be a manifestation of CD in the paediatric population.⁵ Ebach *et al.* reported that LE was identified in 28% children with CD.⁵ We did not identify lymphocytic oesophagitis in our patient cohort ($n = 85$, combining study and control groups). The non-specific chronic inflammation in the oesophageal mucosa was seen in a proportion of these patients. However, the histological findings did not meet the diagnostic criteria of lymphocytic oesophagitis.

To summarise, upper GI symptoms are seen in one-third of paediatric patients with IBD and concurrent FEG. However, these symptoms are not reliable to distinguish them from IBD patients without FEG. Upper gastrointestinal endoscopic abnormalities, predominantly aphthoid ulcers, are predictive of FEG in paediatric patients with IBD. This information is particularly helpful in patients with atypical presentation, such as patients with isolated small bowel CD. The presence of FEG might prompt gastroenterologists to examine the small intestine of patients who are suspected to have IBD with a negative colonoscopy. Paediatric gastroenterologists and pathologists should be aware of the clinical significance of FEG in children with IBD.

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1. Ushiku T, Moran CJ, Lauwers GY. Focally enhanced gastritis in newly diagnosed pediatric inflammatory bowel disease. *Am J Surg Pathol* 2013; 37: 1882–8.
2. Oberhuber G, Püspök A, Oesterreicher C, *et al.* Focally enhanced gastritis in patients with Crohn's disease. *Gastroenterology* 1997; 112: 698–706.
3. McHugh JB, Gopal P, Greenson JK. The clinical significance of focally enhanced gastritis in children. *Am J Surg Pathol* 2013; 37: 295–9.
4. Brown IS, Miller GC, Bettington ML, *et al.* Histopathological findings of extra-ileal manifestations at initial diagnosis of Crohn's disease-related ileitis. *Virchows Arch* 2016; 469: 515–22.
5. Ebach DR, Vanderheyden AD, Ellison JM, *et al.* Lymphocytic esophagitis: a possible manifestation of pediatric upper gastrointestinal Crohn's disease. *Inflamm Bowel Dis* 2011; 17: 45–9.

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A French point of view about the cost effectiveness of RASQ61R immunohistochemistry in colorectal cancer

Sir,

The determination of *KRAS* and *NRAS* mutation status is mandatory for treatment choices in patients with metastatic colorectal carcinomas (mCRC). Indeed, *RAS*-mutated mCRC are resistant to anti-EGFR therapies which are in this manner

usable only in patients with mCRC lacking *KRAS*/*NRAS* mutations. Therefore, the choice of an efficient and accurate method for detecting *RAS* mutation in formalin fixed, paraffin embedded tumour samples is important for the therapeutic management of patients with mCRC. Several different methods can be used searching for *KRAS* and *NRAS* mutation in tumour samples, mainly analysing tumour DNA using next generation sequencing or other *KRAS*-*NRAS* targeted methods, but also as studied by Jhuang *et al.*, SP174-RASQ61R immunohistochemistry detecting both *NRAS*Q61R and *KRAS*Q61R mutant proteins, with excellent sensitivity and specificity.¹ *HRAS* mutations, being extremely rare in mCRC, are not classically investigated for the therapeutic management of patients with mCRC, and the *HRAS*Q61R mutant which is also detected by SP174 antibody has not been reported in mCRC to our knowledge (COSMIC database).² Non-RASQ61R mutant proteins are not detected by SP174 immunohistochemistry and additional molecular analyses are required in samples with SP174 negative results, searching for other *RAS* mutations. In their article, Jhuang *et al.* argued that mutation-specific immunohistochemistry is a cost-effective method helping to diagnose *KRAS* and *NRAS* mutations in patients with mCRC.¹ An alternative point of view can be considered in this field, taking into account different healthcare systems and conditions.

In France, the different methods usable for detecting *RAS* mutations also have different prices.^{3–5} As a widely used method to date and considered as the gold standard, a next generation sequencing (NGS) analysis is proposed at 882.9€ per sample.³ A *KRAS*-*NRAS* mCRC-dedicated restricted analysis using a non-NGS molecular method is proposed at 440.1€ per tumour sample.⁴ Finally, immunohistochemistry using one anti-*NRAS*Q61R SP174 antibody costs 48€ per sample.⁵ We intend to evaluate the costs incurred by different diagnostic strategies to diagnose *KRAS* and *NRAS* mutations in patients with mCRC.

