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ORIGINAL ARTICLE

Breast cancer chemotherapy treatment monitoring based on serum sample Raman spectroscopy

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Abstract

In this paper, breast cancer patients were monitored throughout their chemotherapy treatments (CHT), with blood serum sample Raman spectroscopy and multivariate analysis, approximately for a year. First of all, we discriminate between healthy and clinically diagnosed breast cancer patients. Breast cancer detection in terms of sensitivity and specificity were 87.14% and 90.55% respectively. Although no shifts of peaks in mean spectrum of samples from breast cancer patients were found with respect to the mean spectrum from control patients, some peaks did show clear differences in intensity, the greatest disparities found at 509, 545, 1063, 1103, 1338, 1556, 1083 and 1449 cm^{-1} are associated with amino acids and phospholipid, 1246 and 1654 cm^{-1}, corresponding to amide III and I, respectively. Other peaks of interest encountered at 450, 661, 890, 917 and 1405 cm^{-1} are associated to glutathione. Then, 6 breast cancer patients were monitored during their chemotherapy treatments, the results were in complete correspondence with their medical records, enabling a detailed study of the evolution of each patient's cancer. A special interest arose in the possible correlation between the intensity of Raman peak, 450 cm^{-1}, corresponding to glutathione and evolution of cancer throughout CHT, i.e., glutathione appears to be a good candidate as breast cancer biomarker. The results confirmed that Raman spectroscopy and PCA are, not only a good support to current breast cancer detection techniques, but could also be excellent techniques to monitor more efficiently breast cancer patients undergoing CHT, using blood serum samples which are a lot less invasive than other methods.

Keywords Blood serum · Breast cancer · Chemotherapy treatment · Raman spectroscopy · PCA

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Introduction

According to Pan American Health Organization (PAHO), breast cancer is a disease that affects a considerable number of people worldwide, only in America, around 462 thousand new cases are detected with almost 100 thousand deaths [1]. Only in 2020, breast cancer was the most prevalent cancer, worldwide, with 7.8 million diagnosed alive women and 685,000 deaths [2]. These numbers alone justify the great interest of the public health system in its study.

With early detection, breast cancer treatment can be highly effective, with a high chance of survival. The treatments more frequently used are surgery and radiation therapy, to prevent disease from progressing to chest and lymph nodes, and CHT to reduce risk of metastasis and spreading. Breast cancer pharmacological treatment can be given before (neoadjuvants) or after (adjuvants) surgery, depending on

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cancer biological subtyping. These treatments are administered either orally or intravenously over a long period of time, reducing by half the possibility of cancer recurrence. The purpose of neoadjuvant chemotherapy is to eliminate micrometastases and to target the tumor itself (small but palpable, smaller than 2 cm), in the hope, that it will reduce its size, so the patient can be operated on and in the best scenario, it may even disappear. The surgeon will assess whether a conservative or radical surgery is performed by removing part or the totality of the breast.

The main objective of all drugs used in CHT is to affect the cancer cells DNA, preventing their rapid division and ultimately eliminating breast cancer all together. There is a wide variety of chemotherapy drugs that can be used depending on the type of cancer, its advance or spread status, and the general health of the patient. Most of the time, no changes will be observed on the cancer cells after just one dose, and the procedure will have to be repeated. A CHT is made up of those applications and rest time in between, the patient's standard of living depends on the monitoring quality. The first-line drug can be changed if the cancer is not subsiding or it causes serious side effects.

Raman spectroscopy has proven to be an excellent support technique for cancer detection (with a high sensitivity and specificity) and follow-up treatments already accepted by the WHO, because is fast, low-invasive and low-cost. Raman spectroscopy is an inelastic photon scattering technique that allows knowing the chemical composition of materials and, in recent years, it has been widely used to study biological samples such as cells and tissues for its non-destructive property [3]. Furthermore, this technique has made possible to assess the malignancy of cancer cells [4] and tissues during surgery [5], reducing the procedure's duration and cost. Because each molecule has unique vibrations, the Raman spectrum of a tissue provides molecular fingerprints conveying its biochemical composition. In a Raman spectrum, position, polarization, width and intensity of Raman peaks provide information about composition, internal symmetries, quality and amount of material, respectively.

Nevertheless, these Raman spectroscopy achievements, discriminating between control and cancer samples, would not be as impressive if it was not for multivariate statistical analysis methods, that allow spectral differences of a large number of measured Raman spectra to be viewed in a simple way. PCA is a multivariate method that allows analysis of Raman spectra in a space of orthogonal dimensions in descending order of variance, known as principal component space [6, 7]. Multivariate analysis has been applied to Raman spectroscopy to classify epithelial precancers and cancers [8]. In particular, PCA has been used to differentiate between epithelial precancers and cancers [9]. Raman and PCA have become essential tools in clinic and biomedical research.

In recent years, the detection of degenerative diseases has acquired a particular interest, especially, detection of cancer based on Raman spectroscopy of biological sample obtained by less invasive methods (different from biopsies). This new approach seeks to obtain the same biochemical information that is obtained by analyzing the biological samples used in conventional diagnostic methods. Thus, a new diagnostic method based on Raman spectroscopy of blood serum samples is expected to be admitted as a support technique for official diagnostic methods by the WHO. Among main reported results have been found: detection of diabetes [10], leukemia [11], breast cancer [12, 13] and cervical cancer [14, 15].

The scope of Raman and PCA spectroscopy goes further than cancer detection, it has also proven to be an excellent support technique in monitoring of patients under CHT, allowing an improvement in life quality [11]. In this paper, we report serum sample based Raman spectroscopy and PCA monitoring of breast cancer patients undergoing CHT. To the best of our knowledge, this is the first report of preliminary results evaluating usefulness of Raman spectroscopy in monitoring of breast cancer patients undergoing CHT.

Methodology

Blood samples were obtained by Centro de Investigación Biomédica de Occidente (CIBO), Instituto Mexicano del Seguro Social (IMSS) from 8 patients clinically diagnosed with breast cancer and 14 healthy volunteers. All patients were from Mexico central region and had similar ethnic and socioeconomic backgrounds. The age range for breast cancer and control patients was between 35 and 54 years. Written consent was obtained from subjects and the study was conducted according to the Declaration of Helsinki. To obtain the serum, the blood samples were centrifuged using a speed of 3500 rpm for 10 min, they were then placed in a − 82°C ultra-refrigerator, to keep them in good condition.

Six out of the total 8 breast cancer patients underwent CHT and were monitored throughout the procedure. All breast cancer patients undergoing CHT were also treated surgically, thus their CHT were monitored before (neoadjuvant) and after (adjuvant) surgery. The number of neoadjuvant and adjuvant chemotherapy doses varied according to each patient's clinical record. The main chemotherapy drugs given to patients during treatment were docetaxel, epirubicin, ondansetron, cyclophosphamide, and fluorouracil. Table 1 presents the most relevant clinical information for each breast cancer patient.

