Histopathology of human intestinal anastomosis

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الملامح الباثولوجية النسيجية للمفاغرة المعوية عند الإنسان

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الخلاصة: يُعَدُّ غلق المفاغرة المحكم من الجوانب الهامة في الجراحة البطنية، إلا أن فرص القيام بتقييم نسيجي لمواضع المفاغرة المعوية عند الإنسان كانت نادرة. وقد فحصنا في هذه الدراسة الاستعادية ثلاثين من المفاغرات التي استؤصلت بعد حدوث المضاعفات أو بعد الموت. وقد كان الهدف من ذلك وصف التغيرات النسيجية في مختلف مراحل الاندمال، التي تراوَحَتْ بين 4 أيام و3.5 سنة بعد إجراء الجراحة. وقد أظهرت تشققات المفاغرات ملامح النموت المحاطي الواسع مع تصاقب في الطبقة تحت المخاطية. وتَوَاصَل ظهور الخلايا الالتهابية المزمنة في المفاغرات القديمة الاندمال، مع تقطع في العضلات ومساحات من التليف المتداخل. وسيكون من المفيد فهم المزيد حول محتويات اللمعة التي تنطرح من خلال هذه المناطق المتندبة.

ABSTRACT The faultless seal of the anastomosis is an important aspect of abdominal surgery but opportunities to make a histological evaluation of human intestinal anastomosis sites are rare. This retrospective study examined 30 anastomoses that had been resected following complications or postmortem; the aim was to describe the histological changes at various stages of healing ranging from 4 days to 3.5 years post-surgery. *Anastomosis dehiscence showed features* of extensive mucosal necrosis and bad submucosal apposition. Old healed anastomoses continued to have chronic inflammatory cells and muscular discontinuity with areas of intervening fibrosis. It would be useful to understand more about how the intraluminal contents are propelled through this scarred area.

Histopathologie de l'anastomose intestinale chez l'homme

RESUME La fermeture hermétique de l'anastomose est un aspect important de la chirurgie abdominale mais les occasions de procéder à une évaluation histologique des sites d'anastomose intestinale chez l'homme sont rares. Cette étude rétrospective a examiné 30 anastomoses ayant fait l'objet d'une résection suite à des complications ou post mortem ; le but était de décrire les changements histologiques survenus aux différents stades de la cicatrisation entre 4 jours et 3,5 années après l'intervention chirurgicale. La déhiscence de l'anastomose montrait des caractéristiques de nécrose muqueuse étendue et *de mauvaise apposition sous*-muqueuse. Les anciennes anastomoses cicatrisées continuaient d'avoir des cellules inflammatoires chroniques et une discontinuité musculaire avec fibrose. Il serait utile de mieux comprendre la manière dont le contenu intraluminal est propulsé pour traverser cette zone cicatricielle.

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Received: 31/07/02; accepted: 22/12/02

Introduction

Throughout medical history there have been efforts to develop an ideal method of performing intestinal anastomosis surgery. Over a century ago Nicholas Senn of Chicago, in his presidential address to the Association of Military Surgeons in 1893, said 'We have reasons to believe that the technique of intestinal suturing remains an unfinished chapter and the ideal method of uniting intestinal wounds has yet to be devised' [*1*]. Despite improvements in suture materials and mechanical aids, intestinal anastomoses continue to be complicated by dehiscence and stricture formation.

Further improvement in the outcome of intestinal anastomosis can be achieved through a better understanding of the basic mechanisms of intestinal healing. The mechanisms and pattern of healing following intestinal anastomosis have been extensively studied in animal models using different suture materials, mechanical aids and techniques. It is on the basis of these experimental studies that improvements in human intestinal anastomoses have been made.

The importance of end-to-end apposition of the intestinal layers, particularly the submucosal layers, is essential for primary intestinal healing [*2–4*]. A definitive repair of the underlying layers is necessary before restoration of the epithelium [*5*]. The epithelial healing takes place as neovascularization occurs from the submucosal plexus, which is the major collateral circulation in the bowel wall [*6*].

The histological evaluation of human intestinal anastomoses is difficult as the only source of material is when the anastomosis site is resected following a complication or at the postmortem examination. This reduces the number of specimens that can be examined and also increases the range of the age of anastomoses. To date, we are not aware of any study in the literature describing histopathological changes of human intestinal anastomoses obtained from a site of resection of anastomoses after complications or postmortem. This study aimed to describe the histological changes in early, intermediate and old intestinal anastomosis sites.

