RESEARCH ARTICLE

Total and Intratumoral CD8+ T Cell Expressions are Correlated with Miller Payne Grading and WHO Clinical Response of Neoadjuvant Chemotherapy

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Abstract

BACKGROUND: Chemotherapy has reported to stimulate immune system through direct activation of cluster of differentiation (CD)8+ T cells. Neoadjuvant chemotherapy (NAC) is known to improve the clinical response of locally advanced breast cancer (LABC) patients. However, the immune response-related factor evaluation of NAC in LABC patients has not been routinely performed. Therefore, current study was conducted to evaluate the correlation of NAC-induced CD8+ T cell with chemotherapy response based on Miller Payne grading and World Health Organization (WHO) criteria.

METHODS: LABC patients were recruited and data regarding age, gender, tumor, nodal stages, histopathological grade, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 were obtained. Biopsy and mastectomy tissues were collected and processed for hematoxylin-eosin and CD8 immunohistochemical staining. CD8+ T cell expression in peritumoral and intratumoral areas were documented and measured. Clinical responses based on Miller Payne grading and WHO were analyzed and correlated with CD8+ T cell expression.

RESULTS: There were more subjects with high expression of total (80%), intratumoral (82.5%) and peritumoral (65%) CD8+ T cell expressions. The total (p=0.013) and intratumoral (p=0.015) CD8+ T cell expression, but not peritumoral CD8+ T cell expression, were significantly correlated with Miller Payne Grading. The total (p=0.009) and intratumoral (p=0.001) CD8+ T cell expressions were also significantly correlated with WHO clinical response.

CONCLUSION: Total and intratumoral CD8+ T cell expressions are correlated with Miller Payne grading and WHO clinical response of NAC. Therefore, total and intratumoral CD8+ T cell expressions could be suggested as a predictive marker for clinical response of NAC.

KEYWORDS: breast cancer, neoadjuvant chemotherapy, CD8, clinical response, Miller Payne, intratumoral, peritumoral

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Introduction

Based on data of Global Cancer Observatory in 2020, breast cancer is the most prevalent type of cancer among female in the world, with incidence of more than two million cases annually, and predicted to keep increasing each year.(1-4) Breast cancer is the most prevalent cause of death in women globally, responsible for 15% mortality rate worldwide, whereas Indonesia is ranked as the country with highest mortality rate due to breast cancer in South East Asia.(5-8) In Indonesia, approximately 57.1% locally advanced breast cancer (LABC) patients seek for treatment. LABC is an invasive breast cancer limited to the breast and regional lymph nodes.(9,10) Conventionally, the standard chemotherapy were done after the surgery. Neoadjuvant chemotherapy (NAC) is proven to be more beneficial by increasing breast conservation rates in the resectable breast cancer cases. With NAC, micro-metastasis can be eradicated, therefore can prevent metastasis. For LABC patients, NAC can improve clinical response up to 70-90%.(11,12)

Conventional Chemotherapy agent has been reported to stimulate immune system to attack cancer cells through direct activation of cluster of differentiation (CD)8^+ T cells that could significantly eliminate tumor cells. T cells have an important role to produce interferon gamma which has cytotoxic effects by inhibiting cell cycles as well as inducing apoptosis and tumoricidal activity. Earlier studies showed that high number of $CD8^+$ T cell was independently correlated with pathological complete response.(13,14)

Precise assessment of certain chemotherapy response can be evaluated through microscopic examination of the residual tumor on surgical resection after chemotherapy. Current evaluation of breast cancer prognosis is limited to biological tumor characteristics such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER)2, and Ki67 expressions. However, clinical response to chemotherapy does not always correlated with those markers, thus additional factors should be considered.(13) Since immune response has been reported to play an essential role in chemotherapy response, assessment of immune response-related factor such as $CD8^+$ T cell (15), could be suggested. However, immune response-related factor evaluation is not routinely performed since it has not been well-established. Therefore current study was conducted to evaluate the correlation of NAC-induced $CD8^+$ T cell with chemotherapy response based on Miller Payne grading and world health organization (WHO).

