Correlation Expression Of Beclin-1 With Histopathological Grading And Molecular Subtypes In Invasive Breast Carcinoma Of No Special Type

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Abstract: Background: Breast cancer is most common cancer in women. Most breast cancers are invasive. Invasive breast carcinoma (IBC) is breast cancer that has infiltrated breast tissue. Beclin-1 is an important component in initiation stage of autophagy. Decreased levels Beclin-1 protein due to Beclin-1 gene deficiency, lead to poor prognosis and decreased overall survival in breast cancer cases, where Beclin-1 protein acts as a tumor suppressor.
Objective: To analyze correlation expression of Beclin-1 with histopathological grading and molecular subtypes in invasive breast carcinoma of no special type (IBC-NST).
Methods: This study was an analytic study with a cross sectional approach on 40 samples paraffin block with histopathological diagnosed as IBC-NST. Slides were made with routine staining of hematoxyllin eosin and immunohistochemistry Beclin-1. Beclin-1 expressed on cell membrane and cytoplasm of tumor cells. Scores for Beclin-1 based on multiplication proportion and intensity of staining. Correlation expression of Beclin-1 with histopathological grading and molecular subtypes in IBC-NST was statistically tested.
Results: Most patients with IBC-NST occur age 40-49 years, with average age 50 years, youngest age 27 years and oldest age 73 years. Most tumor size according to T2 criteria. Most molecular subtypes were luminal. Most histopathological grading was grade 3. Immunohistochemical expression of Beclin-1 in IBC-NST was found to be highest with strong expression.
Conclusions: The study showed a significant correlation between immunohistochemical expression of Beclin-1 with histopathological grading (p-value 0.009). There was a significant correlation between immunohistochemical expression of Beclin-1 with molecular subtype (p-value <0.05).

Keywords: Breast cancer, IBC-NST, Beclin-1, histopathological grading, molecular subtype

I. INTRODUCTION

The term IBC refers to a heterogeneous group of malignant epithelial neoplasms originating in mammary glands.[1] The term IBC-NST refers to a large and heterogeneous group of IBCs that cannot be classified morphologically as one specifichistological type. IBC-NST does not have typical features of allspecific subtypes of IBC. There is no difference in risk factors between breast carcinoma in general and IBC-NST.[2]

Breast cancer is most frequently diagnosed cancer in women, accounting for about 24% of all cancers that occur in women andleading cause of cancer death in women worldwide.[1] Based on data from Global Cancer Observatory (GLOBOCAN) in Indonesia 2020 reported that incidence of breast cancer which is first malignancy case with an estimated 65,858 new cases (16.6%) and 22,430 deaths (9.6%). Total number of breast cancerpatients was 201,143 cases within 5 years calculated from all ageranges.[3]

Histopathological grading has become a simple and inexpensive method for assessing tumor behavior and prognosis in IBC, it can also be used to identify possible poor outcomes in patients.[4] Elston–Ellis modification of Bloom and Richardson grading classification, also known as Nottingham grading system (NGS), has been used globally for determination of histopathological grading system of IBC.[4,5] Histopathological grading shows degree of differentiation, which reflects similarity of tumor cells to normal breast cells. NGS is a semiquantitative assessment of three morphological characteristics, namely tubular/glandular formation, nuclear pleomorphism, and mitotic frequency. Overall, they are divided into three grades, grade 1 (well-differentiated tumor), grade 2 (moderately-differentiated tumor) and grade 3 (poorly-differentiated tumor).[4]

Breast carcinoma is heterogeneous at molecular level, with different gene expression patterns leading to different behavior and prognosis. Over past few years, there have been many attempts to characterize and classify breast carcinomas at molecular level for effective treatment. There are four molecular subtypes in breast carcinoma, namely luminal A, luminal B, Human epidermal growth factor receptor 2 (HER2)-enriched, andTriple negative breast cancer (TNBC).[1]

