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Novel physiological analysis using blood flow velocity for colonic polyps: Pilot study

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Abstract:
Real-time visualization of red blood cell flow inside subepithelial microvessels is performed with magnifying endoscopy. However, microvascular blood flow velocity in the colorectum has not been investigated. Here, we aimed to evaluate the blood flow velocity of microvessels of colonic polyps and to compare it with that of surrounding mucosa. We examined 50 lesions, including 30 adenomas (ADs) and 20 hyperplastic polyps (HPs). Blood flow velocities of lesions and their surrounding mucosa were evaluated using magnifying blue laser imaging (BLI) prior to endoscopic resection. Calculation of mean blood flow velocities was based on mean movement distance of one tagged red blood cell using split video images of magnifying BLI. Mean microvascular blood flow velocity was significantly lower in ADs (1.65 ± 0.66 mm/sec; range 0.46–2.90) than in HPs (2.83 ± 1.10 mm/sec; 1.07-4.50) or the surrounding mucosa (3.73 ± 1.11 mm/sec; 1.80-6.20; P < 0.001). The blood flow velocity rate compared with the surrounding mucosa was significantly lower in ADs (0.41 ± 0.16; 0.10-0.82) than in HPs (0.89 ± 0.25; 0.46-1.51; P < 0.001). We found that mean microvascular blood flow velocity was significantly lower in ADs than in HPs and the surrounding non-neoplastic mucosa. These findings indicate that a novel dynamic approach with microvascular blood flow velocity using magnifying endoscopy may be useful in assessing physiological differences between ADs and HPs.

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Introduction:

Colorectal polyps are classified as neoplastic (adenomatous) or non-neoplastic (hyperplastic, hamartomatous, or inflammatory) based on their histology. Adenomas (ADs) are recognized as precursor lesions of colorectal cancer (CRC) in the traditional colorectal carcinogenesis theory: the adenoma–carcinoma sequence. As a cornerstone of effective prevention, colonoscopy with polypectomy has led to a reduction in the incidence of, and mortality from, CRC [1,2]. In contrast to ADs, hyperplastic polyps (HPs) are usually regarded as harmless, non-neoplastic lesions with no malignant potential.

Recently, image-enhanced endoscopies (IEEs), such as blue laser imaging (BLI) and narrow band imaging (NBI), have been developed. Many studies have highlighted the usefulness of IEEs in the detection and diagnosis of colorectal lesions [3]. Blue laser imaging uses two monochromatic lasers (410 and 450 nm) rather than xenon light. Vascular microarchitecture is visualized by a 410-nm laser and a 450-nm laser that supply white light by excitation. Blue laser imaging is useful for a detailed observation of microvascular architecture for a differential diagnosis and yields a unique image that emphasizes the capillary pattern and surface structure [4]. Microvascular density in colorectal tumors can be determined by magnifying BLI. The endoscopic microvascular

density in carcinoma is significantly higher than that in adenoma [5]. Furthermore, magnifying BLI not only enables clear visualization of microvascular structures of the colorectum but also allows real-time visualization of red blood cell flow within subepithelial microvessels (Video 1). Mean microvascular blood flow velocity was reported as significantly lower in an early gastric neoplasm than in the surrounding non-neoplastic mucosa [6]. However, microvascular blood flow velocity in the colorectum has not been investigated. Here, we evaluated the blood flow velocity of colonic polyps, including ADs and HPs, compared with that of the surrounding mucosa in a pathophysiological analysis.

Patients/Materials and methods:

Patients and materials

Patients who underwent colonoscopy for screening at our hospital between March 2019 and January 2023 were included. When colorectal polyps were found, we performed video recordings and retrospectively reviewed the videos. Of these, the lesions that could not be assessed due to the poor quality of images were excluded. Only lesions in which the movement of RBCs could be clearly observed were extracted. Finally, 50 lesions, including 30 ADs (from 20 patients) and 20 HPs (from 13 patients), were

assessed.

Endoscopic system and examination

We used high-resolution optical magnifying endoscopes (EC-L600ZP; Fujifilm Corporation, Tokyo, Japan) and an endoscope video system (LASEREO 7000 series; Fujifilm Corporation). Blood flow velocities were evaluated using magnifying BLI. The structure enhancement function and color mode in BLI were set at B8 and C2, respectively. Endoscopy examinations were performed by six endoscopy specialists (E.K., T.M., K.N., K.H., Y.A., H.F.), each with experience in at least 1000 prior colonoscopic procedures. Lesions were carefully evaluated using BLI prior to endoscopic resection. Careful examination with conventional endoscopy was followed by BLI magnification endoscopy. We evaluated magnifying BLI endoscopic findings using a Japan NBI Expert Team (JNET) system [7].

