Received: 22 June 2020 | Accepted: 4 February 2021

DOI: 10.1111/jvim.16078

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Treatment of genitourinary carcinoma in dogs using nonsteroidal anti-inflammatory drugs, mitoxantrone, and radiation therapy: A retrospective study

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Abstract

Background: Locoregional tumor control and prolonged survival for dogs with genitourinary carcinoma (CGUC) reportedly are achievable using treatment with radiotherapy (RT) with or without adjunctive chemotherapy and nonsteroidal anti-inflammatory drugs (NSAIDs).

Objectives: To characterize event-free and overall survival after treatment of CGUC using NSAIDs, mitoxantrone (MTX), and a standardized RT protocol (57 Gy in 20 fractions).

Animals: Fifty-one client-owned dogs treated between 2008 and 2017.

Methods: Dogs were retrospectively categorized into treatment groups: (a) first-line concurrent chemoradiotherapy (≥1 dose of MTX started within 1 month of RT); (b) first-line chemotherapy (MTX administered for >1 month before RT without tumor progression); (c) RT as a salvage procedure (MTX, surgery or both with subsequent locoregional tumor progression before RT). Treatment-induced toxicoses, event-free survival (EFS), and overall survival times (OSTs) were recorded. The influence of demographics, staging, and treatment-related factors on survival was assessed using Cox proportional hazards modeling.

Results: Median EFS and OST for all dogs were 260 and 510 days with no significant differences among groups 1 (n = 39), 2 (n = 4), and 3 (n = 8). Both EFS and OST were shorter in dogs with moderate to severe clinical signs ($P < .001$ and $P < .001$, respectively); OST was shorter in dogs with prostatic involvement ($P = .02$). Permanent urinary incontinence developed in 16 dogs (31%) at a median of 70 days postirradiation; other toxicoses were mild and self-limiting.

Conclusions and Clinical Importance: Mild clinical signs and lack of prostate involvement were associated with favorable prognosis for survival. Client education regarding the risk of urinary incontinence is warranted.

KEYWORDS
definitive-intent, dogs, intensity-modulated, radiotherapy, urogenital carcinoma

Abbreviations: CGUC, canine genitourinary carcinoma; CI, confidence interval; CT, computed tomography; EFS, event-free survival; IM/IGRT, intensity-modulated and image-guided radiotherapy; mOST, median overall survival time; MTX, mitoxantrone; NSAIDs, nonsteroidal anti-inflammatory drugs; OST, overall survival time; RT, radiotherapy.

1 | INTRODUCTION

Carcinomas are the most commonly diagnosed cancer of the canine lower urinary tract. Although certain anatomic sites may confer a worse prognosis, definitive diagnosis of the various types of lower urinary tract carcinoma is difficult, as in the case of prostate carcinoma, where prostatic carcinoma and urothelial carcinoma of the prostatic urethra are not readily distinguishable on histopathology. Thus, transitional cell (urothelial) carcinomas, adenocarcinomas, and solid carcinomas of the urinary bladder, urethra, ureters, vagina, vulva, or prostate are referred collectively to as aggressive canine genitourinary carcinoma (CGUC).[1,2] Biologic behavior and treatment options are broadly similar and treatments include medical management, surgery, radiotherapy (RT), or multimodal combinations thereof.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used for CGUC, rationalized by the fact that many CGUCs express cyclooxygenase enzymes, which are inhibited by NSAIDs. For dogs with prostatic carcinoma, median overall survival time (mOST) is longer with (6.9 months) vs without (0.7 months) an NSAID.[3] Measurable bladder tumor volume reduction is observed in 18% to 20% of dogs with CGUC treated with NSAIDs alone; mOST is approximately 5 to 6 months.[4,5] Addition of chemotherapy to NSAID treatment is also common, and improves survival. In dogs with prostatic carcinoma, treatment with both an NSAID and chemotherapy has been associated with longer survival than use of NSAIDs alone (mOST 106 days vs 51 days, respectively).[6] For dogs with bladder carcinomas, mOST is longer in dogs treated with mitoxantrone (MTX) plus piroxicam (291 days) vs piroxicam alone (181 days).[7]

In a retrospective study of 25 dogs having undergone total prostatectomy for prostatic carcinoma, mOST was 231 days, which is subjectively longer than achievable with medical management alone.[6,8] In another retrospective study of 37 dogs treated with partial cystectomy with or without adjunctive drug treatment for urothelial carcinoma of the urinary bladder, mOST was 348 days (the group that received daily concurrent piroxicam had mOST of 772 days), which is also subjectively longer than survival achieved with chemotherapy and an NSAID without surgery.[9] However, many cases of CGUC affect the trigone of the urinary bladder, and radical surgery (eg, complete cystectomy with urinary diversion) would be required. This approach is technically feasible, but often declined by pet owners because of the potential morbidity associated with the procedure.[10-12] In such cases, RT may provide an alternative means for locoregional tumor control. In a 2012 study describing clinical outcomes of 21 dogs having undergone full-course intensity-modulated and image-guided radiotherapy (IM/IGRT; 54-58 Gy in 20 daily fractions) with or without adjunctive drug treatment for CGUC, the subjective response rate was 60%, with a median event-free survival (EFS) time of 317 days, and mOST of 654 days.[13] More recently, another study reported on 18 dogs that had been treated using full-course RT (48-54 Gy).[14] Local progression was documented in 7 of 18 dogs at a median of 241 days after completing RT, and mOST was 563 days; EFS was longer with chemotherapy and shorter when metastases were identifiable at diagnosis; overall survival was longer in asymptomatic dogs. Results of these studies are promising for definitive intent RT for CGUC but the total number of reported cases remains low. Therefore, our purpose was to report outcomes for a larger series of CGUC cases, after treatment with NSAIDs, MTX, and a standardized IM/IGRT protocol (57 Gy in 20 daily fractions). A secondary objective was to identify factors that may be predictive of survival.

