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The use of Z-scan technique for determination of biochemical parameters in children with solid tumors or leukemias supplemented or not with selenium

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ABSTRACT

Cancer is a disease that effects cell metabolism causing an imbalance in the health of the patient. On the other hand, malnutrition, presented by oncological patients, is caused by both the disease and its treatment. Some serum biochemical parameters cannot be determined by the traditional method of laboratory blood analysis (spectrophotometry). Among the various techniques that could be used for blood biochemical analysis, we opted for the Z-scan technique, due to its sensitivity to the reading of blood components. Our objective in this work was to compare the data obtained by the Z-scan technique and the spectrophotometry of the serological samples of children with solid tumors and leukemia under treatment, receiving or not selenium supplementation in a randomized, double-blind clinical trial. The biochemical parameters were read based on blood. These blood sampling made at different stages of chemotherapy and selenium supplementation. At each of these stages, the cholesterol, glucose and triglycerides parameters were read using the Z-scan and spectrophotometry techniques. We observed that selenium helps in balancing the health of these patients, and corroborates with our hypothesis that the Z-scan technique may be an alternative for the determination of biochemical parameters.

1. Introduction

The number of patients affected by cancers and its complications has been increasing year after year. Many cases are the result, among other factors, of radioactive effects people are daily exposed to, which cause cell mutations. These cell alterations bring forth many problems regarding the use of macronutrients and metabolic disorders, such as an increase in glucose, triglycerides and cholesterol levels. Besides, tumorigenesis may cause the oxidation of free fatty acids and inhibit lipoprotein lipase (LPL) activity. This metabolic dysfunction leads to loss of quality of life, acute weight loss (anorexia) as well as emotional issues like depression.

Although malignant tumor patients have nutritional deficiencies, they do not qualify as undernourished. Such deficiencies depend not only of age variability but also other factors like the nutritional status of the individual prior to the emergence of the tumor, the type of tumor and the chemotherapeutic protocol. Therefore, metabolic problems vary from patient to patient [1]. Two of the most frequent conditions observed regarding the intake of macronutrients by oncological patients are glucose intolerance and hyperglycemia, which are triggered by glucose turnover complications. These complications cause energetic alterations and, as a result, affect cell metabolism and bring about an increase in fatty acid release even when patients decrease food intake. This is due to the imbalance between lipolysis and lipogenesis, which is caused by the inflammatory and oxidative stress characteristic of the disease [2].

Owing to the complications mentioned above, many studies have been focusing on the mitigation and control of nutritional losses in oncological patients. Some of them discuss the effects of selenium supplementation in patients for the control of metabolic alterations. In some articles, it was observed that children who were supplemented with selenium had the side effects of systemic/chemotherapeutic treatment attenuated [3].

One of the reasons why selenium supplementations are proposed is due to the fact it is an antioxidant micronutrient and has antimutagenic action [4,5]. Moreover, some other studies reveal that besides being an antioxidant, selenium contributes to the conversion of T4 into T3, protects the body from the toxic actions of heavy metals and

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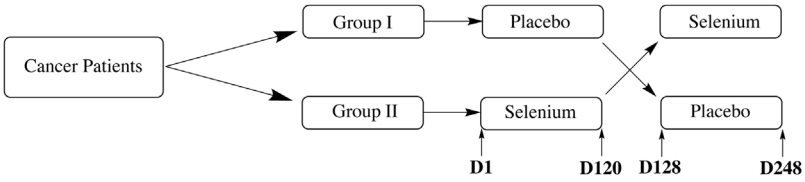

Fig. 1. Scheme of the protocol for patient inclusion.

Table 1
Dietary reference intakes, toxic intake and selenium supplementation limits.

Age group	Dietary reference intakes[a]	Selenium supplementation of 80%	Toxic intake[b] description
0 to 6 mos	15	27	45
7 to 12 mos	20	36	60
1 to 3 yrs	20	36	90
4 to 8 yrs	30	54	150
9 to 13 yrs	40	72	280
14 to 19 yrs	55	100	400
≥19 yrs	55	100	400

[a] Source: WHO.
[b] Source: Institute of Medicine/Food Nutrition board, US National Academy of Sciences.
Washington DC: 2000 (with authorization).

xenobiotics, prevents the development of chronic diseases and increases immune response [6].

