Ki67 indexes in paired cytological smears and cell blocks of canine lymphoma: preliminary results

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Introduction

Ki67 is a protein expressed in all cell cycle phases, except G0 and is used as a proliferation marker in canine lymphoma (CL). Ki67 protein can be immunodetected on biopsies and cytology smears of CL. Ki67 index cut-offs with prognostic significance have been defined for histopathology and flow-cytometry. Our goal was to compare Ki67 indexes estimated on previously-stained cytology smears (PSCS) and on matched cell tube blocks (CTB) of CL.

Methods

- PSCS and matched CTB of 20 cases CL (18 nodal, 1 intestinal and 1 cutaneous) were retrospectively selected (Fig. 1).
- Immunocytochemistry was performed using MIB-1 antibody for Ki67 immunolabelling, and Ki67 index was assessed.
- The Ki67 indexes determined in PSCS and paired CTB were used to classify the CL as low or high proliferative using the cut-off of 12.2% (defined by flow cytometry)[1].

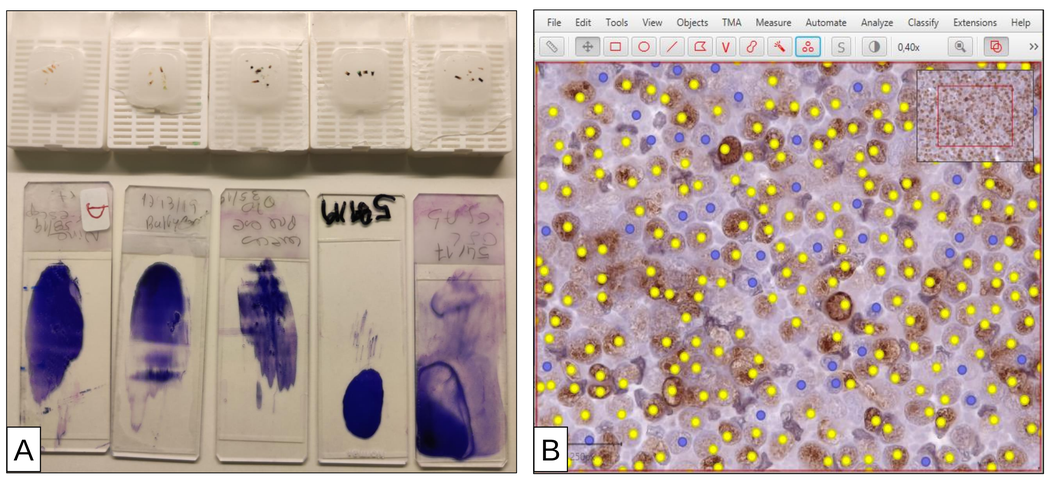

Figure 1. (A) Selection of previously-stained cytology smears and matched cell tube blocks from canine lymphoma cases. (B) Ten fields of each paired case were microphotographed. Ki67 index estimation was performed using a counting tool program by one observer (FS).

