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# Dimensional Analysis of $CD44^{High}$ $CD24^{Low}$ and Ki67 in Triple Negative Breast Cancer

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**Abstract**

**AIM:** To study the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 in triple negative breast cancer (TNBC).

**METHODS:** This cross-sectional study was performed on patients with breast cancer in Haji Adam Malik Hospital Medan from 2013 to 2016 to determine the frequency and pathologic features of TNBC by immunohistochemistry stained.

**RESULTS:** By using immunohistochemistry staining panel of CD44, CD24, Twist, Claudin 7, CK5, CK8/18, EMA, E-Cadherin, Ki-67, a total 67 breast tumour samples with TNBC were classified as 9 stem-cells like 1 basal, 22 baso-luminal, and 23 luminal subtypes

**CONCLUSION:** By using immunohistochemical staining panel, TNBC can be differentiated into stem cells like basal, baso-luminal and luminal subtypes. Didifferentiation and EMT can produce heterogeneity in TNBC subtypes and this will affect in handling TNBC. Stemness in stem cells- like subtypes are resistant to therapy. Therefore, TNBC needs special attention in order to assist in more optimal handling.

## Introduction

Breast cancer is thought to derive from the stem or progenitor cells having abnormalities in the self-renewal process [1]. Mammary stem cells (MaSCs) play an important role in the growth and development of breast cancer, resistance to therapy, and metastasis [2]. Various stem cell markers are used to identify and isolate CSC from various solid tumours, such as CD44 and CD24 [3]. CD24 is a little more expressed in progenitor cells compared to differentiated cells [4]. Therefore, for therapy to be effective, CSC must be recognised and must be differentiated from normal breast stem cells.

The increasing level of Ki-67 shows aggressiveness of tumour growth and indicates a poor prognosis. Triple-negative breast cancer (TNBC) is also correlated with high Ki-67 level [5]. Hence, we were interested in studying about the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 TNBC.

We aimed to study the dimensional analysis $CD44^{high}$ $CD24^{low}$ and Ki67 in triple negative breast cancer.

## Material and Method

This descriptive study with the cross-sectional design was conducted from March to October 2017 and was carried out after getting permission from the Ethical Committee of Medical Faculty USU Medan.

The population were patients diagnosed as breast cancer based on histopathology (mastectomy/biopsy) at RSUP Haji Adam

Malik/Departement of Anatomical Pathology Medical Faculty of USU Medan. TNBC tumours were further stained with CD44 (DF1485, Novocastra Laboratories Ltd., dilution 1:100), CD24 (C-20, Santa Cruz Biotechnology, dilution 1:100), TWIST-1 (H-81, Santa Cruz Biotechnology, dilution 1:100), CK5 (XM26, Novocastra-Vision Biosystems, dilution 1 : 25), CK8/18 (5D3, Lab Vision, dilution 1:300), Claudin-7 (NBPI-35677, Rabbit polyclonal antibody, Novus Biological, dilution 1:100), E-Cadherin (NCH-38; M3612, monoclonal primary antibody, DakoCytomation, Denmark, dilution 1:50), EMA (E29, monoclonal antibody, DAKO, dilution 1:400), and Ki-67 (clone SP6, biomarkers, dilution 1:100).

CD44, CD24 and Twist were stained in membrane cells, with score 0 if < 10% positive tumour cells; 1 if 10-25%; 2 if 25-50%; and 3 if > 50%. Intensity was scored as 0 if unstained, 1 if weakly stained, 2 if intermediate, and 3 if strong. Interpretation of CD44, CD24, and Twist staining was determined based on multiplication of the percentage of positive cells and the intensity of staining. CD44 and CD24, were scored as 0 if (-), 1-3 (+1), 4-6 (+2), and 7-9 (+3) [6]. While Twist was considered weak if total score < 6 and strong ≥ 6 [7]. Claudin-7 staining was scored as 0 if no membranous staining; 1+ (1-10% tumour cells); 2+ (10-30%); and 3+ (> 30%) [6].

For E-cadherin staining, the interpretation of staining is divided into 0 if (-); +1 if weakly and heterogenous stained; +2 if weakly but homogenous stained; +3 if moderately stained, or if strongly but heterogenous stained; and +4 if strongly and homogenous stained. Percentage of tumour cells were scored as 0 if (-); 1 if < 10% membranous stained; 2 if 10-50%; and 3 if > 50%. Interpretation of E-cadherin was determined based on multiplication of percentage of positive cells and intensity of staining, which is negative (scored as 0); weakly stained (total score 1-4); moderately stained (5-8); strongly stained (9-12) [8]. CK5 and CK8/18 were stained in the cytoplasm and positive if ≥ 10% tumour cells. EMA was stained positive in membranous/ cytoplasm cells.

