

Rectal suction biopsy versus incisional rectal biopsy in the diagnosis of Hirschsprung Disease

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Abstract

Background

Hirschsprung disease is one of the most common congenital anomalies that affects colorectal function. Rectal biopsy demonstrating the absence of ganglion cells in the affected bowel is the gold standard for diagnosis. Suction and incisional rectal biopsies are appropriate methods for obtaining diagnostic tissue. The goal of this study is to determine if any differences in adequacy exist between suction and incisional rectal biopsies at our institution.

Methods

We conducted a retrospective review of suction and incisional rectal biopsies for inadequacy per procedure at a tertiary pediatric hospital. Each procedure for rectal biopsy was also evaluated by number of biopsies per procedure. We used two-sample test of proportions to compare inadequacy of suction vs. incisional biopsies.

Results

133 rectal suction biopsy procedures (227 biopsies) and 125 incisional biopsy procedures (140 biopsies) were analyzed. In patients 6 months of age and older, the percentage of inadequate procedures was substantially higher in the suction biopsy group (24.1% vs 0.9%, $p < 0.01$).

Conclusions

A substantially higher proportion of inadequacy was found in suction rectal biopsy group compared to the incisional cohort among the older patient cohort, suggesting incisional biopsies should be strongly considered as primary rectal biopsy method in patients older than 6 months.

Introduction

Hirschsprung disease (HSCR) is characterized by the congenital absence of ganglion cells in the myenteric (Auerbach's) and submucosal (Meissner's) plexuses.[1] [2] Aganglionosis always involves the rectum and extends proximally involving a variable length of contiguous bowel.[1] The aganglionic segment is unable to relax during peristalsis, resulting in a functional obstruction that often presents in infancy with symptoms of abdominal distension, feeding difficulties, failure to pass meconium, and often bilious emesis.[3] [4] With an incidence of 1 in 5000 live births, [4] [5] [6] HSCR represents one of the most common congenital anomalies of colorectal function.[7] Although commonly diagnosed in the neonatal period, a proportion will develop symptoms later in life as infants or during childhood.[1] [8] While other studies can be used for diagnostic screening, rectal biopsy for histologic confirmation of absence of

ganglion cells in the myenteric and submucosal plexus of the affected bowel is the gold standard for diagnosing HSCR.[1] [5] The procedure for biopsy typically involves sampling a segment of the posterior rectal wall by either suction or open incisional biopsy methods.

Suction rectal biopsy and incisional rectal biopsy are both considered appropriate methods for obtaining tissue for diagnosis of HSCR. However, the rate of inadequate suction rectal biopsy has been demonstrated to be significant with a recent systematic review of the literature estimating 12% diagnostic insufficiency associated with suction biopsy.[9] Practice at our institution has generally been to perform suction rectal biopsy in infants and younger children, and incisional biopsy on older children based on the potential likelihood of increased inadequacy rate associated with suction biopsy with older age patients. [10] [11] Currently, no universal standard or protocol exists for age at which suction vs incisional biopsy should be performed and the literature is variable. Our institutional practice has been to perform incisional rectal biopsies in patients older than 12 months and in some patients age 6-12-months depending on specific surgeon preference. This preference towards incisional biopsies in older children derives from the assumption that this practice may reduce inadequacy rates and therefore avoid repeat procedures.

The primary aim of this study was to investigate HSCR diagnostic practices and to determine if the frequency of inadequate rectal suction biopsies indeed increases with patient age, and if so, if any guidance for age at which incisional over suction rectal biopsy as the initial diagnostic procedure should be performed. A secondary aim was to determine the percentage of our “full thickness” biopsies that were truly pathologically full thickness (including myenteric and submucosal plexuses) and to compare any adequacy differences between true full thickness and partial thickness incisional biopsies. Finally, suction rectal biopsies were historically performed by both surgeons and gastroenterologists at our institution and we sought to determine if any variability existed depending on the provider performing the procedure.

