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Original Article

PROGNOSTIC SIGNIFICANCE OF OCCULT LYMPH NODE MICROMETASTASIS IN GASTRIC CANCER: A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY BASED ON 1997 UICC TNM AND 1998 JGCA CLASSIFICATIONS

Significância prognóstica das micrometástases ocultas em linfonodos no câncer gástrico: estudo histoquímico e imunoistoquímico baseado nas classificações UICC TNM de 1997 e JCGCA de 1998

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Dell'Aquila Jr. NF, Lopasso FP, Falzoni R, Iriya K, Gama-Rodrigues J. Prognostic significance of occult lymph node micrometastasis in gastric cancer: a histochemical and immunohistochemical study based on 1997 UICC TNM and 1998 JGCA classifications. ABCD Arq Bras Cir Dig 2008;21(4): 164-9

ABSTRACT – ***Background*** - Micrometastasis is a single or a cluster of malignant cells inside the lymph node that are not detected by routine histopathological sections. Micrometastasis is related to poorer prognosis in many gastric cancer studies the real significance of these cells is still controversial. ***Aim*** – To evaluate if lymph node micrometastasis is a significant independent prognostic factor and important risk factor for recurrence in gastric cancer. ***Methods*** - A total of 1290 lymph nodes from 28 patients with gastric cancer, since 1998 until 2003, treated by radical resection (D2 and modified D3 lymphadenectomies) were studied. Three sections per lymph node were stained by Hematoxilin-Eosin, histochemical (AB-PAS) and immunohistochemical (AE1-AE3) techniques. Kaplan-Meier's survival curves and Log-rank/Cox tests were used in order to compares lymph node micrometastasis positivity, depth (pT) and location of tumor in gastric wall, histologic type, lymphatic, vascular and perineural invasion, lymph node status (pN) and stage. ***Results*** - There were worse prognosis and recurrence in patients with positive lymph node micrometastasis related to vascular and perineural invasions, advanced lymph node status and advanced stages. ***Conclusion*** - Lymph node micrometastasis seems to be a significant independent prognostic factor and important risk factor for recurrence in gastric cancer, in a context of radical D2 lymphadenectomy

HEADINGS - Stomach neoplasms. Clinical-dynamic prognosis. Classification.

INTRODUCTION

Prognosis of patients with gastric cancer is influenced by the presence of metastasis[2,12,13,24], lymph nodal status has been correlated as the most important individual prognostic factor[20]. There wereearly gastric cancer patients, treated radically, who died of recurrence[19]. There also are reports that D2 lymphadenectomy improves survival even in pN0 patients[19]. These may be due at least in part to the presence of lymph node micrometastasis at the time of resection. Although it is not consensual, micrometastasis can be defined as one single cell or a cluster of malignant cells inside of the lymph node that are not visualized by routine methods, but only by special dies, as immunohistochemical AE1-AE3 techniqu es[1,4,6,11,18,29,33,35,36,38]. In order to clarify this issue we studied 28 patients prospectively, treated by radical surgery, minimum of 30 lymph nodes resected from D2 lymphadenectomy and 43 lymph nodes from modified D3 lymphadenectomy.

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METHODS

One hundred eighteen patients were treated by surgery at Department of Gastroenterology, at the University of São Paulo School of Medicine, São Paulo, Brazil, from February 1998 to October 2000. The inclusion criteria were: 1. endoscopic confirmed biopsy of gastric adenocarcinoma, without previous treatment; 2. no previous malignancies or abdomi-

nal surgery or neoadjuvant treatment; 3. no distant metastasis detected by X-ray, ultrasound, CT-scan or visible, palpable or frozen sections biopsies confirmed from liver, peritoneum or lymph nodes classified as M (JGCA 1998) (or M1 LYM) (UICC TNM 1997) or even other location different from esophagus, duodenum, greater or lesser omentum; 4. no severe systemic illness. The main objectives in this coort of patients are select patients with endoscopic and biopsy proved diagnose of gastric cancer, to be submitted to total or subtotal radical gastrectomy D2 lymphadenectomy (JGCA 1998), staged previously or during surgery free of peritoneal, liver and distant lymph node metastasis. We prospectively correlate the histochemical and immunohistochemical (AB-PAS/AE1-AE3) detection of lymph node micrometastasis with clinical and pathological features as: age, gender, tumor size, depth and location of the tumor in the gastric wall, type of lymphadenectomy, type of gastrectomy, histologic type of tumor, lymphatic, vascular and perineural invasion, lymph nodal status, stage studied by (1997 UICC TNM) and 1998 JGCA classifications and follow-up survival curves.

