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Comparison between May Grünwald-Giemsa and rapid cytological stains in fine-needle aspirates of canine mast cell tumour: diagnostic and prognostic implications

Introduction. Mast cell tumours (MCTs) are often diagnosed by cytology based on the identification of purple intracytoplasmic granules with methanolic Romanowsky stains, including May-Grünwald-Giemsa (MGG). In clinical practice, aqueous rapid Romanowsky stains (RS) are commonly used, but mast cell granules may not stain properly. Aim of this prospective study was to investigate the frequency of MCT hypogranularity with RS and its potential implications in tumour identification, cytological grading assessment and recognition of nodal metastatic disease.

Materials and Methods. Cytological preparations of canine primary MCTs and metastatic lymph nodes with subsequent histopathological confirmation were included. For each case, good-quality smears were stained with both MGG and RS and comparatively assessed.

Results. Eleven of 60 (18.3%) primary MCTs were hypogranular with RS; 9 of them were histologically high-grade tumours and in 3 cases (5%) a definitive MCT diagnosis could not be made. Accuracy in cytological grading assessment (85%) did not differ between RS and MGG. Thirteen of 28 (46.4%) metastatic lymph nodes were hypogranular with RS and three independent observers failed to identify nodal MCT metastases in 7-18% of RS-stained smears.

Conclusions. This study confirms that, in a limited number of cases, RS can be ineffective in staining MCT granules, particularly in high-grade tumours, thus making diagnosis more dependent on experience and quality of preparations. In dubious cases, methanolic stains should be applied. The use of RS is discouraged for the search of nodal metastases, as the identification of isolated mast cells can be more challenging.

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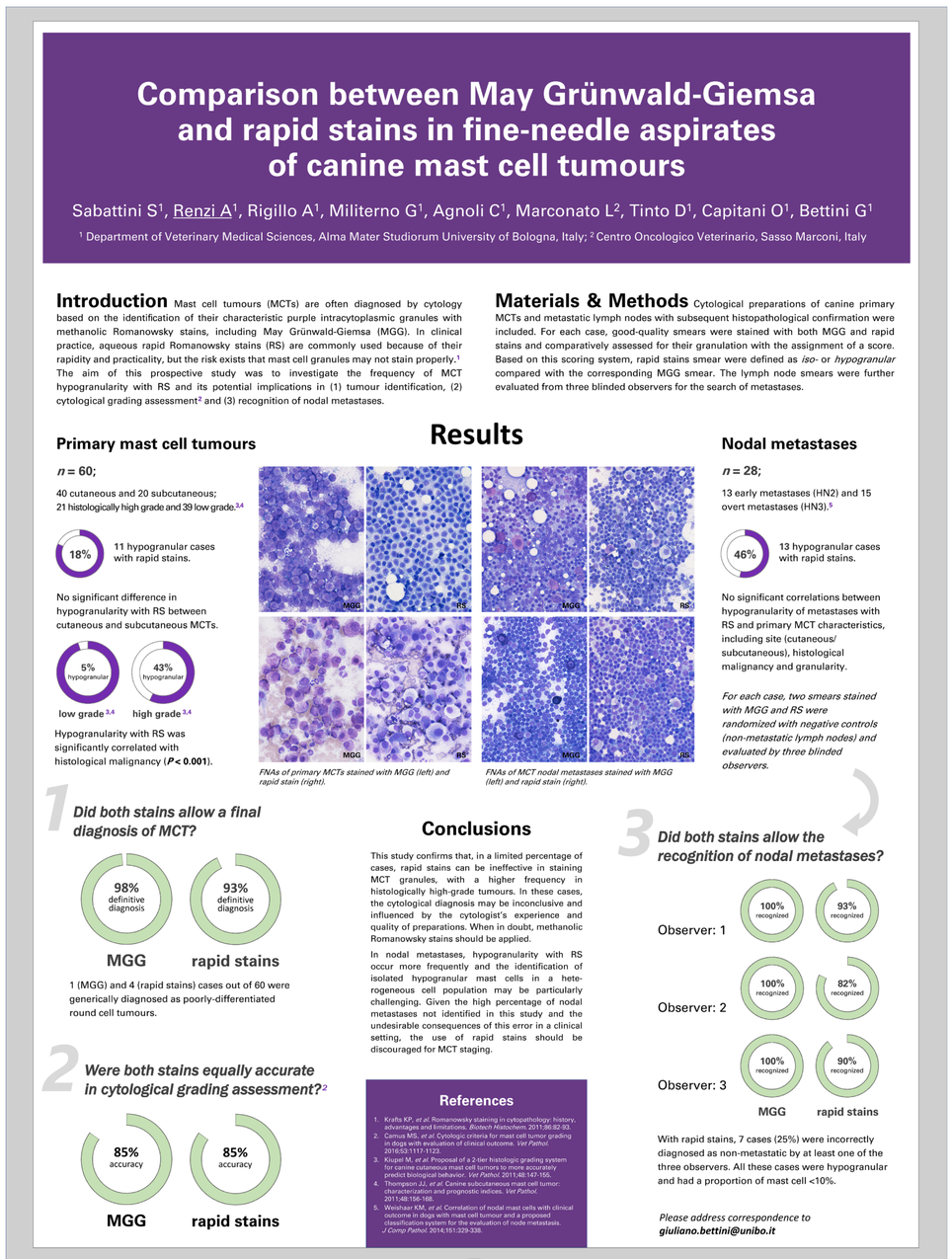