Methods

This study was a retrospective evaluation of the sites of intestinal anastomoses which were resected owing to anastomotic complications of dehiscence, stricture and intestinal obstruction due to fibrosis or recurrence of a tumour. Only 1 anastomotic site was from a postmortem. A total of 30 anastomoses sites were examined from 30 patients. The series included 12 females and 18 males with an age range of 19–75 years. The anastomoses ranged from 4 days to 3.5 years post-surgery.

The cases were divided arbitrarily into three subgroups based on the duration of the anastomosis: Group A, less than 10 days; Group B, more than 10 days and less than 6 months; and Group C, more than 6 months.

The histology samples were examined microscopically following routine haematoxylin and eosin staining techniques. Polarized microscopy was used to identify suture materials. Histological examination was done for the features that have been fully described before in animal models of intestinal anastomosis [*2–4*]:

- Mucosal layer: abnormal crypt architecture: branching, distortion, and degeneration in the intestine; paneth cell metaplasia in the colonic anastomoses.

Villous height and crypt depth were measured.

- Submucosal layer: end-on apposition; vascularity; mucosal herniation.
- Muscular layer: muscle discontinuity; muscle thickening; presence of fibrosis.
- Serosal layer: continuity.
- Inflammation: acute and chronic inflammation, ulceration and oedema. The depth of the inflammatory infiltrate within the layers described above and whether diffuse or patchy in distribution were recorded.
- Fibrosis: mucosal and submucosal fibrosis.
- Foreign body granulomas: presence of foreign body giant cells and stitch granulomas in the submucosal and muscular layers.

Results

The age and sex distribution of the patients, the duration and type of anastomoses, and the reason for resection are summarized in Tables 1–3. In group A (Table 1) there were 7 patients, 5 patients with anastomoses resected following dehiscence and 1 patient where resection was due to small intestine perforation from strangulation of the bowel. The histopathological examination of the bowel wall at anastomotic sites in these patients showed full thickness necrosis of the mucosa with formation of a surface membrane composed of fibrin, acute inflammatory cells, pus and necrotic tissue. There was marked vascular congestion, oedema and areas of haemorrhage in the submucosal layer. The submucosal layer apposition was distorted. There was discontinuity of the muscular layer with areas of focal ischaemic necrosis and neutrophilic infiltrate with features of early granulation tissue formation. However, no fibrosis or foreign body giant cell reaction was present.

The last patient in the above group had a colonic anastomosis resected at postmortem. The cause of death was cardiac arrest secondary to cardiomyopathy. The histological features showed well-aligned mucosal and submucosal layers. However, at the site of anastomosis there was a full

Table 1 **Group A patients: anastomosis duration less than 10 days**

Patient age (years)	Sex	Duration of anastomosis	Reason for resection	Diagnosis
75	M	5 days	Dehiscence	Carcinoma colon
70	M	6 days	Dehiscence	Carcinoma colon
54	F	7 days	Dehiscence	Carcinoma rectum
66	F	7 days	Dehiscence	Carcinoma colon
58	M	8 days	Dehiscence	Carcinoma colon
68	M	4 days	Small intestine perforation	Strangulated small intestine
72	M	5 days	Postmortem examination	Cardiac arrest/ Carcinoma colon

Table 2 **Group B patients: anastomosis duration more than 10 than days and less than 6 months**

Patient age (years)	Sex	Duration of anastomosis	Reason for resection	Diagnosis
65	F	21 days	Bowel dysmotility	Carcinoma of caecum
60	M	21 days	Bowel dysmotility	Carcinoma transverse colon
55	F	58 days	Small intestine dysmotility	Idiopathic megacolon
45	F	28 days	Second operation	Carcinoma rectum
50	M	6 months	Recurrence	Malignant carcinoid tumour
19	F	5 months	Recurrence	Non-Hodgkin lymphoma
25	M	5 months	Recurrence	Non-Hodgkin lymphoma
60	M	4 months	Recurrence	Carcinoma rectum (adinocarcinoma)