Data of 28 patients who underwent potentially curative resection for gastric cancer from February 1998 to October 2000 were admitted to study by fulfilling the inclusion criteria. These patients were closely followed until November 2003; the median length of follow-up was 25 months. Routine follow-up consisted of physical examination, laboratory test (including CEA, CA19.9 levels), endoscopy, chest radiography, abdominal and pelvic ultrasonografy and abdomen (CT) computed tomography or diagnostic videolaparoscopy in case of suspicion of recurrence. Follow-up intervals vary from one, to six months depending on the stage and months of follow-up or as needed.

Radical gastrectomy and lymphadenectomy were performed in all cases.

However immunohistochemical analysis was performed exclusively in negative H&E staining technique concerning metastatic invasion. Lymph nodes were studied based in three sections by H&E, AB-PAS and AE1-AE3 techniques. Mucines were treated by 1% periodic acid, schiff reagent and alcian blue in pH 2.5 and the malignant cells were detected in purple. Antibodies against human cytokeratins AE1-AE3 (Dako Corporation, Carpinteria, CA 93013 USA) were used in order to identify the cytokeratins of malignant cells inside lymph nodes (peroxidase-antiperoxidase technique). Endogenous peroxidasis was blocked. Dark brown insolvable precipitate was formed peripherically around malignant cells (Figure 1).

Statistical analysis

Lymph nodes micrometastasis positivity and clinicopathological factors were compared, with special reference to prognosis and survival curves. Statistically significant differences were analyzed with the two-tailed χ^2 test, Student´s t test or Mann-Whitney test. Kaplan-Meier's survival curves and Log-rank/Cox tests were used. The risk factors were determined by means of logistic regression analysis. Forward stepwise selection with a likeness ratio test was used for selecting variables. The odds ratio in logistic regression analysis was defined as the ratio of the probability that an event will occur to the probability that it will not occur. The prognostic power of variables was expressed by calculation of a relative risk or odds ratio with 95% confident interval. $P<0.05$ was considered statistically significant. All statistical analyses were carried out with the SPSS 12.0 for Windows program (SPSS, Chicago, Illinois, USA).

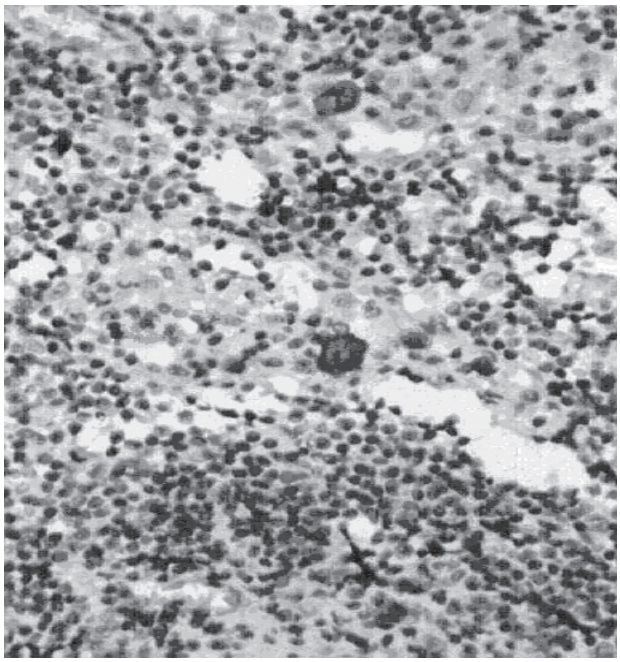

FIGURE 1 - Lymph node micrometastases detected by immunohistochemistry (AE1/AE3 x400)

RESULTS

The mean age of the patients was 61.6 years (range, 35-77 years), with peak incidence in the sixth decade. The total number of resected lymph nodes was 1290, the average of D2 lymphadenectomy was 37.2, median 33 (30-50), the average of modified D3 (D2+ α) was 62.1, median 61.5 (43-86) (Table 1).

