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Original Scientific Paper

HISTOPATHOLOGICAL CHARACTERIZATION AND BIOLOGICAL ASSESSMENT OF TESTICULAR TUMORS IN DOGS WITH CRYPTORCHIDISM

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Abstract: The descent of the testes (*descensus testis*) is a crucial process in the embryonic development of male animals, the disruption of which results in cryptorchidism—a condition representing a significant risk factor for the development of testicular neoplasms. The aim of this study was to determine the prevalence, type, and biological behavior of testicular tumors in dogs with confirmed cryptorchidism, as well as their association with breed and age. The research encompassed a total of 311 biopsies of pathologically suspicious testes collected between September 2019 and September 2024, of which 72 samples (23.15%) were diagnosed as tumors. Histopathological analysis was performed using the standard hematoxylin-eosin (HE) staining method. Of the total diagnosed tumors, 80.56% were benign and 19.44% malignant, with metastases recorded in 42.86% of the malignant cases. The most common tumor types were Leydig cell tumor (33.3%), seminoma (31.9%), and Sertoli cell tumor (25%). The breed most frequently affected by benign tumors was the Siberian Husky, while malignant tumors were most commonly observed in Boxers and Siberian Huskies. The average age of dogs with tumors was 11 years. The results indicate that cryptorchidism, in combination with age and breed predisposition, significantly increases the risk of testicular tumor development in dogs.
Keywords: benign tumors, dogs, malignant tumors, testes

INTRODUCTION

The descent of the testes (descensus testis) begins during embryonic development, when the male gonads are initially located in the abdominal cavity within the vaginal

process. The mesenchymal cord (gubernaculum testis) facilitates their passage through the inguinal canals into the scrotum. Failure of this process results in cryptorchidism (Cooley and Waters, 2001). Undescended testes are exposed to elevated temperatures, impairing spermatogenesis and testicular function (Refsal et al., 1983; Oettlé et al., 1988). Cryptorchidism also affects hormonal balance, increases the risk of trauma and torsion, and may influence behavior and skin changes in dogs (Toyama et al., 2000).
This condition, combined with aging, represents a major risk factor for testicular diseases, particularly testicular tumors, the most frequent neoplasms of the canine reproductive system. Genetic predisposition in certain breeds further increases the risk, which also rises with the age of the animals (Meuten, 2002). Testicular tumors in dogs are most often benign, with limited malignancy or metastasis, though cryptorchid dogs show a higher risk of developing malignant Sertoli cell tumors and seminomas (Masserdotti et al., 2005; Larsen, 1998). Histologically, these tumors originate from germ cells or stromal elements of the testicular cord.
For this study, histopathological records of dogs from September 2019 to September 2024 were analyzed. General data about the dogs were collected, and tumor types were classified according to World Health Organization (WHO) criteria. A total of 311 biopsies from cryptorchid dogs were examined. Seventy-two testicular tumors (23.15%) were diagnosed, of which 58 (80.56%) were benign and 14 (19.44%) malignant. Among the malignant cases, six dogs (42.86%) exhibited metastases in lymph nodes, the spermatic cord, kidneys, mesentery, and other organs.
The average age of affected dogs was 11 years. Unilateral tumors occurred in 60 dogs (83.33%), and bilateral tumors in 12 dogs (16.67%). Most dogs (94.44%) had a single tumor type, while mixed tumors were found in 4 cases (5.56%). The most common tumors were Leydig cell tumors, seminomas, and Sertoli cell tumors. Histopathological evaluation was performed using hematoxylin-eosin (H&E) staining.
The aim of this study was to investigate the association between previous cryptorchidism in dogs and the occurrence of testicular tumors, as well as the differences in tumor types according to breed. It is hypothesized that dogs with cryptorchidism have an increased risk and a different distribution of tumor types depending on breed.

MATERIALS AND METHODS

The study was conducted on a total of $n = 311$ biopsies of pathologically suspicious testes in dogs with confirmed cryptorchidism, of which $n = 72$ samples were testicular tumors collected between September 2019 and September 2024.
For the purpose of the research, records of histopathological analyses of the dogs were maintained. Data were obtained from testicular tumor samples from dogs presented to local veterinary clinics, where a series of general and specialized clinical examinations were performed and testicular tumors were diagnosed. The analysis included general

data on the dogs and classification of tumor types according to the histological criteria established by the World Health Organization (WHO).

