COMPARION OF DIGITAL COUNTING METHODS FOR KI67 STAINED MAST CELL TUMORS

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Introduction: Automated analysis of Ki67 expression is more and more commonly used in the daily routine diagnostics and prognostics of canine mast cell tumors. The purpose of this study was to compare different methods within the same tumour cohort and to find a practicable approach for the daily diagnostic routine

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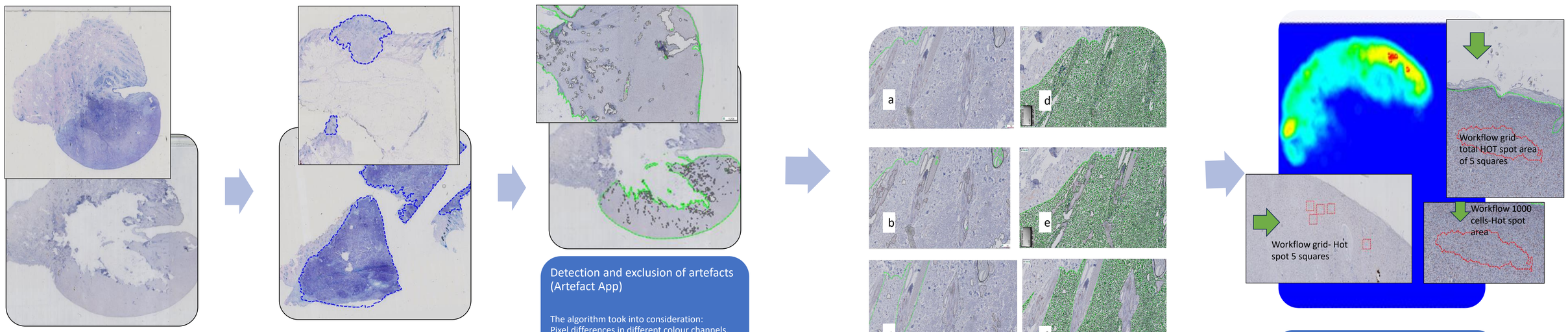
Materials and Methods: FFPE tissue from 78 archived canine mast cell tumors served as material. Slides were reviewed and separated in two groups (cutaneous and subcutaneous), cutaneous were graded according to Patnaik and Kiupel [1,2]. Routinely Giemsa and Ki67 stained slides were scanned and aligned using Visiopharm® for selection of tumour tissue. Ki67 positive nuclei were counted on the whole slide (output: % of total nuclei) using different methods followed by construction of a heat map. Positive cells were then counted in the hot spots according to published literature for manual and automated counting.

Table 1: Workflow of Ki67 counting



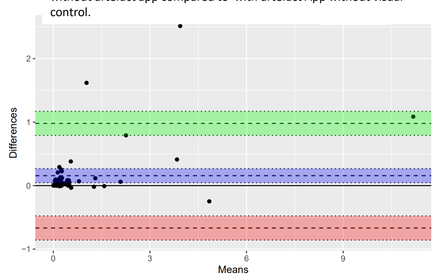
Results:

Table 2 Comparison of percentage of Ki67 positive cells over the whole tumor with/ without artefact exclusion (image a-f table 1)