The overall average of resected lymph nodes was 46.1, median 46 (30-86). According to 1998 JGCA (2nd edition) stage classification11 stained by H&E 17.9% of patients were stage IA, 14.3% were stage IB, 10.7% were stage II, 21.4% were stage IIIA, 28.6% were stage IIIB and 7.1% were stage IV. According to 1997 UICC TNM (5th edition)37 stage classification stained by H&E 17.9% of patients were stage IA, 14.3% were stage IB, 10.7% were stage II, 32.1% were stage IIIA, 10.7% were stage IIIB and 14.3% were stage IV (Table 2).

TABLE 1 - Clinical and pathological characteristics of gastric cancer patients with and without lymph node (Ln) micrometastasis, univariate analysis results

Variable	Positive Ln micrometastasis		Negative Ln micrometastasis		*P* Value
Staining	AB-PAS	AE1-AE3	AB-PAS	AE1-AE3	
Age (years)	59.3±12.6	61.0±12.6	63.4±10.5	62.2±10.6	ns
Gender					
Male (18)	8	10	10	8	
Female (10)	5	5	5	5	ns
Tumor size (max diameter in cm)	6.2±3.2	5.8±3.2	4.7±3.0	4.5±2.9	ns
pT (depth of invasion)					
pT1 (5)	1	2	4	3	
pT2 (7)	2	2	5	5	
pT3 (15)	10	11	5	4	
pT4 (1)	0	0	1	1	
Total (28)	13	15	15	13	ns
Location of tumor in stomach					
Upper (4)	2	2	2	2	
Middle (6)	4	5	2	1	
Lower (14)	4	5	10	9	
Whole (4)	3	3	1	1	ns
Lymphadenectomy					
D2 (18)	7	9	11	9	
D3 (10)	6	6	4	4	ns
Type of Gastrectomy					
Total (15)	9	10	6	5	
Subtotal (13)	4	4	9	9	ns
Histological type					
Differentiated(7)	5	4	2	3	
Undifferentiated (21)	11	11	10	10	ns
Lymphatic invasion					
Positive (10)	7	8	3	2	
Negative (18)	6	7	12	11	ns
Vascular invasion					
Positive (4)	4	4	0	0	
Negative (24)	9	11	15	13	$P<0.05$
Perineural invasion					
Positive (14)	10	11	4	3	
Negative (14)	4	3	11	10	$P<0.01$
pN (lymph node invasion/JGCA)					
pN0 (8/7)*	0	0	8	7	
pN1 (7)	3	3	4	4	
pN2 (9/8)*	7	6	2	2	
pN3 (4/6)*	3	6	1	0	
Total (28)	13	15	15	13	$P<0.01$
pN (lymph node invasion/UICC)					
pN0 (8/7)*	0	0	8	7	
pN1 (10/9)*	5	4	5	5	
pN2 (4/6)*	3	6	1	0	
pN3 (6)	5	5	1	1	
Total (28)	13	15	15	13	$P<0.01$
Stage (JGCA)					
IA (4/3)	0	0[a]	4	3[a]	
IB (5)	1	1[a]	4	4[a]	
II (1)	0	0[a]	1	1[a]	
IIIA (6/5)	4	3[a]	2	2[a]	
IIIB (8)	5	5[a]	3	3[a]	
IV (4/6)	3	6[a]	1	0[a]	
Total (28)	13	15	15	13	$P<0.05$[a]

* - pN variable changes according to staining techniques (AB-PAS/AE1-AE3)
[a] - Stage is related to statistical difference ($P<0.05$) between positive and negative Ln micrometastasis only in AE1/AE3 staining
ns – not significant

From 1290 lymph nodes retrieved of 28 patients, 152 (11.8%) lymph nodes were positively detected by hematoxilin-eosin staining (H&E) and 1138 (88.21%) were negative. After H&E technique all 1228 remaining lymph nodes were sectioned three times again and 197 (16%) lymph nodes were positive for AB-PAS histochemical staining. After AB-PAS technique, AE1-AE3 was performed and 1216 lymph nodes were studied by three sections and 225 (18.5%) lymph nodes were positive for AE1-AE3 technique. There were 4.8% and 5.7% losses in lymph nodes number, respectively in histochemical and immunohistochemical techniques, mostly by technical staining/sectioning problems and small size of lymph nodes. From 28 patients studied, there were 13 patients (46.4%) who had at least one positive lymph node for micrometastasis stained by histochemical technique among H&E negative lymph nodes.