Many laboratory methods can be used to measure the biochemical parameters in oncological patients. Nevertheless, whenever alterations are detected, it is observed that the techniques applied are not diagnostically effective when it comes to the classification of alterations in such parameters. Most of these methods are based on linear optical techniques along with enzymatic colorimetric assays.

Nonlinear optical methods have been widely studied, allowing a more precise molecular-spectrum analysis rather than routine laboratory biochemical tests. Differently, these methods use relative values in the analysis instead of absolute values from techniques like spectrophotometry. Therefore, they define absorbance and transmittance variances in relation to a reference value emitted by the same light source, which is a monochromatic pulsed light beam with high power and voltage that generate thermal influence to the optical reading of the nonlinear refractive index.

The Z-scan technique stands out among others [7,8]. In recent years, this technique has been applied to differentiate between benign and malignant oral tissues [9], differentiating normal and carcinogenic ovarian cells [10], and differentiating tumor cell lines [11]. In addition, it has and it has been used to study the oxidative stress of plasma lipoproteins in humans [12–15] in order to analyze the relation between atherosclerosis and periodontal disease [16], quantify total cholesterol [17], triglycerides [17], protein [18], albumin [18], glucose [19] and creatinine [20] in blood. It is important to point out that Gomez et al. [12] revealed the optical nonlinearity of lipoproteins through this technique. Besides, the researchers showed that the nonlinearity of

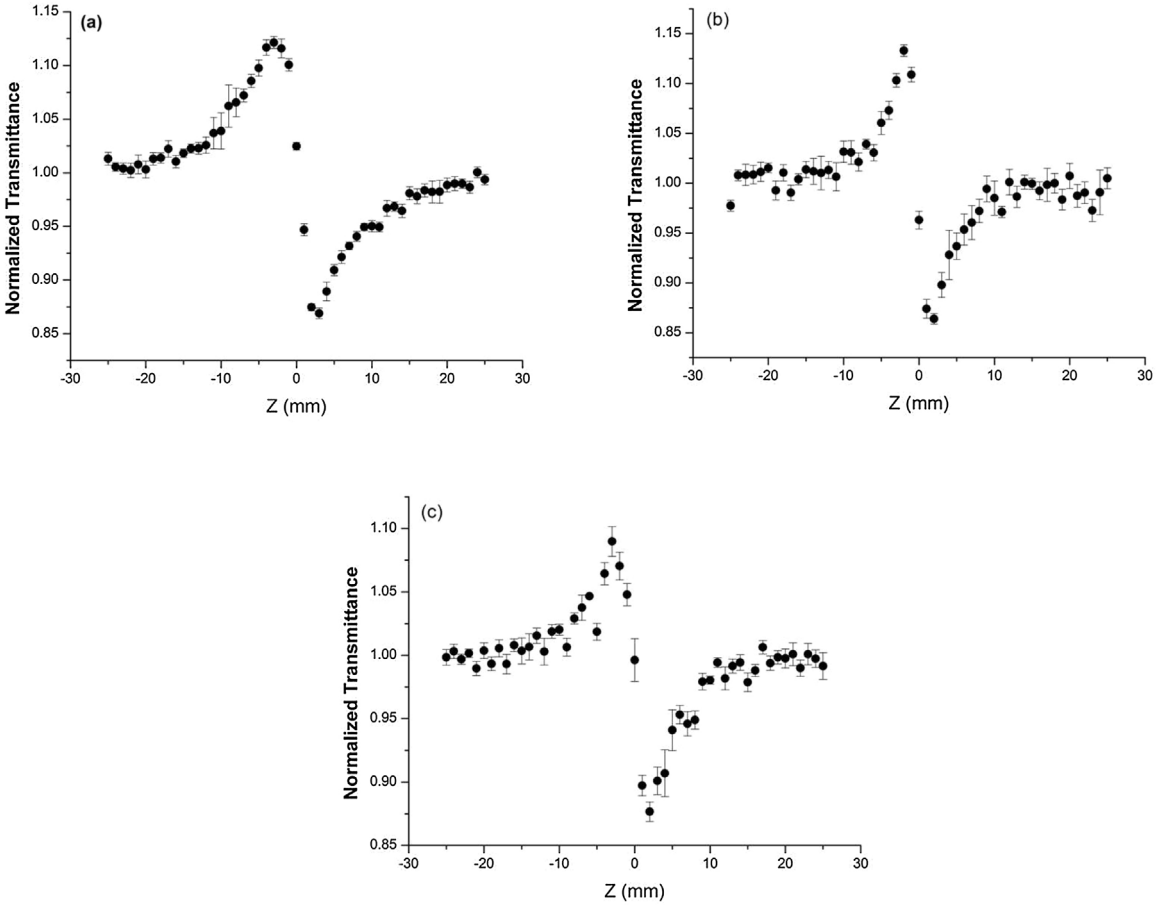

Fig. 2. Typical Z-scan curves for glucose, triglycerides and cholesterol samples.