Autophagy is a catabolic pathway meaning "self-eating" that facilitates recycling of nutrients from damaged, aging organelles and other damaged cellular components through lysosomal degradation. Regulation of this process has been associated with cancer development. This autophagy can play different roles in different tumors and stages of tumor development. In breast cancer, autophagy functions as a mechanism that promotes survival or causes death, so it is very important to define role of autophagy which is expected to be useful for effective treatment strategies in breast cancer cells.[6] Recent data have reported that mutations in genes involved in autophagy play an important role in pathogenesis of various diseases, including cancer. Mechanism of autophagy has not been fully elucidated, emerging evidence suggests that autophagy plays a dual role in breast cancer.[7] In early stages of cancer, autophagy has an inhibitory

effect on tumorigenesis. In advanced stages, autophagy promotes tumor cell growth.[8,9]

Beclin-1 is an important component in initiation stage of autophagy, which takes part in early stages of autophagosome formation (nucleation phase).[6,10] It was reported that Beclin-1 may be a tumor suppressor in sporadic breast cancer and that autophagy may play a role in preventing development of these tumors.[11] Decreased levels of Beclin-1 protein due to Beclin-1 gene deficiency, lead to poor prognosis and decreased overall survival in breast cancer cases, where Beclin-1 protein acts as a tumor suppressor.[6,12] In particular, deletion of Beclin-1 allele has observed in TNBC cells and weak Beclin-1 expression correlated with TNBC subtype.[6] Whether its role is positive or negative has not been fully elucidated. Therefore, researchers are interested in examining whether there is a correlation between immunohistochemical expression of Beclin-1 with histopathological grading and molecular subtypes in IBC-NST.

II. MATERIAL AND METHODS

Sample selection

This analytical study with a cross-sectional approach was conducted at Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan and Anatomic Pathology Unit, H. Adam Malik Hospital, Medan. This research was conducted from January 2022 to May 2022, after obtaining approval from Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara.

Sample of this study was a paraffin block from patients who had been diagnosed histopathologically as IBC-NST that met inclusion and exclusion criteria. Samples were taken using consecutive sampling technique. Inclusion criteria in this study were: (1) Adequate clinical data in medical records (included age, tumor size, and molecular subtype). (2) Representative paraffin block preparations, derived from results of mastectomy or lumpectomy surgery tissue that have been diagnosed histopathologically as IBC-NST. Exclusion criteria in this study were: (1) Paraffin blocks recorded according to data storage devices were not representative so they could not be reprocessed. (2) Preparation of paraffin blocks derived from minimal tissue biopsy results.

Histopathological grading of IBC-NST was performed using Notthingham grading. Tumor grade was obtained from a microscopic assessment of 3 categories, namely: (1) Tubular or glandular formations were given a score of 1 if tubular formations were found >75% of all tumors, a score of 2 if tubular or glandular formations were found in 10-75% of all tumors, and a score of 3 if tubular or glandular formation is <10% of all tumors; (2) Nuclear pleomorphism is given a score of 1 if cells are small, regular and uniform, a score of 2 if nucleus is enlarged and nucleus is moderately varied, a score of 3 if size and shape of nucleus are highly variable; (3) Number of mitoses is based on sum of all mitotic figures in 10 large visual fields (400x). Microscope used in this study has a field diameter of 0.5 mm, so mitotic assessment with a field area of 0.196 mm2 is a score of 1 for number of mitoses 7/10 LPB, a score of 2 for number of mitoses 8-14/10 LPB, and a score of 3 for mitotic count 15/10 LPB. Scores from each category will be summed and interpreted as follows: 1 = Grade 1, if total score is 3-5; 2 = Grade 2, if total score is 6 or 7; 3 = Grade 3, if total score is 8 or 9.[1]

Molecular subtype is a classification of breast carcinoma based on gene expression patterns at molecular level. Obtained from secondary data using an assessment based on results of ER, PR, HER2, and Ki-67 immunohistochemical examinations, secondary data obtained from medical records. Based on this immunohistochemical profile IBC was divided into several molecular subtypes and categorized as follows: 1 = Luminal (luminal A, luminal B HER2 negative and luminal B HER2 positive); 2 = HER2 positive (non-luminal); 3 = TNBC.[1]

