REVIEW

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Clinical Applications of Raman Spectroscopy in Inflammatory Bowel Diseases. A Review

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ABSTRACT

Background & Aims: Inflammatory bowel diseases are still difficult to diagnose and differentiate in some cases despite the serological, imaging, endoscopic and histopathological armamentarium. Raman spectroscopy is a technique that could help with these shortcomings. The aim of this paper is to present the accuracy of Raman spectroscopy in the diagnosis and monitoring of patients with inflammatory bowel diseases.
Methods: We identified the published manuscripts and abstracts up to the 31st of December 2017 by a systematic search of Medline, Embase, Cochrane and other trial registries.
Results: Eight publications were found, showing sensitivities and specificities of Raman spectroscopy in diagnosing and differentiating inflammatory bowel diseases ranging from 82 to 99% and 57 to 99%, respectively, and accuracies of up to 95%.
Conclusion: The technique has so far proven its potential in the positive and differential diagnosis of Crohn's disease or ulcerative colitis, allowing for very rapid results with high sensitivity and specificity.

Key words: Endoscopy – Raman spectroscopy – ulcerative colitis – Crohn's disease – inflammatory bowel disease.

Abbreviations: CD: Crohn's disease; IBD: inflammatory bowel diseases; UC: ulcerative colitis.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are the main well-characterized forms of inflammatory bowel disease (IBD). Their diagnosis is based on the combination of clinical, endoscopic, histopathological, serological and radiological features, but the precise distinction between UC and CD remains a challenge in 5-15% of cases despite the technical advances in recent years. At the same time, diagnosis may be difficult due to clinical overlapping between these two conditions, incomplete IBD expression, or as a result of a reduced histological response of the intestinal mucosa, so that in 5-15% of the patients the initial diagnosis is changed over time from UC to CD or vice versa. Moreover, it has been reported that serological markers, such as anti-Saccharomyces cerevisiae and p-ANCA antibodies, introduced in the positive and differential diagnosis of IBD, have a high specificity but a relatively low sensitivity. The differential diagnosis between UC and CD is mandatory due to distinct approaches in surgical treatment, differences in terms of prognosis, recurrence rate and the risk of development of neoplasia. For these reasons, it is necessary to seek new approaches in the diagnosis of IBD.

Raman spectroscopy is a technique used to quantify vibrations in a system and is based on the non-elastic diffusion of photons. It was originally developed in chemistry and used in the characterization of various molecules and their relationship, and in the physics of the solid, to describe the various characteristics of different materials.

PRINCIPLES OF RAMAN SPECTROSCOPY

The basic principle behind Raman spectroscopy relates to changing the frequency of photons after the interaction of light with an obstacle, which may be liquid, solid or gaseous in nature. After encountering the material of interest, the light is dispersed, this being determined by the characteristic absorption of the sample. If the frequency of the radiation does

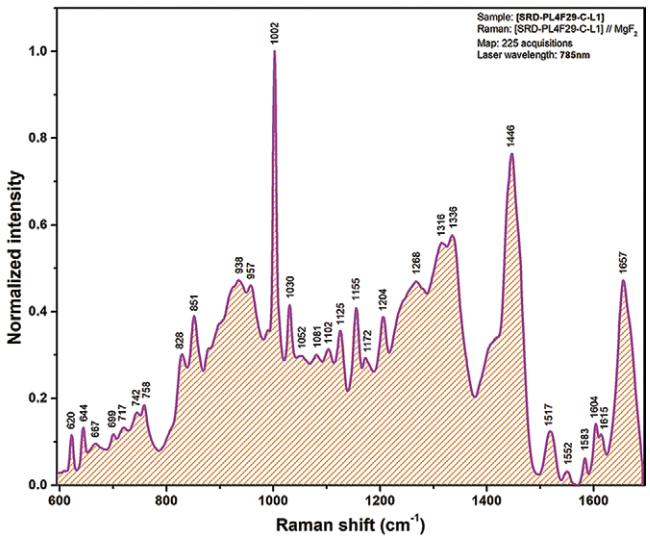
not change, a molecular equilibrium occurs, the dispersion being called Rayleigh. When the dispersion causes a photon frequency shift, the molecular vibrations undergoing changes due to the polarization of the molecules, the dispersion is called Raman, this being quantifiable in cm^{-1}. The most intense dispersion occurs at frequency levels that are lower than the incidence of incident radiation

The importance of this technique lies in the fact that vibrational frequencies are specific to molecular chemical bonds and their symmetry. The region of interest for organic molecules is between 500 and 1500 cm^{-1} so that, using specific tables, reliable conclusions can be drawn on the composition of the measured sample, and the protein-lipid composition of the biological tissues can be characterized. For example, phenylalanine ring breathing corresponds to 1003 cm^{-1}, 1305 cm^{-1} to CH2-twisting and 1660 cm^{-1} to amide I [5].

