MicroRNA-320a Monitors Intestinal Disease Activity in Patients With Inflammatory Bowel Disease

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OBJECTIVES: **In patients with inflammatory bowel disease (IBD), a treat-to-target treatment strategy requires tight monitoring of disease activity. Noninvasive biomarkers may help to monitor the intestinal disease activity. We demonstrated recently that peripheral microRNA (miR)-320a expression in mice follows the course of experimental colitis. The aim of this study was to evaluate the potential of miR-320a to monitor the disease activity in patients with IBD, to predict the course of disease, and to distinguish IBD from infectious colitis.**

METHODS: **The miR-320a levels were prospectively assessed by quantitative real-time polymerase chain reaction analysis of peripheral blood samples from 40 patients with Crohn's disease (CD) and 37 patients with ulcerative colitis (UC) as well as from 19 healthy control individuals and 7 patients with infectious colitis. Disease activity was quantified by appropriate clinical disease indices and endoscopic scoring systems.**

RESULTS: **When compared with healthy controls, miR-320a blood levels were significantly increased in patients with active CD and UC (16.1 ± 2.6 vs 2,573 ± 941; vs 434 ± 96; both $P < 0.001$) and patients with IBD in remission (316 ± 251 [CD] and 91 ± 29 [UC]; both $P < 0.001$). In patients with CD, miR-320a levels showed a strong correlation with the endoscopic disease activity ($r^2 = 0.76$; $P < 0.001$). Similarly, in patients with UC, we detected a significantly enhanced miR-320a expression, which was highest in patients with severe endoscopic disease activity (eMayo = 0–1: 66 ± 16 vs eMayo = 2: 352 ± 102; vs eMayo = 3: 577 ± 206; both $P < 0.001$). Finally, miR-320a blood expression in patients with active CD and UC significantly increased compared with patients with infectious colitis (63 ± 13, $P < 0.001$).**

DISCUSSION: **MiR-320a expression in peripheral blood from patients with IBD follows the clinical and endoscopic disease activities and may help to distinguish IBD from infectious colitis.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A196, http://links.lww.com/CTG/A197, http://links.lww.com/CTG/A198,http://links.lww.com/CTG/A199,http://links.lww.com/CTG/A200

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INTRODUCTION

Patients suffering from Crohn's disease (CD) and ulcerative colitis (UC) can develop disabling complications such as fistulas and strictures or colitis-associated cancer because of uncontrolled inflammation (1,2). Effective medical treatment that results in mucosal healing is associated with improved clinical outcomes (3–7). Endoscopy is highly appropriate to assess mucosal healing; however, this modality is also invasive, expensive, and associated with complications such as abdominal pain, complications owing to sedation, or perforation in up to 0.02%–0.2% (8,9).

By contrast, clinical disease activity indices are easy to assess, but it was demonstrated that only one-third of patients with clinical remission are in endoscopic remission (10). Conversely, symptoms assessed by clinical activity indices such as diarrhea or abdominal pain are not specific to inflammatory bowel disease (IBD) (10–12). Serum markers such as C-reactive protein (CRP) or blood sedimentation rate are of some help to screen for ongoing inflammation and are associated with strong intestinal disease activity in UC or complicated disease in CD (13,14). Nevertheless, both these markers are also not specific to IBD.

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Thus, immediately available tools to noninvasively monitor intestinal inflammation would be desirable.

MicroRNAs (miRNAs), small noncoding, post-transcriptional gene-regulating RNAs, are currently investigated as biomarkers for IBD (15). Previously, we could identify several miRNAs being involved in the barrier-enhancing effect of probiotic *Escherichia coli* Nissle 1917 (16). Of these, we identified microRNA (miR)-320a to strengthen the intestinal epithelial barrier *in vitro* and to follow the disease activity in colitic mice (17).

Therefore, the aim of this prospective study was to evaluate miR-320a as a biomarker to monitor the disease activity in patients with IBD and to distinguish UC and CD from infectious colitis. We hypothesized that miR-320a has the potential to specifically follow intestinal disease activity of patients with IBD.

MATERIALS AND METHODS

Patients

Seventy-seven patients (CD, n = 40; UC, n = 37) with histologically confirmed CD and UC were recruited for our study. Patients with infectious diseases or autoimmune comorbidities such as primary sclerosing cholangitis or autoimmune hepatitis were excluded. Peripheral blood samples per patient were collected at acute flare before treatment escalation or at treatment response and in remission. Patient's characteristics were retrieved from medical records and are summarized in Table 1, Supplementary Digital Content 3, http://links.lww.com/CTG/A198 and the Supplemental Methods, Supplementary Digital Content 2, http://links.lww.com/CTG/A197. Medication at study inclusion is summarized in Table 2, Supplementary Digital Content 4, http://links.lww.com/CTG/A199.

Clinical disease activity in patients with UC was assessed using the Mayo score (18) and in patients with CD in accordance with the Crohn's disease activity index (CDAI) (19). Clinical response was defined as a decrease in CDAI of ≥70 points (20) compared with baseline in patients with CD and reduction in Mayo score ≥3 points with rectal bleeding subscore of 0 in patients with UC (21,22). Clinical remission was defined as clinical Mayo Score 0–1 in patients with UC including rectal bleeding subscore of 0 and CDAI <150 in patients with CD. In addition to the clinical score, disease activity was assessed by endoscopy. Therefore, the Mayo score was used in patients with UC and the simple endoscopic score for Crohn's disease (SES-CD) score in patients with CD (active disease: endoscopic Mayo score ≥2 (21,23); CD: SES-CD ≥3 (24,25). Blood samples obtained from healthy volunteers matched for age and sex (n = 19) served as control samples. Furthermore, patients without IBD but with confirmed diagnosis of *Clostridium difficile*-associated infectious colitis were included before the start of treatment (n = 7). Diagnostic criteria for study inclusion comprised a 2-stage algorithm including testing for both glutamate dehydrogenase and toxins A and B as well as distinct clinical evidence of diarrhea (26). See Table 3, Supplementary Digital Content 5, http://links.lww.com/CTG/A200 for patients' characteristics. The Ethics Committee of the University of Münster approved the study (file number 2013-070-f-S). Written informed consent was obtained from all patients. The study was registered at ClinicalTrials.gov (NCT03698500).

RNA isolation from small volumes of total blood

RNA isolation was performed as described earlier (17). A detailed protocol can be found in the Supplemental Methods, Supplementary Digital Content 2, http://links.lww.com/CTG/A197.

Quantitative real-time polymerase chain reaction

Changes in miRNA expression in analyzed blood were determined by quantitative real-time polymerase chain reaction (miScript SYBR Green PCR Kit [Qiagen, Hilden, Germany]) using the LightCycler 480 system and quantified using the "Light Cycler 1.5.0 software" sp.4 (Roche, Mannheim, Germany). MiRNA-specific miScript Primer Assays (HS_RNU6-2_11 and HS_miR-320a_1) were purchased from Qiagen.

Statistical analysis

Data were compared by using the Mann-Whitney *U* test. Potential correlation between variables were assessed according to Pearson. Data are expressed as mean values ± SEM. The local significance level is set to 0.05. Statistical analyses were performed using IBM SPSS Statistics 13 for Windows (IBM Corporation, Somers, NY).

RESULTS

MiR-320a blood levels are elevated in patients with CD with acute flare and reflect clinical disease activity and treatment response

In a first set of analyses, we investigated miR-320a blood levels in patients with active and quiescent CD. We detected significantly increased miR-320a levels in patients with flare compared with patients in remission (2,573 ± 941 vs 316 ± 251; $P < 0.001$) or healthy control individuals (2,573 ± 941 vs 16.1 ± 2.6; $P < 0.001$; Figure 1a). Next, we analyzed miR-320a levels to reflect severity of clinical disease activity in active CD. We found that in patients with mild disease activity (CDAI 150–220), miR-320a levels were significantly lower compared with moderate and severe activities (CDAI > 220) but not significantly different compared with patients in clinical remission with a CDAI < 150 (242 ± 92 vs 3,374 ± 1,226; $P = 0.016$; vs 350 ± 275; $P = 0.28$; Figure 1b).

In addition, we analyzed the potential of miR-320a to follow the course of inflammation. For this aim, we examined the peripheral expression in 10 patients with CD with acute flare and after reaching clinical response in a paired analysis. Our data revealed that treatment response was accompanied by significant decrease after successful medication compared with untreated patients (647 ± 314 vs 4,878 ± 2,270; $P = 0.004$, Figure 1c). Furthermore, paired analysis of 11 patients with CD revealed significantly lower miR-320a expression after achieving remission compared with that in samples obtained during acute flare in these patients (564 ± 501 vs 2,897 ± 1,985; $P = 0.001$; Figure 1d).

