

Clinical Surgery

Increased skin autofluorescence after colorectal operation reflects surgical stress and postoperative outcome

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Abstract

BACKGROUND: Abdominal surgery is a major oxidative stress effector. The increase in oxidative stress has been related to postoperative complications. Oxidative stress leads to the formation and accumulation of oxidation protein end products, which exhibit autofluorescence (AF) and induce inflammatory reactions.

METHODS: Skin AF was assessed perioperatively in 40 consecutive colorectal surgery patients until discharge. Duration of surgery, estimated blood loss, and urinary production per hour were analyzed as measures of surgical stress. The clinical occurrence of anastomotic leakage, systemic infections, and cardiopulmonary complications within 30 days of surgery were analyzed.

RESULTS: A perioperative increase in skin AF of $19 \pm .2\%$ was observed. Duration of operation and blood loss were independently associated with the perioperative increase in skin AF. Skin AF correlated with C-reactive protein levels postoperatively. American Society of Anesthesiologists classification, duration of operation, and preoperative and perioperative increases in AF were independently associated with postoperative complications.

CONCLUSIONS: This is the first study to demonstrate an association between skin AF and surgical stress and outcomes, which may rate the condition of a patient after operation.

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Surgery of any kind, but especially that of the abdominal viscera, is recognized as a major oxidative stress effector.^{1,2} Oxidative stress relates to the imbalance between the production of free radicals and the antioxidant defense mechanisms. Increased levels of xanthine oxidase, reduced antioxidant status, and changes in mitochondrial function of enterocytes all increase oxidative stress during abdominal surgery (Fig. 1).^{3–5} Inflammation and abdominal ischemic-

reperfusion effects further aggravate oxidative stress.⁶ Oxidative stress has an important role in the development and manifestations of systemic inflammatory reactions.^{7–9} These reactions have been related to the occurrence of anastomotic leakage and systemic complications such as pneumonia, myocardial injury, and mortality.^{3,10–12} In other words, increased oxidative stress after operation reflects surgical stress and the risk for complications. A simple measure of oxidative stress could rate the condition of a patient after operation.

Biochemical reactions between reactive oxygen species and proteins may lead to the formation and accumulation of

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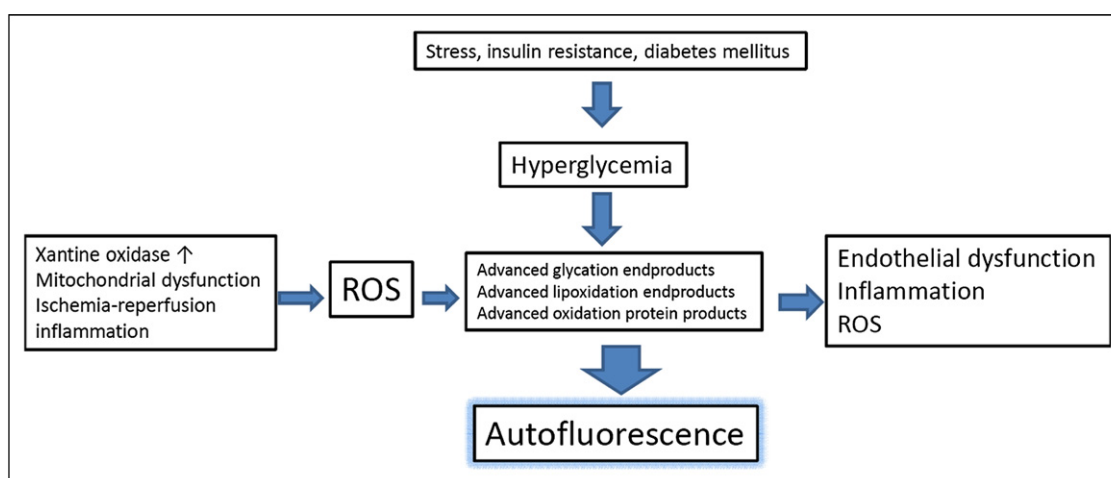


Figure 1 Reactive oxygen species (ROS) form during colorectal surgery because of ischemia-reperfusion effects and antioxidant changes in the enterocytes. Formation of ROS induces the formation and accumulation of oxidation protein end products. Also, metabolic changes during surgery may lead to the formation of these protein end products. Oxidation protein end products play an important role in endothelial dysfunction and inflammation, which has been related to systemic complications such as pulmonary and myocardial injury.

