

Prospective pilot study to detect dogs with non food-induced canine atopic dermatitis using Fourier transform infrared spectroscopy

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Background – The diagnosis of canine atopic dermatitis (CAD) remains challenging due to the lack of a simple biomarker or metabolic profile. In human medicine, Fourier transform infrared spectroscopy (FTIR) is an analytical technique used for several diseases. It requires a small amount of sample and allows the identification of structural moieties of biomolecules on the basis of their infrared absorption, with limited sample pretreatment.

Hypothesis/Objectives – The aim of the study was to evaluate the diagnostic value of FTIR.

Animals – Three groups were tested: 21 dogs with non food-induced CAD (NFICAD), 16 dogs with inflammatory conditions of various origins but without allergic dermatoses (OD) and 10 healthy dogs (H).

Methods – Peripheral blood was collected and spectra were acquired with a FTIR spectrophotometer. A principal component analysis (PCA) was performed on the full wavenumber spectra (4000–600/cm), followed by a Fisher discriminant analysis (DA) to assess the differences between the three groups.

Results – The PCA followed by the DA of whole spectra showed significant differences between the three groups. These results suggest that by using the FTIR method, dogs with NFICAD can be differentiated from healthy dogs and dogs with nonallergic inflammation. There was no overlap between the spectral data of the three groups indicating that NFICAD dogs were correctly segregated from the H and OD groups.

Conclusions – A study on a larger cohort including common pruritic skin diseases is necessary to confirm these initial results and the relevance of this diagnostic technique.

Introduction

Despite its high prevalence in dogs, the diagnosis of canine atopic dermatitis (CAD) remains challenging due to mimicry by other pruritic diseases and the lack of pathognomonic clinical features. Due to the lack of definitive clinical indicators or biomarkers, the diagnosis is assigned based upon epidemiological and clinical criteria which when combined provide a satisfactory degree of sensitivity and specificity.^{1–3} However, these criteria can be applied only after excluding other pruritic diseases, a process which may be time-consuming and costly to execute. Allergen-specific IgE identified by serology and intradermal testing is generally considered useful for selecting allergens for allergen-specific immunotherapy, but not for the diagnosis of CAD. A rapid biological test that could increase the clinician's confidence in a clinical diagnosis of CAD would be very helpful. The main criteria for a reliable and easy to use clinical test include: a readily accessible sample source (blood, urine), sufficient

sensitivity to accurately identify affected individuals and sufficient specificity to limit incorrect labelling of unaffected patients.⁴

Fourier transform infrared spectroscopy (FTIR) is a sensitive, replicable analytical technique with numerous practical advantages.^{5,6} It requires only a small sample of biological material with minimal sample pre-treatment; it is fast, effective, reagent-free, noninvasive and inexpensive. This technique identifies the structural moieties of biomolecules on the basis of their absorption of infrared radiation (IR).⁶ Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. A sample is irradiated by infrared radiation of wavelength between 500 and 4000 nm. Parts of the radiation are absorbed by the sample due to the vibrations of the molecules; such vibrations are dependent upon the structure of the molecule and are thus unique to the particular chemical substance. The intensity of the reflected IR is plotted against its wavelength and such a plot is called an infrared spectrum. For a complex mix of molecules (such as a tissue) there is a specific infrared spectrum resulting from all molecules present. The FTIR absorbance spectrum is one of the most information-rich and concise ways to represent the whole "omics" of a cell or of a medium and, as such, fits all the

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characteristics for the development of a clinically useful biomarker.⁷

The aim of the present study was to evaluate the diagnostic value of FTIR spectroscopy, with principal component analysis and discriminant statistical treatment, on plasma samples from dogs with a clinical diagnosis of CAD compared to healthy dogs with dogs with skin inflammation not associated with an allergic aetiology.

