Evaluation of CD10 positivity in colorectal polyps in neoplastic transformation

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BACKGROUND: *CD10 is a metalloprotein that is potentially associated with greater tumour growth.*
MATERIALS AND METHOD: *We have correlated CD 10 positive in carcinomatous polyps with tumour size, grade, patient age and sex, postoperative TNM staging and Asler - Coller classification. We have matched these cases with a control group that showed presence of polypoid adenomatous tissue with mild to moderate dysplasia.*
RESULTS: *We have divided these in a group of 39 cases, characterised by the presence of carcinoma arising in adenomatous polyps, and a control group of 16 cases, characterised by the presence of colorectal polyps with mild to moderate dysplasia. In the first group, we have discarded three cases for incomplete data.In the remaining 36 cases we have identified 28 patients testing positive for CD10 with positivity values and 8 cases negative for CD10. In CD10 positive cases, we have confirmed the presence of increased incidence of lymph node involvement compared to CD10 negative cases, with high specificity and high predictive value and a higher incidence of cases attributable to group C (Asler-Coller) and grading 3.*
CONCLUSIONS: *CD10 positivity should be assessed in terms of increased progression.*

KEY WORDS: CD10, Colorectal cancer, Colorectal polyps, Neoplastic transformation.

Introduction

Recent studies aimed to immunohistochemically investigate the correlation between early colorectal polyp transformation and depth of invasion and metastasis of colorectal carcinoma led to identification of CD10 a 90 to 110-kDa cell surface zinc-dependent metalloprotease as a marker of severe progression in colorectal neoplasms. Moreover novel sophisticated endoscopic techniques, the identification of the flat polyp- *de novo* cancer sequence and new insight in the *timing* of colorectal polyps transformations , have raised the issue of new pathways and different phenotypic expressions in colorectal cancer histogenesis.
The correlation between CD10 expression in colorectal tumours in relation to differentiation, depth of invasion and metastasis, supports the hypothesis that CD10+ may play a pivotal role in the pathogenesis of colorectal cancer especially in superficial -type carcinoma (Iwase 2005). Several studies have shown CD10+ myofibroblasts in colorectal cancers [1]. These cell lines have been reported to proliferate at the invasive front, which can alter the adhesive and migratory properties of colon carcinoma cells. A link between the presence of CD10+ and p53 has also been observed [2]; 78.9% of CD10 expressing lesions were also positive for p53.

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This study was aimed to examine histogenesis and progression of cancerous polyps and of mild to moderate dysplastic polyps from the viewpoints of CD10 expression.

Materials and methods

Reporting of the differences in phenotypic alterations of non-polypoid lesions and polypoid lesions transformation yielded a review of the case histories of patients underwent surgery for colorectal polyps neoplastic transformation or non-cancerous polyps needing to be surgically removed.
We tested CD10 expression in patients underwent colectomy for histologically defined carcinoma arising from adenomatous tissue in the excised specimen, assuming its presence to be related with development of colorectal carcinoma. We correlated CD10 expression with tumour size, grade, patient age and sex, postoperative TNM staging and Asler - Coller classification.
With regards to TNM classification, we have separated the T value from the lymph node value, examining them separately. We tested our hypothesis on a small control group with adenomatous polyps with mild to moderate dysplasia and without adenocarcinoma.
Immunoperoxidase staining of formalin-fixed, paraffin-embedded tissue sections has been performed using an ordinary biotin-streptavidin method. With the use of polymer chains (Dako, ADVANCE, HRP) tissue sections have been incubated with anti-CD10 primary monoclonal antibody (clone 56C6, dilution 1:50, Novocastra). Sections underwent antigen unmasking by treatment in a thermostatic bath at 98°C for 30 minutes with EDTA buffer pH 9.
The binding sites have been identified with 3,3 diaminobenzidine (DAB) as chromogenic substrate. Finally, sections have been counterstained with Harris hematoxylin. On the other hand, the negative controls have been treated with normal serum to confirm antibody specificity.
We analysed the data by verifying the sensitivity, specificity and the positive and negative predictive value of the tested method.
The term **sensitivity was** taken to imply the proportion of disease positives correctly identified as positives while **specificity** means the proportion of disease negatives correctly identified as test negatives.
Positive predictive value suggested the proportion of patients with positive test results correctly diagnosed as disease patients and **Negative predictive value** meant the proportion of patients with negative test results correctly diagnosed as healthy patients.
We also examined these values based on the probability ratio (**Likelihood Ratio-LR**). Positive LR expressed the probability of a positive result for a disease patient compared to the possibility of a positive result for a healthy patient. Negative LR expressed the analogous result calculated for a negative test.
The data had been analysed also by Student test and Fisher statistical analyses, considering statistically positive values of those with $p < 0.05$.

