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Immunohistochemical analysis of tissue factor expression in gastric carcinoma: correlations with prognosis and survival.

Análise da expressão imuno-histoquímica do fator tecidual no carcinoma gástrico: correlações com prognóstico e sobrevida.

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A B S T R A C T

Objective: to study the expression of the tissue factor (TF) and its correlation with prognosis and survival in patients with gastric carcinoma. **Methods:** we measured the immunohistochemical expression of TF in 50 specimens of gastric adenocarcinomas from patients submitted to curative surgery. We then compared the intensity of its expression with clinical and pathological data, TNM staging, prognostic factors and survival. **Results:** all tumors displayed TF expression; the intensity of TF expression was not associated with TNM stage, clinical or pathological variables or general survival. **Conclusion:** TF has a high expression in gastric carcinoma, but that it is not useful as a prognostic marker.

Keywords: Thromboplastin. Immunohistochemistry. Stomach Neoplasms. Prognosis.

INTRODUCTION

Gastric cancer is the most frequent malignant neoplasm of the gastrointestinal tract. It is the third leading cause of death among all cancers[1]. Although its incidence has been recently reduced in developed countries, it is still a heavy burden for underdeveloped countries in Latin America, and a major issue in some countries in Asia, such as Japan[2]. The mainstay of treatment is still an aggressive surgical approach, which is not devoid of risks and complications. In spite of controversies, adjuvant treatments with chemotherapy and radiation have been increasingly employed[3]. The discovery of novel biomarkers in this disease may lead to developments in therapeutic strategies for these tumors.

In 1865, Armand Trousseau described for the first time the association between cancer, coagulation and thrombosis, in a cohort of patients with gastrointestinal tumors[4]. Apparently, one of the most important agents responsible for these associations is the Tissue Factor (TF)[5], TF is a transmembrane glycoprotein that plays a pivotal role in the extrinsic coagulation pathway by interacting with Factor VII. TF is expressed in fibroblasts in the adventicea of blood vessels, solid organ capsules, epithelial cells in the skin and mucosas, cells of the endometrial stroma, and astrocytes from the central nervous system[6], supposedly acting as a natural haemostatic barrier[7]. When exposed, TF initiates the coagulation cascade, resulting in the production of fibrin[8,9]. An increased expression of TF was also described in a variety of solid malignant neoplasms, including melanoma, breast, prostate and lungs. In some studies, an increased TF expression was also correlated with a worse clinical prognosis[10-13].

Two distinct groups reported the immunohistochemical expression of TF in gastric carcinoma. A Japanese study with 207 patients has shown that an increased expression of TF was associated with a worse prognosis, whereas an Australian study with 160 patients did not reproduce such findings[14,15].

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Our aim was to describe the immunohistochemical expression of TF in neoplastic tissues obtained from patients with gastric adenocarcinomas treated with curative intent in South America. We also wanted to correlate TF expression with clinical and pathologic variables, and with overall survival.

METHODS

Our patients sample is a prospective cohort of patients with gastric adenocarcinoma treated at a university hospital in Southern Brazil who underwent gastric resections at our Institution from 2000 to 2003. Patients were followed until death of for a minimum period of 24 months after surgery.

Exclusion criteria were incomplete resection of the tumor, less than 15 lymph nodes identified at the pathologic report, inadequate pathology specimen, history of previous radiation therapy or chemotherapy, and history of previous diagnosis of cancer.

We compared TF expression with the variables age, gender, location, size and aspect of the tumor in the stomach, tumor stage, tumor grade, Lauren's classification, and follow-up time (defined as the time interval between the date of surgery and the last follow-up visit).

