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ORIGINAL ARTICLE

Association Between Fecal Calprotectin Levels and Small-bowel Inflammation Score in Capsule Endoscopy: A Multicenter Retrospective Study

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Abstract

Background Accurate inflammation reporting in capsule endoscopy (CE) is important for diagnosis and monitoring of treatment of inflammatory bowel disease (IBD). Fecal calprotectin (FC) is a highly specific biomarker of gut inflammation. Lewis score (LS) was developed to standardize quantification of inflammation in small-bowel (SB) CE images.

Goals Multicenter retrospective study aiming to investigate correlation between LS and FC in a large group of patients undergoing CE for suspected or known small-bowel IBD, and to develop a model for prediction of CE results (LS) based on FC levels.

Study Five academic centers and a district general hospital offering CE in UK, Finland, Sweden, Canada, and Israel. In total, 333 patients were recruited. They had small-bowel CE and FC done within 3 months.

Results Overall, correlation between FC and LS was weak (r_s: 0.232, $P < 0.001$). When two clinically significant FC thresholds (100 and 250 μg/g) were examined, the r_s between FC and LS was 0.247 (weak) and 0.337 (moderate), respectively ($P = 0.307$). For clinically significant (LS ≥ 135) or negative (LS < 135) for SB inflammation, ROC curves gave an optimum cutoff point of FC 76 μg/g with sensitivity 0.59 and specificity 0.41. *Limitations*: Retrospective design.

Conclusions LS appears to show low correlation with FC as well as other serology markers of inflammation. FC does not appear to be a reliable biomarker for significant small-bowel inflammation. Nevertheless, FC level ≥ 76 μg/g may be associated with appreciable visual inflammation on small-bowel CE in patients with negative prior diagnostic workup.

Keywords Capsule endoscopy · Fecal calprotectin · Lewis score · Small-bowel inflammation · Monocyte count · C-reactive protein · Multicenter study

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Introduction

Capsule endoscopy (CE) is the prime modality for accurate, non-invasive, and pain-free investigation of the small bowel [1]. In order to standardize reporting of small-bowel inflammation using CE, two scoring indices have been developed: the Lewis score (LS) and the Capsule Endoscopy Crohn's Disease Activity Index (CECDAI) [2–4]. Both scores are based on parameters and descriptors of

inflammatory change and have been externally validated in several reports [5–8]. However, they are of limited discriminatory ability, and it is still unclear how accurately they measure the degree of mucosal inflammation [6, 9].

Calprotectin was first isolated from human granulocyte cells by Fagerhol et al. [10]. Calprotectin is a major component of the cytosol of neutrophils and, to a lesser extent, monocytes and activated macrophages, released in feces upon leukocyte and epithelial activation [11–13]. In the presence of calcium, calprotectin is resistant to degradation and stable in feces at room temperature for up to 7 days [11, 14]. Fecal calprotectin (FC) 'leaks' into the gut lumen through inflamed mucosa therefore reflecting the amount of leukocyte cell activation, migration, and death [15]. Although FC is not disease specific, a recent meta-analysis showed an excellent correlation of FC with the severity of mucosal inflammation. At a cutoff level of 100 μg/g, FC can distinguish inflammatory bowel disease (IBD) from non-inflammatory conditions [16]. Therefore, many experts consider FC a reliable and highly specific biomarker of inflammation [9, 11]. There are conflicting reports suggesting that the correlation between FC and mucosal inflammation may be weaker in small-bowel inflammation in comparison with the colon. Monoclonal, polyclonal, and combination ELISA (quantitative), and bedside immune-chromatographic (semiquantitative) methods have been developed (and validated) for FC measurement [12].

Recently, we showed that measurement of FC levels prior to referral for CE is a useful tool to select patients with possible small-bowel IBD [17]. In this single-center study, FC > 100 μg/g is good predictor of positive small-bowel CE findings, while FC > 200 μg/g was associated with higher CE diagnostic yield (65 %) and confirmed small-bowel inflammation in 50 % of cases. Hence, it is reasonable to consider that strong correlation should exist between FC levels and LS [7–9]. However, in a separate cohort of patients with suspected, isolated small-bowel disease, LS showed strong correlation with FC at levels <100 μg/g [8]. The overall correlation between FC and LS is moderate at best [18]. This is certainly consistent with the high-negative predictive value (NPV) of FC [9]. Nonetheless, in individuals with higher FC levels, LS does not correlate well, and this can have impact on both patient selection for CE as well as with final outcomes.

