DOI 10.7251/VETJ1802370V **UDK 616.5-006.6:636.7.09**

Originalni naučni rad

HISTOLOŠKI I IMUNOHISTOHEMIJSKI PARAMETRI BITNI ZA KLASIFIKACIJU MASTOCITOMA PASA[1*]

Ivana Vučićević[1]*, Darko Marinković[1], Vladimir Kukolj[1], Slađan Nešić[1], Milan Aničić[1], Sanja Aleksić-Kovačević[1]

[1] Katedra za patološku morfologiju, Fakultet veterinarske medicine, Univerziteta u Beogradu, Beograd, Srbija
* Korespodentni autor: Ivana Vučićević, e-mail adresa: ivucicevic@gmail.com

Kratak sadržaj: Mastocitomi su najučestaliji tumori kože pasa. Vode poreklo iz kostne srži, a zbog vrlo promenljivog biološkog ponašanja je predloženo nekoliko kriterijuma za njihovu klasifikaciju, uključujući histološku gradaciju i markere proliferacije ćelija. Mitotski indeks, prisustvo višejedarnih ćelija, ćelija sa bizarnim jedrom i kariomegalija su najvažniji parametri za klasifikaciju mastocitoma pasa na mastocitome visokog i niskog stepena maligniteta. Za potvrdu stepena maligniteta mastocitoma bitno je utvrditi i ekspresiju transmembranskog receptora kinaza tipa (KIT) koji igra značajnu ulogu u rastu i diferencijaciji mastocita, a koga kodira c-KIT protoonkogen. Kod mastocitoma visokog stepena maligniteta, osim membranske ekspresije KIT receptora, može se uočiti i aberantna citoplazmatska ekspresija. Ispitana su ukupno 52 mastocitoma pasa i 12 uzoraka nepromenjene kože pasa. Uzorci kože fiksirani u formalinu i ukalupljeni u parafinu su obojeni hematoksilin-eozinom. Ekspresija KIT receptora je ispitana primenom imunohistohemijske metode bojenja. Klasifikacija mastocitoma na mastocitome visokog i niskog stepena maligniteta je izvršena prema kriterijumima Kiupel-ove dvostepene klasifikacije, uzimajući u obzir i ekspresiju KIT receptora. Od ukupno 52 mastocitoma pasa 16 je bilo niskog stepena maligniteta, dok je 36 klasifikovano kao mastocitomi visokog stepena maligniteta, od kojih 19 mastocitoma pokazuje citoplazmatsku ekspresiju KIT receptora. Kod svih 16 mastocitoma niskog stepena maligniteta ispoljena je samo membranska ekspresija KIT proteina. Činjenica da je kod većine mastocitoma visokog stepena maligniteta ispoljena ekspresija KIT receptora u citoplazmi potvrđuje vezu između aberantne ekspresije KIT receptora i povećane ćelijske proliferacije. Prisustvo mitotskih figura, višejedarnih ćelija, bizarnih jedara i kariomegalije, kao i

[1*] Презентован на 23. Годишњем савјетовању доктора ветеринарске медицине Републике Српске (БиХ). Теслић, 6-9- јуна 2018.

tip ekspresije KIT receptora predstavljaju ključne prognostičke parametre kod pasa sa mastocitomima.

Ključne reči: psi, mastocitom, klasifikacija, KIT receptor

UVOD

Mastocitomi su najčešći okruglоćelijski tumori kod pasa, koji ujedno predstavljaju najučestalije tumore kože kod ove životinjske vrste (Welle i sar., 2008). Prema poreklu, mastocitomi pripadaju grupi mezenhimskih tumora kože i mekih tkiva (Hendrick i sar., 1998). Sastavljeni su od različito diferentovanih mastocita koji se uočavaju kao okrugle ćelije sa različitom količinom granula u citoplazmi, poređane u redove ili grupe (Strefezzi i sar., 2009; Jovanović i sar., 2012). Mastocitomi se kod pasa najčešće sreću na trupu (50-60%), ekstremitetima (25 - 40%), glavi i vratu (10%). Na skrotumu, međici, leđima i repu tumor se ređe sreće (Welle i sar., 2008). Makroskopski izgled mastocitoma varira od stepena diferentovanosti. Dobro diferentovani mastocitomi se najčešće pojavljuju kao nodularne, nekapsulirane mase gumaste konzistencije, iznad kojih su polja kože bez dlake. Veličina varira od 1 do 4 cm u prečniku a klinički podsećaju na lipom. Slabije diferentovani mastocitomi imaju tendenciju bržeg rasta, manje su ograničeni i često praćeni inflamacijom i edemom okolnog tkiva (Jubb i sar., 2007). Na njihovoj površini mogu nastati ulceracije, dok se u blizini mogu razviti manji čvorići, tzv. satelitski noduli. Većina mastocitoma nije pigmentisana, ali se kao povremen nalaz mogu uočiti eritematozni i hiperpigmentisani noduli (Welle i sar., 2008). Lezije na distalnim delovima ekstremiteta, usnama i u području prepona mogu biti u vidu slabo definisanih otoka ili nalik dermatitisu uzrokovanom lizanjem (acral lick dermatitis, eng.) (Gross i sar., 2005; Welle i sar., 2008). Maligni mastocitomi mogu metastazirati putem limfe ili krvi i u većini slučajeva, prvi znak metastaze je uvećanje regionalnog limfnog čvora. Metastaze mastocitoma na plućima su retke, dok se uglavnom javljaju na slezini, jetri i bubrezima, a mogu se često dijagnostikovati i na koži (Morris i sar., 2001).

Etiologija mastocitoma nije u potpunosti poznata, ali se smatra da je, kao i kod većine tumora, multifaktorijalna. Predispozicija pojedinih rasa govori u prilog značaja genetske kompmonente u nastanku mastocitoma (Welle i sar., 2008). Noviji radovi ističu ulogu površinskog receptora tirozin kinaze (KIT protein, CD117) u etiologiji mastocitoma (Vucicevic i sar., 2016; Halsey i sar., 2017).

Dijagnoza mastocitoma postavlja se na osnovu citoloških bojenja preparata dobijenih pravljenjem razmaza dobijenog biopsijom tumora iglom ili

pravljenja otisak preparata tkiva tumora. Od metoda bojenja, najčešće se koriste Romanowsky tip bojenja (npr. *Diff-Quik, Giemsa, Wright's*) ili New Methylene Blue (London i sar., 2003; Strefezzi i sar., 2009). Dok se na osnovu citoloških bojenja može utvrditi da li je u pitanju mastocitom, za klasifikaciju tumora je neophodan histopatološki pregled biopsiranog tkiva (Bostock, 1973; Patnaik i sar., 1984; Kiupel i sar., 2011). Diferencijalno dijagnostički, mastocitomi (naročito slabo diferentovani) se mogu zameniti sa drugim okruglo-ćelijskim tumorima (limfomom, plazmocitomom, histiocitomom i transmisivnim veneričnim tumorom) (Welle i sar., 2008; Oliveira i sar., 2012). U svrhu potvrđivanja dijagnoze mastocitoma i razlikovanja od drugih okruglućelijskih tumora koristi se toluidin plavo (*toluidin blue* - TB, eng.) bojenje za vizuelizaciju citoplazmatskih granula (London i sar., 2003; Strefezzi i sar., 2009).

