Characterization of Cutaneous and Subcutaneous Neoplasms in Canines and Malignancy Prediction Using B-Mode Ultrasonography, Doppler, and ARFI Elastography

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Abstract

Background

Cutaneous and subcutaneous neoplasms are highly prevalent in dogs, ranging from benign to highly aggressive and metastatic lesions. The diagnosis is obtained through histopathology, however it is an invasive technique that may take a long time to obtain the result, delaying the beginning of the adequate treatment. Thus, there is a need for non-invasive tests that can help in the early diagnosis of this type of cancer. The aim of this study was to verify the accuracy of ultrasonography methods to predict malignancy in cutaneous and subcutaneous canine neoplasms. In addition, we aim to propose an ultrasonography evaluation protocol and perform the neoplasms characterization using these three proposed techniques.

Results

Twenty-one types of specific neoplasm were diagnosed, and using B-mode, we verified the association between heterogeneous echotexture, invasiveness, presence of hyperechoic spots, and cavity areas with malignancy. An increased pulsatility was verified in malignant neoplasms using Doppler (cut-off value >0.93). When using the elastography, malignancy was associated with non-deformable tissues and shear wave velocity >3.52 m/s. Evaluation protocols were proposed associating 4, 5, 6, or 7 malignancy predictive characteristics, and characterization was done for all tumors with at least two cases.

Conclusions

We concluded that ultrasonography methods are promising and effective in predicting malignancy in these types of tumors, and the association of methods can increase the specificity of the results.

Background

The cutaneous and subcutaneous neoplasms are frequently observed in the canine species, originating from different cell types, and may present different biological behaviors from benign to highly aggressive and metastatic lesions [1, 2]. These lesions, especially the malignant ones, can promote significant alterations and can cause pain, inflammation, infections, hemodynamic changes, and when metastatic can compromise the function of other organs, which can lead to death [3, 4]. Fast and accurate diagnosis is essential to establish adequate therapy favoring the prognosis and survival of patients [5, 6].

The final diagnosis is obtained via histopathologic analysis, whose samples were collected by incisional or excisional biopsies. Both biopsy techniques are invasive and require anesthesia for the patients [7]. In many cases, there is a delay in obtaining the results, that may slow down the therapeutic approach, which is essential to increase patient survival [8].

The cytopathological evaluation can be used to provide faster results besides being more cost-efficient. However, cytopathology is an invasive technique that cannot promote the final diagnosis, grade some tumor types, have a considerable rate of inconclusive results, and its diagnostic accuracy is variable [9–11]. When considering the limitations of this type of evaluation, fast and noninvasive techniques are required to aid in early diagnosis and therapeutic management.

In human medicine, the ultrasonography technique has been used since the 1990s in skin cancer studies, and it already has applicability in malignancy prediction and differentiation between some tumor types, such as squamous cells and basal cell carcinomas [12, 13]. In veterinary medicine, specifically in the canine species, B-mode, Doppler, and ARFI elastography have already shown promise in malignancy prediction of breast tumors, where malignant neoplasms presented increased dimension and higher systolic and diastolic vascular velocities, as well as high shear velocity values (>2.57 m/s) [14].

As for cutaneous and subcutaneous canine neoplasms, a few studies have demonstrated the applicability of ultrasonographic methods on tumor diagnosis and differentiation. A preliminary study found that predominantly hypoechoic, heterogeneous neoplasms with signs of invasiveness in adjacent tissues were more prone to malignancy [15]. On Doppler, it has been verified that cutaneous mast cell tumors have lower resistivity indices than soft tissues sarcomas and benign lesions [16]. Malignant neoplasms demonstrated greater stiffness on elastography when compared to benign tissues. However, the assessment was only qualitative, without the obtention of quantitative values of this stiffness [17].
Based on the possibility of skin tumors malignancy prediction in canines using ultrasound techniques, this study aimed to evaluate cutaneous and subcutaneous neoplasms using B-mode, Doppler, and ARFI elastography, to determine the accuracy of ultrasonography methods, suggest an evaluation protocol for these neoplasms, and perform the ultrasonographic characterization of the specific tumor types included.

**Results**

**Histopathologic results**

A total of 130 cutaneous neoplasms (98 malignant and 32 benign) were evaluated, resulting in 21 histopathologic classifications (Table 1). The most prevalent malignant neoplasms in this study were the high-grade cutaneous mast cell tumors (18.46%). In comparison, the most prevalent benign neoplasms were lipomas (13.07%).

| Classification                          | Malignancy  | n  |
|-----------------------------------------|-------------|----|
| Adenocarcinoma                          | Malignant   | 1  |
| Sebaceous adenoma                       | Benign      | 6  |
| Basal cell carcinoma                    | Malignant   | 2  |
| Squamous cell carcinoma                 | Malignant   | 15 |
| Mixed carcinoma                         | Malignant   | 2  |
| Apocrine cystadenoma                    | Benign      | 2  |
| Fibrosarcoma                            | Malignant   | 4  |
| Cavernous hemangioma                    | Benign      | 6  |
| Hemangiopericytoma                      | Malignant   | 1  |
| Hemangiosarcoma                         | Malignant   | 5  |
| Cutaneous lymphoma                      | Malignant   | 13 |
| Lipoma                                  | Benign      | 17 |
| Infiltrative lipoma                     | Benign      | 1  |
| High-grade cutaneous mast cell tumor    | Malignant   | 24 |
| Low-grade cutaneous mast cell tumor     | Malignant   | 10 |
| Combined subcutaneous mast cell tumor   | Malignant   | 1  |
| Infiltrative subcutaneous mast cell tumor| Malignant   | 2  |
| Amelanotic melanoma                     | Malignant   | 9  |
| Grade II soft tissue sarcoma            | Malignant   | 7  |
| Grade III soft tissue sarcoma           | Malignant   | 1  |
| Grade II perivascular sheath tumor      | Malignant   | 1  |
| **Total**                               | -           | **130** |