Results

We have retrospectively analysed 55 patients between 2007 and 2008, all treated surgically with colonic resection using both traditional and laparoscopic techniques (41 laparoscopic resections, 14 laparotomic resections) for colorectal polyps. They were neither removed endoscopically nor underwent neoplastic transformation. In all cases there was a surgical indication.
We have divided the patients into one group of 39 cases, characterised by the presence of carcinoma arising in adenomatous polyps, and a control group of 16 cases that were characterised by the presence of colorectal polyps with mild to moderate dysplasia. The latter were not removed endoscopically due to their size or location (problematic polypectomies).
The test for CD10 expression has been conducted on all of them. In the first group, we have discarded three cases at the limits of positivity due to uncertain classification. In the remaining 36 cases we have identified 28 patients testing positive for CD10 with positivity values (Figg. 1, 2), and 8 cases negative for CD10.
The CD10 positive group was characterized by the presence of 15 females and 13 males with mean age of 70.9 ±8.57 years, while the CD10 negative group had 6 females and 2 males with mean age of 72.09 ±10.57 years (Table I, II)
There was no statistically significant difference between the two groups with regards to age and sex.
We have analyzed the mean grading of the CD10 positive and CD10 negative groups undergoing neoplastic transformation, with values of 2.28 ±0.65 in the former and 2 ±0.53 in the latter (we have not repoted any statistical significant differences).
The effect of grading had been analysed for each patient, after they have been subdivided according to CD10 expression (Table II).
In the group of 28 patients, we have identified 3 patients with grading 1, 14 with grading 2 and 11 cases with grading 3. In the latter group, we have identified 2 cas-

TABLE I - *I° group: carcinoma arising in polyp adenomatous*

36 cases	CD 10 POS	CD 10 NEG	P
N°	28	8	
Sex	15 F; 13 M	6 F; 2 M	
Age	70.9+/-8.57 years	72.09+/-10.57 years	N; S

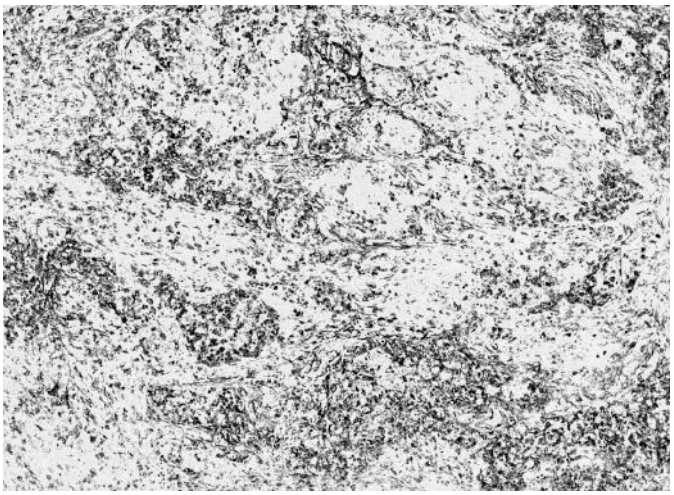

Fig. 1: Antibody anti CD10, magnification 10x, CD10 positive in single neoplastic infiltranting cell.

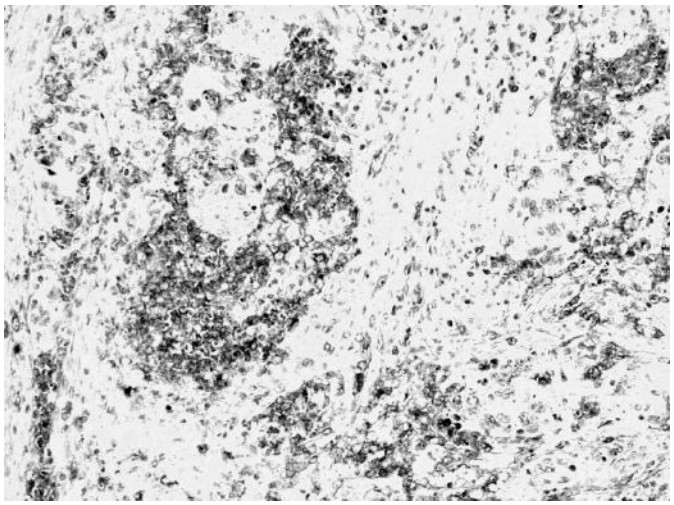

Fig 2: Antibody anti CD10, magnification 20x, CD10 positive in single neoplastic infiltranting cell and microglands.

es of patients that can be attributed to grading 1, 5 to grading 2 and 1 case to grading 3.

