Validation of a Quantitative Image Analysis Algorithm for Ki67 Index in Breast Cancer and Neuroendocrine Tumor

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Background & Objectives

Ki67 index evaluation in Breast Carcinoma (BC) and Neuroendocrine Tumor (NET) depends on the quality of immunohistochemistry (IHC), the interpretation of the staining by the pathologist and the spatial intratumoral heterogeneity of Ki67 expression. Our objective was to design an adapted Quantitative Image Analysis (QIA) algorithm.

Methods

The study used 2 BCs and 2 NETs from 2 external proficiency testing schemes conducted by the french interlaboratory comparison organization (AFAQAP) for Ki67 IHC technique and staining interpretation by the pathologist. In 2021, 55 pathology laboratories received 1 blank slide of a multi-tissue paraffin block composed of 1 BC and 1 NET to apply their routine protocol; in 2022, 66 laboratories received 1 blank slide of another multi-tissue paraffin block made up of 1 other BC and 1 other NET.

Ki67 indexes of the 242 tissue cores were assessed by 2 independent methods: Visually by eyeballing on physical slides by 2 expert pathologists establishing simultaneously a consensus index for each tissue core, and by QIA using the IMSTAR PathoScan Tumor-Marker Ki67 (Ki67-QIA) algorithm (**Fig. 1**). Thus, expert pathologists assessed the quality of the IHC technique for each of the 242 tissue cores according to 4 technique quality levels (optimal, good, borderline, poor) depending on the Ki67 staining, but also on the counterstaining intensity.

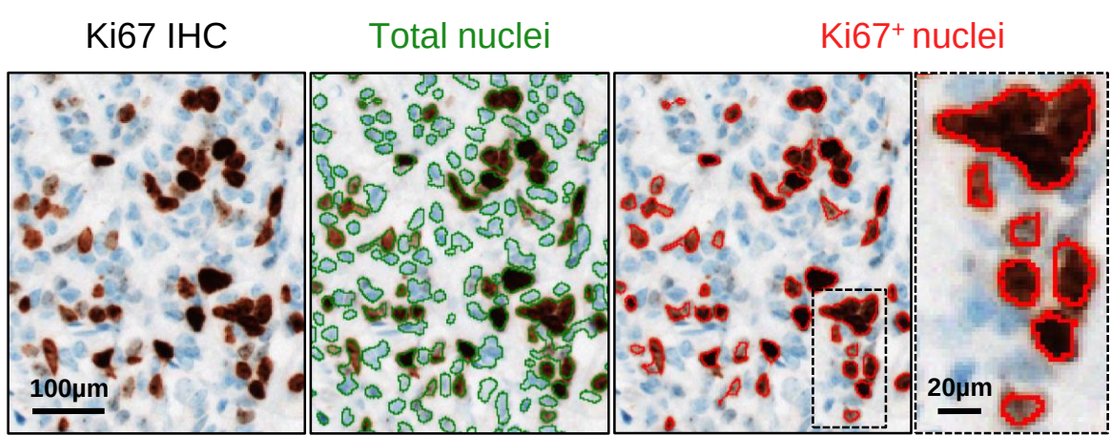


Figure 1: Automated evaluation of Ki67 index by QIA. Specific detection of **total nuclei**, and **Ki67+ nuclei**, isolated or clustered, by the Ki67-QIA algorithm.

Results

46 Ki67 IHC slides received the ”optimal technique” appreciation by the experts for each BC and NET (2021, n= 20 slides; 2022, n= 26 slides), with the Ki67 indexes described in **Table 1**. These 46 slides (out of 121 total slides) are called “optimal” slides.

	Ki67 index (%)	
	2021	2022
BC	12-15	5-10
NET	3-5	4-5

Table 1: Ki67 indexes established by the expert pathologists on the “optimal”. 2 expert pathologists evaluated Ki67 indexes by eye-balling on a multi-head microscope.

The Ki67-QIA algorithm performed an accurate evaluation of Ki67 index with an **overall concordance** with the experts (±1% outside the index classes) of **91% (42/46 cores) for BCs** and **91% (42/46 cores) for NETs** for the 46 ”optimal” slides.

Expert pathologists characterized the 2022 BC and the 2 NETs as **homogeneous tumors** with low spatial intratumoral heterogeneity of Ki67 expression (**Fig. 2**). The Ki67-QIA algorithm confirmed the homogeneity of Ki67 expression, with a **high agreement** with the experts (±1% outside the index classes) of **85% (17/20 cores) for 2021 NET, 96% (25/26 cores) for 2022 NET** and **100% (26/26 cores) for 2022 BC** (**Fig. 3**).