On the basis of previous studies, *RAS*Q61R mutations (i.e., *NRAS*Q61R and *KRAS*Q61R mutations) represent less than 2% of mCRC and, as a result, among 100 analysed tumour samples of patients with mCRC, we estimated that a maximum of two samples would be positive using SP174 immunohistochemistry.^{6–9} To analyse 100 tumour samples using single NGS approach would cost 88,290€, whereas a non-NGS molecular method (e.g., multiplex real-time allele specific amplification or pyrosequencing) would cost 44,010€. A first line mutation screening using SP174 immunohistochemistry in 100 samples would cost 4,800€ but would diagnose only two *RAS*Q61R mutated samples. To diagnose *KRAS*/*NRAS* mutations in the 98 immunohistochemistry-negative samples, performing second line molecular analyses would cost 86,524€ using NGS or 43,130€ using non-NGS molecular methods. As a result, a two-step mutation screening based on first line SP174 immunohistochemistry and second line molecular analyses in case of negative immunohistochemistry would cost between 47,930€ (using non-NGS molecular methods) and 91,324€ (using NGS method) to analyse 100 colorectal cancer samples.

In this manner, adding first line SP174 *RAS*Q61R mutation specific immunohistochemistry testing would result in an increased cost in comparison with single DNA-based analyses and does not seem to be a cost-effective approach according to the prices currently proposed in France for cancer-related molecular tests.^{3–5} Other points of view regarding different healthcare systems in different countries would

merit further consideration as the cost-effectiveness remains poorly reported in the literature in the field of mutation-specific immunohistochemistry. Ancillary studies are also needed to determine which molecular testing strategy is the more cost-effective between NGS and other methods.

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1. Jhuang JY, Yuan CT, Lin YL, *et al.* NRASQ61R immunohistochemistry detects both NRASQ61R and KRASQ61R mutations in colorectal cancer. *Pathology* 2017; 49: 387–90.
2. Reagh J, Bullock M, Andrici J, *et al.* NRASQ61R mutation-specific immunohistochemistry also identifies the HRASQ61R mutation in medullary thyroid cancer and may have a role in triaging genetic testing for MEN2. *Am J Surg Pathol* 2017; 41: 75–81.
3. Le référentiel des actes innovants hors nomenclature de biologie et d'anatomocytopathologie (RIHN). RIHN 2017. http://solidarites-sante.gouv.fr/IMG/xlsx/rihn_v2017_20170606.xlsx
4. Le référentiel des actes innovants hors nomenclature de biologie et d'anatomocytopathologie (RIHN). Liste complémentaire 2017. http://solidarites-sante.gouv.fr/IMG/xlsx/liste-complementaire_v2017_20170607.xlsx
5. Classification Commune des Actes Médicaux v41 2015. <http://www.sfpai.org/media/pdf/copie-de-ccam-v41-acp.pdf>
6. Turchini J, Andrici J, Sioson L, *et al.* NRASQ61R mutation-specific immunohistochemistry is highly specific for either NRASQ61R or KRASQ61R mutation in colorectal carcinoma. *Appl Immunohistochem Mol Morphol* 2017; 25: 475–80.
7. Dias-Santagata D, Su Y, Hoang MP. Immunohistochemical detection of NRASQ61R mutation in diverse tumor types. *Am J Clin Pathol* 2016; 145: 29–34.
8. Uguen A, Gueguen P, Guibourg B, *et al.* Is SP174 immunohistochemistry an interesting ancillary tool to determine RAS mutational status in colorectal carcinoma? *Appl Immunohistochem Mol Morphol* 2017; 25: e25–6.
9. Lasota J, Kowalik A, Felisiak-Golabek A, *et al.* SP174, NRAS Q61R mutant-specific antibody, cross-reacts with KRAS Q61R mutant protein in colorectal carcinoma. *Arch Pathol Lab Med* 2017; 141: 564–8.

