuran Tumor		
< 5 cm	19	26.7
5-10 cm	25	35.2
>10 cm	27	38.0
Status Limfonodi		
N0	23	32.3
N1	38	53.56
N2	9	12.6
N3	1	1.4
Respon NAC		
No Respon/Stable	19	26.76
Partial	52	73.24
CD31		
-	23	32.39
+	48	67.61
VEGF		
-	26	36.62
+	45	63.38

Subject characteristics in this study consisted of age, stage, histopathological grading, estrogen and progesterone hormone receptor expression, Her-2 expression, Ki67 expression, chemotherapy response and CD31 and VEGF expression. The characteristics of the study subjects are shown in Table 1, the average age of patients with locally advanced breast cancer in this study was 48.5 years with the youngest age 41 years and the oldest age 70 years and the most age range in the subjects of this study was 40-50 years with a total of 29 people (40.85%). The epidemiological distribution of this study group is in accordance with research conducted by Campos and Chintamani, where Campos reported an average age of patients with Local Advanced Breast Cancer 49 years while a study by Chintamani et al reported an average age of 43 years with a range of 25-60 years. Based on stadium, 55 people (77.46%) entered stage IIIB, 9 people (12.67%) in stage IIIA and stage IIIC were 7 people (9.85%). Based on histopathology, as many as 40 people (56.34%) of the sample in this study were breast cancer sufferers with a histopathological type of invasive ductal carcinoma (invasive carcinoma of no special type) grade III. Status of involvement of pre-chemotherapy regional lymph nodes in the sample of sufferers of this study were N0: 23 people (32.3%), N1: 38 people (53.56%), N2: 9 people (12.6%) and N3 for 1 person (1.4%). The average tumor size before chemotherapy was 11.1 cm (SD 5.39) with a range between 2.0-15 cm. The biomolecular characteristics of breast cancer in this study mostly expressed estrogen receptors which were 56 positive (78.87%), Progesterone positive 32 (45.7%) and Her-2 negative 38 (53.52%). This shows the lack of Local Advanced Breast Cancer sufferers undergo examinations and diagnostic and therapeutic procedures at an early stage.

Relationship of Research Characteristics with CD31

The relationship between age and CD31 expression has a p value of 0.706 because p = 0.706> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between age and CD31 expression. The relationship between PA Results and CD31 Expression has a p value of 0.161, because the value of p = 0.161> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between PA Results and CD31 Expression. The relationship between ER and CD31 expression has a p value of 0.720, because the value of p = 0.720> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between ER and CD31 expression. The relationship between PR and CD31 expression has a p value of 0.404, because the value of p = 0.404> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between PR and CD31 expression. The relationship between Her2 with CD31 Expression has a p value of 0.371, because the value of p = 0.371> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Her2 and CD31 Expression. The relationship between Ki67 and CD31 expression has a p value of 0.827, because the value of p = 0.827> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Ki67 and CD31 expression. In this study CD31 immunoexpression did not show any relationship with age, regional lymph node metastasis status, tumor size, tumor gradation, estrogen receptor, progesterone receptor. In accordance with

research by Jang et al and Horiguchi et al. where according to research by Horighuchi, CD31 immunoexpression was highest in stage III, this is in line with the results of the study where 72% of the stage III patient group had the most CD31 expression when compared with other groups.

Histopathology and Immunohistochemistry Characteristics

The relationship between age and VEGF expression has a p value of 0.769, because the value of p = 0.769> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between age and VEGF expression. The relationship between PA Results and VEGF Expression has a p value of 0.703, because the value of p = 0.703> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between PA Results and VEGF Expression. The relationship between ER and VEGF expression has a p value of 0.999, because the value of p = 0.999> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between ER and VEGF expression. The relationship between PR and VEGF expression has a p value of 0.736, because the value of p = 0.736> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between PR and VEGF expression. The relationship between Her2 and VEGF expression has a p value of 0.854, because the value of p = 0.854> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Her2 and VEGF expression. The relationship between Ki67 and VEGF expression has a p value of 0.729, because the value of p = 0.729> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Ki67 and VEGF expression. Based on the results of the 2008 Lenderhor study, the high VEGF expression has a significant relationship with the variability in the histopathological and immunohisto chemical features of patients. However, in this study different results were obtained, where the results showed that VEGF had no significant relationship with factors such as age, histopathological features and CPI.