Before starting any treatment, in order to discriminate between serum samples from control and breast cancer patients, samples were obtained, then Raman spectra of all

Table 1 Clinical diagnosis of breast cancer patients

Patient (age)	Cancer stage	CHT	NAD (AS)	AD (AS)	Response to CHT
1 (54)	IIIA	*No*			
2 (45)	IIIA	*No*			
3 (45)	IIIB	*Yes*	1–3 (1)	4–12 (2)	*Pos*
4 (40)	IIIA	*Yes*	1–4 (1)	5–6 (1)	*Pos*
5 (50)	IIIA	*Yes*	1–3 (1)	4–12 (2)	*Neg*
6 (37)	IIIA	*Yes*	1–3 (1)	4–6 (1)	*Neg*
7 (45)	IV	*Yes*	0 (0)	1–8 (3)	*Neg*
8 (41)	IIIB	*Yes*	1–4 (0)	5 (1)	*Neg*

CHT, chemotherapy treatment (*Yes*, on treatment; *No*, none treatment); *NAD*, neoadjuvants doses; *AD*, adjuvants doses; *AS*, number of analyzed samples. Response to CHT (*Pos*, positive response; *Neg*, negative response)

blood serum samples were acquired in the laboratory. To measure the Raman spectrum, a drop of serum, placed onto an aluminum substrate was examined by an Olympus microscope integrated into the Raman system, Horiba Jobin Yvon LabRAM HR800, with 17 mW power laser at 830 nm. In order to ensure statistically good sampling about 10 spectra were collected focusing the laser beam on different points on the surface of serum samples with a 100 × microscope objective and an exposure of 60 s. The analyzed spectral region was from 400 to 1800 cm^{-1}, with a resolution of ~ 0.67 cm^{-1}. Raman system was calibrated with a silicon semiconductor using Raman peak at 520 cm^{-1}. All spectra were measured by the same person and under the same conditions in the laboratory.

Before applying the PCA, raw spectra were first preprocessed carrying out algorithms based on the Savitzky–Golay algorithm [16]. The fluorescence contribution was removed applying a baseline correction using a six spline interpolation method with eleven points. After smoothing and baseline corrections, each spectra were normalized using the each strongest peak. The application of these algorithms, as well as the normalization process, were carried out directly using the LabSpec 6.0 software, which was also used to control the Raman system.

All preprocessed and normalized spectra were stored in a matrix called data matrix. The Raman spectra correspond to the columns of the data matrix, while the rows correspond to each Raman spectra peak. Since the data matrix is used in the PCA to discriminate between the control and breast cancer spectra, the inputs come from healthy and breast cancer patients (before any treatment protocol). All the algorithms for PCA were implemented in MatLab commercial software through the function called *pca* that provides Principal component analysis of raw data, where $coeff = pca(X)$ returns the principal component coefficients, for the n-by-m data matrix X. Rows of X correspond to observations and columns correspond to variables. The coefficient matrix has dimension m-by-m and each of their columns contains coefficients for one principal component in descending order of component variance.

Once the PCA method is applied to the data matrix and the groups in the data are identified (in this case, the cancer and control spectra groups), we checked that this pattern structure is maintained when a given amount of data is removed or added (training data), leaving the other part unchanged (test data), and the PCA is applied to this modified matrix, a process known as cross-validation.

To monitor breast cancer treatments, it is essential, to carry out the discrimination of the Raman spectra of the samples of control and breast cancer patients beforehand. The PCA is then applied to the data matrix constituted by the preprocessed and normalized spectra, expecting to observe two clearly defined groups in space of principal components, one corresponding to Raman spectra from control patients and the other to Raman spectra from breast cancer patients, respectively. PCA results work as breast cancer detection method [12, 13], if a non-diagnosed new patient is included, Raman spectra of its serum sample is taken and the data is added to our data matrix as new columns, then PCA is applied to the new resulting matrix. By observing the position of the new patient's spectrum from in the principal component space, a diagnosis can be offered to the patient; if the spectrum falls between the group defined as control, the patient is healthy, otherwise the patient has cancer, since spectra from patients within the same group have greater spectral similarities (indicating the patients present greater biochemical similarities) than with spectra from the disconnected set. It can be shown that the plot of principal components that allows discriminating between control and cancer patients is also key to carry out the monitoring of CHT for breast cancer patients.

Because the reaction to CHT is often delayed after each dose intake, when patients start CHT, in order to analyze cancer status due to the first dose, blood serum samples are obtained just before receiving the second dose. To know the status of the second dose, serum samples are obtained before receiving the third dose, and so on. The spectra obtained after each dose (mostly about 10 Raman spectra for each sample) are placed on the original data matrix as columns and the PCA is run with this new matrix and the cross-validation process mentioned before is executed. If the patient shows improvement, it would be expected that spectra, initially totally within the cancer group, will approach the control group after each dose, in the hope that once the treatment is finished, the new spectra will be completely within the control group. The results would show, that this technique can be of great support for oncologists in decision making, i.e., to continue, suspend or change the patient's treatment.

Table 2 Details of serum samples used in the study

Spectrum Number	Nature	Number of cases
1–70	Breast cancer	8
71–197	Control	14

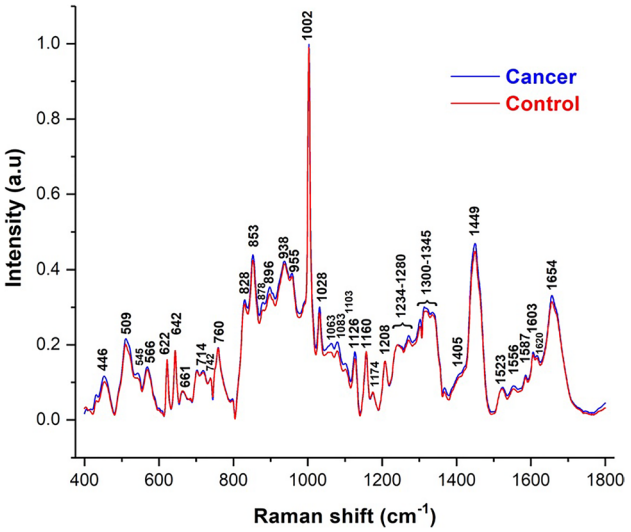


Fig. 1 Mean Raman spectra of control and breast cancer serum samples

Results and discussion

A total of 197 spectra were collected with 127 spectra from 14 control patients (10 spectra for control Patients 1, 2, 3, 9, 10, 11 and 14; 9 spectra for control Patients 5, 6 and 8; 8 spectra for control patient 4; 7 spectra for control patient 7; and 5 spectra for control Patient 13), and 70 spectra from 8 breast cancer Patients (10 spectra for Patients 1, 2, 3, 6 and 7; 9 spectra for Patient 5; 6 spectra for Patient 8; and 5 spectra for Patient 4). All spectra were duly preprocessed by carrying out smoothing, baseline correction and normalization. The data matrix of dimension 2329 × 197 was built and used to implement PCA, where the main information is described by the first principal components. Nogueira et al. [17] have shown that through principal components analysis, the main differences between the sample groups can be found. Details of the main Raman peaks or bands of serum samples used in this study are shown in Table 2.