Klasifikacija mastocitoma je veoma bitna sa prognostičkog aspekta i izbora terapije. Primarna klasifikaciona determinanta je histološka građa mastocitoma. U novije vreme, koristi se dvostepeni sistem klasifikacije na mastocitome visokog i niskog stepena maligniteta. Na osnovu ove podele, mastocitomi visokog stepena maligniteta moraju imati ispunjen najmanje jedan od sledećih kriterijuma: najmanje 7 mitotskih figura na 10 hpf (*High-power Fields* – hpf, eng.), najmanje 3 multinuklearne ćelije na 10 hpf, najmanje 3 bizarna jedra na 10 hpf ili prisustvo kariomegalije kod najmanje 10% neoplastičnih ćelija (Kiupel i sar., 2011). Predloženi dvostepeni sistem gradiranja trebao bi da se koristi pri svakom rutinskom histološkom ispitivanju i dijagnostikovanju mastocitoma. Za mastocitome visokog stepena maligniteta treba primeniti dodatna ispitivanja, kao što su određivanje ekspresije KIT receptora i skrining na c-KIT mutacije, kako bi se odredila adekvatna terapija (Kiupel i sar., 2011, Sabattini i sar., 2014).

KIT protein je receptor za faktor rasta matične ćelije i normalno se nalazi na površini hematopoetskih ćelija i mastocita, igrajući značajnu ulogu u rastu i diferencijaciji ovih ćelija. KIT receptor je transmembranski receptor kinaza tip III koga kodira c-KIT protoonkogen. Ekspresija KIT receptora dokazana je imunohistohemijskim bojenjem, kako na površini normalnih, tako i na površini neoplastično transformisanih mastocita pasa. Povećana ekspresija zabeležena je kod slabo diferentovanih i nediferentovanih mastocitoma (Welle i sar., 2008; El-Agamy, 2012). Izuzev membranske ekspresije, opisana je ekspresija KIT proteina fokalno i difuzno u citoplazmi. Tip I ekspresije KIT receptora se karakteriše prebojavanjem membrane mastocita usled lokalizacije KIT proteina u membrani ćelija, dok je prebojavanje citoplazme prisutno u maloj meri ili potpuno izostaje. Tip II ekspresije se karakteriše difuznim prebojavanjem citoplazme. Tip III ekspresije je takođe vezan za prisustvo KIT proteina u

citoplazmi naoplastičnih mastocita i karakteriše se intenzivnim fokalnim prebojavanjem citoplazme neoplastičnih mastocita. Poremećaji u ekspresiji mogu doprineti neoplastičnoj transformaciji (Misdorp, 2004; Takeuchi i sar., 2010). Povećano prisustvo KIT receptora u citoplazmi (fokalno ili difuzno) je u korelaciji sa kraćim preživljavanjem pasa sa mastocitomom, kao i sa kraćim intervalom do remisije bolesti u odnosu na mastocitome kod kojih je povećana ekspresija membranskih KIT receptora (Webster i sar., 2004).

Cilj našeg ispitivanja je da odredimo vezu između lokalizacije KIT receptora na malignim mastocitima i histološkog stepena posmatranog mastocitoma kod pasa.

MATERIJAL I METODE

Životinje

Ispitivanjima su obuhvaćeni isečci kože 52 psa kod kojih je kliničkim pregledom postavljena sumnja na mastocitom. Isečci nepromenjene kože 12 odraslih pasa, uzorkovani na obdukciji, služili su kao kontrola. Psi različite rase i pola, uzrasta od 3 do 11 godina, poticali su sa teritorije Republike Srbije. Histopatološka dijagnoza kod ovih pasa postavljena je na Katedri za patološku morfologiju, Fakulteta veterinarske medicine, Univerziteta u Beogradu.

Histopatologija

Neposredno nakon biopsije tumora kože pasa, uzorci su fiksirani u 10% neutralnom puferizovanom formalinu ne duže od 48 sati, posle čega su procesovani u automatskom tkivnom procesoru LEICA TP1020. Parafinski blokovi su sečeni pomoću mikrotoma LEICA RM 2235 na tkivne isečke debljine 3-5 µm. Dobijeni preparati su bojeni hematoksilin-eozin (HE) metodom. Na preparatima bojenim hematoksilin-eozinom postavljena je preliminarna patohistološka dijagnoza mastocitoma, a za njenu potvrdu, preparati su dalje bojeni TB metodom.

Histopatološke osobine tumora, kao što su prisustvo mitoza, multijedarnih ćelija, bizarnih jedara i kariomegalije analizirani su na preparatima bojenim hematoksilin-eozinom. Broj navedenih parametara određivan je na deset vidnih polja svakog tumora, pri uveličanju 400 puta primenom metode opisane od strane Romansik i sar. (2007). Na osnovu navedenih parametara, tumori su razvrstani u dva stepena diferentovanosti – tumore visokog stepena maligniteta i na tumore niskog stepena maligniteta.

Imunohistohemijsko bojenje

U formalinu fiksirani i u parafinu ukalupljeni tkivni uzorci sečeni su na 5 mm tanke isečke koji su zatim bojeni primenom višestepene indirektne imunohistohemijske (IHC) tehnika. Isečci su inkubirani sa primarnim antitelom CD117 (polyclonal rabbit anti-human antibody (DAKO, A4502))

razblaženim fosfatnim puferom (PBS) u odnosu 1:400. Imunorekcija je vizualizovana upotrebom DAB + (3,3'-diaminobenzidinom tetrahidrohlorid, DAKO, K3468). Za kontrastiranje korišćen je Majerov hematoksilin. Korišćene su odgovarajuće pozitivne i negativne kontrole.

Morfometrijska analiza

Za morfometrijska ispitivanja korišćen je morfometrijski softver Olympus Cell B, uz upotrebu kamere Olympus Color View III. Lokalizacija KIT proteina je određena na način opisan u istraživanju Webster i sar. (2007), s obzirom na to da se razlikuju membranski KIT proteini i dve vrste citoplazmatskih KIT proteina - fokalno i difuzno raspoređeni u citoplazmi. Koji od KIT proteina preovlađuje, određivano je na osnovu prisustva kod najmanje 10% neoplastičnih ćelija tumora. Ćelije na ivici tumora nisu uzimane u razmatranje.

Statistička analiza

U statističkoj analizi dobijenih rezultata, kao osnovne statističke metode, korišćeni su deskriptivni parametri, kao što su aritmetička sredina i standardna devijacija. Prilikom testiranja i utvrđivanja statistički značajnih razlika korišćen je χ^2 test. Pomoću ovih testova utvrđivano je postojanje statistički signifikantne razlike između ispitivanih parametara na nivou značajnosti od 5% i 1%.

Rezultati

Histološkom analizom isečaka mastocitoma bojenih hematoksilin-eozinom kod 53,85% mastocitoma uočeno je više od šest mitotskih figura na 10 hpf (slika 1a), dok se 46,15% mastocitoma odlikovalo slabijom mitotskom aktivnošću.

Prisustvo više od dve multinuklearne ćelije na 10 hpf bilo je uočeno kod 59,61% mastocitoma (slika 1b). Kod 17,31% mastocitoma su bile su prisutne po dve multinuklearne ćelije, dok je kod 19,23% mastocitoma bila uočena po jedna multinuklearna ćelija na istom broju vidnih polja. Multinuklearne ćelije nisu bile uočene kod 3,85 % mastocitoma.

