Compared to preoperative plasma levels post-operative urokinase-type plasminogen activator-1 levels are persistently elevated for 6 weeks after minimally invasive colorectal resection

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Background: Urokinase-type plasminogen activator-1 (uPA) is a serine protease that converts plasminogen to plasmin after binding to uPA receptor (uPAR). Plasmin catalyzes the regeneration of basement membrane, extracellular matrix, and other tissues. uPA alone and with plasmin leads to activation of angiogenic growth factors that impact tumor cell proliferation, adhesion, and migration. uPA over expression has been noted in colorectal cancer (CRC) and high tissue levels have been correlated with prognosis. uPA/uPAR promotes immune cell activation in healing surgical wounds and may alter perioperative uPA plasma levels. Postoperative (postop) plasma levels, if elevated, may impact the early growth of residual metastases. The impact of minimally invasive colorectal resection (MICR) surgery for CRC on plasma uPA levels is unknown. This study's aim was to measure plasma uPA levels during the first postop month.

Methods: CRC patients undergoing MICR who enrolled in an Institutional Review Board (IRB) approved data/plasma bank for whom adequate plasma was available were included in the study. Patients who had chemotherapy or radiotherapy within 4 weeks, those who received blood transfusions perioperatively and immunosuppressed patients were excluded. Clinical and pathological data were prospectively collected as were blood samples preoperatively, postop day (POD) 1, 3 and at least 1 late time point between POD 7–41. Plasma was isolated and stored at –80 ℃. Late samples were bundled into 7-day blocks and considered as single time points. Total uPA levels (ng/mL) were analyzed in duplicate via enzyme-linked immunosorbent assay (ELISA) and results reported as mean ± standard deviation (SD). The Wilcoxon paired *t*-test was used for analysis.

Results: Ninety-three patients undergoing MICR for CRC [colonic 68%; rectal 32%; average age 65.6 years, laparoscopic 63%, hand-assisted minimally invasive surgery (MIS) 37%] who met criteria were studied. Cancer stage breakdown was; stage I, 30%, stage II, 29%, stage III, 34%, stage IV, 7%. The median preoperative (preop) uPA plasma level (ng/mL) was 529.8 [95% confidence interval (CI): 462.8, 601.1] (n=93). Significant elevations in median levels *vs.* preop were present during POD 3 (542.8, 95% CI: 518.8, 597.3, n=86, P=0.003), POD 7–13 (688.1, 95% CI: 591.7, 753.0, n=72, P<0.001), POD 14–20 (764.9, 95% CI: 704.1, 911.6, n=27, P<0.001), POD 21–27 (685.6, 95% CI: 443.8, 835.8, n=15, P<0.001), and on POD

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28–41 (800.3, 95% CI: 626.9, 940.6, n=21, P<0.001). The colon cancer subgroup's preop and POD 14–20 median results were significantly higher than the corresponding rectal cancer results; otherwise, at the other 5 postop time points there were no significant differences between the rectal and colon cancer subgroups. In addition, no association was found between cancer stage and preop uPA levels and no significant differences were found in postop uPA levels between the hand-assisted laparoscopic group and the lap assisted subgroup at any of the postop time points.

Conclusions: Persistently elevated plasma uPA levels at 5/6 postop time point (P<0.05), in combination with other previously demonstrated long duration proangiogenic plasma protein changes, may render the plasma proangiogenic within the period of the first month post-surgery and may promote angiogenesis within the residual tumor foci. The clinical significance pertaining to these changes, if any, is uncertain and remains to be proven.

Keywords: Urokinase-type plasminogen activator-1 (uPA); postoperative plasma levels; colorectal surgery

