ELISA and Fourier-transform infrared spectroscopy: HER2 gene expression in the blood serum of canines with a mammary tumour

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Abstract

ELISA and FTIR assay techniques were used to identify HER2 gene expression in the blood serum of female dogs and to characterise the biochemical composition. ELISA tests assess the stage of primary tumour development and evolution, while FTIR allows for a complete characterisation of biomolecules associated with the tumoral process. Blood serum samples from 30 female dogs were analysed. Concentrations of the HER2/*neu* protein were detected using ELISA kits specific for canine and human detection. Infrared spectroscopy (IR) was conducted in absorbance mode at a frequency range of 400–4000 cm^{-1} and a resolution of 4 cm^{-1} over 50 scans. The ELISA cut-off for HER2 protein concentration in blood serum was determined using the receiver operating characteristic (ROC) curve and by estimating the area under the curve (AUC) at a 95% confidence interval (CI=95%). The ROC curves in the canine and human ELISA tests were 0.75 and 0.45, respectively. The representative IR spectra for HER2 gene expression corresponded to lipids (1161 cm^{-1}, 1452 cm^{-1}, 2851 cm^{-1}). This study contributes to the knowledge of HER2 through the identification of biochemical features associated with the changes in the HER2/*neu*+ and HER2/*neu*- states.

Key words: *ErbB2; infrared spectroscopy; immunoassay; oncoprotein; blood*

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Introduction

Canine mammary tumour (CMT) is a common pathology in female dogs (Moe, 2001; Zatloukal et al., 2005; Vacellari et al., 2009; Gal et al., 2016) and is characterized by high mortality levels (Silva et al., 2014), where one of four dogs over the age of two years with this tumour may die (Hemanth et al., 2015). Growth factors (GFs) are polypeptides that stimulate cellular proliferation through high-affinity binding to membrane receptors (Goustin et al., 1986). This signalling process leads to autocrine growth phenomena that are typical of neoplastic transformation (Surmacz, 2003; Witsch et al., 2010). Human epidermal growth factor receptors (EGFR/HER) are part of a family of receptor tyrosine kinases (RTK) (Biscardi et al., 2000). The EGFR group includes four members: HER1 (ErbB1), HER2/*neu* (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) (Ceballos & Hernández, 2008). The HER2 proto-oncogene is located on chromosome17q21 in humans (Fukushige et al., 1986) and 1q13.1 in canines (Murua-Escobar et al., 2001). This gene encodes the 185-kDa transmembrane protein HER2/*neu* (Akiyama et al., 1986) that contains an intracellular tyrosine kinase domain (ICD), a transmembrane domain, and an extracellular domain (ECD) with a mass of 105 kDa, which is measurable in blood serum (Ha et al., 2014; Di Gioia et al., 2015). Active HER2 receptors stimulate cellular proliferation and tumour progression (Gutiérrez and Shiff, 2011; Ressel et al., 2013), but not necessarily tumour malignisation (Rungsipipat et al., 1999; Martin de las Mulas et al., 2003; Carney et al., 2004; Ressel et al., 2013; Ferreira et al., 2014; Silva et al., 2014; Burrai et al., 2015; Campos et al., 2015).

HER2 is highly important in the prognosis of human breast cancer (HBC) development (Savino et al., 2009) since it is associated with resistance to chemotherapy and hormonal treatments, leading to reduced survival of patients with overexpression of this gene (Surmacz, 2003; Ceballos & Hernández, 2008). In canines, SNP-based studies have shown that mutations in HER2 are silent and could represent a natural variation in this gene (Hsu et al., 2009). However, silent SNPs can affect the kinetics of protein synthesis and generate diverse configurations of the final proteins (Kimchi-Sarfaty et al., 2007; Komar, 2007), ultimately challenging diagnosis and follow-up. The use of serum and blood-based biomarkers in cancer studies allows achieving a broad perspective of the biochemical characteristics and dynamics of cancer; furthermore, these markers are especially relevant when biopsies are difficult to perform (Jesneck et al., 2009). The enzyme-linked immunosorbent assay (ELISA) is used to evaluate the stage of primary tumour development by enabling the monitoring of changes in HER2 during the evolution of the mammary tumour (Carney et al., 2004). In humans, a serum HER2 (sHER2) concentration of 15 ng/mL is the most appropriate test cut-off value to measure the levels of this protein in blood (Tse et al., 2005; Fehm et al., 2007; Zhang et al., 2018), yet other authors have determined a cut-off of 22 ng/mL (Savino et al., 2009). Despite this, due to the wide variations in sHER2 levels, its clinical value is prognostic, particularly related to tumour progression and therapeutic response (Carney et al., 2004, 2013). In canines, there is still no definitive diagnostic cut-off (Campos et al., 2015).

