# Topical Ankaferd Blood Stopper Administration to Bleeding Gastrointestinal Carcinomas can Decrease Tumor Vascularization


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# Topical Ankaferd Blood Stopper Administration to Bleeding Gastrointestinal Carcinomas can Decrease Tumor Vascularization

**Running title:** Effect of Ankaferd Blood Stopper on Tumor Angiogenesis

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Ankaferd Blood Stopper (ABS) is a standardized herbal extract obtained from five different plants *Thymus vulgaris, Glycyrrhiza glabra, Vitis vinifera, Alpinia officinarum* and *Urtica dioica* [1]. ABS has been approved for the clinical management of external post-surgical and post-dental surgery bleedings in Turkey. ABS also has been used for the management of hemorrhages in difficult clinical conditions [2-4]. ABS represents its unique hemostatic effect by promoting the very rapid (<1 second) 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]. The 'mechanism-of-action' of ABS on tumor tissue has never been explored. One of the hypotheses was the inhibition of tumor angiogenesis. Since tumor microvessel density directly (MVD) reflects tumor neovascularization (angiogenesis) and there is a close relationship between hemostasis and cancer, it is rational to search ABS-effects on neoplastic tissues [5-9]. The aim of this paper is to report observations in two patients with gastrointestinal (GI) cancer, allowing the comparisons to detect local effects of ABS on neoplastic tissues, particularly on the tumor-associated vasculature.

**Examination technique of MVD to compare "de novo" and "ABS-administered" neoplastic tissues**

5 μm thick sections 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 primary antibody against CD34 (mouse monoclonal antibody, clone QBEnd/10, Neomarkers, USA) for 30 min, sections were reacted with 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.

MVD was counted using Nikon E600 microscope. Areas of tissue containing 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. Three different fields were counted with x400 magnification in the most intensely anti-CD34 stained area. Mean numbers were based on these three counts for each biopsy and resection specimens. Operation materials were consisted of the entire tumor including the topically "ABS-administered" local neoplastic tissue sites for comparative histopathological examination with the previous endoscopic tissue, as well as the distant cancer tissue unaffected from the topical ABS application sites.

**Case 1:** A 78-year-old female patient was admitted to our hospital with a chief complaint of rectal bleeding. She had a history of hypertension and congestive heart failure, being treated with metoprolol, furosemide and spironolactone. Digital rectal examination revealed an irregular, non-tender, firm rectal mass and bright red blood coated the exam glove. Flexible rectosigmoidoscopy revealed a 3.5 × 4 cm semipedunculated hemorrhagic, polypoid mass in the rectum. Multiple biopsies were taken from the mass. Following after the endoscopic biopsy procedure, three milliliters of ABS was topically applied onto the bleeding area through a disposable washing pipe (model: PW-205 L, Olympus corporation, Japan). Bleeding was successfully controlled with ABS administration within a few seconds. After nine days of the endoscopy, low anterior resection was performed for the surgical treatment of the rectal tumor based on the clinical indication to treat the patient.

**Case 2:** A 42-year-old male patient was admitted to our hospital with the complaints of epigastric pain, decreased appetite, nausea and vomiting. Upper GI endoscopic examination revealed an ulcerative vegetated lesion arising from the pylorus and extending to

the bulbus implanting a strong suspicion of malignancy. Multiple biopsies were taken from the lesion. Following after the endoscopic biopsy procedure, five milliliters of ABS was administered topically to control bleeding from the tumor. Bleeding was successfully controlled with ABS administration within a few seconds. After sixteen days of the upper endoscopy, gastric resection was performed for the surgical treatment of the carcinoma based on the clinical indication to treat the patient.

The counts of MVD in both cases were summarized in table 1. General histopathological examination together with anti-CD34 stained MVD apparently revealed that the tumor vascularization was decreased in topically "ABS-administered" gastric cancer tissue in comparison to the "de novo" gastric cancer tissue and the resected surgical neoplastic tissue unaffected from the ABS administration at the same specimen (Figure 1).

