Serum soluble Tim-3 is elevated in patients with cervical cancer and is higher in advanced clinical stages

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Abstract. T-cell immunoglobulin and mucin domain-containing protein-3 (Tim-3) is an immune checkpoint molecule that is expressed generally on the cell membrane of immune and cancer cells and is implicated as a negative regulator of anti-tumour immune responses; this occurs through the interaction of Tim-3 with galectin-9. Although the function of membrane Tim-3 is well known, the role of soluble Tim-3 (sTim-3) has been poorly explored. The aim of the present study was to compare the serum levels of sTim-3 in the cervical cancer group of patients vs. the control group, to determine the association between the serum levels of sTim-3 with the clinicopathological characteristics of patients with cervical cancer and with serum galectin-9 levels. The concentrations of serum sTim-3 and galectin-9 were determined using ELISA. A receiver operating characteristic (ROC) curve was performed to determine the diagnostic value of sTim-3. The Mann-Whitney and Kruskall-Wallis tests were used to compare the serum sTim-3 concentrations between the control and cervical cancer groups and among the clinical subgroups. The association between the concentrations of sTim-3 and galectin-9 was determined using Spearman's rank correlation coefficient. sTim-3 expression was higher in patients with cervical cancer compared with control patients. The ROC curve revealed that sTim-3 has diagnostic potential, with a specificity of 95% and a sensitivity of 85.19%. sTim-3 was higher in patients with International Federation of Gynaecology and Obstetrics (FIGO) stage IV compared with those with FIGO stages I, II and III. A moderate positive correlation (ρ=0.41) was identified between sTim-3 and galectin-9. This was the first report of changes in the serum concentrations of sTim-3 in patients with cervical cancer and their diagnostic value. The association between sTim-3 with cervical cancer progression, and the positive correlation between the serum concentrations of sTim-3 and galectin-9 suggested that both proteins might be involved in the immune dysregulation in cervical cancer, but this requires further exploration.

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide, and most cases occur in low- and middle-income countries (1). Prophylactic vaccination significantly reduces the risk of cervical cancer development, and regular screening through Pap test and human papillomavirus detection enable early diagnosis by detecting premalignant lesions or early stages of cervical cancer (2). In México, cervical cancer is the second leading cause of cancer mortality in women, which suggests that, despite the available screening methods, cervical cancer remains a public health problem (3). There are three known histological types of cervical cancer: Squamous cell carcinoma (SCC), adenocarcinoma (AC) and adenosquamous carcinoma (ASC), with SCC and AC being the most prevalent (4). To determine the prognostic outcome and treatment schemes, oncologists consider the clinical stage of cervical cancer, which is determined according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO) (5). For patients with early stages of cervical cancer, the treatment is surgery, while for patients with locally advanced cervical cancer, the treatment is concomitant chemoradiotherapy (2). For patients with recurrent cervical cancer, different therapeutic strategies have been developed, including immune checkpoint inhibitors and DNA damage repair inhibitors (6). The anti-programmed death 1 (PD-1) monoclonal antibody pembrolizumab was the first immunotherapy strategy approved by the US Food and Drug Administration for recurrent or metastatic cervical cancer (6).

The immune system plays crucial roles in both cancer control and development. It has been demonstrated that immune checkpoint proteins that are involved in the negative regulation of the immune response are linked to evasion mechanisms in cancer (7). Exhausted T cells that are unable to eliminate cancer cells overexpress certain immune checkpoints, such as PD-1, cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), lymphocyte activation gene-3 (LAG-3) and T-cell immunoglobulin and mucin domain-containing protein-3 (Tim-3) (7).

Tim-3 is normally expressed on the membrane of several immune cells, including T cells. The binding of Tim-3 to its ligand, galectin-9, results in the suppression of T-cell responses and may lead to $CD8^+$ T-cell exhaustion, which impairs the cytotoxic activity responsible for cancer cell eradication (8,9). Previous studies have shown that membrane Tim-3 expression is significantly greater in the peripheral $CD4^+$ and $CD8^+$ T cells of patients with cervical cancer compared with the controls, with the highest Tim-3 expression found in patients with stage III-IV cervical cancer than in those with stage I-II (10). Compared with premalignant lesions and cervicitis, membrane Tim-3 is also overexpressed in cervical cancer. In addition, the higher expression of membrane Tim-3 was associated with a shorter overall survival in patients with cervical cancer (11). Therefore, the immune checkpoint Tim-3/galectin-9 has been proposed by certain authors as a potential immunotherapy target for patients with cervical cancer (10-12).

Soluble immune checkpoints (sICs) have been identified in the serum, and these soluble forms of some membrane proteins can be produced by alternative splicing, cleavage with proteases or secretion by shedding the cell membrane. Increased levels of sICs have been reported in different cancer types (13). In most cases, the increased expression of sICs has been found to be associated with disease severity and poor overall survival (14).