Materials and methods

Animals and sample collection

Three groups of dogs were included in the study at the dermatology clinics of Oniris, Nantes, France. The first group comprised dogs with CAD: the diagnosis of CAD was achieved by excluding other primary pruritic skin diseases and meeting at least five criteria,¹ combined with a positive response to intradermal testing for aeroallergens. The dogs selected showed no evidence of flea infestation or clinical signs compatible with flea bite hypersensitivity and did not develop positive reactions to intradermal injection of flea extract. In addition, it was required that all dogs (and the animals with which they were in contact) had been treated against fleas every 4 weeks without improvement of pruritus. In addition, the possibility of an adverse food reaction was excluded by feeding an elimination diet for at least 6 weeks without any clinical improvement (commercial novel protein or hydrolysed diet). Dogs were excluded if they had received treatment with glucocorticoids, ciclosporin or antihistamines (ocloacitinib was not available at the time of the study) within 30 days of enrolment, or antimicrobials within 9 days of enrolment. This group was labelled the "NFICAD group" (Non Food-Induced Canine Atopic Dermatitis).

Two control groups were compared to the NFICAD group: dogs without a primary pruritic dermatosis but with variable degrees of cutaneous inflammation due to various diseases processes (designated the OD Group for "Other Diseases"), and a group of healthy dogs without a history of cutaneous or systemic disease and with biochemical analyses and complete blood counts within normal reference ranges (designated H Group for "Healthy").

The study protocol was reviewed and approved by the institutional animal care and use committee, and owner consent was obtained prior to enrolment.

Sample preparation and FTIR spectral acquisition

The dogs were fasted overnight and peripheral blood was collected aseptically from the jugular vein into a heparinized test tube. Samples were centrifuged at 3 g for 15 min and the plasma was collected in 1 mL tubes, frozen immediately and stored at -20°C until FTIR analysis.

For analysis, plasma samples were thawed to room temperature, then centrifuged at 18.32 g for 10 min, then 30 µL of plasma was diluted with 120 µL of distilled pure water in a 0.5 mL cuvette. Cuvettes were vortexed for 1 min and then three aliquots each consisting of 40 µL were placed on 11.5 mm Ø × 2 mm zinc selenide (ZnSe) optical windows (Crystran; Poole, UK). Each sample was spread gently over an entire window surface and then dried at room temperature in a vacuum chamber for 30 min.

Air was used as a blank reference for the background spectrum before each analysis. For each of the three ZnSe window samples, four analyses were performed resulting in 12 spectra for each plasma sample.

Data analysis

Spectra were acquired with a FTIR spectrophotometer (Bruker Vector 33, Bruker Optics; Ettlingen, Germany) equipped with a DGTS detector and a KBr beamsplitter, using Opus software (Bruker Optics) in the 4000–400/cm range, with 32 scans and 2/cm resolution. The processing time was approximately 1 min. The baseline for the raw data was corrected using Opus software (Bruker Optics) in the 4000–400/cm range. The CO₂ absorbance region (2500–2000/cm) was excluded from the analysis in order to avoid any variation

attributable to the CO₂ atmosphere. Raw data were exported from the acquired Opus file to an Excel datasheet. Before multivariate analysis, each spectrum was normalized by the standard normal variates (SNV) transformation, smoothed according to the Savitzky–Golay algorithm and subjected to 2nd derivative transformation;⁷ this transformation allows for decreased sample dispersion.

A principal component analysis (PCA) was performed on the full spectra (4000–600/cm) using XLSTAT (XLSTAT v2011.4.02, Addinsoft; Paris, France) followed by a Fisher discriminant analysis (DA) to assess the differences among the three cohorts of dogs.⁷ PCA is a rigorous statistical method that transforms data into a new coordinate system. The data of each spectrum are replaced by a single new variable called "principal component" which consists of a linear combination of the raw data. Because all of the principal components were orthogonal to each other, no redundant information was produced.⁸ All of the principal components were then submitted to a DA,⁵ which allows for confirmation that groups of observations are distinct and predicts to which group a new observation belongs.

At each spectral frequency, an unpaired Student's *t*-test was performed to determine the difference between spectra of each group. The following analyses were conducted: NFICAD versus (OD+H); NFICAD versus H; NFICAD versus OD, and H versus OD.