Immunohistochemistry

Formalin fixed, paraffin embedded tumor blocks were cut in 3µm sections. Antigen retrieval was performed with Tris/EDTA (20mM Tris/0.65mM EDTA) at a pH of 9.0 and with tap water at 99°C. Endogenous peroxidase blockage was performed with a 3% peridrol solution (H_2O_2 in methylic acid) for 30 minutes, and slides were washed with 5% saline in PBS to reduce background. Slides were then incubated with anti-Tissue Factor mice anti-human type 1 antibody in 1:30 dilution and with anti-CD34 mice monoclonal antibody in a dilution of 1:400. We used the detection system Kit Dako LSAB + peroxidase. Slides were treated with the chromogen 3,3'-diaminoazobenzidine (DAB) and with PBS buffer in a solution of 0.002% hydrogen peroxidase, counterstained with hematoxylin, dehydrated, clarified and mounted.

Tissue Factor Expression

The quantification of TF expression was performed according to the reactivity to the antibody, with an objective lens with 100X magnification. We classified TF expression into four categories: 1 (0% to 25% of cancer cells stained), 2 (26% to 50% of cancer cells stained), 3 (51% to 75% of cancer cells stained) or 4 (76% to 100% of cancer cells stained) (Figure 1).

Statistical Analysis and Ethics

We described quantitative data through means and standard deviations and qualitative data, as percentages. We performed comparisons between quantitative variables using the Student's t test. For qualitative data, we used the Chi-square (x^2) test or Fisher's exact test for the comparisons. We carried out the survival analyses with the Kaplan-Meier method, and applied the log-rank test for the comparison between the curves. We created a multivariable model using variables that were clinically relevant (Cox regression model). The study protocol was revised and approved by the Ethics and Research Committee of our Institution (protocol 345/06).

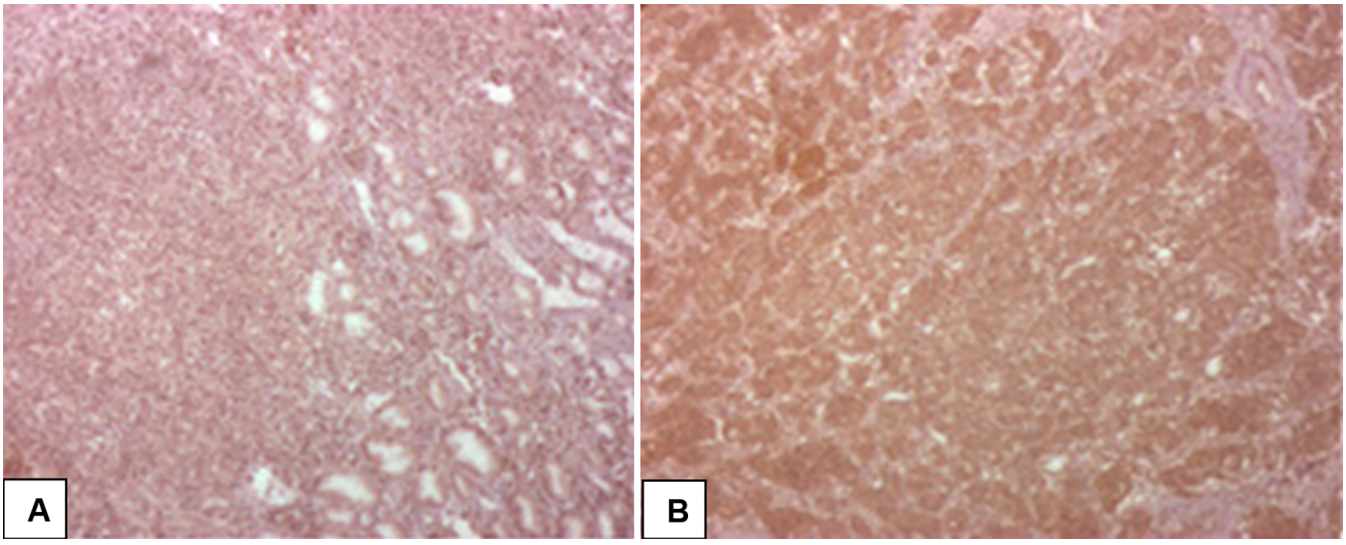

Figure 1. *A) Gastric adenocarcinoma with less than 25% of cells stained, category 1; B) Gastric adenocarcinoma with practically all cells stained, category 4; (50X magnification).*

