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Curriculum: Sanità Animale - Tutor: Prof. Giuliano Bettini

Durante il II anno di Dottorato di Ricerca, sono state svolte le seguenti attività:

Completamento del lavoro **«Canine Splenic Nodular Lymphoid Lesions: Immunophenotyping, Proliferative Activity and Clonality Assessment»**, in collaborazione con l'Università di Padova.
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Canine Splenic Nodular Lymphoid Lesions: Immunophenotyping, Proliferative Activity, and Clonality Assessment

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Abstract

Canine splenic lymphoid nodules are currently classified as indolent lymphomas (marginal zone lymphoma [MZL], mantle cell lymphoma [MCL]) or nodular hyperplasia (lymphoid [LNH] or complex [CNH] type). Their differentiation can be difficult on morphology, because of similar histologic appearance and poorly defined diagnostic criteria. Thirty-five surgical samples of splenic lymphoid nodules were reviewed in order to assess the diagnostic contribution of immunophenotyping, proliferative activity and clonality (PARR) in differentiating between hyperplastic and neoplastic lesions. Proliferative activity was evaluated by double immunolabeling for Ki-67 and CD79a, in order to separately assess the proliferative activity of B cells and non-B cells. Definitive diagnoses were MZL ($n = 11$), MCL ($n = 4$), LNH ($n = 10$), and CNH ($n = 10$). The overall concordance between histology and PARR was above 90%. Lymphomas had a significantly higher percentage of CD79a-positive areas (mean, 36.30%; $P = .0004$) and a higher B-cell proliferative activity (median Ki-67 index, 5.49%; $P = .0012$). The threshold value most accurately predicting a diagnosis of lymphoma was $\geq$28% of B-cell areas, with a Ki-67 index above 3%. Dogs were monitored for a median follow-up time of 870 days (IQR, 569-1225), and no relapses were documented. Overall median survival time was 1282 days. The combination of histology, immunohistochemistry and PARR can improve the diagnostic accuracy for canine splenic lymphoid nodules, although the long-term behavior of these lesions appears similar.

Keywords

dogs, clonality, immunophenotype, indolent lymphoma, hyperplasia, Ki-67 proliferation index, spleen