Introduction

In the United States, colorectal cancer (CRC) is the third leading cause of cancer-related deaths, with 13% 5-year survival in patients with metastasis (1). Surgical resection remains the mainstay of treatment although advances in chemotherapy and radiotherapy have increased the 5-year survival rate notably. Adjuvant chemotherapy, when indicated, is usually delayed for 4 to 6 weeks after surgery to allow for wound healing. Of note, there is anecdotal clinical evidence and strong experimental evidence suggesting that the growth of residual tumor deposits is stimulated in the early postoperative (postop) period (2-6). Although the origin of these tumor-promoting modifications is uncertain, some investigators have implicated surgery-related immunosuppression and/or persistent proangiogenic and tumor stimulatory alterations in plasma protein levels that continue for 2–5 weeks following surgery (7). Thus far, long duration plasma increases have been noted for a total of 14 proangiogenic proteins that include angiopoietin-2 (Ang-2), vascular endothelial growth factor (VEGF), placental growth factor (PLGF), and multiple matrix metalloproteinases (MMPs) (4-11). Also, plasma from weeks 2 and 3 following minimally invasive surgery (MIS) with removal of CRC has been shown to promote endothelial cell (EC) migration and invasion *in vitro* (12). These changes in proangiogenic compositions within the plasma may in fact foster promotion of tumor angiogenesis within remaining tumor deposits early after surgery. To better define surgery's impact on blood composition, investigators continue to assess blood levels of proteins known to play a role in tumor growth and spread. Urokinase-type plasminogen activator-1 (uPA) is one such protein that could, by virtue of its many potential effects, impact tumor cell behavior if significantly elevated levels in the plasma were present.

During and soon after wounding, fibrin plugs play an important hemostatic role. However, during the wound healing proliferative phase, fibrin is degraded by plasmin and other enzymes and is replaced with collagen. uPA and tissue plasminogen activator (t-PA), which convert inactive

Highlight box

Key findings
• uPA plasma levels were shown to be significantly elevated for over one month after MICR in CRC patients.

What is known and what is new?
• Preop blood levels of uPA were noted to be elevated in a wide range of cancers including colorectal tumors. The current study, the first to assess uPA levels postoperatively, shows, for reasons not likely related to cancer, that after bowel resection plasma uPA levels are significantly increased over preop baseline for about 1 month.

What is the implication, and what should change now?
• uPA and the other long duration elevated angiogenic proteins after surgery are capable of promoting tumor development via mechanisms related to angiogenesis. Further investigation to develop anti-cancer treatments to be used perioperatively that are effective but that do not interfere with wound healing appears warranted.

plasminogen to plasmin, play key roles in this process. In addition to its role in fibrin degradation, plasminogen/plasmin and their associated regulatory proteins, including uPA and its receptor, urokinase-type plasminogen activator receptor (uPAR), are capable of breaking down the basement membrane and extracellular matrix (ECM). The uPA-uPAR system also activates MMP, growth factors and other proteases and also interacts with integrin and vitronectin. In the setting of cancer, collectively, these effects break down the physical barrier to tumor cell migration and facilitate tumor invasion and metastasis as well as stimulate tumor growth. These effects of uPA/uPAR also support tumor angiogenesis which is critical to tumor growth. This study of CRC patients concerns uPA which a key component of the plasmin activation system. If uPA levels are persistently increased postop, similar to the above-mentioned proteins with long duration plasma elevations, then further evidence will have been found to support the hypothesis that colorectal resection is associated plasma protein changes that are stimulatory to tumor growth. The level of uPA protein and/or mRNA has been noted to be elevated in a significant proportion of breast, ovarian, bladder, gastric, prostate, and CRCs (13-23). Further, elevated tumor uPA levels have been associated with a worse outcome or more advanced disease for all of these cancers (13-18,21-23). Also, plasma levels of uPA have been shown to be significantly elevated in patients with ovarian, endometrial, non-small cell lung cancer (NSCLC), gastric, hepatocellular, pancreatic, colorectal, and bladder cancers (24-28).

Although preoperative blood levels of uPA have been studied, the influence of surgical resections on levels of postop plasma has not been investigated. The purpose of this study is to determine levels of plasma uPA before, as well as during the first 6 weeks following minimally invasive colorectal resection (MICR) in a group of CRC patients. We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-113/rc).

Methods

Study population

Adult CRC patients at Mount Sinai West Hospital, New York and New York Presbyterian Hospital, Columbia University, New York who had undergone elective MICR and had consented preoperatively to enrollment for an Institutional Review Board (IRB)-approved data and tissue banking protocol for subjects that had sufficient quantities of pre and postop plasma available were eligible for this study. The preoperative plasma levels, determined prior to incision, provide the baseline uPA levels against which the postop results are compared.

