Aims: The aim of this study was to review the use of the on-table “doughnut” biopsy for frozen section assessment of bowel in the operative management of Hirschsprung’s disease (HD).

Methods: This was a single-center retrospective review of doughnut histopathology reports, operation notes, and slides from 2010 to 2017. Data were assessed for the presence of transition zone (TZ) features and the subsequent decision as to the level of pull-through.

Results: Fifty-five patients had a doughnut biopsy taken as part of their intraoperative frozen section histopathology for pull-through for HD during the study period. Forty-eight required a single doughnut, six required a second more proximal doughnut, and one required a third doughnut. Of the 55 first doughnuts, 37 were identified as normal bowel, 17 were TZ, and not defined in the report in one case. Of the 17 TZ doughnuts, 8 were accepted for pull-through and 7 underwent second doughnuts (normal = 4 and TZ = 3). The third doughnut (one case) was normal. TZ was accepted for pull-through in 10/54 (18.5%) patients despite the use of a doughnut. However, TZ was avoided in six (11.1%), where the single-point biopsy was “normal.”

Conclusions: The doughnut allows the entire circumference of pull-through level to be assessed, enabling TZ identification that can be missed by single seromuscular biopsies. This allows identification and avoidance of TZ pull-through, although sometimes, it is accepted for other reasons.

Keywords: Doughnut, frozen section, Hirschsprung’s disease, transition zone

INTRODUCTION

It is well recognized that in Hirschsprung’s disease (HD), the change in bowel innervation from distal, abnormal bowel (aganglionic, thickened nerves) to proximal, normal bowel (ganglionic, no thick nerves) is not abrupt but gradual; this change can be rather variable in degree, in depth, and in its distribution around the circumference and length of bowel affected,[1-4] and the term “transition zone” (TZ) is used to describe this intervening length of bowel. Therefore, bowel from the TZ is occasionally pulled through in error, resulting in a TZ pull-through (TZPT) – one of the well-recognized complications of surgery for HD. This may cause persistent obstruction, necessitate further surgery, and result in poor functional outcome.[5]

In a systematic review of residual aganglionosis after pull-through for HD, Friedmacher and Puri[6] found the incidence of TZPT to be as high as 25.4%. In 2000, Ghose et al.[5] reported a 15% incidence of TZPT in their institution. In order to avoid a TZPT, they suggested that...
the entire full-thickness circumference or “doughnut” of bowel be assessed at frozen section rather than a seromuscular “single-point” biopsy in order to reduce the risk of TZPT. More recently, in 2017, Ghosh et al.\(^7\) published a review of their practice, found an incidence of TZPT of 16%, and reached the same conclusion.

Histologically, although there have been several studies on the features of the TZ,\(^{1,3,8-10}\) standardized diagnostic criteria for defining the features and extent of a TZ during pull-through for HD are lacking. This makes a comparison of results across studies difficult. It is felt that in practice, hematoxylin and eosin (H and E) staining is the only practical method for staining the bowel for intraoperative frozen section (IOFS) assessment during an operation.\(^{11}\)

On the basis of the above surgical and histological considerations, since 2010, we have preferred the routine use of a circumferential “doughnut” biopsy to a “single-point” biopsy during pull-through for HD; the presumption being this would enable us to avoid a TZPT. We report our institutional review of this practice with reference to the suitability and accuracy of this method and relative advantages and limitations.

**Methods**

A retrospective review of case notes and histopathology slides was undertaken for all patients \((n = 55)\) who underwent pull-through for HD at Sheffield Children’s Hospital, UK, between July 2010–July 2017. Data were collected regarding patient demographics, type of pull-through, number and level(s) of on-table intraoperative doughnut specimens taken and sent for IOFS assessment, histological findings (frozen section of “single-point” and “doughnut” biopsies), and histopathology reports. Relevant remarks in the operation notes relating to the IOFS report (telephonic conversation between histopathologist and operating surgeon) and the resulting surgical decision-making relating to the need for further biopsies more proximally or to proceed to pull-through were recorded. In our department, two consultant surgeons were involved in performing all these operations during the period of this study.

**Definition of transition zone**

The presence of any thick nerves, i.e., single or more, was considered to represent a TZ. The presence of ganglion cells and complete absence of thick nerves was considered as normal bowel.

Cases who did not define the characteristics of the doughnut and the intraoperative decision-making regarding level of pull-through in the histopathology report and operation note were excluded.