Histopathological examinations of the samples were performed using the hematoxylin-eosin (HE) staining method on paraffin sections, conducted as follows:

The pathological material was fixed in formalin. The paraffin sections were deparaffinized by immersion in xylene, placed sequentially in four containers for 2-3 minutes each. The samples were then rehydrated by immersion in a descending series of alcohols (100%, 96%, 75%), using two containers for each concentration. The sections were kept briefly (about 30 seconds) in each alcohol to allow them to absorb water and thus take up the aqueous dye (Tkaczyk-Wlizło et al., 2024). The slides were stained with hematoxylin for 5-10 minutes, until the sections became blue and the nuclei dark blue. Hematoxylin is a basic dye that stains nuclei blue. The slides were then rinsed under running water for 10-15 minutes to differentiate the stain until a light blue shade was obtained (checked microscopically). The sections were subsequently stained with eosin for about 2 minutes. Eosin is an acidic dye that stains the cytoplasm light red (Nodtvedt et al., 2011).

The slides were rinsed again in tap water for 1 minute, then passed through a series of ascending alcohol concentrations (75%, 96%, and 100%), spending approximately 30 seconds in each. The purpose of this step was dehydration, allowing the sections to absorb xylene (Fan and De Lorimier, 2007). The slides were then immersed in two xylene baths for 4-5 minutes each to render the tissue transparent. Finally, one to two drops of Canada balsam were applied, covered with a coverslip, gently pressed, and left to harden, resulting in a permanent and high-quality histological preparation suitable for microscopic examination (Nascimento et al., 2020).

The staining results were as follows: nuclei, salts, mucus, and microorganisms stained blue; cytoplasm, connective tissue, and erythrocytes stained red.

The examination of the samples aimed to determine the relationship between the type and biological behavior of testicular tumors analyzed histopathologically (Grieco et al. 2010). Additionally, the study sought to assess the biological behavior of the tumors to facilitate diagnosis and prognosis of the disease. The samples were analyzed using a light microscope.

Histopathological classification of the testicular tumors was carried out in accordance with the histological classification criteria for canine testicular tumors established by the World Health Organization (WHO).