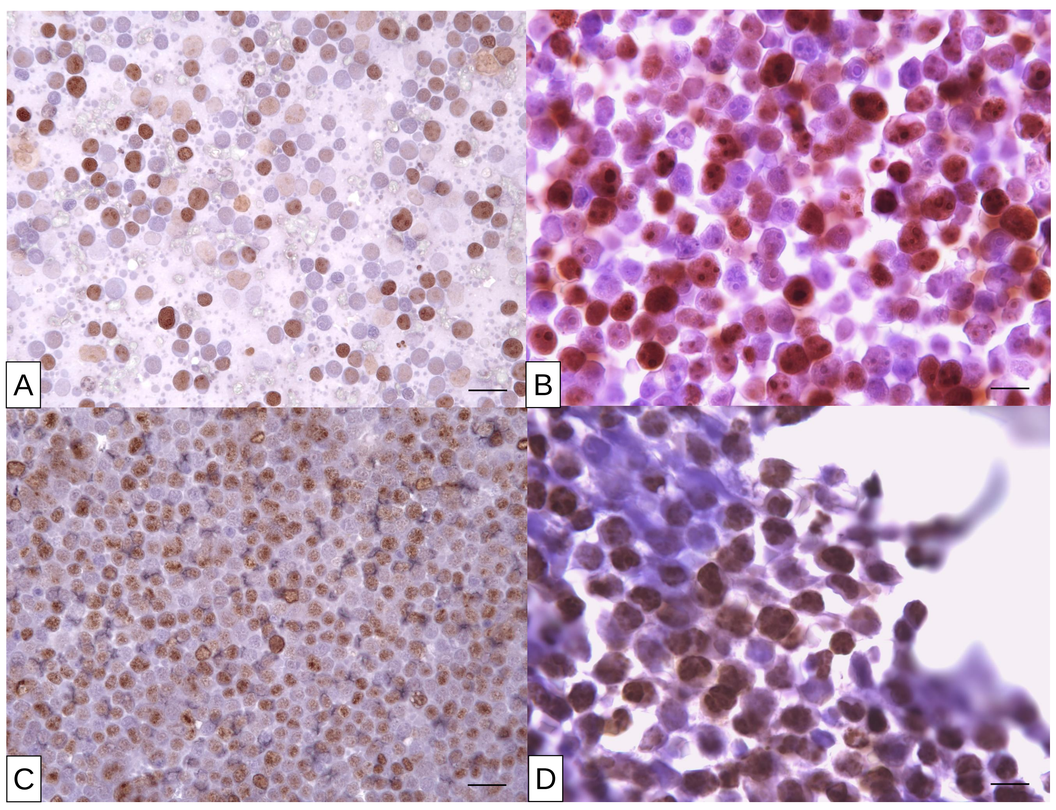

Figure 2. B-cell lymphomas. Immunolabeling with MIB-1 on previously-stained cytology smears (A and C) and on matched cell tube blocks sections (B and D), respectively. More than 60% of the neoplastic lymphocytes showed positive nuclear reactivity for Ki67 in both cases. Diaminobenzidine chromogen, Haematoxylin counterstain; bar = 20 µm (A and C); and 17 µm (B and D).

Results

- In 2 PSCS with long time of archive and 1 CTB out 20 no immunolabelling was detected; excluding these cases, Ki67 index median was 30.5% (SD 22.6%) in PSCS and 44.0% (SD 21.7%) in CTB.
- No significant difference, nor a correlation existed between Ki67 index values on PSCS and matched CTB.
- Moderate agreement existed between PSCS and CTB for the classification in lymphoma with low vs high proliferation (Ki67 index cut-off 12.2%) – Table 1, Figures 2 and 3.

Table1. Ki67 index classification (kappa=0.74).

	Cell-block		
Cytology	Low	High	**Total**
Low	1	4	**5**
High	2	13	**15**
Total	**3**	**17**	**20**

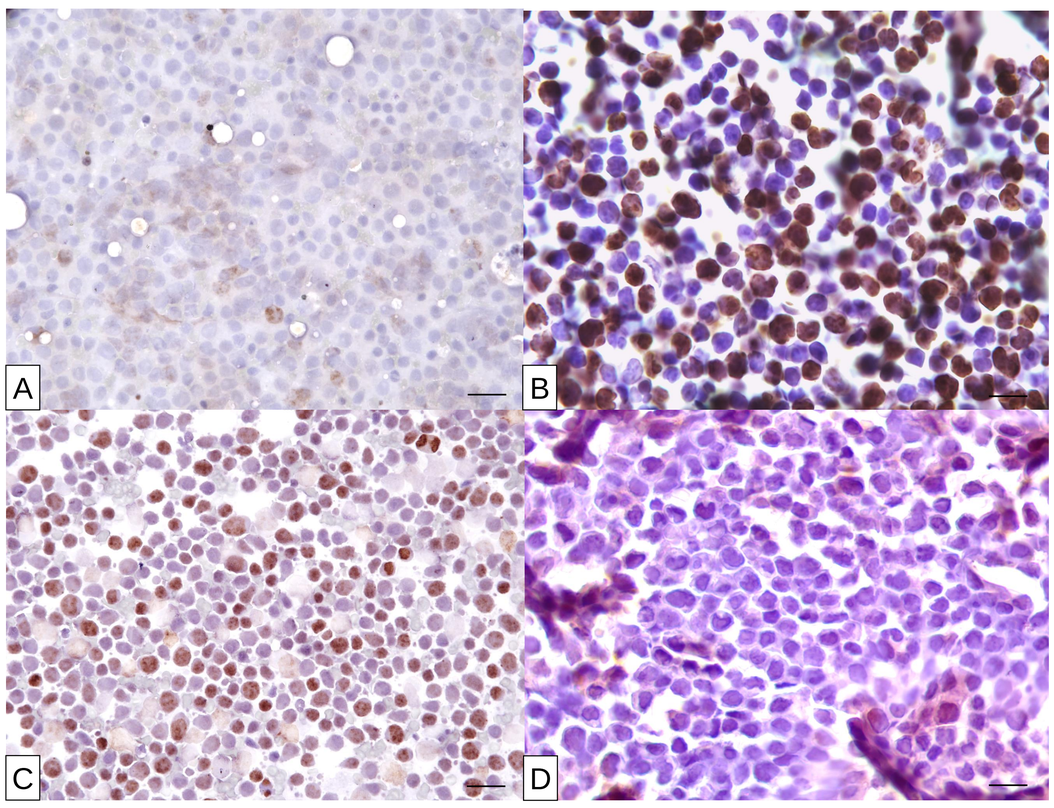

Figure 3. Two different cases (A and B; C and D) from B-cell lymphomas. Immunolabeling applied to previously-stained cytology smears (A and C) and on matched cell tube blocks sections (B and D), respectively. The first case showed low (7%) and high (45%) positive nuclear reactivity for Ki67 for previously-stained cytology smears and matched cell tube blocks, respectively. The second case showed high (45%) and low (1%) indexes for previously-stained cytology smears and matched cell tube blocks, respectively. These cases represent discrepant positive nuclear reactivities in both specimens, according to the reported cut-off of 12,2%. Diaminobenzidine chromogen, Haematoxylin counterstain; bar = 24 µm (A and C); and 17 µm (B and D).

Conclusion: The estimates of the Ki67 index on cytology and on paired CTB in CL cases can vary. However, when a Ki67 index cut-off of 12,2% for defining low vs high proliferative CL is used, the CTB and cytology tend to agree, especially in recent cytology cases.

Bibliography

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