Automatic quantification of airway inflammation on histological images with a new software tool

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The anatomopathological lung alterations developed in the allergic airway inflammation models are difficult to estimate. The eosinophil and T lymphocyte infiltrates as well as the mucus production are classified on an arbitrary intensity scale performed by optical microscopy in a blind fashion. To provide a quantitative assessment of the inflammation in the lung tissue samples, we have developed a software in which cellular infiltration and mucus production are automatically calculated.

Method: Lungs from Olea europaea pollen sensitized BALB/c mice and from control mice were fixed in 4% formaldehyde-PBS.
Tissue blocks were embedded in paraffin and serial sections were stained with hematoxylin and eosin to evaluate cell infiltration, and hematoxylin and periodic acid-Schiff (PAS) for mucus production. Images were acquired with an Olympus IX70 microscope at 20_magnification and captured with a Sony DXC 151P color CCD camera. For the evaluation of the infiltrates, the image was divided into regions of equal size (55_55 pixels, pixel size: 0.45 mm). The regions were then classified into two types (infiltrate-not infiltrate) by means of a segmentation algorithm based on texture analysis. Results were given as percentage of cellular infiltrate area over the total tissue area. For mucus analysis the images were segmented by a threshold technique on the color components. Results were given in terms of percentage of mucus density at the bronchial wall. The same samples were also analyzed manually by optical microscopy by an independent researcher.

Results: The automatic method showed that the allergic mice had a higher total infiltrated area compared to control mice. Specifically, allergic mice showed a 6.6-fold increased inflammatory infiltrated area compare to controls (3374.9% vs. 5.4672%, Po0.001). These automatically acquired findings showed a good positive correlation with those obtained manually by optical microscopy (R250.90, eˆ 50.87). Moreover, the automatic procedure showed a significantly increased mucus recognition in allergic mice compared to controls (9.3971% vs. 0.8470.84%, Po0.001).
Anatomopathological findings were in agreement with immunological and lung function parameters.

Conclusion: The current software developed is a useful tool to automatically quantify airway infiltrating cells as well as mucus production in lung tissue samples. It provides a reliable procedure to measure airway inflammation in lung histological studies.