Therefore, sICs could be potential biomarkers associated with prognosis and treatment response (13). The soluble form of Tim-3 (sTim-3) may be produced in different ways, such as alternative splicing, cleavage from the cell surface by diverse enzymes, including disintegrins and matrix metalloproteases, and passive release from apoptotic cells (15-17). Increased levels of sTim-3 have been reported in different cancer types, such as thyroid carcinoma (7), hepatocarcinoma (18), oral SCC (19) and non-small cell lung cancer (NSCLC) (20). Higher levels of sTim-3 were detected in patients with thyroid carcinoma with lymph node metastasis (7). In contrast to most types of cancer, patients with breast cancer presented lower levels of sTim-3 compared with healthy individuals (21). This indicated that sTim-3 levels are regulated in a tissue-dependent manner.

To the best of our knowledge, the role of sTim-3 in cervical cancer has not been assessed; the only information reported for sTim-3 and cervical cancer corresponds to a study conducted in patients with locally advanced cervical cancer. In this study, the effect of concurrent chemoradiotherapy in the levels of different sICs including sTim-3 were evaluated, and an increase in sTim-3 levels was reported following treatment, emphasizing the importance of designing improved treatment strategies when considering their immunomodulatory effects. Nevertheless, the clinical implications of this increase were not analysed (22).

In a previous study, the presence of galectin-9 was evaluated in the serum of patients with cervical cancer, and it was reported that the serum galectin-9 level was higher in patients with cervical cancer compared with the control group, which increased with advanced clinical stages (23).

The serum levels of Tim-3 need to be explored in patients with cervical cancer to assess whether they are associated with clinicopathological characteristics or clinical outcomes and to evaluate its potential as a diagnostic or prognostic biomarker. Furthermore, the association between the serum levels of sTim-3 and galectin-9 could provide information about their possible role in immune regulation and treatment response.

Materials and methods

Patients and study protocol. A total of 108 patients with cervical cancer and 40 women with a normal cytology report were included in the present study.

Inclusion and exclusion criteria, diagnostic criteria, and description of control subjects. The patients with cervical cancer included in the study were from the High Specialty Medical Unit, Mexican Institute of Social Security (IMSS), in Puebla City, Mexico, and the study was carried out between November 14, 2017 and October 18, 2023, (except for the period from November 14, 2018 to December 17, 2018, in which the project did not have the authorized extension for follow up) in accordance with the Declaration of Helsinki. The ethical regulations were approved by the National Commission for Scientific Research, under the registration number R-2017-785-119. The results presented in the present study correspond to the protocol approved by the Local Committee of Health Research 2106 of the IMSS, under the registration number R-2023-2106-004, in which the use of data and serum samples previously collected, was authorized.

The cervical cancer group included women first diagnosed according to the Bethesda System (24), and the diagnosis was confirmed through the analysis of a biopsy by a pathologist. Women who were previously diagnosed with another cancer type, had acute infections, were pregnant or had autoimmune diseases were excluded from the study. Patients with cervical cancer were staged according to the FIGO criteria (5). The serum samples from patients with cervical cancer were obtained at the beginning of the intervention prior to the commencement of any treatment.

Clinicopathological information was obtained from the clinical records of the patients. The clinical outcomes considered in the present study were complete remission or death during the follow-up period following medical intervention.

Women included in the control group were negative for premalignant lesions or cervical cancer according to their cytology report. Women who were pregnant, had acute infections or had autoimmune diseases were excluded. All the participants were informed about the study and signed informed consent.

Serum sTim-3 concentration. Venous blood (5 ml) was collected from each participant and centrifuged at 2,000 x g for 10 min. The serum was stored at -20˚C until use.

The serum concentration of sTim-3 was determined using a Quantikine Human Tim-3 enzyme-linked immunosorbent assay (ELISA) kit (cat. no. DTIM30; R&D Systems Inc.), according to the manufacturer's instructions. Serum samples were diluted at a 1:5 ratio, and each dilution was performed in duplicate.

The serum galectin-9 concentration was determined using a Quantikine Human Gal-9 ELISA kit (cat. no. DGAL90; R&D Systems, Inc.), according to the manufacturer's instructions. Serum samples were diluted at a 1:2 ratio, and each dilution was performed in duplicate (23).

The microplates were read at absorbances of 450 and 540 nm using a Synergy 4 microplate reader (BioTek Instruments, Inc.). Wavelength corrections were performed by subtracting the reading at 540 nm from the reading at 450 nm. For the analysis, an average of the duplicates of each sample was obtained, and the serum sTim-3 and galectin-9 concentrations were determined by comparison against their respective standard curves, and the values were multiplied by the dilution factor.