Comparison between May Grünwald-Giemsa and rapid stains in fine-needle aspirates of canine mast cell tumours

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Introduction Mast cell tumours (MCTs) are often diagnosed by cytology based on the identification of their characteristic purple intracytoplasmic granules with methanolic Romanowsky stains, including May Grünwald-Giemsa (MGG). In clinical practice, aqueous rapid Romanowsky stains (RS) are commonly used because of their rapidity and practicality, but the risk exists that mast cell granules may not stain properly.[1] The aim of this prospective study was to investigate the frequency of MCT hypogranularity with RS and its potential implications in (1) tumour identification, (2) cytological grading assessment[2] and (3) recognition of nodal metastases.

Materials & Methods Cytological preparations of canine primary MCTs and metastatic lymph nodes with subsequent histopathological confirmation were included. For each case, good-quality smears were stained with both MGG and rapid stains and comparatively assessed for their granulation with the assignment of a score. Based on this scoring system, rapid stains smear were defined as *iso-* or *hypogranular* compared with the corresponding MGG smear. The lymph node smears were further evaluated from three blinded observers for the search of metastases.

Results

Primary mast cell tumours

n = 60;

40 cutaneous and 20 subcutaneous;
21 histologically high grade and 39 low grade.[3,4]

18% — 11 hypogranular cases with rapid stains.

No significant difference in hypogranularity with RS between cutaneous and subcutaneous MCTs.

5% hypogranular — low grade [3,4]
43% hypogranular — high grade [3,4]

Hypogranularity with RS was significantly correlated with histological malignancy (***P*< 0.001**).

FNAs of primary MCTs stained with MGG (left) and rapid stain (right).

FNAs of MCT nodal metastases stained with MGG (left) and rapid stain (right).

Nodal metastases

n = 28;

13 early metastases (HN2) and 15 overt metastases (HN3).[5]

46% — 13 hypogranular cases with rapid stains.

No significant correlations between hypogranularity of metastases with RS and primary MCT characteristics, including site (cutaneous/subcutaneous), histological malignancy and granularity.

For each case, two smears stained with MGG and RS were randomized with negative controls (non-metastatic lymph nodes) and evaluated by three blinded observers.

1 *Did both stains allow a final diagnosis of MCT?*

98% definitive diagnosis — MGG
93% definitive diagnosis — rapid stains

1 (MGG) and 4 (rapid stains) cases out of 60 were generically diagnosed as poorly-differentiated round cell tumours.

2 *Were both stains equally accurate in cytological grading assessment?*[2]

85% accuracy — MGG
85% accuracy — rapid stains

Conclusions

This study confirms that, in a limited percentage of cases, rapid stains can be ineffective in staining MCT granules, with a higher frequency in histologically high-grade tumours. In these cases, the cytological diagnosis may be inconclusive and influenced by the cytologist's experience and quality of preparations. When in doubt, methanolic Romanowsky stains should be applied.

In nodal metastases, hypogranularity with RS occur more frequently and the identification of isolated hypogranular mast cells in a heterogeneous cell population may be particularly challenging. Given the high percentage of nodal metastases not identified in this study and the undesirable consequences of this error in a clinical setting, the use of rapid stains should be discouraged for MCT staging.

3 *Did both stains allow the recognition of nodal metastases?*

	MGG	rapid stains
Observer: 1	100% recognized	93% recognized
Observer: 2	100% recognized	82% recognized
Observer: 3	100% recognized	90% recognized

With rapid stains, 7 cases (25%) were incorrectly diagnosed as non-metastatic by at least one of the three observers. All these cases were hypogranular and had a proportion of mast cell <10%.

