RESEARCH ARTICLE

REVISED Immunoexpression of P63 and SOX2 in triple-negative breast cancers, Indonesia [version 2; referees: 2 approved]

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Abstract

Background: Using immunohistochemical stains to target specific breast cancer markers has become indispensable for evaluation of small diagnostic tissue specimens, and therefore novel marker cocktails for specific breast cancers are required. This study was conducted to assess the immunoexpression of P63 and SOX2 in triple negative breast cancer (TNBC), and to evaluate the predictive diagnostic value of these markers for specific types of TNBC.

Methods: Histological slides and paraffin blocks of TNBC cases were collected from Dr. Hasan Sadikin Hospital, Bandung, Indonesia from 5-years period (2011-2015). Each histological slide was subjected to immunohistochemical staining for P63 (nucleus and cytoplasm) and SOX2 (nucleus), with specific primer antibodies. Immunoexpression of P63 and SOX2 was evaluated using immunoreactivity scoring. Associations between P63 and SOX2 immunoexpression and TNBC types were assessed using Mann Whitney tests. In addition, the predictive diagnostic values of these markers were assessed.

Results: Forty TNBC histological slides were included, and 23 (57.5%) were Basal-like type TNBC and 17 (42.5%) were Non basal-like type TNBC. Immunoexpression of P63 nucleus and SOX2 was not different between types of TNBC. However, immunoexpression of P63 in the cytoplasm in Basal-like type TNBC was significantly higher than in Non basal-like type TNBC (p =0.021). Predictor diagnostic value analysis suggested that immunoexpression of P63 in cytoplasm had 56.5% sensitivity and 70.6% specificity for diagnosing Basal-like type TNBC, with area under curve of 0.64.

Conclusions: Immunoexpression of P63 in the cytoplasm has a relatively weak diagnostic value to discriminate Basal-like and Non basal-like types of TNBC.

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REVISED **Amendments from Version 1**

We revised the introduction focusing on TNBC epidemiology. We emphasised the introduction related to TNBC classification, characteristics and behaviour. We also updated the role of p63 in cancer within the introduction.

In the conclusion, we included more information related to the frequency of Basal and Non-basal type of TNBC and this finding related to the expression of SOX2 and P63 both in Basal and Non-basal type of TNBC.

We also included the acknowledgement section in the current version. We also revised the author list; Harapan Harapan, MD was requested to be mentioned in the acknowledgement section rather than as co-author due to current changing of his research field of interest.

See referee reports

Introduction

Breast cancer, accounting for 25% of all cancer cases and 15% of all cancer deaths among females, is the most frequently diagnosed cancer among female worldwide[1,2]. The incidence of breast cancer increased significantly, approximately by 30% in developed countries[3] and currently it has been also rising in many developing countries[2]. In Asia, 639,824 breast cancer cases and 228,926 deaths were recorded in 2012, from which 48,998 cases and 19,750 deaths occurred in Indonesia[4]. Triple-negative breast cancer (TNBC), a group of breast cancers with the absence of oestrogen receptor and progesterone receptor and no overexpression of human epidermal growth factor receptor 2 (HER2), represents 10%–20% of invasive breast cancer. A global data base, National Cancer Data Base (NCDB), reveals that TNBC was present in 13% of breast cancer patients, ranged from 23.7% in African-Americans to 8.9% in Filipino patients[5]. In Southeast Asia, a study found that TNBC presented in 10.5% among 1227 breast cancer patients[6] . In Indonesia, a study that was conducted between 2010 and 2011 in Bandung found that 11.9% of breast cancer patients were TNBC[7].

Immunohistochemically, TNBC could be divided into two subtypes: Basal-like (positive for the expression of high-molecular-weight/basal cytokeratins 5/6 (CK5/6) and epidermal growth factor receptor (EGFR)) and Non basal-like (negative for the expression of CK5/6 and EGFR). Basal-like TNBC usually has p53 mutation, EGFR overexpression, loss of function of BRCA1, c-MYC amplification, and high histological grade indicating more aggressive characteristics and aggressive behavior[8,9]. In addition, majority of Basal-like cancer cannot be managed effectively with trastuzumab and hormonal treatments[10].