Statistical analysis. The Mann-Whitney test was performed to compare the serum sTim-3 concentration between the control and cervical cancer groups, between the control and cervical cancer groups by the age range and between clinical outcomes. The Kruskal-Wallis test followed by Dunn's test was performed to compare sTim-3 concentration by age ranges in cervical cancer groups and in the different clinicopathological characteristics. $P \leq 0.05$ was considered to indicate a statistically significant difference. In addition, correlation analysis was performed for sTim-3 and galectin-9 concentrations using Spearman's rank correlation coefficient. A normality test was performed on the raw data to evaluate its distribution, and the corresponding test was applied. Statistical analysis was performed using Prism software version 10 (GraphPad software, Inc.). Receiver operating characteristic (ROC) curve analysis was performed following the method by DeLong *et al* (25) using MedCalc software version 22.030 (MedCalc Software bvba).

Results

Characteristics of the study subjects. Serum samples from 108 patients with a diagnosis of cervical cancer and 40 women with a normal cytology report were included in the present study. The patients with cervical cancer had a mean age of 52.3±13.4 (26-87) years, and the control group had a mean age of 31.7±10.2 (21-60) years (Mann-Whitney, $P<0.0001$). The clinicopathological characteristics of the patients with cervical cancer are described in Table I.

Serum sTim-3 concentration is increased in patients with cervical cancer. The serum levels of sTim-3 were significantly higher in the patients with cervical cancer compared with the controls, as shown in Fig. 1. The median concentration in the control group was 1.169 ng/ml compared with 3.146 ng/ml in the cervical cancer group.

As significant differences in age between control vs. cervical cancer samples were obtained, it was determined if there were differences in the concentration of sTim-3 with respect to age. For this, age ranges in the control and in the cervical cancer groups were established and it was determined if there were significant differences between the age groups. The mean concentrations of sTim-3 in control and the median concentrations in cervical cancer groups by age ranges are shown in Table II. No statistical differences were obtained between age groups in the control and the cervical cancer groups (Table II). The concentrations of sTim did not change with respect to age neither in the control group nor in the cervical cancer group.

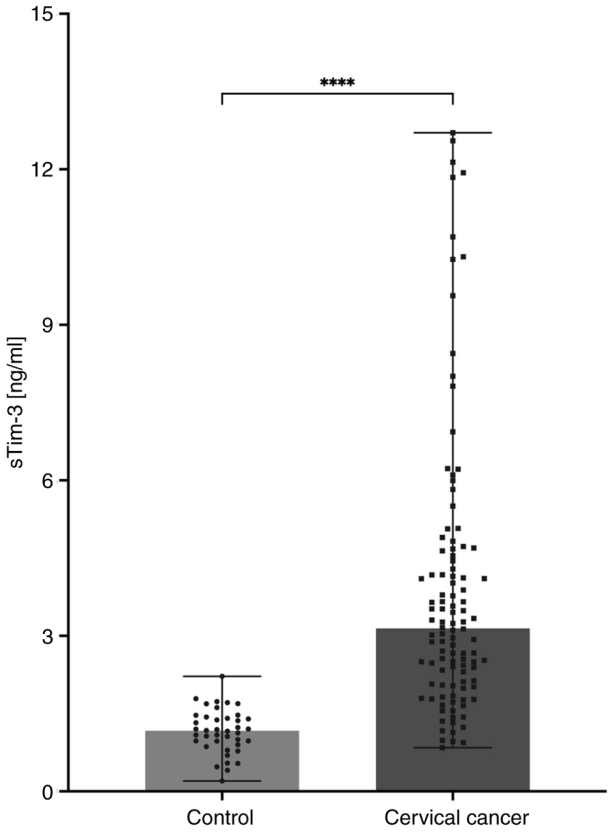

Figure 1. sTim-3 median and range concentration in the cervical cancer and control groups. The Mann-Whitney test was performed to compare the control vs. cervical cancer groups (****$P<0.0001$). sTim-3, soluble T-cell immunoglobulin and mucin domain-containing protein-3.

The levels of sTim-3 by the age range 21-30 years, 31-40 years and 41-60 years between the control and cervical cancer groups were also compared and significant differences were obtained (Table II). The previous analysis supports that the sTim-3 concentration is not modified by age and the concentration changes appeared to be associated with the disease.

ROC analysis and diagnostic efficacy of sTim-3. To determine the diagnostic potential of sTim-3 as a biomarker, ROC analysis was performed for serum sTim-3 in patients with cervical cancer (n=108) vs. the control group (n=40). The area under the curve (AUC) was 0.938 ($P<0.0001$), the Youden index was 0.8019, the optimal cut-off value was >1.7313 ng/ml, specificity was 95.00% and sensitivity was 85.19% (Fig. 2).

