sciendo

10.2478/AMB-2024-0070

ORIGINAL ARTICLE

SERUM LEVELS OF SEMAPHORIN 4C IN BREAST CANCER – ASSESSMENT OF PROGNOSTIC VALUE AND POTENTIAL FOR MONITORING SURGICAL TREATMENT RESPONSE

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Abstract. Introduction. *The global burden of breast cancer (BC) has encouraged ceaseless research in exploring novel biomarkers, aiming to optimize BC management and prognosis.* ***Objective.*** *To explore the prognostic value of serum SEMA4C and investigate its potential for monitoring the response to surgical treatment in patients with BC.* ***Materials and methods.*** *Seventy-five (75) pre-treatment patients from the Clinic of Surgery with invasive BC without any initial treatment prior to blood sample collection were included in the study. The enzyme-linked immunosorbent assay (ELISA) method was used to measure serum levels of SEMA4C in human serum. Participants were divided based on pathological stage, nodal involvement and histological grade. Follow-up blood samples of 35 patients who underwent surgery were collected to investigate if SEMA4C could measure the response to surgical treatment.* ***Results.*** *Postoperative serum levels of SEMA4C were significantly lower than preoperative levels (p p < 0.001). Regarding prognostic value, no statistically significant difference was observed in terms of the pathological stage (p = 0.181), lymph node (LN) status (p = 0.752), and histological grade (p = 0.412).* ***Conclusion.*** *According to our study, serum SEMA4C levels did not differ significantly in terms of pathological stage, LN status and histological grade. Notably, postoperative serum levels of SEMA4C were significantly decreased after surgical treatment compared to preoperative values, which underscores the potential of SEMA4C as a putative candidate biomarker for monitoring response to therapy in patients with BC. However, additional research is mandatory to validate the role of SEMA4C in BC.*

Key words: *breast cancer, serum biomarker, semaphorin 4c, surgical treatment, monitoring*

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Received: *19 June 2024;* ***Accepted:*** *27 June 2024*

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INTRODUCTION

Breast cancer (BC) is one of the most frequently diagnosed malignancies among females and a leading cause of cancer-related mortality globally with 2.3 million new cases and 0.66 million deaths in 2022 [1]. Bulgaria experiences very high incidence rates of BC with over 3500 new cases diagnosed annually [2, 3]. The burden of BC and advances in molecular biology have encouraged ceaseless intensive research in exploring and identifying novel biomarkers, ultimately aiming to improve BC diagnosis, prognosis and treatment.

Semaphorins (SEMAs) comprise a more than 20-member phylogenetically conserved extracellular signaling protein family involved in diverse cellular and metabolic processes. Initially identified as axon guidance molecules during neuronal development, accumulating evidence suggests their essential regulatory functions in the development, homeostasis and cancer progression of many organ systems [4-6]. Semaphorin 4C (SEMA4C), a pivotal transmembrane protein of the fourth class of SEMAs, has been gaining attention for its potential role in BC. Compelling research data indicates that it is overexpressed in the tumor microenvironment (TME) and exhibits tumor progressive properties, accelerating cancer cell proliferation and metastasis [7, 8]. Moreover, besides expressing SEMA4C, BC-associated lymphatic endothelial cells (LECs) have been found to release soluble SEMA4C, reported to be increased in the serum of patients with BC [9-11].

The present study aims to evaluate the prognostic value of serum SEMA4C and investigate its potential for monitoring the response to surgical treatment in patients with BC.

MATERIALS AND METHODS

Patients and study design

The present study includes 75 pre-treatment patients from the Clinic of Surgery with histologically verified invasive BC without any initial treatment prior to blood sample collection. Individuals with concurrent malignancy, previous chemo- and/or radiotherapy, and severe comorbidities (e.g. autoimmune, renal, and liver diseases) were excluded from the study. We explored the prognostic value of SEMA4C based on stage, lymph node (LN) status and histologic grade. Participants were classified into two categories depending on pathological stage – early (Stages IA, IB and IIA) and advanced (Stages IIB, IIIA, IIIB, IIIC, IV). To further investigate the prognostic value of SEMA4C we compared serum levels in terms of lymph node (LN) status and histological grade. In addition, we collected follow-up serum samples of 35 patients between the 2nd and 4th postoperative day (POD) to assess the response to surgical treatment. The study design is presented in Figure 1. The distribution based on molecular subtype is as follows: Luminal A (n = 25), Luminal B Human epidermal growth factor receptor 2 (HER2) negative (n = 38), Luminal B, HER2 positive (n = 3), HER2 overex-