Beclin-1 is a protein that in humans is encoded by BECN1 gene. Assessment of Beclin-1 expression on cell membranes and tumor cell cytoplasm, using Rabbit Anti Beclin-1 Polyclonal Antibody antibody at a dilution of 1:300 (Cat. No. GTX133555; GeneTex, Inc.). Evaluation on immunohistochemical examination was based on percentage of stained cells and intensity of staining. Results were scored as follows: Percentage of stained cells was scored as: (1) 0 = negative or unstained; (2) 1 = <30% of stained positive cells; (3) 2 = >30% of stained positive cells. Staining intensity was graded as: (1) 0 = negative; (2) 1 = weak intensity; (3) 2 = moderate intensity; (4) 3 = strong intensity. Total score is percentage of stained cells times staining intensity: (1) 1 = Total score 0-1 = negative; (2) 2 = Total score 2-4 = weak positive; (3) 3 = Total score 5-6 = strong positive.[13]

Data analysis

Assessment of correlation immunohistochemical expression of Beclin-1 with histopathological grading and molecular subtypes on IBC-NST will be carried out by statistical tests, namely non-parametric associative tests. Assessment of correlation immunohistochemical expression of Beclin-1 and histopathological grading on IBC-NST using Somers'd test because data scale variables are ordinal-ordinal. Assessment of correlation immunohistochemical expression of Beclin-1 with molecular subtypes in IBC-NST using eta test because variables are ordinal-nominal data scales.

III. RESULTS

In this study, obtained as many as 40 preparations of tissue paraffin blocks that have complete medical record data. Most patients with IBC-NST occur at age of 40-49 years (32,5%), with average age of patients with IBC-NST in this study being 50,1 years, where youngest age is 27 years and oldest age is 73 years. Most of tumor size according to T2 criteria, namely tumor size is 2-5 cm (52,5%). Most molecular subtypes are luminal (52,5%) which consists of luminal A, luminal B-HER2 negative, and luminal B-HER2 positive. (Table 1). Most histopathological grading was grade 3 (35%) (Table 2). Immunohistochemical expression of Beclin-1 in IBC-NST was found to be highest with strong expression (62,5%) (Table 3).

Table 1. Distribution frequency IBC-NST based on age, tumor size, and molecular subtype

Variable	(n=40)	Persentase (%)
Age (years old):		
<30	2	5,0
30-39	4	10,0
40-49	13	32,5
50-59	12	30,0
>59	9	22,5
Tumor size:		
<2 cm (*T1*)	9	22,5
2-5 cm (*T2*)	21	52,5
>5 cm (*T3*)	10	25,0
Tumor any size with direct extension to chest wall and/or skin (skin ulceration or nodule) (T4)	0	0
Molecular subtype		
Luminal	21	52,5
HER2 positive (Non luminal)	11	27,5
TNBC	8	20,0

Table 2. Distribution frequency IBC-NST based on histopathological grading

Histopathological grading	(n=40)	Persentase (%)
Grade 1	13	32,5
Grade 2	13	32,5
Grade 3	14	35,0
Total	40	100,0

Table 3. Beclin-1 immunohistochemical expression frequency distribution on IBC-NST

Beclin-1 expression	(n=40)	Persentase (%)
Negative	3	7,5
Weak	12	30,0
Strong	25	62,5
Total	40	100,0

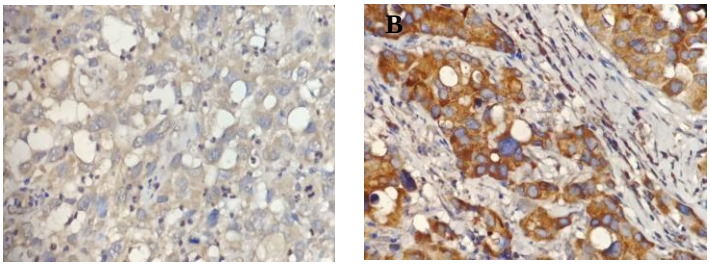

Figure 1. Beclin-1 immunohistochemical expression. A. Weak expression (x400). B. Strong expression (x400).

There is a significant correlation between immunohistochemical expression of Beclin-1 and histopathological grading (p-value= 0,009), where the stronger immunohistochemical expression of Beclin-1, the lower grade (grade 1 and grade 2). On the other hand, the weaker immunohistochemical expression of Beclin-1, the higher histopathological grade (grade 3) (Table 4). There is a significant correlation between immunohistochemical expression of Beclin-1 and molecular subtype (p-value <, where the stronger immunohistochemical expression of Beclin-1, higher probability of occurrence in luminal molecular subtype. On the other hand, the weaker immunohistochemical expression of Beclin-1, higher probability that it will occur in molecular subtype of TNBC (Table 5).

