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GUIDELINES CLINICAL PRACTICE

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New techniques in the tissue diagnosis of gastrointestinal neuromuscular diseases

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Abstract

Gastrointestinal neuromuscular diseases are a clinically heterogeneous group of disorders of children and adults in which symptoms are presumed or proven to arise as a result of neuromuscular (including interstitial cell of Cajal) dysfunction. Common to most of these diseases are symptoms of impaired motor activity which manifest as slowed or obstructed transit with or without evidence of transient or persistent radiological visceral dilatation. A variety of histopathological techniques and allied investigations are being increasingly applied to tissue biopsies from such patients. This review outlines some of the more recent advances in this field, particularly in the most contentious area of small bowel disease manifesting as intestinal pseudo-obstruction.

Key words: Enteric myopathy; Enteric neuropathy; Interstitial cells of Cajal; Intestinal pseudo-obstruction; Visceral pain

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INTRODUCTION

The term gastrointestinal neuromuscular diseases (GINMD) describes a clinically heterogeneous group of disorders of children and adults in which symptoms are presumed or proven to arise as a result of neuromuscular (including interstitial cell of Cajal) dysfunction[1,2]. Common to most of these diseases are symptoms of impaired motor activity which manifest as slowed or obstructed transit[3] with or without evidence of transient or persistent radiological visceral dilatation. Such diagnoses include primary and secondary disorders of the oesophagus to the colon e.g. achalasia, gastroparesis, intestinal pseudo-obstruction and severe constipation. Pathologic abnormalities of the sensorimotor apparatus have been demonstrated in such disorders by a variety of methods since the 1960s; however, this remains an area of evolving interest especially with the increasing availability of newer techniques and more critical appraisal of those more established techniques.

This review outlines some of the more recent advances in this field, particularly in the area of small bowel disease manifesting as intestinal pseudo-obstruction. The area of Hirschsprung disease diagnosis, although numerically important (this being by far the most common GINMD) is not covered here since, although some contention exists, in general the techniques for this diagnosis are long and better established. The review covers the safe acquisition of tissue and advances in histopathological and allied techniques.

SAFE TISSUE ACQUISITION

Tissue may be taken with deliberate diagnostic intent or alternatively come as the by-product of emergency or planned surgical interventions. On this basis, tissues may take the form of mucosal, deep submucosal, seromuscular or full-thickness biopsies or resection

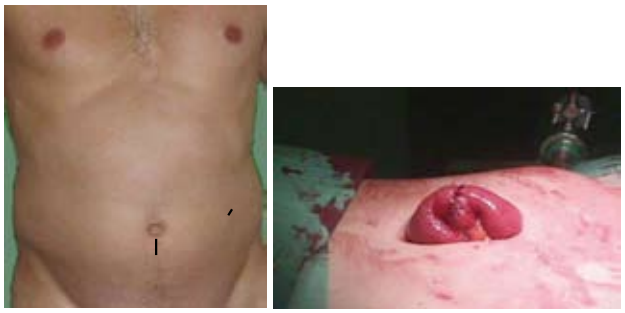

Figure 1 Laparoscopically-assisted full thickness jejunal biopsy. The port sites are shown. After finding a suitable proximal jejunal loop, the bowel is exteriorised by extending slightly the umbilical port incision and biopsy and suture closure performed extracorporeally (Courtesy of B Nyborg, Huddinge, Stockholm).

specimens. Of particular note are recent advances in minimally invasive surgery that have permitted safe access and biopsy of a variety of intra-abdominal tissues including full-thickness bowel biopsy[4]. In the context of GINMD, with some variations, the technique has now been applied to children with colonic dysmotility[5,6] and adults with small bowel dysmotility, predominantly those with proven chronic idiopathic intestinal pseudo-obstruction (CIPO)[7-10]. A very recent study reported on the safety and diagnostic yield of a predominantly laparoscopically-assisted approach (Figure 1) to biopsy the small and large bowel in a cohort of 124 adults with suspected GINMD from 3 European centres. Median operating time was 50 min, conversion rate was 2% and length of stay was 1 d. There was an 8% readmission rate for obstructive symptoms; however, other morbidity was minimal and there were no mortalities. Overall the specific diagnostic yield was 81%, being high for jejunal biopsies (89%), but low for a small number of ileal and colonic biopsies[10]. On this basis, an extracorporeal laparoscopically-assisted procedure appears safe and with acceptable yield if performed in the proximal small bowel for the indications in this study. Completely intracorporeal staple techniques may also be safe, but very little published data exists, at least for the jejunum[10]. Laparoscopic gastric biopsies may also now be taken at the time of gastric pacing[11], and may be important in predicting outcome from this procedure on the basis of ICC pathology (personal communication: Gianrico Farrugia).

