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Are giant ganglia a reliable marker of intestinal neuronal dysplasia type B (IND B)?

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Abstract It has been suggested that giant ganglia are a marker for a developmental bowel disorder, intestinal neuronal dysplasia of the submucosal plexus (IND B), diagnosed in a proportion of patients with severe intractable constipation. Diagnosis of this condition, however, remains controversial with a wide variation in the frequency of diagnosis in different centres. Our aim was to assess the frequency with which giant ganglia could be found in the bowel of individuals who did not give a history of life-long constipation. We also aimed to assess the reproducibility of giant ganglia counts. For this two pathologists independently assessed pieces of normal bowel taken away from the site of the lesion in patients who had undergone surgery for colorectal carcinoma. Giant ganglia containing seven or more ganglion cells were found in 76 and 78% of subjects by each of the two pathologists. There was 1 giant ganglion per 10 ganglia counted in those patients in whom they were identified and 1 giant ganglion per 10.9 ganglia overall. Sections from eight patients in whom there was a history of constipation and/or melanosis coli did not show a greater number of giant ganglia. We conclude therefore that so-called "giant ganglia" are a common feature in the submucosa of normal bowel and that the presence of occasional giant ganglia cannot be considered diagnostic uf IND B.

Key words Intestinal neuronal dysplasia · Giant ganglia · Ganglion cells · Diagnostic criteria

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Introduction

The concept of intestinal neuronal dysplasia of the submucosal plexus (also known as neuronal intestinal dysplasia type B or NID B) was first introduced by Meier-Ruge in 1971 [5]. Reported symptoms include chronic intractable constipation, abdominal distension and overflow incontinence [13], and it is said to be the second-most common cause of primary chronic constipation after Hirschsprung's disease [3, 6, 13]. Although a variety of diagnostic criteria has been suggested, histological diagnosis is usually based upon the detection of giant ganglia within the submucous plexuses of the large intestine. This has been cited as the most relevant age-independent diagnostic feature [8, 9].

However, is the diagnosis of IND B based upon giant ganglia appropriate and reliable? We found that in segments of large intestine resected for carcinoma the resection margins often contained giant ganglia on histological examination. We set out to investigate the frequency of giant ganglia in these specimens and to compare these results with published figures for giant ganglia in cases of IND B.

Materials and methods

Pieces of normal bowel taken away from the site of the lesion in patients who had undergone surgery for colorectal carcinoma were selected. These had previously been fixed in formalin and embedded in paraffin wax. The case notes of each individual were examined and those with a history of life-long constipation were elicited and excluded from the study. Fifty cases were collected.

From the selected blocks, 3-µm sections were cut and stained with haematoxylin and eosin. The sections were examined using a Nikon Optiphot-2 microscope at the ×20 magnification (field diameter 0.932 mm). The ganglion cells were identified by the histological characteristics of a large cell body with extensive, finely granular amphophilic cytoplasm with a large nucleus with dispersed chromatin and a prominent nucleolus. If only part of a cell body with the typical cytoplasm was clearly identified, it was also counted. A ganglion was defined as a group of contiguous nerve cell bodies which were not separated by submucosal connective tissue. If these was any doubt as to the nature of a cell's identity, it

Table 1 Giant ganglia, containing 7 or more ganglion cells, in specimens (total no.=50)

	Specimens with giant ganglia present (%)	Total numbers of ganglia counted	Total number of giant ganglia counted (% of total)
Pathologist 1	39(78)	604	98(16.2)
Pathologist 2	38(76)	883	84(10.2)

Table 2 Melanosis coli cases compared to rest of specimen population

	Total number giant ganglia	Total number of ganglia	Percentage	*P* value vs rest of population
Pathologist 1	9	55	16.4	0.1863
Pathologist 2	7	91	7.69	0.1871

was not included in the count. The whole of the submucosa was examined and the total number of submucosal ganglia counted and recorded. The number of ganglion cells in any ganglia in which seven or more ganglion cells was also documented. The counting was performed by two different histopathologists. Statistical analysis was performed using Minitab Statistical Software, Minitab Inc.

Results

The results of the giant ganglia counts and total ganglia counts are displayed in Table 1. The correlation between both pathologists in the total number of ganglia counted was 0.484 (1.00=perfect correlation), and the correlation between the total number of giant ganglia counted was 0.628 (Table 1). Giant ganglia containing 7 or more ganglion cells were found in 76 and 78% of subjects by each of the two pathologists. There was 1 giant ganglion per 10 ganglia counted in those patients in whom they were identified and one giant ganglion per 10.9 ganglia overall. The number of ganglion cells in the giant ganglia ranged from 7 to 23 per ganglia. Ganglia with more than 7 ganglion cells per ganglion were found in 62 and 54% of subjects by each of the two pathologists.

Eight of the specimens examined showed melanosis coli. For each pathologist, the melanosis coli group was compared to the rest of the specimen population (Table 2), using the non-parametric Mann-Whitney U test. There was no significant difference between the groups ($P > 0.05$).