**B-mode ultrasonography**

In B-mode, measurements of length (3.02±2.85 cm), width (2.58±2.18 cm), and height (1.79±1.71 cm) were not associated with tumor malignancy, as well as echogenicity, capsule, and echotexture pattern (smooth or rough) (Table 2). It was found that echotexture (P=0.007), invasiveness in adjacent tissues (P=0.002), hyperechogenic spots (P=0.031), and cavitary areas (P=0.001) were shown to be predictive characteristics of malignancy. This way, heterogeneous neoplasms with signs of invasiveness, presence of hyperechogenic spots, and
cavitary areas were more likely to be malignant (Figure 1). The predictive values of sensitivity, specificity, accuracy, PPV and NPV are shown in Table 2, and the ultrasonographic characterization of neoplasms is shown in Table 3.

Table 2
Results of association between the mode-B ultrasonographic characterization of malignant cutaneous and subcutaneous canine neoplasms and their predictive values (sensibility, specificity, accuracy, positive predictive value, and negative predictive value) for those with $P < 0.05$.

| Characteristic          | $P$-value | Se (%) | Sp (%) | Ac (%) | PPV (%) | NPV (%) |
|-------------------------|-----------|--------|--------|--------|---------|---------|
| Length                  | 0.780     |        |        |        |         |         |
| Width                   | 0.795     |        |        |        |         |         |
| Height                  | 0.619     |        |        |        |         |         |
| Echogenicity            | 0.059     |        |        |        |         |         |
| Echotexture             | 0.007     | 96.87  | 18.75  | 93.04  | 78.15   | 66.66   |
| Echotexture pattern     | 0.915     |        |        |        |         |         |
| Invasiveness            | 0.002     | 70.83  | 59.37  | 68.14  | 83.95   | 40.42   |
| Capsule                 | 0.099     |        |        |        |         |         |
| Hyperechoic spots       | 0.031     | 50.00  | 71.87  | 55.38  | 84.48   | 31.94   |
| Cavity areas            | 0.001     | 69.38  | 62.50  | 67.69  | 85.00   | 40.00   |

Se – sensitivity; Sp – specificity; Ac – accuracy; PPV – positive predictive value; NPV – negative predictive value.
Table 3

B-mode ultrasonographic characterization (echogenicity, echotexture, echotexture pattern, invasiveness, capsule, hyperechogenic spots, and cavitary areas) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases.