RESULTS

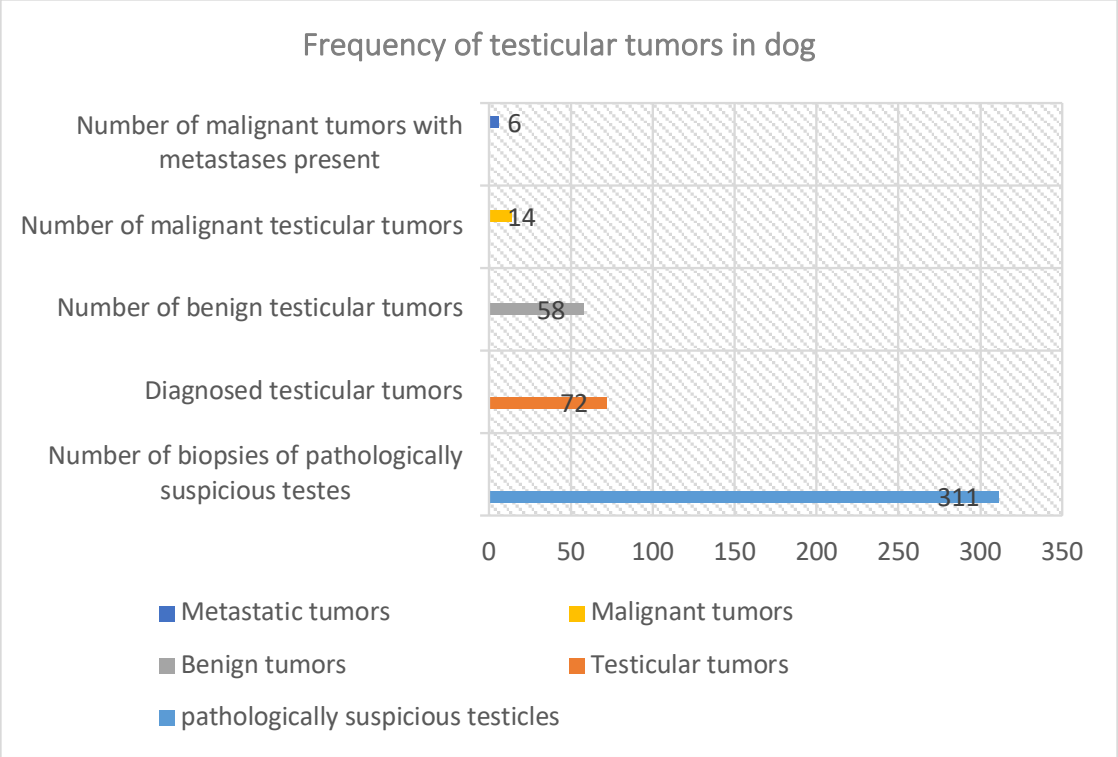

Figure 1. Numerical representation of testicular tumors from the total number of examined dogs.

From Figure 1, the incidence of benign and malignant testicular tumors in dogs confirmed by histopathological analysis can be observed. A total of 311 biopsies were performed on pathologically suspicious testes, of which 72 dogs were diagnosed with testicular tumors. Among these, 58 dogs had benign testicular tumors, while 14 dogs were diagnosed with malignant testicular tumors. Metastases from malignant tumors were observed in 6 dogs.

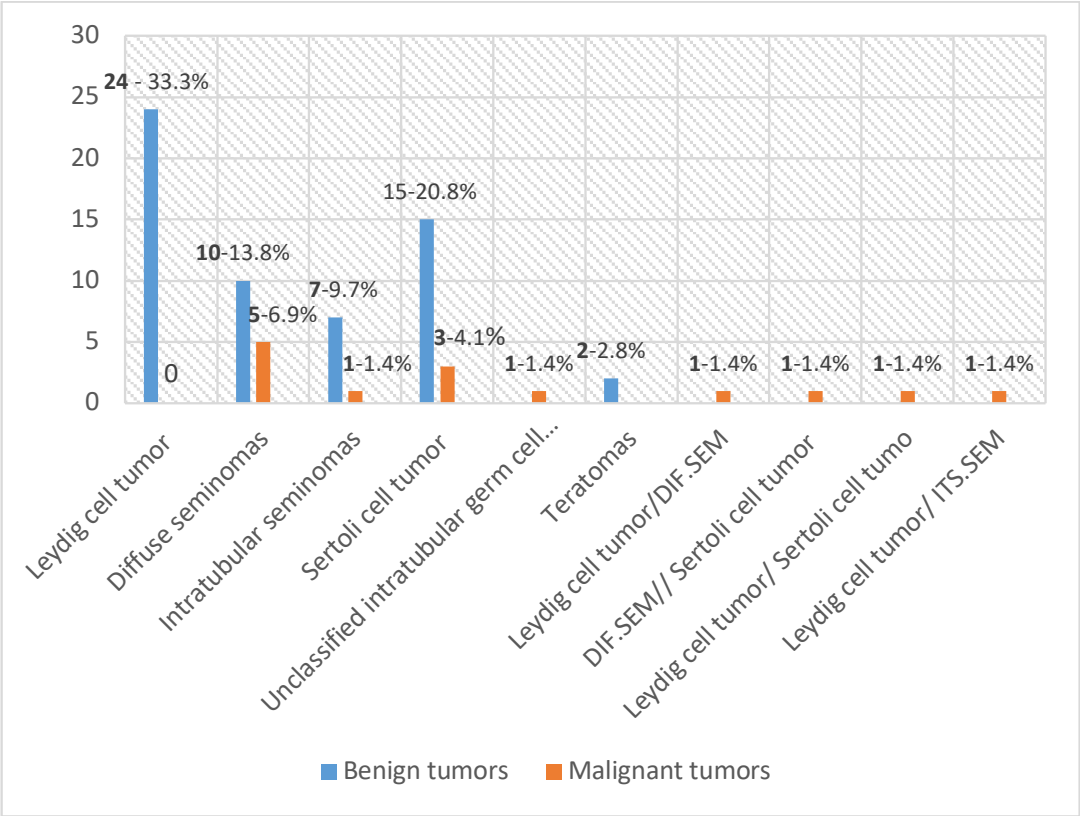

Figure 2. Numerical and percentage distribution of benign and malignant tumors in the selected samples.