Fourier-transformed infrared spectroscopy (FTIR) is a simple and rapid reagent-free method that is non-destructive and requires a small amount of sample (Zelig et al., 2015). Most cancer lesions are identified at the spectral region between 800 and 1800 cm^{-1} (fingerprint); this range

comprises the majority of functional groups, such as carbonyl, carbon-nitrogen, amino, methyl, methylene, carboxy, among others (Lima et al., 2015; Ferreira et al., 2020). However, the spectral region from 2800 to 3000 cm^{-1}, called the first lipid region (Gavgiotaki et al., 2016), is also of interest due to its relevance in the study of HER2.

The ELISA technique is capable of discriminating between specific proteins and can thus identify these molecules in blood serum. This study focused on the gene expression of HER2 in canine serum by using ELISA to identify the expression features of the protein, and to determine the biochemical characteristics based on functional groups analysis by using FTIR.

Materials and methods

Sample collection and conservation

Samples from female dogs diagnosed with CMT (*n*=14) and healthy females (*n*=6) were examined between May 2018 and March 2019. At the time of sample collection, not all patients had a definitive diagnosis of the tumour type. A total of 4 mL blood was collected bv aseptic venipuncture using an evacuated tube without anticoagulant (BD Vacutainer, Becton Drive), with a clot retraction time of three hours at room temperature. Samples were centrifuged at 3000 rpm for 10 minutes (Tuck et al., 2009). The resulting serum was stored at -80°C and multiple cycles of freezing and thawing were avoided. Samples exhibiting any deterioration were discarded.

ELISA – measurement of HER2 protein levels in blood serum

The Canine Epidermal Growth Factor Receptor 2 (Her2Ab) ELISA kit ref. MBS2606515 (MyBioSource, San Diego, CA, USA) and the Human HER2 Platinum ELISA kit ref. BMS207-2 (eBioscience, Vienna, Austria) were used to detect and quantify HER2 protein levels in blood serum. Assays were performed according to manufacturer's instructions. All assays were performed in duplicate, including the control with known concentrations (soluble fragment of the HER2 protein ($p185^{HER2}$) and a canine standard (HER2)). Measurements were performed on a Rayto RT-2600c (Guangming, Shenzhen, China) microplate reader.

The prognostic criteria for classifying patients with overexpression according to the cut-off values were: 1. Patients with CMT and measurement of sHER2 equal to or above the cut-off: true positive (TP = CMT - HER2/*neu*+); 2. Control patients with a measurement of sHER2 below the cut-off: true negative (TN = Healthy - HER2/*neu*-); 3. Patients with CMT and measurement of sHER2 below the cut-off: false negative (FN = CMT - HER2/*neu*); 4. Control patients with a measurement of sHER2 equal to or above the cut-off: false positive (FP = Healthy - HER2/*neu*+).

Acquisition of FTIR spectra

FTIR analysis was performed on 5 μL blood serum at room temperature. The IR spectra were acquired using an Alpha ATR Platinum spectrometer (Bruker Corporation, Germany). The optimal measurement conditions were at room temperature (20°C) and 40% relative humidity. For readings, each sample was placed on a diamond crystal tip. The reading parameters were absorbance mode, frequency range 400–4000 cm^{-1}, and resolution of 4 cm^{-1} with 50 scans per sample. Readings from water were used as blank runs that were subsequently subtracted from the spectra of the samples (Elmi et al., 2017; Santos et al., 2019; Macotpet et al., 2020).