Relationship of Characteristics with NAC Response

The relationship between age and NAC response has a p value of 0.942, because the value of p = 0.942> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between age and NAC response. The relationship between PA Results and NAC Response has a p value of 0.227, because the value of p = 0.227> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between PA Results and the NAC Response. The relationship between ER and NAC response has a p value of 0.327, because the value of p = 0.327> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between ER and NAC response. The relationship between PR and NAC response has a p value of 0.424, because the value of p = 0.424> 0.05 (α = 5%), so it can be concluded that there is no significant relationship between the PR and the NAC response. The relationship between Her2 and the NAC Response has a p value of 0.277, because the value of p = 0.277> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Her2 and the NAC Response. The relationship between Ki67 and NAC response has a p value of 0.466, because the value of p = 0.466> 0.05 (α = 5%), so it can be concluded that there is no significant relationship or relationship between Ki67 and the NAC response. It can be concluded that the patient's response to NAC was not affected by the variability in histopathology and immunohistochemistry of the study subjects.

Relationship between CD31 and VEGF expression with NAC response

The relationship between CD31 expression with NAC response has a correlation coefficient of 0.601 with p value of 0,000, because the value of p = 0,000 <0.05 (α = 5%), so it can be concluded that there is a significant relationship between CD31 expression with NAC response . The direction of the positive correlation shows that the higher the CD31 expression will further increase the NAC response with the moderate correlation category. The relationship between VEGF expression and NAC response has a correlation coefficient of 0.600 with a p value of 0,000, because the value of p = 0,000 <0.05 (α = 5%), so it can be concluded that there is a significant relationship between VEGF expression and NAC response . The direction of the positive correlation shows that the higher the VEGF expression will further increase the NAC response with the moderate correlation category. CD31 physiologically plays an important role in cell-cell, cell-matrix interactions, including proliferation, adhesion, migration, hematopoiesis and lymphocyte activation. Hyaluronic acid (HA) is the main ligand for CD31. CD31 signaling plays an important role in tumor growth and cancer metastasis, and is divided into two major parts namely HA-independent signaling; this process depends on the interaction of CD31 and cytoskeletal proteins or membrane proteins associated with kinases, as well as HA-dependent signaling; this process relies on two CD31 molecules that bind to one another and interact with one another. HA which binds to CD31 on the cell surface triggers various pathways such as PI3K, Akt, PP2A, ERK and Race / Raf / Rac. Several studies have shown a link between CD31 and P-glycoprotein, including a study by Misra et al., Which reported that CD31 through the Phosphoinositide 3-kinase (PI3-K) pathway affects the expression of P-glycoprotein. According to Toole et al, the bond between CD31 and the extacellular matrix of hyaluronan acid on the cell surface will stabilize the bond between Actin, CD31 and P-glycoprotein in cells, this opinion is

strengthened by studies by Ravindranath where CD31 inhibits FBXO21 to degrade P-glycoprotein. This mechanism causes the work of P-glycoprotein to pump cytotoxic materials including chemotherapy drugs can be maintained so that it causes resistance to chemotherapy. The results of this study are consistent with previous studies including studies by Li et al. Which stated an improvement in the response of chemotherapy to patients treated with Lapatinib which resulted in a decrease in cell population with CD31 positive. Research by Yenigun shows that breast cancer cells that express CD31 have a worse response to treatment using Doxorubicin compared to cancer cells that have less or no CD31 expression. Bouelbes and colleagues stated that there was an increased resistance to Traztuzumab therapy in breast cancer cell lines with overexpression of CD31 with positive HER-2. Giving Doxorubicin according to Cheng et al can convert non CSC cells into CSC through the signal transducer and activator of transcription 3 (Stat3) pathway in breast cancer cell lines with positive and triple negative estrogen receptors. According to Cheng et al, as shown in Figure 4.1 above Doxorubicin administration will increase the expression of CD31 and several other CSC marker proteins so that it will increase resistance to Doxorubicin administration itself.

Logistic Regression Equations

Logit regression is one type of analysis used to predict the relationship between the independent variable (X) and the dependent variable (Y), but in logit regression, the main requirement that must be met is that the dependent variable (Y) must consist of two category (in the form of binary). The advantage of logit regression compared to other regressions is that the probability of an event is predictable.

In this study logit regression was used to determine the effect of CD 31 and VEGF independent variables on the neojuvant chemotherapy treatment response.

If the probability value is> 0.5 then this respondent falls into the positive NAC response category. If the probability is <0.5 then the respondent is grouped in a negative NAC response, and so on until the last respondent. The statistical value of exp (Odds Ratio) for the expression variable CD31 (X1) of 7.903 has a positive direction. these results indicate that the probability of a positive NAC response with a positive CD31 is 7.903 times higher than a positive NAC response. Or in other words, a positive CD31 has a greater chance of producing a positive NAC response. The statistical value of exp (Odds Ratio) for the VEGF (X1) Expression variable of 9,080 has a positive direction. These results indicate that the probability of a positive NAC response with a positive VEGF is 9,080 times higher than the psoitf NAC response. Or in other words, a positive CD31 has a greater chance of producing a positive NAC response.

Summary

CD31 and VEGF immunoexpression are associated with a good chemotherapy response in local advanced breast cancer patients receiving anthracycline-based neopjuvant chemotherapy.