RESULTS

In three years, we performed 101 gastric cancer resections at the Department of Surgery of our university hospital. Of these, we included 50 (49.5%) in the study. The main causes for exclusion were <15 lymph nodes identified (in 32 patients) and residual disease (in 16 patients). In three patients, tissue blocks were unavailable. Of the 50 remaining patients, we excluded four of the follow-up analyses due to postoperative deaths during the first admission (early postoperative mortality), being included only in the analyses of prevalence.

Patients mean age was 62.9 years (SD=11.3). Overall, 38 patients were male, and 12 were female. Tumors were located in the middle third of the stomach in 18 cases, in the proximal third in 14 cases, and in the distal third in 13 cases. Mean tumor size was 6.52cm (±4.14), the smallest measuring 0.7cm and the largest measuring 20cm in its greatest diameters. Macroscopically, the most common form of presentation was the ulcerative-infiltrative (Borrmann classification). Concerning tumor differentiation, the most common tumors were moderately differentiated (G2), representing 46% of our sample.

Most patients (96%) presented with tumors infiltrating the muscle layer, characterized as advanced, and in 39 cases (78%) the tumors invaded the serous layer or adjacent organs. In 33 cases (66%) there was at least one metastatic lymph node. Two patients (4%) had distant metastases at the time of surgery, one in the left lobe of the liver and the other a single implant in the small bowel; both lesions were resected along with the gastric lesions.

The patients' main clinical and pathological characteristics are shown in table 1.

Most tumor cells demonstrated intense reactivity for Tissue Factor, and 100% of tumors expressed the protein. In the quantification of TF expression, 39 cases (78%) showed an intense expression (76-100% of cancer cells stained) (Table 2). Due to the small number of cases with a lesser intensity of expression, we joined groups 1, 2 and 3 into a new category (0-75% of positively stained cancer cells). Therefore, for the purpose of statistical analyses, we categorized patients into two groups: low expression (scores 1 to 3) and high expression (score 4).

We compared TF expression with known prognostic factors in gastric cancer such as tumor size, Borrmann and Lauren classifications, invasion of the gastric wall, the presence of positive lymph nodes, pathologic staging and tumor differentiation. There was no statistical correlation between TF expression and any of these prognostic factors (Table 3).

Table 1. *Patients characteristics (N=50).*

Age at admission in years: mean [±SD]	62.9 [±11.3]
Gender [male:female]	3.1:1
Tumor size in cm: mean [±SD]	6.5 [±4.1]
Location in stomach	
Upper	14 (28%)
Middle	18 (36%)
Lower	13 (26%)
Entire	3 (6%)
Gastric stump	2 (4%)
Borrmann type	
0	2 (4%)
1	0 (0%)
2	15 (30%)
3	19 (38%)
4	14 (28%)
Lauren subtype	
Intestinal	20 (40%)
Diffuse	21 (42%)
Mixed	9 (18%)
Tumor differentiation	
G1	1 (2%)
G2	23 (46%)
G3	17 (34%)
G4	9 (18%)
TNM staging	
I	10 (20%)
II	5 (10%)
III	17 (34%)
IV	18 (36%)

Table 2. *Distribution of Tissue Factor expression in our sample.*

TF expression.	Specimens
1 (0-25%)	2 (4)
2 (26-50%)	5 (10)
3 (51-75%)	4 (8)
4 (76-100%)	39 (78)