**Doughnut technique**

Initially, bowel was assessed macroscopically at laparotomy and a “single-point” biopsy taken at the antimesenteric border above the TZ in what was considered to be normal colon. If this was noted to be “normal” (i.e., presence of ganglion cells and complete absence of thick nerves), we proceeded to resecting at least 2 cm length of bowel as the circumferential doughnut biopsy and marked the proximal, antimesenteric margin with a suture to help the histopathology technicians orientate the bowel during IOFS processing of the specimen.

**Doughnut processing – technical considerations**

Doughnut specimens were transferred fresh to the histopathology laboratory. Upon receipt, the doughnut was placed on a strip of filter paper and orientated using a dissecting microscope in such a way as to allow transverse sections of the bowel to be taken [Figures 1 and 2]. The specimen was supported with a cryo-specific embedding medium, such as optimal cutting temperature compound, and snap frozen in liquid nitrogen before transferring directly to the cryostat for sectioning.

Taking a \(<2\) cm thin sliver of bowel can lead to twisting of the bowel during processing for IOFS and loss of bowel orientation around the circumference, with the serosal surface being turned luminal in some areas – making the sectioning and interpretation of the specimen difficult – and increases the possibility of error.

For accurate assessment of ganglion cells across the entire circumference, the doughnut was gently trimmed to full face before the first sections were taken. Further sections at increasingly deeper levels were then cut and picked up on glass slides labeled with the specimen number. Slides were transferred to a rapid fixation solution such as Wolman’s fixative for approximately 1–2 min before staining with H and E.

The study was registered with the local quality and standards department with the number SE1109.

**Figure 1:** Doughnut biopsy showing thick nerves in the submucosa (arrow) (H and E, \(\times10\)). Inset: higher magnification of the thick nerve trunk (H and E, \(\times20\))
**Results**

A total of 55 children underwent pull-through for HD during the study period. There were 45 males and 10 females. The mean age at operation was 8 months (range: 1–152 months). Fifty-two children underwent Duhamel and three transanal pull-through procedures. Twenty-eight patients underwent a primary pull-through and 27 patients a secondary pull-through following an initial defunctioning stoma.

The extent of aganglionosis diagnosed histologically at pull-through surgery was rectosigmoid ($n = 43$), long segment ($n = 7$), total colonic ($n = 4$), and not defined in the report ($n = 1$); the latter was excluded from reporting and analysed. Therefore, 54 patients were analysed in total.

Following the first (i.e., most distal) doughnut biopsy in all 54 children, 37 specimens (68.5%) were identified as “normal” bowel and a pull-through was performed at this level. Seventeen specimens (31.5%) were identified as TZ. In these 17 patients, a TZ was accepted as the level of pull-through in 8 patients (14.8%), without further biopsies. In the other nine patients, a second doughnut was taken at a more proximal level of the colon in seven patients (13.0%), and in the other two patients, the procedure followed in one patient each is as below:

a. A more proximal “single-point” biopsy was taken at upper sigmoid level, shown to be ganglionic, and the surgeon proceeded to a pull-through at this level; the H and E of the proximal margin of the resected bowel was normal with no features of TZ

b. The initial doughnut was in the ascending colon, and hence, the surgeon proceeded to a colonic resection and ileal pull-through for total colonic aganglionosis; it was known that the ileal doughnut taken at the time of defunctioning ileostomy formation was “normal.”

Following the second doughnut biopsy in seven patients, “normal” bowel was found in four patients and the pull-through performed at this level; TZ was identified in the remaining three patients. Of these last three patients, TZPT was undertaken in two patients and a third doughnut biopsy was performed in the remaining one patient. This third doughnut biopsy showed “normal” bowel, and we proceeded to pull-through. These results are summarized in a flowchart [Figure 3].

Therefore, with the help of a doughnut biopsy a TZPT was avoided in 42/54 patients (77.8%). In this group normal bowel was identified following a single doughnut biopsy in 37 patients (68.5%), a second doughnut in four patients (7.4%) and a third doughnut in one patient (1.9%). In 10/54 patients (18.5%) a pull-through was undertaken despite the findings of TZ features. Lastly in 2/54 patients (3.7%) a pull-through was performed without resorting to a further doughnut biopsy on the basis of other findings - we deviated from policy in one patient and in the other patient total colectomy and ileal pull-through was performed on the basis of a normal ileal doughnut taken at the time of earlier stoma formation.