2 | MATERIALS AND METHODS

2.1 | Case selection

A descriptive, retrospective study was performed on cases of CGUC that were treated at 1 of 2 university-based veterinary hospitals between April 2008 and December 2017. Records were reviewed, and we included the outcome of 14 dogs from a previous study.[13] Clinical history, patient demographics, presenting complaint, and clinical signs were recorded. Severity of clinical signs at presentation was classified as either mild (asymptomatic, hematuria, or pollakiuria) or moderate to severe (stranguria, tenesmus, partial or complete urinary tract obstruction with or without medical or surgical intervention). Follow-up information was obtained from the medical records, and by contacting pet owners, referring veterinarians or both.

Inclusion of an individual case required definitive diagnosis of carcinoma by cytology, histopathology, BRAF (B-raf Proto-Oncogene) testing or some combination of these, and complete local and systemic staging, defined as: CBC, serum biochemistry panel, urinalysis, thoracic radiographs, and abdominal ultrasound examination or advanced imaging of the chest or abdomen using computed tomography (CT). In addition to having started definitive-intent full-course IM/IGRT (57 Gy in 20 daily fractions), each included dog also was required to have received treatment with an NSAID and at least 1 dose of MTX. Use of chemotherapeutic drugs other than (and in addition to) MTX did not preclude inclusion. Cases were excluded from analysis if known or suspected lung metastases were present at the time of diagnosis.

Dogs were retrospectively categorized 1 of 3 treatment groups: (a) first-line concurrent chemoradiotherapy, (b) first-line chemotherapy, and (c) IM/IGRT as a salvage procedure after locoregional failure. Group 1 included dogs having been treated with ≥1 doses of MTX administered concurrently with or beginning within 1 month of RT. Group 2 included dogs for which MTX was administered for >1 month and without clinical evidence of tumor progression before starting IM/IGRT. Group 3 included dogs that were first treated by chemotherapy (including at least 1 dose of MTX) with or without surgery, and then IM/IGRT at the time of locoregional progression.

2.2 | Radiation therapy

In all cases, IM/IGRT was planned for delivery on a C-arm linear accelerator using a prescription of 57 Gy delivered in 20 daily (Monday through Friday) fractions to the primary tumor; refer to the

Supporting Information for details of radiation treatment planning and delivery.

2.3 | Chemotherapy

Mitoxantrone was used as the first-line chemotherapeutic agent. Because IM/IGRT was considered to have provided adequate locoregional control, the general recommendation was to give a total of 5 doses of MTX. When given concurrently with IM/IGRT, the initial protocol was to administer MTX at dosages of 5 to 5.5 mg/m^2 IV once every 3 weeks; both institutions observed increased hematologic toxicity and neutropenia associated with fever using this chemoradiation protocol, and beginning in 2015 implemented chemotherapy dose reductions (4 mg/m^2 MTX for dogs weighing <10 kg; 4.5 mg/m^2 MTX for dogs ≥10 kg). If the first dose was well tolerated, then dose escalation was instituted for the next round of treatment. If progressive disease occurred after stopping MTX, additional MTX was recommended; if progressive disease was observed while actively on a MTX protocol, alternative chemotherapeutic agents were considered based on individual clinician discretion. Each dog had prechemotherapy diagnostic testing before administration and a follow-up CBC 1 week after the first dose. Mitoxantrone was given IV by trained oncology technicians in accordance with institutional chemotherapy administration protocols. On days of concurrent chemotherapy and RT, the timing of MTX administration was not controlled and MTX was given either before or after RT, based on staff availability. Data regarding chemotherapy drug dosage, route of administration, adverse events, and dose adjustments were obtained from the records.

2.4 | Treatment-associated toxicities

Radiation toxicoses were retrospectively graded according to the Veterinary Radiation Therapy Oncology Group (VRTOG) classification scheme.[15] Toxicities were deemed acute if first observed within 90 days of RT, and late if first noted >90 days from the time of RT. Chemotherapy toxicoses similarly were recorded based on Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE) criteria.[16] Only hematopoietic toxicities from concurrent chemoradiation were recorded; toxicities resulting from chemotherapy that was given either before or after the course of RT were not recorded. Urinary incontinence after initiating treatment was defined as the involuntary loss of urine during the storage phase, and was assessed according to the medical record, discussion with owners or referring veterinarian, need for diapers or some combination of these. Urinary incontinence was not considered to be a potential chemoradiation complication if it existed before tumor-directed treatment or if it developed immediately after placement of a urethral stent. Information regarding the development, timing, and resolution of urinary incontinence was recorded.