According to the hypothesis that CD10 expression lesions are associated with invasive potentials (grading 3), we tested the sensitivity and specificity of the method for this hypothesis, identifying a sensitivity of 39% and specificity of 87%. The positive predictive value was 91% and the negative predictive value was 29%. There were no statistical significant differences. Positive LR was equal to 3.12 while negative LR was equal to 0.69.

We compared the two previous groups according to size of the cancerous polyp, considering the greatest dimension recorded in the definitive histology report, and based on the postoperative T and N values (all cases were M0) (Table III).

The CD10 positive group showed tumours with mean size equal to 3.82 ±2,21 cm, while the CD10 negative group showed mean size equal to 4.36 ±1.78 cm. There were no statistical significant differences between the two groups for this data.

Evaluation of both groups according to the T value, raised an incidence of T1 equal to 14.2%, T2 equal to 35.7%, T3 equal to 46.4% and T4 equal to 3.7% in the CD10 expression group, while the CD10 negative group T1 was equal to 12.5 %, T2 equal to 12.5% and T3 equal to 75% (Table IV).

Still, there were no statistical significant differences between the two groups.

Regarding the differences in terms of incidence and statistical significance with reference to N, in the CD10 positive group we have recorded 25% of cases as N+ while in the CD10 negative group 12.5% were N+. (p not statistically significant). Again, assuming a greater proportion of lymph node invasion in CD10 expression tumours, sensitivity was equal to 25%, specificity equal to 87%, the positive predictive value equal to 87% and negative predictive value equal to 25%. The positive LR was equal to 2 while the negative LR was equal to 0.89.

We have further verified any differences between the two groups in terms of postoperative classification according

TABLE II - *I° group and grading*

	CD10 pos	CD 10 neg	P
Grading	2.28 ± 0.65	2 ± 0.53	NS
Grading 1	3	2	
Grading 2	14	5	
Grading 3	11	1	NS
Sensitivity	39%		
Specificity	87%		
Positive predictive value	91%		
Negative predictive value	29%		
Likelihood ratio positive	3.12		
Likelihood ratio negative	0.69		

TABLE III - *I° group: size and node ratio*

	CD 10 pos	CD10neg	P
Mean size	3.82 ± 2.21 cm	4.36 ± 1.78 cm	NS
N+	25%	12.5%	NS
Evaluation on Node positivity			
Sensitivity	25%		
Specificity	87%		
Positive predictive value	87%		
Negative predictive value	25%		
Likelihood ratio positive	2		
Likelihood ratio negative	0.89		

TABLE IV - *I° group and postoperative T*

	CD10 pos	CD10 neg	P
T1	14,2%	12.5%	NS
T2	35,7%	12,5%	NS
T3	46,4%	75%	NS
T4	3,7%	–	N

TABLE V - *CD10 and Stage C (1-2)sec. Asler e Coller*

	CD10 pos	CD 10 neg	P
STAGE C1	14,28%	12,5%	NS
STAGE C2	28,57%	–	NS

Sensitivity	42%
Specificity	87%
Positive predictive value	92%
Negative predictive value	30%
Likelihood ratio positive	3.36
Likelihood ratio negative	0.65

TABLE VI - *CD 10 positivity in I° group vs II° group*

	Carcinoma	Adenoma	
CD 10 positive	28/38	0/16	p< 0.001

to Asler – Coller. In the CD10 positive group (Table V) we have recorded 3.57% of cases attributable to class A, 21.42% attributable to class B1, 32.14% to B2, 14.28% to class C1 and 28.57% to C2. In the CD10 negative group we have recorded 37.5% of cases as B1, 50% of cases as B2 and 12.5% as C1. We have not recorded any statistically significant differences. Assuming an increased presence of cases attributable to group C in the Asler and Coller classification, we have shown a sensitivity of 42%, a specificity of 87%, a positive predictive value of 92% and negative predictive value of 30%. The positive LR was equal to 3.36 while the negative LR was equal to 0.65.
Patients subjected to resection of the colon for non-endoscopically removable polyps and making up the control group (absence of cancerous tissue) were all CD10 negative (p <0.001) (Table VI).