Table 4. Correlation expression of Beclin-1 with histopathological grading in IBC-NST

Beclin-1 expression	Histopathological grading						Total		p-value*
	Grade 1		Grade 2		Grade 3				
	n	%	n	%	n	%	n	%	
Negative	2	5,0	0	0,0	1	2,5	3	7,5	0,009
Weak	1	2,5	1	2,5	10	25,0	12	30,0	
Strong	10	25,0	12	30,0	3	7,5	25	62,5	
Total	13	32,5	13	32,5	14	35,0	40	100,0	

* Somers' d test for ordinal-ordinal data scale variables

Table 5. Correlation expression of Beclin-1 with molecular subtypes in IBC-NST

Beclin-1 expression	Molecular subtype						Total		p-value*
	Luminal		HER2 positive (non luminal)		TNBC				
	n	%	N	%	n	%	n	%	
Negative	2	5,0	0	0,0	1	2,5	3	7,5	<0,05
Weak	1	2,5	5	12,5	6	15,0	12	30,0	
Strong	18	45,0	6	15,0	1	2,5	25	62,5	
Total	21	52,5	11	27,5	8	20,0	40	100,0	

* Eta test for ordinal-nominal data scale variables

IV. DISCUSSION

The number of samples diagnosed as IBC-NST in this study were 40 samples, of which 32.5% were aged 40-49 years, with a mean age of 50,1 years, where youngest age was 27 years and oldest age was 73 years. Results of this study are not much different from previous studies. Wais et al. in 2021 reported that age of most IBC patients was 31-60 years.[14] Dalvi et al. in 2021 reported that age of most IBC patients was 61-70 years.[15] Ardian et al. in 2020 reported that age of most IBC-NST patients was 35-49 years.[16] Budzik et al. in 2021 reported that mean age of IBC-NST patients was 60 years with a range of 27-91 years.[17] Most researchers reported that difference in mean age of IBC-NST patients was not very significant, where it was most common in adults. There are several risk factors that can increase likelihood of developing IBC, one of which is age. Older women are reported to have a higher risk. Only about 10% of women diagnosed with IBC are under 45 years of age, and 2 out of every 3 women with IBC are 55 years of age when first diagnosed.[18] Breast cancer at a young age is often familial, and about half of young women with breast cancers under age of 30 have germline mutations in BRCA1, BRCA2, or TP53. Hormonal factors that increase risk of breast cancer among young women include early menarche, oral contraceptives, anovulatory infertility, and late

parity after age 30.[19] Breast cancer in young women (<40 years) is different from breast cancer in women. older (>60 years). The main differences were usually negative estrogen receptor status, multicentric location, higher histological grade, tumors with a TNBC molecular subtype, and higher Ki-67 index. This feature has been found to signal an aggressive disease course with an increased likelihood of poor overall survival and disease-free survival.[20]

In this study, most tumor sizes were in accordance with T2 criteria, namely tumor size was 2-5 cm, as many as 52.5%. Results of this study are not much different from previous studies. Sanges et al. in 2020 reported that tumor size in most cases of IBC-NST was in accordance with T2 criteria (47%).[21] Wais et al. in 2021 reported most tumor size according to criteria T2 (51%).[14,43] Budzik et al. in 2021 reported that tumor size in most IBC-NST cases was in accordance with T2 criteria (41.9%).[17] Contrary to results of study reported by Ardian et al. in 2020 reported that most tumor sizes according to T1 criteria were 0.1- 2 cm (61.1%).[16] Badowska-Kozakiewicz et al. in 2017 reported that most tumor sizes were in accordance with T1 criteria (44.72%).[22] Difference in results of this study may lie in differences in characteristics of study population. System used for staging breast carcinoma is TNM system published by UICC and AJCC. This system includes information about size of primary tumor (T).[1] Size of primary tumor in breast is very important to know because it influences in determining stage, treatment and prognosis.[23]