To obtain and quantify the Raman dispersion, the sample is radiated with monochromatic light, the most commonly used sources being lasers at various wavelengths in visible, ultraviolet, or near infrared spectra (Table I). The dispersing element is in most cases a diffraction network or an interferometer. Thus, a typical Raman system consists of a laser source, a light collection system, a wavelength selector, and a detector. The information obtained is given in the form of wavelength intensity spectra (Fig. 1).

Table I. Types of lasers used in Raman spectroscopy and their advantages

Type	Wavelength	Advantages
Ultra-violet	244 nm, 257 nm, 325 nm, 364 nm	Ultra-violet lasers are best used for detection of proteins, DNA, RNA and for fluorescence suppression.
Visible	457 nm, 473 nm, 488 nm, 514 nm, 532 nm, 633 nm, 660 nm	Blue or green lasers are good for inorganic materials and surface enhanced Raman scattering.
Near infra-red	785 nm, 830 nm, 980 nm, 1064 nm	Near infra-red lasers (660-830 nm) are good for fluorescence suppression.



Fig, 1. A typical Raman spectra.

Compared to other analytical techniques, Raman spectroscopy offers several major advantages. It is a light scattering technique, so it does not require prior preparation of the samples, but only their placement in the irradiation area. This has a very important advantage over histological examinations requiring time for sample processing, sometimes with special and immunohistochemical stains, so that reaching a conclusive result might take a few weeks. Also, it is not necessary to dissolve the solids or otherwise alter the chemical or physical structure of the samples. The tissue samples can be analyzed in various containers, an important element when it comes to infectious materials. At the same time, since the incident light beam can be concentrated on very small regions of the order of 10 microns, the required amount of material is very small.

The major disadvantages of Raman spectroscopy are related to the quality of the laser and its wavelength. Thus, since the Raman dispersion represents 10^{-4} of all the dispersed light, it can sometimes be very difficult to quantify it. An inappropriate increase of laser intensity or acquisition time can lead to the destruction of the sample by burning, an undesirable event especially when working with limited quantities of the material. The use of shorter wavelengths may increase the accuracy of determinations, but sometimes cause fluorescence that masks Raman spectra.

Another thing worth mentioning is that, although the time of acquisition of a spectrum is extremely low, in the order of seconds, the total acquisition time varies greatly depending on the type of material being investigated. Thus, while within a homogeneous inorganic suspension the scanning time may remain low, because wherever the determination is made the results are similar, there is a great variation in the case of tissue samples. In this case, due to the multiple cellular and subcellular elements to be quantified separately, which have significant chemical differences, the total acquisition time may take longer and may be influenced by the size of the sample and the number of acquisitions per sample.

A major drawback of Raman spectroscopy is also the cost of the necessary equipment, which can be very high.

Raman microspectroscopy, an assembly made up of an optical microscope to which the laser system and a spectrometer are connected, has been shown to be promising for cellular and tissue diagnosis in recent years. Raman tissue spectra may be correlated with homologous histopathological examinations because the Raman tissue signature reflects its biochemical composition. The wavelengths of the lasers used in Raman microspectroscopy are 785 or 1064 nm, which reduces the risk of sample burning at the cost of prolonged acquisition times, thus Raman dispersion is decreased towards the infrared spectra.

METHOD

We searched Medline, Embase and the Cochrane library from 1950 to 2017. The search terms used included: "Raman", "spectroscopy", "inflammatory bowel disease", "colitis" and "Crohn's disease". No language restrictions were used. No duplicates were found. We included studies using Raman spectroscopy as a diagnostic means in either UC, CD or both.