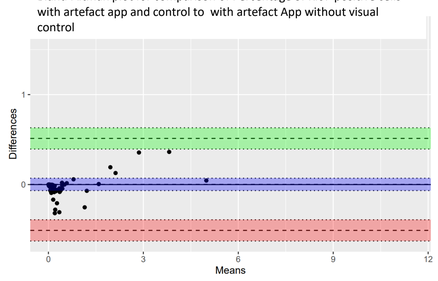
Case Number	Percentage positive cells without artefact APP	Percentage positive cells with artefact APP	Percentage positive cells with artefact APP and visual control
H16-2495.4_ki67	4.719673886	4.967636445	5.012564913
H16-2496.2_ki67	0.400939878	0.37633505	0.316691313
H16-2496.3_ki67	0.734974473	0.354408085	0.0754495
H16-2496.4_ki67	0.16494357	0.091563668	0.026383567
H16-2792.1_ki67	0.113079533	0.114656985	0.112973075
H16-3072.1_ki67	1.842548845	0.223604153	0.139988266
H17-0409.1_ki67	0.3312757	0.212212691	0.15707496
H17-0432.2_ki67	0.25214426	0.137133695	0.044545635
H17-0432.3_ki67	0.377695684	0.151895503	0.069002219
H17-0942.1_ki67	0.025447451	0.025518884	0.0234055
H17-1200.2_ki67	0.180124167	0.095449912	0.034690102
H17-1449.1_ki67	0.310096098	0.26950695	0.195126673
H17-1449.2_ki67	0.523352235	0.470359078	0.469064993
H17-2069.1_ki67	0.235472662	0.136599706	0.062313837
H17-2150.1_ki67	0.374152249	0.122226362	0.078542608
H17-2212.2_ki67	0.466036517	0.461945969	0.41687116
H17-2253.1_ki67	5.197121066	2.683214496	3.039451912
H17-2301.1_ki67	0.539245057	0.572694786	0.587976078
H17-2388.3_ki67	0.481625695	0.396056836	0.314260004
H17-2826.1_ki67	0.194733819	0.204907686	0.190944004
H18-0046.1_ki67	11.71983655	10.63384712	12.38511835
H18-0069.3_ki67	0.382260904	0.36078745	0.042902672
H18-0252.1_ki67	0.399382171	0.366578255	0.325128271
H18-0503.1_ki67	0.00252444	0.001381444	0.001731947
H18-1130.1_ki67	2.118735888	2.057569896	2.18589645
H18-1230.1_ki67	0.089495524	0.01225795	0.012620845
H18-1255.1_ki67	0.014139801	0.013990342	0.013668788
H18-1347.1_ki67	0.07592068	0.072770273	0.07117519
H18-1512.1_ki67	0.038383553	0.019383031	0.006016411
H18-1600.1_ki67	0.423797881	0.37422967	0.166895739
H18-1641.1_ki67	0.11322812	0.109609061	0.082586965
H18-1809.1_ki67	0.152047243	0.112712382	0.038230781
H18-1873.1_ki67	0.264707146	0.264199936	0.207298551
H18-2028.1_ki67	0.52320258	0.440534541	0.430993922
H18-2169.1_ki67	0.433715095	0.418809133	0.43887515
H18-2424.1_ki67	0.505408711	0.506950948	0.507360658
H18-2516.3_ki67	0.018345436	0.017845009	0.018475332
H18-2533.1_ki67	0.018656217	0.016224356	0.012931328
H18-2695.5_ki67	0.023175777	0.021988896	0.016518253
H19-0407.1_ki67	0.019196216	0.018535323	0.011330849
H19-0438.5_ki67	0.053974058	0.049086638	0.045823053
H19-0583.1_ki67	0.462610529	0.463713255	0.463717826
H19-0945.1_ki67	0.244105917	0.036343979	0.023895942
H19-1335.1_ki67	0.170785955	0.142568686	0.136998409
H19-1497.1_ki67	0.032824705	0.022227143	0.021506257
H19-1649.1_ki67	0.138669285	0.049805822	0.026609393
H20-1046.4_ki67	0.461550198	0.415221419	0.347209181
H20-2061.1_ki67	0.117673285	0.021012095	0.013206603
H20-2525.2_ki67	1.254041772	1.272377243	1.019661414
H20-2670.1_ki67	1.362568626	1.2473674	1.177910743
H20-2699.3_ki67	0.28960201	0.235390662	0.067724206
H21-0807.1_ki67	0.831381087	0.76374788	0.822275405
H21-2935.1_ki67	1.578498243	1.58681442	1.592933097
H22-0073.1_ki67	0.496991891	0.49833887	0.191468993
H22-0537.1_ki67	4.046541642	3.634762506	3.99776847
H22-2000.1_ki67	2.653342522	1.860914748	2.053598314

Table 3: Statistical difference of three different workflows

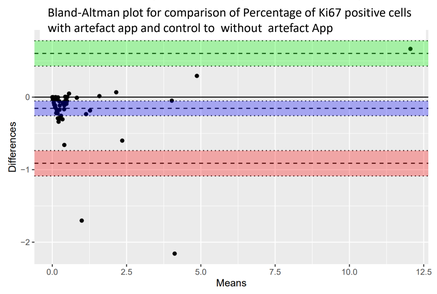
Bland-Altman plot for comparison of Percentage of Ki67 positive cells without artefact app compared to with artefact App without visual control.



