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Serum CD26 levels and fibroblast phenotypic markers in patients with tracheal stenosis secondary to orotracheal intubation

Niveles séricos de CD26 y marcadores fenotípicos de fibroblastos en pacientes con estenosis traqueal por intubación

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ABSTRACT. Introduction: orotracheal intubation stenosis (OTIS) is the most common cause of benign tracheal stenosis. It is the result of a deregulated healing response, a process in which dipeptidyl peptidase-4 (DPP-4 or CD26) has been proposed as one of the molecules with a possible regulatory role. This work aims to evaluate the relationship between serum levels and tissue expression of CD26 with OTIS and its complications, as well as to describe the expression of myofibroblasts by immunofluorescence. **Material and methods:** a case-control study was carried out; serum and tissue CD26 levels were measured. The characteristics of the cases (patients with tracheal stenosis) versus controls (healthy) were compared, as well as associations between serum DPP-4 and surgical variables (bleeding, complications, type of anastomosis, etc.); subsequently, a logistic regression model was performed to evaluate the association of DPP-4-S and the presence of OTIS. The expression of DPP-4 and myofibroblasts in tracheal tissue was also qualitatively evaluated. **Results:** 22 cases and 22 controls were analyzed. In the analysis of the cases, no differences were found between pre-surgical and three months post-surgical DPP-4-S levels. In the logistic regression analysis, DPP-4-S levels did not show adequate sensitivity and specificity to discriminate OTIS; the expression of myofibroblasts in the tracheal tissue analyzed by immunofluorescence revealed an increase in their expression. **Conclusions:** under the conditions of this study, DPP-4-S levels did not adequately discriminate cases of OTIS, although its expression was found to increase in tracheal

RESUMEN. Introducción: la estenosis traqueal secundaria a intubación orotraqueal es la causa más común de estenosis traqueal benigna. Es el resultado de una respuesta desregulada de cicatrización, proceso en el cual la dipeptidil peptidasa-4 (DPP-4 o CD26) se ha propuesto como una de las moléculas con posible papel regulatorio. El trabajo tiene por objetivo evaluar la relación entre los niveles séricos y la expresión tisular de CD26 con la estenosis traqueal secundaria a intubación orotraqueal y sus complicaciones, así como describir la expresión de miofibroblastos mediante inmunofluorescencia. **Material y métodos:** se realizó un estudio de casos y controles; se midieron niveles séricos y tisulares de CD26. Se compararon las características de los casos (pacientes con estenosis traqueal) versus controles (sanos), así como asociaciones entre DPP-4 sérica y las variables quirúrgicas (sangrado, complicaciones, tipo de anastomosis, etcétera); posteriormente, se realizó un modelo de regresión logística para evaluar la asociación de DPP-4-S y la presencia de estenosis traqueal secundaria a intubación orotraqueal. Además, de manera cualitativa, se evaluó la expresión de DPP-4 y miofibroblastos en tejido traqueal. **Resultados:** se analizaron 22 casos y 22 controles. En el análisis de los casos no se encontraron diferencias entre niveles de DPP-4-S prequirúrgicos y tres meses posquirúrgicos. En el análisis de regresión logística los niveles de DPP-4-S no mostraron una adecuada sensibilidad y especificidad para discriminar estenosis traqueal secundaria a intubación orotraqueal; la expresión de miofibroblastos en el tejido traqueal analizado por inmunofluorescencia reveló un aumento en la

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tissue, and it cannot be ruled out that it may serve as a therapeutic target in the early stages of tracheal stenosis secondary to orotracheal intubation or before its formation.

Keywords: tracheal stenosis, CD26, tracheoplasty, DPP-4, myofibroblasts.

expresión de éstos. **Conclusiones:** bajo las condiciones de realización de este estudio, los niveles de DPP-4-S no discriminaron adecuadamente los casos de estenosis traqueal secundaria a intubación orotraqueal, aunque su expresión se encontró incrementada en tejido traqueal; y no se descarta que pueda fungir como blanco terapéutico en etapas tempranas de la estenosis traqueal secundaria a intubación orotraqueal o previo a su formación.

Palabras clave: estenosis traqueal, CD26, traqueoplastía, DPP-4, miofibroblastos.

