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Agreement between immunohistochemistry and flow cytometry in the assessment of Ki-67 index in lymphoma-bearing dogs

In collaborazione con vetmeduni vienna

Introduzione

The prognostic significance of the immunohistochemical assessment of Ki-67 proliferation marker has been largely demonstrated in many tumor types; however, its relevance for canine lymphoma is still debated.[1,2]
Evaluation of Ki-67 by flow cytometry (FCM) in fine needle aspirates has recently shown prognostic relevance in canine high-grade B-cell lymphoma.[3,4]
The aim of this preliminary study was to investigate the agreement between IHC and FCM in the assessment of Ki-67 index in canine lymphoma, in order to evaluate whether these techniques can be used interchangeably for the evaluation of proliferative activity.

Materials & Methods

Dogs referred with a previously untreated lymphoma at the Veterinary Hospitals of the vetmedUni Vienna and Bologna were considered for inclusion in this prospective study. In these patients, Ki-67 index was assessed by FCM on fine-needle aspirates, and the dogs undergoing subsequent diagnostic lymphadenectomy/biopsy with histopathological evaluation were ultimately included.
For each case, Ki-67 index was also assessed immunohistochemically on formalin-fixed and paraffin-embedded tissue sections. By FCM, $10x10^3$ cells were categorized. By IHC, a manual count was performed in five 400x fields, without knowledge of FCM results. With both methods, Ki-67 index was expressed as percentage of positive tumor cells.

Results

Twenty-nine dogs were included in the study. There were 11 males and 18 females; the mean age was 8 years (range, 4-11). Tumors were 26 peripheral nodal, 1 mediastinal, 1 indolent splenic and 1 intestinal lymphoma. According to the final histopathological diagnosis, B-cell lymphomas (n = 21) included 18 diffuse large B-cell lymphoma, 1 lymphoblastic, 1 nodal marginal zone lymphoma and 1 splenic marginal zone lymphoma. T cell lymphomas (n = 8) included 4 peripheral T-cell lymphomas, 1 lymphoblastic and 2 T-zone lymphomas (Table 1). The median time elapsed between FCM and histology was 3.5 days. No therapy was administered in between.
The mean Ki-67 index was 51 ± 27% (range, 2-100%) with FCM and 49 ± 23% (range, 8-86%) with IHC (Figure 1). With both methods, indolent (nodular) lymphomas had a significantly lower proliferative activity compared with diffuse lymphomas (FCM: 25% vs 55%, respectively, P = 0.03; IHC: 16% vs 54%, P = 0.0008). According to the Bland-Altman plot, the bias between the techniques was 2% and 95% limits of agreement were -47% to 52%. Spearman's correlation coefficient was 0.45 (P = 0.014). When stratifying cases according to the growth pattern (nodular vs. diffuse), a significant correlation was not observed among nodular indolent lymphomas (marginal zone and T-zone).

Discussion and conclusions

The assessment of Ki-67 index by FCM in canine lymphoma may represent a valuable alternative to the IHC evaluation since
- it is faster and non-invasive as it does not require a surgical procedure;
- being an automated count, the operator-dependent bias are minimal;
- it allows to assess simultaneously the immunophenotype of the proliferating cells;
- the number of analysed cells is consistently higher than the one assessed immunohistochemically.

Due to the above reasons, FCM might prove more accurate than IHC in the assessment of proliferative activity.
These preliminary data indicate a substantial agreement between the two techniques, although in a subset of cases the discrepancies in Ki-67 index between the two methods were prominent and the overall correlation was modest. This may be due to the above method-related differences in the number of evaluated cells or to difficulties in limiting the assessment of proliferative activity to neoplastic cells in IHC sections. Notably, the lowest agreement was observed in nodular lymphomas, in which neoplastic cells are admixed with a relevant number of resident cells.
Further prospective studies including acknowledged prognostic factors in canine lymphoma should be performed to ultimately assess its prognostic relevance.

