Recent Developments in Understanding Paediatric Barrett's Oesophagus

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Abstract: Barrett's oesophagus (BO) is an acquired pre-malignant condition in which metaplastic columnar epithelium replaces the normal squamous mucosa as a consequence of chronic gastro-oesophageal reflux. The pre-malignant quality of BO is demonstrated in untreated adults with BO which are at risk of Barrett-associated adenoacarcinoma (BAA).

This review addresses the epidemiological, clinical, endoscopic and histological features of paediatric BO. Recent molecular developments and future directions that might lead to a better understanding of this condition are also discussed.

A recent estimate of the prevalence of columnar lined BO in the paediatric population in South Yorkshire (England) found this to be 0.0024% among all children in this geographical area, 0.8% in those children referred for endoscopy and 5.5% in the subgroup of children with GORD. BO was more prevalent in males than in females and risk factors were identified.

Investigations in adult patients with BO have suggested that a key event that leads to the evolution of multiple aneuploid populations and to progression from metaplasia → dysplasia → adenocarcinoma is associated with a multistep process of genetic instability, which leads to a clonal expansion of the abnormal population. Genomic instability has not yet been demonstrated in paediatric BO.

Strong contenders for early events in neoplastic progression in BO include loss of p16 tumour suppressor function, HER2 amplification, loss of function of TP 53 and O6 methylguanine methyl transferase (MGMT) promotor gene silencing.

A clear genetic progression route has not yet emerged for BO. Nevertheless, many of the common abnormalities found in Barrett's associated adenocarcinoma have been shown to be present in BO adjacent to carcinoma.

Keywords: Barrett's oesophagus, gastro-oesophageal reflux disease, intestinal metaplasia, genomic instability, molecular abnormalities.

INTRODUCTION

Barrett's oesophagus (BO) is an acquired pre-malignant condition in which metaplastic columnar epithelium (surface epithelium and glands) replaces the normal squamous oesophageal epithelium as a consequence of chronic GORD [1-4]. Current usage of the term BO is confusing. While some authors at present designate BO as the presence of any glandular mucosa in the oesophagus ("gastric type of BO") [5-7] others require the presence of intestinal metaplasia (IM) to define this condition [8].

The pre-malignant quality of BO is demonstrated in untreated adults with BO. If untreated, BO carries a 30-40 fold increased risk of oesophageal adenocarcinoma and the prognosis for such patients is poor [4, 9]. The incidence of Barrett-associated adenocarcinoma (BAA) increases from 0.2-2.1% in patients with no dysplasia to 70% in those with high grade dysplasia [9]. Despite the high prevalence of GORD in children, BO is rare in this age group [6].

The diagnosis of BO should be confirmed by histological appearances of columnar-lined epithelium. In 1998, the American College of Gastroenterology produced a revised definition of BO which specified the presence of IM with goblet cells [8]. The columnar epithelium in the oesophagus can be of gastric cardia type, gastric fundic type and intestinal metaplastic type with goblet cells [3, 6, 10]. However, only the intestinal metaplastic epithelium confers malignant potential. The more recent definition by the British Society of Gastroenterology defines BO as a segment of columnar metaplasia (whether intestinalised or not) of any length, visible endoscopically above the gastro-oesophageal junction and confirmed or corroborated histologically [11]. This definition considers that "IM can always be identified providing a sufficient number of biopsies are taken over an adequate time-scale". This statement is supported by the fact that the appearance of goblet cells within IM is an age-related process, showing an increase in incidence from 50% in paediatric BO to 84% in adult BO [12]. Furthermore, recent evidence indicate that a small number of biopsies will only demonstrate intestinal metaplasia in 30% of columnar lined oesophagus, whilst a larger number of biopsies will show intestinal metaplasia in 80% of cases [13]. The presence of hiatus hernia is another confounding factor, as it is frequently associated with a long segment BO and it might be difficult to differentiate the two conditions at the endoscopy [6].

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A better understanding of the molecular biology of Barret's metaplasia and of the sequence metaplasia to dysplasia and carcinoma should allow early improved diagnosis, monitoring and therapy and thus modify prognosis [14].

In this review we describe the epidemiological, clinical, endoscopic and histological features of paediatric BO and also discuss recent molecular developments and suggest future directions that might lead to a better understanding of this condition.