Abbreviations:

DPP-4 or CD26 = dipeptidil peptidasa-4.
Serum-DPP-4 = serum dipeptidyl peptidase-4.
TSSOI = tracheal stenosis secondary to orotracheal intubation.
FAP = fibroblast activation protein.

INTRODUCTION

Stenosis caused by prolonged intubation is the most common cause of benign tracheal stenosis. Its incidence has been reported between 0.3-11% and up to 20% of people undergoing tracheal intubation in some studies.[1,2] Severe acute respiratory syndrome due to coronavirus 2 (SARS-CoV-2) and the associated COVID-19 pandemic have caused an increase in critical patients requiring prolonged mechanical ventilation,[3] with an expected increase in the frequency of tracheal pathologies, including tracheal stenosis, in the coming years.[4]

Surgical treatment is the first choice and although it is usually successful in most cases, the recurrence of the disease remains a major obstacle, which has motivated much of the research on the pathogenesis of tracheal stenosis. In this regard, several studies have demonstrated the role of inflammation signaling pathways and infectious processes in the development of laryngotracheal stenosis.[5] Thus, it has been seen in murine fibroblasts and human dermal fibroblasts that soluble dipeptidyl peptidase-4 (DPP-4 or CD26) activates NF-κB and SMAD signaling through PAR2, which leads to the activation of dermal fibroblasts, so it has been suggested that elevated levels of circulating soluble DPP-4 could function as one of the mediators that induce fibrosis in patients.

Currently, there is no universally validated classification that includes a specific therapeutic recommendation and an associated prognosis, however, an integrative classification of the main airway has been proposed that considers the cause of stenosis, magnitude of obstruction, involvement of the mucosa and wall, number of stenotic lesions, presence of fistulas, among other characteristics.[6] This work focuses on tracheal stenosis secondary to orotracheal intubation, which is the most frequent and responsible cause of 48 to 55% of cases.[7] It is often considered that the duration of intubation is the most important risk factor for the development of tracheal stenosis secondary to orotracheal intubation (TSSOI), in both adults and children;[4] although it has also been documented that tracheal stenosis is common even in patients intubated for short periods of time.[8] It is currently believed that aberrant scarring leads to the onset of TSSOI. The normal functional process of wound healing goes through four programmed phases: hemostasis, inflammation, proliferation, and epithelialization or remodeling. These phases are synchronized, temporally controlled, and involve a complex interaction between different cell types, cytokines, mediators, and the vasculature. Phases 1-3 typically last up to three weeks, while the remodeling phase lasts from weeks to years.[9]

Multiple mechanisms involved in the formation of TSSOI have been described, including: the TGF-β superfamily, mucosal trauma, ischemia, biomechanical stress, bacterial translocation, and fibrosis.[10] We particularly focus on the role of myofibroblasts and DPP-4. Current evidence demonstrates that fibroblasts undergo a shift towards myofibroblastic phenotype in response to hypoxia suffered by fibroblasts in TSSOI. This supports the role of hypoxia in the initial pathogenesis of TSSOI, leading to a transdifferentiation of resident fibroblasts into contractile and profibrotic myofibroblasts.[11]

The importance of the CD26 gene family in the regulation of critical biochemical pathways continues to be evident. The two most studied members of the family, CD26 and fibroblast activation protein (FAP), have been investigated as both disease therapeutic targets and diagnostic biomarkers. Their interest as potential biomarkers has been driven mainly by the observation of altered expression profiles in inflammatory diseases and cancer. In addition, the stability and persistence of these soluble proteins in serum make them an attractive proposition as serological markers.[12] The DPP-4 inhibitor linagliptin has been shown to abrogate the expression of fibrotic proteins (such as elastin and a-SMA), and prevent DPP-4-induced activation of transcription factor signaling pathways.[13]