MiR-320a in peripheral blood monitors mucosal disease activity in patients with CD

Next, we compared miR-320a levels to intestinal disease activity of patients with CD. We could demonstrate that patients with severe intestinal inflammation (SES-CD >15) showed significantly higher miR-320a expression levels compared with patients with moderate (SES-CD 7–15) and mild activities (SES-CD 3–6) or quiescent disease (SES-CD < 3; 6,496 ± 3,016 vs 1,188 ± 459, $P = 0.013$; vs 108 ± 21, $P = 0.0012$; vs 56 ± 16, $P < 0.001$; Figure 2a). Patients with mild endoscopic disease activity trended toward higher miR-320a levels compared with patients with quiescent disease with a borderline significance (108 ± 21 vs 56 ± 16; $P = 0.070$). In addition, miR-320a levels revealed a strong correlation with the SES-CD ($r^2 = 0.76$; $P < 0.001$; Figure 2b).

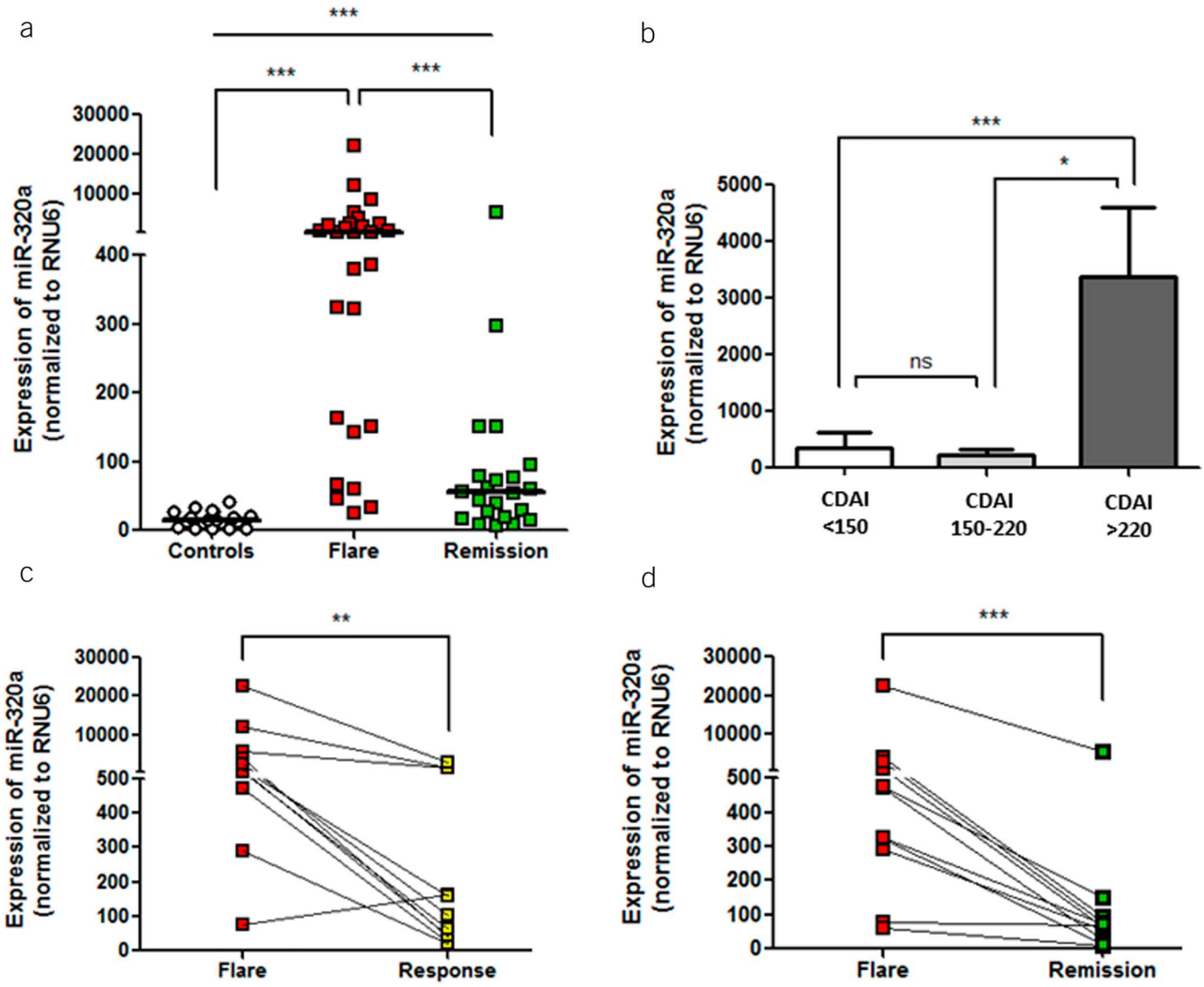

Figure 1. The miR-320a blood levels reflect clinical disease activity and treatment response in patients with CD. Its expression level in blood was assessed in patients with CD with active disease (n = 28) or in remission (n = 23) as well as in healthy controls (n = 19). (**a**) The miR-320a levels were significantly increased in patients with CD with active disease or in remission when compared with healthy controls. (**b**) Patients with CD with mild clinical disease activity (CDAI 150–220; n = 7) revealed significantly lower miR-320a levels compared with those in patients with severe clinical activity (CDAI >220, n = 20), but miR-320a expression was not different when compared with patients in clinical remission (CDAI <150, n = 20). (**c, d**) Paired analysis of patients with CD at acute flare revealed a significant decrease of miR-320a expression after reaching a clinical response (10 pairs) or remission (11 pairs). The miR-320a was normalized to miRNA RNU6 as a reference. Data are (a) median or (b) mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. CDAI, Crohn's disease activity index; CD, Crohn's disease; miR, microRNA; RNU6, Hs_RNU6-2_11.

In addition, we analyzed miR-320a levels depending on the disease manifestation. When compared with patients with isolated manifestation of the small bowel, miR-320a expression in patients with colonic disease manifestation trended toward higher miRNA-320a levels, although without statistical significance ($P = 0.40$; Figure 2c). Both entities revealed distinct elevated miR-320a expression compared with endoscopic remission (both $P < 0.01$).

Medical treatment, including corticosteroid (see Figure 2a,b, Supplementary Digital Content 1, http://links.lww.com/CTG/A196) and biological therapies (see Figure 2c,d, Supplementary Digital Content 1, http://links.lww.com/CTG/A196), showed no effect on miR-320a levels in neither an overall analysis of patients with active CD nor an adjusted analysis for endoscopic disease activity.

Increased miR-320a level in patients with active UC decreases at clinical response

Next, we investigated miR-320a blood levels in patients with active UC disease and in remission. Similar to CD, miR-320a expression significantly increased in patients with UC with flare compared with remission and controls (434 ± 96 vs 91 ± 29; vs 16.1 ± 2.6; both $P < 0.001$; Figure 3a). Next, we analyzed the potential of miR-320a levels to reflect clinical disease activity. When compared with clinical remission, miR-320a levels significantly increased in patients with moderate and severe clinical disease activities (104 ± 36 vs 279 ± 59, $P = 0.005$; vs 499 ± 146, $P < 0.001$; Figure 3b), whereas mild flare revealed no significant miR-320a increase (104 ± 36 vs 93 ± 23; $P = 0.81$).

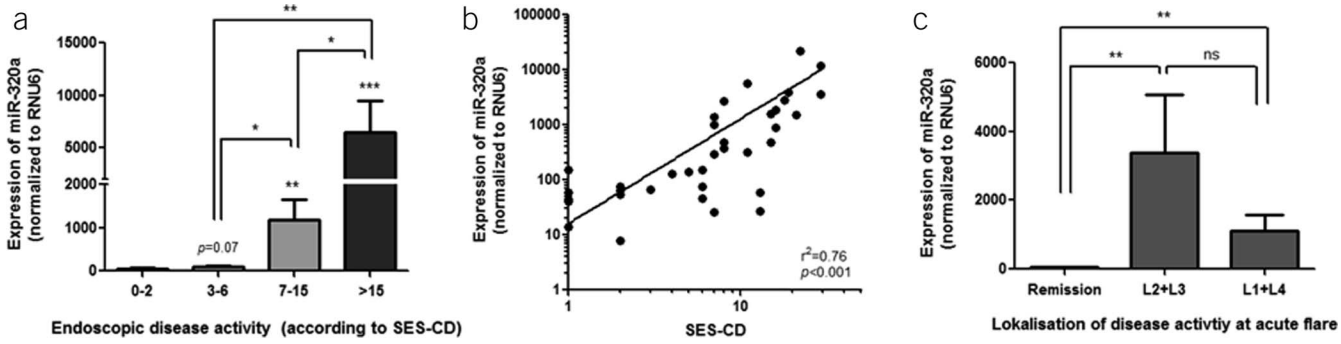

Figure 2. The miR-320a blood levels monitor mucosal disease activity in patients with CD. Its expression level was assessed in patients with mild (n = 6), moderate (n = 12), and severe (n = 7) endoscopic disease activities and in patients with endoscopic remission (n = 8) according to the SES-CD. (**a**) The miR-320a could significantly distinguish patients with endoscopic remission from moderate and severe endoscopic disease activities with a borderline significant difference compared with patient with mild endoscopic disease. (**b**) The miR-320a expression correlated strongly with the endoscopic disease activity assessed by the SES-CD. (**c**) Patients with isolated small bowel disease manifestation (L1 + L4; n = 12) according to the Montreal classification trended toward lower miR-320a expression compared with patients with colonic manifestation (L2 + L3; n = 14) without reaching statistical significance level. The miR-320a was normalized to miRNA RNU6 as a reference. Data are mean ± SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. CD, Crohn's disease; SES-CD, simple endoscopic score for Crohn's disease; miR, microRNA; RNU6, Hs_RNU6-2_11.