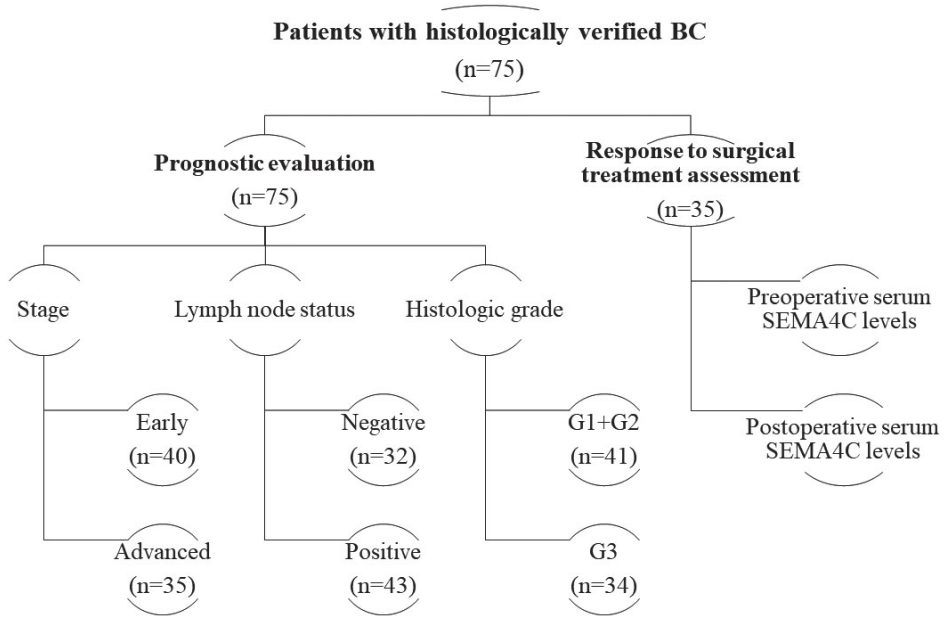

Fig. 1. Study design. Abbreviations: G1 – low grade (well-differentiated). G2 – intermediate grade (moderately differentiated). G3 – high grade (poorly differentiated)

pressed (n = 4) and Triple-negative breast cancer (TNBC) (n = 5). We hypothesized that the observed irregularity in the distribution and the low number of patients with HER2 positive and TNBC would have limited statistical significance, thereby we did not conduct analysis regarding molecular subtype.

Data and sample collection

Clinical characteristics of eligible participants were gathered after signed informed consent was obtained. Data for pathological diagnosis, stage, tumor grade and lymph node status were gathered. Up to 5 ml of venous blood was collected into a vacuum tube from each individual followed by centrifugation at 2600 rpm for 10-12 minutes. The serum samples were stored for up to two weeks at –20◦C in the Clinic of Surgery until transportation to the Clinic of Clinical Immunology and Stem Cell Bank where they were preserved at –80◦C.

Measurement of serum SEMA4c levels

The Enzyme-Linked Immunosorbent Assay (ELISA) method was used to measure serum levels of SEMA4C in human serum quantitatively with Human SEMA4C SimpleStep ELISA® Kit (ab284623). The kit has a sensitivity of 23.5 pg/ml and a range between 93.75 pg/ml and 6000 pg/ml.

Statistical analysis

IBM® Statistical Package for the Social Sciences (SPSS) (Version 25) was used for statistical analysis.

RESULTS

Prognostic value of SEMA4C

Stage

To investigate whether SEMA4C could contribute as a prognostic biomarker in patients with invasive BC, we compared serum levels of SEMA4C between early-stage and advanced cases. The frequency distribution based on the pathological stage is depicted in Figures 2 and 3.

We conducted a non-parametric Mann-Whitney test. While the mean values for advanced cases (650.057 pg/ml) appear higher than those for early-stage cases (445.480 pg/ml), the median values are notably similar (Table 1; Figure 3). The difference between the two variables was not statistically significant (p = 0.181).