EPIDEMIOLOGY OF BO

BO is common in adults, occurring in 8 -15% of patients biopsied for GORD [1, 2, 5] but is much rarer in paediatric practice [1, 6]. The majority of reported young patients are in their second decade; supporting the hypothesis that BO is a sequel of many years of GORD [2].

The prevalence of BO in children is unknown although it has been estimated to be very low [6]. We have recently estimated the prevalence of columnar lined BO in the paediatric population in South Yorkshire (England) and found this to be 0.0024% among all children in our area, 0.8% in those children referred for endoscopy and 5.5% in the subgroup of children with GORD [10].

El-Serag *et al*. [1] found 17 cases of BO out of 6731 patients younger than 20 years who underwent upper GI endoscopy for any indication, giving BO prevalence in this group of 0.25%. Our previously reported case series showed a male predominance (9 males: 3 females) [10]. The median age of our patients was 11.7 years (range 2 to 17 years) [10]. This is in keeping with previous paediatric series, which suggest that 60-90% of affected children are boys [1].

A number of studies has looked at co-morbidities associated with BO in children. Recognised associations include neurological impairment, chronic lung disease (in particular cystic fibrosis), repaired oesophageal atresia and malignancies treated with chemotherapy [6, 15, 16]. In our experience, three of our patients had neurological impairment, two others had asthma, 1 child was obese, an association that has not previously been described in childhood [10]. It has, however, recently been suggested that adult obesity is associated with an increased risk of BO and oesophageal adenocarcinoma [17, 18]. The rapidly burgeoning pandemic of childhood obesity has led to the appearance of other diseases in children that were previously confined to adults, including Type 2 diabetes, dyslipidaemia and non-alcoholic steatohepatitis. It is therefore of concern that these children may also be at increased risk of BO [10].

The estimated prevalence of BO in our patients with oesophagitis is 9% [10]. These figures are similar to the 10% calculated prevalence of BO among all adults with long standing GORD [5].

CLINICAL FEATURES OF BO

The most frequent presenting symptom in our recently reported paediatric cases of BO [10]; was vomiting (9/12); followed by dysphagia (5/12); weight loss (5/12) and abdominal pain (4/12).

ENDOSCOPY

In all 12 cases of BO diagnosed at our institution during 2000 and 2007 [10] the oesophageal appearance at endoscopy was characterised by an erythematous velvet gastric-like mucosa at the distal esophagus (Fig. **1**). Varying degrees of esophageal inflammation with or without ulceration and stricture formation were seen in all the cases.

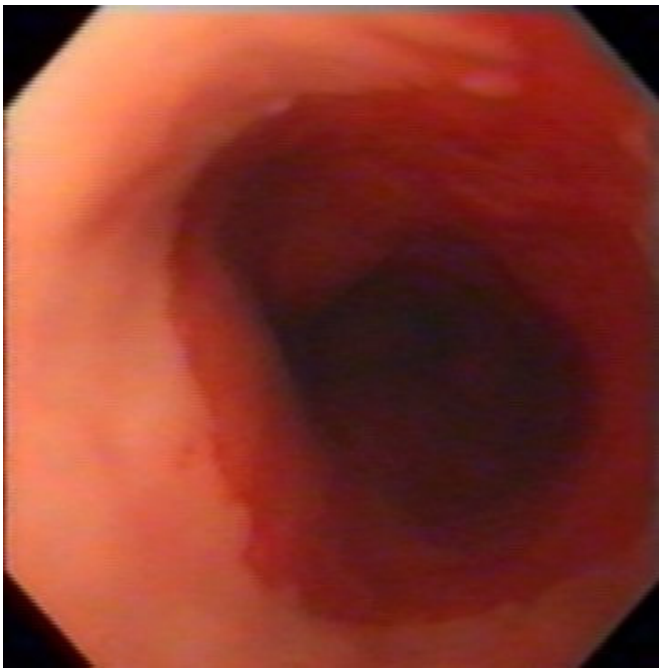


Fig. (1). Endoscopic appearance of BO showing the presence of erythematous adjacent to the normal pale squamous-looking mucosa.

PATHOLOGY

It has been demonstrated that exposure of the distal oesophagus to gastric acid produces injury to the squamous mucosa and ultimately results in replacement by columnar epithelium [2].

