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NARRATIVE REVIEW

Degree of tumor lymphocytic infiltration at diagnosis as a prognostic factor for recurrence in breast cancers of molecular types rich in Her2 and triple negative: Narrative Review.

Tumor infiltrating lymphocyte grade as prognostic factor of recurrence in molecular subtypes of breast cancer (triple negative and Her2): A narrative review.

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Summary

Breast carcinoma is a common disease with significant adverse effects on predominantly female health. Tumor-infiltrating lymphocytes (TILs) manifest the host immune response to cancer. This study reviews and summarizes the literature on the predictive efficacy of a high percentage of tumor-infiltrating lymphocytes in HER2-rich and triple-negative breast cancers. Studies and reviews in English searched in the PubMed database were included. A higher TIL level of TILs corresponds to better disease-free survival in both triple-negative and HER2-rich cancers; therefore, it constitutes a histological marker that should be routinely used in microscopic analyses of breast biopsies.

Keywords:
DeCS: Breast Neoplasms; Tumor Infiltrating Lymphocytes; Triple Negative Breast Neoplasms; ErbB-2 receptor.

DOI: 10.33821/596

Abstract

Breast cancer is a common disease affecting women, with significant health -related adverse effects. Tumor-infiltrating lymphocytes (TILs) are recognized as manifestations of the host's antitumor immun- ity. The following study reviews and summarized bibliographie reports on the effectiveness of prognosis of high levels of tumor-infiltrating lymphocytes on triple-negative and HER2-enriched breast cancer molecular subtypes. Studies and reviews in English from Pubmed's database were included. A higher percentage of tumor-infiltrating lymphocytes is associated with a better prognosis and survival rate of triple-negative and HER2-enriched breast cancer. Consequently, such histological markers should be routinely used in the microscopic analysis of breast biopsies.

Keywords:

MESH: Breast Neoplasms; Lymphocytes, Tumor-Infiltrating; Triple Negative Breast Neoplasms; Recep-tor, ErbB-2.

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Introduction

Breast Breast carcinoma is a common disease with deleterious effects on predominantly female health. It comprises 23% of cancer patients and 14% of cancer-related deaths [1]. It is the most common malignant lesion in women in Guayaquil and Quito [2, 3]; the most common histological type is infiltrating ductal adenocarcinoma, which has been identified in 76% and 84% of the cases collected in Quito and Guayaquil, respectively [1, 3].

In Latin America, few studies relate the lymphocytic response to breast cancer with event-free survival prognosis [4, 5]. In other parts of the world, studies have been published showing that patients with molecular types of poor prognosis (triple-negative and rich in HER2) when they have an increased percentage of tumor-infiltrating lymphocytes (TILs) have better survival prognosis [6, 7].

The historical classification of breast cancer is based on histopathological evaluation of the type and degree of differentiation. Today it is known that breast cancer is a very heterogeneous condition [8, 9]. The most widely used technique to determine the molecular lineage is immunohistochemistry. The expression of estrogen and progesterone receptors (ER and PR, respectively) by tumor cells determines the hormonal status of the lesion and a potential endocrine treatment [10]. In addition, overexpression of the human epidermal growth factor receptor type 2 (HER2) predicts a likely response to Trastuzumab, a humanized monoclonal antibody [9, 11, 12].

Biomarkers

Biomarkers are measurable, quantifiable, detectable biological parameters obtained from a biological sample [13]. There are susceptibility, diagnostic, monitoring, prognostic, and predictive biomarkers. They are critical for the rational development of drugs and medical devices: this has made the concept of personalized and precision medicine [13, 14].

Biomarkers are also used to diagnose challenging lesions, differentiate between benign and malignant entities, in situ and infiltrating tumors, subtyping specific lesions, and determine the primary tissue of less differentiated tumors [11]. The most widely used technique to detect them is immunohistochemistry, frequently using groups of epithelial, lymphoid, or mesenchymal markers [9, 10].

Breast cancer

Morphologically, breast carcinoma is divided into carcinoma in situ (ductal and lobular) and infiltrating. The fourth edition of the World Health Organization (WHO) Classification of Breast Tumors defines at least 26 types of infiltrative disease [15]. These are diverse in their natural history and therapeutic response, and their phenotypic diversity corresponds to the variety of patterns and dimensions of gene expression [16].