The primary aim of this multicenter, retrospective study was to investigate the correlation between LS and FC in a larger group of patients who underwent CE for suspected or known small-bowel IBD.

Our secondary aim was to develop a model for prediction of CE results (LS) based on FC levels.

Materials and Methods

Patients and CE Procedure

This was a retrospective, multicenter study. The study cohort included all consecutive patients who underwent small-bowel CE in five academic referral centers (UK, Finland, Sweden, Canada, and Israel) and a large district general hospital (UK), from January 2010 to December 2013, with clinical suspicion of IBD or for IBD reassessment. Patients having normal ileocolonoscopy, without histological confirmation of Crohn's Disease (CD) on any biopsy material examined, were also eligible. A FC measurement within 3 months from the time of CE was considered necessary for inclusion. The absence of a bidirectional digestive endoscopy in the preceding period (up to a year before CE) was considered an exclusion criterion. Other causes of raised CRP or monocytes were excluded following review of patient case notes. Clinical and demographic data on age, gender, and CE indications were extracted from the patients' files and/or electronic hospital records. A small part of the UK and Swedish data may have been used in a previous publication [25].

The CE was performed with PillCam®SB2/SB3 (Given® Imaging Ltd, Yokneam, Israel) and MiroCam® (IntroMedic Co, Seoul, South Korea), according to local hospital protocols. Technical characteristics of these systems can be found elsewhere in the literature [19, 20]. Bowel preparation, where used, was polyethylene glycol (PEG) 2 or 4 lt. Prokinetics, where used, was in the form of domperidone (5–10 mg orally) and/or metoclopramide (10 mg intramuscularly) [21].

Fecal Calprotectin, C-Reactive Protein, and Monocyte Count

FC was measured with monoclonal/polyclonal ELISA (CALPRO AS, Lysaker, Norway; reference range 0–50 μg/g) or immune-chromatographic assay (Buhlmann's Quantum Blue, Basel, Switzerland; reference range: normal < 50 μg/g; "gray zone" 51–99 μg/g; positive > 100 μg/g) [11]. For the purpose of further statistical analysis, where FC < 20 μg/g, i.e., undetectable, the value 0 was used; for the semi-quantitative assays, for values >300 μg/g, the 300 μg/g was used. The C-reactive protein (CRP) and monocyte count were normal across sites if levels were <5 and <0.8 ng/l, respectively.

Lewis Score Calculation

All videos were reviewed by experienced CE readers (AK, TS, AN, ET, RM, GW, ES and RE). LS was calculated using the integrated LS Calculator (*RAPID*®, Given® Imaging Ltd, Yokneam, Israel) under white light or blue mode review [22]; where the calculator was not available

(MiroView®, IntroMedic Co, Seoul, South Korea), the calculation was performed manually. LS is based on the number and distribution of intestinal segments with villous edema, ulceration, and stenosis. To calculate the LS, the small bowel is first divided into equal transit thirds (tertiles). The final LS represents the highest tertile or the score with stenosis, if demonstrated [23]. Eventually, the LS allows small-bowel inflammatory activity to be classified into three grades: (1) normal or clinically insignificant mucosal inflammatory change (LS < 135); (2) mild disease ($135 \leq$ LS < 790); and (3) moderate-to-severe disease (LS ≥ 790) [2, 5, 6]. The CE date, FC measurement date, and time difference in days between the two was also calculated [8].

Statistical Analysis

Baseline quantitative data are presented as median and inter-quartiles range (IQR). For nominal variables, the Chi-square test or Fisher's exact test were used as appropriate. Student's t test was used for quantitative variables with normal distribution. Spearman's rank correlation coefficient (rho; r_s) was used to assess the correlation between LS and FC. The strength of correlation was defined as follows: r_s values ≤ 0.1 were considered to denote no correlation; 0.1–0.3 weak to modest; 0.3–0.49 moderate; 0.5–0.79 strong; and, ≥ 0.8 very strong correlation [24].