A paired analysis of serial blood samples from patients with UC with acute flare before treatment escalation compared with treatment response (324 ± 107 vs 59 ± 20; Figure 3c) or remission (553 ± 197 vs 116 ± 47; Figure 3d) revealed significantly decreased miR-320a levels after successful treatment induction (both $P = 0.004$).

MiR-320a blood levels monitor mucosal disease activity in patients with UC

When endoscopic disease activity is concerned, miR-320a levels followed the degree of mucosal inflammation according to the endoscopic Mayo score: patients with mucosal healing (eMayo 0–1) showed significantly lower miR-320a levels compared with moderate (eMayo 2) and severe endoscopic disease activities (eMayo 3; 66 ± 16 vs 352 ± 102; vs 577 ± 206; both $P < 0.001$; Figure 4a).

Next, we assessed the amount of miR-320a synthesis for different stages of disease manifestation according to the Montreal classification: when comparing patients with UC suffering from limited colitis, including proctosigmoiditis (E1) and left-sided colitis (E2), with patients with extensive colitis (E3), we detected a trend toward higher miR-320a expression levels in those patients with extensive colonic involvement (739 ± 223 (E3) vs 301 ± 84 (E1/E2); $P = 0.080$; Figure 4b).

Analysis of medication revealed no statistical impact of biological therapy on miR-320a expression levels (see Figure 3c,d, Supplementary Digital Content 1, http://links.lww.com/CTG/A196), whereas corticosteroid treatment was associated with a mild but significant increase of miR-320a levels in patients with active UC with moderate endoscopic disease activity (see Figure 3a,b, Supplementary Digital Content 1, http://links.lww.com/CTG/A196). However, the fact that opposing impact of corticosteroid treatment was observed in patients with endoscopic remission, which revealed a trend toward higher miR-320a levels in corticosteroid-free patients, questions the causality of corticosteroid treatment to be involved in miR-320a regulation.

MiR-320a level in peripheral blood can distinguish infectious colitis from active IBD

As IBD-specific biomarkers are urgently needed, we further investigated the potential of miR-320a to distinguish infectious colitis from IBD. We detected that in patients without IBD but with confirmed *C. difficile*-associated colitis, peripheral miR-320a levels were significantly lower compared with those in patients with CD with mild, moderate, or severe endoscopic inflammation (63 ± 13 vs 108 ± 21, $P = 0.050$; 1,188 ± 459, $P = 0.004$; vs 6,496 ± 3,016, $P < 0.001$; Figure 5a) and those in patients with UC with moderate and severe endoscopic inflammation (63 ± 13 vs 352 ± 102, $P < 0.001$; vs 577 ± 206, $P = 0.002$; Figure 5b).

Taken together, our data point at the potential of miR-320a to differentiate active from quiescent IBD and from patients without IBD but with infectious colitis. Furthermore, miR-320a reflects intestinal disease activity in both active CD and UC and might serve as a marker to treatment response after therapy escalation.

Determination of miR-320a cutoff levels in peripheral blood to distinguish active from quiescent disease

Mucosal healing has become a widely accepted treatment target in IBD with less long-term complications and better maintenance of remission compared with clinical remission as treatment target (27). To define a miR-320a cutoff level that indicates mucosal healing, we assessed patients with endoscopically confirmed absence of endoscopic disease activity. Setting a cutoff level for miR-320a at 120 (relative units), 8 of 10 patients with UC were correctly classified as in endoscopic remission (Figure 6a). Alternatively, 21 of 23 patients with active UC revealed miR-320a levels above 120. The sensitivity estimates were 91.3%, and the specificity estimates were 80%. Clinical and endoscopic disease activities reflected by partial and complete Mayo score was significantly higher in patients with high (>120) miR-320a expression compared with low (<120) miR-320a levels (Figure 6b,c). In CD, 8 of 9 patients with mucosal remission were correctly identified by cutoff value of 120, whereas in patients with active disease, 19 of 24 patients showed miR-320a levels above the cutoff (Figure 6d). This results in a sensitivity rate of 79.2% and a specificity rate of 88.9%. Disease activity indices CDAI and SES-CD were significantly higher in CD patients with high (>120) miR-320a levels compared with low (<120) miR-320a expression (Figure 6e,f).

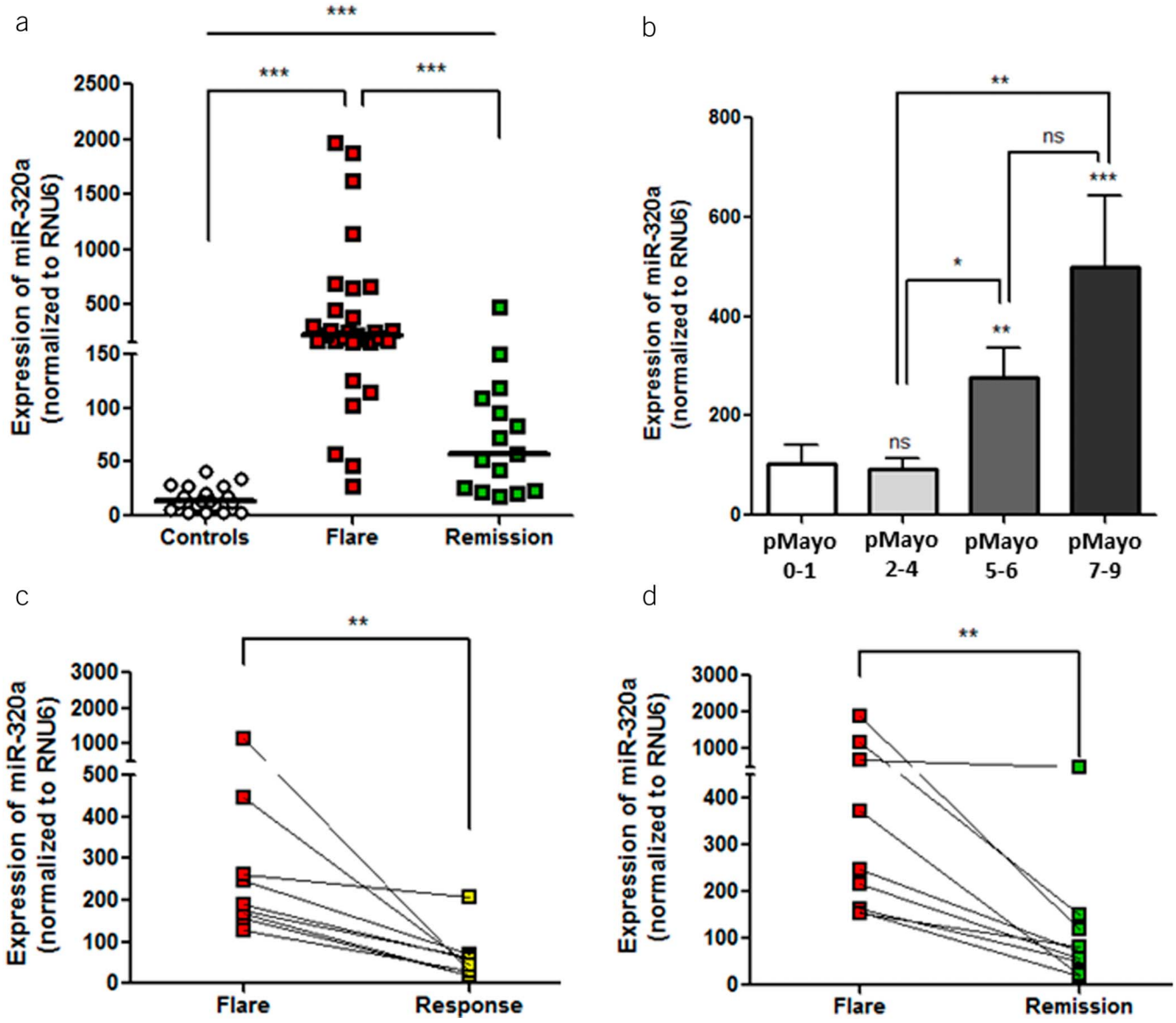

Figure 3. The miR-320a expression in peripheral blood indicates clinical disease activity in UC and reflects treatment response. Its expression level was assessed in patients with UC with active disease (n = 30) or in remission (n = 16) as well as in healthy controls (n = 19). (**a**) In patients with UC, miR-320a expression level was significantly increased in patients with active disease or in remission when compared with healthy controls. (**b**) Analysis of miR-320a to reflect clinical disease activity revealed that miR-320a levels were significantly increased in patients with moderate (pMayo 5–6; n = 10) or severe (pMayo 7–9; n = 11) clinical disease activities compared with patients in clinical remission (pMayo 0–1; n = 12). (**c, d**) Paired analysis of patients with UC at acute flare revealed a significant decrease of miR-320a expression after reaching a clinical response (9 pairs) or remission (9 pairs). The miR-320a was normalized to miRNA RNU6 as a reference. Data are (a) median or (b) mean ± SEM. $*P<0.05$; $**P<0.01$; $***P<0.001$. miR, microRNA; pMayo, partial Mayo score; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