Table 2
Concordance values between conventional and Z-Scan methods in the determination of cholesterol (mg/dL), glucose (mg/dL) and triglycerides (mg/dL).

Markers	ICC (95% CI)	r	C_b	Limits of concordance (lower–upper)[a]
Cholesterol (mg/dL)	0.57 (0.12; 0.81)	0.65	0.87	(−165.0; 169.1)
Glucose (mg/dL)	0.02 (0.00; 0.38)	0.17	0.68	(−61.5; 34.5)
Triglycerides (mg/dL)	0.00 (0.00; 0.36)	0.44	0.33	(−11.0; 270.9)

ICC (intraclass correlation coefficient); 95%CI (confidence interval of 95%);
r (precision) and C_b (accuracy), both obtained from concordance correlation coefficient.
[a] Limits of concordance obtained from Bland-Altman method for the difference of concentrations of the markers according to the applied measurement technique: meandif −1.96*sddif and meandif +1.96*sddif (sd – standard deviation; dif – difference).

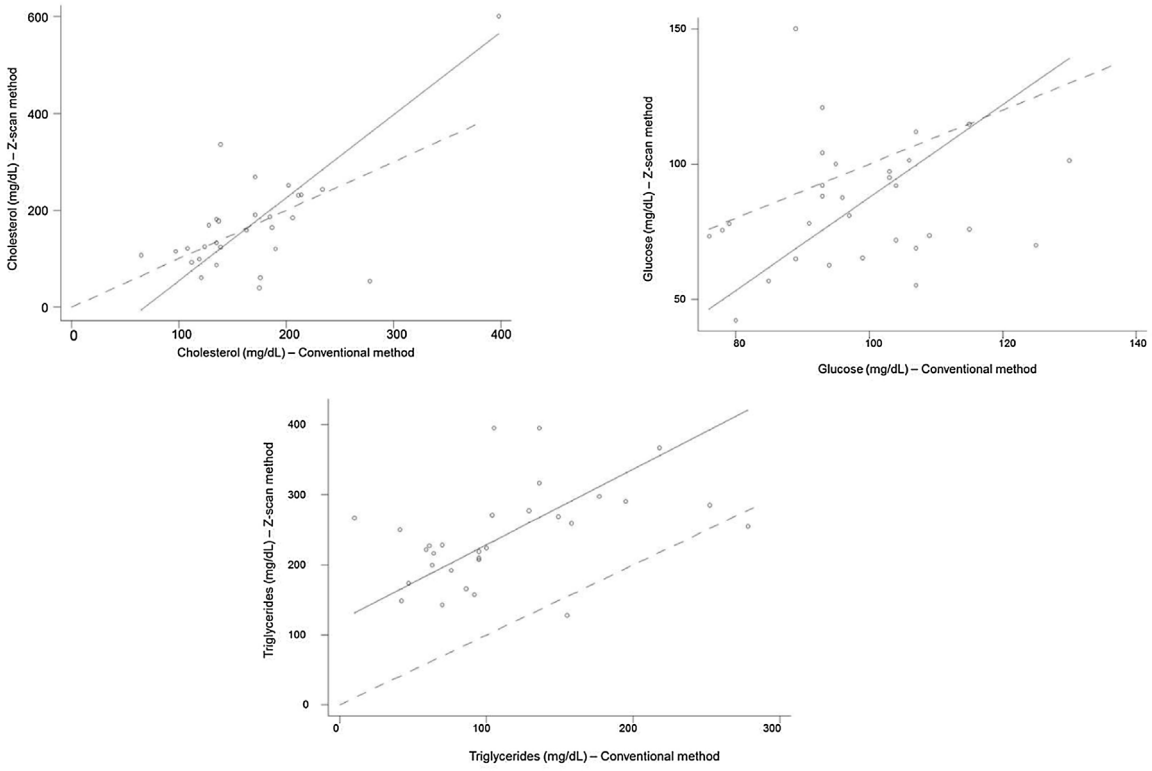

Fig. 3. Values obtained from conventional and Z-scan methods according to the concordance correlation coefficient (n = 30): (a) cholesterol (mg/dL); (b) glucose (mg/dL); (c) triglycerides (mg/dL).

these molecules was associated with their oxidation level.