Methods

Subject Selection and Criteria

LABC patients of Department of Surgery, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo National Central General Hospital from September 2015 to February 2022, were selected and included for this study based on inclusion and exclusion criteria. The inclusion criteria were LABC patients with age of >18 years old, who received full dose of NAC with anthracycline- or taxane-based regimen, prior to mastectomy. Meanwhile, the exclusion criteria were the patients with bilateral or recurrent breast cancer, different/change/inadequate of therapeutic regimen, incomplete medical record and unavailable paraffin block. The protocol of this study was approved by the Ethical Committee of Faculty of Medicine Universitas Indonesia (No. KET-131/UN2.F1/ETIK/PPM.00.02/2022).

Data and Sample Collection

Subject-related data were collected from medical record for information of age, gender, tumor and nodal stages, histopathological grade, as well as immunohistochemical examinations of ER, PR, HER2 and Ki67. Histopathological grade was examined by anatomic pathologist based on haematoxylin-eosin features and divided into 3 categories; grade 1: well differentiated, grade 2: moderately differentiated and grade 3: poorly differentiated. Meanwhile, immunohistochemical examinations of ER, PR, HER2 and Ki67 were carried out with standard immunohistochemical staining procedures in Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia and Dr. Cipto Mangunkusumo National Central General Hospital, with following primary antibodies: anti-ER (Leica Biosystems, Wetzlar, Germany), anti-PR (Leica Biosystems), anti-HER2 (Diagnostic BioSystems, Pleasanton, CA, USA) and anti-Ki67 (Diagnostic BioSystems) antibodies, respectively.

For CD8 immunohistochemical detection, paraffin blocks of biopsy samples were collected, sliced in 4 μm and processed for immunohistochemical staining. Meanwhile for Miller Payne grading, paraffin blocks of mastectomy samples were collected, sliced in 4 μm and processed for hematoxylin-eosin staining.

CD8 Immunohistochemical Staining and Evaluation

Sliced tissues were placed on coated slides, heated, deparaffinized, rehydrated, blocked with 3% H_2O_2, antigen

retrieved with Tris EDTA pH 9.0 and blocked with protein blocking buffer. CD8 (SP16) rabbit monoclonal antibody (Cell Marque, Rocklin, CA, USA) with dilution of 1:200 was used as the primary antibody. Then Starr Trek universal HRP detection system (Biocare Medical, Pacheco, CA. USA) was applied, followed by 3,3′-diaminobenzidine tetrahydrochloride. Counterstaining was performed with hematoxylin. The slide was then dehydrated and coverslipped with Entellan. For positive control, tonsil tissue was used.

Five fields of each sample were randomly selected under a microscope (BX51, Olympus, Tokyo, Japan) with 400x magnification. $CD8^+$ T cell expression in peritumoral and intratumoral areas of each sample were captured and measured by ImageJ (USA National Institutes of Health, Bethesda, MA, USA). Intratumoral and peritumoral areas were defined as inside and outside areas of the tumor stroma, respectively. Then, the results were divided into two groups, low and high expression, based on each's group cut-off.

Miller Payne Grading

Based on the hematoxylin-eosin histopathological features, samples were graded with Miller Payne Grading (15), by 2 calibrated observers, an anatomic pathologist and a surgical oncologist with <10% inter-observer difference. In this study, Miller Payne grading was categorized into two groups, grade 1 was considered as no response, while grade 2-5 were considered as response group.

WHO Clinical Response

WHO clinical response was categorized based on tumor diameter changes, according to WHO criteria. Progression response: >25% increase in tumor size and/or the appearance of new lesion in other site. Stable response: <50% decrease or ≤25% increase in tumor size. Partial response: ≤50% decrease in in tumor size at least for 4 weeks, no appearance of new lesion or disease progression. Complete response: disappearance of the disease during two different observations conducted not less than 4 weeks apart.(16) In this study, subject chemotherapy responses were collected, analyzed based on the WHO Criteria, and divided into two groups. The partial and complete response were considered as response group, while the progression and stable were considered as no response.

Statistical Analysis

Data analysis was done with SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). The cut-offs of intratumoral, peritumoral, and total expression were calculated by area under curve (AUC) analysis and Youden's Index. Fisher Exact and Mann-Whitney tests were used to analyze independent variables and outcomes, with significancy of $p<0.05$.