advanced glycoxidation end products, advanced lipoxidation end products, and advanced oxidation protein products.^{13,14} Many of these end products are fluorescent, and tissue fluorescence is strongly related to oxidative stress.^{15,16} We have developed and validated a noninvasive technique to measure skin autofluorescence (AF) and thus the accumulation of oxidation end products.^{17,18} Skin AF predicts the development and progression of long-term complications in diseases with a chronic state of oxidative stress, such as diabetes mellitus.^{17,19–21} However, acute and profound increases in skin AF and oxidation end products are observed in states of acute oxidative stress, such as coronary events and infections.^{22–25} This pilot study is the first to analyze the variance in skin AF after colorectal operation.

Methods

Patients

This study was a prospective cohort study carried out at the Isala Clinics (Zwolle, The Netherlands) in 2009. Forty consecutive patients who were admitted for elective colorectal operation were analyzed. All patients provided informed consent before operation, and approval was obtained from the medical ethics committee. Patient characteristics are described in Table 1. Demographic characteristics, colorectal disease etiology, and risk factors for surgery such as cardiovascular and pulmonary disease were determined from chart review. An early recovery program has been fully implemented in our hospital. Other factors that are known to influence the accumulation of oxidation end products were analyzed: body mass index, smoking status, diabetes mellitus (according to American

Diabetes Association guidelines), hypertension (defined as blood pressure > 140/90 mm Hg or the use of antihypertensive medication), hypercholesterolemia (defined as the use of statins), renal function, and the presence of coronary artery disease and peripheral artery occlusive disease. Perioperative estimated blood loss, mean arterial blood pressure, duration of surgery, and urinary production per hour were analyzed as measures of overall surgical stress.²⁶ Skin

Table 1 Patient characteristics

Variable	Value
Age (y)	64 ± 11 yrs
Women/men	38%/62%
Diabetes mellitus	18%
Body mass index (kg/m ²)	27.0 ± 6.1
Coronary heart disease	34%
Pulmonary disease	10%
Colorectal cancer	89%
Hyperlipidemia	21%
Smoking	32%
American Society of Anesthesiologists classification	
I	50%
II	26%
III	24%
Colorectal surgery	
Low anterior resection	40%
Left-sided colectomy	33%
Right-sided colectomy	27%
Duration of surgery (min)	110 (70–162)
Blood loss (mL)	300 (102–732)
Mean arterial pressure (mm Hg)	78 (61–87)
Urinary output (mL/min)	60 (12–134)
Preoperative skin AF (AU)	2.15 ± .56
Preoperative skin AF (AU), excluding patients with diabetes	2.11 ± .49

Data are expressed as mean ± SD or as mean (interquartile range).

AF levels were measured the day before operation and every other day after operation until discharge (in the fasting state). C-reactive protein levels were followed postoperatively every other day. We analyzed the clinical occurrence of the following postoperative complications: anastomotic leakage, systemic infections, cardiovascular complications, and pulmonary complications. Additional testing was performed only when postoperative complications were suspected. Thus, chest x-rays, for example, were obtained only when clinical examination suggested pulmonary complications.

Skin AF

Skin AF was measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands). The AGE Reader is a desktop device that uses the characteristic fluorescent properties of some oxidation end products to estimate their level in the skin. Technical details of this noninvasive device concerning the optical technique have been described more extensively elsewhere.¹⁸ In short, the AGE Reader illuminates a skin surface of 4 cm² guarded against surrounding light with an excitation light source with a peak excitation of 370 nm. Emission light (fluorescence) and reflected excitation light from the skin is measured with a spectrometer. In the current series of experiments, the right forearm was positioned on top of the device. A series of 3 consecutive measurements was carried out, which took <1 minute. The mean skin AF of 3 consecutive measurements was calculated and used in the analyses. AF measurements on 1 day and intraindividual seasonal variance showed an Altman error percentage of <6%.

Statistical analysis

Data were prospectively gathered in a database (SPSS version 15.0; SPSS, Inc, Chicago, IL). The Kolmogorov-Smirnov test was used to assess normal distribution of the variables. For comparison between continuous variables, Student's *t* test or the Mann-Whitney *U* test was used. For categorical variables, Fisher's exact test was used. To compare paired groups, Wilcoxon's test was used. Correlations between variables were analyzed by Spearman's correlation. Multivariate regression analyses were performed for determination of independent relationships of variables with AF. Variables shown to be related to AF in univariate analysis ($P < .01$) were tested for their independent effect on AF by multivariate logistic regression analysis. *P* values < .05 were regarded statistically significant.