Ideally, this study would also have included CRC patients who underwent open surgical resection to allow for an assessment of the contribution of surgical method on the results noted, however, the volume of open resections in our centers was insufficient to allow inclusion of an open surgery arm.

The time period across which these patients underwent surgery was 2007 to 2016. One purpose of this banking effort was to determine the impact of major surgery on blood protein composition. Demographic, clinical, peri-operative, and pathologic data was collected for all consenting patients. Immunosuppressed patients and those that had undergone chemotherapy or radiotherapy within 4 weeks of surgery were excluded. Patients who received blood transfusions perioperatively were also not eligible.

Blood samples and enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained from all patients preoperatively and on postop days (PODs) 1, 3, and at least 1 timepoint beyond the first week postop. It was not possible to obtain late blood specimens on set postop days after discharge from the hospital for logistical reasons, therefore, the specimens received late were grouped into 7-day time blocks (POD 7–13, 14–20, and 21–27) which were in turn considered individual points of time for data analysis. Of note, because of the small number of samples obtained during postop weeks 5 and 6, the blood samples from those 2 weeks were grouped together and viewed as a single time point to permit statistical analysis.

Blood samples were obtained in heparin containing tubes (Cat No. 367878Becton Dickinson, New Jersey, USA) and the plasma isolated via centrifugation within 6 hours and stored in 500 µL aliquots at –80 ℃ until utilized.

An ELISA that was available commercially for uPA was utilized for the assays in accordance with manufacturer instructions (Cat No. DUPA00, R&D Systems, Minneapolis, USA). Plasma uPA levels were determined in duplicate and 8 serial dilution standard curve samples were included on each 96 well plate; the results are reported as ng/mL. Briefly, 50 µL three times diluted (with calibrator diluent supplied
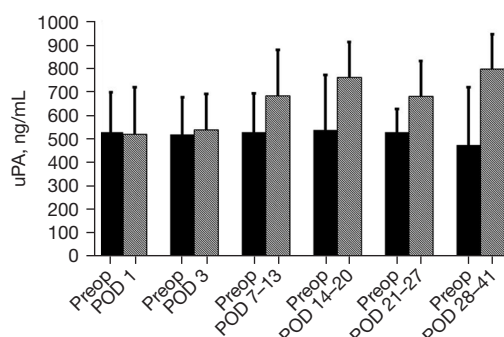
Figure 1 ELISA determined preoperative and postoperative plasma uPA levels of colorectal cancer patients. uPA levels are expressed as median and 75% quartile range [preop *vs.* POD 1 (P=ns, n=93); preop *vs.* POD 3 (P=0.003, n=86); preop *vs.* POD 7–13 (P<0.001, n=72); preop *vs.* POD 14–20 (P<0.001, n=27); preop *vs.* POD 21–27 (P<0.001, n=15); POD 28–41 time point (P<0.001, n=21). ELISA, enzyme-linked immunosorbent assay; preop, preoperative; POD, postoperative day; uPA, urokinase-type plasminogen activator-1.

with the kit) plasma samples in duplicate were used. Diluted human uPA stock solution (20,000 pg/mL) provided with the kit was used to make the standard curve. The standard curve was made using the 2,000 pg/mL uPA as the high standard and calibrator diluent as the zero standards (0 pg/mL). The optical density of ELISA plate was determined at 450 nm at the end of the reaction using an automated microplate reader (Synergy2; Bio-Tek Instruments, Inc., Winooski, VT, USA). The mean plasma uPA concentrations of samples were determined using the log/log curve-fit standard curve.

Statistical analysis

Continuous random variables such as age, surgical time, length of stay (LOS), surgical incision size of group were presented as average and SD whereas for categorical variables, frequencies and percentages were utilized. Because the blood samples obtained after discharge were not obtained on set PODs, and because the specimens received late were spread out over a period of 3 to 4 weeks, the later samples were then grouped into 7-day time blocks (POD 7–13, 14–20, 21–27, and 28–41) and were interpreted as single time points for the sake of data analysis. Because values of uPA for pre-operative and corresponding postop time points were not distributed normally for time points