Methods

This retrospective study was reviewed and approved by the Seattle Children’s Hospital (SCH) Institutional Review Board IRB STUDY#00001322. Patients were identified through review of the SCH pathology database. Archived sections and surgical pathology reports from suction rectal biopsies and “full-thickness”, or incisional, rectal biopsies performed between January 2000 and December 2018 were retrieved. These rectal biopsies included procedures performed at SCH (n = 95), as well as those done elsewhere, but sent to SCH for pathology review (n = 31). At our institution, in 2010 gastroenterologists stopped performing biopsies for diagnosis of HSCR biopsies. Therefore, our analysis of suction biopsies was limited to those acquired between 2000 and 2010, so variability between providers performing biopsy could be assessed. Patients were excluded if they had a diagnosis of HSCR prior to biopsy or if the rectal biopsy was performed for reasons other than evaluation for HSCR.

The medical records were reviewed for the following clinical data: age at time of biopsy, gender, history of prematurity, and indication for biopsy (obtained from operative or procedure report or in documentation immediately preceding the biopsy). Pathology reports were reviewed for stated location of biopsy (cm from dentate line), number of tissue pieces, performing service (surgery or GI) and whether the biopsy was performed at an institution other than SCH. The specimens were assessed for presence of submucosal and myenteric ganglion cells, presence of hypertrophic nerves, whether acetylcholinesterase enzyme histochemistry or calretinin or choline transporter immunohistochemistry was performed, and the original interpretation of the stains. For incisional biopsies, the layer depth of the biopsy was recorded to determine if the biopsy was truly “full-thickness”, consisting of submucosa, muscularis interna, myenteric plexus and, in most cases, muscularis externa versus “partial full-thickness”, consisting of submucosa alone or only submucosa and muscularis interna. Specimens were assessed for adequacy for diagnosis of HSCR, and if inadequate, the reason for inadequacy was noted.

The number of inadequate biopsies and inadequate procedures were determined for both incisional and rectal suction biopsies. An inadequate procedure was defined as having no diagnostically adequate biopsies for that patient during the procedure. The number and percentage of inadequate biopsies and procedures were calculated for age categories < 6 months vs. ≥ 6 months. Two-sample test of proportions was used to compare inadequacy between suction and incisional biopsies in patients 6 months or older at time of biopsy. Unadjusted GEE modeling with logit link function (equivalent to logistic regression) and robust standard errors to account for patient-level correlations was used to compare inadequacy between age categories for suction biopsies and procedures.

Among suction biopsies, 2-sample test of proportions was also used to compare inadequacy of multiple-biopsy procedures to single-biopsy procedures, inadequacy of biopsies with and without calretinin stain, and inadequacy of biopsies performed by a gastroenterologist vs. a surgeon. Among the incisional biopsies, 2-sample test of proportions were used to compare inadequacy for those that were truly full-thickness (included myenteric plexus) to “partial full-thickness” (insufficient depth to include myenteric plexus) biopsies. As many biopsies labeled as “full-thickness” by the surgeon were actually “partial thickness,” the term “incisional biopsy” is used to denote all rectal biopsy specimens procured in this manner and “full-thickness” when referring to histological inclusion of myenteric plexus.

Results

One hundred and twenty-six patients underwent an incisional rectal biopsy and 129 underwent rectal suction biopsy. Of note, 1 patient in the incisional group and 4 patients in the suction biopsy group had multiple biopsy procedures. Gender distribution was similar in the two groups with males comprising 60% of the incisional group and 58% of the suction biopsy group. Median age at biopsy was 62 months (IQR 23, 118) in the incisional group and 26 months (IQR 4, 109) in the suction biopsy group. The most common indication for incisional rectal biopsy was constipation (84%). In the suction biopsy group, concern for bowel obstruction (including abdominal distension and/or vomiting) was the most common indication (67%). One hundred and five (79%) of the suction biopsy procedures were performed by the

surgery team; all others were performed by gastroenterologists. All incisional biopsies were performed by surgeons. (Table 1)