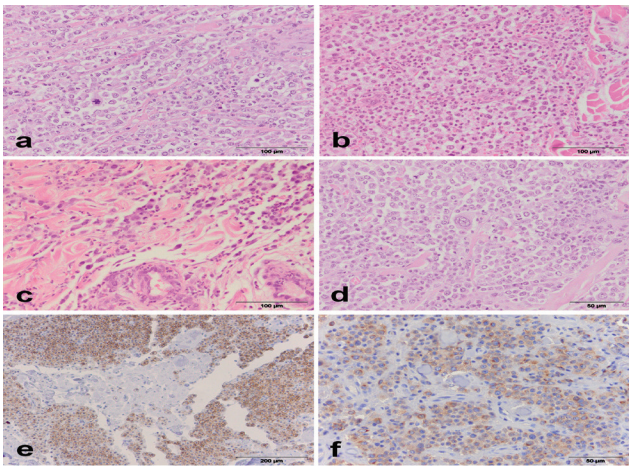
Više od dve ćelije sa bizarnim jedrom na 10 hpf bile su prisutne kod 55,77% mastocitoma (slika 1c). Dve ćelije sa bizarnim jedrom na istom broju vidnih polja bile su uočene kod 7,69% mastocitoma, dok je po jedna ćelija sa bizarnim jedrom bila prisutna kod 26,92% mastocitoma. Kod 9,61% mastocitoma nisu bile uočene ćelije sa bizarnim jedrima.

Više od 10% anaplastičnih mastocita sa jedrima promera oko 8-10 mikrometara bilo je uočeno kod 19,23% mastocitoma, dok je 42,30% mastocitoma sadržalo manje od 10% ćelija sa povećanim jedrom (slika 1d). Kod 38,46% mastocitoma nije bila prisutna kariomegalija

Na osnovu kriterijuma Kiupelove klasifikacije mastocitoma i ustanovljenih parametara (mitotski indeks, multijedarne ćelije, bizarna jedra i

kariomegalija) kod dijagnostikovanih mastocitoma, uočilo se da je 36 (69,23%) mastocitoma odgovaralo mastocitomima višeg stepena maligniteta, dok je 16 (30,77%) mastocitoma bilo nižeg stepena maligniteta.

Imunohistohemijskim bojenjem potvrđeno je prisustvo KIT receptora u isečcima tkiva kože pasa sa mastocitomom. Mikroskopskim pregledom preparata uočavaju se tri tipa imunohistohemijskog bojenja mastocita pozitivnih na KIT protein. Većina mastocitoma u ovom ispitivanju imala je tip I ekspresije KIT receptora, odnosno membransku ekspresiju (63,46%) (Slika 1e). Ovoj grupi su pripadali svi mastocitomi niskog stepena maligniteta i 17 od 36 matocitoma visokog stepena maligniteta. Tip II ekspresije bio je prisutan kod 21,15% mastocitoma (Slika 1f) od kojih su svi bili visokog stepena maligniteta. Nijedan od pregledanih mastocitoma nije imao samo tip III ekspresije, ali su se uočavali kombinovani tipovi ekspresije, i to: 11,54% mastocitoma je ispoljavalo membranski i fokalni citoplazmatski tip ekspresije, dok je 3,85% mastocitoma imalo membranski i difuzni citoplazmatski tip ekspresije KIT proteina.



Slika 1. Koža, pas: a) Prisustvo mitotskih figura kod mastocitoma visokog stepena maligniteta, HE; b) Prisustvo multijedarnih ćelija kod mastocitoma visokog stepena maligniteta, HE; c) Prisustvo ćelija sa bizarnim jedrom kod mastocitoma visokog stepena maligniteta, HE; d) Prisustvo kariomegalije kod mastocitoma visokog stepena maligniteta, HE; e) Ekspresija KIT receprora, tip I (membranski) ekspresije, CD117, LSAB2; f) Ekspresija KIT receprora, tip II (difuzni citoplazmatski) ekspresije, CD117, LSAB2;

DISKUSIJA

Mastocitomi predstavljaju najučestalije tumore kože pasa, naročito kada se radi o pojedinim rasama kao što su bokser, zlatni retriver, labrador retriver i drugi (Welle i sar., 2008). Na osnovu rezultata dobijenih u našem istraživanju najveća učestalost mastocitoma uočena je kod pasa rase zlatni retriver (37,5%), a zatim kod pasa rase bokser (20.7%). Slično ovim rezultatima, većina autora navodi da su mastocitome najčešće dijagnostikovali kod pasa rase bokser (London i sar., 2003; Webster i sar., 2006a; Gil da Costa i sar., 2007; Welle i sar., 2008, Vučićević i sar., 2018). Drugi smatraju da iako bokseri imaju povećan rizik od nastanka mastocitoma, kod njih se uglavnom javljaju mastocitomi niskog stepena maligniteta koji imaju povoljniju prognozu (Thamm i sar., 2007).

Histološka građa se čini najdoslednijim prediktivnim parametrom i u bliskoj je vezi sa dvostepenim sistemom klasifikacije mastocitoma koji je predložio Kiupel (Kiupel i sar., 2011) prema kome se mastocitomi dele na na mastocitome visokog i niskog stepena maligniteta. Prema kriterijumima dvostepene klasifikacije, mastocitomi pregledani u okviru našeg istraživanja klasifikovani su u mastocitome visokog i mastocitome niskog stepena maligniteta. Od ukupno 52 mastocitoma pasa, trideset šest pripada mastocitomima visokog stepena maligniteta, dok šesnaest mastocitoma ima morfološke karakteristike koje ukazuju na nizak stepen maligniteta. Više od šest mitotskih figura na 10 hpf uočeno je kod 77,78% mastocitoma visokog stepena maligniteta, te se prisustvo mitotskih figura smatra statistički značajnim parametrom za klasifikaciju mastocitoma u ovom ispitivanju. Dok pojedini autori smatraju da i mastocitomi nižeg stepena maligniteta, mogu da metastaziraju, te da određivanje samo mitotskog indeksa nije pouzdan način predviđanja ponašanja tumora (Séguin i sar., 2006; Webster i sar., 2007), drugi autori smatraju da je mitotska aktivnost ključna osobina za prognozu ponašanja mastocitoma (Romansik i sar., 2007; Kiupel i sar., 2011). Pojedini autori smatraju da se smrtnost povećava već u slučajevima kada je mitotski indeks veći od 5/10 hpf (O'Connell i Thomson, 2011).

Prisustvo više od dve multinuklearne ćelije na 10 hpf uočeno je kod 86,11% mastocitoma visokog stepena maligniteta, što ukazuje da je ovo statistički značajan pokazatelj klasifikacije mastocitoma. Neka ispitivanja ukazuju da postoji značajna povezanost između broja multinuklearnih ćelija u mastocitomu i vremena preživljavanja pasa, tako da se vreme preživljavanja pasa smanjivalo sa povećanjem broja multinuklearnih ćelija (Thompson, 2012).

U našem ispitivanju uočeno je prisustvo više od dve ćelije sa bizarnim jedrom na 10 hpf kod 86,11% mastocitoma višeg stepena maligniteta, dok kod mastocitoma niskog stepena maligniteta nije zabeleženo prisustvo multinuklearnih ćelija.

Takođe, je uočeno da 27,78% mastocitoma visokog stepena maligniteta sadrži više od 10% anaplastičnih mastocita sa jedrima promera oko 8-10 mikrona, što ukazuje na to da se kariomegalija ne može razmatrati kao značajan prediktivni faktor u ovoj studiji.

Postoji značajna korelacija između mastocitoma visokog stepena maligniteta i bržeg javljanja metastaza, kao i kraćeg vremena preživljavanja pacijenata (Kiupel i sar., 2011; Stern, 2012). Stoga, za mastocitome visokog stepena maligniteta treba primeniti dodatna ispitivanja, kao što su određivanje ekspresije KIT receptora i skrining na c-KIT mutacije, kako bi se odredila adekvatna terapija.