Microvascular blood flow rate measurement

The method used for measuring microvascular blood flow velocity is illustrated in Figure 1. Measurements and analysis were conducted by E.K. and T.M. Magnifying BLI video images were split into 30 frames per second, and mean blood flow velocities were

calculated from the average distance of a single tagged RBC. Before each examination, the endoscope and marker were attached and adjusted for focusing using full zoom. The examination was subsequently conducted. During the video recording of lesions, full zoom was used to adjust the magnification for all cases.

Initially, the segmented images were continuously captured on a large screen. A single tagged red blood cell, identifiable in each lesion, was manually identified by E.K. and T.M. The distance travelled by this red blood cell (Distance A) was manually measured. The distances in 1-mm increments (Distance B) was also manually measured on the same screen. Subsequently, the mean microvascular blood flow velocity was calculated based on Distance A, Distance B, and the number of frames (Mean Blood Flow [μm/s] = Distance A [μm]/Distance B [μm] × 30 [frames]/Frame Number [frames]). Microvascular blood flow velocity in the lesion and surrounding mucosa was evaluated. Furthermore, because of possible the existence of individual differences, such as for example in blood pressure, microvascular blood flow velocity in the lesion was compared with the surrounding mucosa for both ADs and HPs.

Histopathological diagnosis

After appropriate BLI video imaging, each lesion was endoscopically resected and

pathologically diagnosed. The histological diagnosis of each excised lesion was conducted at the Department of Human Pathology at Juntendo University.

Statistical analysis;

Statistical analyses were undertaken using EZR (Easy R; Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). A Mann–Whitney U-test was used to compare continuous data. Fisher's exact test was used in the categorical analysis of variables. Statistical significance was set as $P < 0.05$.

Ethics approval;

The Institutional Review Board and the Ethical Committee of Juntendo University Hospital (#20-219) approved this study. The study was performed in accordance with the principles of the Declaration of Helsinki. This was also an opt-out study. Since the study was conducted using pre-existing data, informed consent from registered patients was not required.

Results:

Baseline characteristics

Table 1 outlines baseline characteristics of the studied polyps. A higher proportion of males was evident in both AD and HP groups (AD, 13 males vs. 7 females; HP, 9 vs. 4) and the median age was 68 and 66 years in AD and HP groups, respectively. Hyperplastic polyps were predominantly located in the distal colon (left-side colon and rectum) rather than in the proximal colon, while ADs were more frequently located in the right-side colon. The median sizes for ADs and HPs were 3.5 mm and 3.5 mm, respectively.

Macroscopically, both ADs and HPs were predominantly sessile (0-Is) or superficial (0-IIa) types. No significant differences in patient's age and sex, tumor location, size, and macroscopic type were found between lesion groups. Regarding the JNET classification, all cases in ADs were classified as Type 2A, while all cases in HPs were classified as Type 1. All excised ADs exhibited low-grade dysplasia and were classified as tubular adenomas.

Microvascular blood flow velocity

Table 2 shows the microvascular blood flow velocity of each lesion and the surrounding non-neoplastic mucosa. The mean microvascular blood flow velocity was found to be significantly lower for AD lesions (median 1.69 mm/sec; range 0.46–2.90) than HP

lesions (median 3.22 mm/sec; 1.07–4.50) or the surrounding mucosa (median 3.82 mm/sec; 1.80–6.20; $P < 0.001$, respectively). However, the mean velocity was not significantly different between HPs and the surrounding mucosa. Video 1 shows actual blood flow in AD and HP cases. In addition, significant differences in mean velocity were not observed with respect to age or sex.

Microvascular blood flow velocity rate

The mean microvascular blood flow velocity rates of ADs and HPs are also presented in Figure 2. The mean velocity rate of the lesion compared to the surrounding mucosa was significantly lower in ADs (median 0.43; 0.10–0.82) than in HPs (median 0.93; 0.46–1.51; $P < 0.001$).