| Histopathological classification (n) | Echogenicity | Echotexture | Echotexture pattern | Invasiveness | Capsule | Hyperechogenic spots | Cavitary areas |
|------------------------------------|-------------|-------------|---------------------|--------------|---------|---------------------|---------------|
| Sebaceous adenoma (6)              | Hypoechoic 83.3% | Homogeneous 16.7% | Smooth 33.3% | Invasive 50% | Present 16.7% | Present 50% | Present 50% |
|                                    | Hyperechoic 16.7% | Heterogeneous 83.3% | Gross 66.7% | Noninvasive 50% | Absent 83.3% | Absent 50% | Absent 50% |
| Basal cell carcinoma (2)           | Hypoechoic 50% | Homogeneous 0% | Smooth 0% | Invasive 100% | Present 50% | Present 100% | Present 100% |
|                                    | Hyperechoic 50% | Heterogeneous 100% | Gross 100% | Noninvasive 0% | Absent 50% | Absent 0% | Absent 0% |
| Squamous cell carcinoma (15)       | Hypoechoic 53.3% | Homogeneous 0% | Smooth 60% | Invasive 66.7% | Present 6.7% | Present 40% | Present 60% |
|                                    | Hyperechoic 46.7% | Heterogeneous 100% | Gross 40% | Noninvasive 33.3% | Absent 93.3% | Absent 60% | Absent 40% |
| Mixed carcinoma (2)                | Hypoechoic 100% | Homogeneous 0% | Smooth 0% | Invasive 100% | Present 0% | Present 0% | Present 0% |
|                                    | Hyperechoic 0% | Heterogeneous 100% | Gross 100% | Noninvasive 0% | Absent 100% | Absent 100% | Absent 100% |
| Apocrine cystadenoma (2)           | Hypoechoic 100% | Homogeneous 50% | Smooth 50% | Invasive 50% | Present 0% | Present 0% | Present 0% |
|                                    | Hyperechoic 0% | Heterogeneous 50% | Gross 50% | Noninvasive 50% | Absent 100% | Absent 100% | Absent 100% |
| Fibrosarcoma (4)                   | Hypoechoic 100% | Homogeneous 0% | Smooth 25% | Invasive 75% | Present 25% | Present 50% | Present 70% |
|                                    | Hyperechoic 0% | Heterogeneous 100% | Gross 75% | Noninvasive 25% | Absent 75% | Absent 50% | Absent 30% |
| Cavernous hemangioma (6)           | Hypoechoic 100% | Homogeneous 33.3% | Smooth 83.3% | Invasive 16.7% | Present 33.3% | Present 33.3% | Present 50% |
|                                    | Hyperechoic 0% | Heterogeneous 66.7% | Gross 16.7% | Noninvasive 83.3% | Absent 67.7% | Absent 50% | Absent 50% |
| Hemangiosarcoma (5)                | Hypoechoic 100% | Homogeneous 0% | Smooth 80% | Invasive 20% | Present 0% | Present 20% | Present 100% |
|                                    | Hyperechoic 0% | Heterogeneous 100% | Gross 20% | Noninvasive 80% | Absent 100% | Absent 0% | Absent 0% |
| Cutaneous lymphoma (13)            | Hypoechoic 84.6% | Homogeneous 0% | Smooth 61.5% | Invasive 100% | Present 15.4% | Present 15.4% | Present 46.2% |
|                                    | Hyperechoic 15.4% | Heterogeneous 100% | Gross 38.5% | Noninvasive 0% | Absent 84.6% | Absent 46.2% | Absent 53.8% |
| Lipoma (17)                        | Hypoechoic 41.2% | Homogeneous 11.8% | Smooth 41.2% | Invasive 41.2% | Present 0% | Present 17.6% | Present 83.5% |
|                                    | Hyperechoic 58.8% | Heterogeneous 88.2% | Gross 58.8% | Noninvasive 58.8% | Absent 100% | Absent 82.4% | Absent 16.5% |
| High-grade cutaneous mast cell tumor (24) | Hypoechoic 79.2% | Homogeneous 4.2% | Smooth 45.8% | Invasive 79.2% | Present 29.2% | Present 58.3% | Present 83.3% |
|                                    | Hyperechoic 20.8% | Heterogeneous 95.8% | Gross 54.2% | Noninvasive 20.8% | Absent 70.8% | Absent 41.7% | Absent 16.7% |
| Low-grade cutaneous mast cell tumor (10) | Hypoechoic 90% | Homogeneous 0% | Smooth 20% | Invasive 80% | Present 20% | Present 90% | Present 90% |
|                                    | Hyperechoic 10% | Heterogeneous 100% | Gross 80% | Noninvasive 20% | Absent 80% | Absent 10% | Absent 10% |
Doppler

The absence of vascularization on Doppler was verified in 59 neoplasms, while 39 had mild vascularization, 21 moderate, and 11 intense. Although an association between vascularization intensity and malignancy was not observed (Table 4), only one benign neoplasm (infiltrative lipoma) presented intense vascularization. There were also no associations between tumor malignancy, location, and vascularization pattern.

| Histopathological classification (n) | Echogenicity | Echotexture | Echotexture pattern | Invasiveness | Capsule | Hyperechogenic spots | Cavitory areas |
|-------------------------------------|--------------|-------------|---------------------|--------------|---------|----------------------|---------------|
| Infiltrative subcutaneous mast cell tumor (2) | Hypoechogetic 100% | Homogeneous 0% | Smooth 0% | Invasive 100% | Present 100% | Present 0% | Present 0% |
|                                      | Hyperechogetic 0% | Heterogeneous 100% | Gross 100% | Noninvasive 0% | Absent 100% | Absent 100% | |
| Amelanotic melanoma (9)               | Hypoechogetic 100% | Homogeneous 0% | Smooth 0% | Invasive 50% | Present 16.7% | Present 44.4% | Present 55.6% |
|                                      | Hyperechogetic 0% | Heterogeneous 100% | Gross 66.7% | Noninvasive 50% | Absent 83.3% | Absent 55.6% | Present 44.4% |
| Grade II soft tissue sarcoma (7)      | Hypoechogetic 100% | Homogeneous 0% | Smooth 100% | Invasive 100% | Present 100% | Present 0% | Present 0% |
|                                      | Hyperechogetic 0% | Heterogeneous 100% | Gross 0% | Noninvasive 0% | Absent 100% | Absent 100% | |

| Characteristic | P-value | Cut-off value | Se (%) | Sp (%) | AUC (%) |
|----------------|---------|---------------|--------|--------|---------|
| Intensity      | 0.211   | -             | -      | -      | -       |
| Location       | 0.617   | -             | -      | -      | -       |
| Patter         | 0.171   | -             | -      | -      | -       |
| Systolic peak  | 0.635   | -             | -      | -      | -       |
| Diastolic velocity | 0.971 | -          | -      | -      | -       |
| Resistivity index | 0.071 | -          | -      | -      | -       |
| Pulsatility index | 0.015 | >0.93         | 90.50  | 55.60  | 75.70   |

| Se – sensitivity; Sp – specificity; AUC – area under the curve. |

Identification of arterial flow using pulsed Doppler was only possible in 51 neoplasms. Of these, 42 were malignant (82.35%) and only nine benign (17.65%). It was found that the peak values of systolic velocity, diastolic velocity, and resistivity index were not predictive of malignancy using the pulsed Doppler. However, the pulsatility index proved to be significant in differentiating between malignant and benign neoplasms (P=0.015), with a cut-off value >0.93 as indicative of malignancy, with 90.5% sensitivity, 55.6% specificity, and 75.7% accuracy (Figure 2). The Doppler ultrasonographic characterization of cutaneous neoplasms is shown in Table 5.
Table 5
Ultrasonographic characterization by Doppler (intensity, location and vascularization pattern, systolic peak, diastolic velocity, resistivity index, and pulsatility index) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases.