Novija istraživanja ukazuju na postojanje, kako normalnih (membranskih), tako i aberantnih citoplazmatskih (fokalnih i difuznih) KIT receptora na mastocitima pasa (Morini i sar., 2004; Gil da Costa i sar., 2007). Za razliku od ranijih istraživanja (Reguera i sar., 2000) koja proučavaju odnos između intenziteta imunohistohemijskog bojenja koji ukazuje na stepen ekspresije KIT proteina i stepena maligniteta tumora, naša studija istražuje povezanost ekspresije aberantnih tipova KIT proteina i histopatološkog stepena mastocitoma. U ispitivanju koje smo sproveli, uočava se da kod svih mastocitoma niskog stepena maligniteta postoji isključivo membranski tip ekspresije KIT receptora, dok se kod mastocitoma visokog stepena maligniteta pored membranskog tipa ekspresije, javljaju i aberantni tipovi ekspresije KIT proteina. Iz naših rezultata se uočava da kod mastocitoma niskog stepena maligniteta nema aberantne ekspresije KIT receptora. Naime, nijedan od aberantnih tipova ekspresije se ne izdvaja kao statistički značajan u odnosu na ostale aberantne tipove ekspresije KIT proteina. Međutim, jasna je povezanost aberantnih tipova ekspresije CD117 receptora i stepena maligniteta tumora. Pojedini autori navode da je KIT receptor transmembranski protein, i kao takav, imunoreaktivnost ovog proteina je lokalizovana na citoplazmatskoj membrani nepromenjenih mastocita. Rezultati ispitivanja potvrđuju da što mastocitomi pasa imaju agresivnije biološko ponašanje, to imaju i veću ekspresiju KIT proteina u citoplazmi mastocita (Kiupel i sar., 2004). Takođe, isti autori smatraju da mastocitomi pasa sa povećanom ekspresijom KIT proteina u citoplazmi neoplastičnih mastocita, imaju povećan rizik od lokalnog recidiva i kraće vreme preživljavanja. Neki autori naglašavaju da postoji jaka korelacija između citoplazmatske (aberantne) imunoekspresije KIT proteina i povećane ćelijske proliferacije, a samim tim i višeg stepena maligniteta, ali da nisu uočili značajne razlike između fokalne i difuzne citoplazmatske ekspresije CD117 receptora, što sugeriše da fokalna i difuzna citoplazmatska ekspresija KIT proteina možda odražavaju slične ćelijske promene. Poznato je da se citoplazmatska ekspresija KIT receptora dovodi u vezu i sa nekrotičnim i ulceroznim promenama. Naime, citoplazmatska ekspresija KIT receptora je u vezi sa povećanom proliferacijom mastocita, a pojava nekroza u mastocitomu može biti

odraz povećane ćelijske proliferacije koju nije u mogućnosti da isprati odgovarajuća angiogeneza. S druge strane, korelacija između citoplazmatska ekspresije KIT receptora i pojave epidermalnih ulceracija može biti posledica KIT – posredovanog oslobađanja histamina i serotonina, što uzrokuje intenzivan svrab (Gil da Costa i sar., 2007).

ZAKLJUČAK

U novije vreme kao najbitniji prediktivni faktori smatraju se prisustvo mutacija c-kit protoonkogena i aberantna ekspresija KIT proteina. Medjutim, pojedini radovi navode da se aberantna KIT ekspresija može uočiti i kod mastocitoma visokog stepena maligniteta kod kojih nije detektovano prisustvo mutacija (Webster i sar., 2006, Vucicevic i sar., 2016). Stoga, bi određivanje ekspresije KIT proteina trebalo da bude deo rutinske dijagnostike i klasifikacije mastocitoma kod pasa.

LITERATURA

1. Bostock D.E., Crocker J., Harris K., Smith P (1989): Nucleolar organiser regions as indicators of post-surgical prognosis in canine spontaneous mast cell tumours. British Journal of Cancer 59(6): 915–18.
2. El-Agamy D.S (2012): Targeting c-KIT in the therapy of mast cell disorders: current update. European Journal of Pharmacology 690(1-3): 1-3.
3. Gil da Costa R.M., Matos E., Rema A., Lopes C., Pires M.A., Gartner F (2007): CD117 immunoexpression in canine mast cell tumours: correlations with pathological variables and proliferation markers. BMC Veterinary Research 21(3): 19.
4. Gross T.L., Ihrke P., Walder E.J., Affolter V.K (2005): Mast cell tumors. Skin Diseases of the Dog and Cat Clinical and Histopathologic Diagnosis. 2nd edn. Oxford, UK: Blackwell Science Ltd 853–65.
5. Halsey C.H.C., Thamm D.H., Weishaar K.M., Burton J.H., Charles J.B., Gustafson D.L., Avery A.C., Ehrhart E.J (2017): Expression of Phosphorylated KIT in Canine Mast Cell Tumor. Veterinary Pathology 54(3): 387-394.
6. Hendrick M.J., Mahaffey E.A., Moore F.M., Vos J.H., Walder E.J (1998): Mast cell tumors. In: World Health Organization International Histological Classification of Tumors of Domestic Animals, Histological Classification of Mesenchymal Tumors of the Skin and Soft Tissues of Domestic Animals. 2nd series, ed. Schulman FY, 28–29. Armed Forces Institute of Pathology, Washington, DC.

7. Jovanović M., Aleksić-Kovačević S., Knežević M (2012): Specijalna veterinarska patologija, Makarije d.o.o., Beograd.
8. Jubb, Kennedy, Palmer (2007): Pathology of Domestic Animals, Saunders Elsevier, 5th edition, 771-73.
9. Kiupel M., Webster J.D., Kaneene J.B., Miller R., Yuzbasiyan-Gurkan V (2004): The use of kit and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors Vet Pathol 41(4): 371-77.
10. Kiupel M., Webster J.D., Bailey K.L., Best S., DeLay J., Detrisac C.J. i sar. (2011): Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior. Veterinary Pathology 48(1): 147-55.
11. London C.A., Seguin B (2003): Mast cell tumors in the dog. Veterinary Clinics of North America Small Animal Practice 33(3): 473-89.
12. Misdorp W (2004): Mast cells and canine mast cell tumours: a review. Veterinary Quarterly 26(4): 156-69.
13. Morris J., Dobson J (2001): Small Animal Oncology. Blackwell Science 59-62.
14. O'Connell K., Thomson M (2011): Evaluation of prognostic indicators in dogs with multiple, simultaneously occurring cutaneous mast cell tumours: 63 cases. Veterinary and Comparative Oncology 11(1): 51-62.
15. Oliveira F.N., Elliott J.W., Lewis B.C., Mathews G.G., Brown R.M., Treadway C.M., Langohr I.M (2013): Cutaneous Mast Cell Tumor With Epitheliotropism in 3 Dogs. Veterinary Pathology 50(2): 234-37.
16. Patnaik A.K., Ehler W.J., MacEwen E.G (1984): Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. Veterinary Pathology 21(5): 469-74.
17. Reguera M.J., Rabanal R.M., Puigdemont A., Ferrer L (2000): Canine mast cell tumors express stem cell factor receptor. Am J Dermatopathol 22(1): 49-54.
18. Romansik E.M., Reilly C.M., Kass P.H., Moore P.F., London C.A (2007): Mitotic index is predictive for survival for canine cutaneous mast cell tumors. Vet Pathol 44(3): 335-41.
19. Sabattini S., Scarpa F., Berlato D., Bettini G (2014): Histologic grading of canine mast cell tumor: Is 2 better than 3?. Vet. Pathol 52(1): 70-3
20. Seguin B., Besancon M.F., McCallan J.L. I sar. (2006): Recurrence rate, clinical outcome, and cellular proliferation indices as prognostic indicators after incomplete surgical excision of cutaneous grade II mast cell tumors: 28 dogs (1994-2002). J Vet Intern Med 20: 933-940.