Association between serum sTim-3 levels, and clinicopathological characteristics and clinical stage. The serum sTim-3 concentration was compared between groups with

Table I. Serum levels of sTim-3 and clinicopathological characteristics of patients with cervical cancer.

A, Clinicopathological characteristics analysed by Kruskal Wallis and Dunn't tests

Clinicopathological characteristics	n	Median concentration (range)	P-value (Kruskal-Wallis test)	P-value (Dunn's test)
Histological type				
SCC	83	3.04 (0.84-12.70)	0.6179	SCC vs. AC >0.9999
AC	11	3.48 (1.32-10.70)		SCC vs. AS >0.9999
ASC	6	2.71 (0.96-5.82)		AS vs. AC >0.9999
Differentiation grade				
G1	4	2.56 (1.35-4.14)	0.6445	G1 vs. G2 >0.9999
G2	56	3.10 (0.94-12.55)		G1 vs. G3 >0.9999
G3	25	3.27 (.84-12.24)		G2 vs. G3 >0.9999
FIGO stage				
I	8	2.16 (0.94-6.93)	0.0371[a]	I vs. II >0.9999
II	27	2.96 (0.84-12.14)		I vs. III 0.7395
III	40	3.29 (1.17-10.26)		I vs. IV 0.0338[a]
IV	13	5.07 (1.23-12.70)		II vs. III >0.9999
				II vs. IV 0.1784
				III vs. IV 0.2562

B, Clinicopathological characteristics assessed by Mann-Whitney U test

Clinicopathological characteristics	n	Median concentration (range)	P-value (Mann-Whitney U test)	N/A
Keratinization				
NKSCC	46	3.01 (0.94-12.14)	0.9495	N/A
KSCC	21	3.26 (0.84-12.70)		
Clinical outcome				
Total remission of disease	15	2.8 (0.98-4.72)	0.1583	N/A
Deceased	14	3.7 (1.17-10.70)		

[a]Statistically significant. G1, well differentiated; G2 moderately differentiated; G3 poorly/non differentiated. SCC, squamous cell carcinoma; AC, adenocarcinoma; ASC, adenosquamous carcinoma; FIGO, International Federation of Gynaecology and Obstetrics; NKSCC, non-keratinizing squamous cell carcinoma; KSCC, keratinizing squamous cell carcinoma; N/A, not applicable.

different histological types of cervical cancer (SCC, ACs and ASCs), differentiation grades, and (for the SCC group) between the keratinization and non-keratinization status. The median concentration of sTim-3 for each group is shown in Table I. Statistical analysis did not reveal differences in these characteristics.

The serum sTim-3 concentration increased progressively with increasing clinical stages; the median concentration and the range of sTim-3 for each group are shown in Table I. The Kruskal-Wallis test results revealed a significant difference among the groups (P=0.0371). Post-hoc Dunn analysis showed that sTim-3 concentration in stage IV was significantly higher compared with that in stage I (P=0.0338; Fig. 3).

ROC curve analysis and diagnostic efficacy of sTim-3 in different clinical stage groups. ROC curve analysis was performed for each clinical stage group vs. the control group. The AUC, the sensitivity and specificity are shown in Fig. 4. The optimal sensitivity values were obtained for clinical stages II, III and IV (Fig. 4B-D), while the specificity values were exceptional for all stages, and notably 100% for stage IV (Fig. 4A-D).

sTim-3 and clinical outcomes. Since serum sTim-3 concentrations were evaluated before any treatment in patients with cervical cancer, the outcome was defined as total remission of disease or death during the follow-up period. No statistical association was observed between sTim-3 levels and patient outcomes; however, a trend towards increasing sTim-3 concentration was observed in the group of deceased patients, as shown in Table I.

Serum galectin-9 concentration in patients with cervical cancer. The serum concentrations of galectin-9 were

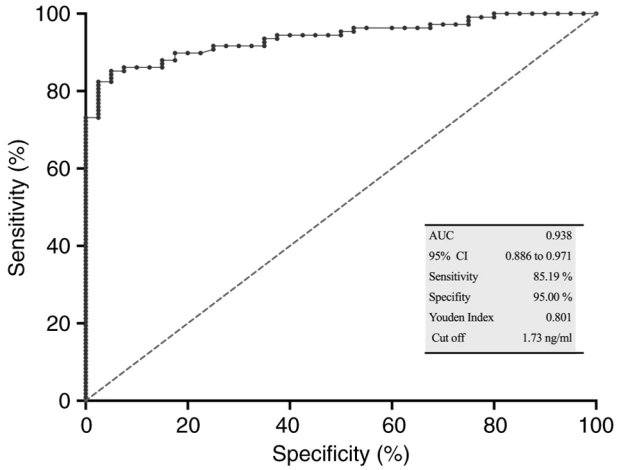

Figure 2. ROC analysis of sTim-3 to determine its diagnostic value for patients with cervical cancer. The ROC curve for serum sTim-3 in patients with cervical cancer compared with the control group was estimated. The graph shows the AUC, 95% CI, Youden index, sensitivity and specificity. ROC, receiver operating characteristic; sTim-3, soluble T-cell immunoglobulin and mucin domain-containing protein-3; AUC, area under the curve; CI, confidence interval.