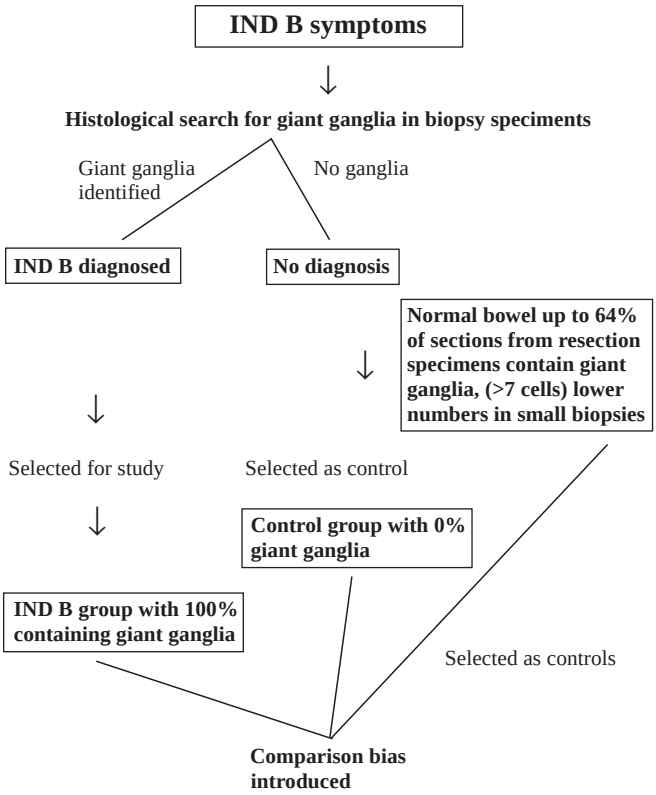

Fig. 1 Introduction of selection bias into studies of IND B

Discussion

Intestinal neuronal dysplasia is a controversial condition. There is a wide variation in the frequency with which it is diagnosed and controversy over the diagnostic features [1, 3, 4]. Some authors have questioned its existence as a diagnostic entity [1, 3]. Others have pointed out technical insufficiencies in many papers published over previous years [7, 10]. Meier-Ruge [11] found that in cases diagnosed as IND B, 10% of the total number of submucosal ganglia contained more than 6 ganglion cells and suggests that diagnosis should be based on examination of 15-µm serially sectioned frozen sections of large bowel stained with lactate dehydrogenase (LDH). We have shown, however, that giant ganglia are demonstrated readily in normal bowel with haematoxylin and eosin staining using only 3-µm sections and that on average 1 in every 10.9 ganglia will contain 7 or more ganglion cells. More than half of the specimens included ganglia with more than 7 cells, and ganglia containing 20 or more cells were identified.

Kobayashi et al. [3] felt that submucosal and myenteric hyperganglionosis was the most consistent finding and that other criteria (giant ganglia and ectopic ganglion

Table 3 Comparison of sources counting giant ganglia in cases in IND

Source	Section thickness	Stains used	Method of staining described	Ganglion cell defined	Counting method described	Percentage of giant ganglia (source described)
Meier-Ruge [11]	15	LDH SDH	Yes	No	No	10% IND patients
Meier-Ruge [12]	15	LDH SDH	Yes	No	No	30% IND patients
Kobayashi [3]	10	H&E AchE	No	No	No	Hyperganglionosis most significant feature
Meier-Ruge [8]	15	AchE LDH	Yes	No	No	20–30% IND patients
Lumb (this study)	3	H&E	Yes	Yes	Yes	10–16% bowel from cases of carcinoma

cells) were more likely to be demonstrated when the biopsy was a full thickness rather than in a suction rectal biopsy. They compared 19 cases that had been diagnosed by histological and histochemical criteria from three children's hospitals in Dublin with post-mortem and live controls. Giant ganglia, defined in the their paper as having more than 7 ganglion cells, were seen in 2 of 23 (9%) controls and 5 of 9 (56%) cases of IND not associated with Hirschsprung's disease. Even hyperganglionosis, considered to be the most reliable feature but which was not clearly defined, was found in 3 of 23 (13%) control cases, all of whom were neonates. Some of the control cases also showed increased acetylcholinesterase staining and ectopic ganglion cells.

Meier-Ruge [6] found that in 358 patients with diagnosis of a dysganglionosis 40.6% showed the features of IND B, characterised by hyperplasia of the submucous plexus and giant ganglia with 7–15 nerve cells. Other findings looked for included nerve cell buds along larger afferent parasympathetic fibres, groups of nerve cells within nerve fibres and heterotopic neurons in the muscularis mucosae or lamina propria. They examined serial frozen sections cut at 15 µm stained with ACHE, LDH and SDH. Morphometic analysis of biopsies from 160 children and 30 adults diagnosed with IND B, compared with 65 paediatric biopsies diagnosed as having normal histology and 15 healthy adult volunteers, was carried out by Meier-Ruge et al. in 1994 [8]. The data suggested to the authors that whereas in the children four criteria, namely increased numbers of nerve cells per ganglion, large ganglion size, large nerve cell size and increased density of the submucous plexuses, were significantly different between affected and non-affected cases, in adults only the number of nerve cells per ganglion was significantly different, with giant ganglia each having 6–11 nerve cells being seen in IND B cases.

When an attempt was made to correlate biopsy findings with clinical outcome [2] by means of clinical questionnaires or interviews, it was found that histological criteria were unhelpful in predicting clinical outcome.

One possibility that must be considered, however, is that selection bias in some other studies may have led to an incorrect impression that giant ganglia are more prevalent in the submucosa of the large bowel in those individuals diagnosed as having IND B. Clearly, if the initial diagnosis of IND B is made on the basis of the presence of frequent giant ganglia, then selection of these cases with comparison to "normal" or with non-selected controls will lead to a selection bias (Fig. 1).

There was poor correlation between the pathologists of counts of total number of ganglia but, as to be expected because of higher visibility, an improved correlation with counts of giant ganglia was obtained. Our sections were thinner than those in some of the previously reported papers, special staining techniques were not used and it is likely that a lower value for the number of giant ganglia was obtained than otherwise might have been had those techniques been employed.

Methodological differences between papers have made an accurate definition of IND B difficult, in particular staining methods, section thickness and counting methods, including magnification and definitions of ganglion cells (Table 3). The frequency with which giant ganglia can be found in control material and the methodological problems inherent in previous studies cast further doubt on the frequency with which this condition is diagnosed.

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