However, Dhinaa et al. [17–19] showed that the Z-scan technique allows for the quantification of total cholesterol, triglycerides, protein, albumin and glucose in the blood. To reach this conclusion, they compared the values obtained through the technique with those analyzed by the enzymatic colorimetric method used in clinical laboratory practice. Among the advantages that the Z-scan technique provides, the fact that it is possible to isolate the object of analysis from the other elements of blood eliminates responses that could interfere with the reading of the sample. Thus, the sensitivity of the Z-scan technique broadens the spectrum of test analyses, granting a greater accuracy in the quantification of analyte concentrations in samples [17–19].

As a result of what was stated above, this study aims to evaluate the use of the Z-scan technique in serum samples of children with solid tumors or leukemias, supplemented or not with selenium, in the following biochemical parameters: glucose, triglycerides and total cholesterol. Moreover, the measurements obtained by the technique were compared with those acquired by the spectrophotometric method so that the laboratory differences regarding the studied parameters could be elucidated.

2. Methodology

2.1. Protocol for the prescription of selenium supplementation

The study was a randomized, double-blind phase II clinical trial with a crossover of groups (selenium vs. placebo) in children with leukemias or solid tumors (Fig. 1). Patients from the pediatric oncology outpatient clinic of Faculdade de Medicina do ABC (FMABC) were recruited. Inclusion criteria comprised age (the youngest patient was 3, and the oldest was 19 years old, with a mean age of 9.48 years) and type of tumor (acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML) of any phenotype) and solid tumors (ST). Patients who had previously been diagnosed with primary immunodeficiency were excluded. In Group I, 11 patients were included and in Group II, 15 patients.

Administration dosage of placebo and selenium, in particular, were based on RDA values for individuals of each age group. Capsules of 27, 36, 54, 72 and 100 µg of selenium were used (selenium glycine). The recommended dosage according to age group and an 80% increase in dosage are shown in Table 1.

Table 3
The Z-scan technique in the classification of patients with abnormal values of cholesterol (≥150 mg/dL) compared with the conventional method.

Cutoff point (mg/dL)	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR−
≥40.02	100.00	0.00	51.72	1.0000	
≥54.38	93.33	0.00	48.28	0.9333	
≥60.86	86.67	0.00	44.83	0.8667	
≥60.95	86.67	7.14	48.28	0.9333	1.8667
≥86.47	80.00	7.14	44.83	0.8615	2.8000
≥92.84	80.00	14.29	48.28	0.9333	1.4000
≥98.53	80.00	21.43	51.72	1.0182	0.9333
≥106.81	80.00	28.57	55.17	1.1200	0.7000
≥114.47	80.00	35.71	58.62	1.2444	0.5600
≥120.23	80.00	42.86	62.07	1.4000	0.4667
≥121.01	73.33	42.86	58.62	1.2833	0.6222
≥123.54	73.33	50.00	62.07	1.4667	0.5333
≥125.18	73.33	57.14	65.52	1.7111	0.4667
≥132.13	73.33	64.29	68.97	2.0533	0.4148
≥159.01	73.33	71.43	72.41	2.5667	0.3733
≥164.83	66.67	71.43	68.97	2.3333	0.4667
≥169.17	60.00	71.43	65.52	2.1000	0.5600
≥177.88	60.00	78.57	68.97	2.8000	0.5091
≥181.83	60.00	85.71	72.41	4.2000	0.4667
≥184.24	60.00	92.86	75.86	8.4000	0.4308
≥186.04	53.33	92.86	72.41	7.4667	0.5026
≥190.81	46.67	92.86	68.97	6.5333	0.5744
≥231.39	40.00	92.86	65.52	5.6000	0.6462
≥231.83	33.33	92.86	62.07	4.6667	0.7179
≥243.01	26.67	92.86	58.62	3.7333	0.7897
≥251.01	20.00	92.86	55.17	2.8000	0.8615
≥268.49	13.33	92.86	51.72	1.8667	0.9333
≥336.52	6.67	92.86	48.28	0.9333	1.0051
≥600.95	6.67	100.00	51.72		0.9333
> 600.95	0.00	100.00	48.28		1.0000

ROC curve = 0.69 (95% CI 0.47–0.90)
LR+: positive likelihood ratio; LR−: Negative likelihood ratio;
Cutoff point for abnormal values of cholesterol: ≥150 mg/dL.