Patients with cancer suffer from various life-threatening hemorrhages, most frequently due to coagulation defects such as thrombocytopenia and disseminated intravascular coagulation [7,9]. ABS-induced formation of the protein network with vital erythroid aggregation covers the entire physiological hemostatic process [1]. Mainly, there are distinct important components of the ABS-induced hemostatic network. Vital erythroid aggregation takes place with the spectrin and ankrin receptors on the surface of red blood cells. Those proteins and the required ATP bioenergy are included in the protein library of ABS [10]. ABS also upregulates GATA/FOG transcription system affecting erythroid functions. [11] Urotensin II is also an essential component of ABS and represents the link between injured vascular endothelium, adhesive proteins, and active erythroid cells [10]. Those concepts have been developed via MALDI-TOF proteomic molecular analyses, cytometric arrays, transcription analysis, and SEM ultrastructural examinations as well as numerous investigations interacting with basic and clinical research facilities [1,10-13]. Therefore, ABS could be effectively used both in individuals with normal haemostatic parameters and in

patients with deficient primary hemostasis and/or secondary hemostasis. Further investigations on the exciting features of ABS as a powerful hemostatic agent are still in progress. Our observations may open new avenues on the upcoming perspectives of ABS.

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

| | The counts of microvessel density* | | |
|---|---|---|---|
| | Biopsy | Surgical resection material | |
| | | ABS-administered | Distal |
| Case 1 | 45, 55, 65 | 25, 37, 26 | 50 |
| Case 2 | 62, 67, 60 | 32, 34, 29 | 60 |

* Three different fields were counted to detect tumor vascularization with x400 magnification in the most intensely anti-CD34 stained area.

**Figure Legends**

**Fig. 1.** Anti-CD34 antibody-labeled MVD observed in the neoplastic tissues of "de novo" rectal cancer (A) in comparison to the following topically "ABS-administered" area of rectal cancer (B) and likewise in "de novo" gastric cancer (C) and in comparison to the following topically "ABS-administered" area of gastric cancer (D) (X200).

## CONFLICT OF INTEREST

**Guarantor of the article:** Mevlut Kurt, MD.

**Specific author contributions:** Nesrin Turhan contributed to manuscript preparation and interpretation of data; Ibrahim C. Haznedaroglu contributed to editing; Mevlut Kurt and Meral Akdogan contributed to manuscript preparation, data collection.

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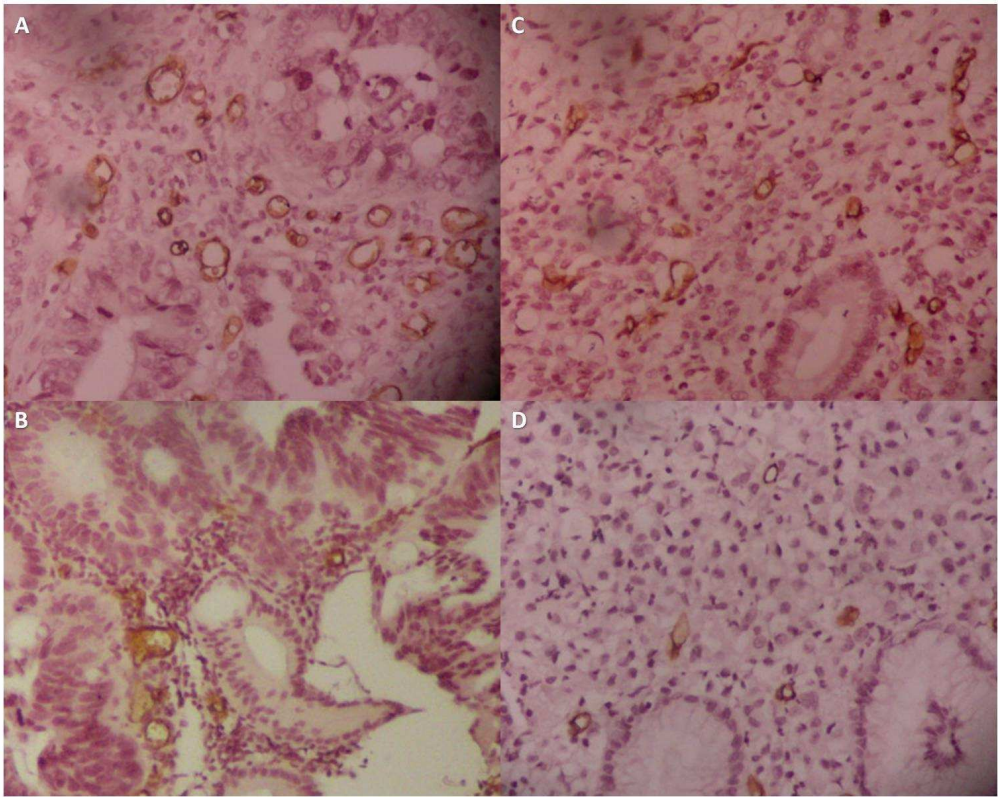


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