2.5 | Outcome and prognostic variables

Event-free survival and overall survival times (OSTs) were used to assess treatment outcome; Kaplan-Meier methods were used. Event-free survival was defined as the time from the first fraction of RT to the first event. Events included progressive local disease, development of metastatic disease or persistent acute or late adverse radiation effects that impacted the dog's quality of life, and death from any cause. Importantly, urinary incontinence was not included as an event (even when permanent) and instead the frequency of and latency to urinary incontinence are described separately (as described above). Overall survival time was defined as time from the first tumor-directed treatment of any sort (surgery, chemotherapy, RT) until death. Cases were censored if they were without events or alive at the time of analysis; loss to follow-up was managed by censoring cases at the date of their last confirmed status update. For most cases, insufficient data was available to allow meaningful evaluation of tumor control using Response Evaluation Criteria in Solid Tumors (RECIST). Instead, comparison of previous radiographic, ultrasonographic, CT, cystoscopy results, or some combination of these was used to subjectively determine whether tumor progression had occurred. All relevant imaging reports were reviewed and approved by an American College of Veterinary Radiology (ACVR) board-certified radiologist. Upon completion of RT, follow-up visits were recommended at 2 weeks, 1 month, and every 3 months thereafter. Abdominal ultrasound examinations were recommended at each restaging visit and thoracic radiographs at every other visit (ie, every 6 months). At detection of disease progression, chemotherapy, RT, surgery, and palliative (eg, stent) options were offered based on the specific problems identified (tumor regrowth, metastatic disease, urethral stricture).

2.6 | Statistical analysis

Kaplan-Meier survival curves were generated using commercially available software (Prism version 8, GraphPad, San Diego, California). Survival estimates also were generated using commercially available software (JMP Pro version 14; SAS Institute, Cary, North Carolina). Univariable analyses were performed using the log-rank test; variables evaluated included treatment group, institution, age, weight, sex, prostatic involvement in male dogs (based on imaging), severity of clinical signs at the time of RT, lymph node irradiation, presence of lymph node involvement at diagnosis, and pursuit of a second course of RT. A multivariable Cox proportional hazard regression model then was constructed to evaluate for potential associations with survival. Variables were entered into the multivariable model if they had a P value <.2 on univariable analysis or if they could be confounding. Notably, for the multivariable analysis, confirmed lymph node involvement was excluded from the analysis; only 2 of 51 dogs had confirmed involvement, and both dogs were included in the larger group of 8 dogs that underwent lymph node irradiation. Similarly, to ensure group size of at least 4 per factor, sex of the animals was condensed

to male vs female for the multivariable analysis. The ENTER method was used for multivariable modeling. Analyses were performed using commercial software (SPSS version 26; IBM Corporation, Armonk, New York). Statistical significance was set at $\alpha < .05$.

3 | RESULTS

3.1 | Patient demographics

During the study period, 124 dogs were treated by RT for CGUC (82 at 1 treatment center and 42 at the other); 51 dogs met the criteria for inclusion (34 at 1 treatment center, and 17 at the other). Demographics data are summarized in Supporting Information. Treatment group 1 included 39 dogs (76%), group 2 included 4 dogs (8%), and group 3 included 8 dogs (16%). All dogs in group 2 had received 2 to 3 MTX doses with no evidence of progressive disease before IM/IGRT. In addition to chemotherapy, 4 dogs in group 3 also had surgical intervention for a bladder tumor. Mild clinical signs were present in 30 (59%) dogs; signs were moderate to severe in the other 21 (41%). Of the 35 male dogs, 12 (34%) had no prostate involvement and 23 (66%) had prostatic involvement on imaging. Twenty dogs (39%) had some degree of lymph node enlargement on evaluation of CT. Six dogs (5 with lymph node enlargement and 1 without) had ultrasound-guided aspirates and cytology performed; 2 had confirmed metastatic carcinoma, 3 did not show evidence of metastasis, and 1 result was inconclusive. Lymph node irradiation was performed in 8 (16%) dogs, including 2 with cytologically confirmed nodal metastasis, 1 with suspected metastasis based on imaging (but a reactive lymph node based on cytology), and 5 that did not have cytologic evaluation of the affected nodes but were suspicious for lymph node involvement based on imaging characteristics. Dosimetric data are summarized in the Supporting Information (Table 1).

3.2 | Treatment outcomes and prognostic factors

When considering all dogs, median EFS was 260 days (95% confidence interval [CI] = 193-343 days) and median OST was 510 days (95% CI = 344-644 days; Figures 1 and 2). Outcomes based on treatment groups are presented in Figures 3 and 4. Eight cases were censored from analysis after completing treatment, with 6 dogs being lost to follow-up and 2 dogs still alive at the time of writing. Median follow-up time for censored cases was 512 days (range, 127-1050 days). Univariate analysis of EFS and OST (summarized in Table 2) indicated that EFS was significantly affected by severity of clinical signs and body weight. Overall survival time was significantly affected by severity of clinical signs, prostate involvement, and lymph node irradiation. These factors in addition to sex, age, treatment group, and use of a second course of RT were included in the multivariable analysis (results summarized in Table 3). Two additional multivariable Cox proportional hazards models were constructed: 1 excluding treatment groups as a variable, and the other combining groups 1 and 2 (Tables S1 and S2); results were similar to those presented in Table 3.