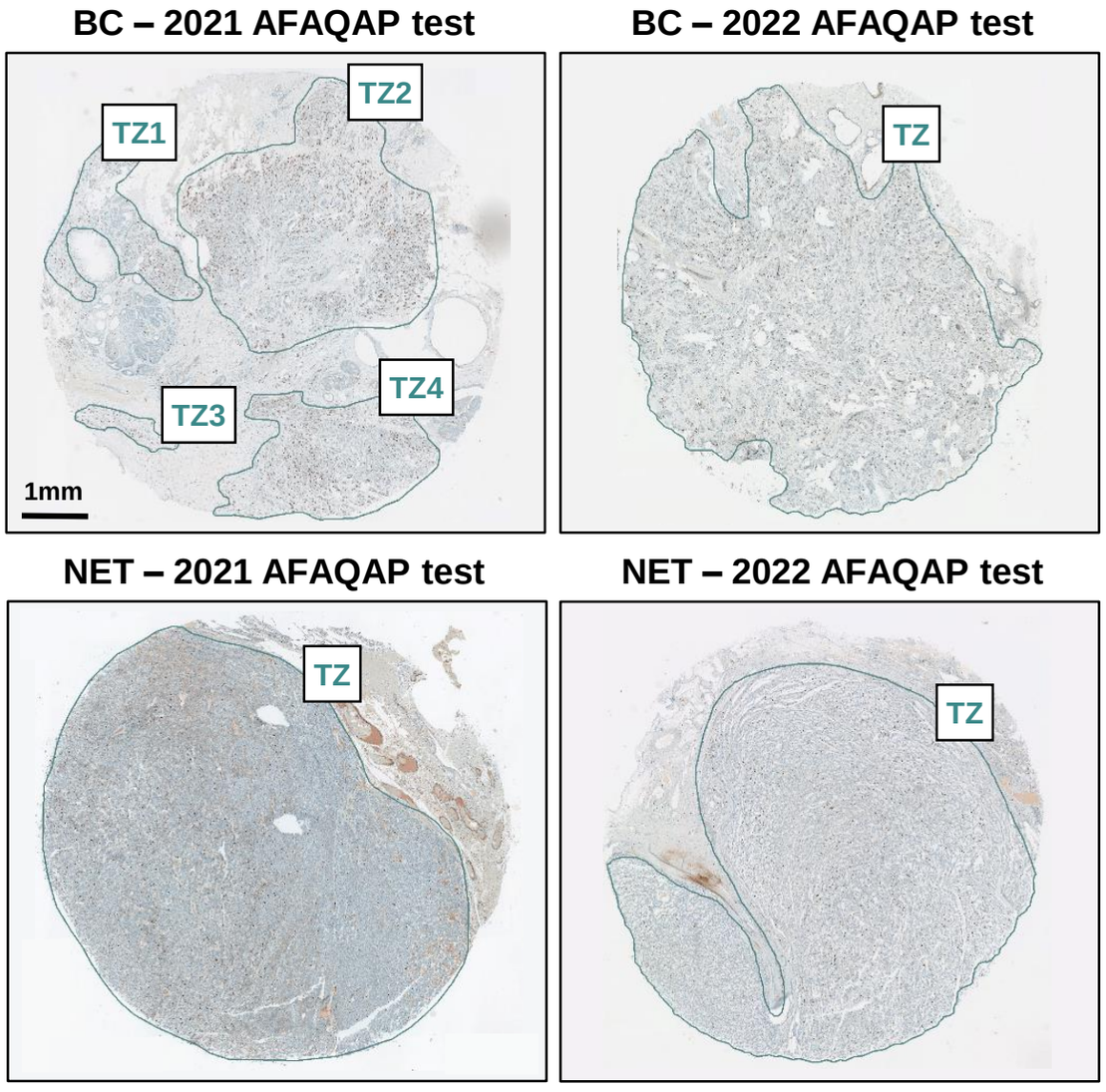


Figure 2: Spatial intratumoral heterogeneity. Experts identified 3 tissue cores as homogeneous tumors (2022 BC, 2021 NET, 2022 NET), and 1 tissue core as heterogeneous with 4 tumor foci (2021 BC). TZ = Tumor Zone.