In 10/54 patients (18.5%) where a TZ was accepted and pull-through was performed without proceeding to further, more proximal doughnut biopsies, this was undertaken following the initial or first doughnut in eight patients and after a second doughnut in two patients. We reviewed the nature of the TZ in these ten doughnut specimens, and the findings are listed in Table 1.

![Figure 2: Thick nerve trunks in the myenteric plexus (arrows). A ganglion cell is also present next to one of the thick nerve trunks (star) (H and E, ×20)](image)

**Table 1: Subjective details of transition zone in doughnut biopsies of 10 patients where this was accepted as level of pull-through**

| Number | Features of minimal transition zone in doughnut biopsy where transition zone pull-through was performed |
|--------|--------------------------------------------------------------------------------------------------|
| 1      | CG with 1 thick nerve                                                                            |
| 2      | CG with some thick nerves                                                                       |
| 3      | CG with 1 thick nerve                                                                            |
| 4      | CG with thick nerves in 1 quadrant                                                               |
| 5      | CG with occasional thickened nerves                                                             |
| 6      | CG but still in keeping with transitional area                                                  |
| 7      | CG with 1 thick nerve                                                                            |
| 8      | CG with 1 thick nerve                                                                            |
| 9**    | CG with 2 thick nerves                                                                           |
| 10**   | CG with 1 thick nerve                                                                            |

CG: Circumferential ganglionosis, **Second, more proximal doughnuts
In other words, where the initial “single-point” biopsy was “normal,” the use of a doughnut biopsy helped to identify a TZPT in 17/54 patients (31.5%). While a TZPT was performed in 10/54 patients (18.5%), it was avoided in 6/54 patients (11.1%); in one patient (1.9%), we deviated from policy.

**DISCUSSION**

Postoperative complications in the form of persistent features of obstruction after a pull-through for HD are well recognized, and a TZPT is always considered in the diagnostic work-up of these patients. Efforts, therefore, have been directed toward avoiding a TZPT at operation by accurate identification of the features of the colon at the level of pull-through and ensuring that “normal” bowel is used. On the contrary, it is well recognized that children can suffer poor outcomes despite avoiding a TZPT due to a variety of other reasons.

Over the decades, while histopathologists have studied the TZ in detail and described its features and complexity,[1,3,8,12] pediatric surgeons have sought ways to provide better samples[5] for the histopathologists to study and more accurately identify and differentiate TZ from aganglionic and normal bowel. This has not proved easy however. Recent reports still show an incidence of TZPT ranging from 16% to 25.4%.[6,7] While some authors[6,7] have recommended the routine use of a doughnut biopsy at pull-through surgery, it would appear that this practice is not widespread as a literature search has not yielded any publications on the institutional or departmental use of this technique for all cases.

**Histopathological considerations**

There have been several studies[1,2,8,9,12] that have studied the histological and immunohistochemical features of the TZ and attempted to establish standardized criteria for its identification and to delineate its extent. The asymmetric nature of the transition from aganglionic to ganglionic bowel in HD was first reported by Gherardi[2] in 1960. In a review of the literature on publications that studied the features of the TZ, Kapur and Kennedy[3] have highlighted the complexity of trying to precisely define the features of the TZ for comparison.
of reports from several studies. In particular, they have highlighted the importance of three features when trying to identify a TZ on IOFS: subcircumferential aganglionosis, hypoganglionosis, and abnormal extrinsic ("hypertrophic") innervations – all of which can only be achieved by taking a circumferential, full-thickness doughnut biopsy of colon [Figure 4]. Other studies[1] have recommended that a length of >3 cm of colon be excised from the proximal extent of TZ bowel in order to minimize the risk of TZPT and described the limitations of performing elaborate studies of gut innervation during a surgical procedure; H and E staining-based approach seems best suited for IOFS.

It is our impression that the role of an experienced histopathologist in interpreting the biopsy material and guiding surgical decision-making is crucial.

**Our results**

We consider this report as the first institutional review of the practice of the routine and consistent use of the doughnut biopsy for IOFS assessment during pull-through surgery for HD. Our review demonstrates a varying response to the finding of a TZ in a doughnut biopsy. Table 1 lists the features of the 10 instances where the doughnut showed ‘minimal’ features of TZ and a pull-through was undertaken despite this finding. In retrospect, where features of TZ were confined to <25% of the circumference or were reported as ‘one or two thick nerves’, this was accepted for pull-through, rather than taking further proximal specimens; ganglion cells were present throughout in all these specimens. Though not recorded for each instance, subjective on-table considerations (duration of operation, level of pull-through, extent of potential bowel resection) have led us to the deliberate performance of a TZPT in some patients where the features of TZ were considered ‘minimal’. In other words, a rigid practice of avoiding all features of a TZ before proceeding to a pull-through has not been practicable; this has to be balanced against the length of colon to be sacrificed in trying to avoid all features of TZ, the circumferential extent and the subjective/descriptive nature of TZ and length of operating time this would entail.