MiR-320a blood levels monitor endoscopic disease activity in patients with mild or absent clinical symptoms

Because especially patients with mild clinical disease activity are at risk for underestimation of intestinal inflammation, we next investigated the potential of miR-320a to indicate intestinal inflammation in patients with IBD with mild or absent clinical symptoms. Before this analysis, we correlated clinical and endoscopic disease activity, which revealed a weak non-significant correlation for CD ($r^2 = 0.28$, $P = 0.11$; Figure 7a) and a significant correlation for UC ($r^2 = 0.55$, $P < 0.001$; Figure 7c). Analysis of miR-320a expression in patients with mild or absent clinical symptoms revealed that miR-320a levels were significantly higher in both patients with UC (n = 16) and patients with CD (n = 17) with intestinal inflammation (defined as SES-CD ≥3 and eMayo score 2–3) compared with patients in endoscopic remission (CD: 682 ± 218 vs 64 ± 17; $P = 0.025$; Figure 7b; UC: 729 ± 315 vs 46 ± 12; $P < 0.001$; Figure 7d). These data indicate that miR-320a reflects intestinal disease activity besides absent or mild clinical symptoms, which further supports our hypothesis of increased miR-320a levels as a surrogate for mucosal inflammation.

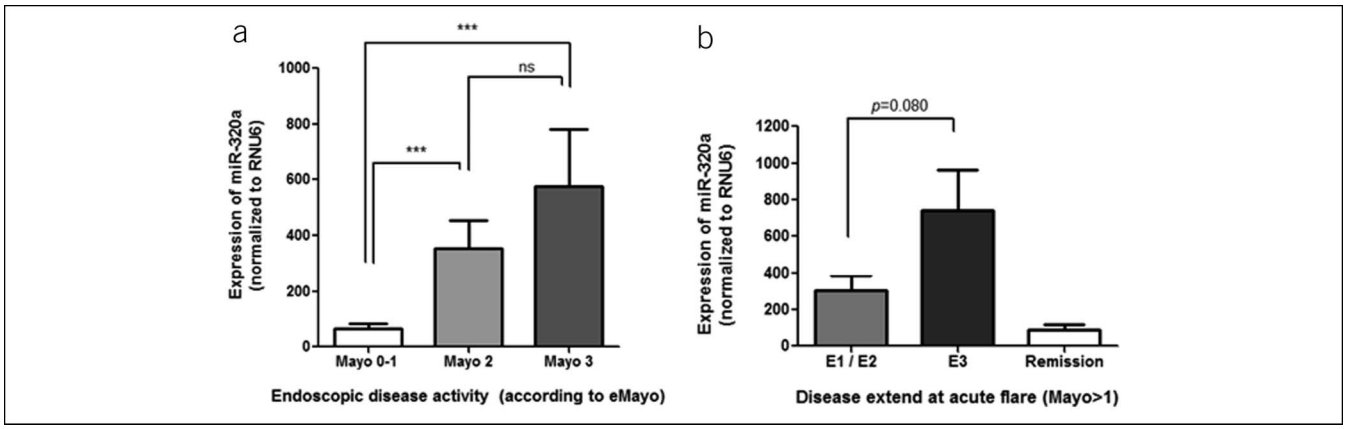

Figure 4. The miR-320a blood levels monitor mucosal disease activity in patients with UC. Its expression level was assessed in patients with moderate (n = 15) and severe (n = 11) endoscopic disease activities and in patients with endoscopic remission (n = 10) according to the endoscopic Mayo score. (**a**) The miR-320a could significantly distinguish patients with endoscopic remission from moderate and severe endoscopic disease activities. (**b**) Patients with more extensive colonic disease manifestation according to the Montreal classification (E3; n = 10) trended toward higher miR-320a expression compared with patients with limited colitis (E1/E2; n = 18) with a borderline statistical significance. The miR-320a was normalized to miRNA RNU6 as a reference. Data are mean ± SEM. $***P < 0.001$. eMayo, endoscopic Mayo score; miR, microRNA; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

Evaluation of the predictive value of miR-320a blood levels

Finally, we prospectively investigated the potential of miR-320a to predict the course of disease. First, initial miR-320a levels at disease flare before treatment escalation were analyzed regarding the prediction of medical treatment response (see Figure 4a,b, Supplementary Digital Content 1, http://links.lww.com/CTG/A196) and the duration of remission during 18 months of follow-up (see Figure 4c,d, Supplementary Digital Content 1, http://links.lww.com/CTG/A196). When concerning medical treatment response, in both CD and UC, equally expressed miR-320a levels were noted in patients with delayed or missing treatment response. Nevertheless, for both CD and UC, we could demonstrate significant higher miR-320a levels at an initial flare in patients with disease recurrence during the follow-up period of 18 months (CD: 5,335 ± 2,184 vs 1,025 ± 579; $P = 0.003$; UC: 932 ± 318 vs 242 ± 51; $P = 0.016$).

Finally, we analyzed miR-320a levels at remission for prediction of flare during the 18-month follow-up. In detail, patients with disease relapse during follow-up flared after a mean duration of 9 months for UC and 7 months for CD. We could demonstrate in IBD (n = 27) that miR-320a levels were significantly higher in patients flaring during the follow-up compared with patients with stable remission (641 ± 548 vs 70 ± 27, $P = 0.042$, Figure 8a). These data could be confirmed in a subgroup analysis of CD (n = 18) because patients with CD with stable remission revealed significantly lower miR-320a levels compared with patients with flare during follow-up (46 ± 11 vs 1,210 ± 1,091; $P = 0.019$; Figure 8b). In patients with UC (n = 9), miR-320a levels in remission revealed no statistical difference in patients with stable remission compared with patients with flare during follow-up (71 ± 21 vs 150 ± 108; $P > 0.99$; Figure 8c).

To sum up, our data hint at a possible predictive value of miR-320a levels for patients with CD in remission to flare during the next 18 months, whereas no predictive potential of miR-320a was observed for patients with UC.

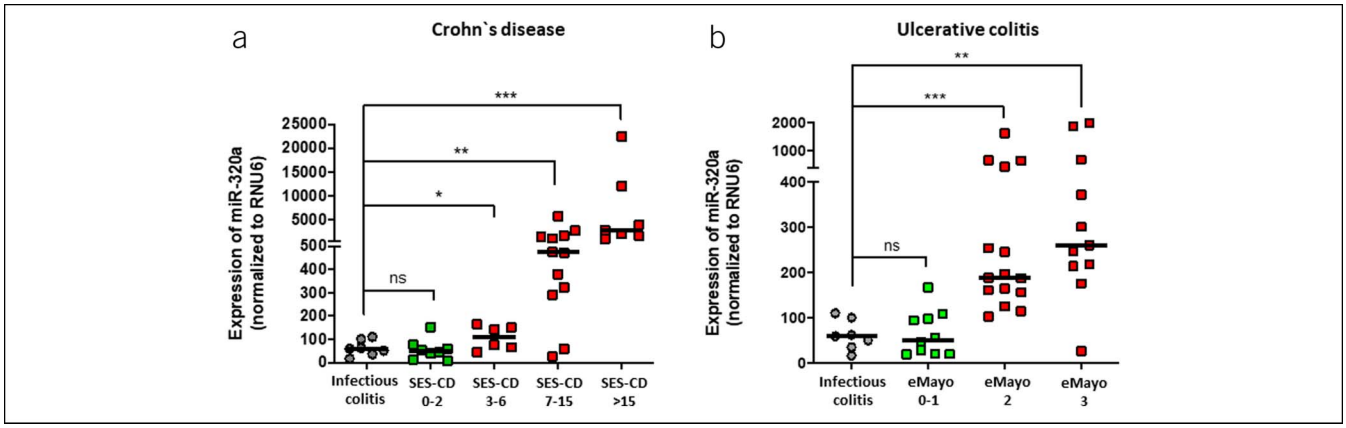

Figure 5. Blood miR-320a level has the potential to distinguish infectious colitis from active IBD. Its expression levels in patients without IBD but with infectious colitis (n = 7) were compared with those in patients with active UC and CD. The miR-320a levels were significantly lower in patients with infectious colitis when compared with mild, moderate, and severe endoscopic disease activities in patients with CD (**a**) as well as compared with moderate and severe endoscopic disease activities in patients with UC (**b**). The miR-320a was normalized to miRNA RNU6 as a reference. Lines present mean. Data are presented as dot plots with mean $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. CD, Crohn's disease; eMayo, endoscopic Mayo score; IBD, inflammatory bowel disease; SES-CD, simple endoscopic score for Crohn's disease; miR, microRNA; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