Spectral data processing

Processing and analysis of the original spectra were performed using OriginPro 9.0.0 (OriginLab Corporation, Northampton, MA 01060 USA). Each spectrum was normalized and baseline

correction performed according to the Savitzky–Golay method with a 2nd order polynomial and 20 data points (Lima et al., 2015; Zelig et al., 2015; Macotpet et al., 2020). The prognostic criteria of cut-off values from the ELISA tests were used to identify the characteristics of the spectra for HER2/*neu* expression. The frequency ranges selected for this analysis included the fingerprint region, 800–1800 cm^{-1} (Ghimire et al., 2020; Macotpet et al., 2020), which was divided into four subregions (800–1100 cm^{-1}, 1100–1380 cm^{-1}, 1380–1700 cm^{-1}, 1700–1800 cm^{-1}), and a spectral region from 2800 to 3000 cm^{-1} since it is relevant for the study of HER2/*neu* (Gavgiotaki et al., 2016; Ferreira et al., 2020).

The segments of the selected spectra were analysed based on second-order derivatives to allow the detection of minor peaks in bands from the main spectral regions and to differentiate between patterns from ill and healthy individuals (Zeling et al., 2015; Ghimire et al., 2020). For this, the spectra were softened using the Savitzky–Golay method with a 2nd order polynomial and 20 data points. The area under the curve (AUC) for each spectrum (original and second-order derivative) was calculated mathematically into a polygon area with an absolute measurement type. The functional groups and vibrational modes were assigned according to previous findings by several authors. The AUC ratio was expressed as the relative area under the curve (RAUC).

Statistical analysis

The following accuracy parameters were analysed from the ROC curve to determine the optimal ELISA cut-off: Youden's index (J) as the maximum vertical distance between the ROC curve and the diagonal (Schisterman et al., 2005), sensitivity (Sn) and specificity (Sp), minimum distance to the top-left corner as the minimum distance between the ROC curve and the left upper corner) (Hajian-Tilaki, 2013). The area under the curve (AUC) was determined to estimate the discriminant capacity of each assay. The prognostic criteria for the classification of patients according to TP, TN, FP, and FN allowed for the establishment of the predictive positive value (PPV) and predictive negative value (PNV).

The absorbance values obtained from the canine and human ELISA tests and the IR spectra (original and second derivative) were checked for normality using the Shapiro-Wilks test. Based on the results from the normality test, parametric tests were used for normal distributions and non-parametric tests for non-normal distributions. A confidence interval of 0.95 was set and a *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using R v.3.2.2, NCSS 11 (NCSS, LLC. Kaysville, Utah, USA), and OriginPro 9.0.0.

Ethical aspects

This study was conducted with the approval of the Ethics Committee on Animal Experimentation, Faculty of Agropecuary Sciences, Universidad de Caldas (CEEA Code-15061601). All dog owners authorised the collection of samples from canine patients through informed consent.

Results

Patients

The samples analysed corresponded to seven breeds: Maltese, Miniature Schnauzer, Cocker Spaniel, French Poodle, English Bulldog, Pitbull, Samoyed, and mixed-breed. The average age was 9.3 years old.

ELISA

Based on the ROC curves, the optimal cut-off and AUC for the canine and

human ELISA tests were ≥0.31 ng/mL and 0.75, ≥9.26 ng/mL and 0.45, respectively (Table 1). The PPV and PNV of canine and human ELISA test was 83% and 50% and 78% and 36%, and the accuracies for each test were 70% and 55%, respectively. Of the 20 female dog samples, the canine ELISA tests discriminated that 14 were HER2/*neu*+ (TP=71%, FP=29%) and six were HER2/*neu*- (TN=67%, FN=33%). Moreover, the human ELISA tests yielded TP=50%, FP=50%, TN=67%, and FN=33%. The Shapiro-Wilks normality test showed statistically significant differences (P<0.05), except for ELISA HER2-negative patients (P=0.13).