Advanced screening and diagnosis methods for breast cancer such as mammograms, ultrasound, magnetic resonance imaging and fine-needle aspiration, have allowed for detection of small lesions at the early stage. Identifying breast cancer at the early stage will increase the potential for curative treatment and therefore increases the survival rate[11–14]. However, smaller lesions are more challenging to diagnose. Therefore, it is essential to use an advanced immunohistochemical approach for evaluation of smaller tissue specimens that target more specific markers[15].

A previous study using a MCF7 breast cancer cell line to produce MCF7-derived tumour xenografts found that P63 and SOX2 immunostainings were two potential markers for breast cancer[16]. P63, involved in cellular differentiation, is a homolog of tumour protein P53 and in normal breast ducts and lobules it is expressed frequently in the nuclei of myoepithelial cells[17]. Mutation of the *p53* gene results in a very high risk of breast cancer[18]. The roles of p63 in tumorigenesis, cancer progression, and metastasis are still being discovered. However, in animal model found that p63 deficiency may be a causative factor for metastatic spread[19,20]. In addition, clinical evidence suggests that a robust correlation between reduced p63 expression and cancer progression[21].

A study revealed that the total percentage of P63-positive cells was related to marked nuclear pleomorphism and the intensity of P63 staining was associated with syncytial growth pattern in TNBC[22]. In addition, data also reveals that *p63* gene expression in breast cancer could be used as a specific marker of metaplastic carcinoma[17], and P63 immunohistochemical staining could improve diagnostic accuracy of breast cancer even in small tissue specimens[23].

SOX2 is a transcription factor belonging to the SOX family and functions as an activator or suppressor of gene transcription[24,25]. Data shows that SOX2 promotes cellular proliferation of breast tissue[26] and regulates self-renewal in cancer stem cells[27]. The scientific evidence reveals that SOX2 acts as an oncogene in epithelial cancers[25] and in the breast, a study found that silencing of *sox2* gene was associated with reduction of the size of the cancer stem cells and restoration of tamoxifen sensitivity[28]. All together, these data indicate that P63 and SOX2 have pivotal role in breast cancer and therefore are potential to be used as specific biomarkers. This study was conducted to assess the immunoexpression of P63 and SOX2 in TNBC cases in order to provide insight regarding their potential diagnostic value (single or in combination) to differentiate TNBC types.

Methods

Study setting and histological slides

A cross-sectional study to assess the immunoexpression of P63 and SOX2 in TNBC cases (negative expression of estrogen and progesterone receptors and c-erbB2) was conducted. Histological slides of TNBC and their paraffin blocks, tested between the 1st of January 2011 and 31st of December 2015, were collected from the Pathology Anatomy Laboratory, Dr. Hasan Sadikin Hospital, Bandung, Indonesia. Each histological slide was examined by two certified pathologists. To classify the type of TNBC morphology, between Basal-like type TNBC and Non basal-like type TNBC, cytokeratin 5/6 (CK 5/6) immunohistochemical staining was carried out on all TNBC histological slides. Concurrently, the immunoexpression of P63 and SOX2 was measured using immunohistochemical stains with specific primer antibodies. The protocol of this study was approved by the Health Research Ethical committee of Sumatera Utara University (approval 103/KOMET/FK USU/2015) and the usage of histological specimens was approved by the Pathology Anatomy Laboratory of Dr. Hasan Sadikin Hospital (LB.02.01/B29/239/X/2015).

Immunohistochemistry

Forty archival paraffin blocks from TNBC cases were subjected to immunohistochemical staining to assess the immunoexpression of CK 5/6, P63 and SOX2. Briefly, 4 µm sections of each paraffin block were prepared using standard procedure[29]. Immunohistochemical staining was conducted using primary antibodies as follows: anti-CK5/6 monoclonal antibody (Biocare Medical, Concord, CA, USA), anti-P63 monoclonal antibody (Biocare Medical, Concord, CA, USA) and anti-SOX2 monoclonal antibody (Abcam, Cambridge, UK). Starr Trek Universal HRP Detection (Biocare Medical, Concord, CA, USA) was used as second antibody. A chromogen 3,3'-diaminobenzidine (DAB) (Biocare Medical, Concord, CA, USA) was used to develop the colour. For each experiment, appropriate controls were used.