21. Stern W.A (2012): Histologic Grading of Canine Cutaneous Mast Cell Tumors: Is There a Good System?. J Vet Sci Med Diagn 1: 1.
22. Strefezzi R.F., Kleeb S.R., Xavier J.G., Catao-Dias J.L (2009): Prognostic indicators for mast cell tumors. Bray J Vet Pathol 2: 110-21.
23. Takeuchi Y., Fujino Y., Watanabe M., Nakagawa T., Ohno K., Sasaki N., Sugano S., Tsujimoto H (2010): Aberrant autophosphorylation of c-KIT receptor in canine mast cell tumor cell lines. Vet. Immunol. Immunopathol 137(3-4): 208-16.
24. Thamm D.H., Vail D.M: Mast cell tumors, In: Withrow SJ, MacEwen EG, editors (2007): Small Animal Clinical Oncology, Saunders, Philadelphia.
25. Thompson J.J (2012): Canine Mast Cell Tumours: Characterization of Subcutaneous Tumours and Receptor Tyrosine Kinase Profiling. PhD Thesis, Guelph, Ontario, Canada.
26. Vučićević, I., Marinković, D., Kukolj, V., Nešić, S., Aničić, M., Aleksić-Kovačević, S (2016): Kit receptor expression in canine cutaneous mast cell tumours (CMCTS) without c-kit mutation. Acta Veterinaria-Beograd 66 (2): 222-233
27. Vučićević I., Marinković D., Kukolj V., Nesić S., Aničić M., Aleksić-Kovačević S (2018): Mast cell tumor within a lipoma in a white toy poodle. Veterinarski Glasnik, 72 (1): 64-67.
28. Webster J.D., Kiupel M., Kaneene J.B., Miller R., Yuzbasiyan-Gurkan V (2004): The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. Vet Pathol 41(4): 371-7.
29. Webster J.D., Yuzbasiyan-Gurkan V., Kaneene J.B., Miller R., Resau J.H., Kiupel M., (2006): The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. Neoplasia 8(2): 104-11.
30. Webster J.D., Yuzbasiyan-Gurkan V., Miller R.A., Kaneene J.B., Kiupel M (2007): Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. Veterinary Pathology 44(3): 298-308.
31. Welle M.M., Rohrer C.B., Howard J., Rüfenacht S (2008): Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. Veterinary Dermatology 19(6): 321-39.

Rad primljen: 30.04.2018.
Rad prihvaćen: 11.10.2018.

Veterinary Journal of Republic of Srpska (Banja Luka), Vol.XVIII, No.2, 370 – 391, 2018
Vučićević et all:
Important histological and immunochemical parameters for classification of mastocytoma in dogs

DOI 10.7251/VETJEN1802370V **UDK 616.5-006.6:636.7.09**

Original scientific paper

IMPORTANT HISTOLOGICAL AND IMMUNOCHEMICAL PARAMETERS FOR CLASSIFICATION OF CANINE MAST CELL TUMORS[2*]

Ivana Vučićević[1]*, Darko Marinković[1], Vladimir Kukolj[1], Slađan Nešić[1], Milan Aničić[1], Sanja Aleksić-Kovačević[1]

[1] Department for Pathological Morphology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia
* Corresponding Author: Ivana Vučićević, e-mail address: ivucicevic@gmail.com

Abstract: Mast cell tumors (MCTs) are one of the most common neoplasms in dogs. They originate from the bone marrow. Several criteria have been proposed for their classification, including histological grading and cell proliferation markers. The mitotic index, multiple nuclei, cells with bizarre nuclei and karyomegaly are the most important parameters for the classification of MCTs on high and low-grade malignancy. For the confirmation of MCT malignancy grade it is also important to determine the expression of the transmembrane kinase (KIT) receptor that has a significant role in the growth and differentiation of mast cell tumors. In high-grade malignancy MCTs in addition to membrane expression of the KIT receptor, aberrant cytoplasmic expression can be also observed. Fifty-two MCTs and 12 control samples of skin from dogs without MCTs were examined. Routinely processed tissue samples were stained with hematoxylin-eosin. Expression of KIT receptor was tested immunohistochemically. The classification of MCTs on high and low-grade malignancy was performed according to the Kiupel's 2-tier classification and the expression of KIT receptors. Sixteen of 52 MCTs were of a low-grade malignancy, while thirty-six were classified as a high-grade malignancy of which 19 MCTs showed aberrant cytoplasm labelling of KIT receptor. All 16 low-grade malignancy MCTs had only membrane expression of KIT receptor. Most high-grade malignancy MCTs showed cytoplasmic KIT expression indicating the link between aberrant KIT expression and increased cell proliferation. The presence of mitotic figures, multinucleated cells, bizarre nuclei and karyomegaly, as well as KIT receptor

[2*] Presented at the 23rd Annual Consulling of Doctors of Veterinary Medicine of Republic of Srpska (B&H). Teslic, June 6-9, 2018

expression pattern are the most important prognostic factors in dogs with mast cell tumor.

Key words: *dogs, mast cell tumor, classification, KIT receptor*

INTRODUCTION

Mast cell tumors are the most common round cell tumors in dogs, and at the same time are the most common skin tumors in this animal species (Welle et al., 2008). Mast cell tumors belong to a group of mesenchymal tumors of the skin and soft tissues (Hendrick et al., 1998). They are composed of differentiated mast cells, which are seen as round cells with different amounts of granules in the cytoplasm, sorted into rows or groups (Strefezzi et al., 2009; Jovanović et al., 2012). Mast cell tumors in dogs are most commonly found on the trunk (50-60%), extremities (25-40%), head and neck (10%). This tumor is less common on scrotum, perineum, back and tail (Welle et al., 2008). The macroscopic appearance of mast cell tumors varies from the degree of differentiation. Well-differentiated mast cell tumors are most commonly present as nodular, nonencapsulated rubber-like mass, above which the areas of the skin are hairless. The size varies from 1 to 4 cm in diameter and they clinically resembles lipoma. Poorly differentiated mast cell tumors have a tendency for faster growth, they are less limited and often accompanied by inflammation and edema of the surrounding tissue (Jubb et al., 2007). Ulceration may occur on their surface, while smaller nodes (satellite nodules) may develop nearby. Most mast cell tumors are not pigmented, but erythematous and hyperpigmented nodules (Welle et al., 2008) can be observed in occasional findings. The lesions at the distal parts of the limbs, the lips and the groin area may be in the form of poorly defined swellings or resembling acral lick dermatitis (eng.) (Gross et al., 2005; Welle et al., 2008). Malignant mast cell tumors can spread throug lymph or blood, and in most cases, the first sign of metastasis is the enlargement of the regional lymph node. Spread to the lungs is not common, while they will most likely spread to the spleen, liver and kidneys. Also they can often be diagnosed on the skin (Morris et al., 2001).

Etiology of mast cell tumors is not fully known, but it is considered, as with most tumors, to be multifactorial. The predisposition of certain breeds shows the importance of genetic in the appearance of MCTs (Welle et al., 2008). Recent papers also emphasize the role of surface receptor tyrosine kinase (KIT protein, CD117) (Vucicevic et al., 2016; Halsey et al., 2017). The diagnosis of mast cell tumors is based on the staining of cytologic smears obtained by fine needle biopsy or by using the impression smears technique. The most commonly used staining methods are Romanowsky

staining (eg Diff-Quik, Giemsa, Wright's) or New methylene blue (London et al., 2003; Strefezzi et al., 2009). While mast cell tumors can be determined on the basis of cytological staining, a histopathological examination of biopsy tissue is indispensable for tumor classification (Bostock, 1973; Patnaik et al., 1984; Kiupel et al., 2011). Mast cell tumors (especially poorly differentiated ones) can be mistaken for other round-cell tumors (lymphoma, plasmacytoma, histiocytoma and transmissible venereal tumour) (Welle et al., 2008; Oliveira et al., 2012). For the purpose of confirming mast cell tumor diagnosis and differentiation from other tumors, toluidine blue (TB) is used for visualizing cytoplasmic granules (London et al., 2003; Strefezzi et al., 2009).