The current management of patients with tracheal stenosis is surgical (tracheal resection with anastomosis),[14] provided that the clinical and anatomical conditions of the patient allow it. Otherwise, there are other alternatives such as laser resection or endoscopic dilation, placement

of stents, interposition grafts, Montgomery splints and, as a last option, tracheostomy.[7,15] Multiple medical interventions have been used with the aim of intervening in the inflammatory process inherent to tracheal stenosis, in order to decrease the rate of restenosis and thereby offer the best alternatives to the patient, these range from the use of non-steroidal anti-inflammatory drugs and steroids, mitomycin C, antibiotics, PPAR receptor agonists (such as lanifibranor), among other therapies that are under investigation.[7,16,17] So a better understanding of the mechanisms underlying the inflammatory and healing process in patients with TSSOI is a relevant field, due to its potential to allow the development of targeted anti-inflammatory therapies. CD26 shows promise in various organs and in various forms of acute and chronic fibrosis.[18] Inhibition of enzyme activity with diprotin A has been shown to result in decreased scarring, making DPP-4 an attractive molecule as a potential therapeutic target or biomarker in TSSOI.[19]

This research work aims to evaluate the relationship between serum levels and tissue expression of CD26 with TSSOI and its complications, as well as to describe the expression of myofibroblasts by immunofluorescence, with the present project being the first in the literature to seek to demonstrate both relationships. Thus, this research seeks to contribute to the understanding of the molecular mechanisms involved in the development of TSSOI.

MATERIAL AND METHODS

Case-control study 1:1. All post-operative tracheoplasty patients diagnosed with TSSOI were included in the National Institute of Respiratory Illness Ismael Cosío Villegas (INER). Non-probability sampling was performed for convenience. Information was collected from 22 cases and 22 controls. The recruitment period was from November 2021 to March 2023.

Table 1: Surgical variables of post-operative patients for tracheal stenosis secondary to orotracheal intubation. N = 22.

Qualitative variables	n (%)
Tracheostomy	
Yes	6 (27)
No	16 (73)
Type of anastomosis	
C-T	6 (27)
T-T	16 (73)
She/He presented complications	
Yes	2 (9)
No	20 (91)
Quantitative variables	**Median [p25-p75]**
Intubation days	14.5 [9-18]
Number of previous dilations	1 [1-2]
Number of tracheal rings resected	4 [3-5]
Bleeding, (mL)	100 [70-120]
Surgical time, (min)	190 [160-210]

C-T= crico-tracheal anastomosis. T-T= tracheo-tracheal anastomosis.

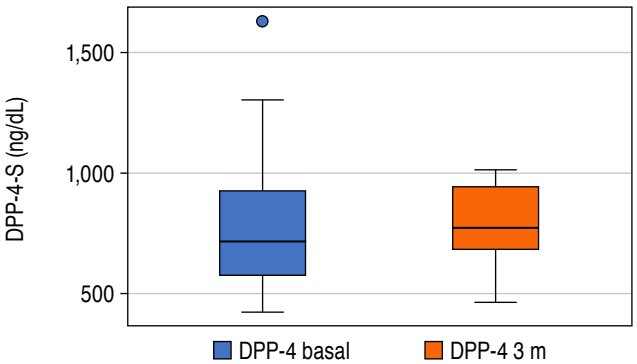

Figure 1: Pre- and post-surgical serum dipeptidyl peptidase-4 levels in cases (patients with tracheal stenosis secondary to orotracheal intubation).

The inclusion criteria for the cases were: patients diagnosed with TSSOI, over 18 years of age, tracheoplasty candidates who had signed informed consent and CRP for SARS-CoV-2 negative upon admission. For controls: INER resident doctors or people who come to the Blood Bank Area to donate blood components, over 18 years of age and who signed the informed consent. Exclusion criteria: patients diagnosed with kidney, autoimmune, liver disease or cancer in the five years prior to their surgical procedure, intake of antibiotics, steroids or anti-inflammatories up to seven days before the surgical procedure or use of DPP-4 inhibitors. Elimination criteria: loss at follow-up, alteration in initial inflammatory markers (CRP > 2 mg/dL, procalcitonin > 1 ng/mL) or patient request for withdrawal from the study.

The following clinical laboratory studies were performed: complete blood biometry, blood chemistry, lipid profile, glycosylated hemoglobin, C-reactive protein, and procalcitonin, as well as base line measurement of serum CD26 on the day of hospital admission. In the surgical procedure, a representative part of the resected surgical piece (stenotic tracheal rings) was taken for the qualitative determination of membrane CD26 staining and the determination of myofibroblast expression by immunofluorescence (FAP, SMA, vimentin and alpha smooth muscle actin). Complications were followed up in the first three months and control serum CD26 was determined between eight and 2 weeks after the surgical procedure.