Table 1
Demographic characteristics

	Full-Thickness Biopsy	Suction Biopsies
n	126	129
Male, n (%)	75 (60)	75 (58)
Age in months at biopsy, median (IQR)	62 (23, 118)	0 (0, 3)
Age in days at biopsy, median (IQR)	N/A	26 (4, 109)
Gestation, n (%)	71 (56)	75 (58)
≥ 37 weeks	18 (14)	39 (30)
< 37 weeks	37 (29)	15 (12)
Unknown		
Indication, n (%)	106 (84)	59 (46)
Constipation		
Failure to pass meconium/delayed meconium	9 (7)	41 (32)
Fecal incontinence	31 (25)	0 (0)
Abnormal barium enema	11 (9)	26 (20)
Signs of Bowel obstruction (including abdominal distension and/or vomiting)	21 (17)	86 (67)
Abnormal Anorectal Manometry	13 (10)	1 (1)
Enterocolitis	0 (0)	1 (1)
Case location, n (%)	31 (25)	
Outside SCH	95 (75)	
SCH		
Biopsy encounter performed by surgery service, n (%)	125 (100)	105 (79)

Suction rectal biopsy

A total of 227 rectal suction biopsies were obtained with 133 individual suction biopsy procedures, as some patients received multiple biopsies during a single procedure. A total of 28 inadequate biopsies (28/227, 12.3%) and 10 inadequate procedures (10/133, 7.5%) were identified in the suction biopsy

group. The proportion of inadequate biopsies was higher for those performed by a gastroenterologist compared to those performed by a surgeon (10/38, 26.3% vs. 18/189, 9.5%, $p < 0.01$). The proportion of inadequate biopsies for those < 6 months was 7.6% compared to 33.3% in those ≥ 6 months, with an odds ratio of 7.3, (95% C.I. 2.8, 18.9), $p < 0.01$. Inadequacy decreased at the procedure level but was still higher in the ≥ 6 -month cohort: 24.1% compared with 2.9%, odds ratio 12.2, (95% C.I. 2.8, 53.3) $p < 0.01$ (Table 2, Fig. 1).

Table 2
Inadequacy vs Age for Rectal Suction Biopsies

Biopsy (N = 227)				
	% (n/N)	OR*	95% CI	p
	inadequate			
< 6 months (reference)	7.6% (14/185)	-	-	
≥ 6 months	33.3% (14/42)	7.3	2.8, 18.9	< 0.01
Procedure (N = 133)				
		OR*	95% CI	p
< 6 months (reference)	2.9% (3/104)	-	-	
≥ 6 months	24.1% (7/29)	12.2	2.8, 53.3	< 0.01
* OR, 95% CI and p values from unadjusted GEE models with robust standard errors to account for patient-level correlation				

Adequacy was also evaluated related to number of suction rectal biopsies per procedure (Table 3). Eighty-one of the suction rectal biopsy procedures only consisted of a single biopsy, 10 procedures consisted of 2 biopsies, and 42 consisted of 3 biopsies during the same procedure. The percent of inadequate procedures were 11.1%, 0% and 2.4% for 1, 2 and 3 biopsy procedures respectively. More than one biopsy during a procedure decreased the percent inadequacy of the suction biopsy procedure (11.1% (1 biopsy) to 1.9% (2 or 3 biopsies per procedure) ($p < 0.05$)). Calretinin staining was performed on 30 of the suction biopsies. None of the biopsies with calretinin were determined to be inadequate, compared to 28 of 197 (14%) without calretinin ($p = 0.03$) (Table 4.)