Discussion:

In this study, the mean microvascular blood flow velocity was calculated for both colorectal lesions (ADs and HPs) and the surrounding non-neoplastic mucosa. We found that the mean microvascular blood flow velocity rate of lesions compared to the surrounding mucosa was significantly lower in ADs than in HPs. To our knowledge, no studies have analyzed the microvascular blood flow velocity of colonic subepithelial microvascular vessels using magnifying BLI. This is the first study to compare the

microvascular blood flow velocity of colorectal polyps. Endoscopically, subepithelial microvessels in ADs are thicker and more tortuous than those in HP [5,7]. Additionally, in this study, blood flow velocity was significantly lower in ADs than in normal mucosa, whereas HPs showed no significant difference with regard to the blood flow velocity of its microvessels compared to those of normal mucosa.

As shown in Figure 3, in ADs, the tumor gland density around the microvasculature was higher compared to that in HPs. The structure of tumor glands was irregular and the stromal architecture surrounding the tumor glands, where microvessels coursed, appeared to be complex and narrow. Therefore, it is hypothesized that differences in the tortuosity of microvessel diameters and variations in tumor gland density around the microvessels may be associated with a decrease in microvascular blood flow. However, further research is needed to elucidate the factors that regulate microvascular blood flow in the colonic mucosa.

The JNET classification evaluates lesions' surface and vascular structure using magnifying NBI [7]. The pit pattern classification by Kudo et al. (1996) assesses the form of pits on the tumor surface according to staining patterns [8]. Both classifications are useful for qualitatively and quantitatively diagnosing colorectal tumors because they are highly reproducible and show good diagnostic accuracy with respect to pathology

and invasion depth [9,10]. However, these classifications are performed using still images. To date, no diagnostic system that uses moving images has been available for colorectal polyps. This analysis remains limited to pathophysiological insights at this stage. However, in the future, if the blood flow velocity in subepithelial microvessels can be measured automatically, this may contribute to the establishment of a new diagnostic system of colorectal lesions using moving images.

In this analysis, lesions were found in which the movement of RBCs could not be captured by magnifying endoscopy. Possible reasons include: 1) the effect of large vessel diameter and high density; 2) bleeding with scope contact when observing the surface structures of lesions; and 3) few flat areas to visualize subepithelial microvessels and ensure a stable view. Therefore, although we attempted to capture blood flow in subepithelial microvessels in some colorectal cancers by magnifying endoscopy, it was difficult to observe blood flow and measure blood flow velocity.

This study has several limitations. First, it was retrospective in nature and used a small cohort of patients at a single institution. Second, this study was exploratory and focused only on ADs and HPs. Future prospective multicenter comparative studies with larger sample sizes should be conducted. Third, we did not specify which area of the lesion to measure microvascular blood flow rate and at what moment microvascular blood flow

was measured. Fourth, because microvessels within the mucosa usually have a disorganized, three-dimensional, microvascular architecture, if these run vertically, blood flow as measured on the surface in two dimensions may become slow. For example, comparing the size of RBC when initially tagged with that when tagged finally may offer a simple estimation of depth. However, this remains a task for future investigation. Fifth, the presence of irregularities in the lesion itself and the observation not necessarily being perpendicular to the mucosa suggest that the distances depicted in Figure 1 may lack reproducibility. Achieving reproducibility might be possible by placing an actual scale on the lesion and recording it in the video footage for distance measurement. Sixth, in this study, the physiological characteristics of two types of lesions were not evaluated. To investigate the relationship between blood flow velocity and physiological differences, it is necessary to compare blood flow velocity under different physiological conditions within the same lesion. And finally, since the researchers who conducted the analysis were not blinded to the data, a potential for bias exists in the measurements. In future, we intend to investigate microvascular blood flow velocity in several types of lesions, including colorectal cancer. We also intend to elucidate the pathophysiological processes that underlie lower microvascular blood flow velocity in colorectal lesions and compare this to that of normal mucosa.

In conclusion, we evaluated the subepithelial microvascular blood flow velocity of colorectal lesions using magnifying endoscopy. We found that the mean microvascular blood flow velocity was significantly lower in ADs than HPs and the surrounding non-neoplastic mucosa. Assessment of the dynamic function of the colonic mucosa by magnifying endoscopy may provide a new approach to elucidating the pathophysiology of colonic tumors. Additional validation is necessary to ascertain the potential applicability of this analysis in future clinical diagnostic practice.