Immunoexpression of CK 5/6 was interpreted as positive or negative, in which positive CK 5/6 indicates Basal-like type TNBC while negative CK 5/6 indicates Non basal-like type TNBC. Immunoexpression of P63 and SOX2 was evaluated using an immunoreactivity scoring system that had been published elsewhere with modification[22]. Staining intensity was scored as follows: 1 (no staining), 2 (weak staining), 3 (moderate staining) and 4 (strong staining). The percentage of positively stained tumour cells was assessed as a proportion of the total number of tumour cells present in the section as follows: 1 (<20%), 2 (≥20–50%), 3 (>50–80%) and 4 (>80%).

Immunoreactivity score was calculated by multiplying staining intensity and the percentage of positivity, and the score therefore ranged from 1 to 16. The immunoreactive score was then divided into low (≤ 5), moderate (≥ 6 – 10) and high (≥11 – 16). Immunoexpression of P63 was measured both in cytoplasm and nucleus while SOX2 immunoexpression was measured in nucleus only.

Statistical analysis

Normality of the data was assessed using the Shapiro-Wilk test and therefore the analysis tests chosen based on the normality of the data. The correlations between immunoexpression of P63 (cytoplasm and nucleus) and SOX2 were assessed using Pearson correlation and Spearman correlation, respectively. The associations of P63 and SOX2 immunoexpression and type of TNBC were assessed using Mann Whitney test. The predictive diagnostic values of P63 cytoplasm for diagnosing Basal-like type TNBC were estimated using several immunoreactivity score cut-off points. Receiver operating characteristic curve (ROC) was plotted and area under the ROC curves (AUC) was estimated. For all analyses, estimates were considered statistically significant for two-tailed values of $p<0.05$. All analyses were conducted using Statistical Package for the Social Sciences software (SPSS for Windows, Version 16, Chicago, IL).

Results

Clinicopathology and classification of TNBC

The histopathology of the TNBC samples used in this study is described in Table 1. Approximately 45% of the samples were classified as metaplastic carcinomas. In addition, immunohistochemical staining for CK 5/6 revealed that 23 (57.5%) of samples were Basal-like type TNBC and while 17 (42.5%) samples were Non basal-like type TNBC.

Immunoreactivity score of P63 and SOX2

Immunoexpression of P63 and SOX2 in samples, categorized by immunoreactivity score, are presented in Table 2. For both types of TNBC (basal and non basal-like type), all immunoreactivity scores for P63 in the nucleus were classified as low grade, while 11 (27.5%) and 7 (17.5%) samples were classified as moderate and high grade, respectively for the P63 in the cytoplasm. The immunoreactivity grade for SOX2 was similar to P63 in the cytoplasm, and therefore correlation analyses were conducted.

Correlation between immunoexpression of P63 and SOX2

There was a strong negative correlation between immunoexpression of P63 in the cytoplasm and immunoexpression of SOX2 in the nucleus in metaplastic carcinoma (a sub-type of TNBC basal-like type) (r=-0.73, p=0.013) (Table 3). In addition, linear regression showed a relatively strong correlation between P63 cytoplasm and SOX2 immunoexpression in metaplastic carcinoma (r=0.49, p=0.012). There was no significant correlation between P63 cytoplasm and SOX2 immunoexpression in Non basal-like type TNBC, and no significant correlation between P63 nucleus and SOX2 immunoexpression either in Basal-like type or Non basal-like type of TNBC.

Table 1. Histopathology types of TNBC samples used in this study (N=40).

Type of histopathology	*n* (%)
Metaplastic carcinoma, spindle cells component	10 (25.0)
Metaplastic carcinoma, producing mucin	3 (7.5)
Metaplastic carcinoma, liposarcoma component	2 (5.0)
Metaplastic carcinoma, squamous cell	1 (2.5)
Metaplastic carcinoma, matrix hyaline	1 (2.5)
Medullary carcinoma	13 (32.5)
Micropapillary carcinoma	1 (2.5)
Invasive ductal carcinoma grade 3	6 (15.0)
Invasive lobular carcinoma grade 3	2 (5.0)
Invasive ductal carcinoma + invasive lobular carcinoma (mixed)	1 (2.5)

Table 2. Immunoreactivity score of P63 and SOX2 in TNBC.