Table 3
Inadequacy by number of suction biopsies per procedure

	1 biopsy	2 biopsies	3 biopsies	> 1 biopsy (2 or 3)	p*
Percent (95% CI, n/N) inadequate procedures	11.1 (6.0-19.8, 9/81)	0.0 (0.0-27.8, 0/10)	2.4 (0.4-12.3, 1/42)	1.9 (0.3-10.1, 1/52)	0.05
*From 2-sample test of proportions comparing > 1 biopsy to 1 biopsy (same as p value from Chi squared test)					

Table 4
Inadequacy of suction biopsies by calretinin staining

	No calretinin	With calretinin	p*
Percent (95% CI, n/N) inadequate biopsies	14.2 (10.0-19.8, 28/197)	0.0 (0.0-11.4, 0/30)	0.03
*From 2-sample test of proportions comparing biopsies with and without calretinin stain (same as p value from Chi squared test)			

Gastroenterology vs Surgeon performing suction rectal biopsy

A sub-analysis was performed to evaluate differences between procedures performed by surgeons compared to gastroenterologists. One hundred and five suction biopsy procedures were performed by the surgery service and 28 were performed by a gastroenterologist. Sixty-one percent of the suction biopsy procedures performed by gastroenterologists were in those 6 months of age or older, versus 11% in those performed by surgeons. The surgeons were also more likely to take multiple biopsies during the procedure (44% compared to 21%). Inadequacy was higher among suction biopsy procedures performed by gastroenterologists (21% vs 4%; $p < 0.01$) (Table 5). Based on the above differences, we conducted an additional analysis omitting the suction biopsies performed by gastroenterologists to see if there was a significant difference in adequacy rates for those older than 6 months. With GI biopsies excluded, the proportion of inadequate biopsies for those < 6 months was 7.7% compared to 25.0% in those ≥ 6 months of age, odds ratio 5.7, (95% C.I. 1.6, 20.7), $p = 0.01$.

Table 5
Patient and biopsy procedure characteristics by service

	Service performing biopsies	
	GI	Surgery
	N = 28 procedures	N = 105 procedures
Male, n (%)	16 (57)	61 (58)
Age in months at biopsy, median (IQR)	12.5 (2.5, 42)	0 (0,1)
≥ 6 months at biopsy, n (%)	17 (61)	12 (11)
Multiple biopsies per procedure, n (%)	6 (21)	46 (44)
Inadequate procedure, n (%)	6 (21)	4 (4)
Procedure dates, median	10/28/2005	12/4/2006
Procedure dates, range	2/25/2003, 12/19/2008	5/14/2003, 12/17/2008
Calretinin stain	1/38 biopsies (3%)	29/189 biopsies (15%)

Incisional biopsy

A total of 140 incisional biopsies were performed (14 of those in patients < 6 months of age) and these corresponded to a total of 126 incisional biopsy procedures (13 encounters for those < 6 months). There was only 1 inadequate procedure (7.7%) in the < 6-month age group, consisting of a single biopsy (7.1%). In those ≥ 6-months there were 3 inadequate incisional biopsies (2.4%) and only 1 inadequate procedure (0.9%) (Fig. 2).

Seventy-five of the incisional biopsies were classified as “true full-thickness”. Among this group were 5 biopsies which included submucosa, muscularis interna, myenteric plexus, but not muscularis externa. Fifty-nine of the biopsies were “partial full-thickness” and for 5 of the biopsies the tissue layers were not defined. None of the “true full-thickness” biopsies were inadequate. 5.1% (3/59), (C.I. 1.7–13.9) of the “partial full-thickness” biopsies were inadequate (p = 0.05) (Table 6).

Table 6
Inadequacy of full-thickness biopsies by depth (“true” vs “partial” full-thickness)

	“True” full thickness	“Partial” full thickness	p*
Percent (95% CI, n/N) inadequate biopsies	0.0 (0.0-4.9, 0/75)	5.1 (1.7–13.9, 3/59)	0.05
*From 2-sample test of proportions comparing biopsies of “true” vs “partial” full thickness			

Rectal suction biopsy vs. Incisional biopsy

In patients 6 months of age or older, the percentage of inadequate biopsies and procedures was substantially higher in the suction biopsy group as compared to the incisional biopsy group for both the biopsy (33.3% vs. 2.4%, p < 0.01) and overall procedure adequacy (24.1% vs. 0.9%, p < 0.01) levels (Table 7).