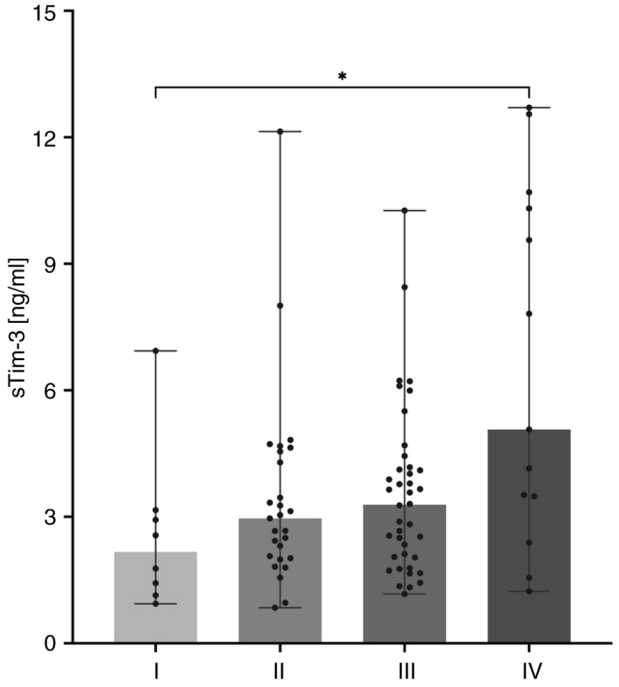

Figure 3. sTim-3 concentration increases with respect to clinical stage. The graph shows the median and range of sTim-3 concentration in different clinical stage groups. The Kruskal-Wallis test followed by Dunn's test was performed to compare FIGO stage groups, (stage I vs. IV; *P=0.0338). sTim-3, soluble T-cell immunoglobulin and mucin domain-containing protein-3; FIGO, International Federation of Gynaecology and Obstetrics.

previously reported in this cohort of patients with cervical cancer (23); however, as new patients were subsequently included, the serum concentrations of galectin-9 were also determined in this study to perform a correlation analysis of the serum levels of galectin-9 and sTim-3. The median concentration of galectin-9 in patients with cervical cancer was 9.64±3.18-34.97 ng/ml. A notably increased concentration of galectin-9 was detected in patients with FIGO stage IV (13.85 ng/ml) compared with stage I (7.73 ng/ml), stage II (8.13 ng/ml) and stage III (8.58 ng/ml) cervical cancer (Table III).

Correlation of the serum levels of sTim-3 and galectin-9. To determine whether there was a correlation between the serum levels of sTim-3 and galectin-9, Spearman's rank correlation coefficient was performed using the data of 102 patients' sera. A moderate positive correlation was identified between sTim-3 and galectin-9 concentrations, with a correlation coefficient of ρ=0.41 and high significance (P<0.0001; Fig. 5).

Discussion

To the best of our knowledge, this was the first time that the serum levels of sTim-3 were compared between patients with cervical cancer and healthy women. The results revealed significantly increased concentrations of sTim-3 in patients with cervical cancer. ROC curve analysis revealed that sTim-3 effectively differentiates patients with cervical cancer from healthy women. These results highlighted its potential use for the diagnosis of cervical cancer, possibly as a complementary cervical cytology test to improve sensitivity.

These results resembled those of other studies that analysed sTim-3 in several types of cancer, including oral SCC, skin basal cell carcinoma, colorectal cancer, gastric cancer, and osteosarcoma, and those studies reported higher levels of sTim-3 in patients with cancer compared with healthy individuals (26-30). By contrast, patients with breast cancer presented lower levels of sTim-3 compared with healthy individuals (21). This indicated that sTim-3 levels were regulated in a tissue-dependent manner.

However, an analysis of sTim-3 levels among several clinical groups revealed that this protein was not associated with the histological type, differentiation grade or keratinizing status; thus, sTim-3 did not appear to be associated with tumour histological characteristics, at least in cervical cancer. Similarly, in a previous study, no association was found between sTim-3 and the histological subtype of the tumour in patients with differentiated thyroid carcinoma (7).