Serum from patients was obtained in a tube without anticoagulant, centrifuged at 1.800 rpm for 15 minutes at 4 °C (Eppendorf 5810R); subsequently, they were

stored in polypropylene tubes at -20 °C. Quantification of plasma CD26 levels was performed by Enzyme-Linked Immunosorbent Assay (ELISA) MyBioSource 96-well MBS2882455 (San Diego; California, United States).

For the immunofluorescence protocol the tissue was cryoprotected with Tissue-Tek and stored at –80 °C. 8 µm thick sections were made on the cryostat, fixed with 4% paraformaldehyde and washed twice with PBS. Sections were washed with 1% blocking serum in PBS-T (PBS with 0.4% Triton X-100). Nonspecific binding was blocked by incubating tissue sections with 5% serum in PBS-T for 30 minutes at room temperature.

Primary antibody diluted in PBS-T from 1% animal serum (CD26 Recombinant Rabbit Monoclonal Antibody [JM11-42], 100 µL) was added. Invitrogen; anti-vimentin antibody [RV202] - Cytoskeleton Marker, 100 µg. Abcam; anti-alpha smooth muscle actin antibody [1A4], 100 µgand Abcam FAPA Polyclonal Antibody, 100 µL. Bioss antibodies). The recommended dilution of the antibody specified in the data sheet was used.

Sections were washed with 1% PBS-T serum, and was added secondary antibody diluted in 1% PBS-T serum (goat anti-Rabbit IgG [H+L] Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 55, 1 mg. Invitrogen and goat anti-Mouse IgG [H+L] Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488, 1 mg invitrogen), was incubated at room temperature for one to two hours, with the recommended dilution of the antibody specified in the data sheet. After application of all primary antibodies, DAPI DNA binding dyes were applied and control was performed in fibroblast culture. Evaluation was performed with a ZEISS Axio Vert A1 microscope.

Healthy controls over 18 years of age who signed informed consent were also included, blood samples were taken for serum CD26 measurement, as well as complementary laboratories for their study (blood biometry, blood chemistry, CRP, procalcitonin, lipid profile and glycosylated hemoglobin).

Table 2: Median difference of serum dipeptidyl peptidase-4 values in categorical surgical variables.

DPP-4-S (ng/dL)	Median [p25-p75]	p
Complications		0.6478
Yes	791 [558-1,023]	
No	708 [528-908]	
Tracheostomy		0.1845
Yes	569 [509-722]	
No	752 [537-980]	
Type of anastomosis		0.507
C-T	822 [538-937]	
T-T	663 [528-841]	

C-T= crico-tracheal anastomosis. T-T= tracheo-tracheal anastomosis.

Table 3: Correlation of serum dipeptidyl peptidase-4 values and quantitative surgical variables.

Variable	Correlation coefficient	p
Intubation days	-0.2154	0.3358
Days with tracheostomy	-0.3201	0.1464
Number of dilations	-0.0799	0.7236
Number of rings resected	-0.1595	0.4784
Bleeding	0.4893	**0.0208**
Surgical time	-0.061	0.7874

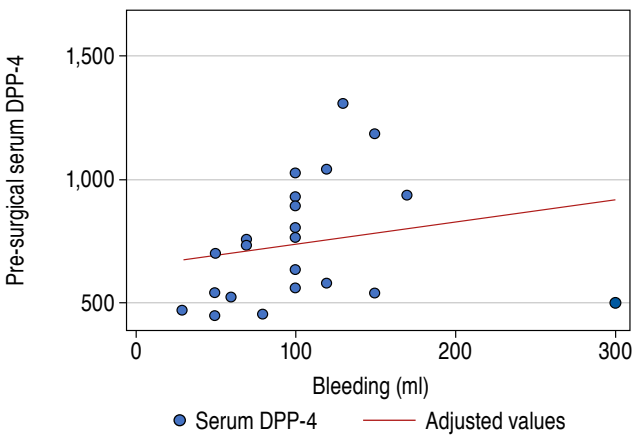

Figure 2: Serum dipeptidyl peptidase-4 levels and transoperative bleeding.

