Clinical Research

Association of P-glycoprotein expression and response to anthracycline-based neoadjuvant chemotherapy in locally advanced breast cancer

Erwin Danil Yulian,[1] Filipus Dasawala,[1] Nurjati Chairani Siregar[2]

pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.225863
Med J Indones. 2022;31:62–9

Received: October 20, 2021
Accepted: April 09, 2022

Authors' affiliations:
[1]Department of Surgery, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [2]Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Corresponding author:
Erwin Danil Yulian
Department of Surgery, Faculty of Medicine, Universitas Indonesia, Jalan Pangeran Diponegoro No. 71, Kenari, Senen, Central Jakarta 10430, Indonesia
Tel/Fax: +62-21-31906548
E-mail: erwin.yulian@ui.ac.id

ABSTRACT

BACKGROUND Neoadjuvant chemotherapy (NACT) has been shown to improve the overall survival of locally advanced breast cancer (LABC) patients with pathological complete response. However, the efficacy may be reduced due to chemoresistance mediated by P-glycoprotein (Pgp). This study aimed to explore the association between Pgp expression and patients' response to NACT.

METHODS A prospective cohort study was carried out from May 2018 to October 2019 at Cipto Mangunkusumo Hospital and Koja Hospital. Treatment-naïve LABC patients were consecutively enrolled in the study. Immunohistochemistry analysis of the biopsy samples was done to semi-quantitatively measure Pgp expression. The clinical response was evaluated after 3 cycles of NACT, while the pathological response was evaluated for subjects who underwent surgery post-NACT.

RESULTS Mean age of the subjects was 46.2 (9.6) years old, and most of the cases were invasive ductal (78%) and luminal B subtype (61%). Pgp was strongly expressed in 21/27 subjects (78%). There were no differences between Pgp-positive and -negative subjects for clinical response (relative risk [RR] 1.1, 95% confidence interval [CI] 0.33–4.01, p = 0.61) and pathological response (RR 1.3, 95% CI 0.8–1.9, p = 0.22). Other clinicopathologic variables were not associated with either clinical or pathological responses.

CONCLUSIONS These results showed that Pgp is expressed in most LABC patients, but its role as a predictive factor could not be established. However, due to the limited subjects and a lack of standardized Pgp measurement, careful consideration must be done when interpreting these results.

KEYWORDS breast cancer, neoadjuvant chemotherapy, p-glycoprotein, treatment outcome

Neoadjuvant chemotherapy (NACT), one of the modalities used in managing locally advanced breast cancer (LABC), has been shown to improve overall survival in patients with pathological complete response (pCR).[1] However, its occurrence rate is low and can be attributed to chemotherapy resistance.[1,2] One of the mechanisms of resistance in breast cancer cells is through the expression of P-glycoprotein (Pgp).[2] Pgp is an ATP-binding cassette (ABC) transporter encoded by the ABC subfamily B member 1 (ABCB1). They are found in normal tissues, such as the intestine, liver, kidney, placenta, and the blood-brain barrier, where it functions as a cellular protector by transporting exogenous substrates. However, the expression of Pgp in cancer cells reduces the intracellular concentrations of chemotherapeutic agents, which ultimately reduces the drug's efficacy. Agents affected by Pgp expression include anthracyclines, vinca alkaloids, taxanes, camptothecins, epipodophyllotoxins, and tyrosine kinase inhibitors.[3] In breast cancer, Pgp expression is

found to vary in levels. However, both clinically and pathologically, results concerning its association with response toward chemotherapy are conflicting.[4–7]

According to population-based studies, more than half of all breast cancer patients in Indonesia were diagnosed in advanced stages, making chemotherapy integral to breast cancer management.[8,9] A study in Cipto Mangunkusumo Hospital reported that although more than 70% of LABC patients clinically responded to NACT, only less than 5% expressed pCR. There could be an association between using an anthracycline-based regimen and the expression of Pgp.[10,11] Due to the absence of reliable predictive biomarkers for chemotherapy, this study aimed to explore the association between Pgp expression and response to NACT in Indonesian LABC patients and its role as a predictive biomarker.[12]