collected later, comparisons of uPA levels for the preop *vs.* Postop time points were performed with the use of non-parametric (Wilcoxon signed rank paired) test, after which the data were reported utilizing median along with confidence interval (CI) values. Also, a bar graph was used to depict the data expressing levels of uPA as median with a 75% quartile range (*Figure 1*). We believe this graph is more suitable to explain the difference of preop *vs.* postop uPA level at each time points. Comparisons of hand assisted procedure subgroup preop and post op values *vs.* laparoscopy assisted procedure subgroup preop and post op values, advancing cancer stage subgroup values, male *vs.* female subgroups values were analyzed by nonparametric Mann and Whitney test since comparisons were done between different groups and numbers (n) of each group were small. Correlation between postop plasma uPA levels and age as well as length of surgery was analyzed by Spearman's rank correlation coefficient (r_s). A P value of P<0.05 was used as statistically significant. All data analysis was performed using SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA). Manuscript is updated accordingly.

Ethical statement

The study was conducted by using material collected from patients who consented preoperatively to participate in the Mount Sinai West Colorectal service's IRB-approved general tissue and data banking protocol (No. GCO1: 16-2619-Institutional Review Board of the Mount Sinai School of Medicine, New York; and IRB reference No. AAAA4473-IRB of the Columbia University Medical Center, New York). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participating CRC patients who were enrolled in an IRB approved data/plasma bank and all patients assented to analysis, present and to publish the paper.

Results

A total of 93 eligible CRC patients who underwent MICR were selected for the study. There were 46 males and 47 females with a mean age of 65.6±13.9 years. Sixty-three (68%) patients had colon cancer whereas 30 (32%) had rectal malignancies. The breakdown of the types of resections carried out is shown is *Table 1*; operations performed most commonly were: right hemicolectomy (29.0%), low anterior or anterior resection (20.4%), sigmoidal resection (21.5%),

Table 1 Clinical and demographic data of the study population

Variables	Values
Age (years), mean ± SD	65.6±13.3
Sex, n (%)	
Male	46 (49.5)
Female	47 (50.5)
Incision size (total study population) (cm), mean ± SD	7.0±4.1
Incision size (laparoscopic procedure group) (cm), mean ± SD	6.96±3.9
Incision size (hand-assisted procedure group) (cm), mean ± SD	9.96±3.2
Operative time (min), mean ± SD	336.5±117.7
Length of stay (days), mean ± SD	7.9±3.7
Type of resection, n (%)	
Right	27 (29.0)
Sigmoid/recto-sigmoid [14/6]	20 (21.5)
LAR/AR [14/5]	19 (20.4)
Proctectomy/total/sub-total [5/3/1]	9 (9.7)
APR	7 (7.5)
Transverse	6 (6.5)
Left	5 (5.4)
Surgical method, n (%)	
Laparoscopic procedure-assisted	59 (63.4)
Hand-assisted/hybrid laparoscopic (HAL)	34 (36.6)
Conversion, lap-assisted to HAL or open (final incision greater than 7 cm)	10 (10.8)
Conversion, HAL to open (incision >12 cm)	6 (6.5)
Colostomies	9 (9.7)
Ileostomies (diverting + permanent)	11 (11.8)

APR, abdominoperineal resection; AR, anterior resection; LAR, low anterior resection; SD, standard deviation.

and abdominoperineal resection (7.5%). As regards surgical method, a laparoscopic-assisted operation was done in 61% [mean incision length (IL) 6.96±3.9 cm] and a hand-assisted laparoscopic operation was done in 39% of patients (mean incision size, 10.0±2.7 cm). There were 10 conversions in the laparoscopy assisted group (defined as incision greater than 7 cm) and 6 conversions in the hand-assisted MIS group (incision greater than 12 cm). The operative time mean value was 336.5±117.7 minutes. The LOS mean value was 7.9±3.7 days. When 4 outliers are eliminated (LOS 35, 16, 15, 15 days) the mean LOS is 6.2 days. The following complications were noted: ileus [9], urinary retention [8], urinary tract infection [3], cardiac [3], pneumonia [2], pulmonary edema [2], sepsis [2], phlebitis [2], wound infection [1], *Clostridium difficile* colitis [1], seroma [1], and seroma [1]. There were no leaks or intrabdominal abscesses. There were no reoperations, however, 1 patient had a percutaneous gastrostomy tube. There were no deaths within 30 days. The final cancer stage breakdown follows; stage I (30%), stage II (30%), stage III (34%) and stage IV (6%).