Nonetheless, in the present study, increased levels of sTim-3 were detected in women with advanced clinical stages of cervical cancer. Indeed, significant differences in sTim-3 were identified between patients with FIGO clinical stage IV disease and those with stage I disease. Of note, this increase could be linked to an immune dysregulation and a blocking immune response that is more noticeable as the disease progresses. However, there were no statistical differences between clinical stages I, II and III; including a larger number of patients per group could make the differences observed statistically significant, but this should be explored in future studies. The ROC curve analysis performed for each clinical stage versus the control group revealed that the concentration of sTim-3 was an effective discriminator for clinical stages II, III and IV, supporting that at the early stage of disease the dysregulation of the immune response is less. In a previous study with patients with cervical cancer, Tim-3 expression was evaluated in peripheral T cells and was significantly increased compared with the control group. The expression of Tim-3 on peripheral $CD4^+$ and $CD8^+$ T cells was significantly

Table II. Serum concentration of sTim-3 with respect to the age range.

A, Concentration of sTim-3 with respect to age range in control and cervical cancer groups

Group	Age range	Median and range concentration of sTim3	P-value (Kruskall-Wallis test)
Control	21-30 years	0.99 ng/ml (0.41-2.21)	[a]0.525
	31-40 years	1.14 ng/ml (0.20-1.73)	
	41-60 years	1.54 ng/ml (0.47-1.69)	
Cervical cancer	21-30 years	4.69 ng/ml (2.88-6.10)	[a]0.6786
	31-40 years	3.26 ng/ml (1.17-10.31)	
	41-60 years	3.10 ng/ml (0.94-12.14)	
	>60 years	3.04 ng/ml (0.84-12.70)	

B, Concentration of sTim-3 in control vs cervical cancer groups by age range

Group	Age range	Mann-Whitney U test P- value
Control vs. Cervical cancer	21-30 years	0.0008
	31-40 years	0.0001
	41-60 years	0.0016

[a]Not significant; Dunn's post hoc test used.

greater in patients with stages III-IV disease than in those with stages I-II disease (12).

The association of sTim-3 with cancer progression has also been observed in gastric carcinoma, where the highest levels of sTim-3 were found in patients with carcinoma, followed by patients with benign gastric disease, and the lowest levels were found in the healthy control group (29). Of note, the serum levels of sTim-3 have not been evaluated in patients with premalignant lesions of the cervix; this is to be the focus of our future studies.

In patients with osteosarcoma, sTim-3 was also found to be associated with the risk of disease progression (30). In patients with colorectal cancer, the levels of sTim-3 are greater in those with postoperative recurrence (28). In clear cell renal cell carcinoma, high levels of sTim-3 were found to be associated with decreased survival (31).

In combination, in the present study, the serum levels of sTim-3 were analysed with respect to the clinical outcome, and higher concentrations were observed in patients who succumbed to disease than in patients with total remission. Given the limited number of samples in the present study, it is important to plan further studies that include a higher number of patients to effectively determine the association of sTim-3 with clinical outcome.

Although the present study did not include patients treated with immunotherapy, it is important to mention that in a study performed on patients with NSCLC and anti-PD-1 immunotherapy, high expression levels of sTim-3 were reported in patients that did not respond to treatment, and an increase in serum levels was linked to tumour progression (32). Considering the anti-PD-1 immunotherapy for patients with metastatic cervical cancer, the study of sTim-3 as a negative factor affecting treatment response highlights the importance of studying this soluble protein in patients with cervical cancer. In addition, it has been proposed to combine anti-Tim-3 therapy with anti-PD-1 therapy, to increase treatment response; therefore, further studying the role of sTim-3 with cervical cancer may provide valuable insights.

Table III. Concentration of serum galectin-9 with respect to the FIGO stage.

FIGO	Median concentration of serum galectin-9	Range concentration
Stage I	7.73 ng/ml	3.18-17.23 ng/ml
Stage II	8.13 ng/ml	3.25-23.45 ng/ml
Stage III	8.58 ng/ml	3.48-23.85 ng/ml
Stage IV	13.85 ng/ml	3.30-34.97 ng/ml

FIGO, International Federation of Gynaecology and Obstetrics.