After preprocessing, the mean spectrum of each group was calculated and analyzed to obtain biochemical information [18, 19].

In Fig. 1, already well-known spectra of a blood serum sample can be observed, with the most characteristic peaks that allow a quick identification of this type of samples, such as the strongest peak in the center of spectra (used to normalize the spectra) at 1002 cm^{-1} corresponding to phenylalanine, two almost identical peaks at 622 and 642 cm^{-1} corresponding to phenylalanine and tyrosine respectively, a doublet at 828 and 853 cm^{-1} assigned to tyrosine, a couple of bumps in the region 1234–1280 cm^{-1} assigned to amide III, a strong and wide band at 1449 cm^{-1} corresponding to phospholipid and the peak at 1654 cm^{-1} assigned to amide I. Nevertheless, other less known biomolecules, but characteristic of these types of samples, can play an important role in the discrimination between samples of patients with some degenerative disease and control, such as β-carotene, amino acid tryptophan, and a molecule of interest in the study of cancer, glutathione [10, 11, 13–15].

Strong peaks at 1002 and 1160 and 1523 cm^{-1} in both spectra indicates the presence of higher amounts of carotenoid [20]. Hata et al. showed that cancer in various organs, such as the skin, mammary gland, lung, liver and colon, is inhibited by the presence of carotenoids [21]; however, in Fig. 1, no differences are observed in the carotenoid peaks, neither in the control nor in the breast cancer spectra. This suggests that carotenoid plays a minor role, in the mechanism of action of breast cancer.

Although, some authors have reported that some peak positions in Raman spectra of the breast tissue from malignant tumors are shifted when compared with the spectra of tissue from benign tumors [22, 23]. However, in the mean spectrum of the serum samples patients, these peaks shifts are negligible or null compared to peaks of the mean spectrum from control patients. Even though mean spectra show that most peaks look similar to each other, some of these peaks show clear differences in intensity, for example, the bands of doublet at 828 and 853 (tyrosine), 1523 (β-carotene), 1587 (tyrosine), 1603 (phenylalanine) and 1620 (tryptophan) cm^{-1}.

The major differences between breast cancer and control spectra can be observed at 509 (tryptophan), 545 (tryptophan), 1063 (phenylalanine), 1083 (phospholipid), 1103 (phenylalanine), 1246 (amide III, overlapping of *α-helix*, *β-folding* and random coil), 1338 (tryptophan, *α-helix* and phospholipid), 1449 (*β-sheet* and phospholipid), 1556 (tryptophan) and 1654 (proteins, amide I, *α-helix*, phospholipid) cm^{-1}, where these peaks were less intense in the control spectrum. Other peaks where greater differences are observed, were 446, 661, 890, 917 and 1405 cm^{-1} assigned to glutathione molecule.

The Raman study of glutathione, an antioxidant molecule that plays an important role in a multitude of cellular processes such as cell differentiation, proliferation, and apoptosis, has been widely neglected. In particular, glutathione and its relationship with many human diseases including cancer [24, 25] have been little studied using Raman spectroscopy

technique. We believe the results of this paper can be a good incentive to focus on this molecule. The following section will enable us to observe, throughout the treatment, the behavior of the glutathione molecule in blood serum samples from breast cancer patients undergoing CHT, and since it allows to detect either improvement or deterioration of the cancer, glutathione appears to be a good candidate as a biomarker in the study of breast cancer.

Table 3 shows observed main bands in control and breast cancer spectra, corresponding assignment of bio-molecules and the comparison of the band intensities in control and breast cancer spectra.

As an alternative to analysis of the mean spectra, PCA method has been applied to discriminate easily between all measured Raman spectra from control and breast cancer patients.

Breast cancer detection

After removing the fluorescence contribution with smoothing and baseline correction, PCA was carried out on the matrix. The main information obtained from the PCA is described by the first principal component loadings, and in the particular case of breast cancer, components that best allowed discriminating between the spectra of serum samples from control and breast cancer patients were, *PC*1, *PC*3 and *PC*9. Figure 2 shows PCA corresponding to all Raman spectra (197 spectra, 127 spectra from control patients and 70 spectra from breast cancer patients), observing a clear division of the data in space of principal components. The two large clusters correspond, one to the serum samples from control and the other to breast cancer patients. The border is represented by the continuous line that separates the two large groups in Fig. 2. Clearly, PCA allowed discriminate between the healthy and breast cancer patients. The differences are due to some of the Raman peaks analyzed above.

The clinical status of the samples is shown in Table 1. In order to test the validity of our prediction classification, a leave-one-out cross-validation was performed. In cross-validation, part of the data is modified, adding and removing sets of spectra, but we found that the structure and the border of the clusters in Fig. 2 remained unchanged. Due to that, only 61 spectra from breast cancer cases were correctly separated from control cases spectra, and 115 spectra from control cases were correctly separated from breast cancer cases spectra, with a sensitivity and specificity given by 87.14% and 90.55%, respectively. When monitoring breast cancer patients undergoing CHT, the original data matrix (made up of the set of spectra from breast cancer and control patients), was modified by adding the sets of spectra obtained during the treatment of the patient being monitored. For every patient undergoing treatment, it was checked that the PCA structure was maintained, even when the set of data added to the original data matrix was changing depending on which patient undergoing treatment was being monitored; this scheme equivalent

Table 3 Main bands observed in control and breast cancer serum spectra, the corresponding assignment of biomolecules and the comparison of the band intensities