| Histopathological classification | Intensity | Location | Pattern       | SP (cm/s) (mean ± SD) | DV (cm/s) (mean ± SD) | RI (mean ± SD) | PI (mean ± SD) |
|----------------------------------|-----------|----------|---------------|-----------------------|-----------------------|----------------|----------------|
| Sebaceous adenoma                | Absent 33.3% | Central 0% | Perinodular 0% | 8.4 ± 2.21           | 2.8 ± 1.22            | 0.67 ± 0.12  | 2.03 ± 1.14   |
|                                  | Discrete 16.7% |          | Mosaic 25%     |                       |                       |                |                |
|                                  | Moderate 50%  |          | Network 75%    |                       |                       |                |                |
|                                  | Intense 0%    | Peripheral 0% |              |                       |                       |                |                |
|                                  |              | Diffuse 100% |              |                       |                       |                |                |
| Basal cell carcinoma             | Absent 0%    | Central 0% | Perinodular 0% | 16.73 ± 29.18         | 4.45 ± 5.88           | 0.7 ± 0.11   | 5.0 ± 9.32    |
|                                  | Discrete 100% | Peripheral 0% | Mosaic 0%     |                       |                       |                |                |
|                                  | Moderate 0%   | Diffuse 100% | Network 100%  |                       |                       |                |                |
|                                  | Intense 0%    |              |               |                       |                       |                |                |
| Squamous cell carcinoma          | Absent 80%   | Central 0% | Perinodular 0% | 10.15 ± 8.55          | 5.5 ± 5.65            | 0.52 ± 0.15  | 1.52 ± 0.91   |
|                                  | Discrete 13.3% | Peripheral 0% | Mosaic 66.7% |                       |                       |                |                |
|                                  | Moderate 0%   | Diffuse 100% | Network 33.3% |                       |                       |                |                |
|                                  | Intense 6.7% |              |               |                       |                       |                |                |
| Mixed carcinoma                  | Absent 100%  | NA        | NA            | NA                    | NA                    | NA            | NA            |
|                                  | Discrete 0%   | NA        | NA            | NA                    | NA                    | NA            | NA            |
|                                  | Moderate 0%   | NA        | NA            | NA                    | NA                    | NA            | NA            |
|                                  | Intense 0%    | NA        | NA            | NA                    | NA                    | NA            | NA            |
| Apocrine cystadenoma             | Absent 50%   | Central 0% | Perinodular 100% | NA                  | NA                    | NA            | NA            |
|                                  | Discrete 50%  | Peripheral 100% | Mosaic 0%    |                       |                       |                |                |
|                                  | Moderate 0%   | Diffuse 0% | Network 0%    |                       |                       |                |                |
|                                  | Intense 0%    |              |               |                       |                       |                |                |

*Only one neoplasm with the arterial flow; SP – systolic peak; DV – diastolic velocity; RI – resistivity index; PI – pulsatility index; SD – Standard deviation; NA – not applicable.
| Histopathological classification (n) | Intensity                      | Location       | Pattern          | SP (cm/s) (mean ± SD) | DV (cm/s) (mean ± SD) | RI (mean ± SD) | PI (mean ± SD) |
|-----------------------------------|--------------------------------|----------------|------------------|-----------------------|-----------------------|----------------|----------------|
| Fibrosarcoma (4)                  | Absent 75%                    | Central 0%     | Perinodular 0%   | NA                    | NA                    | NA             | NA             |
|                                   | Discrete 25%                  | Peripheral 50% |                  |                        |                        |                |                |
|                                   | Moderate 0%                   | Diffuse 50%    |                  |                        |                        |                |                |
|                                   | Intense 0%                    |                |                  |                        |                        |                |                |
| Cavernous hemangioma (6)          | Absent 50%                    | Central 0%     | Perinodular 0%   | 5.85 ± 1.77            | 2.35 ± 0.35            | 0.58 ± 0.06    | 1.06 ± 0.17    |
|                                   | Discrete 50%                  | Peripheral 33.3% |              |                        |                        |                |                |
|                                   | Moderate                      | Diffuse 66.7%  |                  |                        |                        |                |                |
|                                   | Intense                       |                |                  |                        |                        |                |                |
| Hemangiosarcoma (5)               | Absent 80%                    | Central 0%     | Perinodular 0%   | 9.2*                  | 3.4*                  | 0.63*          | 4.14*          |
|                                   | Discrete 20%                  | Peripheral 0%  |                  |                        |                        |                |                |
|                                   | Moderate 0%                   | Diffuse 100%   |                  |                        |                        |                |                |
|                                   | Intense 0%                    |                |                  |                        |                        |                |                |
| Cutaneous lymphoma (13)           | Absent 38.5%                  | Central 0%     | Perinodular 62.5% | 64.7 ± 40.91           | 18.8 ± 17.16           | 0.73 ± 0.18    | 2.18 ± 1.35    |
|                                   | Discrete 53.8%                | Peripheral 62.5% |              |                        |                        |                |                |
|                                   | Moderate                      | Diffuse 37.5%  |                  |                        |                        |                |                |
|                                   | Intense 7.7%                  |                |                  |                        |                        |                |                |
|                                   | Intense 0%                    |                |                  |                        |                        |                |                |
| Lipoma (17)                       | Absent 70.6%                  | Central 20%    | Perinodular 0%   | 18.03 ± 5.71           | 8.67 ± 1.24            | 0.49 ± 1.74    | 0.82 ± 0.58    |
|                                   | Discrete 23.5%                | Peripheral 20% |                  |                        |                        |                |                |
|                                   | Moderate 5.9%                 | Diffuse 60%    |                  |                        |                        |                |                |
|                                   | Intense 0%                    |                |                  |                        |                        |                |                |
| High-grade cutaneous mast cell tumor (24) | Absent 4.2%                 | Central 13%    | Perinodular 4.4% | 17.07 ± 23.89          | 5.02 ± 5.18            | 0.66 ± 0.12    | 3.87 ± 7.2     |
|                                   | Discrete 33.3%                | Peripheral 8.7% |              |                        |                        |                |                |
|                                   | Moderate                      | Diffuse 78.3%  |                  |                        |                        |                |                |
|                                   | Intense 20.8%                 |                |                  |                        |                        |                |                |