When compared to median preop plasma levels of uPA (529.8, 95% CI: 462.8, 601.1), significant elevations in median levels (ng/mL) were noted POD 3 (542.8, 95% CI: 518.8, 597.3, n=86, P=0.003), POD 7–13 (688.1, 95% CI:

591.7, 753.0, n=72, P<0.001), POD 14–20 (764.9, 95% CI: 704.1, 911.6, n=27, P<0.001), POD 21–27 (685.6, 95% CI: 443.8, 835.8, n=15, P<0.001) and on POD 28–41 (800.3, 95% CI: 626.9, 940.6, n=21, P<0.001) (*Figure 1*). The increased percentage from mean baseline at each individual time point was: 8.3% (POD 1), 10.6% (POD 3), 43.6% (POD 7–13), 66.3% (POD 14–20), 30.8% (POD 21–27), and 40.5% (POD 28–41).

Of note, a significant correlation was not found between age and pre-operative or postop uPA levels. Similarly, there were no significance differences noted in the uPA levels between the male and female patients pre- or postoperatively. Of note, there was no association demonstrated concerning cancer stage and preop uPA levels. Also, no significant differences were found in uPA levels between the hand-assisted laparoscopic group (mean IL 9.96±3.2 cm) and the lap-assisted subgroup (mean IL 6.96±3.9 cm) at any of the postop time points. Of note, the colon cancer subgroup's preop and POD 14–20 mean results were significantly higher than the corresponding rectal cancer results; non-significant elevations were also noted in the colon subgroup on POD 7–13, 21–27, and 28–41. There was no relationship found between length of surgery and postop plasma uPA levels.

Discussion

The vast majority of serum protein changes that occur in response to surgery and anesthesia resolve within days (29,30); examples include interleukin (IL)-1 receptor antagonist, TNF, IL-6, and C-reactive protein (CRP) (31). These transient alterations are thought to be related to the acute inflammatory and the hypothalamic-pituitary-adrenal axis responses to tissue trauma and anesthesia. In contrast to these short-lived plasma compositional changes, this study of CRC patients who underwent MICR, found that the mean serum level of uPA was significantly elevated for almost a month after surgery. The peak elevations were noted during the second and third weeks after surgery.

Although similar plasma uPA changes were noted in the colon and rectal cancer subgroups for most time points, the colon group had significantly higher levels preop and at 1 postop time point. The age and sex of the patient as well as the specific method used in surgery utilized (lap-assisted *vs.* hand-assisted MIS) was not significant in impacting the postop results. What is the cause of the uPA plasma increases after surgery?

This study did not assess or consider possible etiologies,

therefore, we can only speculate as to the cause(s) of these changes. Whereas, the acute inflammatory response might contribute to the early increase it cannot account for the week 2 to 4 elevations. Since the cancer has been removed it is highly unlikely that the malignancy is the cause. Of note, there is human evidence regarding etiology for 8 proteins for which plasma levels were similarly increased following MICR for 3 to 5 weeks (32). The study in question simultaneously assessed blood and wound fluid levels of 8 proteins at multiple time points for up to 3 weeks following MICR for both CRC and benign colon pathology. Wound fluid was obtained from the abdomen/pelvis or subcutaneous wounds via Jackson Pratt drains placed at surgery. The median postop plasma levels of the 8 proteins were, again, found to be significantly and persistently elevated from their preop baselines. The wound fluid levels of the same proteins were noted as 3 to 40 times above the correspondingly higher plasma levels at all time points. These results raised the possibility that the increased levels of proteins in the blood originated in healing wounds and had diffused into the blood stream. The fact that all 8 proteins have been shown to play some role in wound healing lends support to this hypothesis. These results also suggest that wound healing is a lengthy process that influences plasma composition. This hypothesis can also be invoked to explain uPA's persistent elevation since uPA's principal action is the activation of plasmin which plays a critical role in the healing of wounds. Plasmin degrades fibrin clots in wounds, and provides access to fibroblasts, ECs, and other cells involved in tissue repair and angiogenesis. What is known about uPA?