The following regions were examined: 3000–3020/cm region (ν = CH vibration): unsaturated fatty acids, cholesterol esters; 2880–2950/cm region (ν asCH₂ vibration): fatty acids, esters, glycerol, phospholipids, triglycerides; 2950–3000/cm region (ν asCH₃ vibration): cholesterol esters, lipids; 1732–1739/cm region (ν C=O vibration): lipids, cholesterol, esters; 1600–1720/cm region (Amide I, ν C=O vibration): β -sheet and α helix from proteins; 1400–1460/cm region (ν C-O-O): amino acids; 1480–1600/cm region (Amide II, δ N-H vibration): α helix from proteins; 900–1300/cm region (ν C-O and ν C-O-C vibrations): saccharides.⁶ A *P*-value < 0.05 was considered significant for all analyses.

Results

Animals

Twenty one dogs with NFICAD (66% males, mean age 4.7 years), 16 dogs with OD (62% males, mean age 4.0 years) and 10 healthy dogs (50% males, mean age 8.2 years) were included in the study. The NFICAD and OD groups comprised primarily pure-bred dogs. West Highland white terriers were over-represented in the NFICAD group (six dogs). All healthy dogs were beagles. Diseases represented within the OD Group were: post-surgical: bone fracture (*n* = 3) and large skin plasty (*n* = 5); infectious conditions: bacterial pyoderma (*n* = 4), and then one each of kerion, sarcoptic mange and demodicosis; and febrile syndrome.

Spectra data

The PCA followed by DA of whole spectra showed a significant difference between the three groups (*P* < 0.001; Table 1 and Figure 1). Fisher distance highlighted that the H Group had the most distinct characteristics compared to the other two groups.

Table 1. Fisher distance among the three groups of dogs

	NFICAD		OD	
	Distance	<i>P</i> -value	Distance	<i>P</i> -value
NFICAD	0	1	19.95	<0.0001
OD	19.85	<0.0001	0	1
H	76.76	<0.0001	92.50	<0.0001

NFICAD Non food-induced canine atopic dermatitis, OD Other disease, H Healthy.

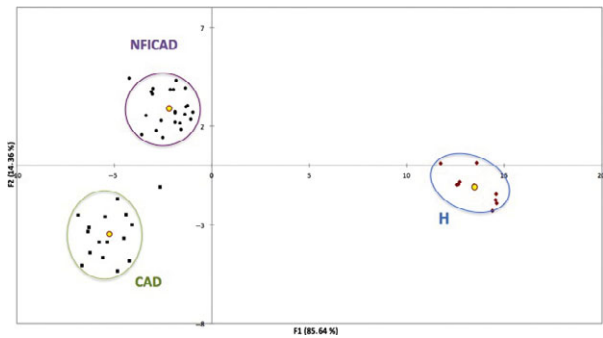


Figure 1. Discriminant analysis of the results of Fourier transform infrared Spectroscopy in three groups of dogs. The three groups were significantly different ($P < 0.0001$). H Healthy, NFICAD Non food-induced canine atopic dermatitis, OD Other disease.

Discriminant analysis was performed for all study subjects to establish profiles for each group, and then a randomly selected sample of seven dogs (five from the NFICAD Group, one each from the OD and Healthy groups) was excluded from this analysis to provide a blinded testing cohort. The DA of the randomly excluded dogs was then analysed and the dogs were blindly classified according to their DA results, into the NFICAD, OD and H groups. The result showed that 100% of the randomly excluded dogs were segregated correctly by their DA.

Data analysis

Student's *t*-tests performed to study differences at each frequency of the spectra showed that in the region corresponding to vibrations of lipid compounds (2880–3000/cm), there was no significant difference between the three groups of dogs. By contrast, the main variations were noted in the regions corresponding to vibrations of proteins and saccharides: 900–1300/cm, 1480–1600/cm, 1400–1460/cm and 1600–1720/cm. In these regions, specific bands corresponding to known biomolecules were examined (Table 2). The absorbance significantly ($P < 0.05$) differed between the groups of dogs (NFICAD versus H+OD) and was higher for NFICAD dogs for those regions corresponding to several identified compounds:

Table 2. Fourier transform infrared spectroscopy – relationship between spectral bands and biomolecules⁶