From Figure 2, the numerical and percentage distribution of the histopathologically diagnosed tumor types can be observed. A total of 24 benign Leydig cell tumors were confirmed, making them the most prevalent tumor in this study, with a representation of 33.3%. No malignant Leydig cell tumors were detected.

Among seminomas, 10 benign diffuse seminomas were diagnosed (13.8%), while 5 malignant diffuse seminomas were identified (6.9%). Benign intratubular seminomas numbered 7 (9.7%), and a single malignant intratubular seminoma was observed (1.4%).

A total of 15 benign Sertoli cell tumors were diagnosed (20.8%), while 3 malignant Sertoli cell tumors were detected (4.1%). From unclassified tumors, 1 malignant tumor was isolated (1.4%), and 2 benign teratomas were identified (2.8%).

All four mixed tumors detected were malignant, each representing 1.4% of the total.

Table 1. Distribution of Benign Testicular Tumors in Dogs

Breed	n	Mean Age (years)	Tumor Classification (Diagnosis)	% within Tumor Type
Siberian Husky	11	10.2	Leydig cell tumor	18.97
Dalmatian	5	12.2	Leydig cell tumor	8.62
Bull Terrier	4	13.5	Leydig cell tumor	6.9

Shetland Sheepdog	2	10.0	Leydig cell tumor	3.45
Fox Terrier	1	11.0	Leydig cell tumor	1.72
German Shepherd	1	10.0	Leydig cell tumor	1.72
Boxer	3	9.0	Diffuse seminoma	5.17
Fox Terrier	2	11.0	Diffuse seminoma	3.45
Siberian Husky	2	14.5	Diffuse seminoma	3.45
Croatian Shepherd	1	12.0	Diffuse seminoma	1.72
Dachshund	1	13.0	Diffuse seminoma	1.72
Yorkshire Terrier	1	8.0	Diffuse seminoma	1.72
Siberian Husky	2	10.0	Intratubular seminoma	3.45
Šarplaninac	2	12.0	Intratubular seminoma	3.45
Saint Bernard	2	10.0	Intratubular seminoma	3.45
Alaskan Malamute	1	10.0	Intratubular seminoma	1.72
Miniature Schnauzer	5	11.2	Sertoli cell tumor	8.62
Pekingese	4	10.0	Sertoli cell tumor	6.9
Airedale Terrier	2	9.5	Sertoli cell tumor	3.45
West Highland White Terrier	1	10.0	Sertoli cell tumor	1.72
Siberian Husky	1	11.0	Sertoli cell tumor	1.72
Cocker Spaniel	1	8.0	Sertoli cell tumor	1.72
Dalmatian	1	11.0	Sertoli cell tumor	1.72
Great Dane	1	9.0	Teratoma	1.72
Fox Terrier	1	14.0	Teratoma	1.72

From Table 1, the percentage distribution of each type of benign tumor for the respective dog breeds, the mean age for each breed, and the type of tumor according to breed can be observed. The most common Leydig cell tumor occurred in the Siberian Husky breed with 18.97%, where the average age was 10.2 years. Diffuse seminomas were most prevalent in Boxers (three dogs) with a representation of 5.17% and an average age of 9 years, while intratubular seminomas were most common in two Siberian Huskies with a mean age of 10 years and two Šarplaninac dogs with an average age of 12 years, each with a total representation of 3.45%. Sertoli cell tumors were most represented in five Miniature Schnauzers (8.62%) with a mean age of 11.2 years. A single teratoma was detected in one Great Dane aged 9 years and one Fox Terrier aged 14 years, each representing 1.72% of the total benign tumors.

Table 2. Descriptive Statistics of Mean Age by Tumor Type

Tumor Type	Mean Age (years)	SD (Standard Deviation)	n (Number of Cases)
Diffuse seminoma	11.25	2.44	6
Intratubular seminoma	10.5	1.0	4
Leydig cell tumor	11.15	1.43	6
Sertoli cell tumor	10.1	1.13	7
Teratoma	11.5	3.54	2

A one-way ANOVA was performed to evaluate potential differences in the mean age of dogs among different types of benign testicular tumors. The results indicated no statistically significant difference in mean age between tumor types ($F(4, 20) = 0.53$, $p = 0.714$). Although there was no significant difference, Leydig cell tumors and diffuse seminomas tended to occur in slightly older dogs (mean ≈ 11.2 years), while Sertoli cell tumors appeared at a somewhat younger mean age (10.1 years). The relatively high standard deviation for teratomas (SD = 3.54) reflects the small number of observed cases (n = 2).