All data was collected in an Excel spreadsheet. The information collected was analyzed with the Stata® version14 program for Mac. Simple frequencies and percentages were calculated for qualitative variables and central tendency and dispersion measures were calculated for quantitative variables. The type of graph was chosen according to the nature of the variables according to whether they were quantitative or qualitative.

Statistical analysis: Differences in DPP-4 levels were analyzed according to the different surgical variables (surgical time, type of anastomosis, bleeding, complications, type of suture). A description of the characteristics of the controls was made and finally the comparison of the characteristics between the cases and the controls was made with the Fisher's exact test for the case of qualitative variables and with the Mann-Whitney or Kruskal Wallis U tests (according to the number of categories of the variable) for quantitative variables. Wilcoxon's sign range test for paired data was also performed, and whether changes in pre and post-surgical Serum DPP-4 levels were statistically significant was evaluated. Additionally, a logistic regression model was performed to evaluate whether Serum DPP-4

Table 4: Qualitative variables of controls divided by subgroup. N = 22.

Variable (category)	Residents (N = 11) n (%)	Non-residents (N = 11) n (%)	p
Gender			0.99
Female	2 (18)	1 (9)	
Male	9 (82)	10 (91)	
Eage			0.99
≤ 33	7 (64)	6 (55)	
> 33	4 (36)	5 (45)	
Weight, (BMI)			0.562
Normal	5 (46)	3 (27)	
Overweight	4 (36)	3 (27)	
Obese	2 (18)	5 (46)	
Comorbidities*			0.476
Yes	2 (18)	0 (0)	
No	9 (82)	11 (100)	
Tobacco use			0.99
Yes	1 (9)	0 (0)	
No	10 (91)	11 (100)	
Alcohol consumption			**0.04**
Yes	7 (64)	2 (18)	
No	4 (36)	9 (82)	
History of COVID-19			**0.002**
Yes	10 (91)	2 (18)	
No	1 (9)	9 (82)	

BMI = body mass index. COVID-19 = coronavirus disease 2019.
* They include: diabetes, systemic high blood pressure, drug addiction, thyroid disease, epilepsy, and asthma.

could be a predictor of tracheal stenosis and a sensitivity and specificity analysis of the test was performed.

The study was approved by the INER Ethics and Research Committee, with approval number C 14-22. Healthy patients and controls were asked to sign the informed consent letter. The study followed the rules of the General Health Law for Research and the Declaration of Helsinki.

RESULTS

A total of 22 cases and 22 controls were included in the study. *Table 1* shows the characteristics of the surgical procedure in patients with TSSOI surgical treatment. About 73% of patients underwent tracheo-tracheal anastomosis and most had no complications. As part of the detailed case analysis, differences in pre-surgical and post-surgical Serum DPP-4 levels were evaluated *(Figure 1)*; no statistically significant differences were found (p = 0.9738). Finally, we evaluated whether one or more surgical features were related to DPP-4 levels. This was performed on both categorical *(Table 2)* and continuous *(Table 3)* variables. The only variable that showed correlation with serum DPP-4levels was transoperative bleeding *(Figure 2)*.

Given the differences in recruitment, in the first part of this research work,19 in which INER resident physicians were recruited as controls (they were recruited from INER Blood Bank donors), a sub-analysis of the controls was performed, comparing residents and non-residentsin search of statistical differences that could point to a selection and information bias in the controls. When analyzing the primary dependent variable, non-significant differences in Serum DPP-4 levels were found between residents and

Table 5: Quantitative variables of the controls divided by subgroup. N = 22.

Variable	Residents (N = 11) Median [p25-p75]	Non residents (N = 11) Median [p25-p75]	p
Serum DPP-4 (ng/dL)	677 [577-700]	927 [771-1,179]	0.3981
Leukocytes (cels/µL)	6.4 [4.9-7.3]	6.8 [5.8-8.3]	0.0956
Linfocitos (cels/µL)	2.2 [1.6-2.8]	2.4 [1.9-2.7]	0.8233
PMN (cels/µL)	2.8 [2.3-4.4]	3.1 [3.7-4.5]	0.0527
Glucose (mg/dL)	93 [90-96]	81 [78-84]	0.1805
HbA1c (%)	5.3 [5.2-5.5]	5.5 [5.3-5.7]	0.9159
Total cholesterol (mg/dL)	183 [143-215]	161 [152-200]	0.4455
Triglycerides (mg/dL)	107 [86-144]	167 [76-211]	**0.05**
Atherogenic index	3.7 [2.5-4.2]	3.5 [2.9-4.9]	0.2358
CRP (mg/L)	0.11 [0.04-0.2]	0.17 [0.1-0.2]	**0.0001**
Procalcitonin (ng/mL)	0.02 [0.01-0.03]	0.02 [0.01-0.02]	0.344

Serum DPP-4 = serum dipeptidyl peptidase-4. PMN = polymorphonuclear. CRP = C-reactive protein.