Table 7
Inadequacy in full-thickness vs. suction biopsies for cases ≥ 6 months of age at biopsy

	Suction	Full Thickness	p*
Percent (95% CI, n/N) inadequate biopsies	33.3 (21.0-48.4, 14/42)	2.4 (0.8–6.8, 3/126)	< 0.01
Percent (95% CI, n/N) inadequate encounters	24.1 (12.2–42.1, 7/29)	0.9 (0.2–4.8, 1/113)	< 0.01
*From 2-sample test of proportions comparing inadequate proportion in suction vs. full-thickness biopsies			

Discussion

This study sought to evaluate and better understand the diagnostic practices surrounding suction and incisional rectal biopsy for diagnosis of HSCR at our institution and to specifically examine adequacy of biopsy related to type of rectal biopsy performed and its relationship to the age of the patient. While in

practice, proceduralists tend to move towards open incisional biopsy in older patient populations, there is no consensus or best practice guideline for determining the specific age of this transition.

Previous investigators have examined the relationship of age and suction vs incisional rectal biopsy. Croffie et al. performed a prospective study in children over 1 year of age, aiming to determine at what age suction rectal biopsy (versus incisional biopsy) was more likely to be inadequate due to insufficient submucosal tissue to detect ganglion cells.[11] However in this study, all biopsies were performed under general anesthesia, which differs from our institutional practice of performing suction rectal biopsies under 6 months old without sedation in clinic. Additionally, while the incisional biopsy was superior to suction biopsy at all ages in terms of providing an adequate sample size, in the 1-3-year-old patient population there was no significant difference between the incisional and suction biopsy with regard to adequacy of the sample. Muise et al. also evaluated suction vs incisional rectal biopsy and the relationship with age of the patient.[12] They conducted a retrospective comparison of suction and full-thickness rectal biopsy in 47 infants and children undergoing work up for HSCR. The results were compared between patients greater vs less than 12 months of age. The authors did not identify a significant difference in adequacy based on technique or age, however, all of the older patients had their biopsies performed in the operating room, regardless of the type of biopsy performed. In contrast, our institution generally uses a 6-month age to move towards open incisional rectal biopsy and again, suction rectal biopsies are performed without sedation or general anesthesia.

In our study, we sought to examine whether our current practice of recommending incisional biopsies for older patients, due to the assumption that a suction biopsy may be more likely to be inadequate in older patients, is warranted. Our review included biopsy results for 225 patients, with an even distribution between incisional and suction rectal biopsies. The patients in the incisional group were, on average, older at time of biopsy, likely reflecting a pre-existing trend at our center towards performing incisional biopsies on older patients. Our study found a substantially higher inadequacy rate in the suction biopsy group compared to the incisional cohort among older patients, those over 6 months of age. Among the suction biopsies alone, inadequacy rates were significantly higher at both the biopsy and procedure level for those patients 6 months and older.

Potential explanations for the increase in inadequacy seen in suction biopsies over 6 months include patient cooperation, as suction biopsies are performed unsedated at our institution and older, larger infants are less likely to comply with the procedure. The findings in our review may also be explained by the decreased density of ganglion cells in the submucosa that is seen with age. Furthermore, older children, especially those with chronic constipation, likely have a larger rectal vault, thus obtaining an adequate mucosal sample can be more difficult when performing a suction biopsy. This may necessitate larger biopsies in older children to achieve an equivalent adequate result.[9] [10] [11]

In the present study we demonstrated that taking more than one biopsy (multiple passes) during a suction rectal biopsy procedure also greatly decreased the chances of an inadequate biopsy. The addition of calretinin staining as a means of reducing inadequacy rates is also important to note, as none of the

suction biopsies where calretinin staining was performed were inadequate compared to 14% of those without calretinin staining. However, most suction biopsies were deliberately selected from an era (prior to 2009), which pre-dated both the simultaneous introduction of calretinin staining at our institution and shift towards incisional biopsies for patients > 6 months of age. Therefore, it is difficult to draw any firm conclusions about the effect of calretinin immunohistochemistry on reducing inadequacy rates for suction biopsies from older patients. Additionally, the reason for inadequacy in almost half of the suction biopsies was that the sample was performed at too distal a location in the anal canal and captured anal mucosa. In these cases, calretinin would not have provided additional value. However this finding further supports the notion that more than one level of suction biopsy would be of benefit to decrease the chance of inadequacy.