TABLE 1 Target and organs-at-risk (OAR) volumes and dosimetric data

	Min	Max	Median
GTV volume	1.27	116.15	23.6
CTV volume	5.95	226.99	50.38
PTV volume	20.99	361.41	113.65
PTV minimum dose	39.04	57.8	50.84
PTV maximum dose	51.69	64.54	60.8
PTV mean dose	56.47	59.88	58.44
PTV D2%	57.7	61.4	59.9
PTV D98%	47.11	60.3	55.97
PTV D50%	53.3	59.96	58.12
Bladder[a] minimum dose	0.43	60.02	57.15
Bladder maximum dose	36.16	62.19	60.18
Bladder mean dose	2.71	60.96	58.75
Bladder D2%	25.87	61.32	59.53
Bladder D98%	0.47	59.48	57.71
Bladder D50%	0.85	60.49	58.75
Colon max dose	47.24	62.2	60.29
Colon >57 Gy (mL)	0	10.95	2.2
Urethra max dose	54.4	64.03	60.34
Urethra >57 Gy (mL)	0	36.27	1.73
Ureters max dose	3	61.13	59.56
Ureters >57 Gy (mL)	0	2.5	0.12
Cauda equina max dose	7.84	54.2	22.7
Cauda equine >57 Gy (mL)	0	0	0

Abbreviations: CTV, clinical target volume; GTV, gross tumor volume; PTV, planning target volume.
[a]Partial bladder irradiation was performed in 8 (16%) dogs and whole bladder irradiation in 43 (84%) dogs.

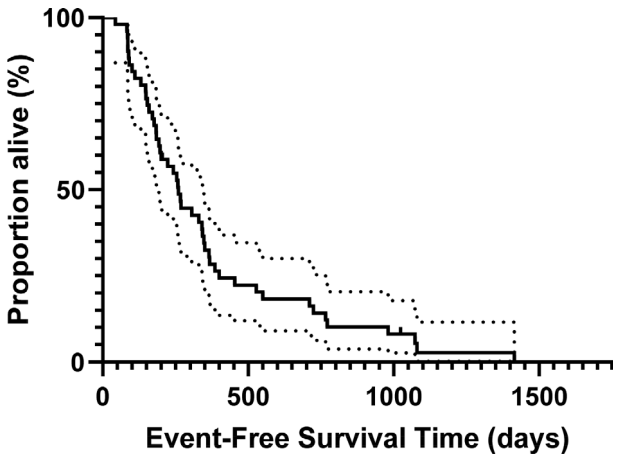

FIGURE 1 EFS for all dogs. EFS, event-free survival

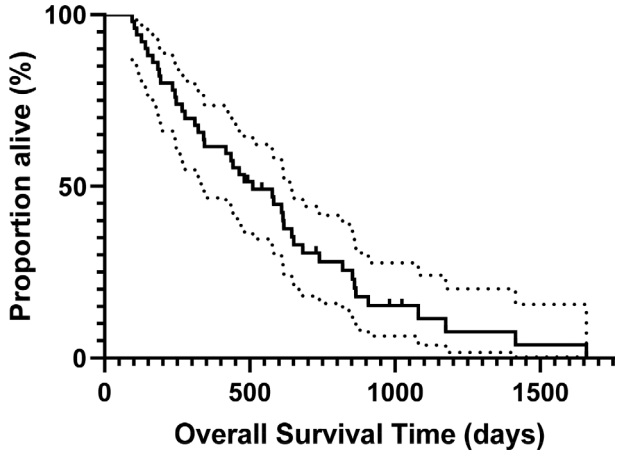

FIGURE 2 OST for all dogs. OST, overall survival time

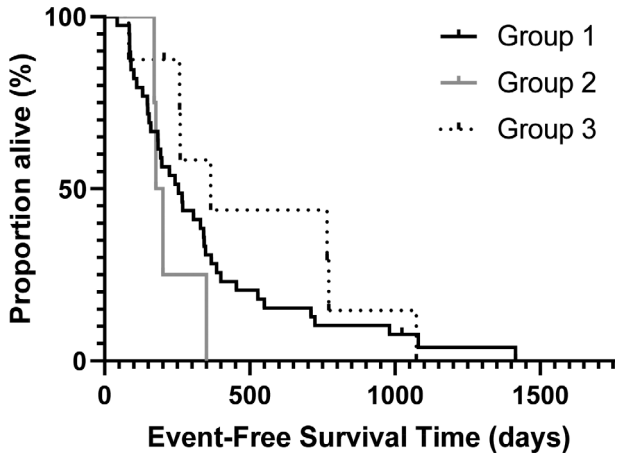

FIGURE 3 EFS by treatment group. Dogs were separated according to treatment groups: (1) concurrent first-line chemoradiotherapy (n = 39); (2) first-line chemotherapy (IM/IGRT started before failing chemotherapy; n = 4); (3) IM/IGRT as a salvage after locoregionally failing first-line chemotherapy (n = 8). EFS, event-free survival; IM/IGRT, intensity-modulated and image-guided radiotherapy