Table 6: Quantitative variables of cases versus controls. N = 22.

Variables	Cases median [p25-p75]	Controls median [p25-p75]	p
Serum DPP-4	708 [536-922]	503 [10-679]	0.3981
Leukocytes	6.9 [6.3-8.5]	6.5 [5.4-7.3]	0.0956
PMN	4.3 [3.7-5.1]	3.6 [2.7-4.4]	0.0527
Glucose	93 [82-104]	87 [81-96]	0.1805
HbA1c	5.4 [5.2-5.8]	5.4 [5.2-5.6]	0.9159
Total cholesterol	166 [138-190]	170 [146-200]	0.4455
Triglycerides	187 [124-212]	121 [78-181]	**0.05**
Atherogenic index	4.2 [3.1-5]	3.7 [2.7-4.6]	0.2358
CRP	0.35 [0.3-0.4]	0.115 [0.1-0.2]	**0.0001**
Procalcitonin	0.02 [0.01-0.03]	0.02 [0.01-0.02]	0.344

Serum DPP-4 = serum dipeptidyl peptidase-4. PMN = polymorphonuclear. CRP = C-reactive protein.

non-residents. The comparison of the categorical variables is shown in *Tables 4 and 5*.

In the statistical analysis of cases versus controls, a higher frequency of comorbidities was observed among patients post-operated by TSSOI, who also showed statistically higher levels of triglycerides and C-reactive protein *(Table 6)*, which corroborated the absence of active inflammatory processes in the controls. In contrast, no statistical differences in serum DPP-4 levels were observed between the groups *(Figure 3)*.

A univariate logistic regression model was performed to evaluate whether Serum DPP-4 could be a predictor of tracheal stenosis, where an OR very close to 1 is observed, and a pseudo R2 of just 1.4%, implying that there is virtually no difference in Serum DPP-4 levels between cases and controls, and that the likely contribution of Serum DPP-4 as a predictor of stenosis is less than 2%.

The detection of FAP-α, CD26, α-SMA and vimentin was performed in trachea tissue, as well as its control. Phase contrast photographs (PH-C) were taken of the markings of each and the images of both markings were spliced (MERGE). Subsequently, the area positive to DAPI, FAP-α *(Figure 4A)*, CD26 *(Figure 4B)*, α-SMA and vimentin labels was quantified. An increase in the area percentage of markers CD26 and FAP-α was observed, suggesting architecture formation of the fibrotic microenvironment in the tissue. The increase in the area percentage of α-SMA suggests the presence of myofibroblasts in the tissue, cells associated with fibrotic tissue formation.

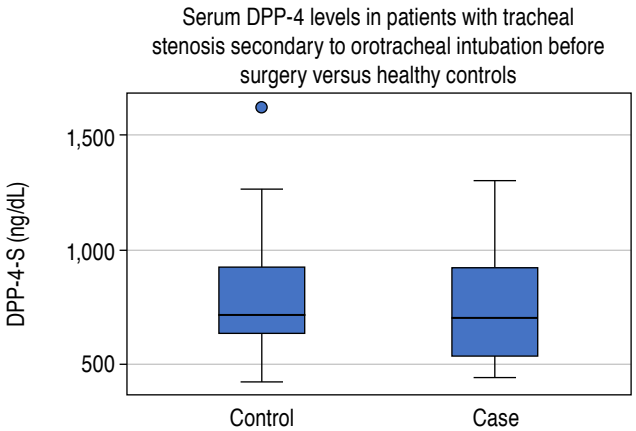

Figure 3: Dipeptidyl peptidase-4 levels in cases (N = 22) and controls (N = 22).