The classification of mast cell tumors is very important when it comes to disease prognosis and type of therapy. The histologicalal examination of mast cell tumors is the primary classification determinant. More recently, a two-stage classification system for mast cell tumors of high and low malignancy has been used. Based on this division, high malignancy mast cell tumors must meet at least one of the following criteria: at least 7 mitotic figures per 10 hpf (High-power Fields – hpf), at least 3 multinucleated cells in 10 hpf, at least 3 bizarre nuclei in 10 hpf or karyomegaly of at least 10% of neoplastic cells (Kiupel et al., 2011). The proposed 2-tier grading system should be used in any routine histological examination and diagnosis of mast cell tumors. For high grade malignancy MCTs, additional studies such as determination of KIT receptor expression and c-KIT mutation screening should be performed in order to determine adequate therapy (Kiupel et al., 2011, Sabattini et al., 2014).

KIT protein is growth factor receptor of stem cell and is normally found on the surface of hematopoietic cells and mast cells, playing a significant role in the growth and differentiation of these cells. This protein is a type III transmembrane receptor encoded by the proto-oncogene c-kit. KIT receptor expression was detected by immunohistochemical staining both on the surface of normal and on the surface of neoplastic mast cells. Increased expression was observed in poorly differentiated and undifferentiated MCTs (Welle et al., 2008; El-Agamy, 2012). Apart from membrane expression, KIT expression was detected focally and diffusely in the cytoplasm.

The KIT-staining patterns are identified as pattern I - membrane-associated staining due to the localization of KIT proteins in the cell membrane while cytoplasmic staining is present in small amounts or completely absent, pattern II - diffuse cytoplasmic staining and pattern III - also relates to the presence of KIT proteins in the cytoplasm of the noplastic mast cells and is characterized by intense focal cytoplasmic staining. Expression disorders can contribute to neoplastic transformation (Misdorp, 2004; Takeuchi

et al., 2010). The increased cytoplasmic expression of KIT receptors (focal or diffuse) is correlated with shorter survival of dogs with mast cell tumors and shorter intervals for remission of the disease compared to mast cell tumors with increased membrane expression (Webster et al., 2004). The aim of our study is to determine the link between the localization of KIT receptors and histologic grade of MCTs observed in dogs.

MATERIALS AND METHODS

Animals

The examinations included skin samples of 52 dogs in which MCT is suspected by clinical examination. Samples of unchanged skin of 12 adult dogs, sampled at autopsy, were used as control. Dogs of different breed and sex, ages 3 to 11, were from the territory of the Republic of Serbia. Histopathologic diagnosis was made at the Department of Pathology, Faculty of Veterinary Medicine, University of Belgrade.

Histopathology

Immediately after the skin tumor biopsy, the samples were fixed in 10% neutral buffered formalin for no more than 48 hours, after which they were processed in the LEICA TP1020 tissue processor. Paraffin blocks were cut using the LEICA RM 2235 microtome into 3-5 μm thick sections. The obtained preparations were stained with hematoxylin-eosin (HE) method. Preliminary pathohistologic diagnosis of MCTs was made on sections stained by hematoxylin-eosin, and for its confirmation, the slides were further stained by toluidin-blue staining kit. Histopathological features of the tumor, such as the presence of mitosis, multinucleated cells, cells with bizarre nuclei and karyomegaly, were analyzed on slaides stained with hematoxylin-eosin. The number of these parameters was determined on ten high power fields of each tumor (400x), using the method described by Romansik et al. (2007). Based on these parameters, the tumors are classified into two levels of differentiation – high grade and low grade tumors.

Immunohistochemical staining

Formalin-fixed paraffin embedded tissue samples were cut into 5 μm thin sections, which were then stained using a multi-step indirect immunohistochemical (IHC) technique. The sections were incubated with a primary antibody CD117 (Rabbit anti-Human Polyclonal Antibody (DAKO, A4502)) diluted with phosphate buffer saline (PBS) in a ratio of 1: 400. Immunoreaction was visualized using DAB + (3,3'-diaminobenzidine tetra-hydrochloride, DAKO, K3468). Mayer's hematoxylin was used for counterstaining. Appropriate positive and negative controls were used.

Morphometric analysis

For morphometric analysis, Olympus Cell B morphometric software and

Olympus ColorView III camera were used. The localization of KIT proteins was determined in the manner described in Webster et al. research (2007), since membrane KIT proteins and two types of cytoplasmic KIT proteins (focally and diffusely distributed in the cytoplasm) differ. -. Dominant KIT protein pattern was determined based on the presence in at least 10% of neoplastic tumor cells. Cells at the edges of the tumor were not taken into consideration.

Statistical analysis

Descriptive parameters, such as arithmetic mean and standard deviation, were used in the statistical analysis of the obtained results. Chi-square test was used to test and determine statistically significant differences. These tests determined the existence of a statistically significant difference between the tested parameters at the significance level of 5% and 1%.

RESULTS

Histological analysis of tissue sections stained with hematoxylin and eosin revealed more than six mitotic figures per 10 hpf in 53.85% of MCTs (Figure 1a), while 46.15% of MCTs were characterized by poor mitotic activity. The presence of more than two multinucleated cells in 10 hpf was observed in 59.61% of MCTs (Figure 1b). In 17.31% of MCTs, two multinucleated cells were present, while one multinucleated cell was observed in 19.23% of MCTs on the same number of visual fields. Multinucleated cells were not observed in 3.85% of MCTs. More than two cells with bizarre nuclei in 10 hpf were present in 55.77% of MCTs (Figure 1c). Two cells with bizarre nuclei on the same number of visual fields were observed in 7.69% MCTs, while one cell with bizarre nuclei was present in 26.92% of MCTs. In 9.61% of MCTs, cells with bizarre nuclei were not detected. More than 10% of anaplastic mast cells with nuclei of about 8-10 micrometers in diameter were observed in 19.23% of MCTs, while 42.30% of MCTs contained less than 10% of the cells with increased nucleus (Figure 1d). In 38.46% of MCTs no karyomegaly was present.

Based on the criteria of Kiupel's classification and established parameters (mitotic figures, multinucleated cells, bizarre nuclei and karyomegaly,) in diagnosed MCTs, 36 (69.23%) of MCTs corresponded to MCTs of higher malignancy, while 16 (30.77 %) of MCTs were of a lower degree of malignancy. Immunohistochemical staining confirmed the presence of KIT receptors in skin tissue sections of the dogs with MCTs. Microscopic examination of the sections revealed three types of immunohistochemical staining patterns for KIT positive cells. The majority of MCTs in this study had KIT expression pattern I or membrane expression (63.46%) (Figure 1e). This group included all low grade malignancy MCTs and 17 out of 36 high grade malignancy MCTs. Expression pattern II was present in 21.15% mast cell tumors

(Figure 1f), all of which were high grade. None of the examined mast cell tumors had only pattern III, but combined patterns were observed, namely: 11.54% of MCTs exhibited membrane and focal cytoplasmic expression, while 3.85% of MCTs had membrane and diffuse cytoplasmic expression of KIT receptors.