IM is characterised by a change in the mucosa, to the extent that it morphologically and histochemically resembles the normal mucosa of either small or large bowel [19]. IM occurring in the stomach can be either complete or incomplete. In complete IM, the metaplastic mucosa resembles the small bowel epithelium and contain goblet cells. The enterocyte-like cells may depict a poorly developed brush border and Paneth cells [19]. In the incomplete type of IM, absorptive cells are not identified and the mucosa shows typical goblet cells intermixed with columnar cells (Fig. **3**).

IM was present in 17/21 (81%) biopsies from all our patients with BO (Fig. **2**) [10]. This number contrast with the 47% reported by El-Serag *et al*. [1]. The differences in the figures may simply reflect different biopsy protocols or sampling error. Oberg *et al*. [13] demonstrated that the chance of detecting IM increases with the length of BO and higher number of endoscopies and biopsies. Studies conducted in adult patients with GORD and BO have

previously shown that IM was present in 50-84% of cases [3, 4, 13]. This is not surprising since IM is an age-related process and therefore more likely to be seen in adulthood than childhood [20].

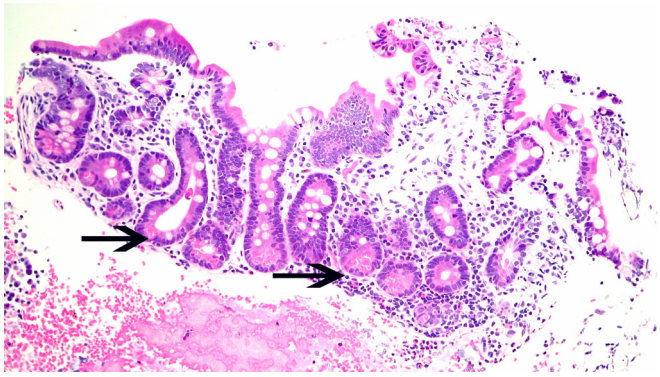


Fig. (2). Microphotography of paediatric case of Barrett's oesophagus depicting incomplete type of intestinal metaplasia, with numerous Goblet cells and Paneth cells (arrows) (H&E x 20).

According to the more recent definition by the British Society of Gastroenterology BO can be diagnosed in absence of IM and with the sole presence of columnar metaplasia on histology of any length, visible endoscopically and above the gastro-esophageal junction [11]. Columnar mucous cells were the most common cell type present in the surface and glandular epithelium in our cases of BO [10]. They are characterised by basal nuclei and clear apical cytoplasm.

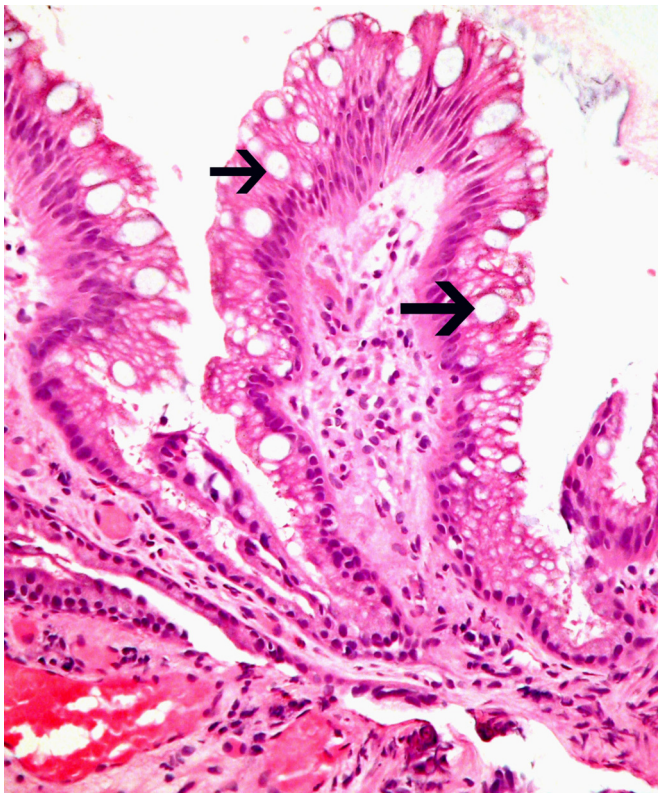


Fig. (3). Goblet cells, hallmark of Barrett's metaplasia, are characterised by basal nuclei and clear apical cytoplasm (arrows) (H&E x 20).