Table 1. Accuracy parameters and optimal cut-off for the canine-ELISA and human-ELISA tests

Parameter	Test	
	Canine ELISA	Human ELISA
AUC	0.75	0.45
Cut-off Value	≥ 0.31 ng/mL	≥ 9.26 ng/mL
Sensibility (%)	71	50
Specificity (%)	67	67
PPV (%)	83	78
PNV (%)	50	36
Accuracy (%)[a]	70	55
95% CI-Sensibility		
Lower	0.419	0.2304
Upper	0.9161	0.7696
95% CI-Specificity		
Lower	0.2228	0.2228
Upper	0.9567	0.9567
J (95% CI)[b]	0.381	0.1667
Sensibility + Specificity	1.381	1.166
Distance to Corner	0.439	0.6009

[a]Accuracy = Proportion of correctly classified patients
[b]J = Youden Index. Sensibility + Specificity-1.

FTIR spectra in blood serum in HER2/neu positive-negative female dogs

The study of HER2-positive and negative states was based mainly on the similar results between the canine and human ELISA tests, which showed that TP and FN individuals display malignant and benign lesions, such as mammary carcinoma and benign adenoepithelioma, whereas TN and FP females are clinically healthy. The original infrared spectra from blood serum (Figure 1) showed six main spectral peaks in the ranges 800 to 1800 and 2800 cm^{-1}, corresponding to proteins, esters, nucleic acids, phospholipids, and lipids.

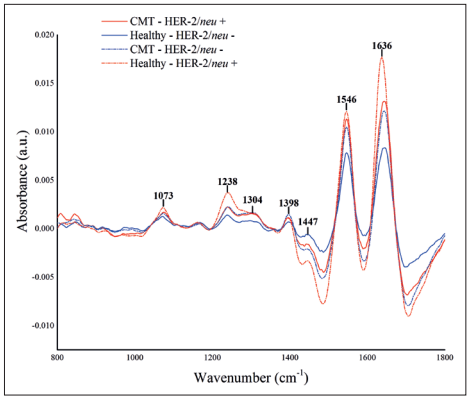

Figure 1. FTIR original spectra for females with CMT and healthy female dogs. Absorbance bands of the main functional groups at frequency ranges from 800 to 1800 cm^{-1} for HER2/neu positive females (red lines) and HER2/neu negative females (blue lines)

The peaks with the highest absorbance are associated with clinically healthy HER2/*neu*-positive females. Table 2 summarizes the vibrational modes assigned and the corresponding blood serum composition according to the literature.

The second-order derivatives of the infrared spectra identified 13 additional representative peaks that were assigned to four subregions. Specifically, subregion 1 (800–1100 cm^{-1}) included phospholipids,

Table 2. Main vibrational modes assigned to the original FTIR spectra in canine blood serum (described in Figure 2) according to various references.

Spectra (cm^{-1})	Organic compound/Vibrational mode assignment	References
1073	Phospholipids: $\nu_s(PO_2)$	Oleszko et al., 2015
1238	Protein: Amide III	Ferreira et al., 2020
1304	Lipids	Bi et al., 2014; Depciuch et al., 2016
1398	Phospholipids/fatty acids, amino acids: $\nu_s(COO^-)$	Kar et al., 2019
1447	Protein (methyl groups), lipids: CH_3 asymmetric bending [δas (CH_3)]	Kar et al., 2019; Ferreira et al., 2020
1546	Protein: Amide II [ν (N–H) ν (C–N)]	Ferreira et al. 2020
1636	Protein: Amide I [ν (C=O), ν(C–N), δ (N–H)]	Ferreira et al., 2020

υ = stretching vibrations, δ = bending vibrations, s = symmetric vibrations, as = asymmetric vibrations

Table 3. FTIR peaks of the average second-derivative spectra of canine HER2/*neu* according to various references