Table 3. Percentage and Numerical Distribution of Malignant Testicular Tumors in Dogs

Breed	n	Mean Age (years)	Tumor Classification (Diagnosis)	% within Tumor Type
Boxer	2	9.0	Diffuse seminoma	14.29
Siberian Husky	2	11.5	Diffuse seminoma	14.29
Samoyed	1	10.0	Diffuse seminoma	7.14
Bulldog	1	12.0	Intratubular seminoma	7.14
Norwegian Hound	1	11.0	Sertoli cell tumor	7.14
Great Dane	1	12.0	Sertoli cell tumor	7.14
Fox Terrier	1	10.0	Sertoli cell tumor	7.14
Pug	1	14.0	IGCNU; carcinoma in situ	7.14
Newfoundland	1	11.0	Leydig cell tumor / Sertoli cell tumor	7.14
Dalmatian	1	12.0	Leydig cell tumor / Diffuse seminoma	7.14
Golden Terrier	1	14.0	Diffuse seminoma / Sertoli cell tumor	7.14
Irish Setter	1	8.0	Leydig cell tumor / Intratubular seminoma	7.14

From Table 2, the percentage distribution of each type of malignant testicular tumor by dog breed, the mean age for each breed, and the tumor type associated with each breed can be observed. The most frequently diagnosed malignant tumors were found in two Boxers with a mean age of 9 years and two Siberian Huskies with a mean age of 11.5 years, each representing 14.29% of all malignant tumors. One case of intratubular seminoma was diagnosed in a Bulldog aged 12 years (7.14%). Sertoli cell tumors were identified in one Norwegian Hound aged 11 years, one Great Dane aged 12 years, and one Fox Terrier aged 10 years, each accounting for 7.14% of the total malignant tumors. In one Pug aged 14 years, an IGCNU (carcinoma in situ) was observed, also representing 7.14%. Mixed tumors were diagnosed in one Newfoundland (11 years old), one Dalmatian (12 years old), one Golden Terrier (14 years old), and one Irish Setter (8 years old), each contributing 7.14% to the overall proportion of malignant testicular tumors.

Table 4. Descriptive Statistics of Mean Age by Tumor Type

Tumor Type	Mean Age (years)	SD (Standard Deviation)	n (Number of Cases)
Diffuse seminoma	10.83	1.80	3
Intratubular seminoma	10.0	2.83	2
Sertoli cell tumor	11.0	1.41	3
IGCNU / carcinoma in situ	14.0	-	1
Mixed tumors (various combinations)	11.25	2.50	4

A one-way ANOVA was conducted to assess potential differences in the mean age of dogs with different types of malignant testicular tumors. The analysis revealed no statistically significant differences in mean age among tumor types ($F(4, 8) = 0.72$, $p = 0.59$). Although not significant, carcinoma in situ (IGCNU) appeared in the oldest dog (14 years), whereas diffuse seminomas and Sertoli cell tumors occurred at mean ages between 10.8 and 11 years. Mixed tumors were observed across several breeds (Newfoundland, Dalmatian, Golden Terrier, Irish Setter) with an average age of 11.25 years. The small sample size ($n = 12$) limits the statistical power, but the data suggest that malignant testicular tumors can occur across a wide range of ages and breeds, with no clear age-related predisposition.

DISCUSSION

The results obtained in this study regarding the frequency of testicular tumors in cryptorchid dogs, breed predisposition, and mean age are consistent with several previous studies. The incidence of diagnosed tumors among a total of 311 biopsies of pathologically suspicious testes was 23.15%, corresponding to 72 dogs.

Regarding tumor type, histopathological analysis revealed 58 benign tumor samples (80.56%), while histological features indicative of malignancy were diagnosed in 14 samples (19.44%). Furthermore, metastases were observed in six cases, accounting for 42.86% of malignant tumors. Each sample in this study corresponded to a single dog.