Most molecular subtypes of IBC-NST were luminal, as many as 52.5%, consisting of luminal A, luminal B-HER2 negative, and luminal B-HER2 positive. Results of this study are not much different from previous studies. Popat et al. in 2020 reported that of 75 cases of IBC-NST, as many as 41 cases (54.67%) with positive ER and 28 cases (37.34%) with positive PR.[24] Budzik et al. in 2021 reported that cases of IBC-NST were often associated with positive hormone receptor expression, namely ER (63.7%) and PR (59.3%).[17] Howlader et al. in 2018 reported that of 196 cases of IBC, 66.6% were hormone receptor positive and HER2 negative.[25] Incidence of ER-positive breast carcinoma is increasing in all ethnicities in United States.[26] Hormone receptor positive, HER-2 negative is a molecular subtype most common in breast carcinoma.[27] Identification of different molecular subtypes can be accomplished through use of immunohistochemical assays. To date, several studies have shown that biological differences in molecular subtypes are associated with differences in treatment response and patient outcomes. Luminal molecular subtype A is associated with a better prognosis with local recurrence occurring more frequently 5 or 10 years after diagnosis. Expression of ER and PR receptors often overlaps between molecular subtypes of luminal A and luminal B. Loss of hormone receptor status tends to have a poorer prognostic picture. HER2-positive molecular subtype has decreased local and systemic recurrence with targeted therapy. TNBC remains a clinical challenge, and several clinical trials are ongoing in an effort to identify mechanisms to help improve outcomes for molecular subtype of TNBC.[25,28]

Most histopathological grade of IBC-NST was grade 3, which was 35%. Results of this study are not much different from previous studies. Misron et al. in 2015 reported that most cases of IBC-NST were grade 3, namely 65 samples from 144 samples studied (45.14%).[29] This is not in line with results of previous studies by Wais et al. in 2021 reported that most cases of IBC were grade 2 (59%).[14] Budzik et al. in 2021 reported that most cases of IBC-NST were grade 2 (54.2%).[17] Oluogun et al. in 2019 reported that most cases of IBC were grade 2 (71%).[30] Determination of histopathological grading has become a simple and inexpensive method to assess tumor behavior and prognosis in IBC, can also be used to identify possible poor outcomes in patients, thus qualifying for administration of neo-adjuvant therapy. Histopathological grading needs to be performed accurately, on properly fixed specimens, and by a trained pathologist.[4] Elston-Ellis modification of Bloom and Richardson grading classification, also known as Nottingham grading system (NGS), has been used globally for determination of histopathological grading system on IBC.[4,5]

Assessment of immunohistochemical expression of Beclin-1 in IBC-NST obtained most with strong expression, which was 62.5%. Results of this study are not much different from previous studies. Won et al. in 2010 reported that positive immunohistochemical expression of Beclin-1 in IBC was 42.4%.[31] Cha et al. in 2014 reported that positive immunohistochemical expression of Beclin-1 in IBC was 45.1%. In this study, positive immunohistochemical expression of Beclin-1 was reported as an independent poor prognostic factor.[32] In accordance with previous studies, in which there was a correlation between immunohistochemical expression of Beclin-1 and poor prognosis in ovarian cancer, gastric cancer, and laryngeal cancer.[33,34,35] This is not in line with results of previous studies, reported that weak immunohistochemical expression of Beclin-1 is associated with poor prognosis in cancer.[36,37,38] This controversy is thought to stem from dual role of autophagy regulating tumor survival and tumor suppression.

Based on results of analysis, that there was a significant correlation between immunohistochemical expression of Beclin-1 and histopathological grading (p-value = 0.009), where the stronger immunohistochemical expression of Beclin-1, the lower grade (grade 1 and grade 2). On the other hand, the weaker immunohistochemical expression of Beclin-1, the higher histopathological grade (grade 3). Results of this study are supported by Won et al. in 2010 reported that positive immunohistochemical expression of Beclin-1 in IBC grade 1 and grade 2 was 28.8%, while grade 3 was 13.6%.[31] Miracco et al. in 2007 reported that expression of Beclin-1 was significantly lower in high grade brain tumors compared to low grade brain tumors (p-value <0.05).[39] This is not in line with results of a previous study by Amer et al. in 2019 reported a correlation between Beclin-1 expression and histopathological grade of breast carcinoma, where strong Beclin-1 expression was found to be 100% in grade 3 (p-value=0.02). Based on these studies, it can be concluded that strong expression of Beclin-1 is significantly correlated with tumor grade. This suggests that Beclin-1 has a dominant tumor suppressor function in early phase of tumorigenesis, and mostly with promoter function in late phase.[13] Choi et al. in 2013 reported that there was no significant correlation between Beclin-1 and tumor grade.[40] Based on results of this study, it is suggested that previous studies suggest that Beclin-1 may play a role in inhibiting development of breast carcinoma. Inhibition may occur through interaction with BCL2 protein.