Band (cm^{-1})	Biomolecules	Comparison of the band intensities
446	Glutathione	$I_C < I_{BC}$
509	Trp	$I_C < I_{BC}$
545	Trp	$I_C < I_{BC}$
566		$I_C \approx I_{BC}$
622	Phe	$I_C \approx I_{BC}$
642	Tyr	$I_C \approx I_{BC}$
661	Glutathione	$I_C \approx I_{BC}$
714	Polysaccharides	$I_C \approx I_{BC}$
742	Phospholipid	$I_C \approx I_{BC}$
760	Trp	$I_C \approx I_{BC}$
828	Tyr	$I_C < I_{BC}$
853	Tyr	$I_C < I_{BC}$
890	Glutathione	$I_C < I_{BC}$
917	Glutathione	$I_C < I_{BC}$
938	Skeletal str α	$I_C < I_{BC}$
955	CH2 rock	$I_C < I_{BC}$
1002	Phe	$I_C \approx I_{BC}$
1028	Phe	$I_C < I_{BC}$
1063	Phe	$I_C < I_{BC}$
1083	Phospholipids O-P-O and C-C	$I_C < I_{BC}$
1103	Phe	$I_C < I_{BC}$
1126	Protein, Phospholipid C-C str	$I_C < I_{BC}$
1160	β-carotene	$I_C \approx I_{BC}$
1174	Trp, Phe	$I_C \approx I_{BC}$
1208	Trp	$I_C \approx I_{BC}$
1234–1280	Amide III	$I_C < I_{BC}$
1300–1345	Trp, α helix, Phospholipids	$I_C < I_{BC}$
1405	Glutathione	$I_C < I_{BC}$
1449	Phospholipid, C-H scissor in CH2	$I_C < I_{BC}$
1523	β-carotene	$I_C < I_{BC}$
1556	Trp	$I_C < I_{BC}$
1587	Protein, Tyr	$I_C < I_{BC}$
1603	Tyr, Phe	$I_C < I_{BC}$
1620	Tyr, Trp C=C str	$I_C < I_{BC}$
1654	Proteins, Amide I, α helix, Phospholipids	$I_C < I_{BC}$

I_C and I_{BC} are the intensities of the same bands in the breast cancer and control spectra, respectively

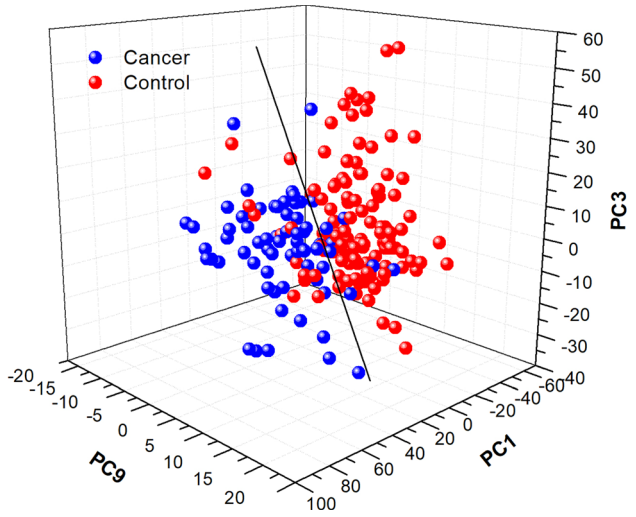


Fig. 2 PCA plot comparing spectra of serum samples from control and breast cancer patients. Plot allows discriminating between control and breast cancer patients

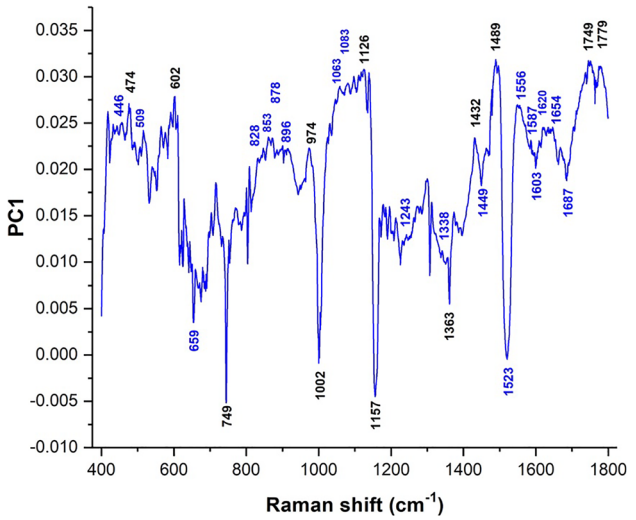


Fig. 3 First loading plot between spectra of serum samples from control and breast cancer patients

to cross-validation is common on PCA studies and the structure maintenance is sign of the method reliability.

High values of sensitivity and specificity and a successful cross-validation are essential to obtain a good CHT monitoring from breast cancer patients, therefore the observed structure in Fig. 2 will be the reference point for monitoring of CHT.

Figure 3 corresponds to the plot of first loading against Raman shift. *PC*1 indicates the position of the peaks, at which Raman shifts show the main differences between the groups of spectra (correlated with the variations in the molecular composition).

Considering all 197 measured spectra, Fig. 3 shows that the most significant differences (determined by the most intense positive and negative peaks), that defined the two large clusters (control and breast cancer), appear at 828, 853 and 1587 (tyrosine); 1523 (β-carotene); 1063 and 1603 (phenylalanine); 509, 545, 1338, 1556, 1620 (tryptophan); 1246 (amide III); 1083 and 1449 (phospholipid); 1654 (amide I), and 446, 661, 890 and 917 (glutathione) cm^{-1}. These differences (labeled in blue peaks) between control and breast cancer samples obtained using *PC*1 plot [19] are consistent with the naked eye observation in Fig. 1, where only the mean control and breast cancer spectra were analyzed. The rest of the peaks (labeled in black peaks) shown in Fig. 3 correspond to other differences, not visible before PCA, between the 197 measured Raman spectra, they are due to the intensity variation, shifting of the band positions, or a mixture of both features. Both Table 3 and the plot of first loading showed that the most significant differences between serum samples from control and breast cancer patients were glutathione, tryptophan, tyrosine, phenylalanine, β-carotene, phospholipid, amide I and amide III.

Monitoring breast cancer patient's chemotherapy treatment

Serum samples were taken from breast cancer patients undergoing CHT. From each sample, about 10 spectra were obtained. Each acquired raw spectrum was preprocessed (smoothing and baseline correction) to remove noise, sample fluorescence, and shot noise from cosmic rays and was normalized to the highest peak. It should be noted that because of hospital logistics, the samples were not obtained for each and every dose.

In addition to breast cancer detection, the present paper proposes Raman spectroscopy and PCA to be used as methods to monitor breast cancer patients under CHT, offering a faster alternative technique by decreasing subjectivity to human error. Monitoring is based on observing the biochemical changes for the CHT duration. Throughout treatment, patient improvement is observed as the breast cancer patient's spectra gets closer to the control group (see Fig. 2).