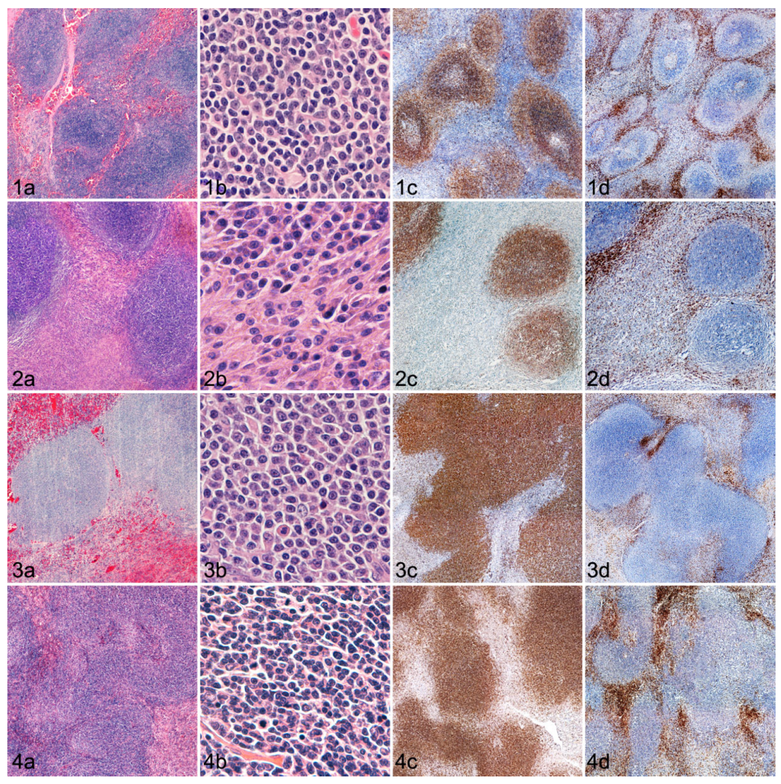


Fig. 1. Lymphoid nodular hyperplasia, spleen, dog. Discrete to merging follicular structures, composed of both small and intermediate-sized B lymphocytes; persistence of an outer ring of T cells surrounding follicles. (a, b) HE, (c) immunohistochemistry (IHC) for CD79a, (d) IHC for CD3. **Fig. 2.** Complex nodular hyperplasia, spleen, dog. Follicular structures are separated by stromal septa composed of collagen and fibroblasts admixed with histiocytes, plasma cells and lymphocytes. (a, b) HE, (c) IHC for CD79a, (d) IHC for CD3. **Fig. 3.** Marginal zone lymphoma, spleen, dog. Irregular and coalescing follicular structures composed predominantly of intermediate-sized B lymphocytes with a moderate amount of cytoplasm and single, large nucleoli; loss of T cells between merging follicles. (a, b) HE, (c) IHC for CD79a, (d) IHC for CD3. **Fig. 4.** Mantle cell lymphoma, spleen, dog. Irregular and coalescing follicular structures composed predominantly of small B lymphocytes with a scant amount of cytoplasm and hyperchromatic nuclei lacking obvious nucleoli. Although there is loss of T cells between merging follicles, they appear globally increased in the section, along with the stromal reaction. (a, b) HE, (c) IHC for CD79a, (d) IHC for CD3.

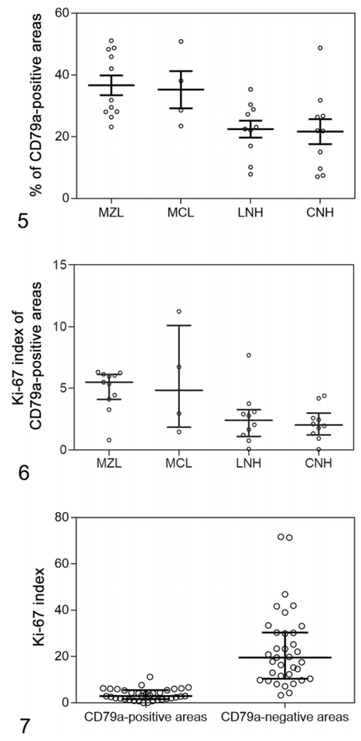
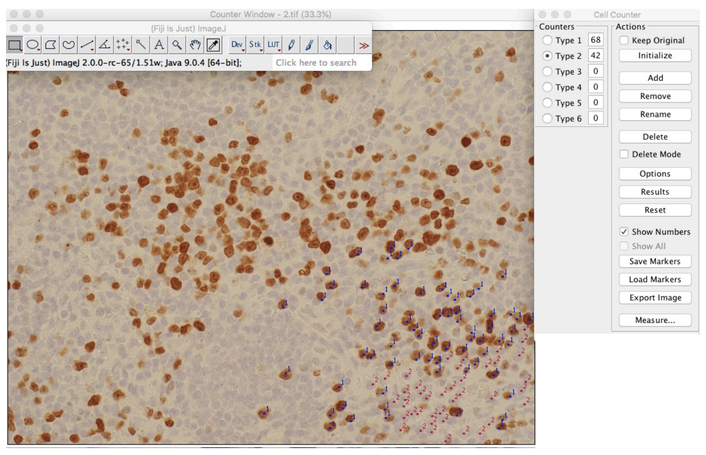


Fig. 5. Percentage of CD79a-positive areas in 35 cases of canine splenic nodular lymphoid lesions. The value was significantly higher in cases diagnosed as nodular lymphoma (mean, 36.3 $\pm$ 10.6%) compared with hyperplasia (mean, 22.1 $\pm$ 10.7%; $P < .001$). MZL, marginal zone lymphoma; MCL, mantle cell lymphoma; LNH, lymphoid nodular hyperplasia; CNH, complex nodular hyperplasia. **Fig. 6.** Ki-67 index of CD79a-positive areas in 35 cases of canine splenic nodular lymphoid lesions. Ki-67 index was significantly higher in cases classified as nodular lymphoma (median, 5.5%; IQR, 3.3-6.2%) compared with hyperplasia (median, 2.1%; IQR, 1.2-3.1%; $P = .001$). **Fig. 7.** Ki-67 index in 35 cases of canine splenic nodular lymphoid lesions. The mean Ki-67 index of CD79a-negative areas was significantly higher than in the CD79a-positive areas ($P < .001$).