METHODS

Study design and population

A prospective cohort study was carried out at Cipto Mangunkusumo Hospital and Koja Hospital from May 2018 to October 2019. In the outpatient clinic, treatment-naïve subjects with stage III (8th edition of American Joint Committee on Cancer staging guideline) breast cancer confirmed histopathologically and had no distant metastasis based on clinical work-up were consecutively enrolled.[13] Subjects who were ≤70 years old, had normal cardiac, liver, and kidney functions, had good performance status (Karnofsky score of ≥70 or Eastern Cooperative Oncology Group score of ≥70 or Eastern Cooperative Oncology Group score of ≤2), and had given their consent were included in this study. Subjects who were unfit for chemotherapy were excluded from this study. Besides the routine immunohistochemistry (IHC) panel, IHC analyses of the biopsy samples were also done to detect the expression of Pgp. Eligible subjects were then given three cycles of 5-fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2 (FAC) NACT at 3 weeks interval.[11] During the treatment period, subjects were closely monitored and treated accordingly to prevent chemotherapy delay. Post-NACT subjects, who responded partially or completely and were deemed operable according to Haagensen and Stout's criteria of operability, underwent modified radical mastectomy 4 weeks after the last dose of chemotherapy and received three cycles of adjuvant chemotherapy.[14] Meanwhile, subjects with inoperable breast cancer or progressive disease underwent another biopsy and received a different chemotherapy regimen. This study was approved by the Ethics Committee of the Faculty of Medicine Universitas Indonesia (No: 731/UN2.F1/ETIK/VII/2018).

IHC analysis for Pgp

IHC was performed on 3 µm sections of formalin-fixed paraffin-embedded (FFPE) biopsy tissue using JSB-1 monoclonal antibody (MAb) (GeneTex Inc., USA). Tissue sections were deparaffinized in xylol and ethanol, and the endogenous peroxidase was quenched in methanol peroxide (3%, 10 min). Slides were pretreated in tris-EDTA buffer (pH 9.0) for 10 min at 96°C, cooled off, and washed with phosphate-buffered saline (PBS) (pH 7.4). Afterward, slides were incubated overnight at 4°C with a primary antibody (1:50). MAb was detected with N-Histofine Simple Stain Max PO (Multi) (Nichirei Biosciences Inc., Japan) for 30 min and then washed with PBS (pH 7.4). Bound peroxidase was developed with 3,3-diaminobenzidine tetrahydrochloride and hydrogen peroxide and counterstained with hematoxylin. Then, the slides were dipped in saturated aqueous lithium carbonate, dehydrated with ethanol, cleared with xylol, and covered with deck glass. Sterile water was used as a negative control.

Pgp expression was evaluated blindly by one pathologist (NCS) and done in duplicates. Semiquantitative measurement of Pgp expression was based on the percentage of Pgp positive cells and the intensity of staining. Frequency was divided into <50% and ≥50% groups. Meanwhile, staining intensity was divided into no, weak, and strong staining groups. Furthermore, we defined the expression as negative if no cells were stained, or <50% of cells were stained with weak intensity, and positive if the percentage of stained cells was ≥50% of cells, or <50% of cells were stained but with strong intensity. The semiquantitative IHC scoring was done in 10 high-power fields of tumor cells.

Response to NACT

The primary endpoint was a response to NACT, both clinically and pathologically. Clinically, the tumor dimensions were measured using a caliper twice: pre-NACT and 4 weeks post-NACT. Assessment of

clinical response was based on the World Health Organization criteria.[15] Complete response (cCR) was defined as complete disappearance of tumor mass, partial response (cPR) was defined as ≥50% reduction in the product of two perpendicular dimensions of the tumor mass, while progressive disease (cPD) was defined as ≥25% increase in the product of two perpendicular dimensions of the tumor mass. When the change did not fall into any categories, it would be defined as stable disease (cSD). cCR and cPR were grouped as responders, while cSD and cPD were non-responders. Meanwhile, the pathological response was determined by comparing tumor cellularity in surgical specimens to biopsy specimens based on the Miller-Payne (MP) criteria. Grade 1 was defined as no significant reduction; grade 2 was defined as a minor reduction (<30%); grade 3 was defined as a 30–90% reduction in tumor cellularity; grade 4 was defined as >90% reduction of tumor cellularity with small clusters or widely dispersed individual cells; grade 5 was defined as no malignant cells identifiable although ductal carcinoma in situ might still be present.[16] In this study, we defined pCR as MP grade 5 with no invasive/in situ residuals in the breast and no invasive residual nodes (ypT0ypN0 or ypTisypN0), while MP grades 1–4 were considered as non-pCR.[1]