Table 4
The Z-scan technique in the classification of patients with abnormal values of glucose (≥100 mg/dL) compared with the conventional method.

Cutoff point (mg/dL)	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR−
≥42.29	100.00	0.00	43.33	1.0000	
≥55.12	100.00	5.88	46.67	1.0625	0.0000
≥56.92	92.31	5.88	43.33	0.9808	1.3077
≥62.55	92.31	11.76	46.67	1.0462	0.6538
≥65.06	92.31	17.65	50.00	1.1209	0.4359
≥65.32	92.31	23.53	53.33	1.2071	0.3269
≥68.89	92.31	29.41	56.67	1.3077	0.2615
≥69.90	84.62	29.41	53.33	1.1987	0.5231
≥71.81	76.92	29.41	50.00	1.0897	0.7846
≥73.26	69.23	29.41	46.67	0.9808	1.0462
≥73.60	69.23	35.29	50.00	1.0699	0.8718
≥75.67	61.54	35.29	46.67	0.9510	1.0897
≥75.93	61.54	41.18	50.00	1.0462	0.9341
≥77.91	53.85	41.18	46.67	0.9154	1.1209
≥78.13	53.85	47.06	50.00	1.0171	0.9808
≥80.87	53.85	52.94	53.33	1.1442	0.8718
≥87.70	53.85	58.82	56.67	1.3077	0.7846
≥88.09	53.85	64.71	60.00	1.5256	0.7133
≥91.96	53.85	70.59	63.33	1.8308	0.6538
≥92.07	46.15	70.59	60.00	1.5692	0.7628
≥94.94	46.15	76.47	63.33	1.9615	0.7041
≥97.21	38.46	76.47	60.00	1.6346	0.8047
≥100.06	30.77	76.47	56.67	1.3077	0.9053
≥101.26	30.77	82.35	60.00	1.7436	0.8407
≥101.39	23.08	82.35	56.67	1.3077	0.9341
≥104.18	15.38	82.35	53.33	0.8718	1.0275
≥112.02	15.38	88.24	56.67	1.3077	0.9590
≥114.81	7.69	88.24	53.33	0.6538	1.0462
≥120.87	0.00	88.24	50.00	0.0000	1.1333
≥150.09	0.00	94.12	53.33	0.0000	1.0625
> 150.09	0.00	100.00	56.67		1.0000

ROC curve = 0.56 (95% CI 0.35–0.78)
LR+: positive likelihood ratio; LR−: Negative likelihood ratio;
Cutoff point for abnormal values of glucose: ≥100 mg/dL.

2.2. Z-scan technique

The method was chosen owing to the fact it is a precise and sensitive optical technique that has been widely used in studies of optical nonlinearities of biological substances [12–14,17–19] and in many materials over the past few years [7,8]. It measures the changes in the intensity of a focused beam that travels through the analyzed sample whenever there is a variation in the sample position while the beam is being delivered. The thermal responses that occur due to the laser excitation must be taken into consideration in regard to the reading of samples because such responses can modify the refractive index and generate gradients, also known as the thermal lens effect. In the current study, a CW laser (Verdi V2 – Coherent®) was used, with wavelength λ of 532 nm, incident power of 88 mW, and a mechanical shutter that supplied a quadratic periodical profile at the wavefront for successive periods of 30 ms [15].

For the obtained data analysis, the aberrant thermal lens model was used [21] given by Eq. (1), in which T_N defines the normalized transmittance in the Z-scan curve:

$$T_N(z, t) = 1 + \theta \arctan\left[\frac{2z/z_0}{(9 + (z/z_0)^2)(1 + (z/z_0)^2)/2\tau + (3 + (z/z_0)^2)}\right] \quad (1)$$

and

$$\theta = \frac{0.24\alpha P}{\kappa\lambda}\frac{b}{dT}\frac{dn}{} \quad (2)$$

θ is the thermally induced phase, z_0 Rayleigh length, α the absorption, P the incident power, b sample effective length, λ the laser wavelength, $\tau = t/t_c$ normalized time and t_c characteristic thermal lens time.