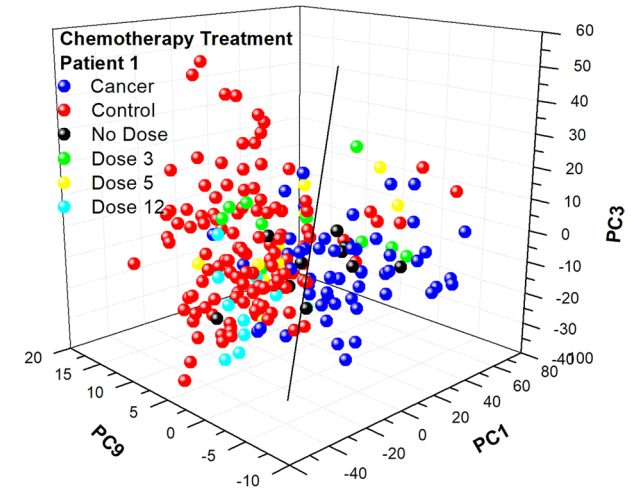
Serum samples from six patients under CHT were studied. A protocol of CHT was assigned to each patient according to their clinical record. The most used chemotherapy drugs in the six studied breast cancer patients were docetaxel, epirubicin, ondansetron, cyclophosphamide and fluorouracil. Serum samples were taken after several chemotherapy doses and analyzed using Raman spectroscopy and PCA until the patients finished CHT. Raman measurements for monitoring of CHT from the six patients complemented the data matrix obtained for breast cancer detection. If this method is admitted, it could give support to the oncologist

to recommend a change on medication at any time of CHT, if no improvement is observed on the patient.

In the following subsections, for all CHT monitoring cases, the black points in Figs. 4, 5 and 6 correspond to the spectra when each patient was diagnosed with cancer and before starting CHT.

CHT monitoring from patient 1

Figure 4, Patient 1, shows PCA plot corresponding to CHT monitoring of the first breast cancer patient. As we mentioned before, black points correspond to the spectra when patient was diagnosed with cancer and without CHT. Green points correspond to the spectra of serum sample from the third neoadjuvant dose, observing that only some green points are already inside the cluster from healthy patients, i.e., patient observed a partial improvement. After the second adjuvant dose (fifth dose), again some spectra (yellow points) persisted inside the cluster from healthy patients, patient remained stable. When patient received the ninth adjuvant dose, all spectra (cyan points) were completely located within the cluster from control patients, i.e., patient was completely healthy after that dose.



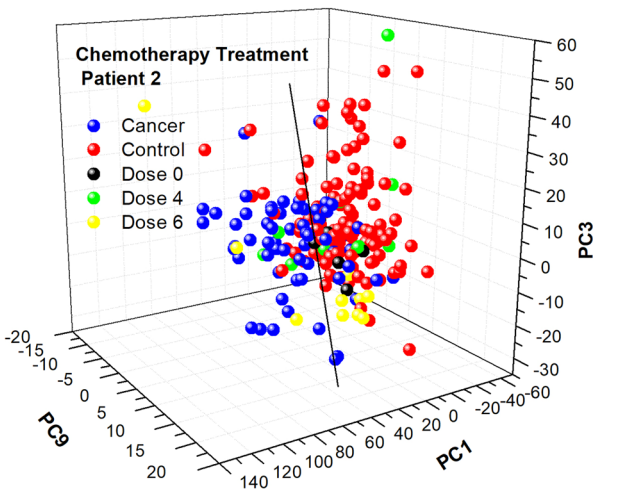
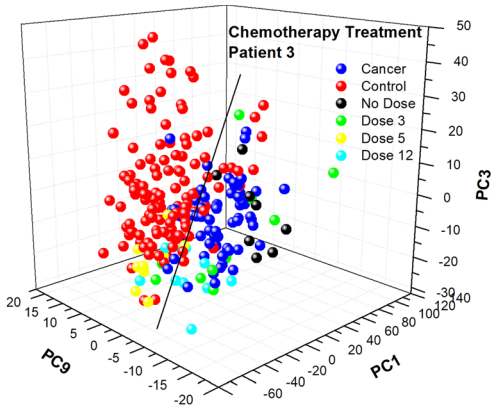


Fig. 4 PCA plot corresponding to monitoring of CHT of the first and second breast cancer patient



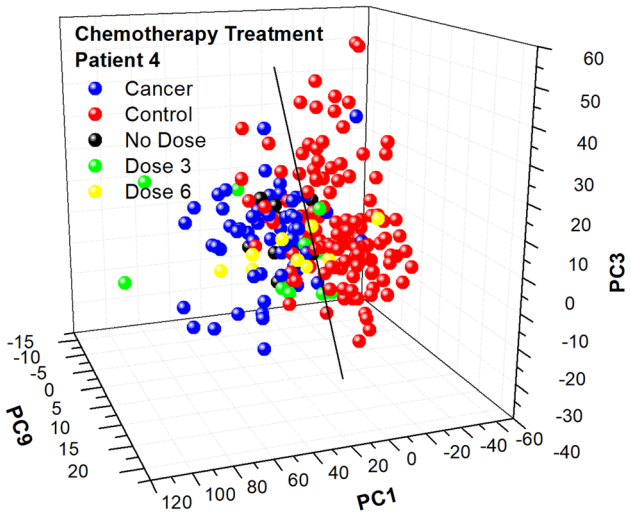


Fig. 5 PCA plot corresponding to monitoring of CHT of the third and fourth breast cancer patient

On the other hand, according to clinical record, the first from the breast cancer patients under CHT (45 years old and stage IIIB) received 3 neoadjuvant doses and 9 adjuvant doses throughout CHT (see Table 1), but blood serum samples were only obtained from the third (neoadjuvant), fifth (adjuvant), and twelfth (adjuvant) dose. After third neoadjuvant chemotherapy dose, patient observed a partial response, i.e., traces of tumor cells or cancer were still found, in complete agreement with Raman and PCA diagnosis (some green points in control cluster). After the third dose, patient underwent radical surgery to continue CHT, receiving nine adjuvant additional doses. After the second adjuvant dose (or fifth dose), patient