Sample size calculation

The sample size was calculated to detect the difference in the proportion of response toward chemotherapy between two independent groups. The following values and information were considered: the proportion of Pgp positive group that did not respond to chemotherapy as 0.9, and the proportion of Pgp negative group that did not respond to chemotherapy as 0.4 based on Veneroni et al,[17] type-I error (α) = 0.05, and sampling power (1-β) = 80%. The sample size needed was 12 individuals in each group; however, considering a 10% dropout rate, the overall sample size needed was 13 individuals in each group.

Statistical analysis

Statistical analyses were carried out using SPSS software version 20.0 (IBM Corp., USA). Descriptive analysis was used to summarize the baseline characteristics of the subjects. Categorical data were analyzed using Pearson's chi-square or Fisher's exact tests where appropriate. The level of significance was set to <0.05 for all statistical tests.

RESULTS

Thirty subjects were eligible and willing to participate in this cohort study. However, three subjects were excluded due to loss of follow-up (Figure 1). Pgp expression was determined immunohistochemically in biopsy specimens before NACT (n = 27) and surgical specimens after NACT (n = 23). Examples of IHC staining intensity are shown in Figure 2.

Table 1 displays the demographics and clinicopathological data of the subjects. The subjects' mean age and body mass index (BMI) were 46.2 (9.6) years and 23.4 (3.2) kg/m^2, respectively. Histopathologically, most of the cases were invasive ductal (78%). The molecular subtype could not be determined in nine subjects due to the unavailability of reagent. Luminal B subtypes were predominant in the remaining subjects (61%). Pgp was positive in 21 subjects (78%) and negative in six subjects (22%). The proportion of Pgp positivity was higher in subjects with BMI <25 kg/m^2, mixed or lobular histopathology, positive hormone receptors, human epidermal growth factor receptor 2 (HER2)-positive, and topoisomerase-2 alpha >15%.

Although most subjects responded clinically, none of them was cCR. Tumor size reduction ranged from 4.2 to 71.4%. Clinical responders were observed in both Pgp positive and negative subjects in a similar proportion (61.9% and 66.7%).

After three cycles of NACT, 23 subjects (85%) were deemed operable and underwent a modified radical mastectomy. Histopathological analysis of the surgical specimens showed that only one subject, who was Pgp negative, achieved pCR. There was no difference between Pgp positive and negative subjects for attaining clinical response (relative risk [RR] 1.1, 95% confidence interval [CI] 0.3–4.0, p = 0.83) or pathological response (RR 1.3, 95% CI 0.8–1.9, p = 0.22) (Table 2).

DISCUSSION

In contrast with previous studies,[6–7] no association was found between pre-NACT Pgp expression and either clinical or pathological responses. However, these data must be interpreted with caution due to several reasons. Therefore, the potential role of Pgp as a predictive biomarker should be considered as there is clear evidence of Pgp limiting drug accumulation in