Suggestions

In patients with locally advanced breast cancer receiving neoadjuvant chemotherapy with an anthracycline-based regimen, CD31 and VEGF immunoexpression tests can be considered as predictors of chemotherapy response. Further research needs to be done on CD31 and VEGF at the mRNA level using the RT-PCR method so that more sensitive and objective examination results can be obtained.

References

[1]. Ardiansyah AO. 2015. Surgical Mapping Oncology Series :Breast Cancer. Surabaya: Airlangga University Press. Pp 7-25.

[2]. Ashariati, A. 2006. *Ekspresi Her-2/neudan mdr-1 BerkorelasiDengan Gen MDR-1 PadaPenderitaKankerPayudara Locally Advanced PascaPengobatanAntrasiklin.*

[3]. Białas, M, Dyduch, G, Dudała, J, et al. 2014. Study of Microvessel Density and the Expression of Vascular Endothelial Growth Factors in Adrenal Gland Pheochromocytomas. International Journal of Endocrinology; 1–9.

[4]. Charpin C, Garcia S, et al.1997. CD31/PECAM automated and quantitative immunocytochemical assay in breast carcinomas: correlation with patient follow up. AM Journal Clinical Pathology

[5]. Chen JH, Feig B, Agrawal G, et al. 2008 MRI evaluation of pathologically complete response and residual tumors in breast cancer after neoajuvant chemotherapy. Journal of Cancer 2008; 112(1): 17–26.

[6]. Comsa S, CimpeanAM, Ceausu R, et al. 2012. Correlation between vascular endothelial growth factor expression, microvascular density in tumor tissue and TNM staging in breast cancer.Archives of biological science. Victor Babes University of Medicine and Pharmacy of Timisoara 64(2), 409-417

[7]. Depkes. 2016. KementerianKesehatanAjakMasyarakatCegah Dan KendalikanKanker. Available at: http://www.depkes.go.id/article/print/17020200002/kementerian-kesehatan-ajak-masyarakat-cegah-dan-kendalikan-kanker.html, accessed

[8]. DeVita, Hellman, dan Rosenberg. 2008. Cancer: Principles & Practice of Oncology, Volume 2. Philadhelphia :Wolters Kluwer Health

[9]. Ellisen LW, Isakoff SJ. 2010. Incorporating Translational Research in the Treatment of Locally Advanced Breast Cancer. In: Taghian et al. Breast Cancer: A Multidisciplinay Approach to Diagnosis and Treatment. USA: Demos Medical Publishing. pp 195-8.

[10]. Folkman, 2007. Antibodies, kits and reagent for angiogenesis research.www.cellsignal.com/angio

[11]. Fox, S B, Generali, DG and A.L Harris. 2007. Breast tumor angiogenesis. Breast cancer research. 9(6),216.