2.3. Samples

In order to obtain the serum for both laboratory analysis and Z-scan measurement, blood samples were collected via peripheral venipuncture, distributed into tubes, and centrifuged at 2100 rpm for 10 min at room temperature (300 K). For both methods, enzymatic colorimetric reagents from ELItech® were used according to the analyzed blood compound (glucose, triglycerides, and cholesterol). All samples were placed in 200 μm cuvettes for Z-scan measurement.

2.4. Calibration

Calibration protocol for the analysis of each compound was followed. It started out with the blank measurement, which is the use of the reagent alone, in order to confirm the scanning conditions. Next, three curves of three distinct dilutions were generated according to the recommendations established by the Brazilian Society for Clinical Pathology/Laboratory Medicine (SBPC/ML). The first measurement was taken from undiluted samples, the second from a 1:2 dilution, and the third from a 1:4 dilution.

1. Calibration curve (ELItech® calibrator)
2. Curve of normal reference sample (according to the normal control established by SBPC/ML)
3. Curve of pathological reference sample (according to the pathological control established by SBPC/ML)

Table 5
The Z-scan technique in the classification of patients with abnormal values of triglycerides (≥100 mg/dL) compared with the conventional method.

Cutoff point (mg/dL)	Sensitivity (%)	Specificity (%)	Correctly classified (%)	LR+	LR−
≥127.50	100.00	0.00	46.67	1.0000	
≥142.99	92.86	0.00	43.33	0.9286	
≥148.76	92.86	6.25	46.67	0.9905	1.1429
≥157.41	92.86	12.50	50.00	1.0612	0.5714
≥166.17	92.86	18.75	53.33	1.1429	0.3810
≥173.63	92.86	25.00	56.67	1.2381	0.2857
≥191.96	92.86	31.25	60.00	1.3506	0.2286
≥199.67	92.86	37.50	63.33	1.4857	0.1905
≥207.01	92.86	43.75	66.67	1.6508	0.1633
≥209.57	92.86	50.00	70.00	1.8571	0.1429
≥216.23	92.86	56.25	73.33	2.1224	0.1270
≥218.88	92.86	62.50	76.67	2.4762	0.1143
≥222.00	92.86	68.75	80.00	2.9714	0.1039
≥224.32	92.86	75.00	83.33	3.7143	0.0952
≥227.13	85.71	75.00	80.00	3.4286	0.1905
≥228.04	85.71	81.25	83.33	4.5714	0.1758
≥250.01	85.71	87.50	86.67	6.8571	0.1633
≥254.88	85.71	93.75	90.00	13.7143	0.1524
≥258.93	78.57	93.75	86.67	12.5714	0.2286
≥266.73	71.43	93.75	83.33	11.4286	0.3048
≥268.49	71.43	100.00	86.67		0.2857
≥270.51	64.29	100.00	83.33		0.3571
≥277.02	57.14	100.00	80.00		0.4286
≥285.53	50.00	100.00	76.67		0.5000
≥290.58	42.86	100.00	73.33		0.5714
≥297.88	35.71	100.00	70.00		0.6429
≥317.00	28.57	100.00	66.67		0.7143
≥367.17	21.43	100.00	63.33		0.7857
≥395.09	14.29	100.00	60.00		0.8571
≥395.39	7.14	100.00	56.67		0.9286
> 395.39	0.00	100.00	53.33		1.0000

ROC curve = 0.91 (95% CI 0.75–1.0)
LR+: positive likelihood ratio; LR-: Negative likelihood ratio;
Cutoff point for abnormal values of triglycerides: ≥100 mg/dL.

After the reading of the three curves, a random reading of the patients' samples was performed so that they could be compared based on the initial curves and the sample concentration could be defined in relation to each analyte. According to the results obtained by means of spectrophotometry, linearities, sensitivities, specificities and maximum and minimum detection limits were analyzed.

2.5. Statistical analysis

In order to evaluate the concordance between both methods, the correlation coefficient (CC) was used (95% CI) [22]. The concordance correlation coefficient was also used [23,24] to assess precision and accuracy values [25,26]. The association between outcome and independent variables was analyzed through the Chi-square test for qualitative data, and through the Mann-Whitney test for quantitative data.