Practice at our institution has shifted over time to rectal suction biopsies being universally performed by a surgical provider. As seen in our results, higher inadequacy rates were noted among gastroenterologists performing suction biopsies. This may be explained by an older median patient age in the group of patients who had biopsies performed by gastroenterologists. Gastroenterologists were also less likely to obtain multiple biopsies during the procedure when compared to surgeons. This is consistent with results reported by Stewart et al., who demonstrated children undergoing a suction biopsy by a pediatric surgeon were significantly younger when compared to the age of the patients biopsied by a gastroenterologist and surgeons typically yielded a higher biopsy adequacy rate.[13] In our current practice, the suction rectal biopsy technique is performed uniformly by general surgeons with a specialized rectal biopsy instrument. Biopsies are also always taken at three levels and utilizes a specific cartridge that allows for measurement of the biopsy site within the anal canal.

For incisional biopsies, inadequacy was low for both the < 6-month age-group and for the older cohort. Inadequacy was more frequent in the “partial full-thickness” group (containing only submucosa or submucosa plus muscularis interna) compared to the “true full-thickness” group. However, the numbers were relatively small and therefore these results may not be broadly generalizable. Our analysis only focused on evaluation for HSCR and the potential additional diagnostic value (e.g., exclusion of non-Hirschsprung neuromuscular pathology) offered by a true full-thickness versus partial full-thickness biopsy was not addressed.

Limitations of the study include the retrospective design and therefore, the reliance on documentation in the medical record. While the specific technique described previously for suction rectal biopsy is generally accepted at our institution, due to the retrospective nature we are unable to determine if the suction biopsies were performed with the correct method. However, the goal of this study was to assess outcomes at our institution and to determine if these results support current diagnostic practices, particularly recommending incisional biopsies in older patients.

A secondary limitation lies with the patient populations identified spanning slightly different time periods, 1/2003-12/2008 for suction rectal biopsy patients and 1/2000-12/2018 for incisional rectal biopsy patients. The suction rectal biopsy patients were intentionally limited to the pre-2009 era after which our

institution shifted to surgeons only performing these biopsies. Additional changes at this time at our institution included three biopsies performed per suction rectal biopsy procedure, routine calretinin staining, and a shift to primarily full thickness rectal biopsies after 6 months of age. As our aim was to investigate the role of many of these variables independently, we elected to evaluate suction biopsies prior to the establishment of these standards. Incisional biopsies both pre- and post-2009 were performed by surgeons from a single site, and therefore, extending the inclusion period forward through 2018 allowed us to increase the number of incisional biopsies without compromising the investigation. In order to address the limitation of retrospective data collection, a single pathologist re-reviewed all the inadequate biopsies to mitigate any potential confounding that could have resulted from pathologic analysis being performed by multiple pathologists. While our initial sample size was moderately large, inadequacy rates, especially for the incisional biopsies were low, making it difficult to draw firm conclusions. It is worth noting that most of our suction biopsies came from one institution (a tertiary pediatric care center) with high surgical and pathology expertise relating to HSCR. It is likely that experience at other centers and standards for number of biopsies, number of histologic sections, use of ancillary studies and diagnostic threshold for inadequacy may differ, which may reduce the generalizability of some of our results and conclusions.

In conclusion, adequacy of rectal biopsies for the diagnosis of HSCR was influenced by the age of the patient, the service performing the biopsy, and the type of biopsy (incisional vs. suction). The rate of inadequate rectal biopsies was low with incisional biopsies in all age groups. At our institution, incisional rectal biopsies were superior to rectal suction biopsies in children greater than 6 months of age. Prospective longitudinal studies with a larger sample size are needed to further validate these findings.