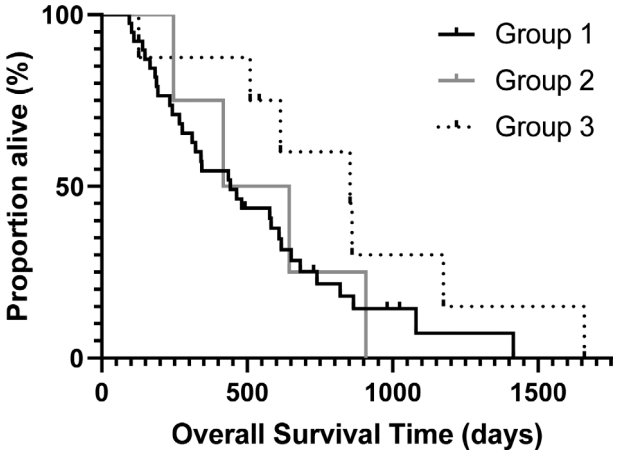

FIGURE 4 OST by treatment group. Dogs were separated according to treatment groups: (1) concurrent first-line chemoradiotherapy (n = 39); (2) first-line chemotherapy (IM/IGRT started before failing chemotherapy; n = 4); (3) IM/IGRT as a salvage after locoregionally failing first-line chemotherapy (n = 8). IM/IGRT, intensity-modulated and image-guided radiotherapy; OST, overall survival time

Six dogs were treated with additional RT after locoregional disease progression; see Supporting Information for details. No significant difference in EFS or OST was detected for dogs that underwent 1 vs 2 courses of RT.

The rate of locoregional tumor progression after completing IM/IGRT was 59% (27/46). The median time to locoregional tumor progression was 343 days (95% CI = 234-442 days). Median time to distant metastasis was 365 days (95% CI = 262-481 days). As previously mentioned, 8 dogs underwent nodal irradiation; 2 (25%) of those dogs subsequently developed progressive nodal disease. The first dog had suspected nodal progression based on ultrasound examination 55 days after RT; the other had progressive nodal disease at necropsy 552 days after RT. The remaining 6 dogs (75%) did not have progressive disease in the irradiated nodal bed after treatment. Of the 43 dogs that did not undergo nodal irradiation, 8 (19%) subsequently developed evidence of locoregional (sublumbar) lymph node metastasis (4 had cytologic confirmation and the remaining 4 diagnoses were based on imaging features), and median time to lymph node metastasis in those cases was 442 days (95% CI = 322-609 days). Based on treatment group allocation, locoregional failure rates were 56%, 50%, and 75% for groups 1, 2, and 3, respectively. Of the dogs with locoregional failure for which the cause of euthanasia was documented, 75% (15/20) were euthanized because of locoregional disease progression, 20% (4/20) because of distant metastatic disease, and 5% (1/20) for both. For dogs with presumed locoregional control at the time of death, 58% (7/12) had been euthanized because of issues unrelated to the tumor, and death was attributed to distant metastasis in the remaining 42% (5/12) of cases. Two necropsies were performed. The first was a dog euthanized for development of functional urinary obstruction. Neither evidence of disease progression nor metastatic lesions were observed. The other dog was euthanized after development of hemoptysis, and necropsy confirmed pulmonary metastasis.

3.3 | Treatment-associated toxicities

Acute radiation effects were reported in 65% of the dogs. Acute colitis was reported in 24 (47%; grade 1 in 23 dogs, grade 2 in 1 dog). Acute dermatitis was recorded in 11 (22%; 7 grade 1, 3 grade 2, and 1 grade 3). Last, 8 (16%) experienced acute genitourinary effects (4 grade 1 and 4 grade 2). Late radiation toxicity was suspected in 3 (6%) dogs that developed urethral obstruction. Diagnostic evaluations in these cases generally included CBC, serum biochemistry, urinalysis, urine culture, ultrasound examination, cystourethrogram, and cystoscopy whenever possible with biopsies of abnormal lesions and normal tissues within the area of interest if no lesions were visualized. One

TABLE 2 Univariate analyses

	Overall survival time						Event-free survival time				
	n	Median	SE	95% CI—Lower	95% CI—Upper	Sig.*	Median	SE	95% CI—Lower	95% CI—Upper	Sig.
All dogs	51	510	61	344	644	—	260	43	193	343	—
Treatment group											
Group 1: Concurrent chemoradiotherapy	39	442	65	277	617	.24	254	53	159	343	.43
Group 2: RT started after chemo, but before progression of disease	4	531	143	246	908		189	42	171	350	
Group 3: RT started after locoregionally failing chemotherapy	8	853	180	127	1174		313	124	83	771	
Treatment center											
Site 1	34	614	67	482	746	.25	260	11	239	281	.9
Site 2	17	418	169	86	750		184	21	144	224	
Sex											
Castrated males	32	418	86	249	587	.16**	243	88	70	416	.83
Intact males	3	651	411	0	1456		385	194	6	764	
Spayed females	16	609	146	323	895		201	57	89	313	
Intact females	0	—	—	—	—		—	—	—	—	
Severity of clinical signs at the time of irradiation (2 categories)											
Mild	30	617	41	536	698	.005***	343	40	264	422	<.001***
Moderate or severe	21	344	141	67	621		193	17	160	226	
Anatomic site											
No prostate involvement	12	651	161	335	967	.02***	330	51	230	430	.28
Prostate involvement	23	341	121	105	577		223	48	129	317	
Confirmed lymph node metastasis?											
No	49	577	91	399	755	.05**	258	31	198	318	<.001***
Yes	2	140	—	—	—		87	—	—	—	
Lymph nodes irradiated?											
No	43	609	80	453	765	.001***	260	17	227	293	.11**
Yes	8	193	35	125	261		153	20	114	192	
Treated with more than one course of IM/IGRT?											
Yes	6	865	402	77	1653	.06**	343	165	19	667	.5
No	45	481	100	285	677		243	38	168	318	
Age (y; median = 10 y)											
≤Median	28	418	97	227	609	.22	184	24	136	232	.14**
>Median	23	614	94	430	798		260	61	141	379	
Body weight (kg; median = 15.6 kg)											
≤Median	26	442	106	235	649	.56	306	64	181	431	.04***
>Median	25	582	88	410	754		197	31	137	257	