Two main groups have been identified, apparently related to ER expression. The ER-enriched group was called “luminal” to indicate molecular similarity to normal luminal cells, while the group called “basal” is ER-negative, corresponding to triply negative cancers (ER, PR, and HER2 negative; table1) [8, 15]. These three markers are used in the routine management of patients with invasive breast cancer. At least five molecular subtypes were revealed: luminal a, luminal B, HER2-enriched, basal-like, and regular breast-like [8]. This classification, however, is not equivalent to the immunohistochemical category [8–10, 17, 18].

Virchow suggested in the 19th century a causal link between cancer and inflammation [20]. In the mid-twentieth century, it was determined that the adaptive immune system could control tumor growth and spread by stimulation, responding to a specific tumor [21, 22]. In addition, it was thought that the immune system could play a role in the disappearance of tumors [23]. The role of TILs in the prognosis of breast cancers has been known since 1967 [24]. It was then elucidated that regressing tumors have highly reactive lymphocytes [25]. Years later, it was described that the predictive value of TILs was associated with rapidly increasing tumors [26].

Table 1. Subtypes of triple negative cancers (8, 18, 19)

subtype	Expression
BL1	Genes related to the cell cycle
BL2	p63, CD10, Ki67
M	Genes related to cell motility. Includes metaplastic carcinomas.
IM	Genes related to the immune response.
LAR	Luminal CK18, androgen receptors

The molecular mechanisms that drive cancer-related inflammation can be summarized in the interference between two pathways: the intrinsic pathway, mediated by oncogene expression, and the extrinsic pathway, where inflammatory mediators and microenvironmental factors are

involved (Figure1). These pathways regulate tumor survival, proliferation, angiogenesis, and immunosuppression [20, 27, 28].

