Topical Ankaferd Blood Stopper Administration to Bleeding Gastrointestinal Carcinomas Decreases Tumor Vascularization

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To the Editor: Ankaferd Blood Stopper ~~(ABS)~~ is a standardized herbal extract obtained from five different plants, namely *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica* (1). ABS has been approved for the clinical management of external postsurgical and postdental surgery bleedings in Turkey. ABS has also been used for the management of hemorrhages in difficult clinical conditions (2–5). ABS represents its unique hemostatic effect by promoting very rapid (< 1 s) formation of a protein network, which acts as an anchor for vital physiological erythrocyte aggregation, covering the classical cascade model of the clotting system without independently acting on coagulation factors and platelets (1). It has been hypothesized that the long-term hemostatic effects of ABS may be due to an inhibitory effect on tumor angiogenesis. The aim of this paper is to report observations in two patients with gastrointestinal cancer for which tumor neovascularization (angiogenesis) before and after the application of ABS was measured as tumor microvessel density (MVD).

A 78-year-old female patient who presented with rectal bleeding was evaluated by flexible rectosigmoidoscopy, which revealed a 3.5×4 ~~cm^3~~ semi-pedunculated hemorrhagic, polypoid mass in the rectum. Multiple biopsy specimens were obtained, followed by a topical administration of 3 ml ABS to achieve hemostasis. After a histopathological diagnosis of adenocarcinoma, low anterior resection was performed 9 days later.

A 42-year-old male patient who was admitted to our hospital with complaints of epigastric pain, decreased appetite, nausea, and vomiting had an ulcerative vegetated lesion arising from the pylorus and extending into the bulbus on upper endoscopy. Multiple biopsy specimens were collected from the lesion after which 5 ml of ABS was administered. A histopathological diagnosis of gastric adenocarcinoma was confirmed followed by total gastric resection 16 days later.

In both cases, the administered ABS was sufficient enough to completely cover the luminal face of the tumors with a "spider-web"-like network.

Sections of 5 µm thickness were prepared for staining with avidin–biotin complex immunoperoxidase. For microvessel staining, sections from each tumor were dewaxed and heated in a microwave oven for 10 min to retrieve the antigens. Endogenous peroxidase was blocked by incubation with 3% hydrogen peroxide in methanol for 10 min. After incubation with a primary antibody against CD34 (mouse monoclonal antibody, clone QBEnd/10, Neomarkers, USA) for 30 min, the sections were reacted with a secondary biotinylated antibody for 15 min and then with streptavidin for 15 min. Each incubation step was followed by thorough washing of the slides in distilled water and phosphate-buffered saline. Finally, all slides were treated with aminoethylcarbazole reagent and counterstained with Mayer's hematoxylin.

The MVD was estimated using Nikon E600 microscope (~~Nikon~~). Areas of tissue that contained the highest density of capillaries and small venules were identified. Large caliber vessels were omitted and even single cells with positive staining were counted as a microvessel.

Measurements were made on the initial biopsy specimens that were obtained before the application of ABS. Similarly, samples collected from the mucosal (luminal) surface that were exposed to ABS (as documented endoscopically) and from deeper regions (muscularis, serosal, and beyond) of the surgically resected material were also evaluated for MVD. Both patients had T4 tumors with invasion beyond the serosa. Great care was taken to avoid sampling from necrotic regions of the tumoral tissue. Three different fields were counted to detect tumor vascularization with ×400 magnification in the most intensely anti-CD34-stained area. Mean numbers were based on these three counts for each biopsy and resection specimen.

Patients' characteristics and MVD results are summarized in **Table 1**.

MVD measurements obtained for the endoscopic biopsy specimens were higher than those for the ABS-exposed superficial tumoral tissue (case 1, median: 55 vs. 26; case 2, median 62 vs. 32). For the deep samples, MVD obtained were 50 and 60 for case 1 and case 2, respectively (**Figure 1**).

A growing number of studies and publications have succeeded in introducing ABS as a novel hemostatic agent, with several potential applications, including in the setting of gastrointestinal bleeding (2–5). Preliminary studies have thoroughly explained the mechanism behind the hemostatic effect of ABS through a protein network, which acts as an anchor for erythrocyte attachment (1). The fact that it does not involve the coagulation cascade, although an advantage in a sense, also suggests that its effect would only be short-lived as the so-called network tends to be washed away soon after application.