Results

Forty LABC subjects were selected. Majority of the subjects were aged ≥40 years old (70%), T4 (87.5%), N0 (42.5%) & N1 (42.5%), invasive histopathological appearance with no special type (82.5%), histopathological grade 2 (60%), luminal B (42.5%), treated with anthracycline-based NAC (60%), ER positive (92.5%), PR positive (55%), HER2 negative (70%) and high Ki67 (67.5%) (Table 1).

Immunohistochemical expression of $CD8^+$ T cell was detected clearly in tonsil tissue (Figure 1A) and breast cancer biopsy (Figure 1B). Based on the AUC analysis and Youden's Index, cut-off for total $CD8^+$ T cell expression was 23.8, with sensitivity of 86.5% and specificity of 100%; cut-off for intratumoral was 6.4, with sensitivity of 89.2% and specificity of 100%; cut-off for peritumoral expression was 14.3, with sensitivity of 67.6% and specificity of 66.7%. By applying the cut-offs, the total, intratumoral and peritumoral immunohistochemical expressions were categorized into low or high expression. Current results showed that there were more subjects with high expression of total (80%), intratumoral (82.5%) and peritumoral (65%) $CD8^+$ T cell expressions (Table 2). Based on Miller Payne grading (Figure 2), mostly subjects were categorized as response (92.5%) (Table 2). Meanwhile based on WHO clinical response, 87.5% of the subjects were categorized as response.

Based on Fisher Exact test, although there was no correlation between total $CD8^+$ T cell expression with age, histopathological grade, immunohistochemical subtype, ER, PR, HER2 and Ki67 (Table 3), the total $CD8^+$ T cell expression was significantly correlated with Miller Payne Grading ($p=0.013$) (Table 4). Intratumoral $CD8^+$ T cell expression, but not peritumoral $CD8^+$ T cell expression, was significantly correlated with Miller Payne Grading ($p=0.015$) as well. When the subject distribution was analyzed, the total ($p=0.006$) and intratumoral ($p=0.004$) $CD8^+$ T cell expressions were significantly correlated with Miller Payne Grading (Table 5).

Clinical responses based on WHO showed similar results with the ones based on Miller Payne Grading. The

Table 1. Subject characteristics (n=40).

Characteristics	n (%)
Age	
≤ 40 years old	12 (30)
≥ 40 years old	28 (70)
Tumor	
T2	1 (2.5)
T3	4 (10)
T4	35 (87.5)
Node	
N0	17 (42.5)
N1	17 (42.5)
N2	3 (7.5)
N3	3 (7.5)
Histopathological Appearance	
Invasive NST	33 (82.5)
Lobular	3 (7.5)
Others	4 (10)
Histopathological Grade	
Grade 1	3 (7.5)
Grade 2	24 (60)
Grade 3	13 (32.5)
Immunohistochemical Subtype	
Luminal A	8 (20)
Luminal B	17 (42.5)
Luminal B & HER2	12 (30)
Triple negative breast cancer	3 (7.5)
NAC	
Taxane-based	16 (40)
Anthracycline-based	24 (60)
ER	
Negative	3 (7.5)
Positive	37 (92.5)
PR	
Negative	18 (45)
Positive	22 (55)
HER2	
Negative	28 (70)
Positive	12 (30)
Ki67	
Low	13 (32.5)
High	27 (67.5)

NST: no special type; NAC: neoadjuvant chemotherapy; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

total (p=0.009) and intratumoral (p=0.001) CD8+ T cell expressions were significantly correlated with WHO clinical response (Table 6). In regards of subject distribution, the total (p=0.003) and intratumoral (p=0.000) CD8+ T cell expressions were significantly correlated with WHO clinical response as well (Table 7).