Lymph node status

We further classified the study population according to the presence of pathologically confirmed LN metastases to investigate whether serum levels of SEMA4C predict nodal involvement in BC (Figure 4).

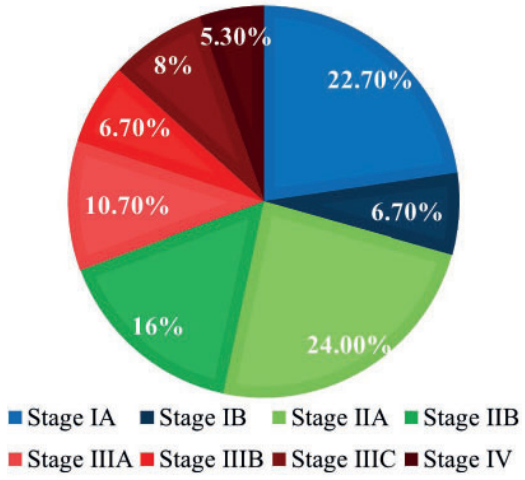

Fig. 2. Distribution of all stages in the study population

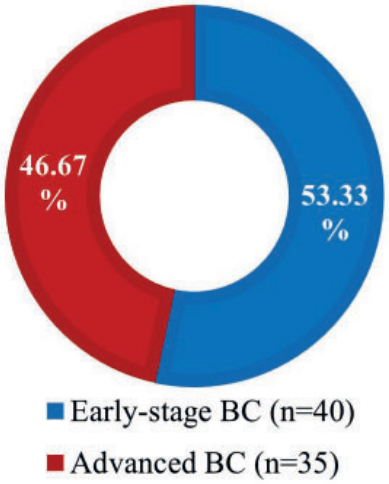

Fig. 3. Frequency of early and advanced BC

Table 1. Descriptive statistical data for early and advanced BC cases

	n	Mean	Min	Max	Median	Standard deviation (SD)
Early BC	40	445.480	86.3	1756.8	336.7	373.957
Advanced BC	35	650.057	137.9	3689	367.8	810.199
Total	75	540.949	86.3	3689	343.0	621.175

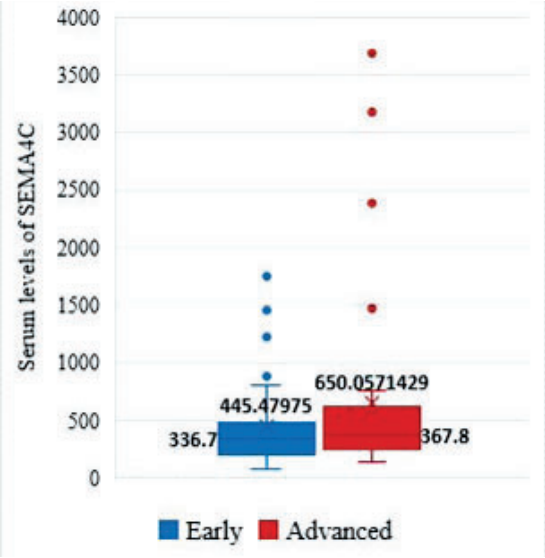

Fig. 4. Boxplot depicting the mean (above) and median values of early and advanced cases

Statistical analysis (Mann-Whitney test) disclosed no statistically significant difference between serum levels of SEMA4C in patients with LN dissemination and those with negative status (p = 0.752) (Mann-Whitney test) (Table 2; Figure 5).

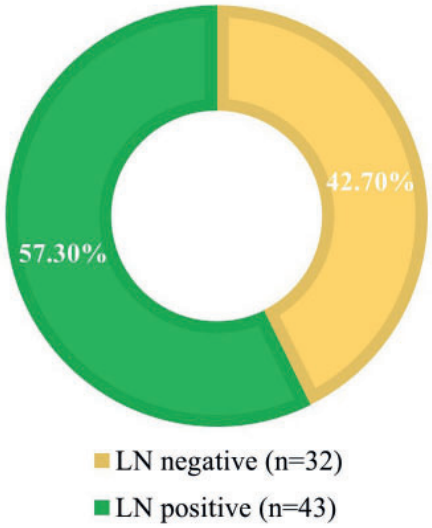

Fig. 5. Lymph node status distribution among the study population

Table 2. Descriptive statistical data of LN status groups

Lymph node status	n	Mean	Min	Max	Median	SD
Negative	32	498.653	139	1756.8	368.5	397.617
Positive	43	572.425	86.3	3689	340.7	748.848
Total	75	540.949	86.3	3689	343.0	621.175