2nd-derivative Spectra (cm^{-1})	Organic compound/Vibrational mode assignment	References
848	Tyrosine proteins	Bi et al., 2014; Zelig et al., 2015; Depciuch et al., 2016
995	Ester bands: vs(C-O)	Elmi et al., 2017; Ferreira et al., 2020
1161	Protein/Carbohydrate/lipid ester bonds: CO–O–C asymmetric stretching (vas (CO-O-C))	Ferreira et al., 2020; Oleszko et al., 2015
1314	Protein: Amide III	Ferreira et al., 2020
1352	Protein: Amide III	Elmi et al., 2017
1452	Lipid and protein: Methylene bending	Bi et al., 2014; Ferreira et al., 2020
1510	Tyrosine proteins: v(C-C)	Kar et al., 2019
1727	Lipids/ester bands:	Gavgiotaki et al., 2016
1768	Ester	Elmi et al., 2017
2851	Lipids, long-chain fatty acids: vs(CH2)	Oleszko et al., 2015
2884	Lipid: CH3 asymmetric stretching (vas (CH3))	Elmi et al., 2017; Ferreira et al., 2020
2920	Nucleic acid/Lipids, long-chain fatty acids: CH2 asymmetric stretching (vas (CH2))	Kar et al., 2019; Ferreira et al., 2020
2989	Phospholipids/cholesterol:	Gavgiotaki et al., 2016

υ = stretching vibrations, δ = bending vibrations, s = symmetric vibrations, as = asymmetric vibrations.

tyrosine-protein, esters; subregion 2 (1100–1380 cm^{-1}) included amide III, carbohydrates, lipids; subregion 3 (1380–1700cm^{-1}) corresponded to phospholipids, fatty acids, lipids, amide I, amide II, and tyrosine protein; and subregion 4 (1700–1800 cm^{-1}) showed lipids and esters. Additionally, the region 2800 to