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First report of Mediterranean stomatocytosis/macrothrombocytopenia in an Indian family: a diagnostic dilemma

Sir,

Mediterranean stomatocytosis/macrothrombocytopenia is a rare autosomal recessive disorder caused by enhanced gastrointestinal absorption of and reduced excretion of plant sterols (sitosterolaemia). It is characterised by short stature, chronic abdominal discomfort, splenomegaly and laboratory features of chronic haemolysis along with the presence of stomatocytic red cells and macrothrombocytopenia on blood films. The rarity as well as protean manifestations of this disorder make it a challenging diagnosis for clinicians as well as pathologists.

A 12-year-old north Indian male, born from a non-consanguineous marriage, was on intermittent follow-up for a presumptive diagnosis of hereditary spherocytosis (HS). His 8-year-old sister too had features of chronic haemolysis with documented cholelithiasis. Clinical findings in both siblings included intermittent jaundice, failure-to-thrive (height and weight 79% and 46% of expected, respectively, i.e., <3rd centile for age) and intermittent epistaxis. Peripheral blood showed the presence of spherocytes, variable numbers of stomatocytes, reticulocytosis and thrombocytopenia. Incubated osmotic fragility (iOFT) was increased in both. However, the flow cytometric eosin-5'-maleimide (EMA) dye-binding test was normal (mean channel fluorescence ratio 1.09; reference value ≤0.8).

The study was approved by the institutional ethics committee of PGIMER, Chandigarh. Samples were collected after obtaining consent of the patient according to approved human subjects' guidelines.

In light of the EMA results, the patient's complete clinical and laboratory records were reviewed. Past history revealed multiple hospital admissions, starting when he was 2 years old for sub-acute intestinal obstruction which was managed conservatively. A 5 mm stone in the common bile duct was noted which passed spontaneously. Later hospital visits were for diarrhoea, failure to gain weight and height, giardiasis, fever, jaundice. Hepatosplenomegaly 4 and 7 cm below right and left costal margins, respectively, were noted. Investigations were negative for malaria, Wilson's disease and chronic liver disease, including viral and autoimmune hepatitis. Based on a single positive report of anti-smooth muscle actin (SMA) antibody, he had received prednisolone and azathioprine at 12 years of age.

The boy's haemoglobin was 117 g/L, total leukocyte count $10.1 \times 10^9/L$, red blood cell count $3.66 \times 10^{12}/L$, mean corpuscular volume 109.2 fL, mean corpuscular haemoglobin 32 pg, mean corpuscular haemoglobin concentration 29.3 g%, red cell distribution width 19.2% and corrected reticulocyte count 9.8%. The automated platelet count (impedance method) was $5 \times 10^9/L$. On a blood film, red cells showed moderate anisopoikilocytosis with a predominant population of stomatocytes and reticulocytosis (Fig. 1A,B). Platelets were reduced ($\sim 50\text{--}60 \times 10^9/L$ on smear estimates) with anisocytosis and several giant forms (Fig. 1C). Mean platelet volumes for the siblings ranged from 11.3 to 12.9 fL (reference range 7.5–11.5).

Biochemical investigations revealed total bilirubin 1.77 mg% with conjugated fraction 0.26 mg%, aspartate aminotransferase 89 U/L, alanine aminotransferase 189 U/L and alkaline phosphatase 189 U/L. Prothrombin time was 13.8 s. Direct Coombs test, G6PD deficiency screening, urine and plasma haemoglobin levels and haemoglobin high-performance liquid chromatography were normal or negative. Flow cytometry based OFT showed increased lysis of red cells with 9.2% residual cells [reference range (RR) $52.3 \pm 20.5\%$]. Serology for HIV, hepatitis B surface antigen, hepatitis C, A and E were negative. Thyroid function was normal: T3 1.71 (0.8–2 ng/mL), T4 12.48 (4.8–12.7 µg/dL) and thyroid-stimulating hormone (TSH) 1.79 (0.27–4.2 µIU/mL).

A duodenal biopsy revealed intraepithelial lymphocytosis with crypt hyperplasia. However, anti-tissue