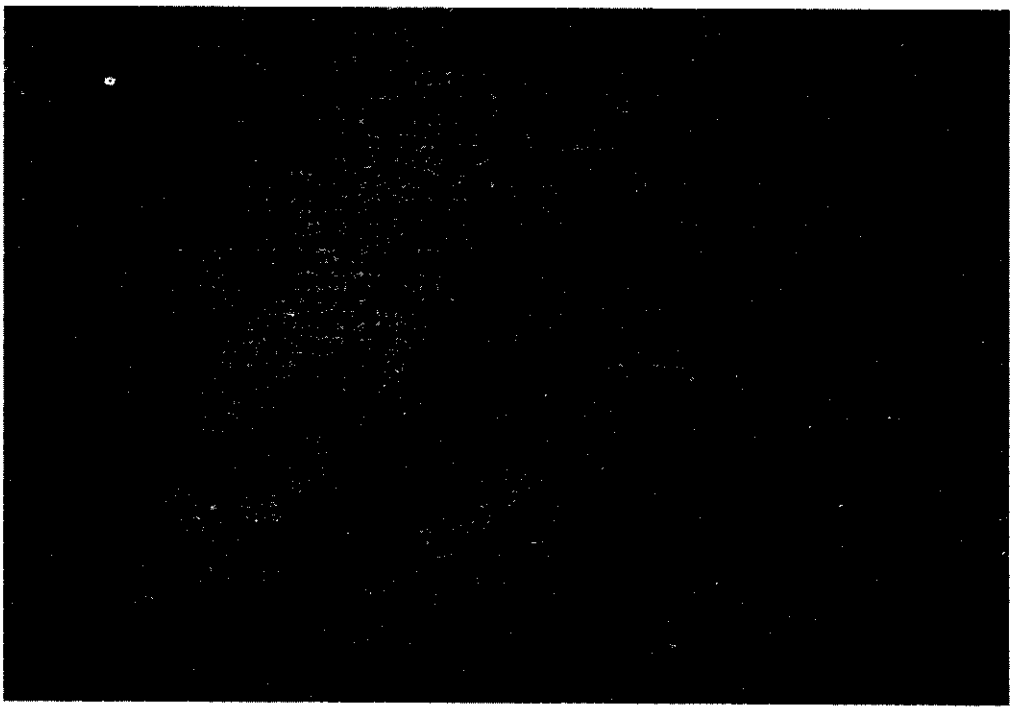

Figure 1 **Dilated and distorted mucosal glands at the site of anastomosis**

Table 3 **Group C patients: anastomosis duration more than 6 months**

Patient age (years)	Sex	Duration of anastomosis	Reason for resection	Diagnosis
65	M	10 months	Recurrence	Carcinoma sigmoid
45	F	6.5 months	Recurrence	Carcinoma rectum
65	F	15 months	Recurrence	Carcinoma caecum
70	M	10 months	Recurrence and pouchitis	Carcinoma caecum
67	M	15 months	Recurrence and pouchitis	Carcinoma colon
65	M	3 years	Recurrence	Carcinoma caecum
56	M	2 years	Recurrence	Carcinoma colon
65	F	2 years	Recurrence	Carcinoma colon
55	M	2.5 years	Recurrence	Carcinoma colon
57	F	2.5 years	Recurrence	Carcinoma rectum
68	M	2 years	Recurrence	Carcinoma rectum
60	M	3 years	Recurrence	Carcinoma transverse colon
60	F	3.5 years	Recurrence	Carcinoma colon
25	M	3 years	Stricture	Crohn disease
27	F	3 years	Adhesions	Crohn disease

thickness necrosis. The submucosa showed numerous dilated blood vessels and early granulation tissue extending to the underlying muscular coat. There was no recognizable evidence of well-formed foreign body granulomas or foreign bodies.

There were 8 patients in group B (Table 2), 2 of them with anastomoses of 21 days duration. Both cases showed histological features of satisfactory healing. The ileorectal anastomoses of these 2 patients showed the ileal mucosa in direct continuity with the colonic mucosa. The mucosa adjacent to the anastomosis site was normal. However, at the site of anastomosis, the glands were distorted and occasional ones were dilated (Figure 1). The lamina propria showed mild active chronic inflammation on the ileal side with focal fibrosis. The colonic side showed similar but less marked changes. There was satisfactory end-on apposition of the mucosal and submucosal layers in both anastomoses. Mucosal herniation, suture granulomas and muscular layer discontinuity were also seen in the anastomosis sites.

Another patient in the series had an ileorectal anastomosis resected for small bowel dysmotility, following a total colectomy performed for idiopathic megacolon. The age of the anastomosis at resection was 58 days. This anastomosis showed mild distortion of crypts with active chronic inflammation in the lamina propria.