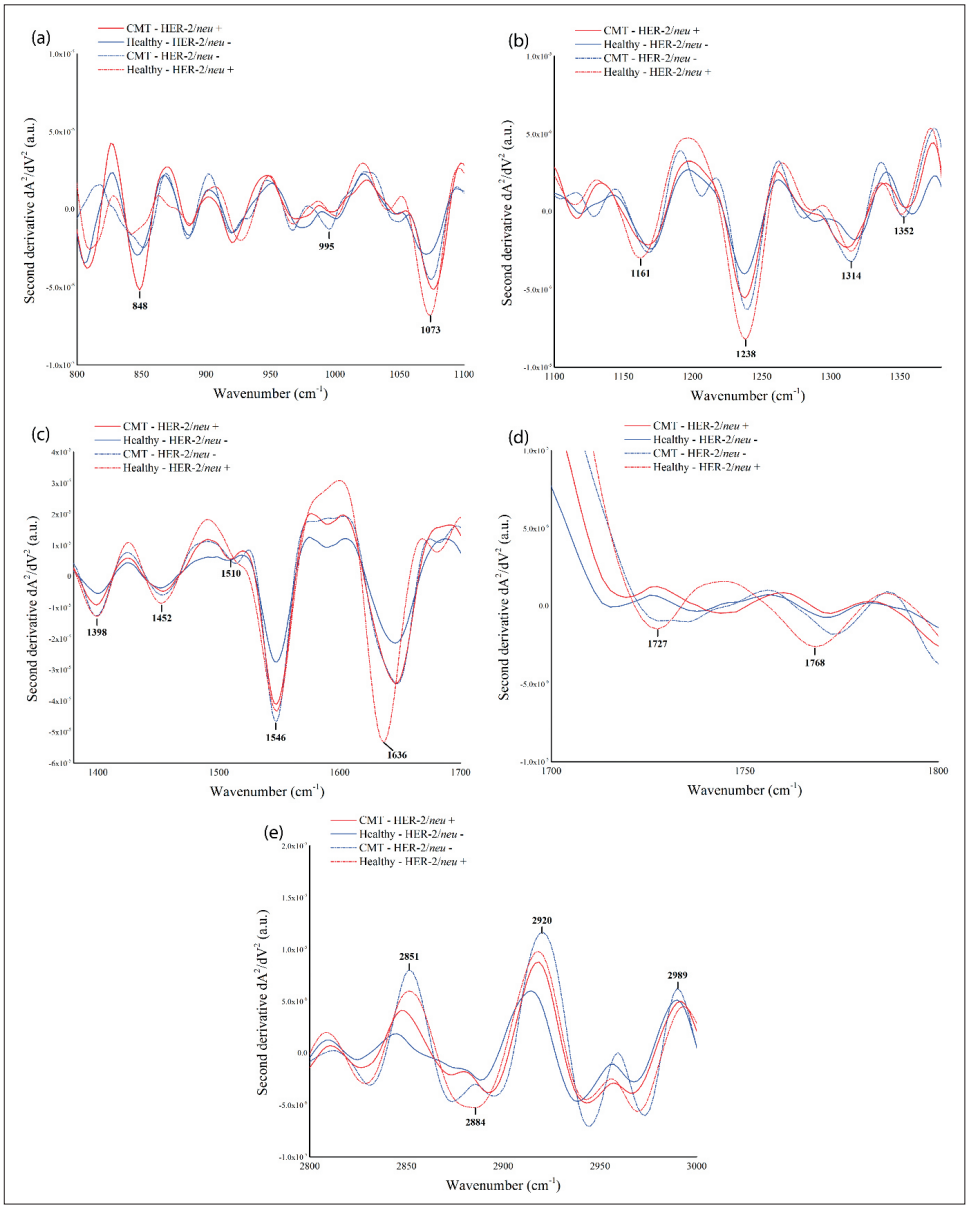

Figure 2. Detail of the second-derivative spectra and most representative frequencies. Spectra (a) 800–1100 cm^{-1}, (b) 1100–1380 cm^{-1}, (c) 1380–1700 cm^{-1}, (d) 1700–1800 cm^{-1}, (e) 2800–3000 cm^{-1} for HER2/neu positive females (red lines) and HER2/neu negative females (blue lines)

Table 4. Statistical analysis of FTIR peaks of the average second-derivative spectra for diagnostic categories and compositional groups

Group	Original-2nd derivative peak (cm^{-1})	TP (Mean±SD)	TN (Mean±SD)	FP (Mean±SD)	FN (Mean±SD)	ANOVA (*p-value*)[1]	t-test (*p-value*)[2]	Tukey-test (*p-value*)[3]	Levene´s-test (*p-value*)[4]
Proteins	848, 1314, 1238, 1352, 1510, 1546, 1636	2.15E-6 ± 3.78E-6	1.34E-6 ± 3.59E-6	3.73E-6 ± 5.96E-6	1.59E-6 ± 5.99E-6	0.21	> 0.05	> 0.05	1.5
Lipids	2851, 2884, 2989	-1.06E-5 ± 1.66E-5	-6.70E-6 ± 1.12E-5	-1.13E-5 ± 1.85E-5	-1.43E-5 ± 2.37E-5	0.14	> 0.05	> 0.05	0.9
Ester	995, 1073, 1304, 1768	-1.68E-6 ± 2.18E-6	-1.08E-6 ± 1.17E-6	-2.12E-6 ± 1.56E-6	-2.56E-6 ± 3.03E-6	0.35	> 0.05	> 0.05	0.8
Mixed	1161, 1398, 1447, 1452, 1727, 2920	-1.56E-6 ± 5.91E-6	-1.41E-6 ± 3.45E-6	-2.38E-6 ± 8.02E-6	-3.96E-6 ± 7.68E-6	0.18	> 0.05	> 0.05	0.6

Abbreviations: TP: CMT - HER-2/*neu*+, TN: Healthy - HER-2/*neu*-, FN: CMT - HER-2/*neu*-, FP: Healthy - HER-2/*neu*+

Note:

[1] Represents $p>0.05$ to comparisons of all prognostic classifications;

[2] Represents $p>0.05$ to comparisons TP vs. TN, TP vs. FP, TN vs. FN

[3] Represents $p>0.05$ indicates that the difference of the means in not significant;

[4] Represents $p>0.05$ homogeneity of variance are not significantly different

3000 cm^{-1} was characterized by lipids, phospholipids, and cholesterol (Figure 2). The description of the vibrational modes and the corresponding functional groups is shown in Table 3.

Four groups were established according to the type of spectra from 20 peaks (original and second derivatives): group 1, proteins; group 2, lipids; group 3, esters; and group 4, mixed. No statistically significant differences were found within each group and the diagnostic categories (Table 4).