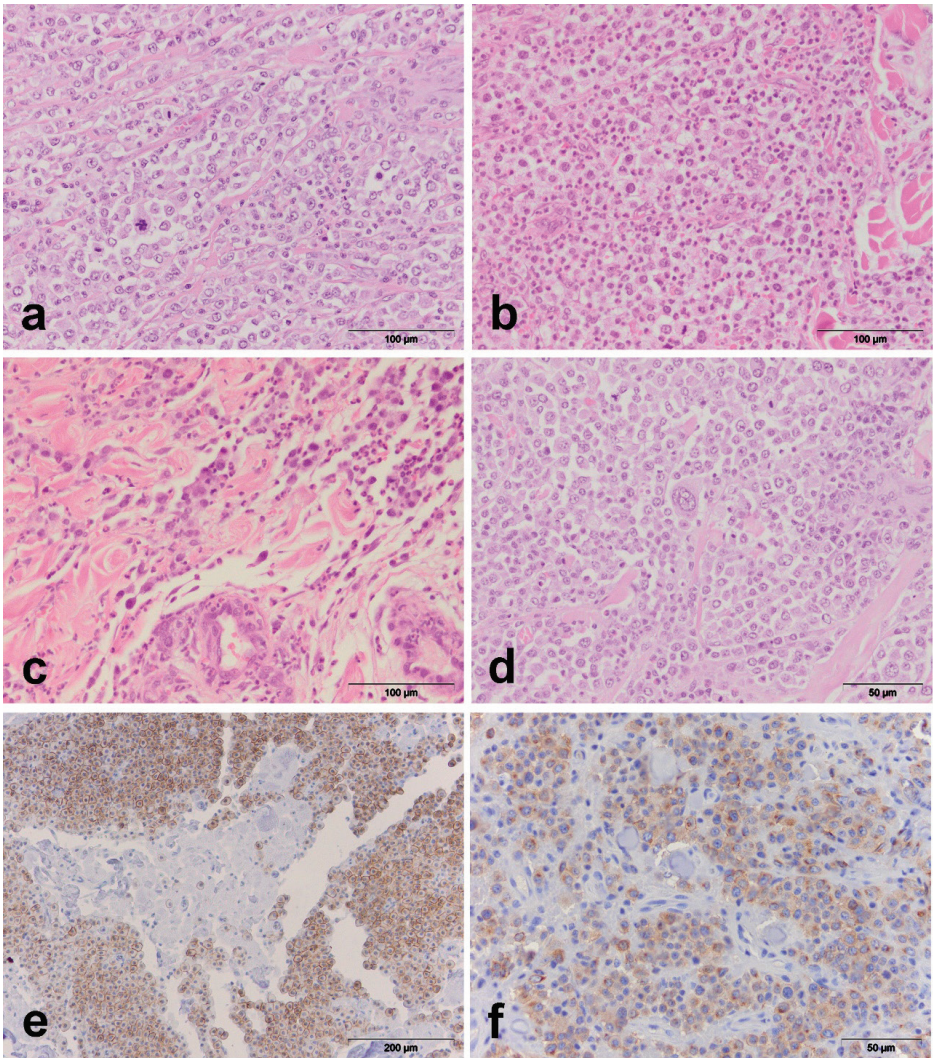


Figure 1. Skin, dog: a) The presence of mitotic figure in high grade MCT, HE; b) The presence of multinucleated cells in high grade MCTs, HE; c) The presence of cells with bizarre nuclei in high grade MCTs, HE; d) The presence of karyomegaly in high grade MCTs, HE; e) Expression of pattern I (membrane expression), CD117, LSAB2; f) Expression of pattern II (diffuse cytoplasmic expression), CD117, LSAB2;

DISCUSSION

Mast cell tumors are the most common dog skin tumors, especially when it comes to certain breeds such as boxer, golden retriever, labrador retriever and others (Welle et al., 2008). Based on the results obtained in our study, the highest incidence of mast cell tumors was observed in golden retrievers (37.5%), followed by boxers (20.7%). Similar to these results, most authors state that mast cell tumors were most commonly diagnosed in boxers (London and Sarah, 2003; Webster et al., 2006a; Gil da Costa et al., 2007; Welle et al., 2008; Vucicic et al.., 2018). Others consider that although boxers have an increased risk of mast cell tumor formation, they are usually of low grade malignancy and have a more favorable prognosis (Thamm et al., 2007).

Histological examination appears to be the most consistent predictive parameter and is closely related to the 2-tire classification system proposed by Kiupel (Kiupel et al., 2011) according to which mast cell tumors are divided into high grade and low grade MCTs. According to the 2-tire system, MCTs examined in our research have been classified in high grade and low grade MCTs. Out of 52 MCTs in dogs, thirty-six were high grade tumors, while sixteen MCTs had morphological characteristics indicating a low degree of malignancy. More than six mitotic figures per 10 hpf were detected in 77.78% of high grade MCTs, and the presence of mitotic figures is statistically significant parameter for classification of MCTs in this study. While some authors consider that low grade MCTs can metastasize, and that determining only the mitotic index is not a reliable way of predicting tumor behavior (Séguin et al., 2006; Webster et al., 2007); other authors consider mitotic activity to be an important feature for the prognosis of mast cell tumor behavior (Romansik et al., 2007; Kiupel et al., 2011). Some authors believe that mortality increases in cases where the mitotic index is greater than 5/10 hpf (O'Connell and Thomson, 2011).

The presence of more than two multinucleated cells in 10 hpf was observed in 86.11% of high-grade MCTs, indicating that this is a statistically significant indicator of MCTs classification. Some studies indicate that there is a significant correlation between the number of multinucleated cells in MCTs and survival time, so that survival time decreases with the increase in the number of multinucleated cells (Thompson, 2012).

In our study, the presence of more than two cells with bizarre nuclei in 10 hpf was observed in 86.11% of high grade MCTs, while the presence of multinucleated cells in low grade MCTs was not observed.

It was also observed that 27.78% of high grade MCTs contain more than 10% anaplastic MCTs with nuclei of 8-10 microns in diameter, indicating that the karyomegaly can not be considered

as a significant predictive factor in this study. There is a significant correlation between high grade MCTs and faster metastases, as well as a shorter survival time of patients (Kiupel et al., 2011; Stern, 2012). Therefore, for high grade mast cell tumors, additional tests such as determining the expression of KIT receptors and screening for mutations of c-KIT should be used in order to determine adequate therapy.

Recent studies indicate the existence of both normal (membrane) and aberrant cytoplasmic (focal and diffuse) KIT receptor expression of MCTs in dogs (Morini et al., 2004; Gil da Costa et al., 2007). Unlike previous studies (Reguera et al., 2000) which examined the relationship between intensity of immunohistochemical staining indicating the degree of KIT expression and the degree of tumor malignancy, our study examines the interconnection of aberrant expression of this protein and histopathological grade of mast cell tumor. In our study, it is observed that in low grade MCTs there is only membrane expression, while in high grade MCTs, in addition to membrane expression, there is also aberrant expression. From our results, it is noted that low grade MCTs have no aberrant expression of the KIT receptor. None of aberrant expression pattern is statistically significant in relation to other aberrant expression patterns.

However, the correlation between aberrant CD117 expression and the degree of tumor malignancy is clear. Some authors state that KIT receptor is a transmembrane protein, and as such, the immunoreactivity of this protein is localized on the cytoplasmic membrane of unchanged mast cells. The results of the study confirm that canine MCTs that have more aggressive biological behavior, they also have greater cytoplasmic KIT expression (Kiupel et al., 2004). Also, the same authors claim that mast cells with increased cytoplasmic KIT expression have an increased risk of local recurrence and shorter survival time. Some authors emphasize a strong correlation between the cytoplasmic (aberrant) immunoexpression and increased cell proliferation, and therefore a higher degree of malignancy, but they did not notice significant differences between the focal and diffuse cytoplasmic CD117 expression, suggesting that focal and diffuse cytoplasmic expression of KIT receptor may reflect similar cell changes. It is known that cytoplasmic KIT receptor expression is associated with both necrotic and ulcerative changes. Cytoplasmic KIT receptor expression is related to increased mast cell proliferation, and the occurrence of necrosis in mast cell tumors can be a reflection of increased cell proliferation which appropriate angiogenesis isn't able to follow. On the other hand, the correlation between the cytoplasmic KIT receptor expression and occurrence of epidermal ulcerations can be due to KIT-mediated release of histamine and serotonin, which causes intense itching (Gil da Costa et al., 2007).