Results

Patient characteristics are described in Table 1. Almost 90% of the colorectal operations were performed for cancer. Approximately 40% of the colorectal operations were low

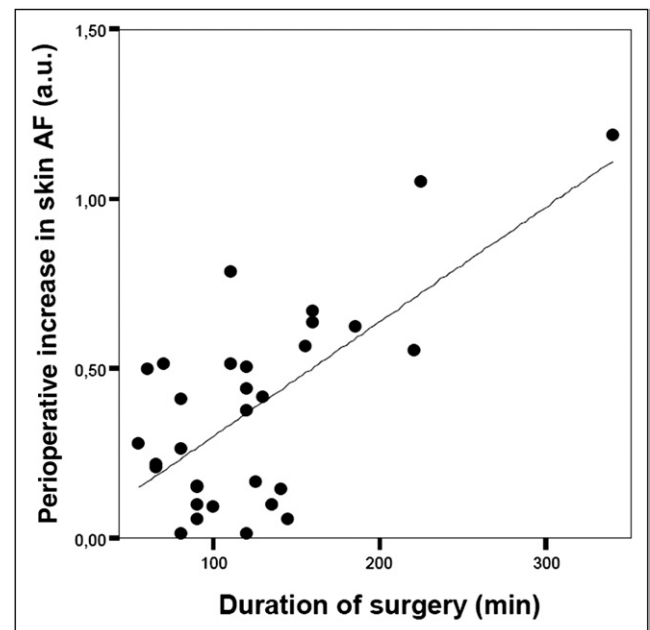


Figure 2 Perioperative increase in skin AF correlates with surgical duration ($r = .68$, $P < .01$).

anterior resections. The mean preoperative skin AF was $2.15 \pm .6$ arbitrary units (AU). Multivariate regression analysis showed that 60% of the variance in preoperative AF was related to age and the presence of diabetes or coronary heart disease ($R = .78$, $P < .01$).

A mean increase in skin AF of $19 \pm .2\%$ was observed on the first day after surgery compared with preoperatively ($2.54 \pm .57$ vs $2.15 \pm .56$ AU, $P < .01$). This increase in skin AF correlated strongly with the duration of surgery ($r = .68$, $P < .01$), estimated blood loss ($r = .69$, $P < .01$), mean arterial blood pressure ($r = -.42$, $P < .01$), and urinary output per hour during surgery ($r = -.37$, $P = .01$) (Fig. 2). There was a negative correlation between the perioperative increase in skin AF and preoperative skin AF levels ($r = -.44$, $P < .01$). Regression analyses for the perioperative changes in skin AF are described in Table 2. Multivariate analysis showed that 75% of the variance in perioperative skin AF levels was related to preoperative skin AF level, the estimated amount of blood loss, and the duration of surgery ($R = .83$, $P < .01$).

All patients were followed until day 30 after surgery. One patient (2.6%) died from a myocardial infarction. Anastomotic leakage was observed in 2 patients (5.2%), pneumonia in 4 patients (10.4%), and myocardial infarction or heart failure in 3 patients (7.8%). Figure 3 shows some examples of skin AF and C-reactive protein variance in patients with and without complications. No complications were observed in 25 patients (65%). Skin AF after operation decreased within several days (mean, 4 days) to levels comparable with preoperative levels in patients without any complications. However, in patients with complications, skin AF levels remained higher or even increased further compared with preoperatively (Fig. 4). Changes in skin AF

Table 2 Variables associated with perioperative increase in skin AF

Variable	Univariate		Multivariate	
	β	<i>P</i>	β	<i>P</i>
Intraoperative characteristics				
Duration of surgery	.51	.001	.31	.04
Estimated blood loss	.52	.002	.29	.04
Urinary output per hour	-.27	.06	-.09	.22
Mean arterial pressure	-.41	.02	-.18	.31
Preoperative characteristics				
Age	.01	.11	.01	.51
Gender	.03	.32	.01	.41
Diabetes	.21	.09	.20	.06
Coronary heart disease	-.24	.09	-.22	.10
Pulmonary disease	.02	.24	.01	.40
Smoking	.20	.11	.11	.49
Body mass index	.04	.26	.01	.59
Statin use	-.23	.08	-.17	.08
American Society of Anesthesiologists classification	.12	.09	.09	.12
Preoperative skin AF	-.42	.02	-.31	.02

levels correlated with changes in C-reactive protein levels ($r = .39$, $P = .03$).