The RAUC showed differences between states, indicated by a higher abundance of peaks corresponding to lipids for HER2/*neu*+ (1161 cm^{-1}, 1398 cm^{-1}, 1452 cm^{-1}, 2851 cm^{-1}, 2920 cm^{-1}), except at 1447 cm^{-1} and 2884 cm^{-1} and 1304 cm^{-1} peak and amide I (1636 cm^{-1}). The RAUC for tyrosin-type proteins (848 cm^{-1}, 1510 cm^{-1}), amide III (1314 cm^{-1}, 1352 cm^{-1}), and amide II (1546 cm^{-1}) were greater for HER2/*neu*-, as were esters (995 cm^{-1}, 1768 cm^{-1}) and phospholipids at 2989 cm^{-1}. These findings indicate that amide I and lipid-related functional groups are a product of HER2 gene overexpression.

Discussion

This study contributes to the knowledge of HER2 regarding gene expression and detection in blood serum, its clinical relevance in CMT research, and its application as a model for human breast cancer (HBC). We demonstrated the likelihood of using canine and human antigens to determine and compare sHER2 concentrations. The canine ELISA tests yielded TP = 71%, TN = 67%, FP = 33%, and FN = 29%, whereas the human ELISA test showed 50%, 67%, 33%, and 50%, respectively. Although there is high homology between the human and canine HER2 antigens (Singer et al., 2012), our findings demonstrate that the use of the human HER2 protein is not adequate to evaluate the concentration of this protein in canine serum, in agreement with Campos et al. (2015). This is likely due to the low capacity of canine antibodies to recognize human HER2. These findings indicate that the antigenic determinants from the two molecules are not the same, perhaps due to differences in the tertiary structure of the proteins (Kimchi-Sarfaty et al., 2007; Komar, 2007; Hsu et al., 2009) or cross-reaction with other members of the HER family (Burrai et al., 2015). Both tests coincided in 29% (4/14) of TP females, TN 33% (2/6), FN 50% (3/6), FP 7% (1/14). Our results on the overexpression of the HER2/*neu* protein are similar to previous reports (Dutra et al., 2004; Ressel et al., 2013; Shinoda et al., 2014; Campos et al., 2015).

A positive correlation has been determined between the presence of HER2 in blood serum and tumour tissue (Campos et al., 2015), tumour mitotic index, high histological grade and size (Muhammadnejad et al., 2012; Silva et al., 2014), although no significant differences have been found between HER2 expression in benign and malignant tumours (Kim et al., 2011; Ressel et al., 2013), indicating that HER2 may participate in tumour formation and rapid progression of CMT (Dutra et al., 2004; Ferreira et al., 2009; Bertagnolli et al., 2011), but not necessarily in malignant transformation, or at least, it is not a good marker of malignancy (Kaszak et al., 2018). The clinical association of HER2 is controversial (Hsu et al., 2009; Ressel et al., 2013; Burrai et al., 2015; Campos et al., 2015). Unfortunately, in this study it was not possible to compare the ELISA and FTIR findings with histological parameters due to limited availability of biopsies.

Based on infrared spectroscopy, we analysed diverse biochemical patterns, such as proteins, lipids, esters, nucleic acids, and carbohydrates. Although we did not obtain significant differences, distinct features between HER2/*neu*-

positive and negative states were observed for certain functional groups, such as the relative intensity at the peak at 1636 cm^{-1}. Furthermore, this study analysed an additional region between 2800 and 3000 cm^{-1} that had not been studied in CMT. Previous research on HER2 using infrared spectroscopy, among other biochemical analysis techniques in cells, tissues, and fluids, reported changes in the compositional profile, especially regarding lipids (CH_2, CH_3), which were found to be higher in the HER2/*neu*-positive state (Hartsuiker et al., 2010; Bi et al., 2014; Gavgiotaki et al., 2016). These reports agree with the findings reported here, except for the peaks at 1304, 1447 and 2884 cm^{-1}. Moreover, high contents of lipids in cytoplasmic organelles are reported in HER2+ HBC cell cultures (Hartsuiker et al., 2010; Gavgiotaki et al., 2016), which are involved in the adhesion and migration of epithelial tumour cells (Murai, 2012). Similarly, several studies have detected an increase in phospholipids, associated with fatty acids and cholesterol synthesis (Menendez and Lupu, 2007; Bi et al., 2014; Elmi et al., 2017; Kar et al., 2019). This pattern was only observed at the peak 1398 cm^{-1} (phospholipid, fatty acid, and amino acid complex). This phenomenon is caused by the regulation of the fatty acid synthase associated with a low regulation of tyrosine kinase receptors (Jin et al., 2010).