The potential to increase yield with multiple biopsies must be balanced against the risk of complications. Clearly, whilst there is some evidence from colectomy and post-mortem small bowel that sections should be taken at fixed intervals to avoid missing 'patchy' abnormalities of muscle or nerve[12], extending this finding to suggest multiple biopsies, even with a small risk for each is not currently advised. On this basis, as well as the potentially increased risks of leakage, laparoscopic full-thickness colonic biopsy is currently not advised, although seromuscular biopsies have been shown to be safe in a large series of paediatric patients[6] and can also be used for determining the HSCR transition zone. The role of appendectomy as a diagnostic surrogate of GINMD has recently been suggested based on preliminary findings in diabetes[13], but needs further exploration[14]. The evolving technique of NOTES (natural orifice transluminal endoscopic surgery) will in the future (in the author's view) have an important role here, with proof of concept already demonstrated in the stomach[15]. Regardless of technique, because of regional differences, whenever full-thickness biopsies are taken, the corresponding intestinal segment(s) should be precisely indicated to the pathologist.

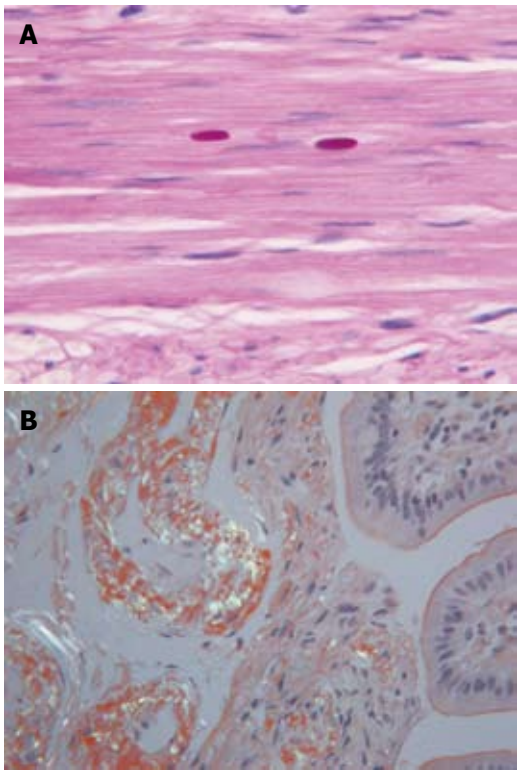

Figure 2 Tinctorial stains used in GI neuromuscular histopathology. A: Periodic acid Schiff staining showing polyglucosan bodies in a patient with intestinal pseudo-obstruction; B: Bifringence from amyloid visualised by Congo red staining (x 25-40).

HISTOLOGICAL TECHNIQUES

Although the histopathological diagnosis of GINMD (and exclusion of other disease) continues to be primarily based upon the analysis of H&E-stained sections with light microscopy, a number of other techniques can also be employed. A critical appraisal of the role of these techniques, particularly in comparison with the 'yield' of H&E, and guidelines for their use is currently being produced by an international working party: www.gastro2009.org/pdf/wp_project_descr07.pdf and is not covered here. Rather, descriptions of some newer diagnostic techniques are presented.