The results of the present study indicated that sTim-3 is associated with cancer progression, but the mechanism remains to be elucidated. Tim-3 is a negative regulator of the anti-tumour immune response, and its overexpression is part of a series of biochemical mechanisms that are activated, favouring cancer development. It was hypothesized that sTim-3 is a soluble signal that suppresses anti-tumour immune response. The soluble form of Tim-3 may be produced in different ways, such as alternative splicing, passive release from apoptotic cells, and proteolytic cleavage by two A disintegrin and metalloprotease metalloproteases (15-17). However, sTim-3 can also be secreted by T cells, as has been reported in differentiated thyroid carcinoma, which highlights that $CD3^+$

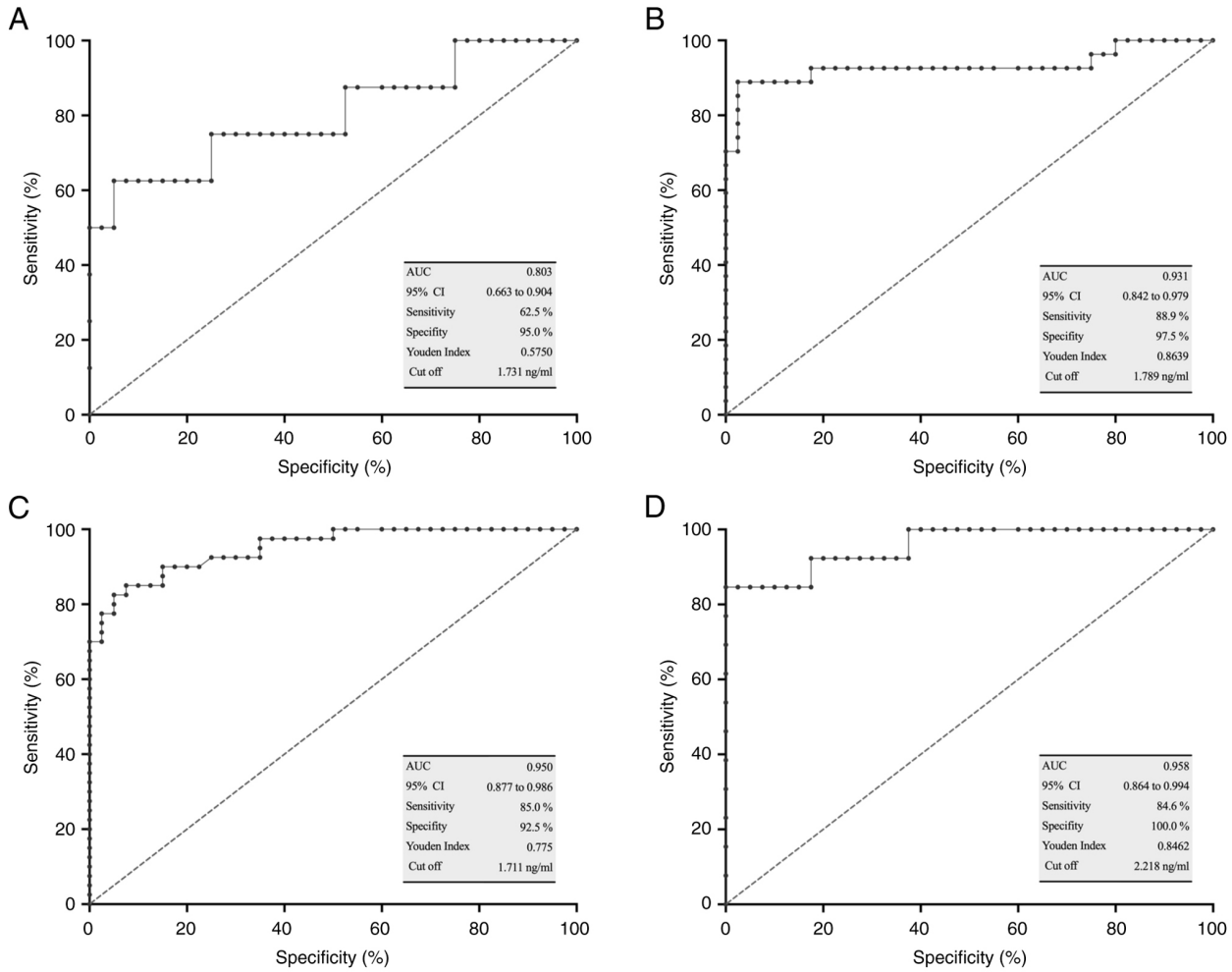

Figure 4. ROC analysis of sTim-3 to determine its diagnostic value for patients with cervical cancer in clinical stage groups. (A) ROC curve for sTim-3 in clinical stage I, (B) clinical stage II, (C) clinical stage III and (D) clinical stage IV groups vs. the control group. The graph shows the AUC, 95% CI, Youden index, sensitivity and specificity. ROC, receiver operating characteristic; sTim-3, soluble T-cell immunoglobulin and mucin domain-containing protein-3; AUC, area under the curve; CI, confidence interval.