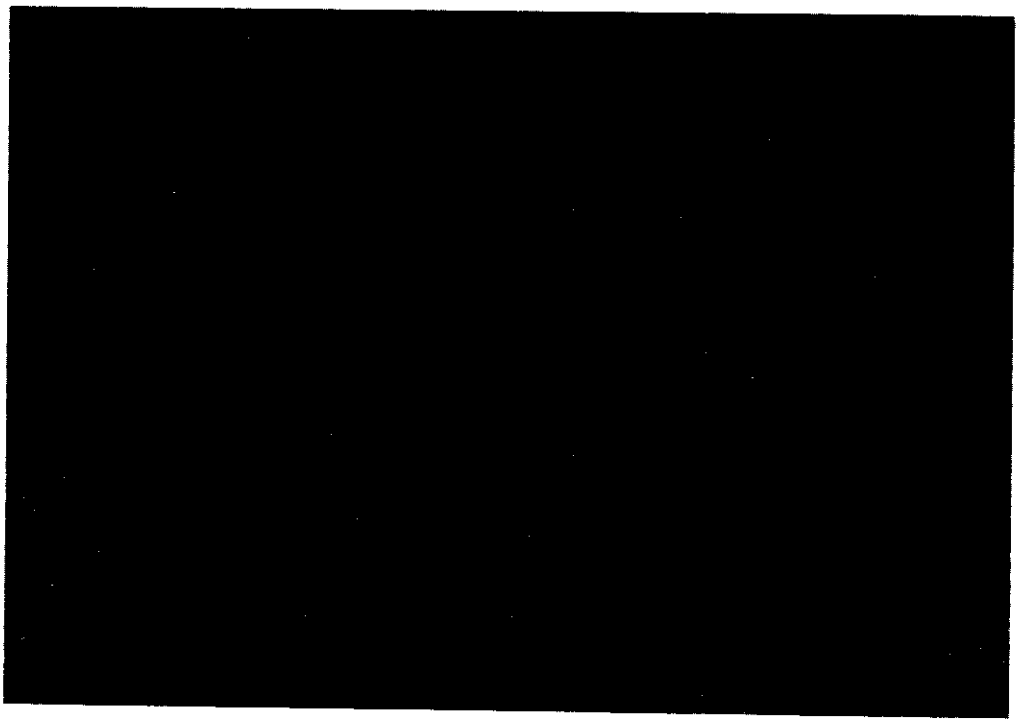

Figure 2 **Stitch granulomas and foreign body giant cell reaction**

Compared with the above 2 anastomoses, there was more fibrosis noted in between the discontinued muscle layers in this older anastomosis. Other features were remarkably similar.

Another patient in Group B had undergone transanal excision of a rectal carcinoma, and following this an abdominoperineal resection as a curative operation. The duration of the anastomosis was 28 days. This yielded an anastomosis site sutured with catgut for examination. There was satisfactory end-on apposition of the mucosal and submucosal layers. Muscular discontinuity and the stitch granulomas were prominent on a background of fibrosis. There was less marked inflammatory response in this anastomosis site.

The remaining 4 patients had had their anastomoses resected due to intestinal obstruction following recurrence of their tumours: 1 had malignant carcinoid tumour, 2 non-Hodgkin lymphoma and 1 adenocarcinoma. The duration of anastomoses were 6, 5, 5 and 4 months respectively. The anastomotic sites showed the involvement of recurrent tumours; however, the adjacent mucosa showed distortion of glands and crypts in addition to chronic inflammatory cell infiltrate composed mainly of lymphocytes and plasma cells in the lamina propria and of fibrosis. The subjacent muscular layers showed discontinuation of muscular bundles with loss and atrophy of the glands and replacement by fibrosis that extended into the

serosa. Multiple stitch granulomas with foreign-body giant cell reaction were present mainly towards the serosal surface (Figure 2).

There were 15 patients in Group C (Table 3) who had anastomoses of duration more than 6 months. Most of these anastomoses were resected owing to recurrence of the tumours except for 2 cases which were known cases of Crohn disease with strictures and adhesions. The anastomotic sites in this group of patients showed very good apposition of all the layers. There was marked prominence of lymphoid tissue in the mucosa in some of the cases, particularly in patients with recurrent pouchitis. The histological features in these anastomotic sites showed a well-aligned mucosa with chronic active inflammation in the lamina propria and focal ulcerations, as typically seen in pouchitis. The submucosa showed foreign body giant cell reaction surrounding suture material. Mucosal herniation was not present. Satisfactory submucosal apposition with muscle discontinuity was also seen. There was mucosal herniation and active chronic inflammatory cells in the submucosal layer in 2 patients.