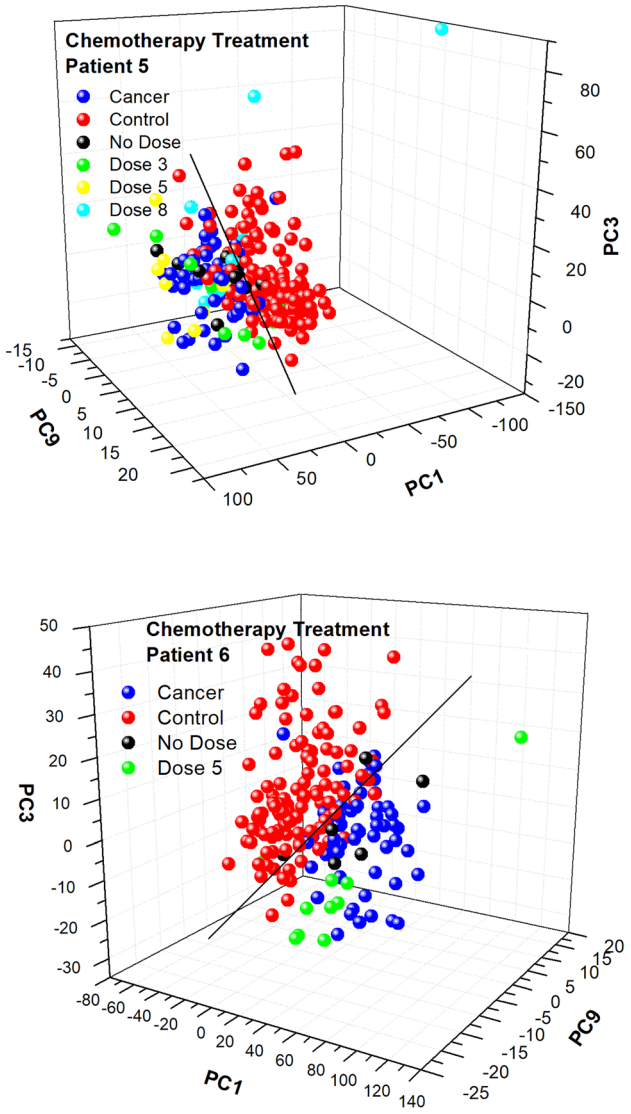


Fig. 6 PCA plot corresponding to monitoring of CHT of the fifth and sixth breast cancer patient

remained stable (some yellow points in control cluster), but after the ninth adjuvant dose (or twelfth dose), patient was declared completely recovered (all cyan points are in the control cluster). CHT from patient 1 was successful. Therefore, results reported by Raman spectroscopy and PCA were in complete agreement with clinical reports shown after each dose of chemotherapy, i.e., Raman spectroscopy and PCA indicated that CHT, particularly for this first patient, was a success. Thus, it can be concluded that, for this patient, Raman spectroscopy and PCA were excellent tools to monitor CHT.

CHT monitoring from patient 2

Figure 4, Patient 2, shows PCA plot corresponding to monitoring of CHT from the second breast cancer patient. Although black point are within cancer cluster, they are very close to the border with the cluster from healthy patients, therefore, it could be thought that patient would have a non-advanced cancer. Observing the green points in Fig. 4 corresponding to the spectra of patient after the fourth neoadjuvant chemotherapy dose, it would seem that there was a patient regression since some spectra or green points moved away from border and into the cancer cluster, even though some points stayed in the control cluster, this data indicates that the patient's health situation was deteriorating. After conservative surgery and the second adjuvant dose (sixth dose), spectra (yellow points) were already completely in control patients cluster, the patient was fully recovered. From the point of view of Raman spectroscopy, surgery and CHT were successful for this patient 2.

According to the second cancer patients clinical record (40 years old and stage IIIA, under CHT), she received 4 neoadjuvant doses and 2 adjuvant doses throughout treatment (see Table 1), but blood serum samples were only obtained from the fourth (neoadjuvant), and sixth (adjuvant) dose. After the fourth neoadjuvant chemotherapy dose, patient observed a partial response, i.e., traces of cancer were still found with conventional methods, in complete agreement with Raman and PCA diagnosis (green points in cancer cluster). After the fourth dose, patient underwent conservative surgery or non-radical surgery to continue CHT, receiving two adjuvant additional doses. After surgery and the second adjuvant dose, patient was declared completely recovered (yellow points are in the control cluster). There was a complete response due to surgery and CHT, since the tumor could be removed and no traces of tumor were found. Therefore, although there could be a discrepancy between official diagnosis and Raman technique about stage of cancer of patient, results reported by Raman spectroscopy and PCA about CHT were in complete agreement with clinical reports shown after each dose of chemotherapy, i.e., Raman spectroscopy and PCA indicated that CHT for the second patient, was a success.

CHT monitoring from patient 3

Figure 5, Patient 3, shows PCA plot corresponding to the CHT monitoring of the third breast cancer patient. The green points correspond to spectra of serum sample from the third neoadjuvant dose, observing that only few green points are already in cluster from healthy patients, i.e., patient observed a partial improvement, not complete. After the second adjuvant dose, all yellow are in cluster control, i.e., patient observed a complete improvement. Nevertheless, after subsequent adjuvant doses, all spectra (cyan points) are observed again in cancer cluster, meaning that patient presented a relapse.

On the other hand, according to clinical record, the third from the breast cancer patients under CHT (50 years old and stage IIIA) received 3 neoadjuvant doses and 9 adjuvant doses throughout treatment (see Table 1), but blood serum samples were only obtained from the third (neoadjuvant), fifth (adjuvant) and twelfth dose. Patient 3 was the one who received the longest follow-up (about a year). Patient received the third neoadjuvant dose, responding positively, since size of tumors decreased considerably and therefore, only a partial response was observed (in PCA some green points are still observed in cancer cluster). After conservative surgery or non-radical surgery, the patient received 9 adjuvant additional doses, and on the second adjuvant dose (fifth dose), a complete response was observed (in PCA all yellow points are completely within control cluster). Nevertheless, after a certain time, a relapse was observed in this patient, for whom radiotherapy was required. Cyan points completely immerse into cancer cluster is an indication of the patient relapse. Patient 3 is a clear example, where Raman spectroscopy was put to test, responding effectively to monitoring of CHT.

CHT monitoring from patient 4

Figure 5, Patient 4, shows PCA plot corresponding to the fourth breast cancer patient CHT monitoring. Green points correspond to spectra of the serum sample from the third neoadjuvant dose, observing that all green points remained completely in cancer cluster, i.e., a null response of patient to CHT is observed. After receiving the third adjuvant dose, it is observed that spectra (yellow points) remained in the cancer patients cluster, i.e., no improvement was ever observed in patient.

According to clinical record, the fourth from the breast cancer patients under CHT (37 years old and stage IIIA) received 3 neoadjuvant doses and 3 adjuvant doses throughout treatment (see Table 1), but samples were only obtained from the third (neoadjuvant), and sixth (adjuvant) dose. After the third neoadjuvant chemotherapy dose, patient observed a partial response, in complete agreement with Raman and PCA diagnosis (all green points in cancer cluster). After, patient underwent radical surgery to continue CHT, receiving 3 adjuvant additional doses, but patient continued with a partial response, cancer never disappeared. Even when patient underwent radiotherapy because her cancer was being resistant to chemotherapy, no positive response was observed. CHT from patient 4 was not successful. Therefore, results reported by Raman and PCA were in complete agreement with clinical reports.