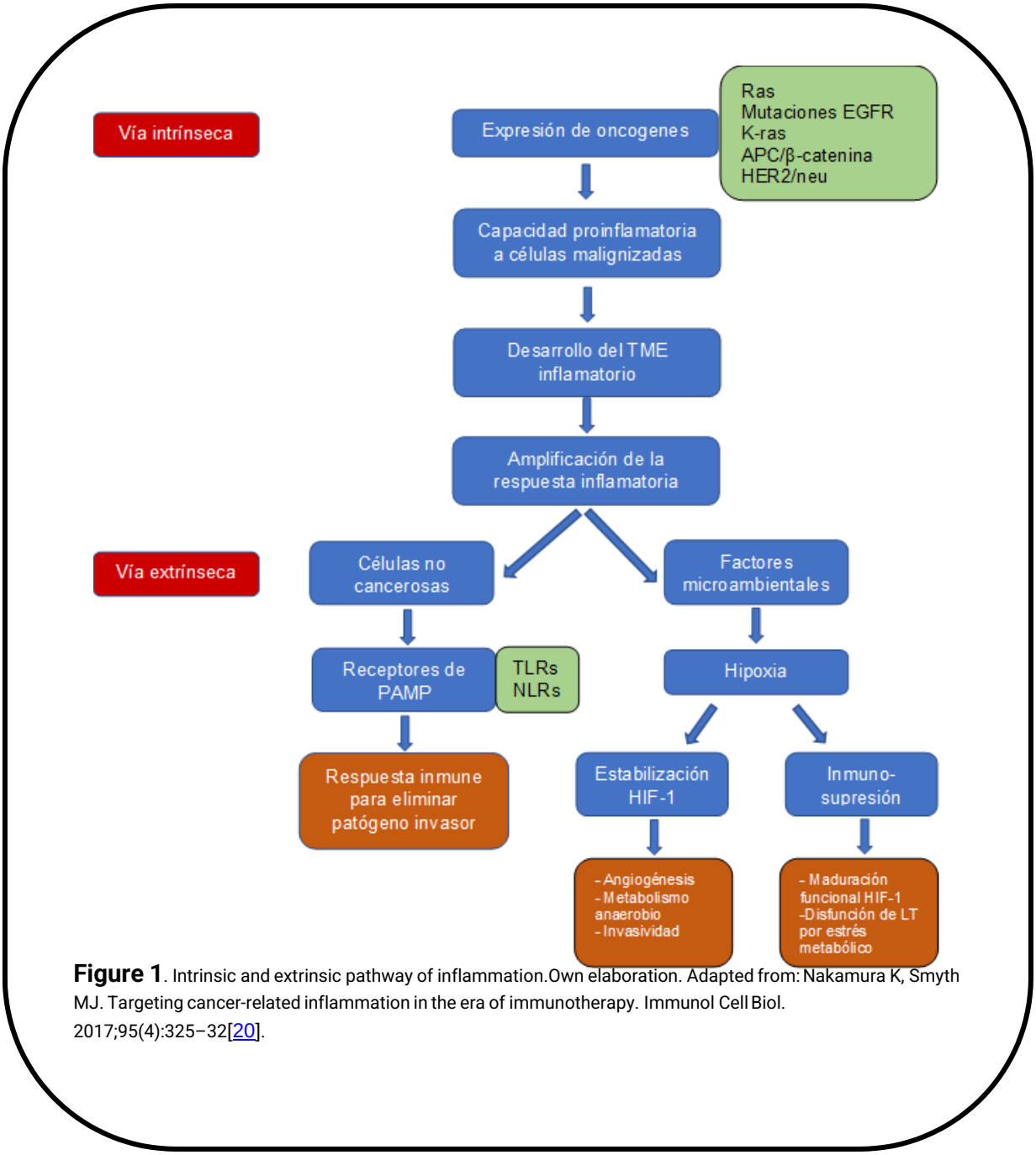

Figure 1. Intrinsic and extrinsic pathway of inflammation.Own elaboration. Adapted from: Nakamura K, Smyth MJ. Targeting cancer-related inflammation in the era of immunotherapy. Immunol Cell Biol. 2017;95(4):325–32[20].

Cells of hematopoietic origin in the tumor microenvironment include [27, 29]. The immune system maintains tissue homeostasis by continuously monitoring and initiating inflammatory reactions, along with coordinated actions of innate and adaptive immunity [30, 31]; In addition, it has a significant role in the control of tumor growth: TILs are a manifestation of the host's

immune response and predict a better outcome in some cancers, such as those of the colon, ovary, lung, and breast. [32–3. 4].

The interaction between the immune system and cancer cells is critical in controlling and eradicating cancer growth and is regulated through homeostasis between activating and inhibitory signals. TILs can kill tumor cells or allow the tumor to escape immune surveillance (cancer immunoediting). It has been postulated that TILs control tumor growth through a cytotoxic mechanism, thanks to the production of cytokines such as interferon-γ, which enhance a cellular immune response. Then, an infiltration of the tumor site by CD8+ lymphocytes would be desirable, in which CD4+ cells would be required to function correctly [22, 35].

There appears to be a significant association between the number of TILs present at diagnosis with therapeutic efficacy and prognosis in early breast cancer in the setting of neoadjuvant and adjuvant therapy. Trastuzumab chemotherapy could alleviate the suppression of antitumor effector immunity by favoring the massive release of tumor-related neoantigens after immunogenic cell death (mediated by cytotoxicity), which can suppress the action of CD4+ lymphocytes, inhibiting the activity of antitumor and restoring the motion of CD8+ lymphocytes [35, 36].

Triple negative immunophenotype

The triple-negative immunophenotype comprises approximately 15-20% of all breast cancers and 30% of deaths. They have a higher histological grade, present at younger ages, with advanced clinical stages, aggressive behavior, and a worse clinical prognosis than the other subtypes [11, 19]. They are not eligible for hormonal or HER2-targeted therapies: treatment is based on cytotoxic chemotherapy with a median survival of 13 to 18 months [35, 37].

This immunophenotype is poorly differentiated and shows a high degree of genomic instability related to mutations in DNA repair genes such as BRCA1 and BRCA2. These processes may allow the development of nonmutant peptides, which could become tumor-specific neoantigens that would be presented to T lymphocytes after being recognized by antigen-presenting cells [35]. Furthermore, this may explain why triple-negative breast cancers are often enriched for inflammatory infiltrates compared to hormone receptor-positive ones [35, 38].

HER2-rich immunophenotype

HER2 belongs to a transmembrane receptor family of four tyrosine kinases that mediate cell growth, differentiation, and survival [10, 39]. In about 20% of breast cancers, amplification of the HER2 gene, located on chromosome 17, is found. This amplification is associated with overexpression of the encoded protein; therefore, it is associated with a more aggressive course of the disease, a higher recurrence rate in localized disease, and a lower survival rate [40, 41].