Grade

Based on the histological grade, we divided the study population into two categories (Figure 6). No statistically significant difference was found between patients with low (G1) and intermediate grades (G2) and patients with high-grade (G3) invasive BC (p = 0.412) (Mann-Whitney test) (Table 3; Figure 7).

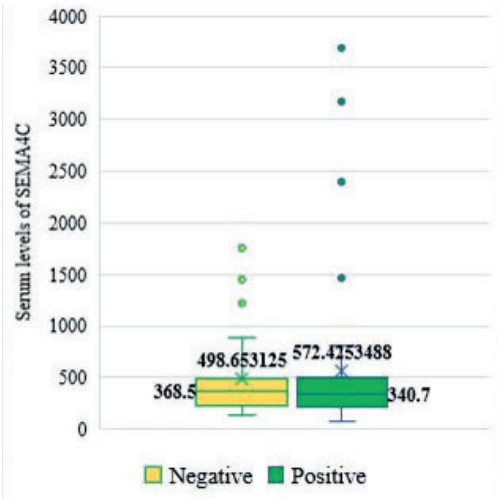

Fig. 6. Boxplot illustrating the mean (above) and median values of SEMA4C according to LN status

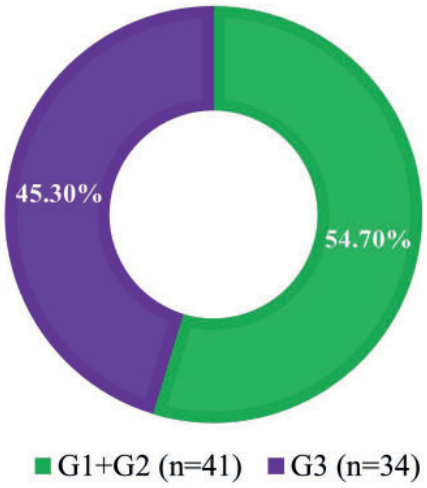

Fig. 7. Distribution of the study population in terms of histological grade

Table 3. Statistical data in terms of histological grade

Histological grade	n	Mean	Min	Max	Median	SD
G1+G2	41	494.155	86.3	2389	343.0	397.617
G3	34	597.376	104	3689	383.7	748.848
Total	75	540.949	86.3	3689	343.0	621.175

Response to surgical treatment assessment

To explore the potential of SEMA4C levels in measuring the response to surgical treatment in patients with invasive BC, we compared serum SEMA4C levels before surgery with those after modified radical mastectomy, quadrantectomy and subcutaneous mastectomy with axillary lymph node dissection. In all patients negative margins (R0) were achieved, confirmed by pathological examination. Statistical analysis (Mann-Whitney test) revealed that postoperative serum levels of SEMA4C were significantly lower than the pre-treatment levels ($p < 0.001$) (Table 4; Figure 8). In addition, the median test showed a statistically significant difference between the two variables ($p = 0.017$).

DISCUSSION

BC is a highly heterogeneous disease in regard to etiology and pathological characteristics. Treatment concepts continue to evolve, focusing on minimally invasive, more precise and effective diagnostic and therapeutic strategies. Contemporary medicine is increasingly adopting the personalized multidisciplinary approach to BC management. Exploring and identifying novel biomarkers is crucial for elucidating the molecular basis of tumor progression and treatment responses, leading to optimized therapy, and reducing the risk of overtreatment, undertreatment, and incorrect treatment [12].