RECENT MOLECULAR DEVELOPMENTS IN BO AND FUTURE DIRECTIONS

Because of the relatively small risk of neoplastic progression in BO, it has been argued that endoscopic surveillance programs aiming at identification of patients with high grade dysplasia are not cost-effective [21, 22]. Thus, the recognition of early and objective alternative risk markers, less susceptible to sampling error, will be of relevance in the management of BO patients. From an early stage, flow cytometry of DNA content of epithelial cells in BO of adult patients suggested that neoplastic progression in this condition was associated with a process of genomic instability resulting in aneuploidy [23-25]. Most tumours have a profile of genetic alterations which are characteristic of that particular neoplasm so the pattern associated with BAA has been eagerly investigated. Early studies of the cytogenetics of BAA showed a massive number of dosage changes with most of the known oncogene and tumour suppressor regions implicated. Over the last 10 years there have been numerous publications using cytogenetics [26, 27], loss of heterozygosity (LOH) techniques [28, 29], comparative genomic hybridisation (CGH) [30, 31] and fluorescence *in situ* hybridisation (FISH) [32, 33] for the study of BAA and its precursor lesions. Regions identified from studies by all these various means as being frequently involved in the genetic profile of BAA included 3p14-21, 4p, 4q, 5p15, 5q21-22,7p12 (EGFR),7q36, 8q24 (CMYC), 9p12(p16), 11q13 (CCND1)17p13.1(TP53), 17q11.2(HER2), 20q13.2 and the Y chromosome.

The endoscopic monitoring of BO patients and the recording of sequential biopsies resulted in an ideal archive for the study of potential sequence of genetic events from BO to BAA. Although it is clear that there is increasing frequency of genetic alterations with advancing neoplasia [34-36]; it is also clear that there is considerable genetic heterogeneity, with abnormal clones at an early stage sometimes expanding, sometimes disappearing with other abnormal clones appearing only at later stages. Investigations in adult patients with BO have suggested that genomic instability is a key event that leads to the evolution of multiple aneuploid populations and that the sequence metaplasia → dysplasia → adenocarcinoma in BO is associated with a multistep process of genetic instability in a heterogeneous population of metaplastic cells leading to a clonal expansion of the abnormal population [26, 36-40].

Unlike some other tumour progression pathways (for example familial adenomatous polyposis to colorectal carcinoma), a clear genetic progression route has not yet emerged for BO to BAA. Nevertheless, many of the common abnormalities found in BAA have been shown to be present in the pre-neoplastic Barrett's epithelium adjacent to carcinoma as well as in the Barrett's mucosa in sequential biopsies performed before the diagnosis of adenocarcinoma [35, 41, 42].

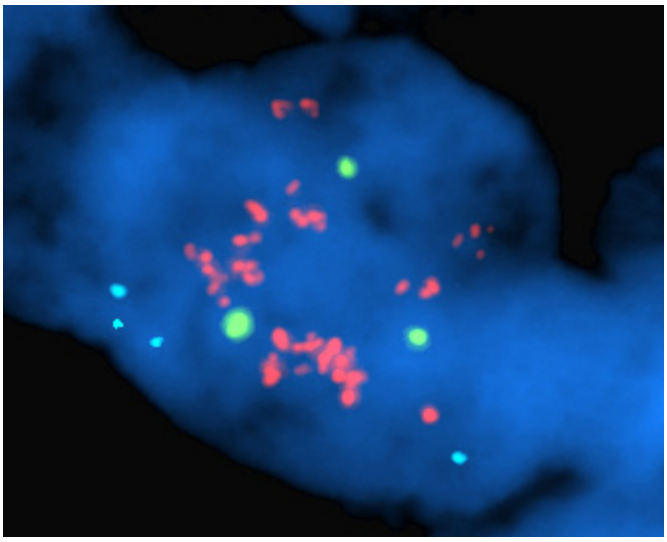
Extreme genetic instability is usually an effect rather than a cause of neoplastic progression, but in this pre-cancerous condition it may be causal [43, 44]. *In vitro* exposure of