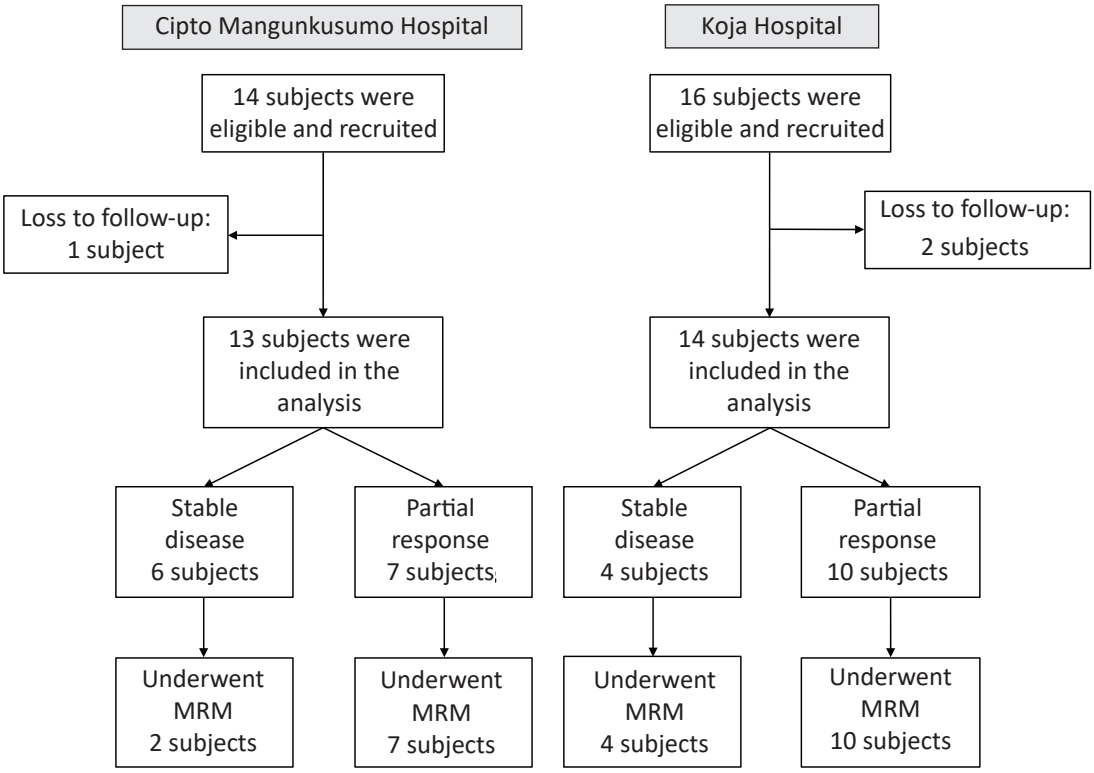

Figure 1. Flow diagram of the subjects throughout the course of the study. MRM=modified radical mastectomy