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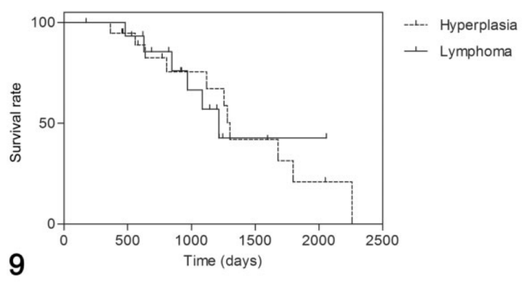
Canine splenic nodular lymphoid lesions: immunophenotyping, proliferative activity and clonality assessment

Table 1. Distribution of Clinical and Pathological Variables According to Diagnosis in 35 Cases of Canine Splenic Nodular Lymphoid Lesions.

Variable	Nodular lymphoma ($n = 15$)	Nodular hyperplasia ($n = 20$)	*P*
Male/female ratio	1:0.9	1:1	.84
Age (years)[a]	9.2 ± 2.2	9.8 ± 3.2	.59
Body weight (kg)	14.2 ± 8.9	20.4 ± 11.6	.093
Presence of clinical signs	8 (53%)	2 (10%)	.008*
Abdominal mass[b]	5 (33%)	2 (10%)	.19
Multiple splenic nodules	3 (20%)	8 (40%)	.28
Diffuse splenomegaly	3 (20%)	7 (35%)	.45
Nodule diameter (cm)[a]	3.4 ± 1.8 cm	1.8 ± 0.9 cm	.002*
Ruptured mass	2 (13.3%)	0 (0.0%)	.17
Hematological abnormalities	6 (40.0%)	3 (15.0%)	.13
CD79a-positive areas[c]	36.3 ± 10.6%	22.1 ± 10.7%	.0004*
CD3-positive areas[c]	14.8 ± 7.3%	13.4 ± 7.2%	.58*
Ki67 index, B cells	5.5% (3.3-6.2%)	2.1% (1.2-3.1%)	.001*
Ki67 index, non-B cells	30.0% (12.4-41.7)	18.0% (9.8-23.4%)	.13
Median survival (days)[e]	1216 (921-1511)	1302 (1231-1373)	.85

*$P<0.05$.
[a]Mean ± standard deviation.
[b]Abdominal mass detected on physical examination.
[c]Percentage of the tissue area occupied by CD79a- or CD3-positive cells.
[d]Median percentage (interquartile range).
[e]Median (95% confidence interval).



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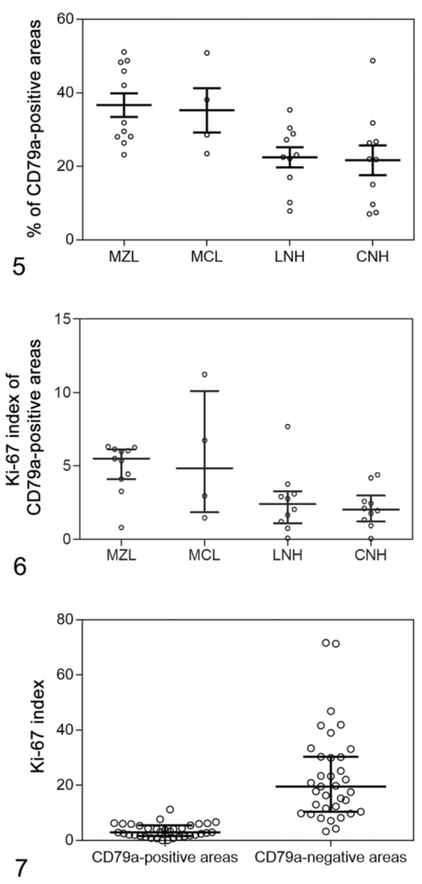

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Fig. 5. Percentage of CD79a-positive areas in 35 cases of canine splenic nodular lymphoid lesions. The value was significantly higher in cases diagnosed as nodular lymphoma (mean, 36.3 ± 10.6%) compared with hyperplasia (mean, 22.1 ± 10.7%; $P < .001$). 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 ($P < .001$).

Introduction. 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.

Materials and Methods. 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.

Results. 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* = 0.0004) and a higher B-cell proliferative activity (median Ki-67 index, 5.49%; *P* = 0.0012). The threshold value most accurately predicting a diagnosis of lymphoma was ≥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, with no significant difference according to final diagnosis (Fig. 9).

Conclusions. 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.

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Publications

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Congress Proceedings

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