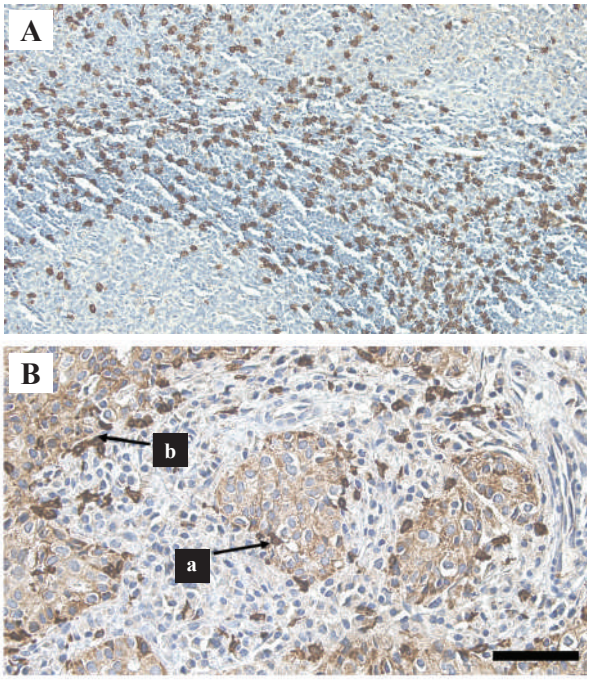

Figure 1. Immunohistochemical expression of CD8. A: tonsil tissue; B: breast cancer biopsy. CD8+ T cells were observed in intratumoral (a) and peritumoral areas (b). Black bar: 100µm.

Discussion

Earlier breast cancer study in Indonesia reported that higher prevalent of female patients in the age of ≥40 than those in the age of <40 (68.9% *vs.* 31.1%). In addition, women in

Table 2. Total, intratumoral and peritumoral CD8+ T cell expression, Miller Payne grading and clinical response subject distribution (n=40).

Characteristics	n (%)
Total CD8+ T Cell Expression	
Low	8 (20)
High	32 (80)
Intratumoral CD8+ T Cell Expression	
Low	7 (17.5)
High	33 (82.5)
Peritumoral CD8+ T Cell Expression	
Low	14 (35)
High	26 (65)
Miller Payne Grading	
No response	3 (7.5)
Response	37 (92.5)
WHO Clinical Response	
No response	5 (12.5)
Response	35 (87.5)