3. Results

Based on the recruitment process criteria, the initial sample comprised 26 patients, 16 males and 10 females. Among them, 17 had been diagnosed with ALL, 02 with AML, 03 with neuroblastoma (NB), 01 with testicular tumor, 01 with rhabdomyosarcoma, 01 with suprarenal tumor and 01 with osteosarcoma. However, throughout the course of the study, three patients died and two with leukemia were discontinued for not following supplementation directions. At the end of the study, analyses were made possible when it was revealed which patients received selenium in the first part of the trial and which ones received it in the second part.

The typical Z-scan curves for glucose, triglycerides, and cholesterol samples are shown in Fig. 2a–c respectively.

Table 2 compares the concentration values of cholesterol (mg/dL), glucose (mg/dL) and triglycerides (mg/dL) obtained by the conventional spectrophotometric method with those measured by the Z-scan technique. It can be observed that the intraclass correlation coefficient lower limits between both methods were 0.12 for cholesterol, 0.02 for glucose and 0.00 for triglycerides. Precision and accuracy of these values were determined based on the coefficient of concordance [26] of 0.65 and 0.87 for cholesterol, 0.17 and 0.68 for glucose and 0.44 and 0.33 for triglycerides.

Table 2 also shows the limits of concordance established by the Bland-Altman method. The concordance between methods for the measurement of cholesterol, glucose and triglycerides ranged between −165.0 and 169.1 mg/dL, −61.5 and 34.5 mg/dL and −11.0 and 270.9 mg/dL respectively.

In Fig. 3, the lines generated from cholesterol (mg/dL), glucose (mg/dL) and triglycerides (mg/dL) measurements deviate from the line of perfect concordance, i.e., the line at 45. The Z-scan method overestimates cholesterol and triglyceride levels (mg/dL) (Fig. 3a and c) and underestimates glucose levels (mg/dL) (Fig. 3b) in relation to the conventional method. Also, through the use of Lin's concordance correlation coefficient (CCC), it was observed that the Z-scan technique had low concordance with the conventional method according to the McBride classification criteria (2005) for cholesterol, glucose and triglycerides measurements.

Tables 3–5 respectively show the classification of patients with abnormal values of cholesterol (> 150), glucose (> 100) and triglycerides (> 100) along with the values of sensitivity (%), specificity (%), positive and negative likelihood ratios and the area under the curve for the Z-scan method.

It can be observed that when Fig. 4 is compared with Tables 3–5, the Z-scan technique showed that the area under the curve for abnormal cholesterol levels was of 0.69 (95% CI 0.47–0.90) (Table 3, Fig. 4a), for abnormal glucose levels was of 0.56 (CI 95% 0.35–0.78) (Table 4, Fig. 4b), and for abnormal triglycerides levels was of 0.90 (CI 95% 0.75–1.0) (Table 5, Fig. 4c)

4. Discussion

The results show that it is possible to use a nonlinear optical method for determining biochemical analytes in patients with solid tumors or leukemias supplemented or not with selenium. However, as observed throughout the analysis, there are certain peculiarities in each analyte when the results from both methods are compared. It is noted that the Z-scan technique has greater sensitivity for the determination of triglycerides. It is known that triglyceride levels are high in patients with tumors, and many studies have reported the relationship of lung, colorectal and other tumors to high levels of triglycerides [28–30]. In such cases, the Z-scan technique is more effective than the spectrophotometric analysis, which is more commonly used in daily laboratory routine.

Studies like Dhinaa and Palanisamy's [17] show the high analytical sensitivity in the measurement of triglycerides concentration in samples by using a laser with a wavelength of 532 nm. Their adopted mathematical model, however, refers to a refined study regarding the thickness of the sample and disregarding relative factors like exposure time and temperature difference in the sample. This difference may be caused by the light energy emitted by the laser, which is transformed into thermal energy according to changes in the refractive index of the sample. It is important to point out that the refractive index is the factor that will determine the concentration of triglycerides in the sample. In the current study, the sample thickness was not taken into consideration.