Discussion

In recent years, the adenoma to carcinoma sequence in colorectal polyps has seen the introduction of novel tumour development models.

Such assumptions of neoplastic degeneration have supported the well-established notions in defining the risk of transformation from adenoma to carcinoma as the association between the histological characteristics of the adenoma (tubular, villous, tubulovillous), its size and grade [3]. The definition of adenoma undergoing neoplastic transformation means an adenomatous polyp in which the cancer has invaded the "muscularis mucosa" and submucosa by direct contact (pT1) [4]. This lesion has metastatic lymph node risk percentages of up to 16% of cases, a risk which increases with submucosal invasion. Approximately 3-10% of all colon polyps removed are identified with that group. But when a polyp is removed endoscopically what cancer risk factors should we analyse with regard to surgical excision of the tract of colon affected? The introduction of screening for colorectal cancer led to increased detection of such neoplastic forms. There were coded parameters used as guidelines in screening for colorectal cancer involving the difference between high vs. low risk in relation to adenomatous polyps transformation; attention to the endoscopic resection margin (<1-2 mm), grade III, lymphovascular invasion of the pedicle , the level of resection margin invasion, the adenoma/carcinoma ratio (> or <50%).
Genetic alterations such as activation of oncogenes and inactivation of tumour suppressor genes can acknowledged the neoplastic transformation process. K ras is mutated in 47% of colorectal cancers, while it is present in 9% of small adenomas and in 58% of adenomas >1 cm. Another significant mutation identified to date is that of APC: this is a "gate keeper" gene on the q arm of chromosome 5 and is involved in the neoplastic transformation process. P53 (p arm, chromosome 17) is mutated [5-6].
The acknowledged adenoma-carcinoma sequence is still valid but new pathways have been included, enriching it and introducing new concepts compared to a few years ago [7-9].
The new concepts have been essentially developed along two lines:
1) Improved characterisation of the lesions identified during colonoscopy with the development of high-resolution endoscopy and chromoendoscopy, with identification of lesions other than polypoid lesions and with independent growth pathways;
2) The second pathway with the development of new knowledge in the molecular biology of non-polypoid lesions of the colon in the form of flat adenoma and sessile serrated adenoma.
In a 1990 publication by Longacre and Fenoglio-Preiser serrated adenoma is verified as a histological definition in 110 (0.6%) of the cases examined, showing 11% severe dysplasia or intramucosal carcinoma [10]. Subsequently, serrated adenoma has been subdivided into (SSA) sessile serrated adenoma and (TSA) traditional serrated adenoma [11].
Sessile serrated adenoma (SSA) appears sessile with tubular cytoarchitecture. It is associated with hyperplastic polyposis and is found in the right colon-cecum.

Traditional serrated adenoma (TSA) is both micro- and macroscopically similar to adenoma, is pedunculated, has tubulovillous and villous cytoarchitecture and is predominantly localised in the distal colon. SSA has the BRAF mutation, DNA methylation and is more frequent in women [12-16].

TSA shows low levels of DNA methylation, the Kras mutation and methylation of the DNA repair gene known as 0-6 methylguanine DNA methyltransferase. Hence, there is a novel pathway for the onset of colorectal cancer known as the serrated pathway, which would be responsible for the onset of colorectal cancer in the absence of pre-existing adenoma. Such lesions would be correlated with the presence of non-neoplastic lesions (hyperplastic polyps) and SSA [6]. Inactivation of the BRAF protein kinase had been reported in this transformation pathway. Progression from SSA to neoplastic forms requires silencing of the hMLH1 gene (MMR mismatch repair gene) [17,18]. Interruption of the mismatch repair cascade results in the accumulation of mutations, the formation of the neoplastic process and microsatellite instability. BRAF, PIK3CA, KRAS are early mutations in the process of carcinogenesis [19-21].

In 2007, Jass altered the chain of events in the adenoma-carcinoma transformation sequence by introducing SSA and TSA along with their mutations [22].

Before Jass classified the transformation time was between 5 and 10 years, in 2007 Jass introduced a real question mark in the temporal definition of cancerous transformation. If confirmed by further studies, these new concepts will be introduced into clinical practice (the prevention of colorectal cancers is based on screening programs, colonoscopy with standard techniques and the removal of any polypoid lesions identified during colonoscopy) [23-26].