DISCUSSION

The levels of Serum DPP-4 did not show adequate sensitivity and specificity to discriminate the disease, although CD26 tissue expression was found to be increased in the cases with respect to the control tissue.

In the analysis of the cases, no statistically significant differences were found between pre and post-surgical levels, a result that contrasts with what was identified in the first part of this study,[19] which can be explained by the significantly lower levels of DPP-4 in the controls included in said work. However, we must consider that the action of DPP-4 is complex and involves multiple signaling pathways and mechanisms that are still poorly understood,[13] while the effects of Serum DPP-4 could be independent of its enzymatic activity, which also plays an important role in inflammation.[20] In addition, tracheal stenosis is a pathology of very heterogeneous etiology,[21] in which the role of multiple pathophysiogenic mechanisms has been described.[22] It has been seen, for example, the clinical impact can vary depending on the type of stenosis,[23] for

which we cannot affirm that the simple values of Serum DPP-4 are the only marker of its activity, and we do not rule out its potential usefulness in other contexts of inflammation and scarring.

Not surprisingly, the positive correlation found between Serum DPP-4 levels and transoperative bleeding *(Figure 2)*, given the high frequency of DPP-4 expression in the endothelium and its recognized role in hemostasis.[11] In the case of the airways, Johnson et al.[24] found that several monoclonal antibodies that recognize CD26/Serum DPP-4 intensely stained the endothelium of the pulmonary capillaries, but not those of other types of large-caliber blood vessels,[20] leading us to think that the airway could have particularities that make it especially susceptible to