oesophageal epithelial cells to bile acids, in particular nitric oxide, has been shown to induce DNA damage [45]. Other *in vivo* and *in vitro* work has supported the hypothesis that reflux agents are genotoxic [46, 47]. Thus, generalised aneuploidy could be an early enough event to predict progression as proposed by several investigations [48, 49]. Flow cytometric methods can be used to measure total cell DNA content but have generally been found inferior to assessment by CGH techniques, due to lack of sensitivity. FISH is generally recognised as the best test for aneuploidy, as it is visualised directly in the tissue of interest, unlike all the techniques based on total DNA. We have examined a series of adult BAA with a panel of FISH probes chosen from the most frequently associated regions of chromosomal gain or loss and found the panel to be informative in 8 out of the 10 cases (unpublished results). We used four different multicolour probe mixes (1) HER2/17cen/4cen (2) p16/9cen (3) TP53/17cen/6cen (4) CCND1/11cen. Fig. (**4**) demonstrates two adult cases of BO with a range of abnormalities detected by our panel.

We have also recently investigated genetic instability by looking into the presence of microsatellite instability in paediatric BO using laser microdissection on paraffin-embedded endoscopic biopsies in 6 paediatric patients harbouring BO and 6 age-matched controls [50]. In our study, we selected laser microdissection technique as it allowed to obtain a single cell and to guarantee the purity and the specificity of the molecular events founded in the tissues under investigation. Histologically all these cases showed the presence of specialised intestinal metaplasia containing goblet cells replacing the squamous oesophageal epithelium. Goblet cells were positive with Alcian Blue pH 2.5, indicating the presence of sialomucins. None of the paediatric cases of BO tested by us showed any evidence of microsatellite instability. The absence of genomic instability in our population could reflect the need for a longer period for BO to develop, i.e., later in life. Also, it might be that BO only arises in a proportion of patients. The fact that we used laser microdissection of the metaplastic cells guaranteed the purity of the tissues to be investigated and the specificity of the negative molecular events we encountered. Nevertheless, the absence of microsatellite instability in our paediatric study group does not exclude other genetic alterations and it is consistent with results from adult series in which low level microsatellite instability is believed to result from generalised genomic instability as defects in mismatch repair system genes are rarely found [51].

Despite the plethora of genetic studies in this condition, the early inference that loss of p16 tumour suppressor function is one of the primary events in neoplastic progression in BO [52] has received the most support from research [53]. Other strong contenders for early events are HER2 amplification, loss of function of TP 53 and MGMT promotor silencing. HER2 is a member of the human epidermal growth factor receptor family of transmembrane receptor tyrosine kinases which promote tumour proliferation in a variety of epithelial neoplasms. Recent work has shown that HER2 amplification, assessed by IHC and FISH, predicted rapid progression from early lesions in BO to full BAA in 8 of 11 patients [54]. This is of great interest as there are currently targeted therapies against overexpression of the HER2 protein and tumours which display amplification of HER2 may be suitable for this treatment.

(**a**)



(**b**)

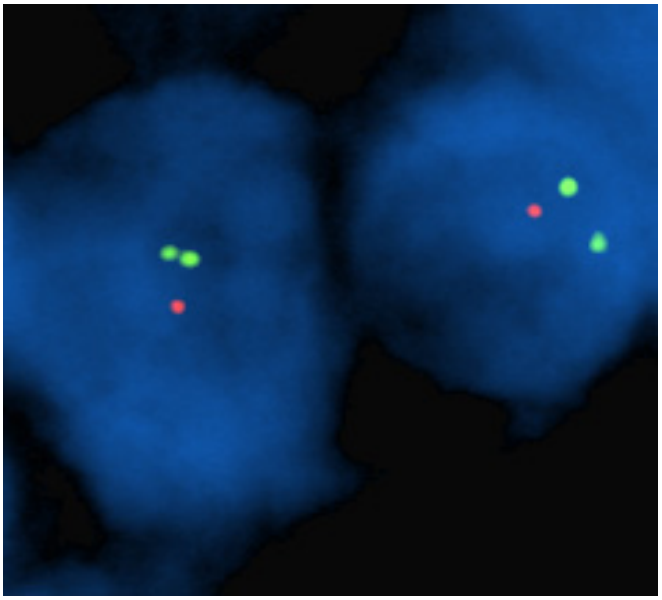


Fig. (4). a: Probe mix HER2 in red, chromosome 17 centromere in green and chromosome 4 centromere in aqua. This cell from a case of Barrett's adenocarcinoma shows amplification of HER2 and gain of chromosomes 4 and 17; **b**: Probe mix p16 in red and chromosome 9 centromere in green. This cell from a case of Barrett's adenocarcinoma shows deletion of p16.