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Figure 1. The method for measuring microvascular blood flow velocity

All magnifying blue laser imaging (BLI) video images were split into 30 fps. The distance covered by one tagged red blood cell was measured manually at continuing split images (distance A) on a large screen, and 1-mm increments (distance B) were also measured manually on the same screen. Subsequently, the mean microvascular blood flow velocity was calculated by distance A, distance B, and the number of frames N (mean blood flow velocity [mm/s] = distance A [mm]/distance B [mm] × 30 [frames]/number of frames N [frames]).

Figure 2. Microvascular blood flow velocity rates of ADs and HPs

ADs (median, 0.43; range; 0.10–0.82) vs. HPs (median, 0.93; range, 0.46–1.51), ***P < 0.001.

Abbreviations: ADs, adenomas; HPs, hyperplastic polyps.

Figure 3. Blue laser imaging and pathological images of a representative adenoma and hyperplastic polyp cases

(a)Blue laser imaging (BLI) in an adenoma (Case #13). (b) BLI image of a hyperplastic polyp (Case #35).

(c) Pathological image of an adenoma (Case #13). (d) Pathological image of a hyperplastic polyp (Case #35). Microvessels in adenomas are larger, denser, and more tortuous than those in hyperplastic polyps. Green arrows indicate dilated and tortuous microvessels.

Table1. The baseline characteristics of the studied lesions.

Lesions, n = 50 (Patients, n = 33)	ADs n = 30 (n = 20)	HPs n = 20 (n = 13)	*P* value
Gender (%) (Male/Female)	13 (65) /7 (35)	9 (69) /4 (31)	> 0.999
Age (range)	68 (45-83)	66 (49-76)	0.416
Location (%)			0.188
Right colon	19 (63)	8 (40)	
Left colon	9 (30)	8 (40)	
Rectum	2 (7)	4 (20)	
Size (mm) median	3.5 (2-16)	3.5 (2-12)	0.908
Macroscopic type (%)			0.682
Ip	0	0	
Isp	1 (3)	0	
Is	20 (67)	13 (65)	
IIa	9 (30)	7 (35)	

ADs, adenomas; HPs, hyperplastic polyps;
Age is represented as median (range).

Table 2. The microvascular blood flow velocity of the studied lesions.

Adenomas	(A) Lesions	(B)The surrounding mucosa	Hyperplastic polyps	(C) Lesions	(D) The surrounding mucosa
1	1.16	3.69	31	3.97	3.72
2	1.59	3.42	32	3.18	3.19
3	2.3	3.81	33	3.44	3.32
4	1.8	3.81	34	3.44	3.32
5	2.2	3.83	35	4.24	4.09
6	2.12	5.11	36	4.5	4.26
7	2.01	6.2	37	3.44	3.71
8	2.3	3.52	38	3.28	3.07
9	2.2	6.03	39	3.71	2.46
10	2.6	5.15	40	3.97	4.29
11	1.85	5.37	41	3.22	3.55
12	2.65	5.96	42	1.38	2.3
13	1.32	3.83	43	1.38	2.3
14	2.12	4.77	44	1.07	2.3
15	1.59	3.22	45	1.84	2.3
16	1.48	1.8	46	2.3	2.3
17	1.06	2.1	47	3.23	5.07
18	2.12	4.77	48	1.09	2.31
19	1.5	4.98	49	1.63	2.31
20	2.1	4.98	50	2.3	2.53
21	2.9	4.88			
22	0.69	2.53			
23	1.38	2.53			
24	0.57	3.69			
25	0.69	3.69			
26	1.84	3.69			
27	0.92	3.69			
28	1.38	3.69			
29	0.46	4.38			
30	0.75	4.48			
Median (range)	1.69 (0.46-2.90)	3.82 (1.80-6.20)	Median (range)	3.22 (1.07-4.50)	3.13 (2.30-5.07)

(A) Adenomas vs (B) The surrounding mucosa, $P < 0.001$; (A) Adenomas vs (C) Hyperplastic polyps, $P < 0.001$; (C) Hyperplastic polyps vs (D) The surrounding mucosa, $P = 0.408$.

Video 1. Dynamic blood flow within subepithelial microvessels by magnifying blue laser imaging.

The first half of the video shows a magnified blue laser imaging video of normal mucosa in the colon. When fully zoomed in, the movement of red blood cells can be clearly seen. The second half of the video shows distinct blood flow in colorectal adenomas (left) and hyperplastic polyps (right). It can be seen that the blood flow of the adenoma is slower than that of the hyperplastic polyp.