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Rapid diagnosis of intrapartum group B streptococcal carriage by fluorescent in situ hybridisation

Group B streptococcus (GBS) still causes considerable neonatal morbidity and mortality. Early-onset infections (appearing within seven days of birth) account for about 80% of infections and are thought to arise from contact with the organism in the maternal genital tract during delivery.¹

Data from the United States in the 1970s estimated an incidence rate of greater than 2 per 1000 live births, and case fatality rates approaching 50%.² However, the use of intrapartum prophylaxis with antibiotics led to a 70% decline in GBS disease during the 1990s.³

In the UK, a recent study found an incidence of early-onset sepsis exceeding 0.72 per 1000 live births, with a mortality of 9.7%, rising to 15% in premature infants.⁴

Controversy remains about the optimal strategy for prevention. In the United States, the delivery of intrapartum antibiotics to at-risk mothers has shifted from a risk-based approach to one based on a universal culture-based screen for vaginal and rectal GBS carriage at 35–37 weeks' gestation.⁵ All women found to be carriers at 35–37 weeks (or labouring before this time) are offered prophylaxis. In the UK, routine screening is not recommended as there are doubts about delivery and cost effectiveness; instead, a risk factor-based approach is advocated.⁶ Intrapartum antibiotic prophylaxis is offered to all women with recognised risk factors for early onset GBS disease, namely, a previous baby affected by GBS, GBS bacteriuria detected during current pregnancy, preterm labour, prolonged rupture of membranes and fever in labour.⁶ In the US, women presenting to maternity services at the onset of labour who have not have been screened are also managed using this approach.⁵

An alternative strategy might be to employ a rapid test for the detection of GBS colonisation at the time of onset of labour or rupture of membranes. If the test was as sensitive as culture, rapid enough to allow prophylaxis to be given promptly and simple enough to be available around the clock in all hospitals, it could replace either a culture-based or risk-based scheme.⁵ However, such tests have proved elusive. Rapid immunoassays have not proved to be sufficiently sensitive, and although molecular techniques are available, they are expensive and require an infrastructure and expertise beyond the reach of many units.^{7,8}

Currently, on our maternity unit, routine GBS screening is undertaken only in high-risk labours, namely women with prelabour rupture of membranes (whether this occurs before or after 37 weeks' gestation) or women in preterm labour or who are suspected of being in preterm labour. Swabs are taken on presentation or at the onset of labour and are processed by conventional culture methods. Although some positive results are available after 18–24 hours, many take up to 48 hours, and they are frequently too late to direct intrapartum antibiotic prophylaxis.

We compared fluorescent in-situ hybridisation (FISH) with conventional culture for GBS to see if this novel method might provide a reliable, rapid alternative.

Methods

Eighty intrapartum GBS screens (paired rectal and vaginal swabs) were processed in parallel by conventional culture (incorporating both direct and broth enrichment steps) and GBS FISH (Crefast, SeaPro Thermanostics, Liverpool, UK) as per manufacturer's guidelines.

Specimens were processed by FISH in batches of 20, which included positive and negative controls.

The paired swabs were applied directly on to the supplied slides, heat fixed through a Bunsen flame, dehydrated using serial alcohol washes and treated with lysozyme. DNA probes were then added and incubated in a humidified chamber to allow hybridisation for 90 minutes. The slides were then allowed to

air dry, anti-fade mounting was applied and they were read using an ultraviolet microscope.

FISH slides were blinded and read independently by two investigators and results were compared with those obtained on culture.

Results

A total of 24 of 80 GBS screens were positive by culture (30%) and 30 of 80 (37.5%) were positive by the FISH method (an average of the results from the two investigators).

A numerical breakdown of the FISH and culture results is as follows (it should be noted that the values given are the averages of the two investigators' results):

- culture positive, FISH positive: 13.5;
- culture positive, FISH negative: 13.5;
- culture negative, FISH positive: 27.5;
- culture negative, FISH negative: 33.

Positive and negative control slides were also examined. These consisted of pure cultures of either GBS (positive controls), or group A streptococci (negative controls). Of these pure cultures:

- positive controls: 4 out of 6 correct;
- negative controls: 8 out of 10 correct.

Using culture as our gold standard, the sensitivity of GBS FISH in our study was 50%; it showed 55% specificity. The calculated kappa value was 0.15. Agreement between the two investigators did not improve with greater experience in slide reading (no improved concordance between later and earlier sample batches).

In our hands, preparation, hybridisation and slide reading took approximately 5 hours per batch of samples.

Conclusions

In order to direct antibiotic prophylaxis for women presenting in labour, any system used to screen for GBS must be rapid enough to generate a result within the first stage of labour so that antibiotics can be given intrapartum as opposed to postpartum. It must also be able to show a high sensitivity and specificity in order to be a useful and reliable diagnostic tool. From the laboratory's point of view, as many investigations will be submitted outside the standard working day and the test is not automated, it should also not be excessively labour intensive to perform.

In our hands, the GBS FISH does not meet these requirements. With poor sensitivity and specificity coupled with a labour intensive specimen work up, we feel that this method is technically inferior to the selective enrichment culture currently employed in our laboratory. In addition, the poor concordance seen between operators adds an unacceptable variability to the interpretation of results. This is despite the fact that the results could be generated more rapidly than by culture and therefore direct clinical management.

With further development, the FISH technique could be more readily incorporated into current working practice, especially if it could be automated and the specimen workup time reduced. However, in order to become a useful investigation for intrapartum GBS screening, the intra-observer variability, sensitivity and specificity would need to be significantly improved.