*Only one neoplasm with the arterial flow; SP – systolic peak; DV – diastolic velocity; RI – resistivity index; PI – pulsatility index; SD – Standard deviation; NA – not applicable.
ARFI Elastography

Both qualitative and quantitative assessments were shown to be significant in predicting malignancy (Table 6). Regarding deformability, it was observed that 11 benign and nine malignant neoplasms were classified as deformable, while 21 benign and 89 malignant were non-deformable. Deformability was shown to be predictive of tumor malignancy with 90.2% sensitivity, 35.48% specificity, 87.09% accuracy, 81.3% PPV, and 55% NPV.

Table 6

| Characteristic                  | P-value | Se (%) | Sp (%) | Ac (%) | PPV (%) | NPV (%) | AUC (%) |
|--------------------------------|---------|--------|--------|--------|---------|---------|---------|
| Deformability                  | <0.001  | 90.2   | 35.48  | 87.09  | 81.3    | 55.00   | -       |
| SWV                            | 0.024   | 54.1   | 68.7   | -      | -       | -       | 62.7    |

SWV – shear wave velocity; Se – sensitivity; Sp – specificity; Ac – accuracy; PPV – positive predictive value; NPV – negative predictive value; AUC – area under the curve.

In the quantitative elastography study, greater rigidity was observed in malignant (3.72±1.94 m/s) compared to benign neoplasms (3.21±1.86 m/s); consequently, SWV above 3.52 m/s was indicative of malignancy (Figure 3), with 54.1% sensitivity, 68.7% specificity, and AUC of 62.7%. Among the benign neoplasms, adenomas had high rigidity (4.12±2.06), hence the most rigid adenoma had a mean SWV of 8.3 m/s. Of the malignant neoplasms, the most rigid were soft tissue sarcomas (4.11±1.81 m/s) and mast cell tumors (3.76±1.92 m/s), however, the largest SWV observed was in a squamous cell carcinoma (9.1 m/s). The characterization of tumor stiffness by ARFI elastography is shown in Table 7.
Table 7
Ultrasonographic characterization by ARFI elastography (deformability and shear wave velocity – SWV) of cutaneous and subcutaneous canine neoplasms for tumor types that presented two or more cases.

| Histopathological classification (n)         | Deformability | SWV (m/s) (mean ± SD) |
|--------------------------------------------|---------------|-----------------------|
| Sebaceous adenoma (6)                      | Deformable 16.7%  | 3.82 ± 1.92            |
|                                            | Non-deformable 83.3% |                       |
| Basal cell carcinoma (2)                   | Deformable 0%    | 3.87 ± 1.84            |
|                                            | Non-deformable 100% |                      |
| Squamous cell carcinoma (15)               | Deformable 0%    | 3.82 ± 1.96            |
|                                            | Non-deformable 100% |                      |
| Mixed carcinoma (2)                        | Deformable 0%    | 2.46 ± 0.19            |
|                                            | Non-deformable 100% |                      |
| Apocrine cystadenoma (2)                   | Deformable 0%    | 3.72 ± 1.81            |
|                                            | Non-deformable 100% |                      |
| Fibrosarcoma (4)                           | Deformable 0%    | 3.89 ± 1.88            |
|                                            | Non-deformable 100% |                      |
| Cavernous hemangioma (6)                   | Deformable 0%    | 3.87 ± 1.79            |
|                                            | Non-deformable 100% |                      |
| Hemangiosarcoma (5)                        | Deformable 20%   | 2.9 ± 1.97             |
|                                            | Non-deformable 80% |                      |
| Cutaneous lymphoma (13)                    | Deformable 0%    | 3.6 ± 1.97             |
|                                            | Non-deformable 100% |                     |
| Lipoma (17)                                | Deformable 64.7% | 3.83 ± 1.84            |
|                                            | Non-deformable 35.3% |                      |
| High-grade cutaneous mast cell tumor (24)  | Deformable 4.2%  | 3.76 ± 1.92            |
|                                            | Non-deformable 95.3% |                    |
| Low-grade cutaneous mast cell tumor (10)   | Deformable 0%    | 3.91 ± 1.79            |
|                                            | Non-deformable 100% |                    |
| Infiltrative subcutaneous mast cell tumor (2)| Deformable 0% | 3.96 ± 1.83          |
|                                            | Non-deformable 100% |                    |
| Amelanotic melanoma (9)                    | Deformable 0%    | 3.72 ± 1.83            |
|                                            | Non-deformable 100% |                    |
| Grade II soft tissue sarcoma (7)           | Deformable 0%    | 3.67 ± 1.8             |
|                                            | Non-deformable 100% |                    |