In order to detect the association between FS and LS adjusted for other factors, a multivariate linear regression analysis was used. The initial model contained age and monocyte count as adjustment factors of time lag between FC measurement and small-bowel CE. The model was subjected to a backwards elimination procedure using a multivariate linear regression analysis using the likelihood ratio test. A two-tailed probability (P) value < 0.05 was considered to be statistically significant. In addition, a receiver operating characteristic (ROC) analysis was conducted in order to determine the optimum cutoff point of FC results using the dichotomization of LS as explained in the previous paragraph. Statistical analyses were carried out in R statistical package.

Ethics Consideration

This study was conducted in accordance with local research ethics guidelines. After review by the local ethics committee(s), further specific ethical review and approval was not required, as the study was considered a service evaluation/clinical audit based on previously collected clinical data, with no additional patient intervention, obtained as part of regular clinical care.

Results

Patients and Capsule Endoscopy Data

In the aforementioned period, 333 (119M/214F; median age: 41 years; IQR: 25) patients who fulfilled the study inclusion criteria were referred for CE due to clinical suspicion of small-bowel IBD ($n = 287$; 98M/189F; median age: 41 years; IQR: 26) or suspicion of small-bowel inflammation reactivation in patients with known CD ($n = 46$; 21M/25F; median age: 34.5 years; IQR: 24). Two different small-bowel CE systems were used (PillCam®SB: 150/MiroCam®: 183); in three patients the capsule endoscope (2 PillCamSB®, 1 MiroCam®) was retained in the stomach for the entire period of the recording, hence no LS data were available. These cases were excluded from further analysis. Symptoms were mainly diarrhea, anemia, weight loss, and/or abdominal pain, Table 1.

Fecal Calprotectin

Clinically Important FC Thresholds

FC measurements were performed with a quantitative ELISA in 280 patients and with semiquantitative assays in the remainder ($n = 50$). Overall, for the entire dataset ($n = 330$), correlation between FC and LS was weak (r_s: 0.232, $P < 0.001$). When the two clinically significant FC thresholds of 100 and 250 μg/g were examined [11, 17], irrespective of the FC assay used, the r_s between FC and LS for the two threshold levels was 0.247 (weak) and 0.337 (moderate), respectively ($P = 0.307$). The median values (with range; IQR) for FC, LS and the time interval between FC measurement and small-bowel CE were 90 (15,255; 240) μg/g, and 0 (0,337.5; 337.5) and 0 (0,62.75; 62.75) days, respectively. Furthermore, no LS/FC correlation difference was recorded between the two small-bowel CE systems, ($P = 0.118$).

In the quantitative FC (ELISA) subgroup ($n = 280$), the correlation between FC and LS was moderate (r_s: 0.385, P: 0.0), as previously shown [8, 25]. The median values (with range; IQR) for FC, LS, and the time interval between FC measurement and small-bowel CE were 28 μg/g (9,220; 211), and 0 (0,339.75; 339.75) and 14.5 days (0,46.75; 46.75), respectively. In this subgroup, 150 CE were performed with MiroCam® and the remainder ($n = 130$) with PillCam®SB. No statistical difference between FC levels (100.37 ± 191.24 vs 90.71 μg/g; $P = 0.649$), time interval between FC/CE (28.4 ± 39.4 vs 20.63 ± 29.5 days; $P = 0.059$), prokinetic use ($P = 0.547$), or bowel prep use ($P = 0.717$) between the two CE subgroups was noted, Table 2a, b.

Table 1 Indications for referral for CE

Indication	Number of patients (% of total)
Diarrhea	112 (33.6)
Abdominal pain	104 (31.2)
Iron deficiency anemia	62 (18.6)
Raised FC	26 (7.8)
Weight loss	23 (6.9)
OGIB	19 (5.7)
Abnormal radiological investigations	11 (3.3)
Background of celiac disease, autoimmune disease or IBD	11 (3.3)
Nutritional deficiencies/malabsorption, e.g., B12/folate, albumin	9 (2.7)
Family history of IBD	6 (1.8)
Perianal fistula	6 (1.8)

Please note that numbers do not add up to study size of 333 as many patients had more than one indication for referral