Bands (/cm)	Name of the identified biomolecule
1600–1488	Albumin
1428–1363	Fibrinogen
1338–1292	Transferrin
1428–1378	Haptoglobin
1337–1279	α 1-acid glycoprotein
1419–1361	IgG1
1652–1622	IgG2
1652–1628	IgG3
1538–1505	IgG4
1418–1372	IgA
1428–1360	IgM
1479–1464	IgD
1484–1427	Apolipoprotein-A
2862–2835	Apolipoprotein-B
1676–1660	Apolipoprotein-C

albumin, transferrin, α 1-acid glycoprotein, haptoglobin, IgA, IgG1, IgG2, IgG3, IgG4, IgM and ApoC. A significant difference ($P < 0.05$) was also noted between NFICAD and OD for six absorption bands with an increased absorption for NFICAD corresponding to the vibrations of peptide areas of amino acids and amide I. Two bands corresponded to the absorbance of known biomolecules (IgG2 and ApoC), one band partially included the absorbance region of IgG4 (1505–1538/cm) and the origin of the final three bands was unknown.

Discussion

Our results suggest that using the FTIR method, dogs with NFICAD can be distinguished from healthy dogs and dogs with nonallergic inflammatory dermatoses. There was no overlap between the spectral data of the three groups, indicating that NFICAD dogs were segregated correctly from the H and OD groups.

To date, no single biological parameter has been identified as an effective predictor of CAD. A promising approach to the development of new diagnostic methods is use metabolomics strategies, where a wide range of metabolites is measured in order to define a metabolic “fingerprint” of the CAD phenotype. The recent and rapid development of metabolomic technologies has changed the way in which new biomarkers are being discovered.^{4,9–11} However, even though these high-throughput techniques are highly sensitive and enable the collection of very detailed information, they are also time-consuming and very expensive. Of these sophisticated techniques, FTIR holds promise as a powerful tool to characterize the chemical composition of very complex probes through the recognition of key chemical signatures. Along with appropriate statistical data processes such as PCA, hierarchical cluster analysis (HCA) or artificial neural network (AAN), FTIR allows the detection of light shifts in the chemical profile of various complex media such as tissues, microorganisms or body fluids and, hence, the means to assess the similarities or differences between samples.^{7,8} The method has been applied in the diagnosis of various human diseases, such as neoplasia,^{8,12–14} inflammatory bowel disease,¹⁵ chronic fatigue¹⁶ and overtraining.¹⁷ The assumption behind FTIR analysis is that these various conditions induce biochemical shifts in tissues or body fluids that may be detected by the technique. In neoplasia, for example, cells grow out of control, consuming carbohydrates, fats and proteins. Thus, the difference between normal and malignant tissue can be identified in a variety of bands in FTIR spectra. Moreover, these differences might be detected at a very early stage of malignancy. Investigators have used this technology to achieve successful discrimination between normal and malignant tissues from the stomach and colon.^{5,8}

In the present study, plasma was used because it is an easily sampled medium and because it has also been used for infrared spectrometric studies of coeliac diseases, chronic fatigue syndrome and in sports medicine.^{9,11,16–18} It can discriminate between patients with Crohn's disease and ulcerative colitis,¹⁵ identify patients suffering from chronic fatigue syndrome¹⁶ and

allow clear discrimination between exercising subjects according to their recovery programme.¹⁸ These proof-of-concept studies suggest that the technology could be useful in diagnostic profiling for a wide range of disease-related “fingerprints”.

In the present study, the cross-validation procedures (prediction set) clearly identified five of 16 dogs with NFICAD with an accuracy of 100%. Figure 1 demonstrates that the NFICAD group and the H group were clearly separated into different regions of the scatter plot, highlighting the homogeneity of spectra obtained by FTIR on these two cohorts. For the OD group, the distribution of spectra is more heterogeneous, perhaps due to the heterogeneity of the skin pathology in this group.

There were several absorption bands that differed between the NFICAD and OD+H groups. Knowledge of what each absorption band corresponds to in dogs is essential to the identification of the metabolites that are represented by the bands. In the present study, conclusions were extrapolated from human data⁶ with the assumption that human and canine molecules are structurally similar. If this assumption is valid, the results suggest that the plasma of NFICAD dogs contains greater amounts of proteins, some amino acids and saccharides, than plasma from healthy dogs and dogs with other inflammatory skin diseases.