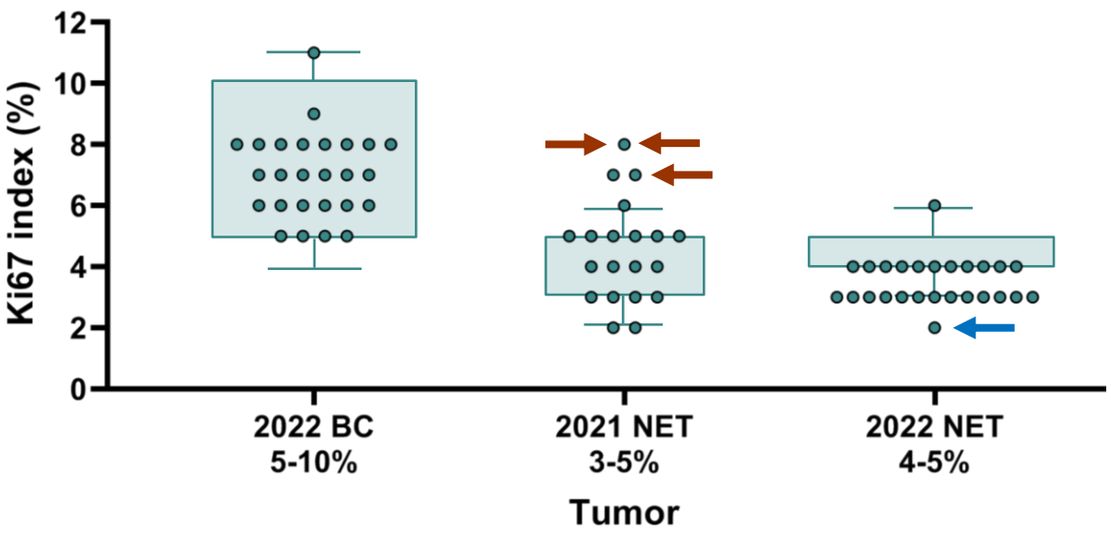
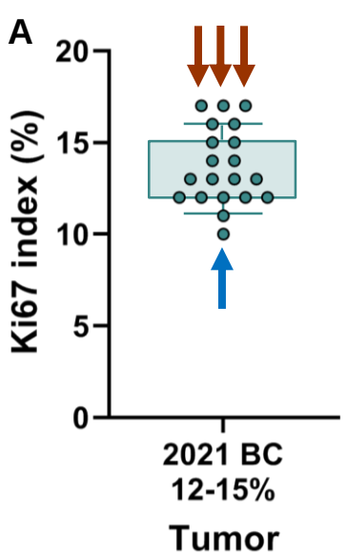


Figure 3: Evaluation of Ki67 IHC by the visual method and by the Ki67-QIA algorithm on the homogeneous tumors. The 3 overvalued cores are indicated by **brown arrows**; the undervalued core by a **blue arrow**.

On the contrary, the 2021 BC was qualified by the expert pathologists as a **heterogeneous tumor** with 4 main tumor foci disseminated in a large stroma, and variable Ki67 index levels per tumor zone (**Fig. 2**). **Agreement** between the Ki67-AIQ algorithm and the experts was **80% for the totality of this tumor taking into account the 4 tumor zones** (**Fig. 4A**). In addition, Ki67-QIA provides an accurate measurement of Ki67 index on a large number of cancer cells, and accounts for the heterogeneity of the Ki67 index by tumor zone (**Fig. 4B**).

A



B

	Area (mm²)	Number of nuclei	Ki67 index (%)
TZ1	2.08	4,200	10.7
TZ2	8.83	17,533	16.4
TZ3	0.46	633	12.9
TZ4	3.83	7,856	16.6

Figure 4: Evaluation of Ki67 IHC by the visual method and by the Ki67-QIA algorithm on the heterogeneous tumor. (**A**) Ki67 indexes are the mean for the 4 Tumor Zones TZ1-4. The 3 overvalued cores are indicated by **brown arrows**; the undervalued core by a **blue arrow**. (**B**) Ki67-QIA algorithm provides quantitative results for each Tumor Zone: area, number of nuclei and Ki67 index.

Conclusions

The QIA solution was efficient to evaluate Ki67 index on technically optimal IHC slides despite multiple laboratories IHC techniques being applied, highlighting the need for quality in IHC to obtain robust digital evaluations. The objective quantification of intra-tumoral heterogeneity opens an additional challenge. Ki67-QIA algorithm allows to evaluate Ki67 index on a large number of cancer cells and to visualize and quantify Ki67 index spatial variations in tumors, enabling to identify tumor zones under or over the clinical utility thresholds.

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