FC fecal calprotectin, *IBD* inflammatory bowel disease

Table 2 Breakdown of results by subgroup

(a) Comparison of subgroups

	Quantitative FC	Semiquantitative FC
N	280	50
Median FC (μg/g) (range IQR)	28 (9–220; 211)	145 (105.75–300; 194.25)
Median LS (range IQR)	0 (0–339.75; 339.75)	135 (0–287; 287)
Median time from FC to CE (days) (range IQR)	14.5 (0–46.75; 46.75)	25 (0–474; 474)

(b) Comparison of MiroCam® vs. PillCam® SB2 subgroups in the quantitative FC group

	MiroCam®	PillCam®SB2	*P* value
N	150	130	
Median FC (μg/g, SD)	100.37 ± 191.24	90.71 ± 166.1	0.547
Time from FC to SBCE (days, SD)	28.4 ± 39.4	20.63 ± 29.5	0.059
Prokinetic use	55	42	0.547
Bowel prep used	54	42	0.717

FC fecal calprotectin, *IQR* inter-quartile range, *LS* Lewis score, *SD* standard deviation, *SBCE* small-bowel capsule endoscopy

In the subgroup of semiquantitative FC ($n = 50$), there was no correlation between FC and LS (r_s: −0.130, *P*: 0.377). In this subgroup, the median values (with range and IQR) for FC and LS were 145 μg/g (105.75,300; 194.25), 135 (0,287; 287), respectively. PillCam®SB was used in 18 and MiroCam® in 32 patients. Furthermore, the median interval between small-bowel CE and FC was 25 days (0–474; 474) (i.e., not significantly different from the quantitative FC group; $P = 0.07$).

Monocytes and CRP

The median (range; IQR) monocyte and CRP counts were 0.535 (0.41, 0.72; 0.31) and 7 (3,15; 12), respectively. The correlation between monocyte count and LS was weakly negative (r_s: −0.019, *P*: 0.732), while the relevant value for CRP was r_s: −0.095, *P*: 0.086. It has been reported that the CRP/monocyte ratio represents the acute phase of inflammation [26]. There were 73 complete datasets (ratio, FC and LS) with measurements obtained ±7 days around the CE (median: 0 days, IQR: 0 days). The median value of the ratio was 12 (5.21, 24.47; 24.25), and the correlation of the ratio with FC and LS was r_s: 0.14 (*P*: 0.235) and r_s: 0.02 (*P*: 0.865), respectively.

Model Creation

In order to investigate the potential association between LS and FC, both variables were log-transformed. The final model for the association of LS and FC was found to be:

$$\log(\mathrm{LS}+1) = -1.05 - 0.0087 \times \text{time lag simplistic} + 1.0471 \times \log(\mathrm{FC}+1)$$

Other predictors such as age ($P = 0.902$) and monocyte count ($P = 0.805$) were eliminated from the initial model during the backwards elimination procedure. The results of the final model are provided in Table 3, where the intercept ($P = 0.269$) was kept as it was found that the normality of the residuals was violated when this was removed. Furthermore, the model is interpreted as an increase of 1 point in FC gives an increase of 1.0471 in log(LS + 1) (95 % CI: 0.679; 1.415). The latter translates to a 0.389 points increase in LS (95 % CI: 0.159; 0.832) for a constant FC/CE time lag simplicity of zero. Also an increase of 1 point in FC/CE time lag gives a decrease of −0.0087 (95 % CI: −0.016; −0.001) in log(LS + 1).

Optimum Cutoff Point of FC

The analysis using ROC curves gave that the dichotomization of LS at 135 for clinically significant (LS ≥ 135) or negative (LS < 135) for SB inflammation gave an optimum cutoff point of FC 76 at µg/g with sensitivity 0.59 and specificity 0.41.

Discussion

FC level in the stool is directly proportional to neutrophils in the intestinal lumen; therefore, its use as biomarker of enteric inflammation and neoplastic lesions has been proposed. One of the main indications for CE is the direct visualization of the extent, location, and severity of small-bowel inflammation [23]. Others suggest that FC could discriminate between organic and functional intestinal pathology and allow selection of patients who are more likely to benefit from a colonoscopy [16]. Recently, we hypothesized that FC can be used as selection tool for performing CE in patients with continuing clinical suspicion for small-bowel IBD, despite preliminary negative diagnostic workup [17]. Currently, healthcare systems worldwide are under significant economic strain to provide high-quality care with shrivelling budgets [26, 27]. Therefore, increasing the diagnostic yield of patient workup with inexpensive, accurate, non-invasive investigations, has multiple benefits [13, 28].