Therefore, by routinely using a doughnut biopsy a TZPT has been avoided in 6/54 patients (11.1%) in whom the initial ‘single point’ biopsy was reported as ‘normal’. Secondly, the surgical team is aware of the nature of the ‘minimal’ TZ where used and this informs expectations of functional outcome, counselling of parents and prognostication. Given that the length of bowel resected in patients who underwent a TZPT in our series was a mean of 6cm, perhaps we could conclude that ‘large’ resections were avoided. This is in line with the recommendations of excising >2cm of proximal bowel by White and Langer[1], or >5cm by Kapur[12] and Coyle[4], on the basis of their studies, in order to avoid a TZPT.

Such a pragmatic approach to the report of “minimal TZ” on IOFS is supported by reports in the literature of the limitations of IOFS in accurately determining the features of HD or normal bowel innervation; Shayan et al.[9] reported a 3% (9/304) discrepancy between IOFS and permanent sections in a review of their HD patients. White and Langer[1] and Gherardi[2] have demonstrated the non-uniform distribution and density of ganglia around the circumference of the bowel and between the submucosal and myenteric plexuses. Given, in addition, the subjective aspect of the technical methods of preparing and reporting IOFS specimens, which are dependent on the expertise and experience of the pathology technician and the pathologist, respectively, a “practical” approach rather than trying to achieve complete avoidance of TZ features makes sense.

Globally, the implementation of a policy of routine use of IOFS assessment may not be feasible. This could be due to lack of resources or infrastructure and/or the non-availability of an experienced pathologist. Even in these situations, if a doughnut biopsy of the bowel is taken at the level of stoma formation – instead of a “single-point” or partial circumference biopsy – and sent for paraffin section processing, the incidence of identifying a TZ would certainly be increased and eventually reduce the incidence of TZPT.

**Figure 4:** Photo of specimen of doughnut of colon taken for intraoperative frozen section assessment. The proximal margin is marked at the antimesenteric point with a suture for orientation
Limitations of this study

• Retrospective study with lack of a standardized definition of TZ—assessment of “thick nerves” and density of ganglionosis
• Different pathologists were involved in the interpretation of IOFS specimens depending on duty rotas introducing an element of individual bias
• The acceptance of “minimal” features of TZ for pull-through was not predefined and subjective

Future considerations

In our review of the histology specimens and reports for this study, we noticed the need for a standardized method of reporting the findings of all histology studies related to the assessment of HD patients. Given this lack of a standardized method and the intrinsic complexity and variations of the histology of HD, especially the features of a TZ, errors in interpretation are likely and would invariably lead to TZPT and clinical morbidity. As in other fields of medicine, the conceptual and practical challenge in interpreting and reporting the histological findings in HD, even in experienced hands, is to quantify the qualitative in order to reduce error and enhance accuracy in decision-making.

Clinicians and parents would be interested to know if such an approach of using the doughnut results in an overall reduction in morbidity related to pull-through surgery for HD. This can only be answered if there is widespread and consistent adoption of this technique and standardization in reporting is achieved.

It would be interesting to compare the functional outcome between those patients in whom TZ was accepted as the level of pull-through and those patients in whom bowel was considered normal at the level of pull-through. This study did not have enough patients available for bowel function assessment to enable meaningful comparison. This is a potential subject for future analysis.

Conclusions

We recommend the routine use of full-thickness, circumferential doughnut biopsy for IOFS assessment in all cases of pull-through for HD. We recognize that, because of other considerations, complete avoidance of TZPT is not always possible. However, when routinely used, the surgeon and histopathologist can work together to minimize the impact of TZPT and are fully aware of the instances of “minimal” TZPT, and this enables a realistic assessment of the situation for each individual patient. We also recommend the use of a doughnut biopsy at level of stoma formation where IOFS facilities are unavailable to reduce the overall incidence of TZPT.

Acknowledgments

We would like to thank Mr Alan Drummond in the histopathology department for providing the reports and slides for review and Mr Paul Arnold for explaining the technical considerations of processing a doughnut biopsy for frozen section assessment.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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