The increase in perioperative skin AF was significantly lower in patients without complications ($.26 \pm .05$ vs $.57 \pm .1$ AU, $P = .01$). Multivariate logistic regression analysis showed that American Society of Anesthesiologists classification (odds ratio [OR], 1.9; 95% confidence interval [CI], .8–3.1), duration of surgery (OR, 2.9; 95% CI, 1.4–4.5), preoperative skin AF level (OR, 3.4; 95% CI, 1.9–6.2), and increase in perioperative skin AF level (OR, 1.98; 95% CI, .89–3.1) were independently associated with the development of postoperative complications.

Comments

Skin AF is a novel technique to noninvasively measure oxidative stress. This is the first study to analyze skin AF levels after colorectal surgery. Skin AF increases after colorectal surgery, and this increase is related to markers of surgical stress, such as estimated blood loss. Increased skin AF is also associated with the development of postoperative complications. Skin AF may (in time) provide an easy tool that rates the condition of a patient both before and after surgery. It may provide immediate and graded feedback on how an operation went for a patient.

We have previously observed increased skin AF in states of chronic oxidative stress as well as in acute increases in oxidative stress.^{17,19,25,27} Skin AF levels increase profoundly in patients with myocardial infarction, and this increase is related to patients' outcomes after 1 year of follow-up.²⁵ Comparable changes in skin AF and patients' outcomes were observed in patients with sepsis (unpub-

lished data). Manipulation of the intestine during surgery has some detrimental effects, including oxidative stress. Increased xanthine oxidase levels and alterations in intestinal epithelial structure induce oxidative stress and inflammatory reactions.¹⁰ Splanchnic vasoconstriction due to loss in local intestinal autoregulation by low cardiac output and anesthetic interventions, as well as surgical manipulation, may induce ischemia-reperfusion effects.⁶ These effects may further aggravate oxidative stress and induce distant organ dysfunction.

Estimated blood loss, duration of surgery, and urinary output per hour during operation have been shown to reflect surgical stress. Gawande et al²⁶ derived a simple score on the basis of intraoperative data that accurately rates the condition of patients after operation. Estimated blood loss, for example, was a strong predictor of postoperative outcome in a cohort of 300 colectomy patients. Blood loss and duration of operation are strongly related to oxidative stress, although this has been predominantly analyzed in cardiac surgery patients. Skin AF, as a marker of protein damage by oxidative stress, was associated with these same markers of surgical stress in the present study. Interestingly, we observed a negative relationship between preoperative skin AF levels and the perioperative increase in skin AF. It seems that preoperative chronic low states of oxidative stress, as reflected by higher preoperative skin AF levels, may to some extent protect against surgical oxidative stress. Others have shown that previous episodes of oxidative stress (preconditioning) trigger protection against subsequent ischemia and oxidative stress injury.^{28–30} Finally, preoperative statin treatment was associated with lower increases in perioperative skin AF. Martinez-Comendador et al³¹ recently observed that preoperative statin treatment reduces systemic inflammatory responses and oxidative stress in cardiac surgery patients.

Increased preoperative skin AF levels were associated with the development of complications. Others have already described that preoperative levels in oxidation protein end products are strongly related with postoperative outcome in cardiac surgery patients.³² We also observed that perioperative increases in skin AF were associated with the development of postoperative complications. Intestinal ischemia-reperfusion has been shown to cause pulmonary and myocardial injury.^{10,33} Oxidative stress levels may be associated with anastomotic healing, and reducing oxidative stress was shown to decrease anastomotic dehiscence rate in animal studies.¹¹ An interaction between oxidative stress and inflammatory reactions plays an important role in these complications.²³ Indeed, we observed a relation between changes in skin AF and C-reactive protein levels.