Declarations

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Figures

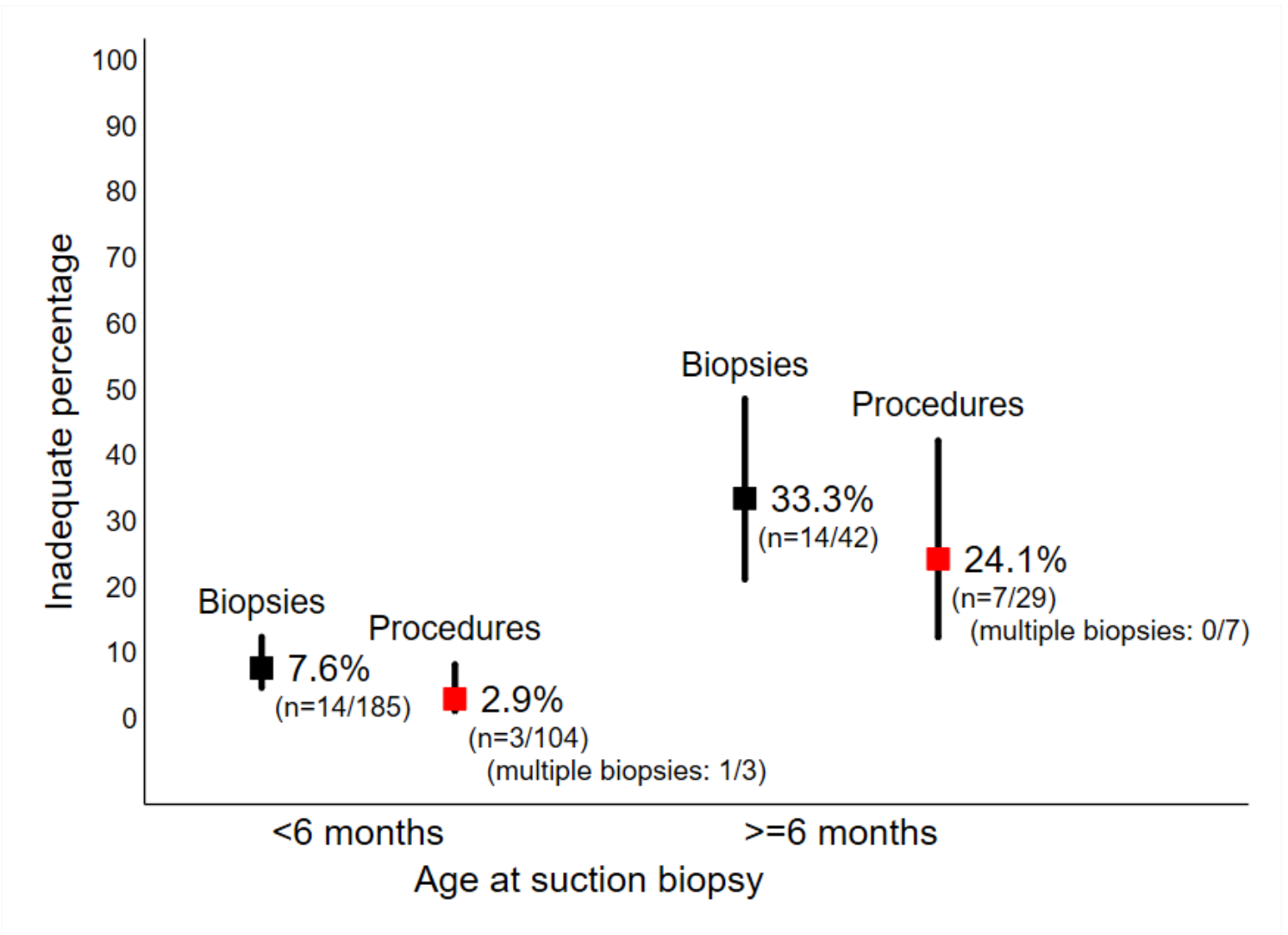


Figure 1

Inadequacy vs. age: suction biopsies: *Inadequacy by age categories <6 months (reference) vs. ≥6 months*