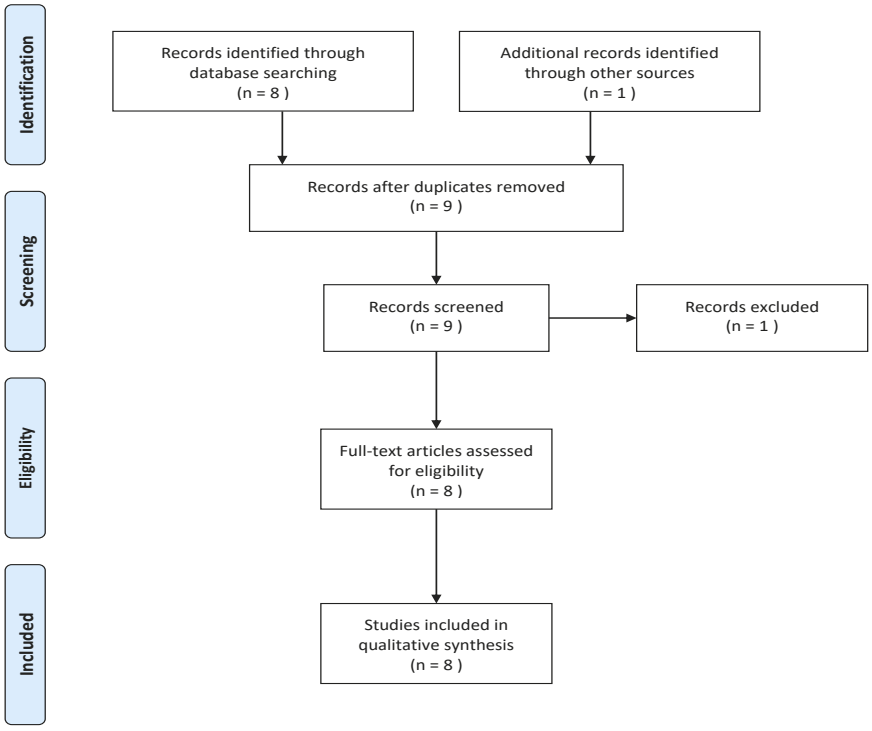


Fig. 2. Flowchart of the studies identified.

We included both animal and human studies. One study was excluded, as it did not use Raman spectroscopy as a diagnostic tool in IBD (Fig. 2).

RESULTS

Eight publications up to 2017 were found using our search criteria. All studies were performed on humans. While 5 studies were performed *in vitro*, using tissue samples from endoscopic biopsies or after colectomy, 3 were performed *in vivo*, using endoscopic-coupled Raman spectroscopy. These studies showed sensitivities and specificities of Raman spectroscopy in diagnosing and differentiating inflammatory bowel diseases ranging from 82 to 99% and 57 to 99%, respectively, and accuracies of up to 95% (Table II).

DISCUSSION

The potential for Raman microspectroscopy in diagnosing multiple pathologies, including the gastroenterological ones,

Table II. Published studies on Raman spectroscopy applied in inflammatory bowel diseases.

Reference	Subjects	Outcomes	Performance indicators
Bi et al., 2011 [9]	12 pts with UC, 9 pts with CD	Significant differences in nucleic acid, phenylalanine and lipid spectra	N/A
Bielecki et al., 2012 [10]	13 pts with UC, 14 pts with CD, 11 healthy controls	Accurate separation between patients with IBD and healthy controls	Sensitivity 99.07%, Specificity 98.81%
Veenstra et al., 2014 [8]	4 pts with UC, patients without UC	Accurate separation between patients with UC and controls	Sensitivity 82% Specificity 89%
Pence et al., 2014 [12]	15 pts with UC, 26 patients with CD, 10 healthy controls	Accurate real-time endoscopic assessment of IBD	Accuracy 79.7%
Wood et al., 2014 [14]	18 pts with UC, 30 healthy controls	Accurate separation of different colorectal pathologies	Accuracy 95.1%
Chernavskaia et al., 2016 [11]	7 pts with UC, 6 pts with CD, 7 pts with infectious colitis	Multimodal images are able to display major indicators of an inflammation	N/A
Pence et al., 2017 [13]	8 pts with UC, 15 pts with CD, 8 healthy controls	Accurate real-time endoscopic assessment of IBD	Sensitivity 86% Specificity 57%
Ding et al., 2017 [16]	18 pts with UC, 31 healthy controls	Accurate real-time endoscopic separation between UC and controls	Sensitivity 83.5%

pts: patients; UC: ulcerative colitis; CD: Crohn's disease; N/A: not available.

has been recently demonstrated [6, 7]. However, there is little data on the use of Raman spectroscopy to detect the molecular changes that occur in epithelial cells during inflammatory or tumoral conditions.