Pro-uPA is released by a large variety of cell types including ECs, fibroblasts, neutrophils, monocytes, and macrophages as a single polypeptide chain (411 amino acids) with minimal plasmin activating function. It is cleaved into a 2 chain "activated" high molecular weight (HMW) form by plasmin, kallikrein, cathepsin, trypsin and other enzymes. uPA can be further degraded into a single chain low molecular weight (LMW) form that maintains plasmin activating activity. Pro-uPA, HMW uPA, as well as LMW uPA can all bind to cell surface bound uPAR. uPA is broken down and regulated by the serine proteases PAI-1 and PAI-2. uPA and the uPA-uPAR complex, besides generating plasmin, activates MMP and other growth factors [basic fibroblast growth factor (bFGF), transforming growth factor β (TGF-β), IL-1β]; uPA-uPAR also impacts cell adhesion by interacting with vitronectin. uPA-uPAR is not capable of independent signal transduction, however,

because it has multiple binding sites it can interact with integrins and initiate intracellular signaling via the Jak-STAT, focal adhesion kinase (FAK), and other pathways that influence transcription, cell growth and survival (33,34). These effects and actions facilitate tissue repair and wound healing. As noted, plasmin and plasmin activator system also figure prominently in cancer development.

Plasmin is the principal mechanism by which ECM and the basement membrane are degraded in solid tumors. ECM breakdown provides tumor cells access to adjacent tissues, lymphatics, and blood vessels which facilitates invasion, metastases, and angiogenesis. uPA, by activating plasmin plays a crucial role in this process (33). Also, uPA, independent of plasmin, and in conjunction with its cell bound receptor uPAR, facilitates tumor growth via multiple pathways (35,36). uPA alone has been shown to positively modulate extracellular regulated kinase's (ERK's) tumor stimulatory effects in breast cancer cells; in addition, when bound to uPAR, it enhances the latter's independent ERK/p38 pro-tumor growth modulating activity (37,38). As regards angiogenesis, it has also been demonstrated that uPA has chemotactic effects on endothelial and smooth muscle cells and is a mediator of the mitogenic effects of PDGF and bFGF on vascular smooth muscle cells (39,40). uPA alone and when bound to uPAR has also been shown to activate and promote several growth factors and to modulate both cell proliferation and apoptosis (34). Also of note, the uPA activation and degradation process generates an amino terminal fragment that has been shown to have mitogenic effects and to stimulate EC movement (41-43).

As mentioned in the introduction, uPA expression is notably increased in many types of tumors and high expression has been shown to be a negative prognostic indicator. Also, preop blood levels were noted to be elevated in a wide range of cancers including colorectal tumors. The current study, the first to assess uPA levels postoperatively, shows, for reasons not likely related to cancer, that after bowel resection plasma uPA levels are significantly increased over preop baseline for about 1 month. Thus, uPA joins the short but growing list of proteins that have been demonstrated to remain elevated up to and beyond one month postoperatively. This list includes angiopoietin-2, chemokine ligand 16, IL-8, chitinase-3 like protein, MMP-3, monocyte chemotactic protein-1, PLGF, PAI-1, soluble vascular adhesion molecule-1, and finally VEGF (4-6,8-12,29).

VEGF plays crucial role in promoting multiple steps early in angiogenesis. VEGF triggers proliferation of