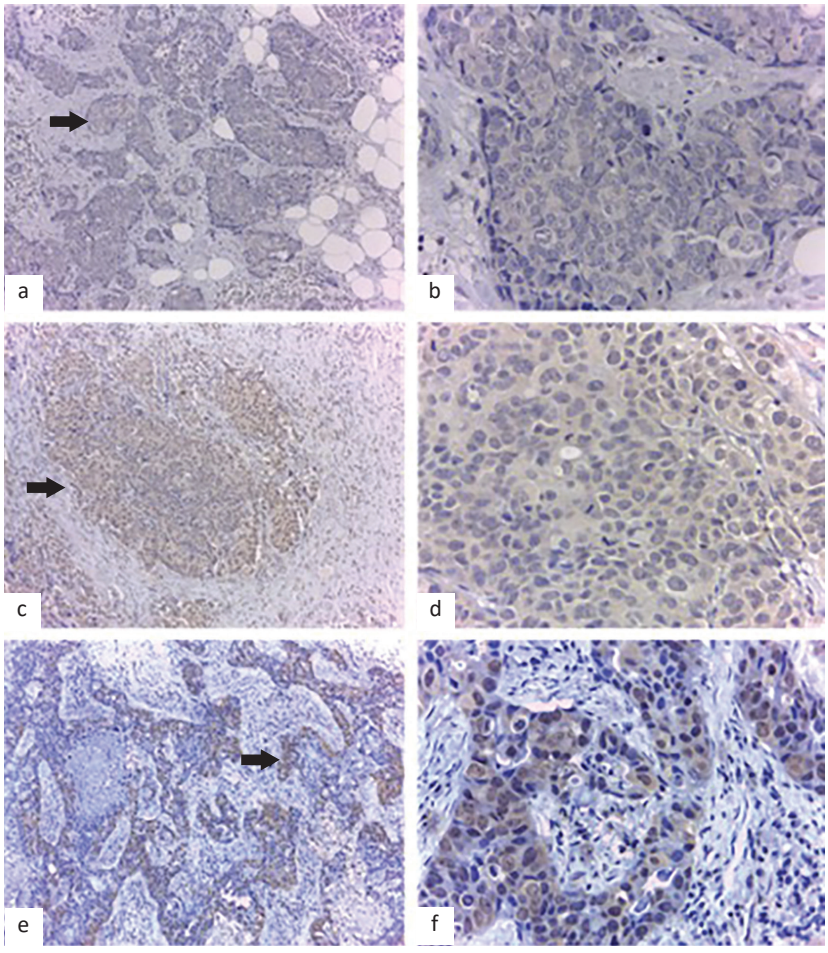

Figure 2. Pgp IHC staining with JSB-1 in breast cancer cells (arrow). (a) No staining (40×); (b) no staining (100×); (c) weak staining (40×); (d) weak staining (100×); (e) strong staining (40×); (f) strong staining (100×). IHC=immunohistochemistry; Pgp=P-glycoprotein

Table 1. Demographics and clinicopathological data of the subjects

Variables	n (%)
Age (years)	
≤40	7 (26)
>40	20 (74)
BMI (kg/m²)	
<25	18 (67)
25–30	9 (33)
Histopathology	
Ductal	21 (78)
Lobular	3 (11)
Mixed	3 (11)
Histopathological grade	
Grade I	3 (11)
Grade II	13 (48)
Grade III	11 (41)
ER (+)	23 (85.2)
PR (+)	23 (85)
HER2 (+)	3 (17)
Ki-67	
<20%	7 (28)
≥20%	18 (72)
TOP2A	
≤15%	13 (48)
>15%	14 (52)
Molecular subtype	
Luminal A	6 (33)
Luminal B	11 (61)
Triple negative	1 (6)
Pgp expression (+)	21 (78)
Clinical response*	
No change	10 (37)
Partial response	17 (63)
Pathological response†	
1 (no significant reduction)	9 (39)
2 (<30% reduction)	7 (30)
3 (30–90% reduction)	4 (17)
4 (>90% reduction)	2 (9)
5 (no residual cancer cells)	1 (4)

BMI=body mass index; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; Pgp=p-glycoprotein; PR=progesteron receptor; TOP2A=topoisomerase-2 alpha
*Based on World Health Organization criteria; †based on Miller-Payne (MP) criteria

selected cancer patients.[3] Firstly, the percentage of Pgp positivity found in this study was much higher than the previous studies.[5,6,18] Nevertheless, in the meta-analysis by Trock et al,[19] high variability of Pgp expression was observed between the included studies, ranging from 0–100%. This variability can be caused by several factors, such as different MAbs and the difference in interpreting and quantifying Pgp expression.

Pgp is a transmembrane protein; hence, its detection through IHC can be done using MAbs directed against surface epitopes, such as MRK16 or UIC2, or cytoplasmic epitopes, such as C219 and JSB1.[20] Furthermore, each MAb shows variations in reactivity.[21,22] Therefore, using two MAbs is recommended to improve accuracy by targeting different epitopes.[23] However, in this study, only MAb that targeted cytoplasmic epitope was used because of the possible alteration of surface epitopes in FFPE tissues. Moreover, JSB1 was used instead of C219 because of the higher concordance rate with MRK16.[24]

Immunostaining of Pgp is expected to occur in the cell's membrane. However, the staining was observed in the cytoplasm (Figure 2), which was also reported in previous studies.[4–7] There have been conflicting views regarding the location of Pgp staining, where one considers it an artifact or a cross-reaction with pyruvate carboxylase. In contrast, others argue that it reflects the transport of Pgp from the Golgi apparatus to the cell membrane.[4,20,23] Therefore, the use of other assays for validation is recommended.[23] However, studies comparing IHC staining and mRNA levels reported discordant results, and functional studies are still limited.[3]

Another cause of the variability is the semi-quantification of Pgp expression. This study used the percentage of stained cells and the staining intensity.[23] However, to date, no standard criteria have been proposed. Different studies used different criteria to measure Pgp expression, resulting in the reported Pgp expression variability.[5,6,25]