Among known biomolecules of particular interest, bands corresponding to human IgG4, IgG2/IgG3 and apolipoprotein C were increased in NFICAD dogs versus OD+H dogs. It is possible that the increased levels of immunoglobulins observed in the area of IgG idiotypes could actually be due to canine IgE. This would be clinically relevant because evidence continues to support IgE as the major antibody associated with CAD.¹⁹ Although the IgE absorption band is unknown in dogs and humans, IgE shares the same basic molecular architecture as antibodies of other classes; the only difference being the heavy ϵ -chain that contains one more domain than the heavy γ -chain of IgG.²⁰ It could therefore be hypothesized that the absorption band of IgE is covered within the IgG bands. Alternatively, the bands could truly represent increased expression of canine allergen specific IgG sub-classes, although their causative role in CAD is questionable.¹⁹

The spectral area of 1660–1675/cm, which corresponds to the apolipoprotein C absorption region in humans, was also elevated in the NFICAD group. The apolipoprotein C group of molecules is involved in lipoprotein metabolism and is expressed predominantly in liver, skin and brain tissue of healthy individuals, primarily within macrophages and keratinocytes.^{21,22} A role for this group of molecules in AD is supported by data collected from transgenic mice which overexpress apolipoprotein C1 (ApoC1) in the skin. Overexpression in these mice disrupts the lipid component of the epidermal barrier, resulting in a mouse with many clinical and histological features of AD.²³ A better identification of this band is necessary to confirm the hypothesis that increased levels of apolipoprotein C could contribute to the pathogenesis of CAD.

Limitations of our study include the small number of dogs and the heterogeneity of the OD group. However, nonoverlap of the data from these groups suggests a

potential difference in the metabolic profiles of dogs with NFICAD, nonallergic inflammation and healthy skin. Further characterization of groups with common pruritic and/or inflammatory skin diseases (e.g. sarcoptic mange, demodicosis, flea bite hypersensitivity, bacterial pyoderma) will be needed for more precise demonstration of metabolomic “maps” for these diseases. Moreover, this technique should also be tested to discriminate between food-induced and non food-induced AD.

In conclusion, this pilot study suggests that FTIR may be a useful technique for segregating dogs with NFICAD from those with some other inflammatory dermatoses. Additional studies with larger cohorts will be required to define the “fingerprints” of these other pruritic diseases and to characterize the diagnostic utility of this technique for differentiating between NFICAD and other allergic dermatoses.

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Résumé

Contexte – Le diagnostic de dermatite atopique canine (DAC) reste un défi en raison du manque d'un simple biomarqueur ou d'un profil métabolique. En médecine humaine, la FTIR (Fourier transform infrared spectroscopy) est une technique analytique utilisée pour différentes maladies. Elle nécessite une petite quantité d'échantillon et permet une identification de la structure des fragments de biomolécules sur la base de leur absorption infra-rouge avec un prétraitement limité des échantillons.

Hypothèses/Objectifs – Le but de cette étude était d'évaluer la valeur diagnostique de FTIR.

Sujets – Trois groupes ont été testés: 21 chiens avec DAC non induite par l'aliment (NFICAD), 16 chiens avec atteinte inflammatoire de différentes origines mais sans dermatoses allergiques (OD) et 10 chiens sains (H).

Méthodes – Le sang périphérique a été collecté et des spectres ont été acquis avec spectromètre FTIR. Un PCA (principal component analysis) a été réalisé sur spectre total (4000–600/cm⁻¹), suivi d'un test de Fischer d'analyse discriminante (DA) afin d'évaluer les différences entre les trois groupes.

Résultats – Les trois groupes ont montrés des différences significatives. Ces résultats suggèrent qu'à l'aide du FTIR, les chiens avec NFICAD peuvent être distingués des chiens sains et des chiens atteints d'inflammation non allergique. Il n'y avait aucun chevauchement entre les spectres des trois groupes indiquant que les chiens NFICAD étaient correctement distingués des groupes H et OD.

Conclusions – Une étude sur une cohorte plus large comprenant des dermatoses prurigineuses fréquentes est nécessaire pour confirmer ces données initiales et la pertinence de cette technique diagnostique.