Grade	P63 cytoplasm, *n* (%)	P63 nucleus, *n* (%)	SOX2, *n* (%)
Low (≤ 5)	22 (55.0)	40 (100.0)	19 (47.5)
Moderate (≥ 6 – 10)	11 (27.5)	0 (0.0)	12 (30.0)
High (≥11 – 16)	7 (17.5)	0 (0.0)	9 (22.5)

Immunoexpression of P63 and SOX2 in Basal-like and Non basal-like type of TNBC

Immunoexpression of P63 cytoplasm, P63 nucleus and SOX2 in Basal-like and Non Basal-like TNBC is shown in Table 4. The data indicates that the immunoexpression of P63 cytoplasm in Basal-like type TNBC was significantly higher compared to Non basal-like type TNBC (p=0.021). Immunoexpression of P63 nucleus and SOX2 was not different between Basal-like and Non basal-like types of TNBC, with p-values of p=0.27 and p=0.17, respectively.

Predictor diagnostic value of P63 in the cytoplasm for diagnosing Basal like type TNBC

As mentioned above, immunoexpression of P63 in the cytoplasm was the only marker that was significantly different between TNBC types. Therefore, immunoreactivity score of P63 cytoplasm was further analysed to determine its ability to predict Basal-like type TNBC. Table 5 shows the predictive values of P63 in the cytoplasm for determining Basal-like type TNBC, using seven immunoreactivity score cut-off values from 3 to 9. It shows that P63 has relatively weak diagnostic value in diagnosing Basal-like type TNBC. The highest sensitivity was achieved at immunoreactivity score 3, while specificity was increasing with a higher immunoreactivity score.

Using the average score of P63 cytoplasm immunoexpression for Basal-like type TNBC in this study, 5.6 or 6, the sensitivity and specificity of P63 cytoplasm immunoreactivity score to predict Basal-like type TNBC was 56.5% and 72.6%, respectively with area under curve 0.64. The receiver operating curve of predictive diagnostic value of P63 cytoplasm for determining Basal-like type TNBC is plotted in Figure 1.

Dataset 1. Immunoexpression and immunoreactivity scores of P63, SOX2 and CK 5/6 in the forty specimens that were analysed

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Table 3. Correlation between immunoexpression of P63 cytoplasm and SOX2 in Basal-like type TNBC.

TNBC basal-like type	*n*	P63	SOX2	r	*p*
		Mean (±SD)	Mean (±SD)		
Invasive ductal carcinoma	2	5.50 (3,54)	5.00 (5.66)	-	-
Invasive ductal carcinoma and invasive lobular carcinoma	1	16.00 (0.00)	9.00 (0.00)	-	-
Invasive lobular carcinoma	1	8.00 (0.00)	1.00 (0.00)	-	-
Invasive micropapillary carcinoma	1	12.00 (0.00)	16.00 (0.00)	-	-
Medullary carcinoma	6	5.50 (4.76)	8.00 (5.48)	0.64	0.172
Metaplastic carcinoma	12	6.67 (4.68)	6.00 (3.77)	-0.73	0.013*

*Significant at 0.05

Table 4. Immunoexpression of P63 and SOX2 in Basal-like type TNBC and Non basal-like type TNBC.

Marker	TNBC type	*n*	Immunoreactivity score Mean (±SD)	*p*
P63 cytoplasm	Basal-like	23	6.96 (4,73)	0.021*
	Non basal-like	17	3.76 (4,16)	
P63 nucleus	Basal-like	23	1.22 (0,52)	0.273
	Non basal-like	17	1.06 (0,24)	
SOX2	Basal-like	23	6.78 (4,69)	0.172
	Non basal-like	17	4.82 (3,61)	

*Significant at 0.05

Table 5. Predictor diagnostic value of P63 in the cytoplasm for diagnosing Basal-like type TNBC.