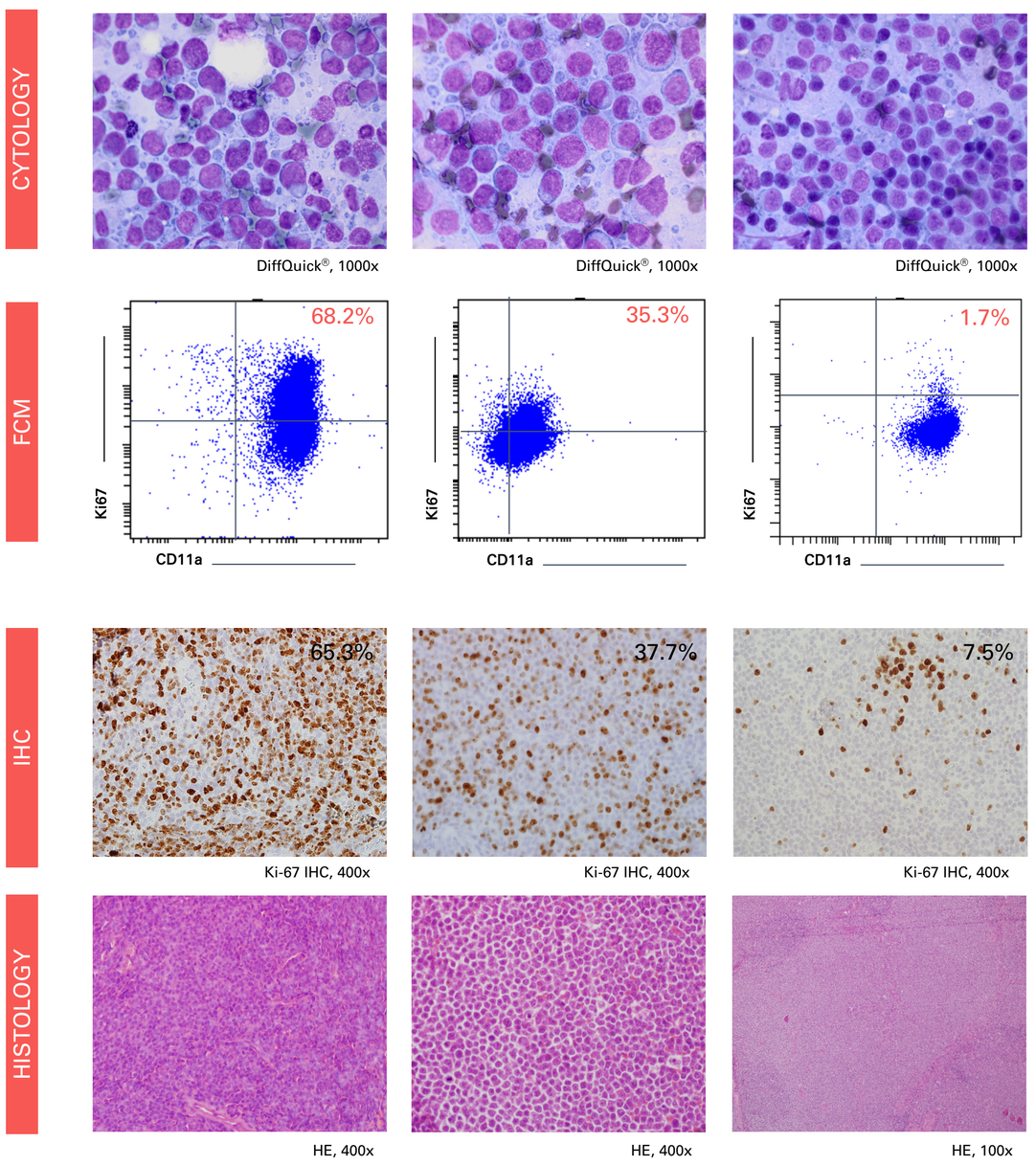

Figure 1
Comparison between flow cytometry and immunohistochemistry in the assessment of Ki-67 index. Corresponding cytological and histological images are also shown. Representative examples of high (case No. 8), intermediate (case no. 23) and low (case No. 1) Ki-67 index. Percentage values are provided.

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