Antibodies developed as anti-HER2 therapy, especially Trastuzumab, have improved the prognosis of this subgroup of patients [42]. Trastuzumab's mode of action is related to the inhibition of oncogenic signaling by binding to HER2 receptors on tumor cells and the stimulation

of antibody-dependent cellular cytotoxicity (ADCC) [43]. ADCC is mediated through the activation of Fc receptors (RsFc) on cells of the immune system. Previous animal studies have shown that trastuzumab antitumor activity was significantly reduced in RsFc-deficient mice, demonstrating that ADCC plays a critical role in trastuzumab activity [40, 43].

In addition, an increase in tumor infiltration by NK cells has been associated with the administration of Trastuzumab. Infiltration by other immune cells is also associated with Trastuzumab efficiency and increased survival rates in HER2+ breast cancers [40].

TIL count

TILs TILs are a mixture of different cell types, usually dominated by T lymphocytes, with variable proportions of B lymphocytes, NK (natural killer) cells, macrophages, and dendritic cells. For this reason, the quantification and characterization of TILs have been used as an aid for the evaluation of tumor immunogenicity [31].

As already mentioned, TILs have been correlated with a good prognosis in several cohorts [40, 43, 44]. Tumors infiltrated by immune cells are frequently observed, but the cell composition varies between tumors and organs. Myeloid-derived leukocytes—including macrophages, dendritic cells, and myeloid-derived suppressor cells—have been identified in murine models as shaping the microenvironment through the substances they produce, either as an antitumor immunostimulatory environment or a tumor-promoting microenvironment [36, 45]. Antitumor T cells can be activated or suppressed. These regulate the polarization of macrophages in their functional M2 (protumorigenic) or M1 (antitumor) phenotypes, highlighting the importance of cross-communication in the formation of the tumor microenvironment [45].

The detection of T lymphocytes is carried out by immunohistochemistry: the cytotoxic ones with the CD8 marker; the regulators with the marker FOXP3 [32]. However, the most common method of detecting and quantifying TILs is by light microscopy of hematoxylin and eosin (H&E)-stained histological slides of tumor samples, by direct visualization and measurement of mononuclear cells in representative tumor sections. The International Immuno-Oncology Biomarker Working Group has made recommendations to maximize reproducibility with efforts toward standardization (Figures 2, 3, 4; Table 2) [31, 45, 46].)

Table 2. Criteria for lymphocyte count [45]

	Criteria
1.	Tumor infiltrating lymphocytes (TILs) should be reported by the stromal compartment (=% stromal TILs). The denominator used to determine the stromal %TILs is the area of stromal tissue (the area occupied by mononuclear inflammatory cells over the total intratumoral stromal area), not the number of total cells (the fraction of the total stromal nuclei that represent cell nuclei). mononuclear inflammatory
2.	TILs should be evaluated within the tumor borders of the infiltrating tumor.
3.	Exclude TILs outside the tumor border and around DCIS and normal lobes
4.	Exclude TILs in tumor areas with crush artifacts, necrosis, regressive hyalinization, as well as in the previous core biopsy site.
5.	All mononuclear cells (including lymphocytes and plasma cells) should be scored, but polymorphonuclear leukocytes are excluded.

6.	One section (4-5 micrometers, magnification x200-400) per patient is considered sufficient.
7.	Whole sections are preferred to biopsies whenever possible. Cores can be used in neoadjuvant therapy: at the moment, there is no validated methodology to score TILs after neoadjuvant treatment.
8.	A full pathologist's assessment of the average TILs in the tumor area should be used.
9.	TILs can provide more biologically relevant information if scored as a continuous variable, as it will allow more accurate statistical analyzes that can be categorized into different limits in the future. The pathologist should report your scores in as much detail as your comfort allows.
10.	TILs should be evaluated as a continuous parameter. The percentage of stromal TILs is a semi - quantitative parameter for this assessment. The dissociated growth pattern of lymphocytes needs to be taken into consideration. Typically, lymphocytes do not form solid cell aggregates; therefore, the designation "100% stromal TILs" would allow for some empty tissue space betweenindividual lymphocytes.