Abbreviations: CI, confidence interval; IM/IGRT, intensity-modulated and image-guided radiotherapy; RT, radiotherapy.
*P value for Log-Rank test.
**$P < .2$.
***P value less than .05, and considered statistically significant.

dog was suspected of developing functional urethral obstruction approximately 4 months after RT; no evidence of stricture or progressive disease was observed on cystoscopy. Euthanasia was elected after offering medical management with antibiotics, alpha-adrenergic antagonists, and skeletal muscle relaxants. The remaining 2 dogs developed urethral obstruction >600 days post-RT. One dog was euthanized shortly after developing obstruction, and it is unclear whether obstruction resulted from presumed radiation-induced stricture or tumor

TABLE 3 Multivariable Cox proportional hazards model for OST

Variable	Comparison	Overall survival time		Event-free survival time	
		Sig.*	Hazard ratio	Sig.	Hazard ratio
Treatment group	Group 1 vs 2	.05**	5.399	.32	1.935
	Group 1 vs 3	.18	3.742	.12	4.189
Sex	All groups	.3	2.707	.5	1.827
Severity of clinical signs at the time of irradiation	Mild vs moderate/severe	***<.001*** ***	0.093	***<.001*** ***	0.123
Anatomic site	No prostate involvement vs prostate involvement	***.02*** ***	0.280	.33	0.622
Lymph node irradiation?	Yes vs no	.91	0.933	.75	1.212
Treated with more than one course of IM/IGRT?	Yes vs no	.74	1.327	.45	1.680
Age	High vs low	.39	0.681	.16	0.547
Body weight	High vs low	.08**	2.610	.26	1.844

Abbreviations: IM/IGRT, intensity-modulated and image-guided radiotherapy; OST, overall survival time.
Bolded and italicized numbers are for significant prognostic factors and are analogous to the stars listed below.
*P value for multivariable Cox proportional hazards model.
**P value less than .1.
***P value less than .05, and considered statistically significant.

TABLE 4 VCOG adverse events based on MTX prescribed dose

VCOG hematopoietic adverse event	4-4.5 mg/m^2 MTX	5-5.5 mg/m^2 MTX
No adverse event	5 (50%)	9 (39%)
Adverse event	5 (50%)	14 (61%)
• Grade 1	1 (20%)	2 (14%)
• Grade 2	0 (0%)	4 (29%)
• Grade 3	0 (0%)	2 (14%)
• Grade 4	4 (80%)	6 (43%)

Abbreviations: MTX, mitoxantrone; VCOG, Veterinary Cooperative Oncology Group.

progression. The second dog had a more extensive diagnostic evaluation, which included cystoscopy and biopsies. Stricture secondary to RT was suspected based on narrowing of the proximal urethra observed on the cystourethrogram with no evidence of disease on cystoscopy or histopathology. A urethral stent was placed and successfully relieved the obstruction for 2 months, at which point the dog was euthanized because of metastatic disease.

Of the dogs that received concurrent chemoradiation, 20/33 (61%) developed hematopoietic toxicity. The distribution according to VCOG scores was: 15% (3/20) grade 1, 20% (4/20) grade 2, 15% (3/20) grade 3, and 50% (10/20) grade 4. Subjectively, hematologic toxicity was worst in dogs that received the lowest doses of MTX (Table 4). Five dogs were observed to have neutropenia associated with fever during chemoradiation. Most were able to complete radiation protocol with delays ranging from 1 to 4 days. One dog required a total treatment delay of 6 days because of grade 4 neutropenia and concurrent aspiration pneumonia before completing RT.

Permanent urinary incontinence was reported in 31% (14/45) of dogs with documented long-term follow-up. None were known to have had urinary incontinence before RT. Median time to development for incontinence was 70 days; 86% (12/14) of these events occurred within 4 months of RT completion, and the remaining 2 dogs developed incontinence 246 and 370 days after RT.