In the present study, retrospective data on FC, monocyte count, and CRP paired with CE findings (LS was used to quantify small-bowel inflammation in an objective way) [2, 8] were collected from patients with clinical suspicion of small-bowel IBD ($n = 287$), out of which 3% had ileitis on colonoscopy but inconclusive histology, from high-volume CE centers (UKx2, Finland, Sweden, Canada, Israel). The remainder ($n = 43$) had a history of known CD and were referred for small-bowel assessment with CE. Experienced CE reviewers reported the CE results at each site for the purpose of clinical care/need using white light and/or blue mode (depending on preference per reviewer) [6, 22]. In 84.8 % of cases, FC was measured using a commercially available ELISA (range 0–50 µg/g). In these patients, CE was performed using the PillCam®SB in 46.4 % of cases; the remainder was performed with MiroCam®. Based on the CE system used, the two patient subgroups were equivalent in terms of FC levels, time interval between FC measurement and performance of CE, and procedural factors for small-bowel CE such as the use of a prokinetic and/or a bowel purge (or not). Therefore, we are able to confirm that the lack of an integrated calculator in the MiroCam® proprietary software (MiroView®) notwithstanding the calculated LS had the same correlation with FC levels.

Another finding of this study is low correlation of FC with monocyte count, CRP, CRP/monocyte, and LS, Table 2. The former has been previously shown in studies from our group [15, 25]. Furthermore, elevated CRP, FC, or the combination of both was poorly correlated with detectable small-bowel inflammation [18, 29]. Nevertheless, it is worth noting that when the threshold level of significant SB inflammation, as denoted by LS was shifted from 135 to 350, the correlation of FC and LS was similar at r_s: 0.07 (P: 0.637) and 0.09 (P: 0.696) for the suspected and known CD group, respectively.

Others have recently confirmed strong inter-observer agreement in determining LS in CE [6]. Höög et al. [7], in a cohort of 30 patients, showed that there was a significant persistent correlation between endoscopic inflammation and FC (at study inclusion and at a year's follow-up). More recently, Olsen et al showed that the proportion of patients with findings on small-bowel CE increased with increasing FC [30]. Nevertheless, in their cohort, a positive FC (≥50 mg/kg) had a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)

Table 3 Model for the association of FC and LS

Model	Coefficients	SE	t value	Pr(> |t|)	95 % CI
Intercept	−1.0513	0.9466	−1.11	0.269	−2.907; 0.804
Time lag FC/CE	−0.0087	0.0039	−2.24	0.027	−0.016; −0.001
Log (FC + 1)	1.0471	0.1876	5.58	<0.001	0.679; 1.415