Secondly, no cCR was demonstrated for the response to NACT, and only one subject elicited pCR in this study. We believe that it was suboptimal and occurred due to two reasons. First, following the Indonesian Society of Surgical Oncology (ISSO) systemic therapy guideline, only three cycles of NACT were given.[11] Although disease-free survival and overall survival were similar between those who received sandwich chemotherapy (i.e., NACT followed

Table 2. Bivariate analysis between clinicopathological variables and clinical and pathological responses

Variables	Clinical response*		$p^{\dagger}$	Pathological response‡		$p^{\dagger}$
	Non-responder, n (%)	Responder, n (%)		Non-pCR, n (%)	pCR, n (%)	
Age (years)			0.678			0.739
≤40	2 (29)	5 (71)		6 (100)	0 (0)	
>40	8 (40)	12 (60)		16 (94)	1 (6)	
BMI (kg/m^2)			0.561			0.609
<25	7 (39)	11 (61)		13 (93)	1 (7)	
25–30	3 (34)	6 (66)		9 (100)	0 (0)	
Histopathology			0.244			1.000
Ductal	8 (35)	15 (65)		18 (95)	1 (5)	
Lobular	2 (100)	0 (0)		2 (100)	0 (0)	
Mixed	0 (0)	2 (100)		2 (100)	0 (0)	
Histopathological grade			1.000			0.130
Grade I	1 (34)	2 (66)		2 (67)	1 (33)	
Grade II	5 (39)	8 (61)		11 (100)	0 (0)	
Grade III	4 (36)	7 (64)		9 (100)	0 (0)	
ER			0.128			0.826
Positive	7 (30)	16 (70)		18 (95)	1 (5)	
Negative	3 (75)	1 (25)		4 (100)	0 (0)	
PR			0.613			0.826
Positive	8 (35)	15 (65)		18 (95)	1 (5)	
Negative	2 (50)	2 (50)		4 (100)	0 (0)	
HER2 (n = 18)			0.515			0.813
Positive	0 (0)	3 (100)		3 (100)	0 (0)	
Negative	6 (40)	9 (60)		12 (92)	1 (8)	
Ki-67 (n = 25)			0.355			0.318
<20%	1 (14)	6 (86)		6 (86)	1 (14)	
≥20%	8 (44)	10 (56)		15 (100)	0 (0)	
TOP2A			0.695			0.565
≤15%	6 (43)	8 (57)		12 (92)	1 (8)	
>15%	4 (31)	9 (69)		10 (100)	0 (0)	
Pgp expression			0.613			0.217
Positive	8 (38)	13 (62)		18 (100)	0 (0)	
Negative	2 (33)	4 (67)		4 (80)	1 (20)	

BMI=body mass index; ER=estrogen receptor; HER2=human epidermal growth factor receptor 2; pCR=pathological complete response; Pgp=p-glycoprotein; PR=progesteron receptor; TOP2A=topoisomerase-2 alpha
*Based on World Health Organization criteria; †Fisher's exact test; ‡based on Miller-Payne (MP) criteria

by surgery and adjuvant chemotherapy) and full-dose NACT, increased frequency of cCR and pCR was observed with an increased number of chemotherapy cycles and addition of taxane and/or targeted therapy.[1,26] This was long adopted in the international guidelines, such as the National Comprehensive Cancer Network guideline, but it was only recently advocated in the updated ISSO systemic therapy guideline.[27,28] Second, the measurement of the tumor dimensions was not image-assisted, which could have caused measurement bias in the evaluation of clinical response due to the fibrotic process in the breast connective tissue following chemotherapy.

Numerous studies have investigated clinicopathologic factors to predict response to NACT. It was found that triple-negative breast cancer (TNBC)

and HER2-positive breast cancer, if treated with the targeted therapy, were the predictors of the response to NACT.[12,29] However, in this study, the analysis of the breast cancer subtype and response to NACT was not performed because it was not powered.