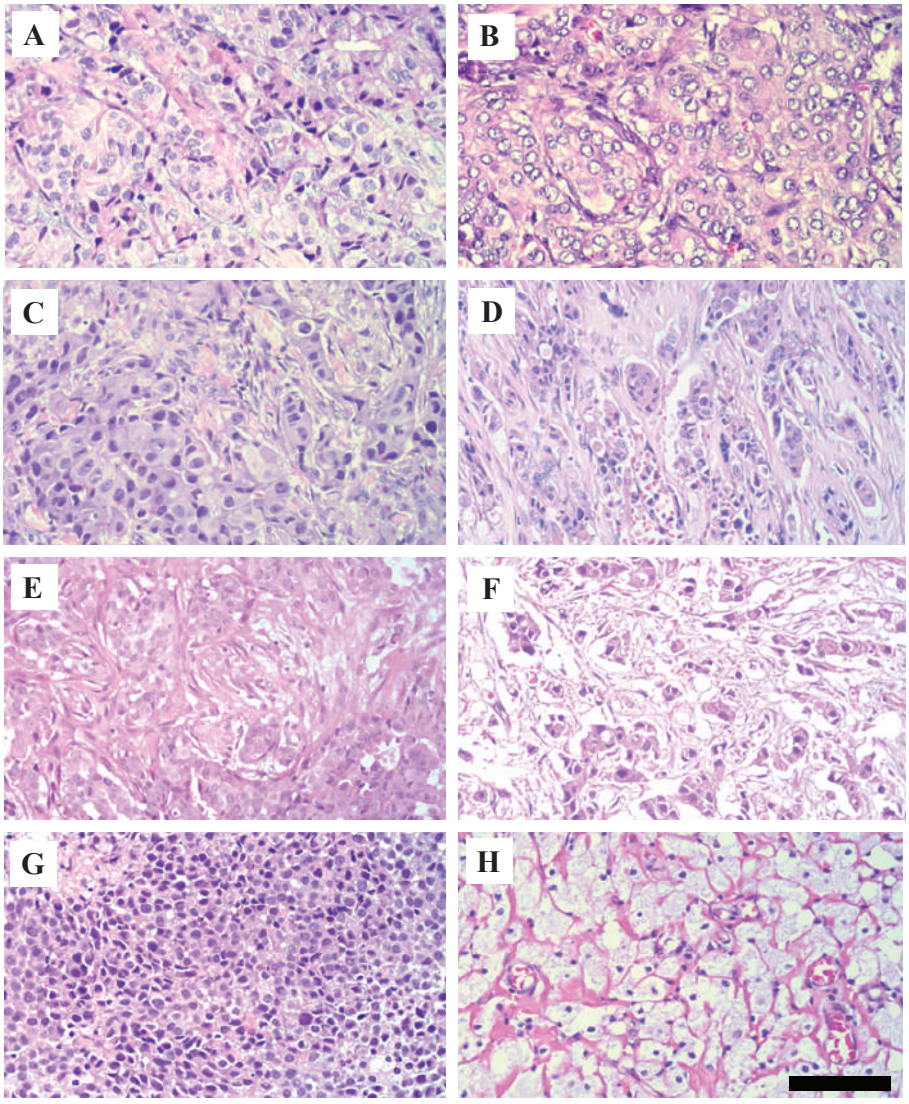

Figure 2. The histopathological expression of biopsy and mastectomy tissue based on Miller Payne grading. Grade 1, from biopsy (a) and mastectomy tissue (b); Grade 2, from biopsy (c) and mastectomy tissue (d); Grade 3, from biopsy (e) and mastectomy tissue (f); Grade 5, from biopsy (g) and mastectomy tissue (h). Black bar: 100 μm.

the age of ≥40 were reported to have an increase of breast cancer risk up to 13.3 times.(17) In the current study, similar population number was included, 70% of the subjects were aged ≥40. Based on the histopathological appearance, most samples of the current study were categorized as invasive carcinoma with no special type (NST) (82.5%), which also has been reported as the most common histopathological appearance of breast cancer in previous reports.(18,19) From the subject characteristics data, majority of subjects had luminal B type (42.5%), which is in accordance with the breast cancer registry data in Indonesia.(18)

In the current study, there was no correlation between $CD8^+$ T cell expression with age, histopathological appearance, histopathological grade, immunohistochemical subtypes, ER, PR, HER2 and Ki67. Factors related to the $CD8^+$ T cell immune profile were found to be multifactorial, including tumor genetics, germline genetics, microbiomes and pharmacological agents.(20,21) However, there were studies reported that $CD8^+$ T cell expression was correlated with higher histopathological grade, triple negative breast cancer subtype, ER negative, tumor grade and size.(22,23)

In the current study, the total $CD8^+$ T cell expressions was significantly correlated with Miller Payne grading and WHO clinical response. This result is in accordance with previous report showing that tumor infiltrating lymphocytes (TIL) was associated with NAC response.(24) In addition, in the current study, intratumoral $CD8^+$ T cell expressions was significantly correlated with Miller Payne grading and WHO clinical response as well. These results supported the recent report suggesting that intratumoral $CD8^+$ was the potential prognostic marker in breast cancer patient, instead

Table 3. Subject characteristics *vs.* total CD8+ expression T cell.

Characteristics	Total CD8+ Expression T Cell		*p*- value
	Low n (%)	High n (%)	
Age			
≤40 years old	2 (5)	10 (25)	0.548
>40 years old	6 (15)	22 (55)	
Histopathological Grade			
Low grade	6 (15)	21 (52.5)	0.479
High grade	2 (5)	11 (27.5)	
Immunohistochemical Subtype			
Luminal	8 (20)	29 (72.5)	0.502
Non-Luminal	0 (0)	3 (7.5)	
ER			
Negative	0 (0)	3 (7.5)	0.502
Positive	8 (20)	29 (72.5)	
PR			
Negative	6 (15)	12 (30)	0.065
Positive	2 (5)	20 (50)	
HER2			
Negative	6 (15)	22 (55)	0.548
Positive	2 (5)	10 (25)	
Ki67			
Low	3 (7.5)	10 (25)	0.521
High	5 (12.5)	22 (55)	

*Tested with Fisher Exact test; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

Table 4. Total, intratumoral and peritumoral CD8+ T cell expression *vs.* Miller Payne Grading (no response (n=3) and response (n=37)).