SEMAs are expressed in all tissues and execute crucial regulatory functions in immune and organ homeostasis, as well as in organ-specific disease development, including cancer [13, 14]. The common structural feature of all SEMAs is a single, cysteine-rich, ~500-amino-acid extracellular “sema” domain, essential for receptor binding. The semaphorin family comprises secreted, transmembrane and cell surface-attached molecules subdivided into eight

Table 4. Descriptive statistical data of pre- and postoperative levels of SEMA4C

	n	Mean	Min	Max	Median	SD
Preoperative levels	35	527.591	86.30	2389.00	340.4	497.314
Postoperative levels	35	216.288	59.15	683.00	201.7	132.699
Total	70	371.939	59.15	2389.00	256.0	393.857

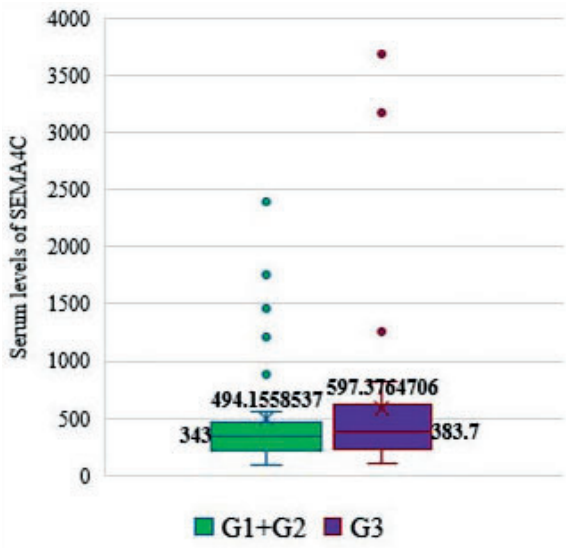

Fig. 8. Boxplot comparison according to histological grade

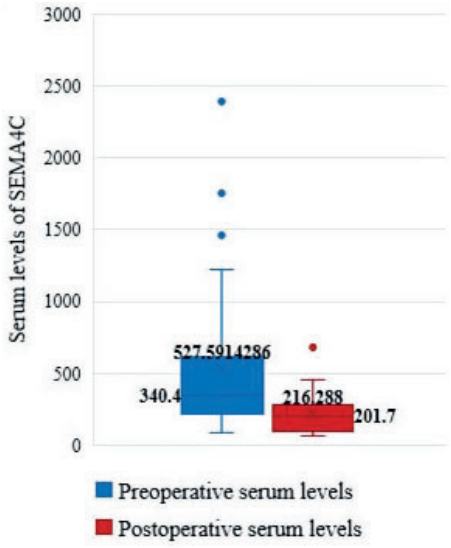

Fig. 9. Comparison between preoperative and postoperative serum SEMA4C levels

classes based on structural similarities. Only classes 3-7 are expressed in humans. Signal transduction is mainly mediated by the plexin and neuropilin receptor family [4, 6, 15]. Literature data suggests that SEMAs and their receptors are extensively expressed in tumor and immune cells. They perform multifaceted dualistic activities in the tumor microenvironment (TME) of different malignancies as stimulators or inhibitors of tumor growth. Moreover, they exhibit regulatory functions in numerous hallmarks of cancer, including proliferation, angiogenesis, metastasis, immune evasion, and tumor-associated inflammation [7, 15-18]. Current literature data has revealed that while some members have tumor proliferative properties in BC (SEMA3C, SEMA3E, SEMA4A, SEMA4C, SEMA4D, SEMA7A), several additional members are discussed to exhibit tumor-suppressive character, such as SEMA3A, SEMA3B and SEMA3F [4, 5, 17, 19]. This underscores their unique expression profiles and context-dependent activities in the complex molecular landscape of BC.

Accumulating research evidence has displayed numerous applications of different SEMAs in clinical medicine. Yang Q et al. highlighted the essential role of microRNAs (miRNA) in chemotherapy-induced epithelial-mesenchymal transition (EMT) in BC. Their findings revealed significant downregulation of miR-125b in paclitaxel-resistant (PR) cells, while ectopic expression of miR-125b reversed the EMT phenotype in PR cells. They have discovered that miR-125b regulated PR-mediated EMT partly by targeting Sema4C, thus concluding that overexpression of miR-125b or depletion of Sema4C sensitized PR cells to paclitaxel [20]. Besides SEMA4C, SEMA7A is also discussed as a prognostic biomarker and therapeutic target in patients with postpartum BC [21]. SEMA4A and SEMA4D are reported to potentiate tumor progression in BC [4, 18, 22]. Given the fact that SEMA4A has been identified as a major regulator in TNBC progression, Paranthaman and Veerappapillai have presented a design of a peptide-based vaccine, intending to stimulate a significant humoral and cellular immune response, targeting SEMA4A in TNBC [23]. The combination of Pepinemab, a humanized anti-SEMA4D antibody observed to stimulate immune cell infiltration into the TME, with immune checkpoint inhibitor Avelumab, is currently being investigated in a phase Ib/II clinical trial in patients with immunotherapy-resistant and PD-L1-negative/low non-small cell lung cancer (NSCLC) [24]. In summary, SEMAs have shown remarkable abilities in different aspects of cancer management, including as diagnostic and prognostic biomarkers, and molecular therapeutic targets. Ongoing research continues to uncover new mechanisms and applications of semaphorins in clinical medicine.