It was interesting to note that one subject who developed pCR was Pgp negative. Moreover, the subject had low-grade luminal A breast cancer, which usually does not respond satisfactorily to chemotherapy. However, in retrospect, pCR probably transpired in this patient because the histopathology of the breast cancer was mucinous carcinoma, a rare subtype of invasive ductal carcinoma, which inherently has an excellent prognosis.[30]

Another interesting observation was that a higher proportion of Pgp positivity was observed in subjects with hormone receptors negative and HER2-positive. A similar observation was reported by Nedeljković et al,[31] where ABCB1 and ABCG2 were expressed more frequently in TNBC. These findings questioned how efflux proteins were found more frequently in breast cancer subtypes that are usually responsive to chemotherapy, whereas animal studies showed that expressions of these efflux proteins lead to chemoresistance.[3]

There were several limitations to this study. Firstly, molecular subtypes could not be determined in some subjects because of the reagent unavailability. Secondly, we did not perform other assays such as mRNA or functional Pgp to validate the IHC results.

Due to the relatively late update on the breast cancer systemic therapy guidelines paired with limited coverage by the national health insurance (*Badan Penyelenggara Jaminan Sosial Kesehatan*), patients might have received substandard care. Therefore, the government and healthcare providers must work together to increase awareness and knowledge of breast cancer, and ensure access to the best possible breast cancer care.

In conclusion, although Pgp is expressed in most LABC patients, its role as a predictive biomarker could not be established. The small sample size and the lack of standardized Pgp measurement must be considered when interpreting these results. Furthermore, the elicited clinical and pathological responses might have been suboptimal due to the outdated NACT regimen employed, obscuring the significance of Pgp. Thus, studies with more sample size and a standardized method of Pgp measurement coupled with a functional assay are warranted to elucidate this matter.

Conflict of Interest
The authors affirm no conflict of interest in this study.

Acknowledgment
None.

Funding Sources
None.

REFERENCES

1. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014;384(9938):164–72.
2. Clarke R, Leonessa F, Trock B. Multidrug resistance/p-glycoprotein and breast cancer: review and meta-analysis. Semin Oncol. 2005;32(6 Suppl 7):S9–15.
3. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of efflux pumps in multidrug-resistant cancer. Nat Rev Cancer. 2018;18(7):452–64.
4. Faneyte IF, Kristel PM, van de Vijver MJ. Determining *MDR1*/P-glycoprotein expression in breast cancer. Int J Cancer. 2001;93(1):114–22.
5. Rudas M, Filipits M, Taucher S, Stranzl T, Steger GG, Jakesz R, et al. Expression of MRP1, LRP and Pgp in breast carcinoma patients treated with preoperative chemotherapy. Breast Cancer Res Treat. 2003;81(2):149–57.
6. Chintamani, Singh JP, Mittal MK, Saxena S, Bansal A, Bhatia A, et al. Role of p-glycoprotein expression in predicting response to neoadjuvant chemotherapy in breast cancer-a prospective clinical study. World J Surg Oncol. 2005;3:61.
7. Mehrotra M, Anand A, Singh KR, Kumar S, Husain N, Sonkar AA. P-glycoprotein expression in Indian breast cancer patients with reference to molecular subtypes and response to anthracycline-based chemotherapy—a prospective clinical study from a developing country. Indian J Surg Oncol. 2018;9(4):524–9.
8. Anwar SL, Raharjo CA, Herviastuti R, Dwianingsih EK, Setyoheriyanto D, Avanti WS, et al. Pathological profiles and clinical management challenges of breast cancer emerging in young women in Indonesia: a hospital-based study. BMC Womens Health. 2019;19(1):28.
9. Gondhowiardjo S, Silalahi M, Manuain DA, Sekarutami SM, Christina N, Octavianus S, et al. Cancer profile in Central Jakarta: a 5-year descriptive study. Radioter Onkol Indones. 2018;9:34–42.
10. Shintia C, Endang H, Diani K. Assessment of pathological response to neoadjuvant chemotherapy in locally advanced breast cancer using the Miller-Payne system and TUNEL. Malays J Pathol. 2016;38(1):25–32.
11. Manuaba IBTW, editor. Indonesian Society of Surgical Oncology (ISSO) 2010 solid cancer management guidelines. Jakarta: Sagung Seto; 2010. Indonesian.
12. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: past, present and future. Semin Cancer Biol. 2018;52(Pt 1):56–73.
13. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, editors. AJCC cancer staging manual. 8th ed. New York: Springer; 2017.
14. Haagensen CD, AP. S. Carcinoma of the breast. II-criteria of operability. Ann Surg. 1943;118(6):1032–51.
15. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer. 1981;47(1):207–14.
16. Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith

I, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast. 2003;12(5):320–7.
17. Veneroni S, Zaffaroni N, Daidone MG, Benini E, Villa R, Silvestrini R. Expression of P-glycoprotein and *in vitro or in vivo* resistance to doxorubicin and cisplatin in breast and ovarian cancers. Eur J Cancer. 1994;30A(7):1002–7.
18. Prajoko YW, Aryandono T. The effect of p-glycoprotein (P-gp), nuclear factor-kappa B (*Nf-κb*), and aldehyde dehydrogenase-1 (*ALDH-1*) expression on metastases, recurrence and survival in advanced breast cancer patients. Asian Pac J Cancer Prev. 2019;20(5):1511–8.
19. Trock BJ, Leonessa F, Clarke R. Multidrug resistance in breast cancer: a meta-analysis of MDR1/gp170 expression and its possible functional significance. J Natl Cancer Inst. 1997;89(13):917–31.
20. van der Heyden S, Gheuens E, De Bruijn E, Van Oosterom A, Maes R. P-glycoprotein: clinical significance and methods of analysis. Crit Rev Clin Lab Sci. 1995;32(3):221–64.
21. Lehne G, De Angelis P, Clausen OP, Egeland T, Tsuruo T, Rugstad HE. Binding diversity of antibodies against external and internal epitopes of the multidrug resistance gene product P-glycoprotein. Cytometry. 1995;20(3):228–37.
22. Lacueva FJ, Teruel A, Calpena R, Medrano J, Mayol MJ, Perez-Vazquez MT, et al. Detection of P-glycoprotein in frozen and paraffin-embedded gastric adenocarcinoma tissues using a panel of monoclonal antibodies. Histopathology. 1998;32(4):328–34.
23. Beck WT, Grogan TM, Willman CL, Cordon-Cardo C, Parham DM, Kuttesch JF, et al. Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. Cancer Res. 1996;56(13):3010–20.
24. Tóth K, Vaughan MM, Slocum HK, Arredondo MA, Takita H, Baker RM, et al. New immunohistochemical "sandwich" staining method for MDRI P-glycoprotein detection with JSB-1 monoclonal antibody in formalin-fixed, paraffin-embedded human tissues. Am J Pathol. 1994;144(2):227–36.
25. Decker DA, Morris LW, Levine AJ, Pettinga JE, Grudzien JL, Farkas DH. Immunohistochemical analysis of P-glycoprotein expression in breast cancer: clinical correlations. Ann Clin Lab Sci. 1995;25(1):52–9.
26. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, et al. Preoperative chemotherapy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol. 2008;26(5):778–85.
27. National Comprehensive Cancer Network. Breast cancer (version 2.2022) [Internet]. [updated 2021 Dec 20; cited 2022 Jan 24]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.
28. Indonesian Society of Surgical Oncology (ISSO). 2020 cancer management guidelines. Handojo D, Haryono SJ, Sudarsa IW, Panigoro SS, Setiaji K, Tanggo EH, et al, editors. Jakarta: PERABOI; 2020. Indonesian.
29. Kuroi K, Toi M, Ohno S, Nakamura S, Iwata H, Masuda N, et al. Prognostic significance of subtype and pathologic response in operable breast cancer; a pooled analysis of prospective neoadjuvant studies of JBCRG. Breast Cancer. 2015;22(5):486–95.
30. Makki J. Diversity of breast carcinoma: histological subtypes and clinical relevance. Clin Med Insights Pathol. 2015;8:23–31.
31. Nedeljković M, Tanić N, Prvanović M, Milovanović Z, Tanić N. Friend or foe: ABCG2, ABCC1 and ABCB1 expression in triple-negative breast cancer. Breast Cancer. 2021;28(3):727–36.