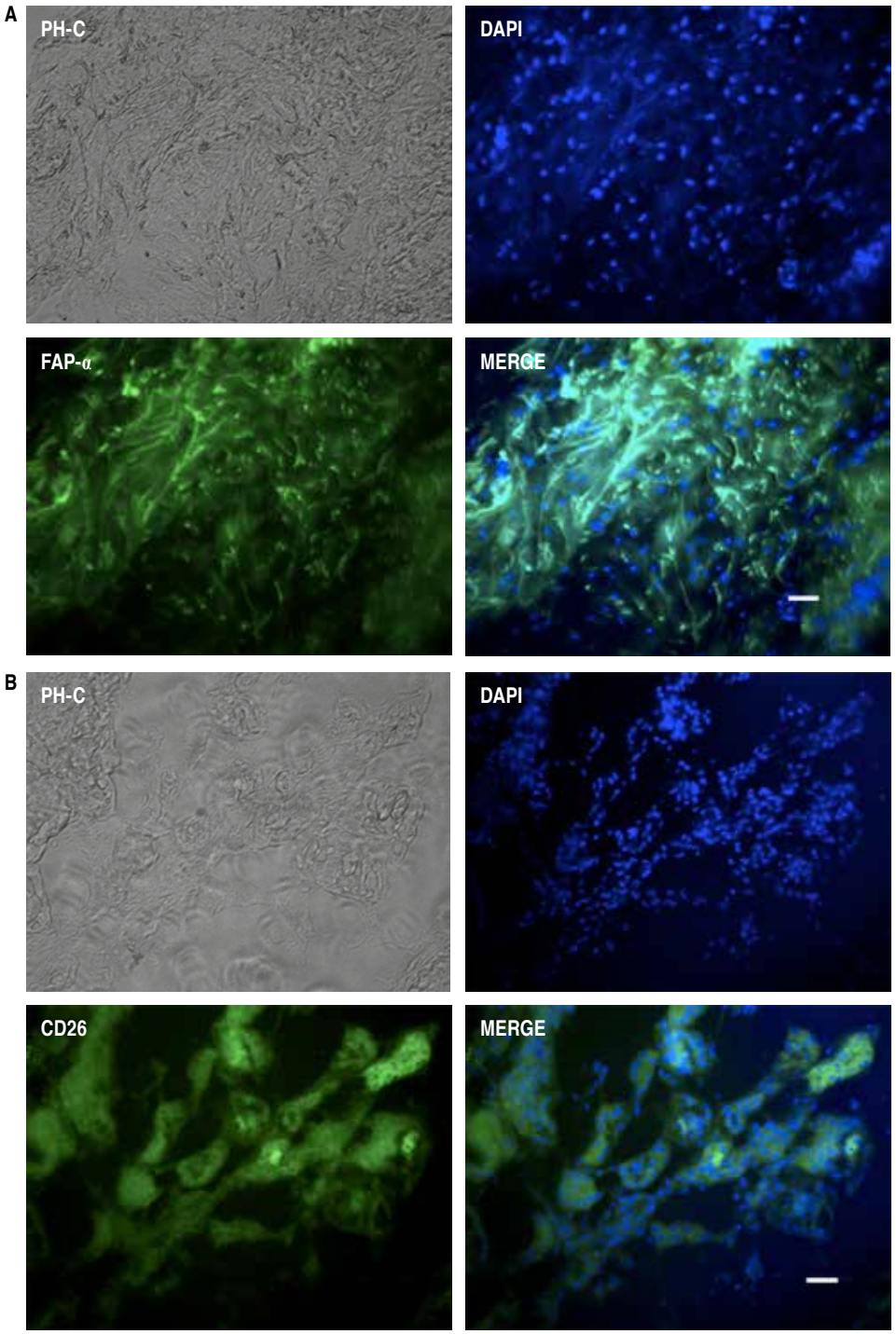

Figure 4:

A) Detection of FAP-α in tracheal tissue. Scale bar 20 μm. **B)** Detection of CD26 in tracheal tissue. Scale bar 20 μm.

altered healing processes, as has been hypothesized in the etiology of trachealstenosis.[18]

Intra-control analysis revealed differences in Serum DPP-4 levels between residents and non-residents. Likewise, when comparing other characteristics between both subgroups, residents had higher blood glucose and higher frequency of alcohol consumption and a history of COVID-19 (all with $p < 0.05$). To explore these results, we performed complementary exploratory linear regression models, in which an inverse relationship was observed between the history of COVID-19 and Serum DPP-4 ($p < 0.001$), while the significance of blood glucose and alcohol consumption was lost when controlling for other variables.

Thus, it is evident that there could be a relationship between SARS-CoV-2 infection and Serum DPP-4 activity. Although this relationship has not been fully elucidated, in one study it was observed that in patients infected with MERS-CoV, the concentration of Serum DPP-4 in plasma decreased significantly and correlated with the severity of the disease, while in 2020 a similar result was reported in patients with severe COVID-19.[25]

The results of this analysis showed no significant differences in sociodemographic and clinical characteristics between cases and controls *(Table 6)*. There was also no difference in the primary end point, Serum DPP-4 levels. However, there was a difference in CD26 expression in tracheal tissue. This could be due to several factors, including the half-life of Serum DPP-4,[20] the stage of tracheal stenosis in which it could have the greatest impact (acute versus chronic phase), or the time when its levels were measured in the cases, which was done prior to surgery, a time that does not necessarily correspond to the stages of stenosis formation.

This study does have its limitations. First, given that most of the patients referred for surgery came from other institutions, the interval between the onset of the pathology and the performance of the surgery is unknown, which has implications for the potential usefulness of the detection of DPP-4 in the initial stages of the pathogenesis of TSSOI. Finally, the immunofluorescence results are descriptive, so the relationship between serum and tissue levels of DPP-4 could not be statistically evaluated.

The investigation of other molecules as possible diagnostic, prognostic or therapeutic targets, especially in humans, is extremely valuable for the progress of scientific knowledge in this field, even if there are no significant differences since, as we know, «the lack of evidence is also evidence» and serves as a guide for future research, increasing the potential for the development of specific immunological, genomic and proteomic therapies for the treatment of inflammatory/fibrotic conditions, including TSSOI.[10,26]

CONCLUSIONS

Under the conditions of this study, Serum DPP-4 levels did not adequately discriminate cases of TSSOI, although its expression was increased in tracheal tissue, so it is not ruled out that it may serve as a diagnostic marker or therapeutic target in early stages of TSSOI or prior to its formation, for example, at times close to intubation.

This article is one of the few studies in humans where serum and tissue levels of CD26 are evaluated, as well as the description of the transdifferentiation of fibroblasts to myofibroblasts in the diseased tissue of patients with ETSIO, serving as a precedent and making an invaluable contribution to the study of its pathophysiology, which opens the way to future research in the search for biomarkers in tracheal pathology.

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