Table 3. *Correlation between TF expression and prognostic factors.*

Variable	Low TF expression (N=11)	High TF expression (N=39)	p
Age, years	64.8±8.5	62.4±12	0.54
Tumor size (cm)	6.8±3.2	6.4±4.5	0.78
Borrmann IV	3 (27.3)	11 (29.7)	0.99
Diffuse Lauren	8 (72.7)	13 (33.3)	0.47
T3-T4	10 (90.9)	29 (74.4)	0.45
Positive lymph nodes	10 (90.9)	23 (59.0)	0.11
G3-G4	8 (72.7)	18 (46.2)	0.22
Stage III-IV	10 (90.9)	25 (64.1)	0.18

Mean follow-up was 28.9 months (range one 1 to 60 months). Only 19 (38%) patients were alive at the time of analysis. Of the 31 deaths, four (8%) were due to postoperative complications, and the remaining due to disease progression. In 22 patients, it was possible to determine the main recurrence site: diffuse peritoneal carcinomatosis in 12, isolated peritoneal recurrence in three, liver in three, anastomotic site in two, lung in one patient and spleen in one. Disease stage influenced overall survival, as represented in figure 2.

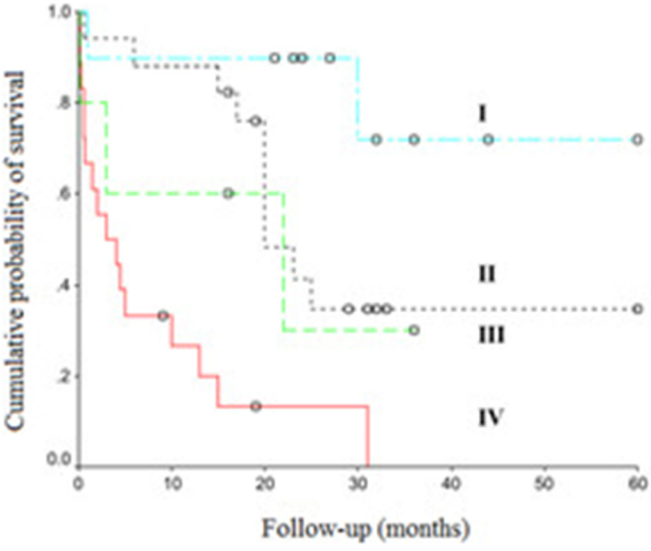

Figure 2. *Overall survival by stage.*

Patients with an increased expression of TF had a mean survival of 33.6 months (95%CI=25.3-41.9; SE=4.2), whereas those with low expression had a mean survival of 9.74 months (95%CI=4.51-14.97; SE=2.6). This result was statistically significant (p=0.0017). Figure 3 depicts the survival curves of patients with low and increased TF expression.

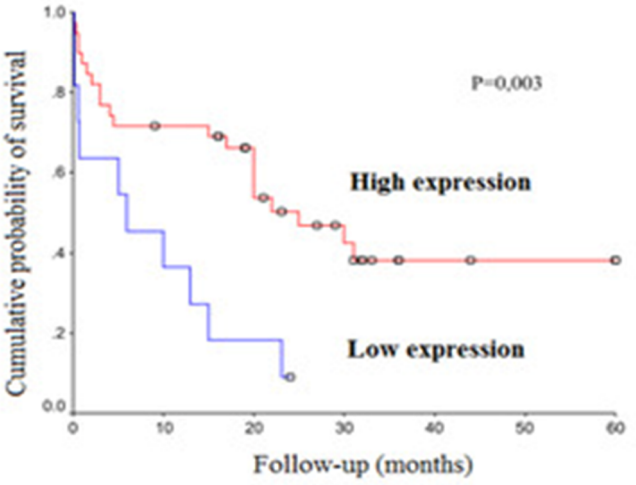

Figure 3. *Survival curves for patients with high intensity (>75% of cancer cells) and low intensity (<75% of cancer cells) TF immunohistochemical expression.*

As TF expression was a statistically significant factor influencing overall survival, we performed a multivariate regression analysis adjusted for the most well known prognostic factors in gastric

cancer. In this analysis, TF expression was not an independent prognostic factor when adjusting for age, tumor stage, grade, and Lauren and Borrmann classifications (HR 0.58; 95%CI=0.21-1.56; p=0.28).