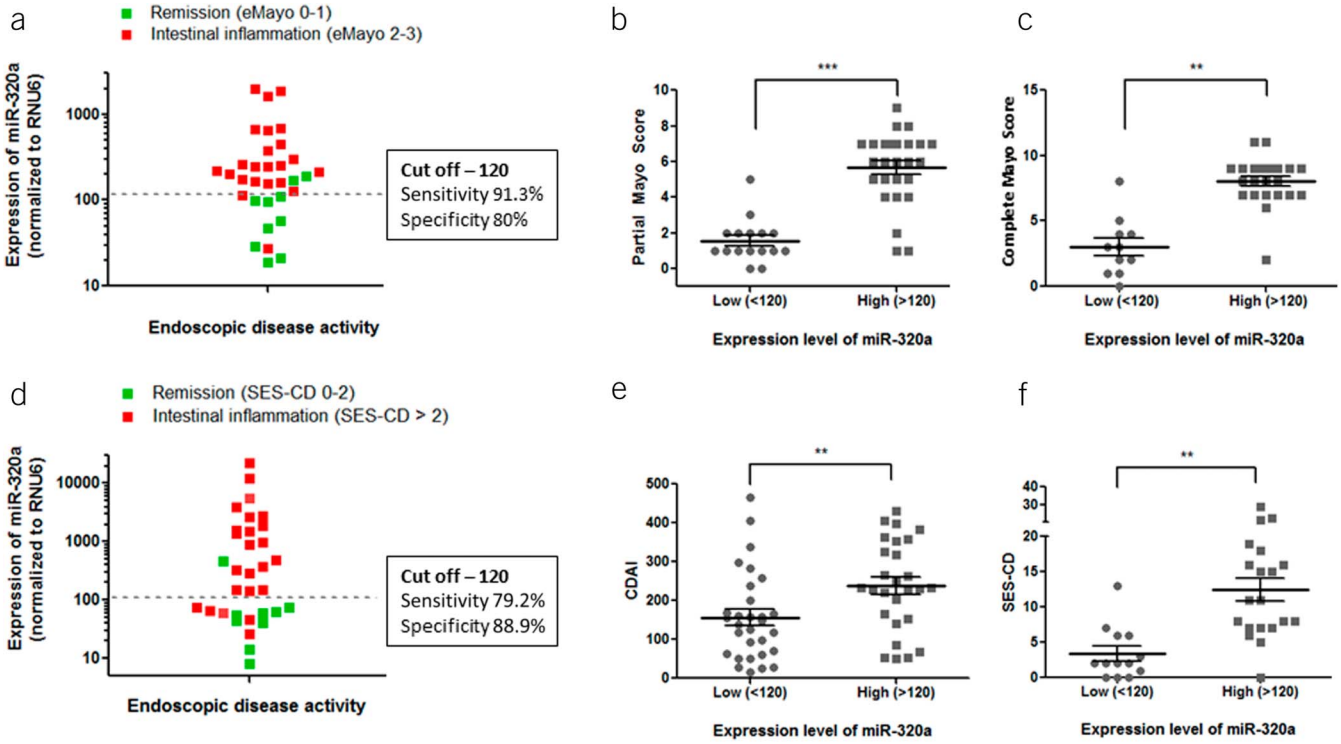

Figure 6. The miR-320a blood cutoff level can distinguish active from quiescent disease. (**a**) In UC, a cutoff level at 120 (relative units) for miR-320a could identify 8 of 10 patients correctly as in endoscopic remission and 21 of 23 patients as in active UC. Resulting sensitivity and specificity estimates were 91.3% and 80%, respectively. The disease activity indices partial Mayo score (**b**) and complete Mayo score (**c**) were significantly higher in patients with UC with a miR-320a cutoff level > 120. (**d**) In CD, 8 of 9 patients with mucosal remission and 19 of 24 patients with active disease were correctly identified by a cutoff value of 120, with resulting sensitivity and specificity rates of 79.2% and 88.9% respectively. The disease activity indices CDAI (**e**) and SES-CD (**f**) were significantly different in patients with CD with high (>120) miR-320a levels compared with low (<120) miR-320a expression. The miR-320a was normalized to miRNA RNU6 as a reference. Data are mean ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$. CD, Crohn's disease; CDAI, Crohn's disease activity index; miR, microRNA; SES-CD, simple endoscopic score for Crohn's disease; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

DISCUSSION

Our study demonstrates that miR-320a expression in peripheral blood follows the course of endoscopic disease activity in patients with CD and UC. We found moderate to high sensitivity and specificity estimates, thereby suggesting a miR-320a cutoff value of 120 relative units to be indicative for an acute flare in patients with IBD, especially in UC. Finally, detection of miR-320a blood level facilitates the differentiation of infectious colitis from IBD activity.

Assessment of initial IBD diagnosis is challenging because many patients present with unspecific symptoms such as diarrhea and abdominal pain, which can also be associated with a large number of non-IBD disorders including infectious colitis, irritable bowel disease, food intolerances, hypersensitivity, or other intestinal and digestion disorders. Although some biomarkers have been suggested as promising noninvasive markers for IBD diagnosis, still no biomarker could replace performance of invasive endoscopy and histological confirmation to assess IBD diagnosis in daily clinical practice (28,29).

In clinical practice, the most promising blood biomarker to work up IBD disease course is the inflammatory marker CRP (30), which has been shown to significantly correlate with the endoscopic disease activity score SES-CD (31) and to indicate complications such as perforations, abscesses, and fistula tracts in CD, whereas, in UC, CRP levels display disease severity in patients with severe inflammation (32,33). Nevertheless, these markers still rather unspecifically indicate ongoing inflammation (34–37), and it is impossible to confirm IBD diagnosis owing to the low sensitivity of CRP for active IBD, which was at 71% in CD and 42% in UC according to the recent study by Chen et al. (38). Furthermore, various studies could demonstrate that CRP levels are not elevated in all patients with active IBD and up to 50% of patients with UC reveal normal CRP levels at acute flare, depending on the study (32,37). More promising results concerning noninvasive specific assessment of intestinal inflammation have been shown with fecal biomarkers including fecal calprotectin, lactoferrin, and S100A12 (39–43). Among these markers, the most used marker is fecal calprotectin, which has been shown to be elevated in up to 100% of patients with active colonic IBD (39,44). However, analog with CRP, calprotectin possesses low specificity and is also elevated in non–IBD-related intestinal inflammation (e.g., infectious colitis, diverticulitis, or celiac disease) (45–48), in gastrointestinal bleeding, and after ingestion of blood-mimicking gastrointestinal bleeding (49). Furthermore, fecal calprotectin levels are elevated in up to 71% of patients with colorectal cancer (CRC) or polyps (50).