Association of malignancy predictive characteristics

All characteristics that presented significant results in malignancy prediction were considered for the association between the different types of ultrasound techniques. Thus, seven tumor characteristics were considered: heterogeneous, invasive, presence of hyperechogenic points, presence of cavitory areas, PI above 0.93, non-deformable, and above 3.52 m/s SWV.
It was observed that 85 neoplasms had at least four malignancy predictive characteristics (Table 8). Seventy-two neoplasms were indeed malignant, and only 13 were benign. Five or more malignancy predictive characteristics were found in sixty neoplasms, where 53 were malignant and seven were benign. When considering all seven malignancy predictive characteristics, 16 neoplasms were computed, where 14 were malignant, and only two were benign.

| Predictive characteristics | Total (n) | Malignant (n) | Benign (n) | P-value | Se (%) | Sp (%) | Ac (%) | PPV (%) | NPV (%) |
|---------------------------|-----------|---------------|------------|---------|--------|-------|-------|--------|--------|
| Four or more              | 85        | 72            | 13         | 0.001   | 73.46  | 59.37 | 70    | 84.7   | 42.22  |
| Five or more              | 60        | 53            | 7          | 0.002   | 54.08  | 78.12 | 60    | 88.33  | 35.71  |
| Six or more               | 45        | 41            | 4          | 0.002   | 41.83  | 87.5  | 53    | 91.11  | 32.94  |
| All seven                 | 16        | 14            | 2          | 0.230   |        |       |       |        |        |

n – total number; Se – sensitivity; Sp – specificity; Ac – accuracy; PPV – positive predictive value; NPV – negative predictive value.

Discussion

This study provides important information regarding the diagnosis and classification of cutaneous and subcutaneous canine neoplasms, as it was possible to establish malignancy predictive characteristics by all techniques used (B-mode, Doppler, and ARFI elastography). In addition, it was possible to determine an ultrasound examination protocol that could contribute to lesions diagnosis and prognosis and provide individual ultrasound characteristics for each studied tumor type. Because it is a complementary method, its characteristics are highly sensitive and have a positive predictive value. These results were obtained in all three ultrasound techniques that were performed.

Given the high number of cutaneous and subcutaneous neoplasm types, it should be considered that they have different structural components and biological behaviors. They can range from benign to highly aggressive and metastatic lesions [18], which justifies the moderate results observed. The authors would like to emphasize the importance of studies regarding specific cancer types, as the present study results differed from previous canine mammary tumors findings. In another study, with breast tumors, different characteristics and predictive values of malignancy were found [14].

There were no associations between malignancy and tumor measurements in this study, which can be explained by the fact that neoplasms were diagnosed at different stages. There were no associations with echogenicity, which may be related to the different pathological processes involved, such as active inflammation or tissue necrosis in different tumor types [19]. A preliminary study involving 42 cutaneous neoplasms showed an association between malignancy and hypoechogenicity [15]. A greater number of neoplasms and specific types of skin cancer that were included in the present study may explain the discrepancy between the two studies.

The heterogeneous echotexture indicative of malignancy seen in cutaneous and subcutaneous tumors is explained by the different structural components, such as the presence of cavitation areas, points of fibrosis, or microcalcifications. The association between heterogeneous echotexture and malignancy was already demonstrated in previous studies with different types of neoplasms (cutaneous and mammary) in both humans and animals [13, 15–17, 20].

It was possible to identify the signs of invasiveness in adjacent tissues because of their reactivity or the difficult tumors delimitation and then associate it with malignancy. This association is justified because malignant neoplasms tend to be more aggressive and invasive than benign ones, even requiring a greater safety margin when surgically removed [21].

On Doppler, it was not verified any qualitative characteristic with malignancy. It is known that tumor growth, both in malignant and benign lesions, is dependent on the blood supply [22]. Therefore, it is reasonable the fact that no significant results were obtained in neoplasm differentiation through these characteristics even though other researchers showed associations with malignancy in other tumor types, such as breast cancer in women and canine mast cell tumors [23, 24].