[12]. Franceschini G, Terribile D, Magno S et al. 2007. Update in the treatment of locally advanced breast cancer: a multidisciplinary approach. Eur Rev Med PrahmacolSci; 11: 283-9.
[13]. Gao, et al. 2017. Extramural Portfolio Supported by the National Heart, Lung, and Blood Institute, 2008–2015. The NHLBI page.1-6.
[14]. Garg PK. 2015. Current definition of locally advanced breast cancer. Current Oncology: e 409-10.
[15]. Haudokiset al.1994. Inhibition of Angiogenesis by Antracyclin and Titanocen.PharmacheteucalDepartement of Yunani University.http://www.ncb.nlm.niv.gov/pubmed.97535
[16]. Hortobagyi, GN, Heim, W, Hutchins, L, et al. 2010. A Phase 2 Study of A Fixed Combination of Uracil and Ftorafur (UFT) and Leucovorin Given Orally in A 3-times-daily Regimen to Treat Patients With Recurrent Metastatic Breast Cancer. Cancer. 1440-1445
[17]. International Agency for Research on Cancer.Latest word cancer statistics.World Health Organization Press Release. 2018 (Cited 2018 Sept 18). Diaksesmelaluihttp://globocan.iarc.fr/Pages/fact_sheets_population.aspx
[18]. Jones, DT, Lechertier, T, Mitter, R. 2012. Gene Expression Analysis in Human Breast Cancer Associated Blood Vessels. PlosOne. 7(10); e44294: 1-14
[19]. Kemenkes RI. 2015. InfodatinKanker. Kemenkes RI Pusat Data danInformasi.
[20]. Kleer, CG, Zhang Y, Pan Q, Merajver SD. (2004). WISP3 (CCN6) is a secreted tumor-suppressor protein that modulates IGF signaling in inflammatory breast cancer. Neoplasia.6, 179–185
[21]. Kresno, Siti Boedina.2012. IlmuDasarOnkologi.BadanPenerbit FKUI Jakata
[22]. Lertkiatmongkol, P., Liao, D., Mei, H., Hu, Y., & Newman, P. J. (2016). Endothelial functions of platelet/endothelial cell adhesion molecule-1 (CD31). Current Opinion in Hematology, 23(3), 253–259
[23]. Lo, YL. (2012). A Potential Daidzein Derivative Enhances Cytotoxicity of Epirubicin on Human Colon Adenocarcinoma Caco-2 Cells. International Journal of Molecular Sciences. 14(1), 158–176
[24]. Londero V, Bazzocchi M, Del Frate C, et al. Locally advanced breast cancer: comparison of mammography, sonography and MR imaging in evaluation of residual disease in women receiving neoajuvant chemotherapy. EurRadiol 2004; 14: 1371–1379.
[25]. Manuaba TW. 2010. KankerPayudaradalam: PanduanPenatalaksanaankanker solid PERABOI. Jakarta: CV SagungSeto. pp 17-45.
[26]. Masood, S. Neoajuvant chemotherapy in breast cancers. Women's Health 2016, Vol. 12(5) 480–491.
[27]. Mohamed, SY, Mohammed, HL, Ibrahim, HM, et al. 2017. Role of VEGF, CD105, and CD31 in the Prognosis of Colorectal Cancer Cases. Journal of Gastrointestinal Cancer: 1-12
[28]. Mousa, S.a.2000. Mechanism of Angiogenesis : Potential TheraupeticTargets,In Angiogenesis inhibitors and stimulator : Potensial therapeutics implication (SA Mousa),1-12. Landes bioscience, Georgetown.
[29]. National Comprehensive Cancer Network (NCCN). 2015. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Ver. 2.2015. Fort Washington, PA: nccn. Current version available online at http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.Cited on Jan 12th, 2018.
[30]. Ng T,et al. Impact of Adjuvant Anthracyclin Based and Taxane Based Chemoterapy on Plasma VEGF Level and Cognitive Function in Breast Cancer Patient : A Longitudinal Study. Clin Breast Cancer,2018
[31]. Nussenbaum, F and Herman, IH. Tumor Angiogenesis: Insights and Innovations. Journal of Oncology. 2010: 1-25
[32]. Paddock, C., Lytle, B. L., Peterson, F. C., Holyst, T., Newman, P. J., Volkman, B. F., & Newman, D. K. (2011). Residues within a lipid-associated segment of the PECAM-1 cytoplasmic domain are susceptible to inducible, sequential phosphorylation. Blood, 117(22), 6012–6023
[33]. Paddock, C., Zhou, D., Lertkiatmongkol, P., Newman, P. J., & Zhu, J. (2015). Structural basis for PECAM-1 homophilic binding.Blood, 127(8), 1052–1061 Page, R and Takimoto, C.2012 Principles of chemotherapy. Cancer Management: A Multidisciplinary Approach.
[34]. Pavlakis K, Messini I et al. 2008. The Assessment of angiogenesisand fibroblastic stromagenesis in hyperplastic and pre invasive breast lesion. BMC Cncer,8,88.
[35]. Pauty, 2017. A Vascular Endothelial Growth Factor-Dependent Sprouting Angiogenesis Assay Based on an In Vitro Human Blood Vessel Model for the Study of Anti-Angiogenic Drugs. www.ebiomedicine.com
[36]. PharmKGB. 2010. Cyclophosphamide pathway, pharmacodynamics.. Available at: https://www.pharmgkb.org/pathway/PA2035. Cited on Mar 22th, 2019
[37]. Relf.M, Lejeune.S,Scott.PA,et al. 1997. Expression of the Angiogenic : Factors Vascular Endhotelial Cell Growth Factor, Acidic and Basic Fibroblast Growth Factor, Cancer research, 57, 963-969.
[38]. Righi, L, Deaglio, S, Pecchioni, C. 2003. Role of CD31/Platelet Endothelial Cell Adhesion Molecule-1 Expression in in Vitro and in Vivo Growth and Differentiation of Human Breast Cancer Cells.CD31 and Breast Cancer 1165 American Journal of Pathology. 162(4): 1163-1174
[39]. Said, G, Guilbert, M, Morjani, H. (2012). Extracellular Matrix Proteins Modulate Antimigratory and Apoptotic Effects of Doxorubicin. Chemotherapy Research and Practice. 268681, 1-10
[40]. Sapino, A, Bongiovanni, M, Cassoni, P, et al. Expression of CD31 by cells of extensive ductal in situand invasive carcinomas of the breast.Journal of Pathology.2001; 194: 254–261.

Dr.HeruPurwanto, et. al. "Expressions of CD 31 and VEGF as Predictors Anthracycline-Based Neoadjuvant Chemotherapy Response in Local Breast Cancer Patients." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS),* 19(7), 2020, pp. 12-18.