The same results were not found in the determination of glucose levels. Maybe the factors mentioned above influenced the reduction of

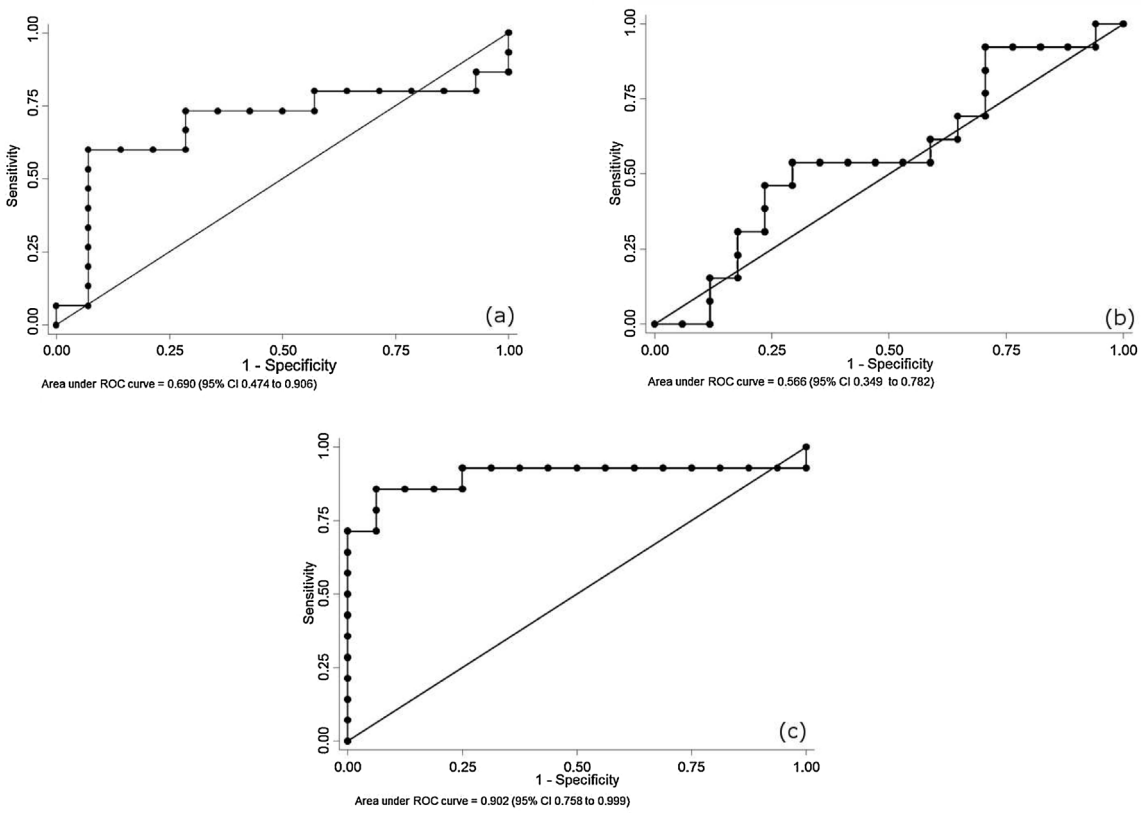

Fig. 4. ROC curve in relation to the Z-scan technique compared with the conventional method. In Fig. 4a, the classification of patients with abnormal cholesterol levels (cutoff point ≥150 mg/dL); in Fig. 4b, abnormal glucose levels (cutoff point ≥100 mg/dL); in Fig. 4c, abnormal triglycerides levels (cutoff point ≥100 mg/dL). The cut-off point used was based on the Brazilian Pediatric Society (SBP) [27].

these levels in the serum samples. This fact was not observed by Dhinaa and Palanisamy [19] since the calibration curve was obtained from a standard sample of glucose. It is known that there are differences in the application of the Z-scan technique whenever the complexity of the tested sample is taken into consideration.

Finally, when it came to the determination of total cholesterol levels, the obtained results were those with greater concordance between both tested methods. Maybe, when the Z-scan method is applied for cholesterol determination, no high differences in concentrations is observed, a fact that was not detected in the determination of triglycerides and glucose in the serum samples.

In summary, the Z-Scan technique can be used for laboratory analyses in serum samples of children with solid tumors or leukemias. Each determination must be analyzed separately so that the best method may be applied according to the patient's clinical condition and the type of sample to be tested.

Disclosures

The authors report no conflict of interest related to this study. All procedures performed in this work involving human participants were in accordance with the ethical standards of the Ethical Committee of ABC Medical School. Informed consent was obtained from all individual participants included in this study.

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