In our study, miR-320a blood levels are significantly increased in both patients with UC and patients with CD when compared with healthy controls. Noteworthy, we can demonstrate that miR-320a levels are not elevated in patients with infectious colitis compared with those in patients with active IBD. Our study did not analyze the expression of miR-320a serum levels in patients with IBD with CRC. However, 2 recent studies reported significantly reduced miR-320a levels in patients with CRC (51,52). It was also

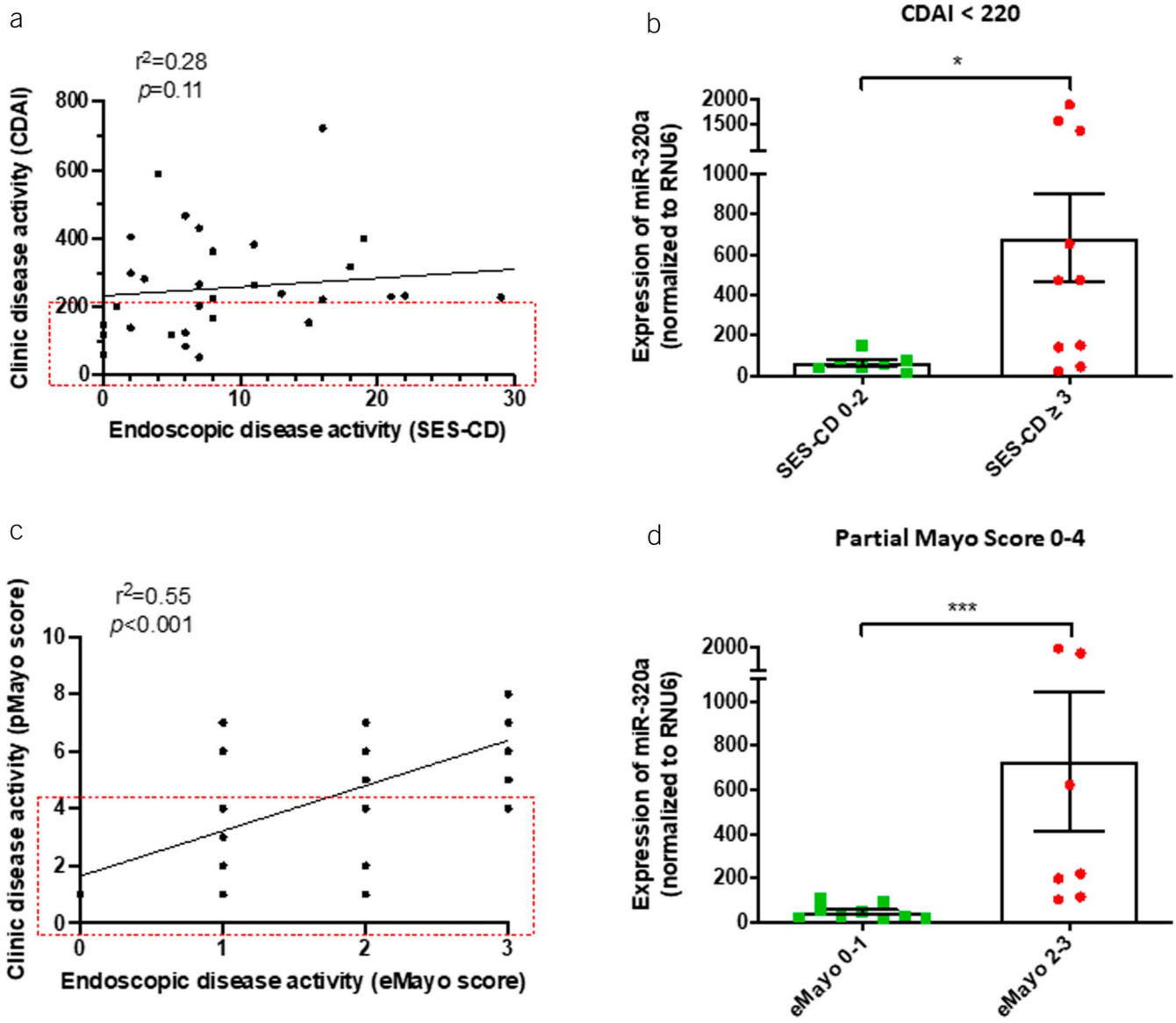

Figure 7. The miR-320a monitors intestinal inflammation in patients with mild or no clinical symptoms. Correlation of clinical and endoscopic disease activity scores is shown in (**a**) for CD and (**c**) for UC. Red squares indicate patients who were further included in analyses of miR-320a expression in peripheral blood of patients with CD (**b**) and UC (**d**) with mild/absent clinical symptoms (CDAI <220; pMayo score 0–4) and absence (SES-CD 0–2; eMayo 0–1) compared to presence (SES-CD ≥ 3; eMayo 2–3) of intestinal inflammation. The miR-320a was normalized to miRNA RNU6 as a reference. Data are mean ± SEM. $*P < 0.05$; $***P < 0.001$. CD, Crohn's disease; CDAI, Crohn's disease activity index; eMayo score, endoscopic Mayo score; miR, microRNA; pMayo score, partial Mayo score; SES-CD, simple endoscopic score for Crohn's disease; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

shown that in another autoimmune disease such as rheumatoid arthritis, miR-320a regulates osteoblast differentiation. In this disease, miR-320a expression was detected in synovial tissues, but miR-320a levels were not found to be increased in peripheral blood samples (53). Thus, our data and the current literature strongly hint at miR-320a to serve as a noninvasive biomarker for IBD diagnosis and a certain potential of miR-320a for IBD specificity. Larger studies are needed to further prove the potential of miR-320a as an IBD-specific biomarker.

Recently, the treat-to-target paradigm has been widely accepted (27), and tight control of disease has been shown effective in randomized-controlled trials (7). As a prerequisite, appropriate markers are required to trigger treatment escalation. Because repetitive endoscopy is not possible to guide clinical decision-making owing to invasiveness of the procedure, inconvenience for patients, and not at least costs, noninvasive biomarkers are desirable to provide tight control in patients with IBD. In this context, it is noteworthy that besides clinical assessment of disease activity, appropriate biomarkers to monitor endoscopic disease activity in patients with IBD are of special importance as endoscopic remission and mucosal healing have been proven to be associated with an improved long-term disease course when compared with clinical remission only (6,54,55).

In this regard, various studies demonstrate calprotectin as a promising biomarker to reflect endoscopically assessed severity of intestinal inflammation and mucosal healing (35,56–58). Furthermore, calprotectin seems to be of predictive value because levels at baseline remission were significantly higher in patients

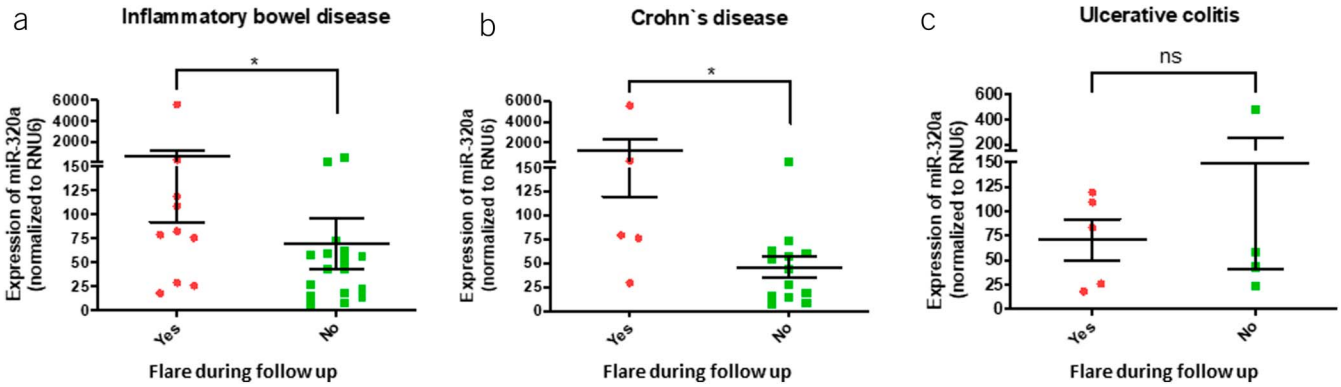

Figure 8. Predictive value of blood miR-320a levels for disease relapse in patients with inactive CD. The miR-320a levels at remission were analyzed regarding the prediction of flare during 18 months of follow-up. The miR-320a expression was significantly higher in (**a**) patients with IBD (n = 27) with disease relapse during follow-up compared with patients with sustained remission. Subgroup analysis confirmed higher miR-320a levels in patients with inactive CD (**b**) (n = 18) with flare during follow-up, whereas no significant differences were observed in patients with inactive UC (**c**) (n = 9) with or without flare during follow-up. The miR-320a was normalized to miRNA RNU6 as a reference. Data are mean ± SEM. $^*P < 0.05$. CD, Crohn's disease; IBD, inflammatory bowel disease; miR, microRNA; RNU6, Hs_RNU6-2_11; UC, ulcerative colitis.

with relapse during follow-up compared with patients with sustained remission (59). Nevertheless, calprotectin is unspecific in IBD. In addition, correlation between small bowel disease inflammation and calprotectin levels, which vary from r_s0.247–0.337 depending on the thresholds, were weak, and an optimal cutoff analyzed in ROC curve (76 μg/g) just revealed a sensitivity of 0.59 and a specificity of 0.41 (60).

We demonstrate that miR-320a blood levels accurately discriminate active flare from remission revealing strong correlation with endoscopic activity scores in both CD and UC. Interestingly, there is distinctly weaker association between miR-320a expression and mild clinical disease activity in both entities. Regarding that, our analyses show that miR-320a blood levels reflect intestinal disease activity in patients with mild or absent clinical symptoms. These data further support our hypothesis of increased miR-320a levels as a surrogate for mucosal inflammation and may indicate that miR-320a reflects subclinical intestinal inflammation in asymptomatic patients. This is of particular importance because subclinical intestinal inflammation is often not adequately treated and can lead to long-term complication, including colorectal carcinoma, strictures, and fistulas, whereas mucosal healing is associated with less severe long-term complications (4,61–63). Moreover, within a follow-up period of 18 months, we found that increased miR-320a levels in patients with inactive CD were associated with higher risk of relapse rates, indicating a possible predictive value of miR-320a in CD.