Intensity of staining was scored as 0 if < 25%, 1 if 26-50%, and 2 if 51-100%. Score 0 and 1 was considered low and scored 2 as high [9]. For Ki-67, 300 cells were counted (include proliferating and non-proliferating cells), and the percentage of proliferation were counted with cut-off point 10% positively nuclear cells [10]. After that, molecular classification of TNBC was done and classified as Claudin low (stem cell-like) subtypes if $CD44^{+}$ $CD24^{-}$, Claudin-low, Twist-$1^{high}$; basal-like subtypes if $CK5^{+}$ and $EMA^{+}$, and luminal subtypes if $CK8/18^{+}$ and $E\text{-cadherin}^{+}$.

The results of this study were processed using statistical software and displayed in frequency distribution in tables.

## Results

To determine the ontogeny and differentiation of TNBC subtypes in stem cells stages, we used CD44 and CD24 immunohistochemical stains. The classification of stem cells-like type into SC-1 to SC-3 were arbitrary (Figure 1).

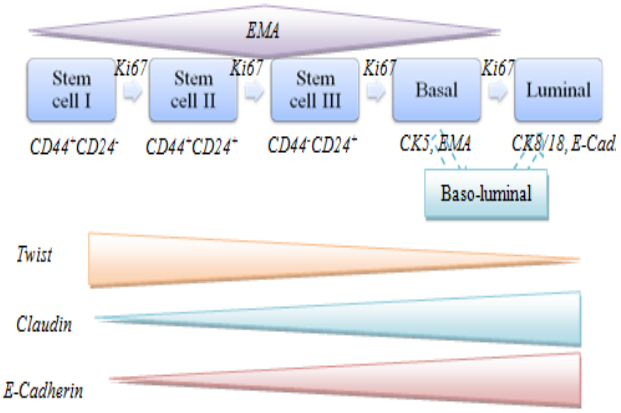


*Figure 1: Schema of ontogeny differentiation breast epithelial from stem cells to luminal cells associated with a panel of various molecular markers for TNBC*

From 67 TNBCs in this study, there were 10 cases of $CD44^{+}CD24^{-}$, 36 cases of $CD44^{-}CD24^{+}$, 9 cases of $CD44^{+}CD24^{+}$, and 12 cases of $CD44^{-}CD24^{-}$. With Twist, Claudin 7, Cytokeratin 5 dan 8/18 (CK5 dan CK8/18), EMA, E-Cadherin, and Ki67 immunohistochemical stain panel, TNBC was classified as 9 cases of stem-cell-like, 1 basal, 22 baso-luminal and 23 luminal subtypes. After classified, Tumours would then be categorised as low and high proliferation based on Ki67 staining. Both low and high Ki67 were more commonly found in TNBC with $CD44^{-}CD24^{+}$ (both 18 cases, 50%).

## Discussion

MaSCs are marked with high CD44 and negative/low CD24 ($CD44^{+}CD24^{-/low}$) adhesion molecule expressions. $CD44^{+}CD24^{-/low}$ phenotype is often related to poor prognosis [11]. In this study, CD44, CD24, Claudin-7 and Twist-1 were used as molecular markers of TNBC stem cell-like subtypes; CK5, EMA for basal-like sub-types; and CK8/18 for luminal sub-types. Results from 67 TNBC cases showed marked heterogeneous and overlapping profiles.

After immunohistochemistry staining of 67 TNBC cases was seen, we concluded that the clinical application of dividing stem cell-like subtypes into SC-1, SC-2, and SC-3 would not be useful. The importance of this study was the identification of

stemness which will influence therapy. The classification of SC-1 to SC-3 can only help to facilitate understanding these complicated problems of ontogeny. In this study, $CD44^{-}CD24^{+}$ groups were very heterogeneous.

In conclusion, by using immunohistochemical staining panels, TNBC can be classified into stem cell-like basal, baso-luminal, and luminal subtypes. Differentiation signs (EMT) in basal, baso-luminal and luminal subtypes can be recognised with CD44, CD24 and Twist. Differentiation and EMT can cause heterogeneity in TNBC subtypes, and this influence in TNBC therapy. Stemness behaviour in stem cell-like subtypes is resistant to therapy. Besides that, Ki-67 expression shows the aggressiveness of tumours. In controlling the aggressiveness of tumours, effective medicines must be used to manage the cell cycle. Therefore, TNBC needs special attention to assist in more optimal handling.

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