Tinctorial stains (Figure 2)

Although there is vast variation in current practice, tinctorial stains can supplement H&E with particular use in the assessment of specific structures and

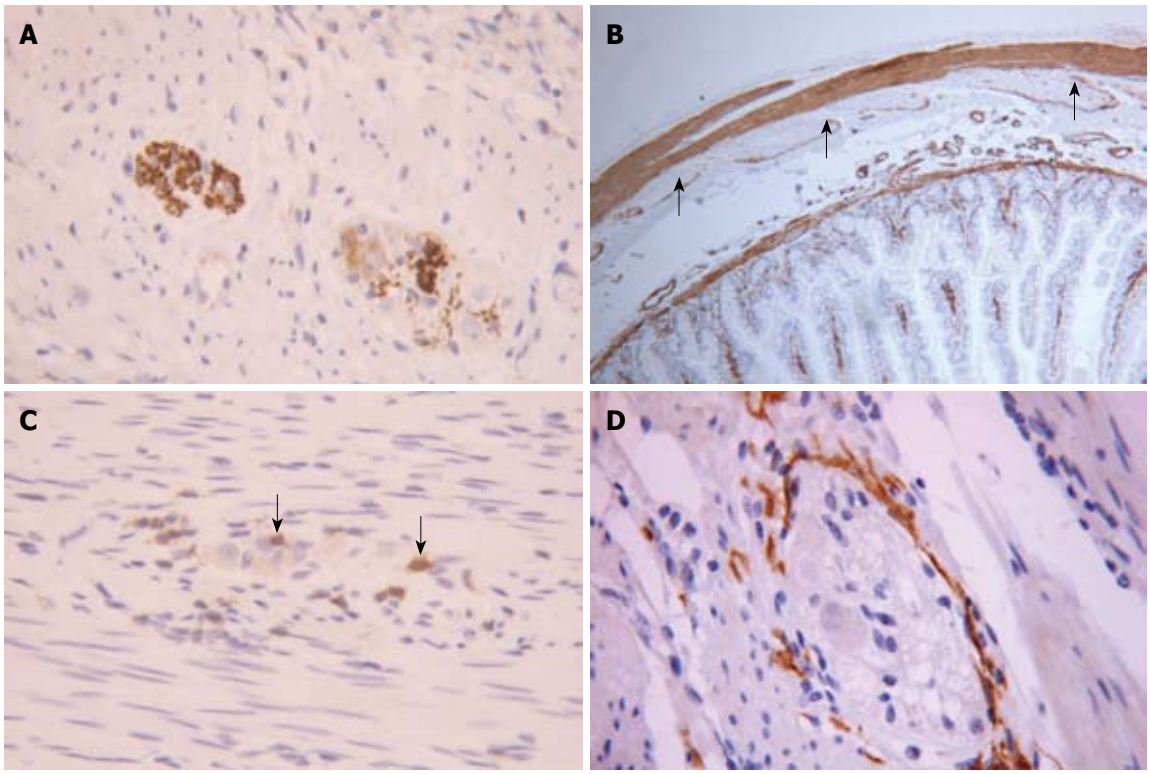

Figure 3 Immunohistochemistry using antibodies to. A: Neuron specific enolase allowing clear visualisation of myenteric ganglia, neuronal number and size; B: Smooth muscle alpha actin showing absent staining in the circular muscle layer of the jejunum (arrows) in a patient with enteric dysmotility; C: CD3 showing small numbers of periganglionic T lymphocytes (arrows) in numbers that most would deem abnormal and indicative of ganglionitis; D: CD117 staining showing normal myenteric plexus interstitial cells of Cajal (ICC-MP). (Original magnification x 40-100).

cell types. With periodic acid Schiff (PAS) staining, inclusion bodies e.g. polyglucosan, lipofuscin granules (secondary autophagic lysosomes), and glycogen can be observed, and PAS combined with diastase treatment can differentiate between glycogen and other structures (glycogen disappears after diastase pretreatment), which may be of value where a glycogenosis or related metabolic disorder are suspected. Polyglucosan inclusion body myopathy has recently been described in GINMD[16] and cannot easily be identified without use of PAS staining. Amyloid is a rare secondary cause of GINMD and can be detected with ease using Congo red staining. With Giemsa staining, mast cells and eosinophils can be seen easily, and the condition of the neuronal cytoplasm assessed (marginalization of the Nissl and chromatolysis). Various types of trichrome staining assist in the establishment of fibrosis and in differentiation from interstitial oedema (both cause increases in the distance between cells, and in early fibrosis this can be difficult to differentiate). Relevant to some rare cases of GINMD, Gomori trichrome staining is also used to diagnose mitochondrial neurogastro-intestinal encephalomyopathy (MNGIE) on the basis of finding 'ragged red fibres' on skeletal muscle biopsy[17].