DISCUSSION

Surgical resection is the most effective treatment for gastric adenocarcinoma. However, the aggressiveness of this neoplasm and its propensity for local, nodal and systemic progression makes early diagnosis difficult, and a potentially curative surgery is not an option for roughly half of the patients at presenation[3]. The poor prognosis of the disease and the elevated levels of recurrence and deaths with conventional treatment justify the search for novel therapeutic targets.

Most of our patients were male and older than 60 years, which is accordance with the literature. In spite of the recent decline in incidence of gastric cancers in developed countries, it remains one of the main causes of morbidity and mortality in Brazil, according to data published by the Brazilian National Cancer Institute (INCA)[16].

Differently from American data, in which most tumors were proximal[17], in our study most tumors occurred in the distal third of the organ. The localization of the lesion is important, since it will define the type and extension of the resection, the extent of the lymphadenectomy and the type of reconstruction, all factors that may influence the procedure's morbidity and the mortality[18].

The most important prognostic factor at the time of the diagnosis in gastric cancer is tumor stage[3]. Unfortunately, most gastric cancers in our series were advanced at the time of diagnosis. In only two patients the tumors were classified as early. In the Brazilian literature, in more than half of the cases there were distant metastases at diagnosis[19]. The percentage of metastatic cases at diagnosis is also high in the international literature[17]. In our series, most patients were classified as stage III or IV, which may explain the increased postoperative mortality (8%) observed. In the published literature, the main determinants of postoperative mortality are the presence of concomitant disease, the presence of lymph node metastases, tumor size, surgical experience and patient's age. Patients with stages III or IV tumors have a five times greater mortality than patients with stages I or II tumors[20].

The extension of the tumors across the gastric wall and the involvement of lymph nodes are the main factors that will define TNM staging in gastric carcinomas[21]. In figure 2, we confirm that survival is directly related to staging in our sample. Cure is often possible for patients diagnosed with tumors at early stages. As the incidence of gastric carcinoma in Brazil does not justify screening the whole population through radiographic or endoscopic exams, health policies that help identify individuals at increased risk for these neoplasms are necessary.

Other important factor of poor prognosis in gastric cancer is the Borrmann classification, which evaluates the penetration across the gastric wall. The fact that 66% of our patients were classified as having tumors Borrmann III or IV may explain their poor survival rates. Lauren's classification stratifying tumors into intestinal and diffuse types is often used clinically, since it is simple and reproducible. Epidemiologically, the intestinal type is more common in areas where the risk of developing gastric carcinoma is higher, whereas

the diffuse type has a uniform distribution across areas of high and low risk of gastric cancers. It is speculated that the intestinal and diffuse types of gastric cancers are really two distinct diseases, with peculiar characteristics and specific risk factors[22]. In Brazil, a study by the National Cancer Institute (INCA) suggested a decrease in the incidence of intestinal type tumors in Rio[23], whereas another study with more than 600 patients in São Paulo has shown that the intestinal type is present in more than 60% of cases[24]. In our study, the small percentage of patients with intestinal type tumors (40%) contrasts with the national literature from Brazil, in which the intestinal type is usually the predominant cancer.

The expression of TF and its value as a prognostic factor in cancer has been studied in a variety of tumors, but not in gastric cancer. In other tumor types, an increased TF expression is usually associated with greater tumor aggressiveness and with worse prognosis[25]. The relationship between cancer and coagulation is complex and implies that the TF produced by malignant neoplasms may be involved with a system of intracellular signalization that acts independently from the coagulation cascade in promoting tumor development[26]. In our study, there was immunohistochemical expression of TF in the gastric cancer cells of all 50 patients. This degree of expression suggests that it may be a useful therapeutic target in the management of this disease. Although in most cases TF expression was high, for the purpose of analyses we have divided patients into groups of high and low TF expression to try to correlate the findings with known prognostic factors and with survival.