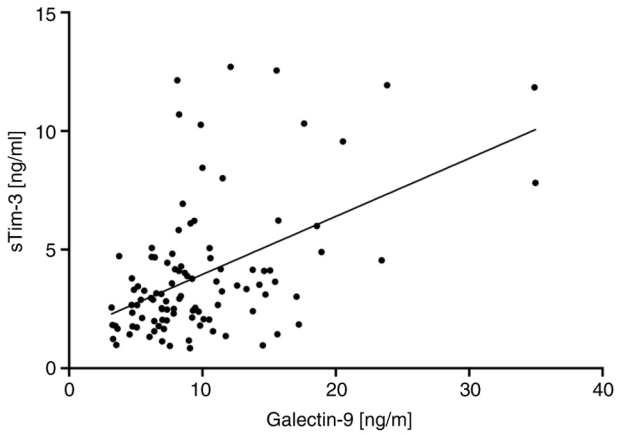

Figure 5. Spearman's rank correlation coefficient of the serum concentration of sTim-3 compared with that of galectin-9. The analysis results revealed a moderate correlation (ρ=0.41 and P<0.0001). sTim-3, soluble T-cell immunoglobulin and mucin domain-containing protein-3.

and $CD8^+$ T-cell subsets isolated from metastatic lymph nodes exhibit increased secretion of sTim-3 compared with those isolated from normal lymph nodes (7).

The major ligand of Tim-3 is galectin-9, which participates in multiple cellular processes through interaction with carbohydrates in glycoproteins (33,34). It has been reported that the binding of galectin-9 to membrane Tim-3 induces T-cell apoptosis (35,36). In our previous study, serum galectin-9 concentrations were evaluated, and higher levels were reported in patients with cervical cancer compared with those in healthy women. The highest serum galectin-9 levels were reported in patients with advanced clinical stage disease (23).

The previous analysis of serum galectin-9 compared with the results obtained for sTim-3 suggested that sTim-3 is a better diagnostic biomarker, as the sensitivity to discriminate between control and patients with cervical cancer was 85.19% for sTim-3 compared with 68.2% for galectin-9 (21). With respect to disease progression, both proteins exhibited a similar behavior, with disease progression being linked to an increase in concentration; sTim-3 showed significant differences between clinical stage I vs. clinical stage IV, and serum galectin-9 showed significant differences between clinical stage I vs. IV, and clinical stage II vs. VI (23). The difference in sensitivity of these soluble proteins could be the result of their biological roles; Tim-3 is a receptor that has different ligands (galectin-9, phosphatidylserine, carcinoembryonic

antigen-related cell adhesion molecule 1, and high mobility group protein B1), these ligands have been studied in cancer and the results have shown that they are related with disease progression. Thus, in addition to galectin-9, other ligands could be participating in cervical cancer progression through their interaction with sTim-3, which could explain the difference in sensitivity observed between sTim-3 and serum galectin-9 (37-39).

Given that new patients were subsequently included in this cohort and considering the relevant interactions of galectin-9 with Tim-3, the serum concentrations of galectin-9 were also determined, and a correlation analysis of the serum levels of both proteins was performed. These results revealed a positive moderate correlation, suggesting a possible role of this immune checkpoint complex in the progression of the disease. Tim-3/galectin-9 signalling was involved in immune regulation, and changes in serum levels could affect the development, prognosis and response to cancer, which highlighted the importance of Tim-3/galectin-9 as potential biomarkers and therapeutic targets (13).

sICs play important roles in immune modulation and are involved in the development and prognosis of cancer; thus, they are considered potential biomarkers for improving cancer diagnosis, as well as therapeutic targets (13). To date there is no biomarker that is routinely used for the diagnosis and follow-up of patients with cancer, but different serum biomarkers have been proposed for prognosis and follow up of patients with cervical cancer. One of the most studied is squamous cell carcinoma antigen (SCC); the serum levels of this antigen have been reported to be increased in patients with cervical cancer, with higher levels in advanced clinical stages. This antigen has been proposed for monitoring the response to treatment (40,41). One of the limitations of the present study is the lack of evaluation of other serum biomarkers studied previously (42), which would have been useful to compare the results obtained for sTim-3. This would have enriched the insights of the present findings.

In conclusion, sTim-3 may be an effective discriminator of patients with cervical cancer and a potential diagnostic biomarker. In addition, the increased expression of sTim-3 in patients with advanced clinical stage highlights its potential as a biomarker of progression. Serum immune checkpoints must be studied to elucidate their functions in cancer progression, treatment response and survival.

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Availability of data and materials

All data generated or analysed in this study are included in this published article.

Authors' contributions

VVR and JRL designed the study. VJVZ, ICR and SPP performed the investigation and data collection. VVR and JRL drafted the manuscript and provided approval of the final version of the manuscript. ICR, JRL and VVR performed the data analysis, and created the figures and tables. ICR and SPP confirm the authenticity of all raw data. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Human Ethics and Local Committee of Health Research number 2106 from the Mexican Institute of Social Security with the registration number R-2023-2106-004. Written informed consent was obtained from all the participants in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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