Immunohistochemistry (IHC) (Figure 3)

The past thirty years has seen the use of IHC evolve in many areas of GI practice including that of GINMD diagnosis. With respect to mainly diagnostic rather than research applications, neuronal markers such as PGP9.5 and neuron specific enolase (NSE) may be employed to assist in the determination of neurons particularly if quantitation is considered important. This latter point is very contentious because heterogeneity of methods has meant that few normative data exist for any single method, especially when age and regional specificity are considered[18]. Nevertheless, diagnoses reliant on decreases in numbers of neurons and ganglia[19] have complemented findings made previously using the more laborious technique of silver staining[20]. Alpha smooth muscle actin deficiency has been demonstrated by IHC in some children[21] and adults[9] with GINMD, and stresses the importance also of regional specificity-this being a normal variant in the ileum[9]. Inflammatory neuropathies[8,10,22] and much less commonly leiomyopathies[23] may be best diagnosed by immunocyte IHC when large infiltrates (visible on H&E) are not apparent. This finding may prompt further autoimmune investigation (below) and dictate important changes in therapy[22-24]. Finally, c-kit (CD117) IHC has now become established in detecting changes in ICC numbers that certainly accompany, and may be causative of some GINMD[25].

Research applications of IHC have predominantly addressed disease mechanisms and pathways. In GINMD, many studies have attested to alterations in neurochemically-stained subsets of neurons allied to their differing functions. Changes said to underlie abnormal neuronal development[26], retarded colonic transit e.g. reduced substance-P[5], failure of sphincter

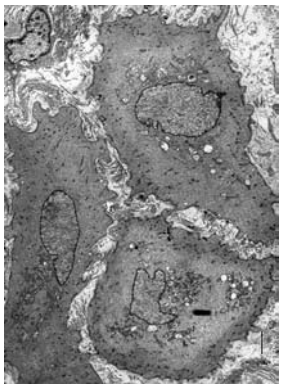

Figure 4 Electron micrograph of smooth muscle cells showing increased Golgi indicative of transition to a more secretory phenotype in a patient with enteric myopathy and pseudo-obstruction. (x 50 000).

relaxation e.g. decreased nitric oxide[27], or visceral hyperalgesia e.g. increased transient receptor potential channels[28] have been variously reported. Beyond mechanism and target identification, whether such changes may become clinical biomarkers of disease or guide treatment is the subject of much ongoing study, particularly if identifiable on endoscopic mucosal biopsy[29].

Electron microscopy (Figure 4)

Ultrastructural examination of neurons, muscle and interstitial cells of Cajal can be a useful adjunct to the above assessments in certain patients. These include some rare childhood myopathies where H&E findings are absent or equivocal (e.g. subtle fibrosis, atrophy of myocytes or myocyte vacuolation)[30], the identification of rare inclusion bodies[31] suggestive of mitochondrial disorders and some ultrastructural changes of ICC[32] and myocytes, including a transformation to more secretory phenotypes.

ADJUNCTIVE INVESTIGATIONS

Proteomic investigations

In cases characterized by clinical (adult onset, personal or family history of autoimmunity) and histopathological findings (especially inflammatory neuro or myopathies) an autoimmune pathogenesis may be suggested. A variety of antibodies directed to nuclear proteins[33,34] and, to a lesser extent, membrane-bound receptors[35,36] of the enteric neuromuscular compartment have been found in patients with secondary GINMP, especially of paraneoplastic origin. The presence of some of these autoantibodies in patients with idiopathic disorders affecting gut motility[22] has prompted their attempted identification in several recent studies[23,35,37,38]. In nearly all cases, proof of pathogenicity remains weak in comparison with established autoimmune diseases of the neuromuscular junction[39]. For a very recent full review, see Kashyap & Farrugia, 2008[40]. If clinically suspected, it is, however, reasonable to take a sample of serum for antibody testing. This should be sent to an established neuroimmunology unit where a variety of methods such as radioimmunoprecipitation assays may be employed[39] (Figure 5). Established antibody tests include those for anti-Hu[34] and anti voltage-gated calcium channels (particularly in paraneoplasia)[41], anti smooth muscle (particularly in myopathies and scleroderma), anti-ganglionic acetylcholine receptor (particularly if associated with dysautonomia)[35,39] and anti voltage-gated potassium channel antibodies[38,39]. One other blood-based investigation occasionally indicated in the investigation of pseudo-obstruction is the thymidine phosphorylase leucocyte activity assay[42] in patients suspected on the basis of clinical findings to have MNGIE[17].