Diagnostic test	Cut-off of P63 cytoplasm immunoreactivity score						
	3	4	5	6	7	8	9
Sensitivity (%)	78.3	73.9	56.5	56.5	52.2	52.2	30.4
Specificity (%)	58.8	58.8	70.6	70.6	82.4	82.4	88.2
Positive predictive value (%)	72.0	70.8	72.2	72.2	80.0	80.0	77.8
Negative predictive value (%)	66.7	62.5	54.5	54.5	56.0	56.0	48.4
Positive likelihood ratio (%)	190.1	179.5	192.2	192.2	295.7	295.7	258.7
Negative likelihood ratio (%)	37.0	44.3	61.6	61.6	58.1	58.1	78.8
Accuracy (%)	70.0	67.5	62.5	62.5	65.0	65.0	55.0

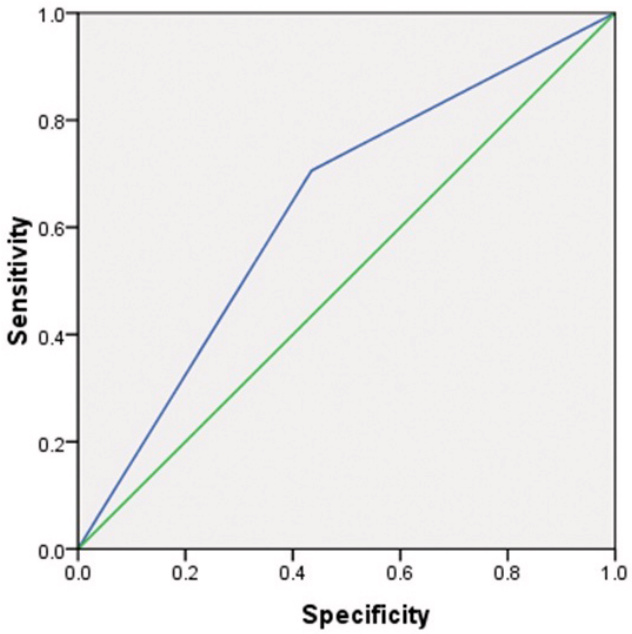

Figure 1. Receiver operating curve of P63 cytoplasm immunoexpression for determining TNBC basal-like type.

Discussion

To the best of our knowledge, this is the first study conducted to assess the immunoexpression of P63 and SOX2 in TNBC cases in Indonesia. Some studies have been conducted to assess the predictive values of P63 as specific marker for breast cancer[17,30]. In addition, the idea of utilization of a cocktail of specific markers has been proposed previously to provide higher sensitivity and specificity for diagnosing specific breast cancers[15,22,31]. However, none of the previous studies had been conducted to assess the diagnostic value of immunoexpression of P63 and SOX2 in combination. This study, at the beginning, sought to assess predictive value of combination both of those markers for specific type of TNBC. However, we found that there was no difference in immunoexpression of SOX2 between Basal-like type TNBC and Non basal-like type TNBC. Nevertheless, we found that immunoexpression of P63 cytoplasm, but not P63 nucleus, was higher in Basal-like type TNBC compared to Non basal-like type TNBC.

P63 has been proposed as a breast cancer marker for a long time, but with conflicting results. A study demonstrated that immunoexpression of P63 was associated with breast cancers, for example

the metaplastic carcinoma type of breast cancer[17], but there was no difference in immunoexpression of P63 between medullary breast carcinomas and atypical medullary breast carcinomas of TNBC[30]. In our study, we found that the sensitivity and specificity of P63 cytoplasm immunoexpression to diagnose Basal-like type TNBC was 56.5% and 72.6%, respectively, with area under curve of 0.64. This sensitivity and specificity seems higher compared to a previous study, with 14% and 94%, respectively in determining a Basal-like type in infiltrative ductal carcinomas (TNBC)[22]. All together, these data indicate a weak predictive value of P63 immunoexpression as marker for Basal-like type TNBC. However, a study found that P63 is a specific marker for metaplastic carcinomas of the breast (a sub-type of Basal-like type TNBC)[17]. In our study, we could not assess the predictive value of P63 cytoplasm immunoexpression for determining metaplastic carcinomas, due to the small sample size (see Table 3).