TABLE 2 - Stage and upstage based on H&E, AB-PAS and AE1-AE3 staining and 1998JGCA/1997 UICC TNM classifications

	CLASSIFICATION	JGCA	UICC	JGCA	UICC	JGCA	UICC
	STAINING	H&E	H&E	AB-PAS	AB-PAS	AE1-AE3	AE1-AE3
STAGE	IA	5(17,9%)	5(17,9%)	4(14,3%)	4(14,3%)	3(10,7%)	3(10,7%)
	IB	4(14,3%)	4(14,3%)	5(17,9%)	5(17,9%)	5(17,9%)	6(21,4%)
	II	3(10,7%)	3(10,7%)	1(3,6%)	1(3,6%)	1(3,6%)	1(3,6%)
	IIIA	6(21,4%)	9(32,1%)	6(21,4%)	8(28,6%)	5(17,9%)	6(21,4%)
	IIIB	8(28,6%)	3(10,7%)	8(28,6%)	3(10,7%)	8(28,6%)	5(17,9%)
	IV	2(7,1%)	4(14,3%)	4(14,3%)	7(25%)	6(21,4%)	7(25%)

Numbers in bold show stage changing

From same 28 patients studied, there were 15 patients (53.6%) who had been stained positive, for micrometastasis at this time by immunohistochemical technique among H&E negative lymph nodes cases.

The final stage, after detection of lymph node micrometastasis by special techniques: AB-PAS and AE1-AE3 that was not identified in routine staining H&E were upstaged (Table 2).

The overall survival average rate was 47.1%, limited to 69 months. Regarding to AE1-AE3 technique, for 15 patients with detected lymph node micrometastasis the survival average rate was 21.5% (28.2 months, limited to 63 months) For 13 patients with not detected lymph node micrometastasis the survival average rate was 69.2% (51.6 months, limited to 69 months). At the time of the final follow up 11 patients (39.3%) were still alive, 3 (10.7%) were lost to follow-up and 14 (50%) died from recurrence. Occult lymph node micrometastasis in routine H&E detected by immunohistochemical technique (AE1-AE3) had worse prognosis (Log-rank/Cox tests $p<0.05$). The presence of lymph node micrometastasis had prognostic significance in patients with gastric cancer in this coort (Figure 2).

Vascular and perineural invasions, lymph nodal status, stage and survival were correlated to positive lymph node micrometastasis. The survival curves were performed and patients with lymph node micrometastasis had poorer prognosis (Figure 2).

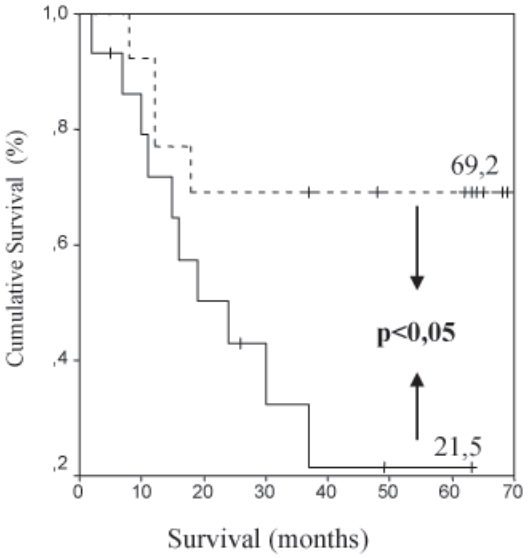

FIGURE 2 - Survival curve for gastric cancer patients with lymph node micrometastasis (continuous line) was lower compared with those without lymph node (interrupted line) detected micrometastasis (P<0.05) (Cox)

DISCUSSION

It is well established that depth of tumor invasion in gastric wall (pT), lymph node invasion (pN) and distant/peritoneal metastasis (pM) are important prognostic factors in gastric cancer[2,12,13,24]. Lymph nodal invasion can be detected from 36% to 54% of all cases of gastric cancer[19,20,33,38]. Prognosis is related to lymph nodal status[19]. "Residual tumors" and remnant positive lymph nodes left behind can lead to "recurrence" in gastric cancer. Recurrence can occur even in early gastric cancer treated with radical resections, as reported in 1996, by Maehara et al.[19], 34 patients died of recurrence, part of them due to lymph node micrometastasis.