In patients whose ileocolic anastomosis was resected owing to recurrence of Crohn disease, the anastomotic sites were obscured with features of Crohn disease, some with recurrence of disease, others with strictures or adhesions. Muscle discontinuity and stitch granulomas were present in these patients. Fibrosis involving the bowel layers was most prominent in this group of patients with hyalinization evident more prominently with increasing age of the anastomoses.

Discussion

The faultless seal of the anastomosis is an important aspect of abdominal surgery. The anastomotic complications of dehiscence and stricture formation are the major cause of morbidity and mortality after gastrointestinal surgery. The sequence of events in the intestinal anastomosis is the same as the healing of wounds elsewhere in the body [*7*]. However, unlike cutaneous healing, where progress can be observed on a daily basis and intervention made early if necessary, healing of intestinal anastomoses cannot be inspected directly and any complications usually require re-operation. Inflammation is followed by mobilization of the cells that form granulation tissue. Fibroblasts then proliferate with synthesis and extrusion of collagen and a ground substance that is involved in the extracellular maturation of the collagen [*8,9*].

Several investigations have shown that the method of apposition influences the repair process of the epithelium. In this regard, satisfactory apposition of submucosal layers is most important [*2,3*]. Jansen et al. have described the concept of primary intestinal healing with good submucosal apposition and secondary intestinal healing with bad submucosal apposition [*2*]. General factors affecting intestinal healing include patient's age, nutritional status, diabetes, malignancy, exposure to radiation [*10*] and steroid consumption. Local factors include blood flow [*11*], bacterial contamination at the site of the anastomosis, distraction of the anastomosis and use of drains. The suture materials and, more importantly, the suture technique affect the outcome of anastomosis [*12*].

This study aimed to describe the histological changes in human intestinal anastomosis at various stages of healing in order to understand the associated complications such as dehiscence, which usually requires re-operation with the associated morbidity of a laparatomy and additional general anaesthesia. The histological features of early anastomoses where satisfactory healing is occurring shows good apposition of all the intestinal layers and increased acute inflammatory cells, particularly neutrophils. Presence of increased concentrations of neutrophils at the early stages of anastomosis healing in humans has been shown by indium-111-labelled granulocyte scanning [*13*]. Granulocytes continue migrating into the area of anastomoses for at least 20 days following surgical trauma. This plays a significant role in healing and prevention of infection. In the early phase of anastomotic healing, the granulocyte collagenase is mainly responsible for the observed lysis of collagen after intestinal anastomoses in the animal experiments [*14*]. However, the healing process is largely dependent on the ability and sufficiency of collagen synthesis at the site, which in turn needs a profound understanding of the molecular and biochemical pathways and the factors that control them. The increased neutrophil congregation at the site of a leaking anastomosis probably further weakens it [*14*]. Neutropenia prevents a decrease in strength in rat intestinal anastomosis [*15*]. Mucosal herniation is a manifestation of direct bridging of the anastomotic submucosal defect and was observed in all anastomotic sites in our Group B and C patients, except occasionally in patients with stapled anastomoses. Crypt architecture was distorted in the early stages of healing; this improved in older anastomoses and was less severe in stapled anastomoses.

Stitch granulomas were observed in slightly older anastomoses as seen in patients in Groups B and C. Chronic inflammatory cells, mainly lymphocytes and plasma cells, were observed in old anastomoses. Muscular layer discontinuity was constantly observed, irrespective of the duration and technique of anastomosis. The area between the interrupted muscular layers was filled with fibrous tissue.

In animal experiments, a generalized increase in collagen synthesis has been observed from the second day of small bowel anastomosis, whereas in colonic anastomoses adequate laying down of collagen occurs between days 10 and 14. For colonic anastomoses, therefore, sutures that dissolve slowly should be used [*16*].

In conclusion, we have described a spectrum of histological changes in early, intermediate and old intestinal anastomosis sites. The limitations of this study lie in the small number of specimens examined because of the difficulty of procurement. Anastomosis dehiscence shows features of extensive mucosal necrosis and bad submucosal apposition. Old healed anastomoses continue to have chronic inflammatory cells and muscular discontinuity with areas of intervening fibrosis. It would be useful to understand more about how the intraluminal contents are propelled through this scarred area.

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