4 | DISCUSSION

Our study reaffirms that, in cases of unresectable CGUC, a multimodal treatment approach including definitive-intent IM/IGRT is associated with prolonged EFS and OST. Although direct comparison among studies is precluded by their retrospective nature, results of our study generally are similar to those of other reports in which full-course radiation prescriptions were delivered using modern radiation equipment for macroscopic CGUC. In our study, median EFS and OST were 260 days and 510 days, respectively, from the time of initiating any tumor-directed treatment. Comparatively, a previous study reported median EFS and OST of 317 and 654 days from the first day of irradiation in a group of dogs with CGUC[13] and another study reported median EFS and OST of 220 and 563 days from the first day of irradiation for prostatic carcinomas.[14] Together, these studies suggest that inclusion of definitive intent RT in a multimodal treatment regimen results in a prognosis for survival that is superior to medical management (chemotherapy plus NSAIDs) alone, where median OST is approximately 10 months.[7,17-19]

We intentionally used different starting points to calculate EFS and OST as compared to previous studies. To standardize and critically evaluate outcomes and events after irradiation in all treatment groups, EFS was defined as time of first RT to time of first event. Similarly, to assess OST, time from first tumor-directed treatment (surgery, chemotherapy, or radiation) to death was used. This approach allowed unbiased comparison of RT outcomes across the different treatment

groups, especially in group 3 dogs that had received and failed alternative treatments. As such, these definitions provide the most accurate assessment of radiation outcomes for CGUC treatment and also should allow for direct comparison to previously published studies.

A secondary goal of our study was to identify factors that are predictive of survival after IM/IGRT treatment for CGUC. Our results differ from those of a previous IM/IGRT study that indicated a trend toward increased survival in dogs with primary prostatic disease.[11] We believe our results to be more reliable because our study included a larger number of dogs with prostatic disease (including 2 from a previous study[13]), and because of significant findings in both the univariate and multivariable statistical analyses. These new results also are in accordance with previous literature reporting that prostatic involvement is associated with worse outcomes in male dogs[17]. The specific reason for worse outcomes in dogs with prostatic involvement is not known. Potential explanations include the statistical approach used, or perhaps a different biological behavior of prostatic carcinoma vs urothelial carcinoma. For dogs in our study, it is unclear whether the finding of better outcome with mild clinical signs is a result of early detection, lower tumor stage (ie, lead-time bias), or less aggressive tumor phenotype. Regardless, knowledge that advanced clinical signs are associated with increased hazard of early events and death should be useful to clinicians when advising pet owners. The lack of significant difference in EFS and OST between treatment groups is not surprising, and likely reflects small sample sizes in groups 2 and 3. Although not significantly different from other treatment approaches, survival in dogs treated with IM/IGRT after failing first-line chemotherapy with or without surgery (ie, the 8 dogs in group 3) was excellent (median OST, 853 days). Thus, veterinarians should not withhold definitive intent RT and reserve it as a salvage treatment. The available data suggest that incorporation of radiation into a treatment plan improves locoregional tumor control, and in our study 42% of dogs with durable local tumor control were known to have been euthanized because of tumor-related problems (all from metastatic disease). Thus, a recommended approach would be to treat with NSAIDs and 1 to 2 doses of chemotherapy after diagnosis, and then initiate IM/IGRT in dogs that have stable disease, and finally, resume chemotherapy (eg, for 5-6 doses given at the maximally tolerated dose). Restaging using CT of the chest and abdomen should be considered to maximize the likelihood of detecting patients with early metastatic disease before RT. Doing so would allow clinicians to assess patient response to first-line chemotherapy and provide early antimetastatic treatment. Recognizing that some cases of CGUC unpredictably exhibit particularly aggressive biologic behavior characterized by early distant metastasis, this approach also would mitigate risk of initiating intensive and expensive combination chemoradiotherapy in such a situtation.

Consideration of target volumes is important for CGUC, in regard to both lymph nodes and the amount of urinary tract tissue included. Because only a few dogs in our study (8) received nodal irradiation, we cannot conclude that it would delay or prevent locoregional disease progression. This approach, however, should be considered for future studies because in an intensity-modulated, targeted treatment plan, it is not likely to increase morbidity. Similar consideration should be given to inclusion of the bladder or prostate in the treatment plan. Although it is possible that a field cancerization effect could result in topographical exclusion of part of the urinary tract, concern also exists about irradiating parts of the urinary tract that may not be affected, which could increase morbidity. Because in our study the median time to locoregional failure was long (343 days), and a 31% risk of permanent urinary incontinence was observed with treatment, our current recommendation is to only treat regions with known or strongly suspected disease. It remains critical that careful staging (ideally with cystoscopy) be performed before the onset of IG/IMRT for CGUC to try and identify all affected regions of the urinary tract and ensure that all abnormal tissue is included.

Because of the targeted nature of IM/IGRT, it was expected that concurrent use of chemoradiation for CGUC would be well tolerated. However, given that dogs undergoing definitive intent RT are known to experience clinically relevant decreases in total white blood cell count, it is not surprising that severe cytopenias might result from the combination of relatively high dose RT with cytotoxic chemotherapy.[20,21] Indeed, in our study, 56% of dogs that received concurrent MTX and RT developed hematopoietic adverse events, and 64% were classified as grade 3 or 4 complications. This rate of moderate to severe hematopoietic toxicity is subjectively higher than has been reported with MTX alone.[17,22] Considering this observation, and in the absence of any clear benefit from giving concurrent (vs sequential) chemoradiation, we consider it reasonable to abandon the idea of using concurrent MTX and IM/IGRT for CGUC. An alternative approach would be drug dose reduction, change in the antineoplastic chemotherapeutic agent, or both.