The analyses of our results were surprising in many ways. As shown in table 3, although statistical significance was not reached, most known factors of poor prognosis in gastric cancer were more associated with a low expression of TF, except Borrmann's classification. This finding may explain the results of the survival analysis shown in figure 3, in which we describe a significantly worse survival for patients with a low TF expression compared with that of patients with an increased TF expression. In multivariate analyses, however, adjusting for other prognostic factors, the degree of TF expression did not independently predict prognosis.

TF was present in tissues from all gastric cancers in our cohort. Although the degree of expression did not correlate with other prognostic factors or with survival, we believe that it may be a useful target for anticancer therapies in the future.

RESUMO

Objetivo: *estudar a expressão do fator tecidual (FT) e sua correlação com o prognóstico e sobrevida em pacientes com carcinoma gástrico.* **Métodos:** *verificamos a expressão imuno-histoquímica do FT em 50 espécimes de adenocarcinomas gástricos de pacientes submetidos a tratamento cirúrgico com intenção curativa. A intensidade da sua expressão foi comparada com dados clínicos e patológicos, estadiamento TNM, fatores prognósticos e sobrevida.* **Resultados:** *houve expressão do FT em todos os tumores; a intensidade de expressão do FT não foi associada com estágio TNM, variáveis clínicas ou patológicas ou sobrevida geral.* **Conclusão:** *este estudo mostra que o FT tem uma expressão elevada em carcinoma gástrico, mas que este não é útil como marcador de prognóstico.*

Descritores: *Tromboplastina. Imuno-Histoquímica. Neoplasias Gástricas. Prognóstico.*