Bland-Altman plot for comparison of Percentage of Ki67 positive cells with artefact app and control to with artefact App without visual control



Bland-Altman plot for comparison of Percentage of Ki67 positive cells with artefact app and control to without artefact App

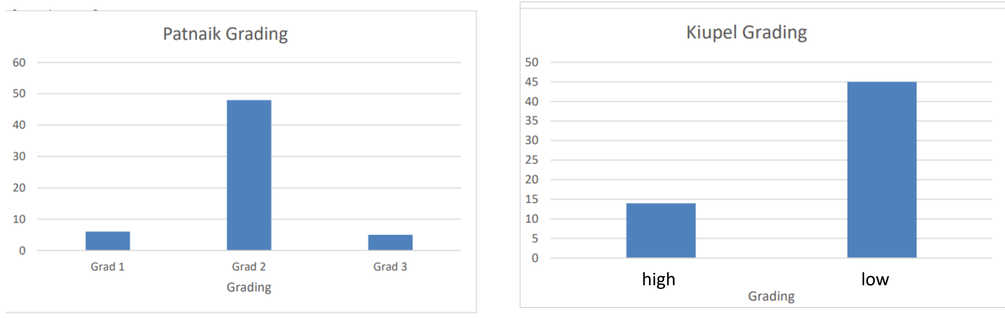


Results: The workflows allowed standardised collection and comparison of the Ki67 index as well as selection of Hotspots. Alignment of Giemsa and Ki67 stained slides was useful for the detection of tumour tissue. Additional visual exclusion of artefacts did not have a significant effect on the counting of positive cells within the whole tumour after application of the artefact APP (Bland Altman plot 1 and 2). Visual control of the hot spots appeared necessary to avoid that melanophages and blood vessels might lead to false positives. The selection of the method (square versus area) in the workflow grid influences the outcome of cases above the threshold.

Table 4: Comparison of cases above the threshold in different Ki67 positive counting methods

Legend: red line shows threshold for a worse prognosis according to data in literature.

Table 5: Distribution of tumor grades according to Patnaik and Kiupel among cutaneous tumors



Conclusions:

This study underlines the need of a standardized approach for the automated Ki67 counting in mast cell tumours among pathologists, which allows comparison of different methods[3,4] with fewer resources. Examination of a larger cohort with clinical data is now needed to compare the different methods.
Techniques published for the microscopic evaluation pose some difficulties when transferred to the automatic counting.
In addition, transparent communication and close collaboration with the laboratory is needed to minimize preanalytical issues and thereby saves pathologists time and costs.

Literature:

1. **Patnaik** AK, Ehler WJ, MacEwen EG. Canine Cutaneous Mast Cell Tumor: Morphologic Grading and Survival Time in 83 Dogs. Vet Pathol. 1984;21(5):469-474. doi:10.1177/030098588402100503
2. Kiupel M, Webster JD, Bailey KL, et al. Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior. Vet Pathol. 2011;48(1):147-155. doi:10.1177/0300985810386469
3. Webster JD, Yuzbasiyan-Gurkan V, Miller RA, Kaneene JB, Kiupel M. Cellular Proliferation in Canine Cutaneous Mast Cell Tumors: Associations with c-KIT and Its Role in Prognostication. Vet Pathol. 2007;44(3):298- 308. doi:10.1354/vp.44-3-29
4. Abadie JJ, Amardeilh MA, Delverdier ME. Immunohistochemical detection of proliferating cell nuclear antigen and Ki-67 in mast cell tumors from dogs. J Am Vet Med Assoc. 1999;215(11):1629-1634.