Micrometastasis can be defined by a single malignant cell or a cluster of malignant cells inside lymph node not detected by routine techniques as H&E, but only detected by special techniques as histochemical and immunohistochemical staining[1,4,6,11,18,29,31,33,35,36,37,38]. There are others definitions for micrometastasis as malignant cells cluster smaller than 2 mm in diameter[32], smaller than 0.5 mm[27] and 5 or less cells[15], all of them with stromal reaction such as granulation or desmoplastic tissue. In the other hand microinvolvement can be described as individual tumor cells without stromal reaction[32].

There are authors that do not agree that might be a statistical difference in prognostic significance of patients with and without lymph node micrometastasis[3,5,14,22,23,30,34]. Stachura et al.[34], in 1998, after had studied retrospectively 40 patients with early gastric cancers for the presence of micrometastasis in regional lymph nodes by cytokeratin 18 immunostaining related that micrometastasis had no effect on patients survival and takes more than the diagnosis of micrometastasis alone to estimate its real prognostic significance in theirs series. Fukawaga et al.[5], in 2001, reported a rate of 35.5% micrometastasis among 107 patients classified as pT2pN0M0 (JGCA 1998) detected by immunohistochemical AE1-AE3 staining, treated by D2, there was difference between infiltrative and expansive tumors (P=0.02) related to detected micrometastasis, greater in the first one, but not in survival or prognosis between patients with and without detected micrometastasis. Morgagni et al. in 2001[22] and 2003[23] reported large number of patients with early gastric cancer, treated radically, that were not influenced by micrometastasis presence at all, in other words, there was no difference in survival or prognosis for such early gastric cancer patients.

Reversely, there are several authors that agree that there is significant difference in prognostic and survival among patients with and without lymph node micrometastasis [8,9,10,17,19,26,28,39].

Ishida et al.[9], in 1997, reported 2446 lymph nodes removed from 109 gastric cancer patients stained with hematoxilin-eosin, Alcian blue in combination with periodic acid-Schiff stain and antibodies against cytokeratin and carcinoembryonic antigen (CEA). Metastases were confirmed in 230 lymph nodes (9.4%) stained with H&E, an additional 201 lymph nodes (17.6%) had micrometastasis identified only by immunostaining for cytokeratin (197 lymph nodes) and/or CEA. Adenocarcinomas with micrometastasis had significantly worse prognoses at Stage II. Between the two histologic types of gastric carcinoma established by Lauren16, the diffuse type had more micrometastasis than the intestinal type. The presence of micrometastasis in lymph nodes is an indispensable factor in determining the prognosis of gastric carcinoma patients. Cai et al.[2], in 2000, examined 1945 lymph nodes of 79 patients with submucosal gastric cancer by two consecutive sections for H&E and immunostaining with anticytokeratin antibody (CAM 5.2), and demonstrated a lesser 5-year survival in the group of patients with micrometastasis in lymph nodes. Harrison et al.[8], in 2000, related a retrospective review of 25 patients, from 1981 to 1998, resected for T1-4N0M0 gastric and gastroesophageal junction adenocarcinoma who underwent immunohistochemical analysis with CAM 5.2, the median number of lymph nodes resected was 7(1-33), the median follow-up time was 25 months (range 4-195) with an overall survival rate of 55%. For patients with evidence of lymph node micrometastasis (n=9), the 5-year survival rate was significantly decreased (35%), compared to a 66% 5-year survival rate for negative patients (n=16, P=0.05). The presence of immunohistochemical detected lymph node micrometastasis correlates with worse prognosis for patients with histologic negative gastric cancer lymph nodes and immunohistochemical techniques may be a useful additional staging modality in this subset of patients. Ishigami et al.[10], in 2003 reported 4203 lymph nodes examined retrospectively, from 180 gastric cancer patients and not detected any micrometastasis in submucosal gastric cancer patient who underwent lymph node dissection. Gastric cancer patients with more than six

metastatic lymph nodes all had nodal micrometastasis. Patients who were detected positive for micrometastasis had a significant poorer survival rate than those without micrometastasis ($P<0.05$)