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Histopathological features of gastrointestinal mucosal biopsy specimens in children with epidermolysis bullosa

Epidermolysis bullosa (EB) is a term used to represent a group of conditions characterised by cutaneous blistering due to abnormalities of ultrastructural components anchoring epithelial cells to either each other or to the basement membrane.^{1–3} There are a range of subtypes,

which were traditionally classified on the basis of the level of the epidermal separation, more recently delineated on the basis of the underlying genetic defect.⁴ The vast majority of the clinical literature in these conditions is based around the cutaneous complications. There is a reported association between some subtypes of epidermolysis bullosa and pyloric stenosis,⁴ but there are no previous reports of mucosal histopathological features, despite these patients sometimes exhibiting significant gastrointestinal symptoms.

We report the gastrointestinal mucosal histopathological features in a series of patients with epidermolysis bullosa who underwent endoscopic evaluation for gastrointestinal symptoms at a single specialist centre.

Methods

Since 2003 it has been our policy to investigate all children seen in the epidermolysis bullosa clinic with significant abdominal symptoms by upper and lower gastrointestinal endoscopic examination with mucosal biopsies. During this three-year period, all cases that underwent endoscopic examination with this indication were identified. The findings at the time of endoscopy were reviewed in addition to other clinical details such as basic demographic information, symptomatology, and the specific type of EB. The histopathological specimens were reviewed by a consultant paediatric pathologist who was blinded to the endoscopic findings and clinical details regarding the type of EB. Histological features of significance were recorded and compared to the clinical information. The study was approved by the local research ethics committee.

Results

During the study period there were nine patients identified in whom endoscopic examination had been carried out and mucosal biopsies performed. Table 1 presents demographic information, clinical details and a summary of the histopathological gastrointestinal findings. The mucosal biopsy specimens showed features ranging from no histological abnormalities to moderately severe inflammatory changes, such as an increase in lamina propria inflammatory cell density, including predominant eosinophils, and focal active

inflammation with neutrophils present in surface epithelium. In addition, a striking finding, noted in four of the nine cases, was an abundance of karyorrhectic cellular debris located within the lamina propria beneath the surface epithelium of the colonic biopsy specimens. All these changes were exhibited in biopsy specimens from children with recessive dystrophic EB subtype. Furthermore, in three cases, patchy granular brown, pigment-containing macrophages were also identified within the lamina propria, which stained positively with Perl's stain, indicating haemosiderin pigment deposition. In no case was morphologically apparent epidermal separation or clefting identified, and no morphological features indicating vasculitis could be seen.

Discussion

The findings of this study have documented, for the first time, the specific histological abnormalities identifiable on routine gastrointestinal mucosal biopsies in children with EB who experience gastrointestinal symptoms.⁵ The predominant pathological finding in the abnormal cases was varying degrees of inflammatory change. The mechanism of this inflammatory process in this clinical setting remains uncertain, but it is possible that the abnormal epithelial cell adhesion, both to other mucosal epithelial cells and to the underlying basement membrane, may facilitate passage of antigenic molecules across the mucosal surface to stimulate an inflammatory process. Alternatively, the underlying mechanism may be an autoimmune-type process, with basement membrane splitting resulting in exposure of normally "hidden" antigenic components and induction of autoimmunity. Furthermore, although only present in a proportion of the cases, where identified, a most striking finding was a florid increase in the amount of colonic subepithelial lamina propria karyorrhectic debris, with or without associated haemosiderin deposition. Such a feature may occasionally be seen in other clinical settings⁶ in otherwise unremarkable colonic mucosal biopsy specimens or those with inflammatory changes, but not to the extent encountered in this scenario. It is likely that this material represents cellular debris, presumably related to increased cell turnover at the epithelial–basement membrane junctional zone; such a feature may be

Table 1 Epidermolysis bullosa (EB) subtype, clinical gastrointestinal symptoms and summarised endoscopic and positive histopathological findings in a gastrointestinal mucosal biopsy series from nine children with EB

Case (age, sex)	EB type	Symptoms	Endoscopic findings	Histopathological findings
1 (14, M)	JEB	Diarrhoea, GOR, FTT	No villous structures seen	Duodenum: partial villous atrophy Colon: neutrophil polymorphs in epithelium and lamina propria. Mixed inflammatory infiltrate including eosinophils
2 (7, F)	EBS	FTT	Mild colitis	Colon: lamina propria debris ++
3 (6, F)	RD	Diarrhoea/constipation, GOR	Gastritis Mild colitis	Stomach: mild chronic inactive gastritis Colon: lamina propria debris ++
4 (7, F)	RD	Diarrhoea, GOR	Mild colitis	Colon: lamina propria debris ++
5 (7, F)	RD	Diarrhoea, GOR, FTT	Gastritis Normal colon	Stomach: chronic inactive gastritis Duodenum: increased lamina propria plasma cells and eosinophils Colon: borderline inflammatory change
6 (6, F)	RD	Diarrhoea, GOR	Mild pancolitis	Colon: increased eosinophils. Prominent lymphoid aggregates. Haemosiderin-laden macrophages
7 (6, F)	RD	Diarrhoea, abdominal pain	Mild pancolitis	Stomach: mild chronic inactive gastritis Colon: haemosiderin-laden macrophages, lamina propria debris++
8 (4, F)	Kindlers	Diarrhoea	Mild distal colitis	Colon: increased eosinophils
9 (7, F)	RD	Diarrhoea, abdominal pain	Mild colitis	Colon: increased eosinophils, haemosiderin-laden macrophages

EBS, EB Simplex; M, male; F, female; RD, recessive dystrophic; JEB, junctional; FTT, failure to thrive; GOR, gastro-oesophageal reflux.