Inflammatory bowel diseases have been only recently studied by means of Raman spectroscopy, with the idea that this technique could reduce the number of uncertain or inaccurate diagnoses. At the same time, the possibility of developing very small optical fiber probes that can be used during endoscopic examinations and the reduced time required to obtain *in vivo* results could lead to a revolution in the positive and differential diagnosis and the monitoring of IBD. Thus, some authors reported promising results in diagnosing these conditions using endoscopic probes.

In one of the first studies published on the topic, using colorectal biopsy specimens from 8 patients who underwent surgery, Veenstra et al. [8] managed to differentiate subjects with UC from healthy subjects with a sensitivity of 82% and a specificity of 89%, with significant differences in the spectral regions associated with nucleic acids (1,044 cm^{-1}), lipids (1,102 and 1,656-1,658 cm^{-1}) and proteins (755, 1,030-1,032, 1,102, 1,170-1,173, 1,246, 1,613 and 1,656-1,658 cm^{-1}), explained by the development of inflammation and increased cell turnover.

Bi et al. [9] examined colon specimens taken from 21 patients with CD and UC who underwent partial or total colectomy, in an attempt to establish differences between the two entities. Raman spectra were obtained using a laser probe emitting at 785 nm applied to the mucosal surface. After statistical quantification of the results, an increase in the amount of lipids (regions 1255-1314 cm^{-1}, 1423-1479 cm^{-1}, 1657 cm^{-1}) was demonstrated in subjects with UC versus those with CD, more likely due to loss of lipid deposits from the submucosa in those suffering from CD. At the same time, the levels of structural proteins (996-1013 cm^{-1}) and nucleic acids (1559-1576 cm^{-1}) were lower in those with UC, differences that could be explained by the destruction of colon morphology and reduced cellular integrity in the examined areas. The study limits could be the lack of a control group and the use of a univariate statistical test as opposed to a multivariate one [9].

Perhaps one of the most important studies using Raman spectroscopy in the diagnosis of inflammatory bowel diseases is that of Bielecki et al. [10]. They compared spectral images obtained from 13 subjects with UC, 13 subjects with CD and 11 healthy control subjects and, using the appropriate morphological images from the same subjects in haematoxylin-eosin staining, developed a computerized system capable of the computational analysis of histological and spectral structures, differentiating epithelial elements from conjunctival or mucosal ones. Subsequently, using this mapping system, they were able to establish statistically significant differences between the groups of healthy and diseased subjects, especially in the regions corresponding to the heme groups (743, 1245 and 1580 cm^{-1}), which can be explained by hyperemia in the context of inflammation. Significant differences were also found in bands 1003, 1245, 1305, 1450 and 1660 cm^{-1}, thus reinforcing the idea that spectral analysis of colon epithelium in IBD, even in clinical and endoscopic remission, can be separated from healthy epithelium.

Chernavskaia et al. [11], using multimodal nonlinear imaging techniques, including Raman spectroscopy, on human tissue biopsies from 7 patients with UC and 6 patients with CD, highlighted important elements of inflammation of the colonic epithelium, such as the distortion of crypts, epithelial ruptures, basement membrane thickening, presence of lymphocytic infiltrates or mucosal scarring. The authors argued that with the development of sufficiently small probes that could be used in endoscopic equipment, these multimodal imaging techniques could be used *in vivo* as a surrogate for the histopathological examination, allowing for a rapid and independent diagnosis by the examiner. But, before an immediate spectroscopic diagnosis could be made, it is necessary to develop precise scoring methods