Based on results of analysis, that there was a significant correlation between immunohistochemical expression of Beclin-1 and molecular subtype (p-value <0.05), where the stronger immunohistochemical expression of Beclin-1, the higher probability that it would occur in luminal molecular subtype. On the other hand, the weaker immunohistochemical expression of Beclin-1, the higher probability that it will occur in molecular subtype of TNBC. Referring to previous research, by Nurdinov et al. in 2014 reported that a deletion of Beclin-1 allele was observed in TNBC cells and that Beclin-1 expression was weakly correlated with TNBC subtype.[6] Cicchini et al. in 2014 reported that weak Beclin-1 expression and activation of WNT pathway gene signature correlated with molecular subtype of TNBC, activation of TNF Receptor Superfamily Member 11a (TNFRSF11A) axis and poor prognosis in human breast carcinoma. His results suggest that Beclin-1 may have a non-autophagy-associated role in breast development, providing insight into apparently paradoxical role of Beclin-1 in tumorigenesis.[41] Not in line with results of a previous study by Amer et al. in 2019 reported that all samples with strong positive Beclin-1 expression were TNBC (6%).[13] Matthew-Onabanjo et al. in 2020 reported that screening of patients with weak Beclin-1 expression could identify a subgroup of patients who would be more sensitive to transferrin drugs (TFR1).[42] TFR1 is also of clinical interest both as a targeted therapy and because of its potential for drug delivery.[43] Chemotherapy drug conjugates - transferrin transported intracellularly by endocytosis of TFR1 is more effective in tumors expressing low levels of Beclin-1 and elevated TFR1.[44] Beclin-1 may be a clinically relevant biomarker for many cancer patients.[42] A further study at scale A larger scale is needed to determine expression of Beclin-1 especially in molecular subtype of TNBC. Referring to a previous study, it was suggested that screening of patients with weak Beclin-1 expression could identify a subgroup of patients who would be more sensitive to TFR1 drugs. Based on results of previous studies and results of this study, immunohistochemical examination of Beclin-1 can be used as an additional panel in cases of breast cancer, especially TNBC in future, in order to take advantage of possibility of this pathway for benefit of treatment and prognosis.

V. CONCLUSION

A study was conducted on patients with IBC-NST at Department of Anatomic Pathology, USU Medical Faculty and Anatomic Pathology Unit, H. Adam Malik Hospital Medan with following conclusions:

1. Most patients with IBC-NST are 40-49 years old, average age of IBC-NST sufferers is 50.1 years, youngest is 27 years old and oldest is 73 years old. Most tumor size T2 criteria, ie tumor size 2-5 cm. Most molecular subtypes were luminal which consisted of luminal A, luminal B-HER2 negative, and luminal B-HER2 positive.
2. Most histopathological grading is grade 3.
3. Immunohistochemical expression of Beclin-1 in IBC-NST was found to be highest with strong expression.
4. In accordance with research hypothesis, a significant correlation was found between immunohistochemical expression of Beclin-1 and histopathological grading, where the stronger immunohistochemical expression of Beclin-1, the lower grade (grade 1 and grade 2). On the other hand, the weaker immunohistochemical expression of Beclin-1, the higher histopathological grade (grade 3).
5. In accordance with research hypothesis, a significant correlation was found between the immunohistochemical expression of Beclin-1 and molecular subtype, where stronger immunohistochemical expression of Beclin-1, the higher probability of occurrence in luminal molecular subtype. On the other hand, the weaker immunohistochemical expression of Beclin-1, higher probability that it will occur in molecular subtype of TNBC.

VI. COMPETING INTERESTS

Author has no financial interests relevant to product or company described in this article.

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VII. ETHICAL APPROVAL

Research has been approved by Health Research Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara with No: 312/KEPK/USU/2022.

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