CHT monitoring from patient 5

Figure 6, Patient 5, shows PCA plot corresponding to the fifth breast cancer patient CHT monitoring. After a radical surgery, patient received 8 adjuvant doses, but spectra of the samples corresponding to the third, fifth and eighth dose are represented by green, yellow and cyan points, respectively (see Fig. 6), remained completely within the cancer patients cluster, i.e., no improvement was ever observed.

According to clinical records, the fifth from the breast cancer patients under CHT (45 years old and stage IV) received 8 adjuvant doses throughout treatment, no neoadjuvant doses were given (see Table 1), blood serum samples were only obtained from the third, fifth and eighth dose. After a radical surgery, patient received a third chemotherapy dose, observing a partial response, in complete agreement with Raman and PCA diagnosis (all green points in cancer cluster). After, patient received the fifth and eighth dose, the patient continued with a partial response, cancer never disappeared (the yellow and cyan points stayed in cancer cluster). The patient had to be hospitalized after the fifth session due to a fever that progressed to neutropenia. CHT from patient 5 was not successful. Patient's health situation was very critical. Nevertheless, reported results by Raman spectroscopy and PCA were also in complete agreement with clinical reports.

CHT monitoring from patient 6

Figure 6, Patient 6, shows PCA plot corresponding to the sixth breast cancer patient CHT monitoring. Patient received 4 neoadjuvant doses, but no serum samples were obtained. After, patient received one adjuvant dose, observing that all green points corresponding to this dose remained completely in cancer cluster, i.e., no improvement was observed in the patient. After, patient underwent radical surgery and radiotherapy, still no improvement was observed.

On the other hand, according to clinical record, the sixth from the breast cancer patients under CHT (41 years old and stage IIIB) received 4 neoadjuvant doses and 1 adjuvant dose throughout treatment (see Table 1), but blood serum samples were only obtained from the fifth dose (adjuvant). For 20 years, patient had a nodule always reported as benign, but about six months before starting chemotherapy treatment patient was diagnosed with breast cancer. This patient began treatment with 4 chemotherapy neoadjuvant sessions, but samples were unavailable. Later, patient underwent radical surgery and received 1 adjuvant dose, and possibly, later also underwent radiotherapy, nevertheless, there was no positive

response neither to surgery nor dose. Green points in the cancer cluster after surgery show that reported results by Raman spectroscopy and PCA were also in complete agreement with clinical reports from patient.

It would have been very interesting to continue monitoring the patients who responded positively to CHT, 5 years after the treatment was over, to ensure the non-return of cancer, but it was no longer possible. Nevertheless, after analyzing six interesting and varied cases of CHT, there is no doubt about the promising effectiveness of Raman spectroscopy and PCA techniques to monitor CHT from breast cancer patients. These techniques could be excellent support methods for breast cancer oncologist to offer a more effective and less aggressive CHT for patients.

A fact of particular interest observed in the monitoring of CHT was the possible correlation between the intensity of the Raman peak, 450 cm^{-1}, corresponding to the glutathione molecule, and the evolution of cancer in patients during CHT. In Fig. 7, it can be seen how the intensity of the peak, possibly associated with glutathione [19], varies according to the health status of the cancer patient, if the patient improves, the peak decreases (see Fig. 7, Patient 1, red spectrum; Patient 3, green spectrum and Patient 5, red spectrum) or if the patient relapsed, peak increases (see Fig. 7, Patient 1, green spectrum; Patient 2, red spectrum and Patient 3, green spectrum). It means that glutathione could possibly play the role of a biomarker in the study of breast cancer. Nevertheless, more studies than the existing ones, about the relationship between glutathione and cancer are necessary [24, 25], and Raman spectroscopy could be the tool that will allow studying such a relationship.

Conclusion

In this study, we present the results of Raman spectroscopy and PCA monitoring of breast cancer patients undergoing chemotherapy. Raman spectroscopy was performed on blood serum samples and then principal component analysis was used to process the data. In order to follow the treatment, a successful breast cancer diagnosis, before CHT, was obtained using the same Raman spectroscopy technique (same laser, conditions and software). For breast cancer diagnosis, a total of 197 spectra from 8 breast cancer patients and 14 control patients were measured, which were duly preprocessed. The already well-known mean spectra of a blood serum sample from control and breast cancer patients were shown, with the most characteristic peaks that allow the immediate identification of this type of samples and their constituent molecules, such as amino acids, β-carotenes, phospholipid and amide I and III. The major differences between breast cancer and control spectra were identified with special interest in those peaks corresponding to glutathione molecule. PCA was used to better appreciate the differences between all spectra, identifying two large groups associated to healthy and breast cancer patients. PCA results were validated by leave-one-out cross-validation method. Therefore, it was possible to confirm, once again, that based on serum sample Raman