The most frequently diagnosed tumors in this study were Leydig cell tumors, seminomas, and Sertoli cell tumors, which aligns with findings reported by (Nodtvedt et al., 2011). The numerical and percentage distribution of diagnosed tumors was as follows: Leydig cell tumors were identified in 24 samples, representing 33.3% of all tumors. This is consistent with previous research by (Masserdotti et al., 2005), where over 50% of examined testicular tumors were of this type. No malignant Leydig cell tumors were observed in this study (Câmara et al., 2014).

Seminomas were the second most common tumor type, diagnosed in 23 samples (31.9%), in agreement with who classify this tumor type as the second most frequent in dogs (Dugat et al., 2015; Spugnini et al., 2000). Among these, 10 cases were benign diffuse seminomas (13.8%), and five cases were malignant (6.9%). Benign intratubular seminomas accounted for seven cases (9.7%), while one malignant intratubular seminoma was observed (1.4%).

Sertoli cell tumors were the third most frequent, with a total of 18 tumors (25% of all cases). Of these, 15 were benign (20.8%), and three were malignant (4.1%), which is consistent with reports by (Herndon et al., 2012), indicating that malignant Sertoli cell tumors occur in 10-15% of cases, while the remainder are benign (Fadok et al., 1986; Herndon et al., 2012).

One unclassified malignant tumor (IGCNU) was diagnosed, representing 1.4% of all tumors. This tumor type is a predisposing factor in cryptorchid dogs, as described by Grieco et al. (2008), who also reported that IGCNU can occur in conjunction with testicular dysgenesis syndrome in certain dogs.

Two benign teratomas were also identified, representing 2.8% of all tumors. Mixed tumors were observed in combinations of Leydig cell and Sertoli cell tumors, Leydig cell and diffuse seminoma, diffuse seminoma and Sertoli cell tumor, and Leydig cell and intratubular seminoma. All mixed tumors in this study exhibited malignant characteristics, although Leydig cell tumors were benign in three out of four mixed cases. This finding suggests that, under certain conditions, tumors that are initially benign may undergo malignant transformation (Dzimira et al., 2017). However, this study did not determine whether mixed tumors initially arose as benign Leydig cell tumors that later became malignant, or as seminomas or Sertoli cell tumors that subsequently incorporated Leydig cells.

Regarding breed predisposition for benign tumors, Siberian Huskies were the most affected, with a total of 16 dogs (27.59%) diagnosed with benign testicular tumors. The mean age of affected Siberian Huskies was 11.4 years, consistent with (Cooley and Waters, 2001), who reported an association between Leydig cell tumors and conditions such as cryptorchidism, prostatic enlargement, and perineal hernia, which are more frequently observed in this breed.

Concerning breed predisposition for malignant tumors, the most affected breeds were Boxers and Siberian Huskies, each presenting two tumors, with mean ages of 9 and 11.5 years, respectively, and representing 14.29% of the total malignant tumor cases. These findings support the breed predisposition described by (Cooley and Waters, 2001).

The mean age for all dogs with testicular tumors in this study was 11 years, indicating a close association between age and the occurrence of testicular neoplasms.

CONCLUSION

- The present study confirms a high correlation between cryptorchidism in dogs and the occurrence of testicular tumors, which were diagnosed in 23.15% of the total suspicious samples.
- Tumor identification was performed using histopathological examination with hematoxylin-eosin (HE) staining. Diagnosed tumors included Leydig cell tumors, seminomas, Sertoli cell tumors, teratomas, and IGCNU (carcinoma in situ) as single tumor types, as well as mixed tumors such as Leydig cell/Sertoli cell tumors, Leydig cell/diffuse seminoma/Sertoli cell tumors, and Leydig cell/intratubular seminoma.
- Leydig cell tumors were the most frequently diagnosed tumor type, accounting for one-third of all samples (33.3%), and all were benign. This confirms the association of this tumor type with a benign character.
- The mean age of dogs with testicular tumors was 11 years, confirming that testicular neoplasms predominantly occur in adult dogs.
- Regarding breed predisposition, genetic factors in correlation with cryptorchidism represent an additional risk for tumor development in certain breeds. In this study, Siberian Huskies were the most affected, with tumors diagnosed in 18 dogs, representing 25% of the total number of tumors.

Conflict of interest: The authors declare that they have no conflict of interest related to this work.

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