However, our experience, as conveyed in our previous study on tumoral gastrointestinal bleeding, has shown that the effect of ABS may actually be a sustained one (2,3). This, combined with the data on ABS-related proteomic analysis and its unique effects on critical transcription factors, led us to speculate on the antiangiogenic effects of ABS, particularly in

Table 1. MVD in the biopsy and surgical materials of the patients

No.	Age/sex	Endoscopic finding	ABS (ml)	Time to surgery (day)	MVD (×400 field)		
					Endoscopic biopsy	Superficial tumor tissue	Deep tumor tissue
1	78/F	A 3.5×4 ~~cm²~~ semi-peduncu-lated hemorrhagic, polypoid mass in the rectum	3	9	45, 55, 65	25, 37, 26	50
2	42/M	Ulcerative vegetated lesion arising from the pylorus and extends to the bulbus	5	16	62, 67, 60	32, 34, 29	60

ABS, Ankaferd Blood Stopper; F, female; M, male; MVD, microvessel density.

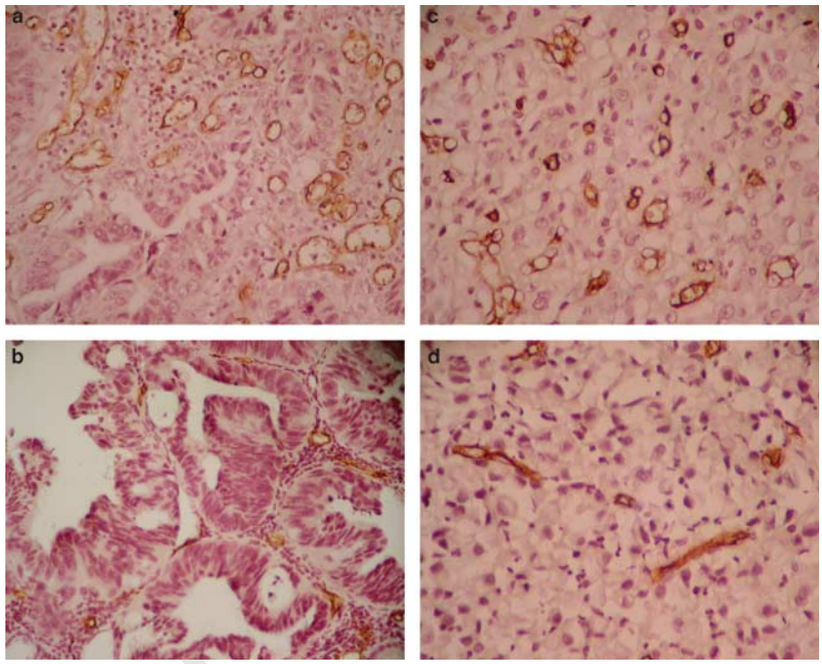

Figure 1. Anti-CD34 antibody-labeled microvessel density (MVD) observed in the neoplastic tissues of: (**a**) Ankaferd Blood Stopper (ABS)-unexposed rectal cancer in comparison with the following (**b**) topically ABS-exposed area of rectal cancer and similarly in (**c**) ABS-unexposed gastric cancer and in comparison with the following (**d**) topically ABS-exposed area of gastric cancer (magnification, ×200).

the setting of neoplasia, where neovascularization is quintessential.

Over the years, MVD has been shown to be a reliable measure of angiogenesis in neoplastic tissue, a notion not wholly supported by all authors (6,7). Nevertheless, many studies on the efficacy of antitumor treatment modalities (chemotherapy and radiotherapy), particularly antiangiogenic agents, used MVD as a measure of response to treatment, this being the main incentive behind preferring MVD measurement in samples obtained from our two patients.

To increase the reliability of our measurements, sampling was performed by an experienced pathologist, with particular care given to establishing tissue orientation of the surgically resected material, to ensure that MVD measurements were correctly performed on ABS-exposed (mucosa) and ABS-unexposed (muscularis propria outwards) areas, respectively.

The results from these two cases, although presumptuous, demonstrated meaningful decreases in MVD measurements in the tissue exposed to ABS, compared with MVDs from biopsy specimens before ABD administration and from deeper (unexposed) neoplastic tissue. These findings suggest the presence of a secondary, more sustained, mechanism of hemostasis, besides the initial protein network.

A major drawback of this investigation is that MVD measurements were made from "hotspots" of CD34 staining, and only three such areas were evaluated for each specimen, which may not accurately reflect angiogenesis. However, we strongly believe that these findings

warrant further investigation of the antiangiogenic property of ABS under more controlled experimental conditions, not only to help establish the exact mechanisms behind its hemostatic effect but also to help further understand and refine the antitumor effects of this agent.

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