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Detection Of Automatic Digital Image Analysis Problems For The Evaluation Of Immune Markers In Breast Cancer Biopsies

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Introduction/ Background

Automatic digital image analysis has increased in recent years. There are several applications for image analysis in pathology, among them, immunohistochemistry biomarkers quantification. This is a simple, economic and fast method to quantify stained biomarkers in digitalized biopsies enhancing sensitivity and objectivity. However, automatic procedures do not always work satisfactorily in all digitalized samples.

Aims

To quantify the number of incorrectly analyzed images as a result of errors on the automatic procedure developed for quantifying immune markers in breast cancer biopsies, to evaluate the amount of time spent in this reanalysis and to define which kind of biopsy produces more errors.

Methods

10,770 cores of breast tumor (intra-tumoral and peri-tumoral areas) and negative and positive axillary lymph nodes areas from ductal invasive breast cancer were included in different tissue microarrays (TMAs). Slides of each TMA were immunohistochemically stained for CD4, CD8, CD57, CD68, S100, LAMP3, CD83, CD1A, CD123 and CD21 immune markers and were digitalized at 40X with the Apperio Scanscope XT scanner. Each core was extracted as an individual digital image in TIFF format. The stained area was automatically quantified using procedures developed with Fiji (Image J) software in a HP Intel Inside Core i.7 computer with 16GB of RAM memory. Firstly, the whole area of each core was evaluated in pixels by using the luminance channel, applying the median filter and gray-scale segmentation. The second step evaluated the positive pixel number stained in brown for obtaining a brown color channel and then, a gray scale and size segmentation for positive objects, including holes inside the segmented area. Finally, this selected brown area was automatically surrounded by an overlay.

Results

2,751 had to be reanalyzed (25.5%). Specifically, intra-tumoral and peri-tumoral cores were those with higher reanalysis levels (35.6% and 34.9%, respectively) while axillary lymph nodes cores present lower levels (Negative Nodes 13.5% and Positive Nodes 16.4%). Regarding the immune biomarker, CD21, LAMP3 and CD123 were those with higher reanalysis levels (38.4%, 35.2% and 34.2%, respectively) in contrast with CD8, CD68 and CD83 that were those with lower levels (16.4%, 17.7%, 18.4%, respectively). The reanalysis levels of the remaining biomarkers were 22% in CD4, 23.9% in S100, 26.% in CD1A and 27.4% in CD57. Each core took a mean of 1.56 minutes to be analyzed so the total time spent in the reanalysis of the images was 71.5 hours. The principal reasons for reanalysis were problems in the TMAs assembly accuracy and

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presence of adipose tissue, hemosiderin, artifacts, unspecific staining and background noise. Several TMAs presented glue bubbles and different types of dirt, as hairs or dust that were quantified as positive area. Intratumoral and peritumoral cores are those with higher levels of adipose tissue. These adipocytes cause alterations in the automatic quantification due to their different cellular structure. Specifically, in cores stained for S100 marker in which membranes of adipocytes were also stained in brown and, thus, quantified as immune marker.

Conclusions: It could be possible to reduce the time of analysis and to obtain more exact values of immune quantification in digital images improving the accuracy of TMAs assembly, overcoming unspecific staining and background and adapting the parameters of the procedures.