Even though no vascularization points were observed in some tumors by color Doppler, the lack of vascularization should not be ruled out. It is known that the color Doppler technique has limitations at microvascular level and tissue perfusion, requiring other methods for diagnostic complementation, such as contrasted ultrasound [14]. Nevertheless, this technique was not available and could not be tested in the present
study. This Doppler technique limitation contributed to the impossibility of evaluating all neoplasms by pulsed Doppler, with the Doppler velocimetry indices being calculated for only a portion of those who presented vascularization in color Doppler.

The lack of association between RI, systolic peak, and diastolic velocity with malignancy could be because it was only possible to identify the arterial flow in 9 benign neoplasms, predominantly in malignant lesions (82.35% of cases). However, a PI increase in malignant neoplasms was verified. The increase in this index has already been associated with malignancy in other types of lesions, such as ovarian and thyroid tumors in humans and metastases in canine lymph nodes. These may be related to the compressive effect tumor, the angiogenesis process, and the presence of arteriovenous shunts, which promote turbulent flows with high perfusion rates [25–27].

In the same way, as B-mode observed heterogeneity, the increased rigidity observed in malignant neoplasms can also be explained by tissue components they may present. In a previous study, greater stiffness was found in malignant mammary tumors in female dogs compared to benign ones, and this increase in stiffness was justified by the presence of areas of fibrosis, microcalcifications, and even collagen deposition [14].

The study of the rigidity of skin neoplasms in dogs has already been carried out qualitatively and semiquantitatively (through scores) through elastography, with greater rigidity being observed in malignant tissues, however no real quantitative values of the shear wave velocity were obtained. only subjective analysis [17]. On the other hand, this study provides more detailed information regarding neoplasms stiffness since it was possible to verify that an SWV greater than 3.52 m/s was indicative of malignancy. In addition, the elastography method used (ARFI method) allows more reliable results that are easy to perform, with greater reproducibility and less interobserver variability than sonoelastography [28].

Some benign neoplasms, such as adenomas, showed high tissue stiffness, justified by the accumulation of keratin and predominantly lymphoplasmacytic inflammatory infiltrate [29], that cause rigidity alterations in the keratinocytes and extracellular matrix [30, 31].

Because ultrasonography is a complementary exam and should not be used alone to diagnose neoplasms, in this study, we demonstrate the importance of the association between the findings of the different techniques performed. These have been already described for evaluating breast tumors in women, where an increase in accuracy was found when elastography and Doppler findings were associated [24]. In our study, as we increased the number of malignancy predictive characteristics, there was a decrease in the number of false positives, increase in protocol specificity, and positive predictive value.

Among the study's limitations, it should be considered that some tumor types had a low experimental number, and as noted in this discussion and we had some values discrepancies (e.g., adenomas), which may be responsible for the low specificity and accuracy values that were observed.

Conclusions
Findings from this study indicate that ultrasonography has good applicability in the malignancy prediction of cutaneous and subcutaneous canine neoplasms through different techniques. It provides quick and noninvasive results and can be used as a complementary method for this diagnosis. Furthermore, we found that the assessment protocol by associating the findings of different ultrasound techniques allows for greater reliability in diagnosing malignancy in this type of cancer.

Methods

Experimental design
This study was carried out according to the ARRIVE guidelines 2.0 (2020). Prospective data collection was conducted between September 2019 and June 2021. Sixty-six dogs of different breeds and ages (9.45±2.58 years) from the hospital routine presented cutaneous or subcutaneous neoplasms were enrolled in the study. The Veterinary Oncology sector previously evaluated all patients.

Ultrasound evaluation
Trichotomy of the tumor region was done with up to two centimeters of the peritumoral region. In order to maintain the patient's comfort during the examination and without sedation or anesthesia, patients were positioned in decubitus according to the anatomical location of the neoplasms. ACUSSON S2000™ equipment (Siemens®, Munich, Germany) was used for all the techniques performed, with a linear transducer and frequency ranging from 8 to 10Mhz. In addition, an ultrasonographic conductive gel was used throughout the examination.

B-mode ultrasound
The transducer was positioned in the central superficial region of the neoplasms, adjusting the focus, gain, and depth as needed. After adjusting the device, the nodules and masses were measured in longitudinal (length and height) and transversal (width) sections. The characteristics of echogenicity (hypoechogenic or hyperechogenic), echotexture (homogeneous or heterogeneous), echotexture pattern (coarse or smooth), invasiveness in adjacent tissues (presence or absence), capsule (presence or absence), cavitary areas (presence or absence), and hyperechogenic points (presence or absence) were evaluated.

**Doppler**

The color Doppler function was activated to identify neovascularization, and the pulse repetition frequency (PRF) was adjusted to 977 Hz. When necessary, changes were made to the pre-established PRF. Tumor neovascularization was characterized according to its intensity (absent, mild, moderate, or intense), location (central, peripheral, or diffuse), and pattern (perinodular, mosaic, or network).

The pulsed wave Doppler was activated and used only for those neoplasms that presented vascularization at color Doppler examination. At this stage, the PRF used in the qualitative assessment was maintained, and the caliper was adjusted to cover 2/3 of the vessel's caliber, and using an angulation towards the vessel when necessary, respecting the limit of 60º degrees. At least three spectral traces were obtained [14] to get the peak values of systolic velocity (m/s), diastolic velocity (m/s), resistivity index (RI), and pulsatility index (PI).