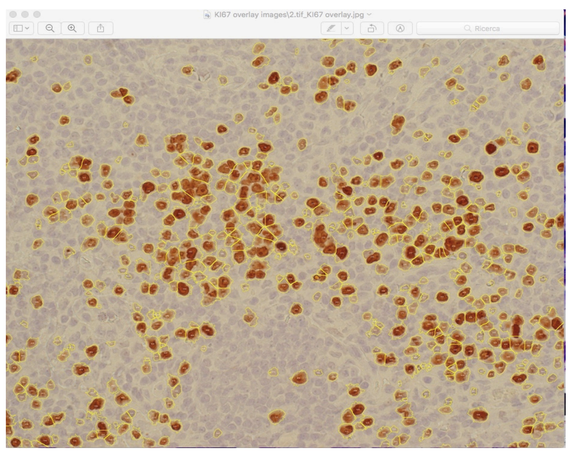
In collaborazione con vetmeduni vienna

In corso: **Validazione dell'indice di proliferazione cellulare (Ki-67 index) con citofluorimetria a flusso come marker prognostico precoce in cani e gatti portatori di linfoma.**

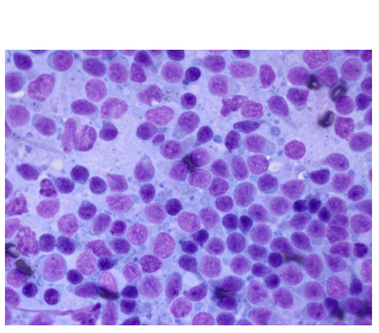
L'indice di proliferazione cellulare (Ki-67 index) ha acquisito in Medicina Veterinaria, come in umana, un importante valore prognostico nell'indagine del comportamento biologico di molti tumori, soprattutto in linfoma, mastocitoma e melanoma. Ki-67 è un antigene nucleare espresso in tutte le fasi attive del ciclo cellulare, ad eccezione di G0, e consente pertanto l'evidenziazione delle cellule in fase replicativa sul totale delle cellule neoplastiche, rendendo così possibile il calcolo di un indice. Ad oggi, la tecnica *gold standard* per la formulazione di questo indice prevede, sia in patologia umana che in veterinaria, l'esame immunoistochimico su sezioni di tessuto fissato in formalina e incluso in paraffina, e la successiva la conta manuale o mediante metodiche semi-automatiche di analisi di immagine delle cellule positive in una determinata area o su un totale di 100 cellule.
La citofluorimetria a flusso è una tecnica computerizzata già molto diffusa in ematologia, che consente in tempi rapidi l'identificazione di cellule vitali marcate con anticorpi secondari cromofori e la stimolazione di questi attraverso luce polarizzata. Attualmente questa tecnica, applicabile su cellule raccolte mediante agoaspirazione, ha assunto un ruolo predominante nella diagnosi e immunofenotipizzazione dei linfomi di cane e gatto, sostituendo progressivamente l'esame istologico e immunoistochimico, anche a causa del fatto che il trattamento del linfoma non prevede un approccio chirurgico. Questo studio mira a validare la citofluorimetria anche nella determinazione del Ki-67 index, tramite comparazione con la classica metodica immunoistochimica seguita da conta manuale o di analisi di immagine.



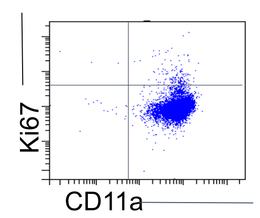
Conta manuale



Conta in analisi di immagine



T-cell lymphoma
CD3+CD4-CD8-,
CD45-MHCII+
CD5+CD21+
PTZL



$Ki67^+$ 1.7%

Flow cytomery assay

Pubblicazioni

Sabattini S, Lopparelli RM, **Rigillo A**, *et al*. Canine splenic nodular lymphoid lesions: immunophenotyping, proliferative activity and clonality assessment. *Veterinary Pathology* 2018. Accepted.

Sabattini S, Renzi A, Marconato L, Militerno G, Agnoli C, Barbiero L, **Rigillo A**, Capitani O, Tinto D, Bettini G. Comparison between May Grünwald-Giemsa and rapid cytological stains in fine-needle aspirates of canine mast cell tumour: diagnostic and prognostic implications. *Veterinary and Comparative Oncology* 2018. Accepted.

Atti di Congresso

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