The transmembrane SEMA4C has been reported to be overexpressed in a multitude of malignancies: breast, cervical, ovarian, colorectal, esophageal, gastric, and lung cancers [15, 22, 25]. Increased levels of SEMA4C in BC are found to be associated with tumor growth, metastasis, poor prognosis, and resistance to endocrine therapy and chemotherapy [4, 5, 7, 10]. A preclinical study by Yang J et al. has demonstrated that SEMA4C with its corresponding receptor plexin-B2 promotes cancer cell proliferation, macrophage recruitment, and angiogenesis, thus reshaping the TME in BC [8, 18]. Furthermore, SEMA4C has been identified as the most upregulated gene in tumor-associated LECs in BC, stimulating lymphangiogenesis through the activation of PlexinB2-ERBB2 signaling cascades. This promotes the proliferation and migration of tumor cells, thereby accelerating lymphatic dissemination [9]. Based on experimental evidence, Gurrapu et al. have provided evidence that blockade of SEMA4C/PlexinB2 signaling results in cell cycle inhibition in the G2/M phase, growth arrest, cell senescence and upregulation of tumor-suppressor genes [7].

The expanding exploration of SEMA4C has opened up new horizons for future research on developing novel diagnostic and therapeutic approaches. A multicenter retrospective study by Wang Y et al. encompassing 6213 individuals has explored the diagnostic value of SEMA4C in BC. No association between pre-treatment serum SEMA4C levels with clinicopathological features (tumor size, tumor grade, LN status, and molecular subtype) was found. Moreover, post-surgery serum levels were lower than pre-treatment levels ($p < 0.001$) [11]. Our study shows no correlation between serum SEMA4C levels and pathological stage, LN status and histologic grade, which is consistent with data from the aforementioned research. We also report identical results in the measurement of serum SEMA4C levels before and after surgical treatment, confirming that postoperative serum levels measure significantly lower than preoperative levels ($p < 0.001$). Additionally, Wang Y et al. have demonstrated the performance of serum SEMA4C as a candidate diagnostic biomarker in early BC [11, 26].

Measurable serum protein biomarkers remain the most suitable candidates for routine clinical assessments and population-based studies due to the less invasive sample collection, high reproducibility, ease of operation, and low cost [11]. Regarding surgical treatment, their importance can be perceived through several aspects. Firstly, biomarkers can be informative about the effectiveness of a surgical intervention and guide clinicians in the assessment of whether

the operation has successfully removed or altered the targeted pathology. In some cases of oncologic surgery, a significant reduction in approved tumor markers in the POD indicates radical tumor removal. The ideal biomarkers should provide prognostic information and optimize the need for adjuvant therapies. Furthermore, it should be reliable for long-term monitoring of patients and detection of recurrence. Biomarkers are crucial in the realm of surgical treatment and research. Recent studies have opened up new perspectives for the exploration of novel biomarkers, with the ultimate goal of improving patient care and outcomes.

CONCLUSION

According to our study, serum SEMA4C levels did not differ significantly in terms of pathological stage, LN status, and histological grade. Notably, postoperative serum levels of SEMA4C were significantly decreased after surgical treatment compared to preoperative values, which underscores the potential of SEMA4C as a putative tool for monitoring response to therapy in patients with BC. Further research is mandatory to validate the role of SEMA4C as a biomarker in patients with BC.

Funding. *The present study is financed by the Medical University of Sofia (Contract Nr. D-142/2023)*

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