Resumen

Introducción – El diagnóstico de dermatitis atópica canina (CAD) sigue siendo un reto debido a la falta de un biomarcador simple o un perfil metabólico. En medicina humana, la espectroscopía infrarroja transformada según Fourier (FTIR) es una técnica analítica utilizada para varias enfermedades. Se requiere una pequeña cantidad de muestra y permite la identificación de restos estructurales de biomoléculas sobre la base de su absorción en el infrarrojo, con pretratamiento de la muestra limitado.

Hipótesis/Objetivos – El objetivo del estudio fue evaluar el valor diagnóstico de FTIR.

Animales – Se probaron tres grupos: 21 perros con enfermedad alérgica no alimentaria (NFICAD), 16 perros con enfermedades inflamatorias de origen diverso, pero sin dermatosis alérgicas (OD) y 10 perros sanos (H).

Métodos – se obtuvo sangre periférica y los espectros fueron realizados con un espectrofotómetro FTIR. Un análisis de componentes principales (PCA) se realizó en los espectros completos (4000–600/cm⁻¹), seguido de un análisis discriminante de Fisher (DA) para evaluar las diferencias entre los tres grupos.

Resultados – El PCA seguido de la DA de los espectros completos mostraron diferencias significativas entre los tres grupos. Estos resultados sugieren que al utilizar el método FTIR, los perros con NFICAD pueden diferenciarse de los perros sanos y perros con la inflamación alérgica. No hubo superposición entre los datos espectrales de los tres grupos indicando que los perros con NFICAD fueron correctamente separados de los grupos H y OD.

Conclusiones – Se necesita un estudio sobre una población de animales más amplia, incluyendo enfermedades comunes pruriginosas, para confirmar estos resultados iniciales y la relevancia de esta técnica de diagnóstico.

Zusammenfassung

Hintergrund – Die Diagnose der atopischen Dermatitis (CAD) des Hundes bleibt aufgrund fehlender einfacher Biomarker oder eines metabolischen Profils eine Herausforderung. In der Humanmedizin ist die Fourier-Transform-Infrarotspektroskopie (FTIR) eine analytische Technik, die bei verschiedenen Krankheiten angewendet wird. Sie benötigt eine kleine Probenmenge und erlaubt eine Identifizierung der strukturellen Teile von Biomolekülen auf der Basis der Infrarotabsorption, bei limitierter Vorbehandlung der Proben.

Hypothese/Ziele – Das Ziel dieser Studie war eine Evaluierung des diagnostischen Wertes der FTIR.

Tiere – Es wurden drei Gruppen getestet: 21 Hunde mit nicht durch Futter induzierter CAD (NFICAD), 16 Hunde mit entzündlichen Zuständen unterschiedlichster Ursache aber ohne allergischen Dermatosen (OD) und 10 gesunde Hunde (H).

Methoden – Es wurde peripheres Blut entnommen und die Spectra mittels FTIR Spektrophotometer gewonnen. Eine Hauptkomponentenanalyse (PCA) wurde an den Gesamtspectra durchgeführt (4000–600/

cm^{-1}), gefolgt von einer Diskriminanzanalyse nach Fisher (DA), um die Unterschiede der drei Gruppen festzustellen.

Ergebnisse – Die PCA, gefolgt von der DA der Gesamtspectra zeigte signifikante Unterschiede zwischen den drei Gruppen. Diese Ergebnisse weisen darauf hin, dass mit der FTIR Methode Hunde mit NFICAD von gesunden Hunden und von Hunden mit nichtallergischer Entzündung unterschieden werden können. Es bestand keine Überlappung zwischen den Spektraldaten der drei Gruppen, was darauf hinweist, dass NFICAD Hunde korrekt von den H und OD Gruppen isoliert wurden.

Schlussfolgerungen – Eine Studie mit einer größeren Studiengruppe mit häufigen juckenden Erkrankungen ist nötig, um diese anfänglichen Ergebnisse und die Relevanz dieser Diagnosetechnik zu bestätigen.

