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 (r² = 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, Supplemental 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, Supplemental 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-5). 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 168 ± 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 (r² = 0.76; P < 0.001; Figure 2b).
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 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.
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 (Figure 6b,c). In CD, 8 of 9 patients with mucosal remission were correctly identified by cutoff level of 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 level 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 6c,f).

**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 6c,f).
miR-320a Monitors IBD Activity

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: $682 \pm 218$ vs $64 \pm 17$; $P = 0.025$; Figure 7b; UC: $729 \pm 315$ vs $46 \pm 12$; $P < 0.001$; 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 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.
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 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.

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.
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
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 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...
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 $r=0.247-0.337$ depending on the thresholds, were weak, and an optimal cutoff analyzed in ROC curve (76 $\mu$g/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 (46-63). 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 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
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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.

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