We found that SOX2 immunoexpression grade was classified as moderate and high grade in 55% of TNBC cases (Table 2,) and it has been indicated previously that SOX2 has strong roles in promoting breast cancers[26–28,32]. However, there was no different in immunoexpression between Basal-like type TNBC and Non basal-like type TNBC. This result indicates that SOX2 expression is not different amongst TNBC types. This finding was in line with a previous study that indicated that SOX2 was expressed across different breast cancer subtypes[33]. A study found that SOX2 antibody in the sera was is higher in patients with breast cancer compared to healthy women and therefore it could be used to discriminate between breast cancer patients and healthy controls[34]. In addition, a meta-analysis found that SOX2 expression was associated with tumor size, histological grade, the aggressiveness and lymph node metastasis in TNBC patients[35]. All together, these results indicate that there was a possibility SOX2 expression could be used for diagnosing breast cancers, but there was no difference in expression amongst breast cancer types, and therefore it could not be used as specific marker for differentiating TNBC types.

There are some limitations to this study. The sample size was relatively small, and therefore some analyses could not be conducted. In addition, the diagnostic specimens were collected from different procedures such as from biopsy, mastectomy or lumpectomy, and this might influence the immunoexpression of the markers.

Conclusions

Immunoexpression of P63 cytoplasm is higher among Basal-like type TNBC compared to Non basal-like type TNBC. However, the predictive diagnostic value of P63 immunoexpression in the cytoplasm for Basal-like type TNBC is relatively low, with 56.5% sensitivity and 72.6% specificity.

Data availability

Dataset 1: Immunoexpression and immunoreactivity scores of P63, SOX2 and CK 5/6 in the forty specimens that were analysed. DOI, 10.5256/f1000research.12671.d179131[36]

Competing interests

No competing interests were disclosed.

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Data Source

Open Peer Review

Current Referee Status: ✔ ✔

Version 2

Referee Report 20 February 2018

doi:10.5256/f1000research.14420.r29568

✔ **Diah Rini Handjari**
University of Indonesia, Jakarta, Indonesia

This revised version is good and sufficient for indexing.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 06 February 2018

doi:10.5256/f1000research.14420.r29569

✔ **Irianiwati Widodo**
Department of Anatomical Pathology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

This revised version is good and already sufficient for publication.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 20 October 2017

doi:10.5256/f1000research.13722.r26782

? **Diah Rini Handjari**
University of Indonesia, Jakarta, Indonesia

I. - In introduction there is only little information regarding of nuclear expression of p63.
- It doesn't mention about cytoplasmic p63 expression.
What is significance function of p63 in tumor progression or disease progression?
It written in result and analysis.
- In introduction you haven't discussed yet about the incidence of triple negative breast cancer in the worldwide, Asia or Indonesia ?

II. In the methods, to classify TNBC morphology into Basal like and non Basal like, based on only, antibody (CK 5/6) but you didn't perform EGFR staining. Which is one of the marker of TNBC.
- Is there any reference about the scoring of intensity and the percentage of positivity of expression of p63?
- Is there any reference about imunoreactive score?

III. In discussion, there is also no information about cytoplasmic p63 and its role in disease progression.
- Researcher only stated that cytoplasmic p63 expression has predictive value to classify breast cancer into TNBC and there is no comparison of p63 expression sensitivity and specificity with other TNBC marker as like CK 5/6 and EGFR which has been routinely used.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Pathologist with major interest in colorectal cancer

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Nov 2017

Harapan Harapan, Syiah Kuala University, Indonesia

Thank you for comments and suggestion. We have deleted some sentences related to the general introduction of breast cancer and added specific information related to TNBC. We would like to confirm that predictive diagnostic analysis in this study was conducted using qualified statistician. There is no evidence to support the importance of the negative correlation between immunoexpression of P63 cytoplasm and immunoexpression SOX2 nucleus in metaplastic carcinoma, and therefore we are unable to discuss this finding in depth. In conclusion section, we have added some additional principal finding as suggested by the reviewer.

Competing Interests: There is no competing interest in this study.

Author Response 25 Nov 2017

Harapan Harapan, Syiah Kuala University, Indonesia

Thank you for comments and suggestion. We have deleted some sentences related to the general introduction of breast cancer and added specific information related to TNBC. In the revised manuscript, the data of the incidence from global, Southeast Asian countries and Indonesia are included.