It has also been demonstrated that loss of p16 on chromosomes 9p and TP53 on 17p are already present in a high proportion (61%) of patients with BO without evidence of cancer or dysplasia [55]. The loss of function of both of these tumour suppressor genes is a common cause of genomic instability in many tumours and fits well as early events with the model of BO to BAA, characterised as it is by extreme genetic instability. The loss of function of these genes is by chromosomal loss or deletion of the specific region, mutation or silencing by methylation. Epigenetic causes of tumourigenesis such as gene silencing by

methylation are amongst the newest areas in cancer investigations. Aberrant promoter methylation of the 0(6) methylguanine methyl transferase (MGMT) gene, which acts to repair DNA damaged by alkylation of guanine residues, is seen in a variety of cancers and precancerous changes [56]. The effect of this methylation is the accumulation of G to A transition mutations that can affect genes required for genomic stability. There is recent evidence that this is a frequent and early event in the progression of BO to BAA [57, 58]. This, again, is not just of interest for the potential indication of neoplastic progression, but for the implication of increased sensitivity of the lesion to treatment by alkylating agents.

It now seems likely that a mixture of FISH for deletion/amplification status, methylation testing for gene silencing and gene mutation study for these important cancer genes may yet give guidance for the follow-up of patients.

Barrett's metaplasia is definitively recognised as a step in the development of the BAA and the prognosis for invasive oesophageal adenocarcinoma is very poor despite advances in multimodality therapy. An improved understanding of the molecular biology of this disease may allow early improved monitoring, diagnosis and therapy and thus alter prognosis. If BO develops at a paediatric age, the condition might have a continuous span until adulthood and therefore the identification of risk markers at an early stage seems attractive [50]. Although the only type of metaplasia which carries an increased risk for adenocarcinoma is the one associated with the intestinal type of BO [8], the presence of goblet cells may not be the earliest indicator of it. The Caudal Homeobox genes (CDX) are thought to have a seminal role in the transformation of normal squamous epithelium to metaplastic columnar cells [59]. CDX2 is a regulatory gene involved in intestinal differentiation and its expression is an early event in this process. We have recently used immunohistochemistry to detect the encoded protein of CDX2 and revealed it to be a sensitive marker of intestinal differentiation that may be useful in the diagnosis of histological equivocal cases of BO [10]. Furthermore, CDX2 expression has not been found in the normal esophageal mucosa [10, 60]. CdX2 mRNA gene expression has also been demonstrated (using reverse transcriptase polymerase chain reaction) in BO mucosa and also at the stage of oesophagitis [61]. This would support the view that CdX2 expression appears to be related with the transition from GORD to BO.

One of the problems the paediatric team faces after a diagnosis of BO is made is how to plan follow-up. The value of endoscopic surveillance biopsy for dysplasia and carcinoma in patients with BO is controversial. One reason is that patients at risk might not yet have developed dysplasia or this might be focal or difficult to recognise histologically [7]. The diagnostic difficulties that may hamper the histologic diagnosis of dysplasia are related to sampling error, difficulty in distinguishing reactive changes from changes due to dysplasia, inter-observer variability in the diagnosis of dysplasia and in differentiating high-grade dysplasia from invasive carcinoma [7, 14]. Therefore, although epithelial dysplasia is still the "gold-standard" for increased risk of malignancy and its presence would indicate the need for surveillance [4, 25, 62], molecular markers are gaining clinicians' confidence for distinguishing patients at lower or higher risk of progression to malignancy, even before the morphological changes become evident.

Finally, it is clear that molecular biology has not yet been able to identify a single molecular marker that would allow recognition of those patients at risk of BAA. More prospective studies using the molecular markers that have emerged most strongly are required to inform accurate, appropriate intervention and treatment for this condition, both paediatric and adult.

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