Most of the dogs in our study developed acute radiation effects, which generally were mild and self-limiting. Late radiation effects (ie, functional urinary obstruction or urethral stricture) were infrequent. A novel finding in our study was the high rate and rapid onset of permanent urinary incontinence after IM/IGRT. Given the close temporal proximity between completion of RT and development of urinary incontinence, this complication was presumed to be radiation-associated. This conclusion is supported by the fact that other common potential differential diagnoses for incontinence were considered unlikely after thorough review of case histories. Although urinary incontinence has been reported after high dose single fraction intraoperative RT in dogs, it has not been reported as a common complication of CGUC treatment when modern irradiation protocols are utilized.[23,24] Urinary incontinence is known to be an uncommon complication of pelvic irradiation in humans. In humans, incontinence may be an acute or late effect of radiation, and when acute, it typically resolves within a few weeks.[25-29] Risk factors in humans include age, radiation protocol, prostatic volume, dose to the trigone, maximum radiation dose to a target (hot spots), and concurrent chemotherapy.[26,27,30-34] The pathophysiology of radiation-induced urinary incontinence remains incompletely defined.[26,27,31] An improved mechanistic understanding likely will aid in development of effective therapeutic and preventative strategies. From a therapeutic perspective, first-line treatment for this problem in our clinics generally has consisted of alpha-1 adrenergic antagonists, and such treatment has met with variable success.

Potential mitigation strategies worthy of further consideration include attempts to resolve lower urinary tract infections before initiation of RT for CGUC, radiation dose reduction, and identification of efficacious radioprotectors. Use of sequential rather than concurrent chemoradiation is another option, which seems logical given the aforementioned increased risk for incontinence in humans treated with concurrent chemoradiation, and in light of our observation of apparently increased risk of moderate to severe hematologic toxicity when MTX is given together with IM/IGRT.

Despite the relatively long EFS and OST reported in our study, most treated dogs ultimately did succumb to progressive locoregional tumor growth. The radiation protocol used in our study (57 Gy in 20 fractions) was designed as a modest treatment intensification compared to a previous study (median RT prescription dose of 54 Gy and highly variable adjuvant chemotherapy regimen)[13] with a goal of increasing tumor control while maintaining relatively low rates of late radiation effects. Considerations for how to improve locoregional tumor control without increasing risk of toxicity may include: (a) delivery of higher total doses of radiation with smaller fraction size; (b) a more hypofractionated course of RT because some preclinical data support that this tumor type with low radiosensitivity (ie, α-to-β ratio) may benefit from larger fractional doses of radiation; (c) mechanically debulking the tumor before irradiation (eg, surgical cytoreduction, laser ablation); (d) use of a radiosensitizer (eg, high atomic number nanoparticles, DNA damage response inhibiting drugs); [31,32] or (e) early screening and detection to facilitate treatment of smaller disease burdens and early stage disease. These strategies must take into account the benefit to risk ratio for urinary tract function.

Our retrospective study had several limitations. Recall bias (among clinicians and pet owners) may have contributed to possible underreporting of certain toxicities. Not all pet owners adhered to the recommended follow-up protocols, and even when they did, restaging of bladder tumors using ultrasonography is difficult and often unreliable, which limited our ability to estimate EFS and precluded use of standardized response evaluation criteria for robust classification of oncologic outcomes.[35] Patient classification into different treatment groups may allow for selection or treatment bias. However, treatment groups were not statistically significant on multivariable analysis, including 2 separate analyses that excluded treatment groups and another that combined groups 1 and 2. Similarly, dogs with prostatic disease were not subdivided into separate tumor types (urothelial carcinoma, adenocarcinoma, or carcinoma with mixed urothelial and glandular phenotypes)[31] given the difficultly in distinguishing between these subtypes. Similar concerns arise when considering carcinomas that arise in other lower urinary tract sites. It remains unclear if detection of BRAF dysregulation is specific to carcinomas of urothelial origin, and histology is rarely utilized for diagnosis confirmation. Thus, we made no attempt to distinguish among prostatic, bladder, or urethral tumor subtypes. To avoid imprecise definition and classification of cases, and to avoid improperly implying any specific biology or histology for the included cases, we opted to describe cases as fitting within the broad category of CGUC, although that general term is not commonly used in veterinary medicine and includes some disease sites not represented in our study (eg, vagina, cervix). The terminology CGUC also has the benefit of being consistent with a previous IM/IGRT study, which described a smaller group of similarly treated dogs.[13]

In summary, use of an IM/IGRT protocol with MTX and NSAIDs for treatment of CGUC was associated with prolonged locoregional tumor control and survival. Mild clinical signs and lack of prostate involvement were associated with decreased risk of death. Pet owners also must be counseled about the risk of permanent urinary incontinence. Additional investigation is required to optimize the clinical management and potential outcomes of dogs with lower urinary tract carcinomas.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Clerc-Renaud B, Gieger TL, LaRue SM, Nolan MW. Treatment of genitourinary carcinoma in dogs using nonsteroidal anti-inflammatory drugs, mitoxantrone, and radiation therapy: A retrospective study. *J Vet Intern Med*. 2021;35:1052–1061. https://doi.org/10.1111/jvim.16078