Taking things one step farther, Pence et al. [12] evaluated several spectra from patients with CD or UC by the Raman technique with optical fiber probes coupled to a laser using a wavelength of 785 nm, and compared them to spectra obtained from healthy patients. All the spectra were obtained by the colonoscopic evaluation of the subjects, the probes being advanced on the endoscope operative channel. The subsequent morphological examination of the tissues in the areas of interest confirmed the subtype of IBD. There were statistically significant differences between healthy subjects and those with IBD at 425 cm^{-1} (δ (CCC) skeletal backbone), 610 cm^{-1} (p (CH) wagging in proteins), 1080 cm^{-1} Of the lipids, 1440 cm^{-1} shoulder (δ (CH2) deformation of proteins and lipids), 1160 cm^{-1} and 1525 cm^{-1} (β-carotene), and 1741 cm^{-1} (ν(C = O) in lipids). Overall, subjects with active disease had reduced spectra intensity, more likely in the context of parietal edema with attenuation of the intensity of incident laser beam, and widening spectra corresponding to protein elements most likely related to an increase in fibrin concentration at the level of the bowel wall. Thus, Raman spectroscopy had a sensitivity of 86% in differentiating subjects with IBD from healthy subjects with a fairly low specificity of 39%, but which increased to 57% when considering the differentiation between subjects with IBD in clinical and endoscopic remission from those with a flare. At the same time, the spectroscopic examination succeeded in discriminating between the two subtypes of IBD with a sensitivity of 83% and a specificity of 55%. Of course, the results can be largely influenced by the lack of homogeneity of the examined mucosa due to the presence of pathological products (blood, mucus, fibrin deposits) on its surface. This technique required great precision in handling the probe, and it was advisable to avoid exaggerated pressure on the examined mucosa.

In an attempt to improve the endoscopic diagnosis of IBD, Wood et al. [14] developed a Raman probe to be used with endoscopic equipment. However, the study was not conducted *in vivo*, but colonoscopic biopsy fragments from the large bowel were collected from various pathologies, including from 18 patients with UC, which were subsequently subjected to Raman spectroscopy using an 830 nm laser system. After applying the appropriate statistical methods, healthy tissue was differentiated from diseased tissue with an accuracy of 95%.

However, in current clinical practice, macroscopic endoscopic differentiation between normal and pathological colorectal mucosa is readily accomplished. There are instances, among others, in some types of IBD, when the macroscopic appearance of the mucosa is perfectly normal, but there is an inflammatory activity at the microscopic level. This is important, because persistent microscopic inflammation, both acute and chronic, in patients with UC has been associated with increased relapse rate, hospitalization, colectomy and the risk of colorectal neoplasia. Depicting the microscopic inflammatory status in a macroscopically normal mucosa could lead to treatment escalation in order to reduce the risks mentioned above. Ding et al. [16] tried to achieve this goal in 18 patients with UC, using endoscopic-coupled Raman spectroscopy. They demonstrated substantial decreases in the content of total lipids and phosphatidylcholines in the inflamed colon tissue as compared to controls, as well as to those with quiescent disease, with a sensitivity of 83.5% and 97.1%, respectively.

Endoscopic Raman spectroscopy could therefore be routinely added to the standard gastrointestinal endoscopy. As each Raman spectrum acquisition can be completed within 0.5 to 3 seconds, examining the whole colon adds just less than 5 minutes to the procedure, with no need for more invasive procedures, such as biopsy sampling. This is important as it greatly reduces the time it takes to provide a proper diagnosis. In addition, developing a computational system that can independently analyze samples *in vivo* and differentiate between healthy and diseased tissue might make sample interpretation by a pathologist or gastroenterologist unnecessary, thus reducing costs. However, with the technology presently available, the endoscopist could perform the interpretation, as long as he or she undergoes training in Raman spectra interpretation.

Another possible indication in using Raman spectroscopy is the endoscopic surveillance of patients with colonic IBD, as we know that colorectal cancer has a higher incidence in these patients compared to healthy subjects. In a study including 65 patients with Barrett's esophagus, Raman spectroscopy identified lesions containing high-grade dysplasia or early cancer with an overall diagnostic accuracy of 89% [18]. As such, the same method could be applied to patients with IBD. Highlighting areas with high-grade dysplasia in the colon before they can be visualized with the current techniques could completely change the management of these patients, and drastically reduce the need for high-risk endoscopic or surgical interventions.

CONCLUSION

We are at an important crossroads in the development of positive and differential diagnosis of IBD. Raman spectroscopy demonstrates important advantages in relation to the speed and accuracy of the results, with sensitivities and specificities ranging from 82 to 99% and 57 to 99%, respectively. In the future, histopathological examination might become unnecessary, as endoscopic tissue spectral analysis yields immediate results. In addition, Raman spectroscopy can be performed on fluids, so that in the future we might not even require endoscopic exploration in patients with IBD, establishing the diagnosis and performing the monitoring by using simple blood tests.

Conflict of interests: No conflict to declare.

Authors' contributions: C.T. drafted and wrote the article. M.T. revised the article.

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