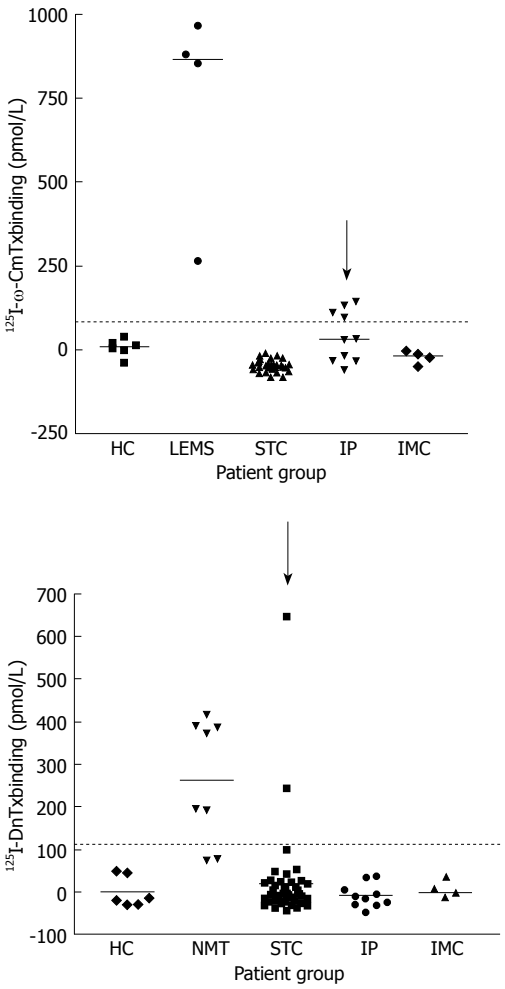

Figure 5 Radioimmunoprecipitation assays of sera from patients with GINMD and negative and positive controls. Assays for antineuronal antibodies directed to anti-voltage-gated calcium (anti-VGCC P/Q-type) and potassium channels (VGKC) are shown. Four IP sera are weakly positive for anti-VGCC P/Q-type antibodies, and 2 STC sera strongly positive for anti-VGKC (arrowed). Dotted line: mean + 3SD; HC: Healthy controls; LEMS: Lambert-Eaton myasthenic syndrome; STC: Slow transit constipation; IP: Intestinal pseudo-obstruction; IMC: Idiopathic megacolon; NMT: Neuromyotonia.

Genomic investigations

Recent history has witnessed a colossal expansion in data regarding the human genome in health and disease. In keeping with this, several studies have demonstrated molecular genetic changes that accompany, and in some instances, contribute to various forms of intestinal dysmotility. Whilst offering interesting research insights, few presently have great value in clinical practice, and these are in the most part limited

to quite characteristic clinical syndromes. Most utilised are candidate single gene approaches, and these have been applied to screening for RET mutations in patients with Hirschsprung disease[43] or suspected multiple endocrine neoplasia (MEN) 2 syndromes[44], and thymidine phosphorylase mutation analysis in patients with MNGIE[42]. A variety of tests may also be appropriate in patients in which GI dysmotility may accompany other systemic diseases such as muscular dystrophy, cystic fibrosis and neurofibromatosis. In most cases, the information delivered is used to guide genetic counselling, and prognosis rather than influence diagnosis (except prenatally) or treatment (except in MEN where prophylactic surgery may be required to prevent subsequent neoplasia[45]).

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