However, a proportion of patients, not insignificant in percentage terms, remains excluded, since they are still out with the diagnostic capability of conventional colonoscopy, indeed, certain adenomas have a flat, depressed shape and are not easy to detect or remove by standard techniques [27].

It is just these depressed, non-polypoid lesions that may be one of the carcinogenic pathways.

The above reported lesions have been classified by Kudo in accordance with the ***Japanese Research Society of the colon*** as polypoid type (type I), flat (type II), depressed (type III), further broken down into type 1p (pedunculated), 1ps (subpedunculated) and 1s (sessile). Flat elevated type IIa, flat elevated IIa + IIc with depression.

Flat type IIb and depressed with type IIc and type IIc + IIa slightly depressed (28,29). This scheme had been further revised by introducing new classifications [30]. In 2008 Soetikno et al. [31] published data from the first study conducted on the western population describing how in 1819 patients using staining with *carmine indigo* during colonoscopy, non-polypoid colorectal neoplasms were identified in 14.8% of the population, responsible for 54% of the cancers identified.

The development of staining during endoscopy and assessment of *pitt pattern* has opened up new avenues of study and treatment of lesions that until recently were not detectable and not identified [30]. It is precisely within the scope of this reclassification of colorectal polyps that the search is underway for biological markers that can highlight new pathways of carcinogenesis or the greater/lesser aggressiveness of certain colorectal cancers at the same stage.

Among them, CD10 metalloproteinase has a prominent role [1,2,32]. It is important to identify CD10 positive colorectal tumours at an early stage and treat them in an intensive, customised manner since several reports [2,32,33] have shown that they have a higher incidence of venous invasion or liver metastasis. Furthermore, in the flat adenoma-carcinoma sequence, CD10-expression is raised since the diagnosis of low grade dysplasia, with an incidence still higher than CD10 expression in non-polypoid lesions with mild dysplasia than polypoid [2]. CD10 expression is assumed in cancerous lesions arising *de novo.*

In CD10 positive cases, we have confirmed the presence of increased incidence of lymph node involvement compared to CD10 negative cases, with high specificity and high predictive value.

The CD10 positive group also showed a higher incidence of cases that can be attributed to group C according to the Asler and Coller classification and a higher incidence of cases attributed to grading 3 with respect to cancerous transformation that however were attributed to the CD10 negative group.

Postoperative histological verification has been essential for confirming the correlation between CD10 positivity and tumor progression.

If validated by additional future studies, CD10 expression in non-polypoid and *de novo* neoplastic transformation should be assessed in terms of increased progression.

Aside from that, CD10 expression in endoscopically removed polyps which show evidence of neoplastic transformation without resection margins infiltration may be worthing of further consideration . Evaluation of CD10 expression in this group of polyps may be used for defining surgical planning on side of the existing criteria used for indication of surgical treatment. The potentials expressed by new diagnostic techniques now available, may be reviewed in the light of CD10 expression [34,35]. Further studies should be conducted on future prospective trials.

Riassunto

PREMESSA: Il CD10 è una metalloproteina potenzialmente associata con il maggior accrescimento di un tumore.

MATERIALE E METODO: Abbiamo correlato la positività CD10 nei polipi carcinomatosi con la grandezza del tumore, il grado, sesso ed età dei pazienti, stadiazione

TNM postoperatoria e classificazione di Asler-Coller. Abbiamo confrontato questi casi con un gruppo di controllo che presentava tessuto polipoide adenomatoso con displasia da lieve a moderata.
RISULTATI: Abbiamo diviso i pazienti in un gruppo di 39 casi caratterizzati dalla presenza di tumore insorto su polipi adenomatosi, ed un gruppo di controllo di 16 casi caratterizzati dalla presenza di polipi colorettali con displasia da lieve a moderata.
Nel primo gruppo abbiamo scartato tre casi per l'incompletezza dei dati. Nei rimanenti 36 casi abbiamo identificato 28 pazienti con test positivo per CD10 ed 8 casi con test negativo. Nei casi con test per CD10 positivo abbiamo confermato l'esistenza di una accresciuta incidenza di coinvolgimento linfonodale rispetto ai casi con CD10 negativo, con alta specificità ed elevato valore predittivo, ed una maggiore incidenza di casi attribuibili al gruppo C (Asler-Coller) ed al grado 3.
CONCLUSIONI: La positività per CD10 dovrebbe essere considerata in termini di accresciuta progressione neoplastica.

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