Because most of studies were done retrospectively and patients submitted to several adjuvant treatments, it is possible that there is still a role for a rigid quality-controlled lymphadenectomy and prospective study to elucidate this issue. Even after curative resections, recurrence can occur in patients with early gastric cancer[16]. Gotoda et al.[7] studied more than 5,000 early gastric cancer patients who had undergone gastrectomy with lymph node dissection at the National Cancer Center Hospital and the Cancer Institute Hospital in Tokyo and none of the 1230 well differentiated intramucosal of less than 30 mm diameter regardless of ulceration findings were associated to metastases nor the 929 early lesions without ulceration regardless tumor size. None of the 145 differentiated adenocarcinomas of less than 30-mm-diameter without lymphatic or venous permeation were associated with metastases. Based in risk factors and field evidence by large number of patients a gastric cancer guideline in Japan was built[25].

Lymphatic system has an important role in spread of gastric cancer metastasis, as well as of lymph node micrometastasis, mainly in undifferentiated histological types, restricted to blue nodes and lymph nodes within the dye flow area of patent blue used in sentinel node navigation surgery for early gastric cancers[21].

Apart from the clinical and pathologic prognostic factors in gastric cancer, others molecular biological studies have suggested new prognostic factors that are now being extensively investigated: growth factors and receptors (epithelial growth factor, transforming growth factor T-ß1 receptor); oncogenes and suppressor genes (c-erb B2, ras, HST-1, K-sam, c-met, p53, DCC, Rb P21 and nm23); cell adhesion molecules (integrins, cadherin, immunoglobulin superfamily, CD44); chromosome deletion of 1q, 5q, 7p, 17p; tissue inhibitor of metalloproteinasis and urokinasis plasminogen activator and microsatellite instability among others.

CONCLUSION

Lymph node micrometastasis seems to be a significant independent prognostic factor and important risk factor for recurrence in gastric cancer, in a context of radical lymphadenectomy. Immunohistochemical AEI-AE3 techniques may disclose occult lymphnode micrometastasis and so contribute to refine pathological staging in gastric cancer.

Dell'Aquila Jr. NF, Lopasso FP, Falzoni R, Iriya K, Gama-Rodrigues J. Significância prognóstica das micrometástases ocultas em linfonodos no câncer gástrico: estudo histoquímico e imunoistoquímico baseado nas classificações UICC TNM de 1997 e JCGCA de 1998. ABCD Arq Bras Cir Dig 2008;21(4):164-9

RESUMO – ***Racional*** – Micrometástases são um conjunto de células malignas dentro de linfonodo que não são detectadas pelos exames histopatológicos de rotina. Elas são relacionadas a prognóstico mais pobre em muitos estudos sobre câncer gástrico, mas a real significância dessas células permanece controversa. ***Objetivo*** – Avaliar se micrometástase linfonodal é um fator independente de prognóstico e importante para detectar a recurrência do câncer gástrico. ***Métodos*** - Um total de 1290 lifonodos de 28 pacientes com câncer gástrico, de 1998 a 2003, tratados com operações radicais (D2 e D3 modificadas) foram revistos. Três secções por linfonodo foram corados por Hematoxilina-Eosina, histoquímica (AB-PAS) e imunoistoquímica (AE1-AE3). Curvas de sobrevida de Kaplan-Meyer e teste de Log-rank/Cox foram usados para comparar positividade das imcrometástases, profundidade (pT) e localização tumoral na parede gástrica, tipo histológico, invasão linfática, vascular e perineural, estado linfonodal (pN) e estádio onde se encontra a doença. ***Resultados*** – Houve pior prognóstico e recurrência nos pacientes com linfonodos com micrometástases relacionadas às invasões vascular e perineural , avançado estado de invasão linfática e estadiamento mais elevado. ***Conclusão*** – Micrometástase parece ser importante e independente fator de risco para recurrência no câncer gástrico no contexto das linfadenectomias radicais D2.

DESCRITORES – Câncer gástrico. Prognóstico Clínico Dinâmico. Classificação.

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