On the other hand, we report an increase in amide I, especially in HER2/*neu*+. However, amide I not necessarily is associated with HER2 overexpression. Several authors report an increase in this protein in HBC (Elmi et al., 2017; Chrabaszcz et al., 2018), but a consistent variation is not observed in HER2+ cell cultures (Bi et al., 2014). Amide I and amide II are reported in greater amounts in hyperplastic tissues (Tian et al., 2015); however, a reduction of amide II and an increase of amide I suggest a possible malignisation process (Simonova & Karamancheva 2014). A reduction of amide III has been observed in HER2+ cell cultures (Bi et al., 2014), which is consistent with our findings.

FTIR enables the non-invasive recognition of tissue alterations through markers of cellular activity. In this study, we detected Sn 93% and Sp 100%. This agrees with recent studies in dogs that identified the most important spectra in cancer using blood serum and reported Sn and Sp of 76.7% and 87.5%, respectively (Macotpet et al., 2020) and Sn 100% and Sp 100% in conformational proteins in HBC (Ghimire et al., 2020).

Given the analogies between both species (Rowell et al., 2011; Pinho et al., 2012), and the homology between human and canine HER2 antigens, immunotherapy seems to be promising in canine patients with HER-2 expression (Singer et al., 2012; Kaszak et al., 2018). However, additional markers associated with the expression of this gene must be considered in order to elucidate the prognostic value of HER2 overexpression in female dogs.

This study has enabled the recognition of important features of HER2 through discriminant ELISA tests and several biochemical characteristics based on spectral patterns in a biological fluid, such as blood serum. Future studies should address the amplification of the IR spectra described here using nanoparticles in blood serum from ELISA HER2/*neu*-positive or negative patients. Finally, the results of the FTIR identified two possible control peaks of HER2 overexpression, corresponding to the regions surrounding the peak 1636 cm^{-1} and a band between 2800–3000 cm^{-1}, which were associated with amide I and the corresponding lipids (CH_2, CH_3), respectively.

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ELISA i infracrvena spektroskopija s Furierovom transformacijom: ekspresija HER2 gena u krvnom serumu pasa s tumorom mliječne žlijezde

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Ova studija primijenila je ELISA i FTIR tehnike za identifikaciju ekspresije HER2 gena u krvnom serumu kujica i za karakterizaciju biokemijskog sastava. ELISA testovi procjenjuju fazu razvoja i evolucije primarnog tumora, a FTIR omogućuje potpunu karakterizaciju biomolekula povezanih s tumorskim procesom. Analizirali smo uzorak krvnog seruma 30 kujica. Detektirali smo koncentracije HER2/*neu* proteina uporabom dva ELISA kompleta za detekciju u pasa, odnosno ljudi. Infracrvena spektroskopija (IR) je provedena u apsorpcijskom načinu pri frekvencijskom rasponu od 400-4000 cm^{-1} i rezoluciji od 4 cm^{-1}, 50 skenova. Odredili smo ELISA graničnu vrijednost (*cut-off*) za koncentraciju HER2 proteina u krvnom serumu uporabom krivulje karakteristika primatelja-operatora (ROC) i procjenom površine ispod krivulje (AUC) uz interval pouzdanosti od 95 % (CI=95 %). ROC krivulje u ELISA testovima za pse i ljude bile su 0,75, odnosno 0,45. Reprezentativni IR spektri za ekspresiju HER2 gena odgovarali su lipidima (1161 cm^{-1}, 1452 cm^{-1}, 2851 cm^{-1}). Ova studija doprinosi poznavanju HER2, putem identifikacije biokemijskih svojstava povezanih s promjenama u HER2/*neu*+ i HER2/*neu*- stanjima.

Ključne riječi: *ErbB2, infracrvena spektroskopija, imunoanaliza, onkoprotein, krv*