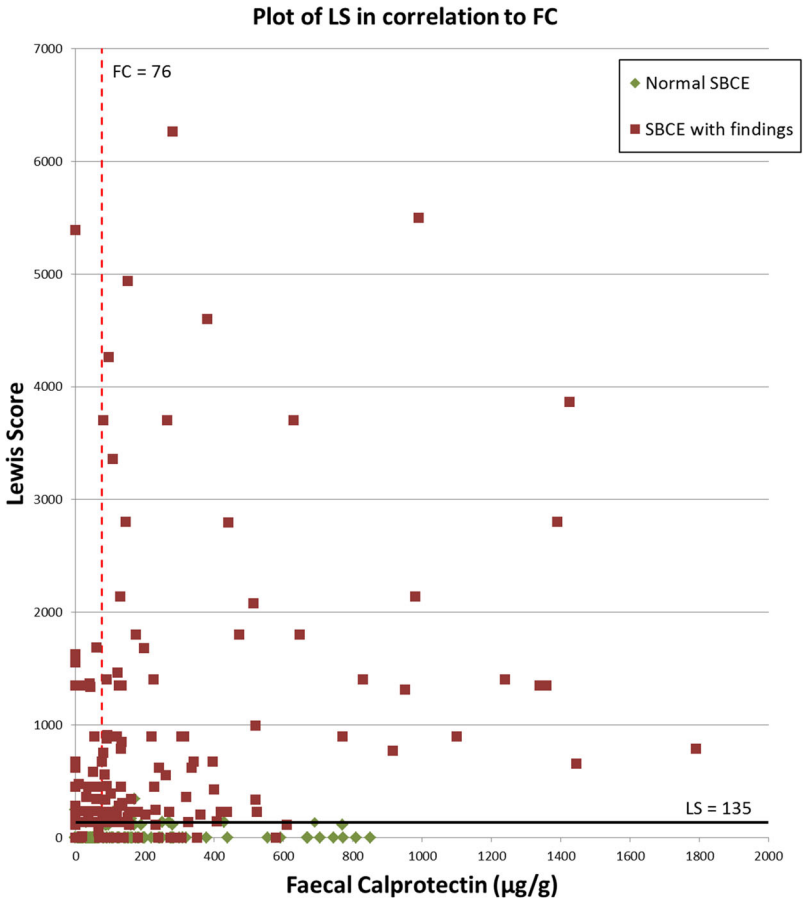

Fig. 1 Plot of LS in correlation to FC

of 54.2, 69.9, 43.3, and 78.2 %, respectively. The correlation of FC values with presence of active small-bowel inflammation as detected by magnetic resonance enterography (MRE) was similar to that of CE [29].

Limitations of this study include the lack of formal assessment of the extent of mucosal visualization. As not all patients underwent bowel preparation prior to CE, it is possible that LS could in part be altered by the degree of small-bowel visualization. However, there is a lack of data on LS correlation with the quality of SB visualization. The fact that the CEs in this study were each reviewed by a single reviewer only, despite substantial cumulative experience in CE, could be a further limitation leading to lower diagnostic yield.

This study did not establish a correlation between endoscopic severity, as measured by the LS, and FC or other biomarkers of inflammation. This is likely to reflect deficiencies of the scoring system [25] as well as the study's inherent limitations such as the cutoff level selected. FC may also be a marker of subclinical inflammation; Gisbert and McNicholl [31] found that FC was higher in asymptomatic first-degree relatives of patients with IBD, and FC has been seen to predict relapse in asymptomatic or quiescent CD [32]. Another study has found that FC does not reliably distinguish IBD from malignancy [33], which may—indirectly—suggest that FC is not as good at distinguishing generalized inflammation from foci of inflammation. Furthermore, some studies show FC is a more reliable indicator of colonic than SB inflammation, i.e., usefulness of FC varies with location of inflammation within the gut, and there is difficulty in establishing correlation due to the heterogeneity of presentations in CD [34, 35]. Figure 1 shows how LS is generally low in patients with normal SBCE; however these patients have a wide range of FC. Conversely our study also had patients with low FC but high LS, which could have been indicative of a single large lesion, such as an isolated stenosis, yielding a diagnosis. Further prospective studies should be performed to investigate the difference between the equivocal results of our study and other studies which show positive correlation between LS and FC.

Our findings suggest that in patients with strong clinical suspicion of small-bowel CD and negative bidirectional endoscopy, CE should not be limited to patients with elevated biomarkers only. Especially, CRP and the ratio in particular were not associated with SB inflammation on CE. Moreover, the correlation was moderate for FC, and if this biomarker was used to guide the decision to perform

CE, at least 40 % of patients will be misdiagnosed. However, the use of single FC measurement per patient for the purpose of this study [36, 37], its retrospective nature and the use of different laboratories and FC kits should be considered as additional limitations of this study. Nevertheless, FC $\geq$ 76 μg/g may be associated with appreciable inflammation on CE in patients with negative prior diagnostic workup.

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Authors' contributions All authors contributed to data collection. Dr. A Koulaouzidis created the first draft. All authors critically reviewed the document and provided changes. All authors approved the final version of the article, including the authorship list.

Compliance with ethical standards

Conflict of interest Dr. Koulaouzidis received an Given Imaging Ltd/ESGE Ltd research Grant in 2011. He has also accepted material support for research from SynMedUK Ltd. Dr. Seidman has received in-kind research support from Given Imaging/Medtronic Inc., 2011–2015. Rami Eliakim received consultation fees from Given Imaging. The rest of the authors have no disclosure to make.

Competing interests None.

Patient consent None.

Ethics approval Clinical Audit Department at the Royal Infirmary of Edinburgh.

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