The tumor area is divided into a stromal compartment and an intratumoral compartment, and most of the studies are performed in both chambers. Still, it is considered that the stromal compartment is more representative and reproducible because the lymphocytes are more abundant, more homogeneous, and more visible. Consequently, the associations with clinico- pathological parameters and disease prognosis are better established with stromal TILs [31].

There is no consensus in the literature to determine the cut-off point for a degree of the high or low density of TILs. However, although there is no difference in the prognosis of breast carcinomas with “high grade” cut-off points at 25%, 35%, and 50% of TILs, [47], it is suggested that pathologists score the percentage of TILs, as a continuous variable, as precisely as possible [48].

TILs in HER2 cancers

A high level of lymphocytic infiltration is associated with a significantly higher rate of pCR (complete histopathologic response), overall survival, and disease-free survival. In addition, the degree of TILs increases after treatment with Trastuzumab, a recombinant monoclonal antibody directed against HER2 with clinical activity in advanced breast cancer with overexpression of HER2, which significantly improves clinical prognosis. A high grade of TILs in the residual tumor is associated with a considerably better prognosis than residual tumors with a low TIL grade of TILs [41, 49, 50].

TILs in HER2 cancers are located more prominently at the margin of invasion than at the center of the tumor, contrary to colorectal cancer, where TILs at the center of the tumor are associated with a better prognosis [31, 51, 52]. The CD8 marker has a better predictive value, which confers importance to cytotoxic T lymphocytes in tumor control [32, 33, and 48]. It is not yet clear whether TILs in this cancer subtype are involved in the cytotoxicity of anticancer drugs [40]. See an example in Figure 2.

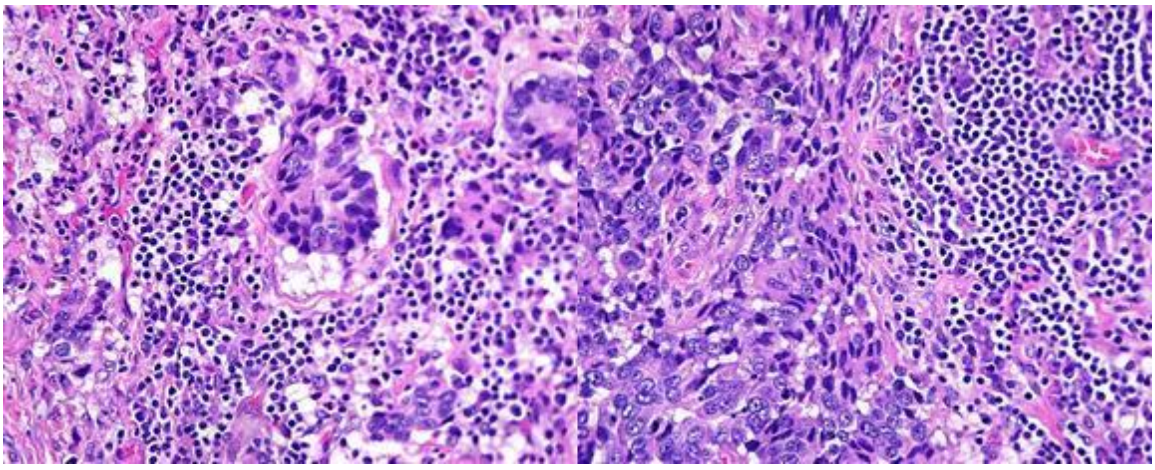

Figure 2. Breast medullary carcinoma with a count of approximately 40% of TILs. Source: Pathological Anatomy Service, Hospital Carlos Andrade Marín, Quito.

TILs in TNBC

It has already been mentioned that TNBC shows a high degree of genomic instability associated with mutations in DNA repair genes, such as BRCA1 and BRCA2. These processes would lead to developing a series of tumor neoantigens recognized by antigen-presenting cells and

presented to T lymphocytes; thus, TNBC is more frequently enriched for TILs than the other hormone-positive types [35].