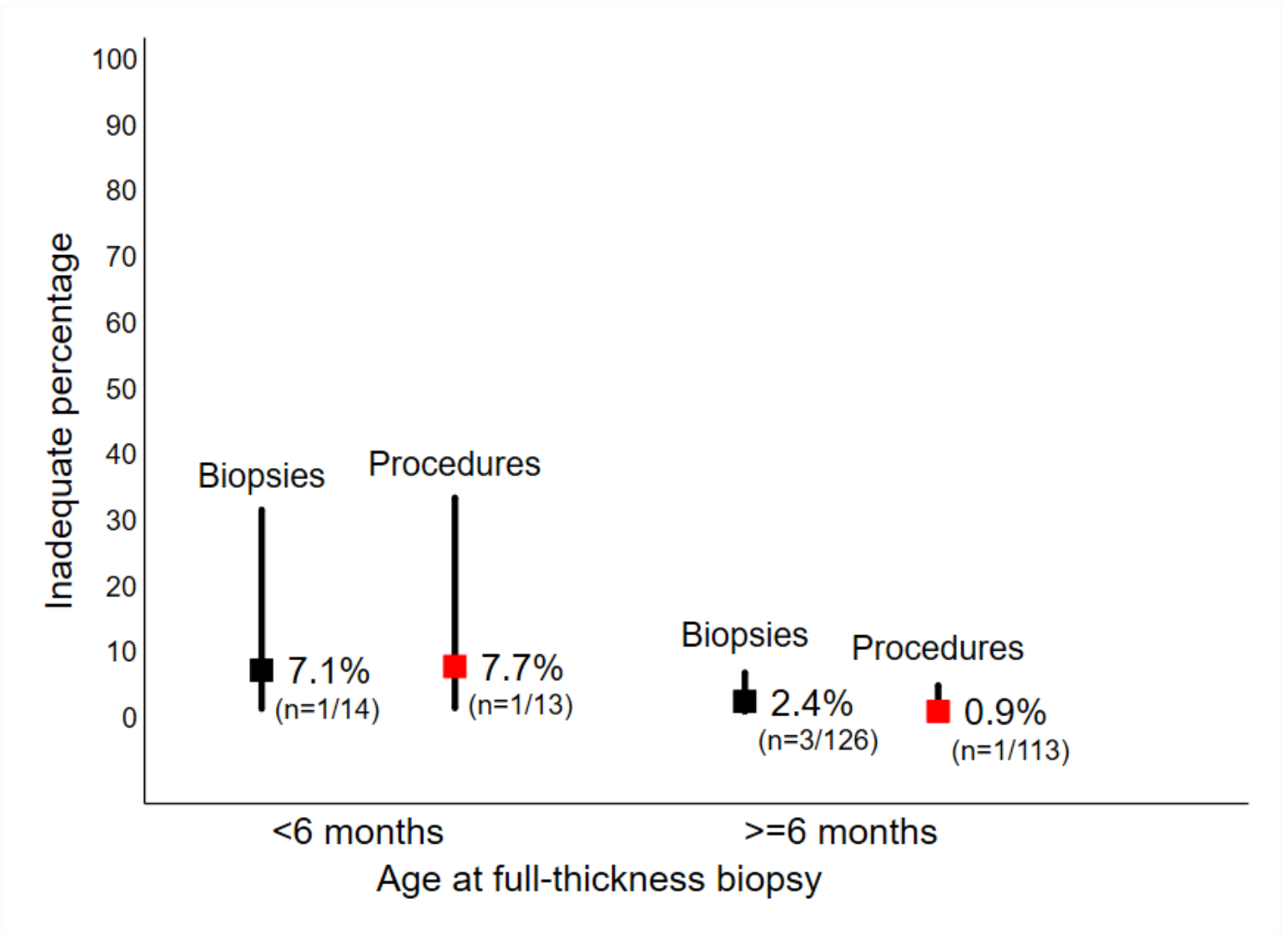


Figure 2

Inadequacy vs Age for Full-Thickness Biopsies