Characteristics	Miller Payne Grading		*p*- value
	No Response (Mean±SD)	Response (Mean±SD)	
Total CD8+ T cell expression	15.80±7.27	40.57±20.98	0.013*
Intratumoral CD8+ T cell expression	3.93±2.88	18.56±12.18	0.015*
Peritumoral CD8+ T cell expression	11.86±5.31	22.00±14.08	0.248

*Tested with Mann-Whitney test, significant at $p<0.05$

Table 5. Subject distribution of total, intratumoral and peritumoral low/high CD8+ T cell expression *vs.* Miller Payne Grading (no response (n=3) and response (n=37)).

Characteristics	Miller Payne Grading		*p*- value
	No Response n (%)	Response n (%)	
Total CD8+ T Cell Expression			
Low	3 (7.5)	5 (12.5)	0.006*
High	0 (0)	32 (80)	
Intratumoral CD8+ T Cell Expression			
Low	3 (7.5)	4 (10)	0.004*
High	0 (0)	33 (82.5)	
Peritumoral CD8+ T Cell Expression			
Low	2 (5)	12 (30)	0.276
High	1 (2.5)	25 (62.6)	

*Tested with Fisher Exact test, significant at $p<0.05$

Table 6. Total, intratumoral and peritumoral CD8+ T cell expression *vs.* WHO clinical response (no response (n=5) and response (n=35)).

Characteristics	WHO Clinical Response		*p*- value
	No Response (Mean±SD)	Response (Mean±SD)	
Total $CD8^+$ T cell expression	18.92±10.25	41.54±21.01	0.009*
Intratumoral $CD8^+$ T cell expression	4±2.05	19.39±12.00	0.001*
Peritumoral $CD8^+$ T cell expression	14.92±9.77	22.14±14.22	0.357

*Tested with Mann-Whitney test, significant at $p<0.05$

of peritumoral expression.(25) In addition, another study from Indonesia reported that $CD8^+$ might be a predictive factor for clinical response of NAC in breast cancer patients. (13) However, there were also reports suggesting that NAC in breast cancer patients were related with $CD8^+$ T cell expression in both intratumoral and tumor parenchyma, high $CD8^+$ T cell expression in both areas could result in good clinical response.(21) Taken together, current study has strengthened the importance of total and intratumoral $CD8^+$ T cell expressions for achieving good NAC clinical response based on both Miller Payne and WHO. Nevertheless, further long-term observational study with more numbers of study subjects should be conducted.

Conclusion

Total and intratumoral CD8+ T cell expressions are correlated with Miller Payne grading and WHO clinical response of NAC. Therefore, total and intratumoral CD8+ T cell expressions could be suggested as a predictive marker for clinical response of NAC.

Authors Contribution

SSP, SCM, and PR were involved in concepting and planning the research. SSP, SCM, and HH performed the data acquisition/collection. SSP, SCM, HH, and FS conducted the data analysis and interpreted the results. SSP, SCM, and FS edited the manuscript. AK, DJP, PR, and FS designed the figures and tables. All authors took parts in giving critical revision of the manuscript.

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Table 7. Subject distribution of total, intratumoral and peritumoral low/high CD8+ T cell expression *vs.* WHO clinical response (no response (n=5) and response (n=35)).

Characteristics	WHO Clinical Response		*p*- value
	No Response n (%)	Response n (%)	
Total $CD8^+$ T Cell Expression			
Low	4 (10)	4 (10)	0.003*
High	1 (2.5)	31 (77.5)	
Intratumoral $CD8^+$ T Cell Expression			
Low	5 (12.5)	2 (5)	0.000*
High	0 (0)	33 (82.5)	
Peritumoral $CD8^+$ T Cell Expression			
Low	3 (7.5)	11 (27.5)	0.222
High	2 (5)	24 (60)	

*Tested with Fisher Exact test, significant at $p<0.05$

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