CONCLUSION

More recently, the presence of c-kit proto-oncogene mutation and aberrant KIT expression are considered to be the most prominent predictive factors. However, some papers state that aberrant KIT receptor expression can be seen in high grade MCTs in which no mutation was detected (Webster et al., 2006, Vucicevic et al., 2016). Therefore, determination of KIT receptor expression should be part of routine diagnostics and classification of mast cell tumors in dogs.

LITERATURE

1. Bostock D.E., Crocker J., Harris K., Smith P (1989): Nucleolar organiser regions as indicators of post-surgical prognosis in canine spontaneous mast cell tumours. British Journal of Cancer 59(6): 915–18.
2. El-Agamy D.S (2012): Targeting c-KIT in the therapy of mast cell disorders: current update. European Journal of Pharmacology 690(1-3): 1-3.
3. Gil da Costa R.M., Matos E., Rema A., Lopes C., Pires M.A., Gartner F (2007): CD117 immunoexpression in canine mast cell tumours: correlations with pathological variables and proliferation markers. BMC Veterinary Research 21(3): 19.
4. Gross T.L., Ihrke P., Walder E.J., Affolter V.K (2005): Mast cell tumors. Skin Diseases of the Dog and Cat Clinical and Histopathologic Diagnosis. 2nd edn. Oxford, UK: Blackwell Science Ltd 853–65.
5. Halsey C.H.C., Thamm D.H., Weishaar K.M., Burton J.H., Charles J.B., Gustafson D.L., Avery A.C., Ehrhart E.J (2017): Expression of Phosphorylated KIT in Canine Mast Cell Tumor. Veterinary Pathology 54(3): 387-394.
6. Hendrick M.J., Mahaffey E.A., Moore F.M., Vos J.H., Walder E.J (1998): Mast cell tumors. In: World Health Organization International Histological Classification of Tumors of Domestic Animals, Histological Classification of Mesenchymal Tumors of the Skin and Soft Tissues of Domestic Animals. 2nd series, ed. Schulman FY, 28–29. Armed Forces Institute of Pathology, Washington, DC.
7. Jovanović M., Aleksić-Kovačević S., Knežević M (2012): Specijalna veterinarska patologija, Makarije d.o.o., Beograd.
8. Jubb, Kennedy, Palmer (2007): Pathology of Domestic Animals, Saunders Elsevier, 5th edition, 771-73.
9. Kiupel M., Webster J.D., Kaneene J.B., Miller R., Yuzbasiyan-Gurkan V (2004): The use of kit and tryptase expression patterns as prognostic tools for canine

cutaneous mast cell tumors Vet Pathol 41(4): 371-77.

10. Kiupel M., Webster J.D., Bailey K.L., Best S., DeLay J., Detrisac C.J. i sar. (2011): Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior. Veterinary Pathology 48(1): 147-55.
11. London C.A., Seguin B (2003): Mast cell tumors in the dog. Veterinary Clinics of North America Small Animal Practice 33(3): 473-89.
12. Misdorp W (2004): Mast cells and canine mast cell tumours: a review. Veterinary Quarterly 26(4): 156-69.
13. Morris J., Dobson J (2001): Small Animal Oncology. Blackwell Science 59-62.
14. O'Connell K., Thomson M (2011): Evaluation of prognostic indicators in dogs with multiple, simultaneously occurring cutaneous mast cell tumours: 63 cases. Veterinary and Comparative Oncology 11(1): 51-62.
15. Oliveira F.N., Elliott J.W., Lewis B.C., Mathews G.G., Brown R.M., Treadway C.M., Langohr I.M (2013): Cutaneous Mast Cell Tumor With Epitheliotropism in 3 Dogs. Veterinary Pathology 50(2): 234-37.
16. Patnaik A.K., Ehler W.J., MacEwen E.G (1984): Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. Veterinary Pathology 21(5): 469-74.
17. Reguera M.J., Rabanal R.M., Puigdemont A., Ferrer L (2000): Canine mast cell tumors express stem cell factor receptor. Am J Dermatopathol 22(1): 49-54.
18. Romansik E.M., Reilly C.M., Kass P.H., Moore P.F., London C.A (2007): Mitotic index is predictive for survival for canine cutaneous mast cell tumors. Vet Pathol 44(3): 335-41.
19. Sabattini S., Scarpa F., Berlato D., Bettini G (2014): Histologic grading of canine mast cell tumor: Is 2 better than 3?. Vet. Pathol 52(1): 70-3
20. Seguin B., Besancon M.F., McCallan J.L. I sar. (2006): Recurrence rate, clinical outcome, and cellular proliferation indices as prognostic indicators after incomplete surgical excision of cutaneous grade II mast cell tumors: 28 dogs (1994-2002). J Vet Intern Med 20: 933-940.
21. Stern W.A (2012): Histologic Grading of Canine Cutaneous Mast Cell Tumors: Is There a Good System?. J Vet Sci Med Diagn 1: 1.
22. Strefezzi R.F., Kleeb S.R., Xavier J.G., Catao-Dias J.L (2009): Prognostic indicators for mast cell tumors. Bray J Vet Pathol 2: 110-21.

23. Takeuchi Y., Fujino Y., Watanabe M., Nakagawa T., Ohno K., Sasaki N., Sugano S., Tsujimoto H (2010): Aberrant autophosphorylation of c-KIT receptor in canine mast cell tumor cell lines. Vet. Immunol. Immunopathol 137(3-4): 208-16.

24. Thamm D.H., Vail D.M: Mast cell tumors, In: Withrow SJ, MacEwen EG, editors (2007): Small Animal Clinical Oncology, Saunders, Philadelphia.

25. Thompson J.J (2012): Canine Mast Cell Tumours: Characterization of Subcutaneous Tumours and Receptor Tyrosine Kinase Profiling. PhD Thesis, Guelph, Ontario, Canada.

26. Vučićević, I., Marinković, D., Kukolj, V., Nešić, S., Aničić, M., Aleksić-Kovačević, S (2016): Kit receptor expression in canine cutaneous mast cell tumours (CMCTS) without c-kit mutation. Acta Veterinaria-Beograd 66 (2): 222-233

27. Vučićević I., Marinković D., Kukolj V., Nesić S., Aničić M., Aleksić-Kovačević S (2018): Mast cell tumor within a lipoma in a white toy poodle. Veterinarski Glasnik, 72 (1): 64-67.

28. Webster J.D., Kiupel M., Kaneene J.B., Miller R., Yuzbasiyan-Gurkan V (2004): The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. Vet Pathol 41(4): 371-7.

29. Webster J.D., Yuzbasiyan-Gurkan V., Kaneene J.B., Miller R., Resau J.H., Kiupel M., (2006): The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. Neoplasia 8(2): 104-11.

30. Webster J.D., Yuzbasiyan-Gurkan V., Miller R.A., Kaneene J.B., Kiupel M (2007): Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. Veterinary Pathology 44(3): 298-308.

31. Welle M.M., Rohrer C.B., Howard J., Rüfenacht S (2008): Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. Veterinary Dermatology 19(6): 321-39.

Paper received: 30.04.2018.

Paper accepted: 11.10.2018.