ECs along with, migration and invasion thus encouraging continued development of microvasculature, whereas Ang-2 promotes destabilization of connections between endothelium and perivascular cells, which enrich the effects of VEGFs (8). In addition to the proliferation and survival of ECs, PLGF stimulates recruitment of the smooth muscle precursors enveloping developing vessels in tumors. Taken together along with VEGF, these factors produce more stable and mature vessels (10). Soluble vascular cell adhesion molecule-1 (sVCAM-1) supports a vital beginning step in the development of angiogenesis via binding to VLA4 *in vitro* which is EC-bound and induces chemotaxis of ECs via both FAK as well as p38 mitogen-activated protein (MAP) signaling pathways (9,44,45). Monocyte chemoattractant protein-1 (MCP-1), via the recruitment of proangiogenic proteins, promotes angiogenesis by generating both plasma monocytes as well as tissue macrophages and ECs into wounds and tumors (5). In tumor stroma, macrophages have shown in previous studies to express CHi3L1, which likely stimulates tumor angiogenesis (6,46). Protease family protein MMPs 2, 3, and 7 are key regulators of cell matrix interactions and support angiogenesis by way of promoting stimulation of budding vessels as well as migrating cells (47-49). CXCL-16, a transmembrane chemokine, and its receptor CXCR-6 pair activity associated with pro-inflammatory stimuli, signaling induced recruitment and angiogenesis of endothelial precursor cells (4,50-52). IL-8 offers a function in angiogenesis, often by healing surgical wounds tissues (11) and uPA, by activating plasmin plays a crucial role in ECM breakdown, invasion, metastases, and angiogenesis. Studies utilizing ECs housed *in vitro* suggest the end effect of plasma make-up changes during postop weeks 2 through 3 is proangiogenic. uPA is also part of the group of proteins that exude blood levels after MICR. It is apparent from the biological role of these proteins including uPA that they all have some effect in the process of angiogenesis.

As mentioned, all of these proteins have some effect in the process of angiogenesis. Further, human plasma from the postop weeks 2 through 3 after colorectal resection has been previously shown to stimulate proliferation, invasion, and migration of ECs *in vitro* (8,12). Thus, the postop plasma is proangiogenic for up to a month. Also of note, uPA and the other long duration elevated proteins after surgery are capable of promoting tumor development via mechanisms related to angiogenesis. Are these changes sufficient and capable of stimulating tumor angiogenesis and growth in residual metastases? If so, then the immediate postop period

may be a dangerous time in the life of cancer patients because adjuvant chemotherapy is routinely not started for 4 to 6 weeks. In that case, it would make sense to develop anti-cancer treatments to be used perioperatively that are effective but that do not interfere with wound healing. Immunomodulators and other immunotherapies are options that warrant assessment for this time period. It is the authors view that further study of surgery's systemic impact on the patient's physiology is indicated. Also, investigations focused on elucidating the downstream manifestations and effects of wound healing would also be of value.

This study has a number of shortcomings. First the late postop time point sample sizes are notably less than the overall sample size. Unfortunately, it is not possible to obtain weekly samples from all patients after discharge for logistical reasons. Patients are geographically scattered and have, at most 2 office visits during the first 4 to 6 weeks at which time samples can be obtained. A second limitation is that the late samples are bundled into 7-day time periods. This is a necessity because it is not possible to schedule postop office visits such that they fall on the same postop day. A third weakness is that we did not assess baseline uPA levels in a control group of cancer free patients and thus cannot comment on whether the cancer patients preop levels are elevated preoperatively. Finally, the study patients' tumors were not analyzed for uPA expression. Finally, we have no intermediate or long term oncologic follow up data.

Conclusions

uPA plasma levels were shown to be elevated for over one month after MICR in CRC patients. The size of the increase peaked during the second postop week and the percent change from baseline for postop weeks 2 through 6 ranged from 30 to 66 percent. Of unclear importance and for unclear reasons the uPA levels of the subgroup of colon cancer patients were higher than the subgroup of rectal cancer patients at 2/7 time points. Of note, age, sex, and the use of laparoscopic assisted *vs.* hand assisted methods did not impact postop levels. The pathogenesis of the postop change in uPA is not unknown and was not addressed in this study. However, based on a prior study done on a group of proteins that demonstrated similar persistent increases in blood levels after CRC surgery, it is postulated that the healing wounds may be one cause. Because all 15 of the proteins shown to elevated for 3–6 weeks after MICR promote angiogenesis and/or tumor growth, further investigation of this time period and the advancement of anti-cancer therapies for early postop use appears warranted.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-113/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-113/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-113/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The present study was conducted by using material collected from patients who consented preoperatively to participate in the Mount Sinai West Colorectal service's IRB-approved general tissue and data banking protocol (No. GCO1: 16-2619-Institutional Review Board of the Mount Sinai School of Medicine, New York; and IRB reference No. AAAA4473-Institutional Review Board of the Columbia University Medical Center, New York presbyterian hospital). Written informed consent was obtained from all participating colorectal cancer patients who were enrolled in an IRB approved data/plasma bank and all patients assented to analysis, present and to

publish the paper.

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196

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