Concerning procedural aspects, the group of miRNAs provides some general advantages as a biomarker. The miRNAs have been demonstrated to be very stable in frozen peripheral blood of humans (64). Because commercially available calprotectin test assays have been shown to lack assay standardization leading to variation of calprotectin levels between test assays, miR-320a measurement is performed by RT-PCR analysis, a widely established standardized and sensitive method for gene expression analysis (39,65,66). Furthermore, fecal biomarkers show a circadian variability (67,68), which may be overcome by stool sampling that is, however, limitedly accepted by patients with IBD. Accordingly, biomarkers obtained from peripheral blood appear advantageous, especially regarding their easy availability and standardized measurement along with routine laboratory tests.

The miRNAs have been extensively investigated as potential biomarkers and therapeutic targets in various diseases. To our knowledge, the first-known diagnostic panel was launched in 2012 to identify cancer of unknown primary origin consisting of 64 miRNAs that could indicate up to 42 different tumor types with a sensitivity of 85% (69). Furthermore, a potential of circulating miRNA as a biomarker for cancer entities, e.g., in lung (70), pancreatic (71), colorectal (72), and breast cancers (73), has been shown. However, certain challenges include the great heterogeneity of miRNAs in each cancer type and incomplete understanding of its precise role in oncogenesis (74). In addition, technical challenges include difficulty of isolation and purification as well as storage time and conditions as potential sources of errors (75,76). Concerning treatment options, a promising role of miRNA as therapeutic targets has been shown: e.g., Miravirsen is an antagonist of miR-122, which plays an important role in hepatitis C virus pathogenesis (77). Hepatitis C virus replication is significantly reduced by Miravirsen, which is currently investigated as a treatment option in clinical trials (78). Taken together, miRNAs reveal a promising use as biomarker and therapeutic targets in future clinical practice.

Our study is limited by the small sample size included, representing preliminary data without a direct comparison with established noninvasive biomarker such as calprotectin. Nevertheless, our study is the first trial investigating the potential of miR-320a to serve as a biomarker for diagnosis and disease monitoring of IBD. In addition, included patients were well characterized concerning clinical and intestinal disease activities. A further limitation is the fact that besides infectious colitis, no other non-IBD disorder with symptoms of diarrhea and abdominal pain such as irritable bowel disease or food intolerance was included. Furthermore, the direct impact of medication on miR-320a expression could not finally be elucidated because of small patient cohorts, although our data indicate no major miR-320a regulation by corticosteroid or biological therapy. Additional studies are needed to specifically address these topics and to further validate the potential of miR-320a to serve as an IBD-specific biomarker.

In conclusion, we demonstrate the potential of miR-320a to be helpful in initial diagnosis and differential diagnosis as well as in tight control of disease activity with a predictive value for maintaining remission in CD. Larger prospective studies are warranted

to further elucidate miR-320a as a preferred disease-specific biomarker in patients with IBD.

CONFLICTS OF INTEREST

Guarantor of the article: Friederike Cordes, MD.

Specific author contributions: Friederike Cordes, MD, Claudia Demmig, MD, Christoph Cichon, PhD, and Dominik Bettenworth, MD contributed equally to this work. F.C. has designed and performed the research study, performed literature research, and wrote the paper. C.C. and D.B. contributed to the study design and performance, the literature research, the manuscript writing, and the final revision of the article. C.D. performed the sample collection and the data documentation. A.B. M.B., F.L., P.L., T.N. and P.T. contributed to the performance of the study, the literature research, and the final revision of the article. M.A.S. and H.H.S. contributed to the study design, the literature research, and the final revision of the article. All authors have read and approved the submitted version of the manuscript.

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Study Highlights

WHAT IS KNOWN

- ✓ Appropriate biomarkers are a prerequisite to facilitate a treat-to-target approach in patients with IBD.
- ✓ Neutrophil markers such as fecal calprotectin can help to monitor colonic inflammation but are not IBD specific.
- ✓ MicroRNA-320a has been shown to strengthen intestinal epithelial barrier function *in vitro* and blood levels in colitic mice correlate with the follow-up of intestinal inflammation.

WHAT IS NEW HERE

- ✓ MicroRNA-320a blood levels are increased in patients with IBD with acute flare compared with those in healthy controls or patients with infectious colitis.
- ✓ Expression levels of microRNA-320a in peripheral blood adequately reflect intestinal disease activity of both UC and CD.
- ✓ Patients with IBD with intestinal disease activity reveal significant higher microRNA-320a expression levels when compared with patients with infectious colitis.

TRANSLATIONAL IMPACT

- ✓ MicroRNA-320a has the potential to noninvasively assess intestinal inflammation in patients with IBD.

REFERENCES

1. Sartor RB. Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Prac Gastroenterol Hepatol 2006;3:390–407.
2. Solberg IC, Vatn MH, Høie O, et al. Clinical course in Crohn's disease: Results of a Norwegian population-based ten-year follow-up study. Clin Gastroenterol Hepatol 2007;5:1430–8.
3. Baert F, Moortgat L, Van Assche G, et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. Gastroenterology 2010;138:463–8. quiz e10-1.
4. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. Gastroenterology 2011;141:1194–201.
5. Khanna R, Bressler B, Levesque BG, et al. Early combined immunosuppression for the management of Crohn's disease (REACT): A cluster randomised controlled trial. Lancet 2015;386:1825–34.
6. Shah SC, Colombel JF, Sands BE, et al. Mucosal healing is associated with improved long-term outcomes of patients with ulcerative colitis: A systematic review and meta-analysis. Clin Gastroenterol Hepatol 2016;14:1245–55.e8.
7. Colombel JF, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): A multicentre, randomised, controlled phase 3 trial. Lancet 2018;390:2779–89.
8. Lüning TH, Keemers-Gels ME, Barendregt WB, et al. Colonoscopic perforations: A review of 30,366 patients. Surg Endosc 2007;21:994–7.
9. Arora G, Mannalithara A, Singh G, et al. Risk of perforation from a colonoscopy in adults: A large population-based study. Gastrointest Endosc 2009;69:654–64.
10. Baars JE, Nuij VJ, Oldenburg B, et al. Majority of patients with inflammatory bowel disease in clinical remission have mucosal inflammation. Inflamm Bowel Dis 2012;18:1634–40.
11. Berrill JW, Green JT, Hood K, et al. Symptoms of irritable bowel syndrome in patients with inflammatory bowel disease: Examining the role of sub-clinical inflammation and the impact on clinical assessment of disease activity. Aliment Pharmacol Ther 2013;38:44–51.
12. Gracie DJ, Williams CJ, Sood R, et al. Poor correlation between clinical disease activity and mucosal inflammation, and the role of psychological comorbidity, in inflammatory bowel disease. Am J Gastroenterol 2016;111:541–51.
13. Henriksen M, Jahnsen J, Lygren I, et al. C-reactive protein: A predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. Gut 2008;57:1518–23.
14. Solem CA, Loftus EV Jr, Tremaine WJ, et al. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. Inflamm Bowel Dis 2005;11:707–12.
15. Kalla R, Ventham NT, Kennedy NA, et al. MicroRNAs: New players in IBD. Gut 2015;64:504–17.
16. Veltman K, Hummel S, Cichon C, et al. Identification of specific miRNAs targeting proteins of the apical junctional complex that simulate the probiotic effect of E. coli Nissle 1917 on T84 epithelial cells. Int J Biochem Cell Biol 2012;44:341–9.
17. Cordes F, Brückner M, Lenz P, et al. MicroRNA-320a strengthens intestinal barrier function and follows the course of experimental colitis. Inflamm Bowel Dis 2016;22:2341–55.
18. Lewis JD, Chuai S, Nessel L, et al. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. Inflamm Bowel Dis 2008;14:1660–6.
19. Best WR, Becktel JM, Singleton JW, et al. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology 1976;70:439–44.
20. Sandborn WJ, Ghosh S, Panes J, et al. A phase 2 study of tofacitinib, an oral Janus kinase inhibitor, in patients with Crohn's disease. Clin Gastroenterol Hepatol 2014;12:1485–93 e2.
21. D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology 2007;132:763–86.
22. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462–76.
23. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. New Engl J Med 1987;317:1625–9.
24. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for crohn's disease: The SES-CD. Gastrointest Endosc 2004;60:505–12.
25. Peyrin-Biroulet L, Panes J, Sandborn WJ, et al. Defining disease severity in inflammatory bowel diseases: Current and future directions. Clin Gastroenterol Hepatol 2016;14:348–54 e17.
26. Debast SB, Bauer MP, Kuijper EJ, et al. European Society of Clinical Microbiology and Infectious Diseases: Update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect 2014;20(Suppl 2):1–26.
27. Peyrin-Biroulet L, Sandborn W, Sands BE, et al. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): Determining therapeutic goals for treat-to-target. Am J Gastroenterol 2015;110:1324–38.