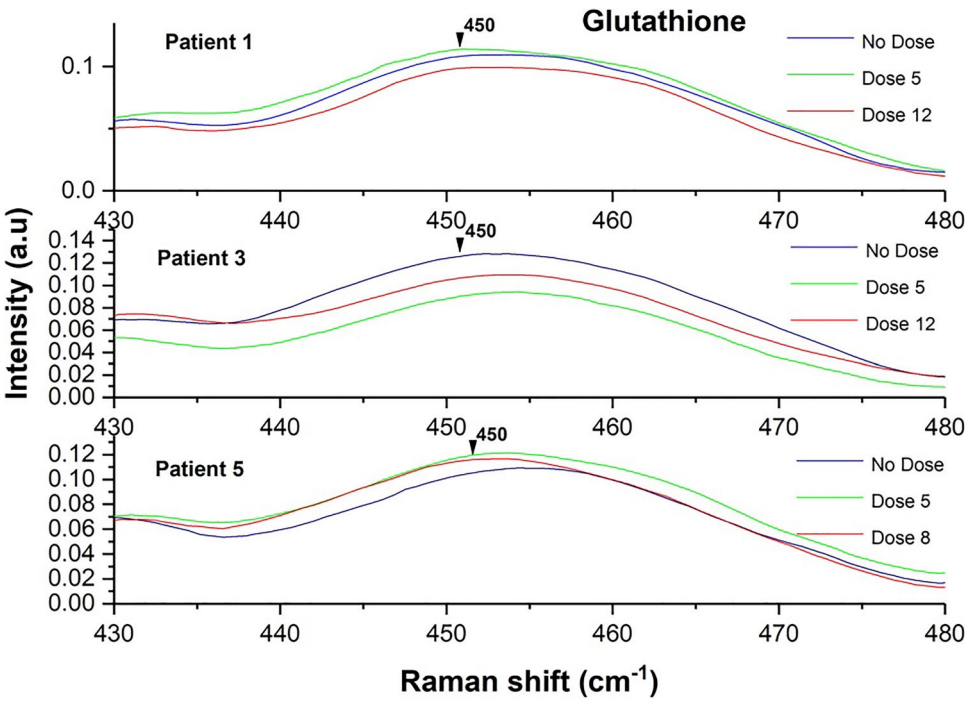


Fig. 7 Glutathione behavior in CHT from breast cancer patients

spectroscopy, it is possible to establish a diagnostic method for breast cancer with a sensitivity and specificity of 87.14% and 90.55%, respectively. This diagnostic analysis was the central idea of monitoring, since it was taken as the reference point to observe behavior of breast cancer from patients after a chemotherapy dose, this is, the patient's condition was inferred as spectra got closer or further to the control group (improvement or relapse, respectively). Six patients with different stages of breast cancer were successfully monitored throughout CHT (up to 12 doses), according to the official clinical records of Instituto Mexicano del Seguro Social (main social health center in Mexico, of which, 2 patients observed complete improvement, but others observed only partial improvements or relapse. A special interest arose in the possible correlation between intensity of Raman peak, 450 cm^{-1}, corresponding to glutathione and evolution of cancer throughout CHT, i.e., glutathione appears to be a good candidate as breast cancer biomarker. The results confirmed that Raman spectroscopy and PCA are, not only a good support to current techniques to detect breast cancer, but also could be excellent techniques to monitor more efficiently breast cancer patients chemotherapy treatments using blood serum samples, with the added convenience that it is minimally invasive.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Pan American Health Organization (PAHO) Breast cancer. https://www.paho.org/hq/index.php?option=com_content&view=article&id=5041:2011-breast-cancer&Itemid=3639&lang=es. Accessed 24 Dec 2021
2. World Health Organization (WHO) Breast cancer. https://www.who.int/news-room/fact-sheets/detail/breast-cancer. Accessed 24 Dec 2021
3. Choo-Smith LP, Edward MHG, Endtz HP, Kros JM, Heule F, Barr H, Robinson Jr. J S, Bruining HA, Puppels GJ (2002) Medical applications of Raman spectroscopy: from proof of principle to clinical implementation. Biopolymers 67:1–9
4. Chan JW, Taylor DS, Lane SM, Zwerdling T, Tuscano J, Huser T (2008) Nondestructive identification of individual leukemia cells by laser trapping Raman spectroscopy. Anal Chem 80:2180–2187
5. Haka AS, Volynskaya Z, Gardecki JA, Nazemi J, Lyons J, Hicks D, Fitzmaurice M, Dasari RR, Crowe JP, Feld MS (2006) In vivo margin assessment during partial mastectomy breast surgery using Raman spectroscopy. Cancer Res 66:3317–22
6. Kroonenberg PM (1983) Principal component analysis: Theory and applications. DSWO, Leiden
7. Smith LI (2002) A tutorial on Principal Components Analysis. Computer Science, ID:60161425
8. Mahadevan-Jansen A, Richards-Kortum RR (1996) Raman spectroscopy for the detection of cancers and precancers. J Biomed Opt 1(1):31–70
9. Stone N, Kendall C, Shepherd N, Crow P, Barr H (2002) Near-infrared Raman spectroscopy for the classification of epithelial precancers and cancers. J Raman Spectrosc 33:564–573
10. González-Solís JL, Villafán-Bernal JR, Martínez-Zérega BE, Sánchez-Enríquez S (2018) Type 2 diabetes detection based on serum sample Raman Spectroscopy. Lasers Med Sci 33:1791–1797
11. González-Solís JL, Martínez-Espinosa JC, Palomares-Anda P (2014) Monitoring of chemotherapy leukemia treatment using Raman spectroscopy and principal component analysis. Lasers Med Sci 29:1241–1249
12. Pichardo-Molina JL, Frausto-Reyes C, Barbosa-García O, Huerta-Franco R, González-Trujillo JL, Ramírez-Alvarado CA, Gutiérrez-Juárez G, Medina-Gutiérrez C (2006) Raman spectroscopy and multivariate analysis of serum samples from breast cancer patients. Lasers Med Sci 10103:432–438
13. Vargas-Obieta E, Martínez-Espinosa JC, Martínez-Zérega BE, Jave-Suárez LF, Aguilar-Lemarroy A, González-Solís JL (2016) Breast cancer detection based on serum sample surface enhanced Raman spectroscopy. Lasers Med Sci 31:1317–1324
14. González-Solís JL, Martínez-Espinosa JC, Torres-González LA, Jave-Suárez LF, Aguilar-Lemarroy AC, Palomares-Anda P (2014) Cervical cancer detection based on serum samples Raman spectroscopy. Lasers Med Sci 29:979–985
15. Sánchez-Rojo SA, Martínez-Zérega BE, Velázquez-Pedroza EF, Martínez-Espinosa JC, Torres-González LA, Aguilar-Lemarroy A, Jave-Suárez LF, Palomares-Anda P, González-Solís JL (2016) Cervical cancer detection based on serum sample surface enhanced Raman spectroscopy. Rev Mex Fis 62:213–218
16. Chalmers JM, Griffiths PR (2002) Handbook of vibrational spectroscopy, vol. 5. Application in life pharmaceutical and natural science. Wiley, New York
17. Nogueira VG, Silveira L (2005) Raman spectroscopy study of atherosclerosis in human carotid artery. J Biomed Opt 10:031117–1–031117-7
18. Stone N, Kendall C, Smith J et al (2004) Raman spectroscopy for identification of epithelial cancers. Faraday Discuss 126:141–57
19. De Gelder J, De Gussem K, Vandenabeele P, Moens L (2007) Reference database of Raman spectra of biological molecules. J Raman Spectrosc 38:1133–1147
20. Schultz H, Baranska M, Baranski R (2005) Potential of NIR-FT-Raman spectroscopy in natural carotenoid analysis. Biopolymers 77:212–221
21. Hata TR, Schlz TA, Ermakov IV et al (2000) Non-invasive Raman spectroscopic detection of carotenoids in human skin. J Invest Dermatol 115:441–8
22. Alfano RR, Liu CH et al (1991) Human breast tissue studied by IR Fourier transform Raman spectroscopy. Lasers in Life Sci 4:23–28
23. Frank CJ, McCreery RL, Redd DCB (1995) Raman spectroscopy of normal and diseased human breast tissues. Anal Chem 67:777–783
24. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, Marinari UM, Domenicotti C (2013) Role of glutathione in cancer progression and chemoresistance. Oxidative Medicine and Cellular Longevity Volume 2013, Article ID 972913
25. Bansal A, Simon MC (2018) Glutathione metabolism in cancer progression and treatment resistance. J Cell Biol 217(7):2291–2298