**ARFI Elastography**

The elastographic evaluation was performed using the VTIQ method (virtual touch tissue imaging quantification, 2D-SWE technique). Color elastograms were performed in the qualitative study. Where blue colors represented more elastic areas, green and yellow represented intermediate stiffness, and red corresponded to more rigid areas. Based on the color pattern, neoplasms were classified according to their deformability (deformable or non-deformable). The same elastograms were used for quantitative analysis, and at least three areas of interest were selected. Those areas were randomly chosen to obtain the mean shear wave velocity (SWV - m/s), quantified by the VTIQ software, and using total stiffness as a representative value [14].

**Histopathological evaluation**

After ultrasound examinations, clinical care was continued, and biopsies (incisional or excisional) were performed to obtain the definitive diagnosis. Patients were individually anesthetized, and surgical protocols were defined under the recommendation of the responsible veterinarian. These tumor samples were fixed in 10% formalin and sent to the veterinary pathology laboratory within the university, where histological cuts were performed to make slides stained with hematoxylin and eosin and, in cases of mast cell tumors, with toluidine blue. After histopathological diagnosis, neoplasms were classified as benign or malignant, as established by the World Health Organization (WHO).

**Statistical analysis**

All data were analyzed using the SPSS Statistics 20 package (IBM®, New York, United States), and a significance level of 95% was used for all tests (P < 0.05). Echogenicity, echotexture, texture pattern, invasiveness, capsule, hyperechogenic spots, cavitary areas, and deformability were associated with malignancy using the Chi-square test, and sensitivity, specificity, accuracy, and positive (PPV) and negative (NPV) predictive values were calculated. Logistic regression was performed to differentiate malignancy according to the intensity, location, and pattern of vascularization. The other characteristics were submitted to the Kolmogorov-Smirnov normality test. The Mann-Whitney test was performed to analyze length, width, height, systolic peak, diastolic velocity, and pulsatility index. While for the resistivity index and SWV, a t-test was performed for independent samples. A ROC curve was used to obtain the cut-off point, sensitivity, specificity, and area under the curve for significant results.

Afterward, the variables with significant results were selected, and a descriptive analysis of the association between the different ultrasound techniques was performed. Furthermore, they were grouped into four groups: 1) presence of at least four predictive malignancy characteristics; 2) at least five characteristics; 3) at least six characteristics; 4) seven characteristics. Thus, the chi-square test verified an association with malignancy, and the values of sensitivity, specificity, accuracy, PPV, and NPV were calculated. Additionally, descriptive analysis was performed and expressed in percentages of the qualitative ultrasonographic characteristics and the mean and standard deviation of the quantitative characteristics for each tumor type included in this study, except for single cases.

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Animal Care and Use Committee of Universidade Estadual Paulista “Júlio de Mesquita Filho”, Jaboticabal, Brazil (Protocol 010047/19) and the owners formally agreed, through signing a term of responsibility, to enroll their animals in this study. All
methods were performed in accordance with National Animal Testing Control Board (CONCEA).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare having no competing interests.

Funding

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Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions

ICKC, MARF, RARU and ABN planned the experimental design. ICKC and RKC performed the ultrasound exams. ICKC and RARU performed a statistical analysis. ICKC and EMB performed the writing and translation of the manuscript. MARF and ABN reviewed the writing of the manuscript. All authors approved the final version of the article.

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Figures
Figure 1

B-mode ultrasound images in longitudinal section of canine cutaneous neoplasms. a Apocrine cystadenoma - predominantly hypoechoic, homogeneous, non-encapsulated, noninvasive, lacking hyperechogenic spots and cavitary areas; b lipoma - predominantly hyperechogenic, homogeneous, non-encapsulated, noninvasive, with absence of hyperechogenic points and cavitary areas; c Low-grade mast cell tumor - predominantly hypoechogenic, heterogeneous, partially encapsulated, with signs of invasiveness in adjacent tissues, presence of hyperechogenic spots (arrows), and cavitary areas (arrowheads); d high-grade mast cell tumor - predominantly hypoechogenic, heterogeneous, encapsulated, with signs of invasiveness in adjacent tissues, presence of hyperechogenic spots (arrows), and cavitary areas (arrowheads). Note reactivity of adjacent musculature (asterisk).
Figure 2

Ultrasonographic images of canine cutaneous neoplasms obtained by pulsed Doppler, showing spectral tracings and Doppler velocimetry indices calculations. a Benign neoplasm (cavernous hemangioma) with 0.73 (not indicative of malignancy) pulsatility index (PI); b Malignant neoplasm (grade II soft tissue sarcoma) presenting 1.97 PI (indicating malignancy).

Figure 3

B-mode (a, c) and ARFI elastography (b, d) ultrasound images of canine cutaneous neoplasms. In (a) and (b), hemangioma – elastogram image showing predominance of blue colors (less rigid) and with mean shear wave velocity (SWV) of 2.65 m/s (not indicative of malignancy); In (c) and (d), high-grade cutaneous mast cell tumor – elastogram showing a predominance of green and yellow colors (intermediate stiffness) and with an average SWV of 4.77 m/s (indicating malignancy).

Supplementary Files

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- data.xlsx