REFERENCES

1. World Health Organization, I.A.f.R.o.C [Internet]. Estimated number of deaths, both sexes, worldwide (top 10 cancer sites) in 2012. Available from: https://gco.iarc.fr/today/online=-analysis-multi-bars?mode-cancer&mode_population=continents&population=900&sex=0&cancer=29&type=1&statistic=0&prevalence=0&color_palette=default
2. Park JY, von Karsa L, Herrero R. Prevention strategies for gastric cancer: a global perspective. Clin Endosc. 2014;47(6):478-89.
3. The Society for Surgery of the Alimentary Tract [Internet]. Patient Care Guidelines. Surgical Treatment of Gastric Cancer. Beverly (MA): SSAT. c2004 [cited 2017]. Available from: http://www.ssat.com/cgi-bin/guidelines_SurgicalTreatmentGastricCancer_EN.cgi
4. Anand M, Brat DJ. Oncogenic regulation of tissue factor and thrombosis in cancer. Thromb Res. 2012;129 Suppl 1:S46-9.
5. Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. J Clin Oncol. 2009;27(29):4834-8.
6. Carmeliet P, Collen D. Molecules in focus: tissue factor. Int J Biochem Cell Biol. 1998;30(6):661-7.
7. Kocatürk B, Versteeg HH. Tissue factor isoforms in cancer and coagulation: may the best isoform win. Thromb Res. 2012;129 Suppl 1:S69-75.
8. Higashi S, Iwanaga S. Molecular interaction between factor VII and tissue factor. Int J Hematol. 1998;67(3):229-41.
9. Osterud B. Tissue factor: a complex biological role. Thromb Haemost. 1997;78(1):755-8.
10. Mueller BM, Reisfeld RA, Edgington TS, Ruf W. Expression of tissue factor by melanoma cells promotes efficient hematogenous metastasis. Proc Natl Acad Sci U S A. 1992;89(24):11832-6.
11. Ueno T, Toi M, Koike M, Nakamura S, Tominaga T. Tissue factor expression in breast cancer tissues: its correlation with prognosis and plasma concentration. Br J Cancer. 2000;83(2):164-70.
12. Abdulkadir SA, Carvalhal GF, Kaleem Z, Kisiel W, Humphrey PA, Catalona WJ, et al. Tissue factor expression and angiogenesis in human prostate carcinoma. Hum Pathol. 2000;31(4):443-7.
13. Sawada M, Miyake S, Ohdama S, Matsubara O, Masuda S, Yakumaru K, et al. Expression of tissue factor in non-small-cell lung cancers and its relationship to metastasis. Br J Cancer. 1999;79(3-4):472-7.
14. Yamashita H, Kitayama J, Ishikawa M, Nagawa H. Tissue factor expression is a clinical indicator of lymphatic metastasis and poor prognosis in gastric cancer with intestinal phenotype. J Surg Oncol. 2007;95(4):324-31.
15. Lo L, Valentine H, Harrison J, Hayes S, Welch I, Pritchard S, et al. Tissue factor expression in the metaplasia-adenoma-carcinoma sequence of gastric cancer in a European population. Br J Cancer. 2012;107(7):1125-30.
16. Instituto Nacional de Câncer José Alencar Gomes da Silva. Coordenação de Prevenção e Vigilância. Estimativa 2014: Incidência de câncer no Brasil. PDF file. Rio de Janeiro: INCA; 2014. Available from: http://www.inca.gov.br/bvscontrolecancer/publicacoes/Estimativa_2014.pdf
17. Carneiro F. Stomach cancer. In: Steward BW, Wild CP, editors. World Cancer Report 2014. PDF file. Lyon: International Agency for Research on Cancer; 2014. p. 383-391. Available from: https://inovelthng.files.wordpress.com/2016/11/world-cancer-report.pdf
18. Kunisaki C, Makino H, Takagawa R, Oshima T, Nagano Y, Kosaka T, et al. Tumor diameter as a prognostic factor in patients with gastric cancer. Ann Surg Oncol. 2008;15(7):1959-67.
19. Lourenço LG, Hamada GS. Gastric cancer in Brazil. Gastric Cancer. 2001;4(2):103-5.
20. Toneto MG, Moreira LF, Jeckel Neto E, Souza HP. Gastrectomia em pacientes idosos: análise dos fatores relacionados a complicações e mortalidade. Rev Col Bras Cir. 2004;31(6):373-9.
21. Chae S, Lee A, Lee JH. The effectiveness of the new (7th) UICC N classification in the prognosis evaluation of gastric cancer patients: a comparative study between the 5th/6th and 7th UICC N classification. Gastric Cancer. 2011;14(2):166-71.
22. Howson CP, Hiyama T, Wynder EL. The decline of gastric cancer: epidemiology of an unplanned triumph. Epidemiol Rev. 1986;8:1-27.

23. Abib AR, Oliveira IM, Koifman S. Histopatologia do câncer de estômago (classificação de Lauren) em amostra de pacientes hospitalares no Rio de Janeiro, 1980-1995. Cad Saúde Pública. 1997;13(Supl 1):S99-S104.
24. Marigo C, Okuyama MH, Santo GC. Tipos histológicos e mortalidade por câncer gástrico em São Paulo. Cad Saúde Pública.1997;13(Supl1):S93-7.
25. Kakkar AK, Chinswangwatanakul V, Tebbutt S, Lemoine NR, Williamson RC. A characterization of the coagulant and fibrinolitic profile of human pancreatic carcinoma cells. Haemostasis. 1998;28(1):1-6.
26. Shoji M, Hancock WW, Abe K, Micko C, Casper KA, Baine RM, et al. Activation of coagulation and angiogenesis in cancer: immunohistochemical localization in situ of clotting proteins and vascular endothelial growth factor in human cancer. Am J Pathol. 1998;152(2):399-411.

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