Stromal lymphocytic infiltration constitutes a robust and independent prognostic marker in TNBC treated with neoadjuvant therapy: a high immune response is predictive of a significantly lower risk of recurrence or death, distant recurrence, and overall mortality [53, 54]. Chemotherapy can promote the massive release of tumor-associated neoantigens, followed by cytotoxicity-induced cell death, suppresses regulatory T cells, and restores cytotoxic T cells. These CD8 and FOXP3 cells have a substantial role in neoadjuvant therapy and lead to a better PCR after it [35, 53, 54]. In contrast, patients with TNBC and a low level of TILs have a higher rate of recurrence [55].

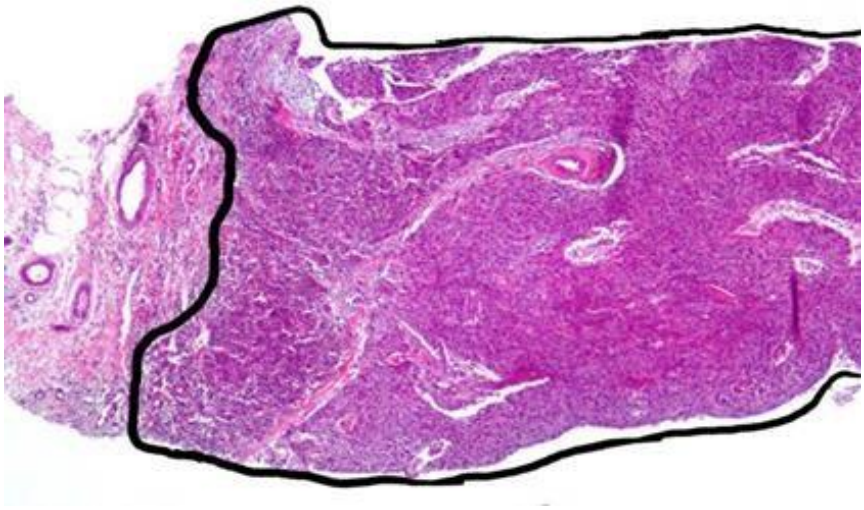

Figure 3. Evaluation of TILs within tumor borders (area outlined with a black line). Source: Pathological Anatomy Service, Hospital Carlos Andrade Marín, Quito

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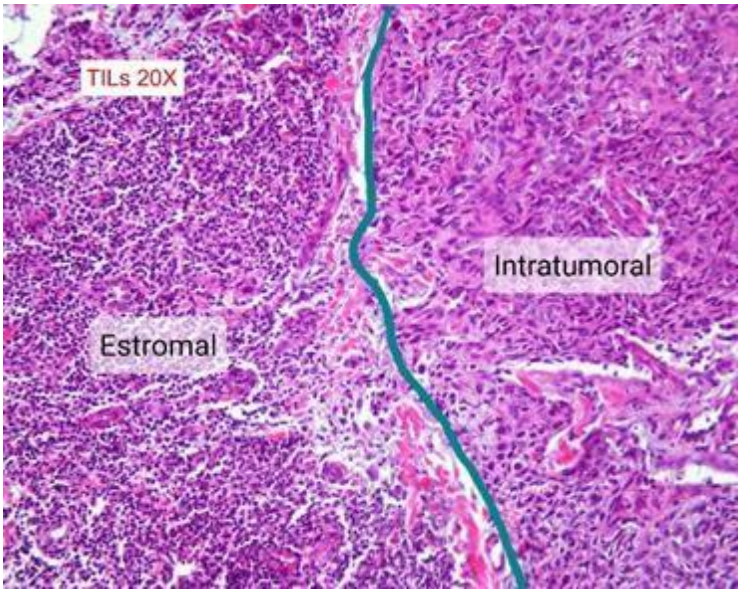

Figure 4. Distribution of TILs according to intratumoral and stromal compartments (area delimited by a green line). Source: Pathological Anatomy Service, Hospital Carlos Andrade Marín, Quito.

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administrative information

abbreviations

CBC:Basal cell carcinoma. CT:Trichoblastoma. TE: trichoepithelioma. CK: Cytokeratins. WHO: World Health Organi-zation. UVA: Ultraviolet.

Additional Files

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Availability of data and materials

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Author contributions

MABP and DVPR alike performed: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Method- ology, Project Management, Resources, Software, Supervision, Validation, Visualization, Writing - Original Draft, Writ- ing: Review and Editing. The authors read and approved the final version of the article.

Consent to publication

Does not apply for a narrative review.

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