要約

背景 – 犬のアトピー性皮膚炎(CAD)の診断には、簡単なバイオマーカーや代謝プロファイルが存在せず、いまだに困難である。人医療域では、フーリエ変換赤外線分光分析(Fourier transform infrared spectroscopy; FTIR)が様々な疾患の診断に用いられている。検査には少量のサンプルとわずかな前処置のみが必要で、赤外線吸収能に基づいて生体分子の構造部位を同定することができる。

仮説/目的 – 本研究の目的は、FTIRの診断的価値を評価することである。

供与動物 – 非食物誘発性CAD(NFICAD)の犬21頭、アレルギー性皮膚疾患以外の様々な臓器の炎症性疾患(OD)の犬16頭、健康犬(H)10頭の3群について検査した。

方法 – 末梢血を採取し、FTIR分光光度計を用いて、周波数範囲を測定した。全周波数範囲($4000\text{--}600/\text{cm}^{-1}$)にて、主成分分析(PCA)およびフィッシャーの判別分析法(DA)を実施し、3群間の差を比較した。

結果 – 全周波数範囲にてDAを実施したPCAにおいて、3群間に有意差が認められた。このことは、FTIR法はNFICADと健康犬および非アレルギー性炎症性疾患を鑑別可能であることを示唆している。これら3群間の周波数範囲データにオーバーラップはなく、NFICAD犬がH群およびOD群から、正確に区別されたことが示唆された。

結論 – 今回の初期成果および本診断法の妥当性を裏付けるためには、一般的な掻痒性皮膚疾患を含むより大規模なコホート研究が必要である。

摘要

背景 – 由于缺乏单个生物标记物或代谢概况,诊断犬异位性皮炎(CAD)仍是挑战。在人医上,傅里叶变换红外光谱(FTIR)可用于多种疾病的分析技术。它只需要少量样本,在红外吸收预处理下,可以识别有限样本的生物分子结构基团。

假设/目的 – 本次研究目的为评估FTIR的诊断意义。

动物 – 实验分为三组:21只无食物过敏的CAD患犬(NFICAD),16只患有多来源炎症但没有过敏性皮肤病(OD)的犬只,以及10只健康犬(H)。

方法 – 采集外周血,通过FTIR分光光度计检测区光谱。对所有光谱($4000\text{--}600/\text{cm}^{-1}$)进行主成分分析(PCA),然后使用费舍尔判别分析(DA)评估三组间的区别。

结果 – PCA以及之后的全光谱DA显示三组间差异显著。这结果表明,使用FTIR方法,NFICAD患犬可与健康犬和非过敏性炎症区分。三组光谱数据没有重叠,表明NFICAD患犬和H组以及OD组完全不同。

总结 – 还需要对更多常见瘙痒性皮肤病患犬进行研究,来确定本次研究结果,以及与诊断技术的关联。

Resumo

Contexto – O diagnóstico de dermatite atópica canina (DAC) permanece desafiador devido a ausência de um biomarcador simples ou um perfil metabólico. Na medicina humana, a espectroscopia infravermelha transformada de Fourier (FTIR) é uma técnica analítica utilizada para diversas doenças e requer uma quantidade pequena de amostra. A FTIR permite a identificação de segmentos estruturais de biomoléculas baseado em sua absorção infravermelha, necessitando de preparação limitada da amostra.

Hipótese/Objetivos – Este estudo tem como objetivo avaliar o valor diagnóstico da FTIR.

Animais – Três grupos foram testados: 21 cães com dermatite atópica não induzida por alimentos (DAC-NIA); 16 cães com doenças inflamatórias diversas, mas sem dermatopatias alérgicas (OD) e 10 cães saudáveis (S).

Métodos – Sangue periférico foi coletado e o espectro foi adquirido por meio de espectrofotômetro de FTIR. A análise componente principal (PCA) foi realizada em espectro completo ($400\text{--}600/\text{cm}^{-1}$), seguido de análise discriminatória de Fisher (DA) para avaliar as diferenças entre os três grupos.

Resultados – A PCA seguida pela DA do espectro total demonstrou diferença significativa entre os três grupos. Os resultados sugerem que através da utilização do método de FTIR, cães com DACNIA podem ser diferenciados de cães saudáveis e de cães com inflamação não alérgica. Não houve sobreposição entre os dados de espectro dos três grupos, indicando que cães com DACNIA foram corretamente segregados dos grupos S e OD.

Conclusões – Um estudo com uma coorte mais extensa incluindo doenças pruriginosas corriqueiras é necessário para confirmar estes resultados iniciais e a relevância desta técnica diagnóstica.