28. Annese V, Daperno M, Rutter MD, et al. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis 2013;7:982–1018.
29. Khanna R, Levesque BG, Sandborn WJ. IBD: Measuring what counts–endoscopic assessment in IBD. Nat Rev Gastroenterol Hepatol 2014;11:9–10.
30. Franks I. IBD CRP is a good long-term biomarker. Nat Rev Gastroenterol Hepatol 2011;8:359.
31. Schaffer T, Schoepfer AM, Seibold F, et al. Serum ficolin-2 correlates worse than fecal calprotectin and CRP with endoscopic Crohn's disease activity. J Crohns Colitis 2014;8:1125–32.
32. Rogler G, Biedermann L. Clinical utility of biomarkers in IBD. Curr Gastroenterol Rep 2015;17:26.
33. Fagan EA, Dyck RF, Maton PN, et al. Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. Eur J Clin Invest 1982;12:351–9.
34. Schoepfer AM, Trummler M, Seeholzer P, et al. Discriminating IBD from IBS: Comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflamm Bowel Dis 2008;14:32–9.
35. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162–9.
36. af Björkesten CG, Nieminen U, Turunen U, et al. Surrogate markers and clinical indices, alone or combined, as indicators for endoscopic remission in anti-TNF-treated luminal Crohn's disease. Scand J Gastroenterol 2012;47:528–37.
37. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: Useful, magic, or unnecessary toys? Gut 2006;55:426–31.
38. Chen JM, Liu T, Gao S, et al. Efficacy of noninvasive evaluations in monitoring inflammatory bowel disease activity: A prospective study in China. World J Gastroenterol 2017;23:8235–47.
39. Brookes MJ, Whitehead S, Gaya DR, et al. Practical guidance on the use of faecal calprotectin. Frontline Gastroenterol 2018;9:87–91.
40. Theede K, Holck S, Ibsen P, et al. Level of fecal calprotectin correlates with endoscopic and histologic inflammation and identifies patients with mucosal healing in ulcerative colitis. Clin Gastroenterol Hepatol 2015;13:1929–36.e1.
41. Mao R, Xiao YL, Gao X, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies. Inflamm Bowel Dis 2012;18:1894–9.
42. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:2218–24.
43. Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: Fecal calprotectin for assessment of inflammatory bowel disease activity. Inflamm Bowel Dis 2014;20:1407–15.
44. Lopez RN, Leach ST, Lemberg DA, et al. Fecal biomarkers in inflammatory bowel disease. J Gastroenterol Hepatol 2017;32:577–82.
45. Sýkora J, Siala K, Huml M, et al. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young children with acute gastroenteritis. Acta Paediatr 2010;99:1389–95.
46. Ertekin V, Selimoğlu MA, Turgut A, et al. Fecal calprotectin concentration in celiac disease. J Clin Gastroenterol 2010;44:544–6.
47. Tibble JA, Sigthorsson G, Foster R, et al. High prevalence of NSAID enteropathy as shown by a simple faecal test. Gut 1999;45:362–6.
48. Shastri YM, Bergis D, Povse N, et al. Prospective multicenter study evaluating fecal calprotectin in adult acute bacterial diarrhea. Am J Med 2008;121:1099–106.
49. Vavricka SR, Heinrich H, Buetikofer S, et al. The Vampire Study: Significant elevation of faecal calprotectin in healthy volunteers after 300 ml blood ingestion mimicking upper gastrointestinal bleeding. United Eur Gastroenterol J 2018;6:1007–14.
50. von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. Am J Gastroenterol 2007;102:803–13.
51. Fang Z, Tang J, Bai Y, et al. Plasma levels of microRNA-24, microRNA-320a, and microRNA-423-5p are potential biomarkers for colorectal carcinoma. J Exp Clin Cancer Res 2015;34:86.
52. Hur K, Toiyama Y, Schetter AJ, et al. Identification of a metastasis-specific MicroRNA signature in human colorectal cancer. J Natl Cancer Inst 2015;107. doi: 10.1093/jnci/dju492.
53. Moran-Moguel MC, Petarra-Del Rio S, Mayorquin-Galvan EE, et al. Rheumatoid arthritis and miRNAs: A critical review through a functional view. J Immunol Res 2018;2018:2474529.
54. Maaser C, Sturm A, Vavricka SR, et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. J Crohn's colitis 2019;13:144–164.
55. Sturm A, Maaser C, Calabrese E, et al. ECCO-ESGAR uideline for diagnostic assessment in IBD Part 2: IBD scores and general principles and technical aspects. J Crohn's colitis 2019;13:273–284.
56. Chang JY, Cheon JH. Fecal immunochemical test and fecal calprotectin measurement are noninvasive monitoring tools for predicting endoscopic activity in patients with ulcerative colitis. Gut Liver 2018;12:117–8.
57. Sipponen T, Kärkkäinen P, Savilahti E, et al. Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. Aliment Pharmacol Ther 2008;28:1221–9.
58. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol 2015;50:74–80.
59. Mooiweer E, Severs M, Schipper ME, et al. Low fecal calprotectin predicts sustained clinical remission in inflammatory bowel disease patients: A plea for deep remission. J Crohn's colitis 2015;9:50–5.
60. Koulaouzidis A, Sipponen T, Nemeth A, et al. Association between fecal calprotectin levels and small-bowel inflammation score in capsule endoscopy: A multicenter retrospective study. Dig Dis Sci 2016;61:2033–40.
61. Magro F, Lopes S, Coelho R, et al. Accuracy of faecal calprotectin and Neutrophil gelatinase B-associated lipocalin in evaluating subclinical inflammation in UlceRaTIVE colitis-the ACERTIVE study. J Crohn's colitis 2017;11:435–44.
62. Frøslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: Results from a Norwegian population-based cohort. Gastroenterology 2007;133:412–22.
63. Rutter M, Saunders B, Wilkinson K, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. Gastroenterology 2004;126:451–9.
64. Balzano F, Deiana M, Dei Giudici S, et al. miRNA stability in frozen plasma samples. Molecules 2015;20:19030–40.
65. Whitehead SJ, French J, Brookes MJ, et al. Between-assay variability of faecal calprotectin enzyme-linked immunosorbent assay kits. Ann Clin Biochem 2013;50:53–61.
66. Labaere D, Smismans A, Van Olmen A, et al. Comparison of six different calprotectin assays for the assessment of inflammatory bowel disease. United Eur Gastroenterol J 2014;2:30–7.
67. Lasson A, Stotzer PO, Ohman L, et al. The intra-individual variability of faecal calprotectin: A prospective study in patients with active ulcerative colitis. J Crohn's colitis 2015;9:26–32.
68. Kristensen V, Malmstrøm GH, Skar V, et al. Clinical importance of faecal calprotectin variability in inflammatory bowel disease: Intra-individual variability and standardisation of sampling procedure. Scand J Gastroenterol 2016;51:548–55.
69. Meiri E, Mueller WC, Rosenwald S, et al. A second-generation microRNA-based assay for diagnosing tumor tissue origin. Oncologist 2012;17:801–12.
70. Zhao Y, Song Y, Yao L, et al. Circulating microRNAs: Promising biomarkers involved in several cancers and other diseases. DNA Cel Biol 2017;36:77–94.
71. Kawaguchi T, Komatsu S, Ichikawa D, et al. Clinical impact of circulating miR-221 in plasma of patients with pancreatic cancer. Br J Cancer 2013;108:361–9.
72. Zanutto S, Pizzamiglio S, Ghilotti M, et al. Circulating miR-378 in plasma: A reliable, haemolysis-independent biomarker for colorectal cancer. Br J Cancer 2014;110:1001–7.
73. Antolín S, Calvo L, Blanco-Calvo M, et al. Circulating miR-200c and miR-141 and outcomes in patients with breast cancer. BMC Cancer 2015;15:297.
74. Cui M, Wang H, Yao X, et al. Circulating MicroRNAs in cancer: Potential and challenge. Front Genet 2019;10:626.
75. Cheng HH, Yi HS, Kim Y, et al. Plasma processing conditions substantially influence circulating microRNA biomarker levels. PLoS One 2013;8:e64795.
76. McDonald JS, Milosevic D, Reddi HV, et al. Analysis of circulating microRNA: Preanalytical and analytical challenges. Clin Chem 2011;57:833–40.
77. Jopling C. Liver-specific microRNA-122: Biogenesis and function. RNA Biol 2012;9:137–42.
78. Titze-de-Almeida R, David C, Titze-de-Almeida SS. The race of 10 synthetic RNAi-based drugs to the pharmaceutical market. Pharm Res 2017;34:1339–63.