In our study, we used the histological paraffin blocks that have been confirmed as TNBC previously by testing ER, PR and HER2. To differentiate Basal like and non Basal like, either CK 5/6 or EGFR staining could be used with no significant different sensitivity and specificity (Livasy *et al.*, 2006; Nielsen *et al.*, 2004). In this study, CK 5/6 was employed to differentiate TNB into Basal like and non Basal like and this procedure was conducted during the study. This method is established method to define basal phenotype (Sasa *et al.*, 2008; Rakha *et al.*, 2007).

In our study, we used the histological paraffin blocks that have been confirmed as TNBC previously by testing ER, PR and HER2. To differentiate Basal like and non Basal like, either CK 5/6 or EGFR staining could be used with no significant different sensitivity and specificity (Livasy *et al.*, 2006; Nielsen *et al.*, 2004). In this study, CK 5/6 was employed to differentiate TNB into Basal like and non Basal like and this procedure was conducted during the study. This method is established method to define basal phenotype (Sasa *et al.*, 2008; Rakha *et al.*, 2007). CK 5/6 and EGFR.

As mentioned Immunohistochemistry Section, the principle of the scoring system for immunoexpression of P63 and SOX2 used in this study was have been elsewhere with modification (Thike *et al.*, 2010). In detailed: Staining intensity was scored as follows: 1 (no staining), 2 (weak staining), 3 (moderate staining) and 4 (strong staining). The percentage of positively stained tumour cells was assessed as a proportion of the total number of tumour cells present in the section as follows: 1 (<20%), 2 (≥20–50%), 3 (>50–80%) and 4 (>80%). Then from staining intensity and percentage of positively stained cells, we created immunoreactivity score by multiplying staining intensity and the percentage of positivity. The score, therefore, ranged from 1 to 16 (divided into low (≤ 5), moderate (≥ 6 – 10) and high (≥11 – 16). However, specifications for staining intensity and the percentage of positivity used in this study are the standard system used by Pathology Anatomy Laboratory of Dr. Hasan Sadikin Hospital Bandung since 1990. These score systems are also adopted in some breast cancer diagnostic centres in Indonesia.

Immunoreactive score system used in this study have been published elsewhere (Thike *et al.*, 2010). We mentioned this in Immunohistochemistry Section of our Methods.
In this study produce all raw data related to: type of TNBC (Basal like and Non Basal-like), subtype of cancer, expression of SOX2, P63 and CK 5/6 including staining intensity, distribution of the

positively cells and immunoreactive score.

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Competing Interests: There is no competing of interest

Referee Report 12 October 2017

doi:10.5256/f1000research.13722.r26921

Irianiwati Widodo
Department of Anatomical Pathology, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Introduction:

- Do not explain the epidemiology of breast cancer too long, but explain more detail about TNBC and its subtypes (Basal-like and non basal-like), such as differences in histological type, behaviour, prognosis, treatment etc. So, the role of p63 and SOX2 on TNBC becomes clearer.

Method:

- Statistical analysis predictive diagnostic value of cytoplasmic p63 expression of basal-like and non basal-like TNBC, as far as I know there should be also negative p63 expression. Therefore I suggest to ask a qualified statistician.

Discussion:

- Explain the importance of negative correlation between p63 expression and metaplastic carcinoma. Compare with previous studies.

Conclusion:

Mention also other important results of this study such as:

- The frequency of non basal-like is higher than basal-like.
- The most common histological type of TNBC is metaplastic carcinoma.
- Negative correlation between p63 expression and metaplastic carcinoma
- SOX2 expression in TNBC, even there is no stattistically significant

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: I am a pathologist with major interest in breast cancer

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Nov 2017

Harapan Harapan, Syiah Kuala University, Indonesia

Thank you for comments and suggestion. We have deleted some sentences related to the general introduction of breast cancer and added specific information related to TNBC. We would like to confirm that predictive diagnostic analysis in this study was conducted using qualified statistician. There is no evidence to support the importance of the negative correlation between immunoexpression of P63 cytoplasm and immunoexpression SOX2 nucleus in metaplastic carcinoma, and therefore we are unable to discuss this finding in depth. In conclusion section, we have added some additional principal finding as suggested by the reviewer.

Competing Interests: No competing interests were disclosed.