Measuring skin AF in surgical patients could serve several purposes. First, it may identify patients at increased risk for surgery. We have previously shown that skin AF is a stronger predictor of survival than the presence of, for example, coronary heart disease.^{17,19} Second, it could also help surgical teams to identify patients coming out of sur-

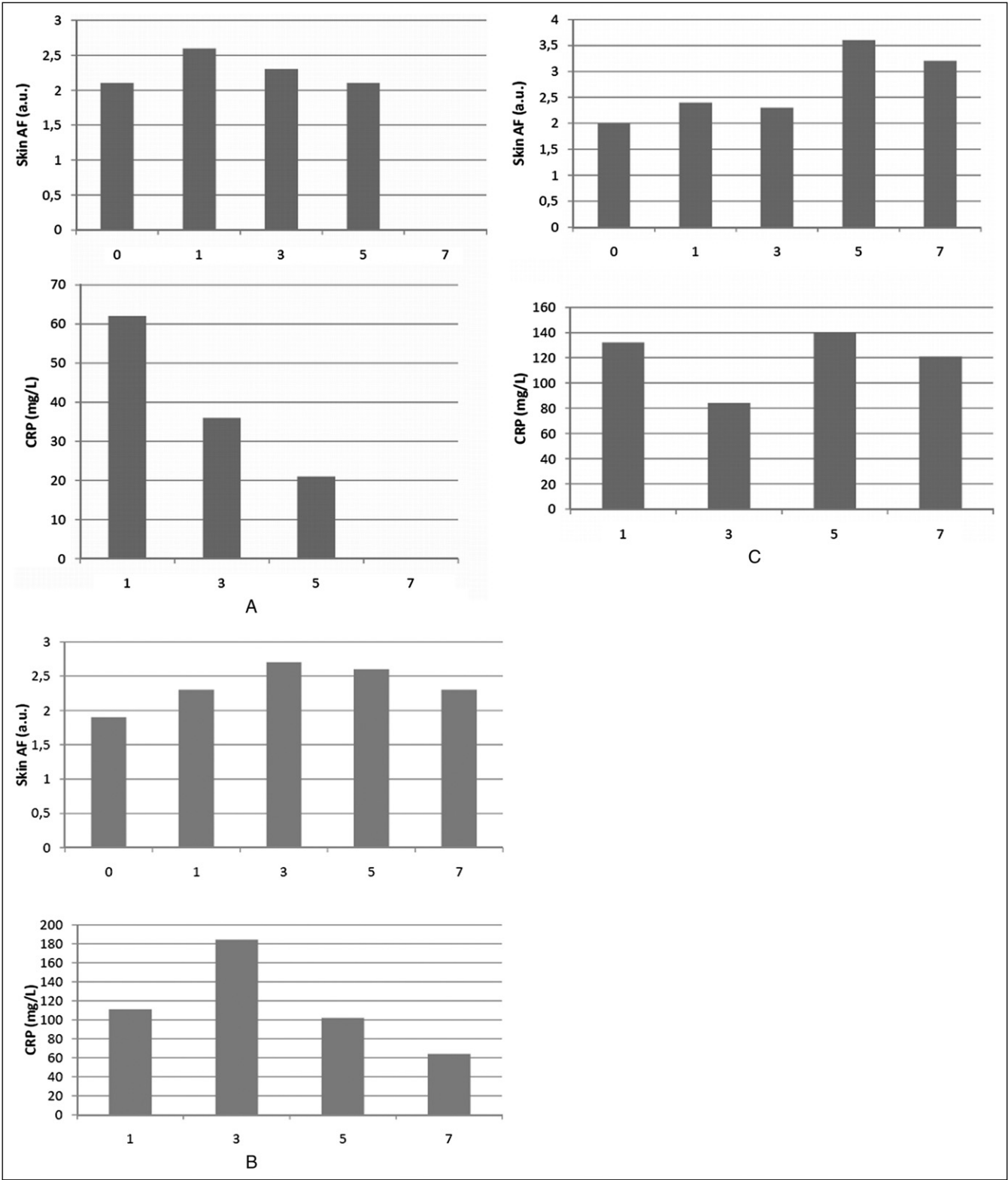


Figure 3 Changes in skin AF and C-reactive protein after colorectal operation in (A) a patient without complications, (B) a patient who developed pneumonia on day 3 after surgery, and (C) a patient who was found to have an intra-abdominal abscess on day 5 after operation.

gergy who are at increased risk for complications. Finally, skin AF may be used to monitor improvement programs, such as enhanced recovery programs.

There were several limitations to our study. The number of patients in this study was limited, and only elective surgery patients were included. To what extent skin AF

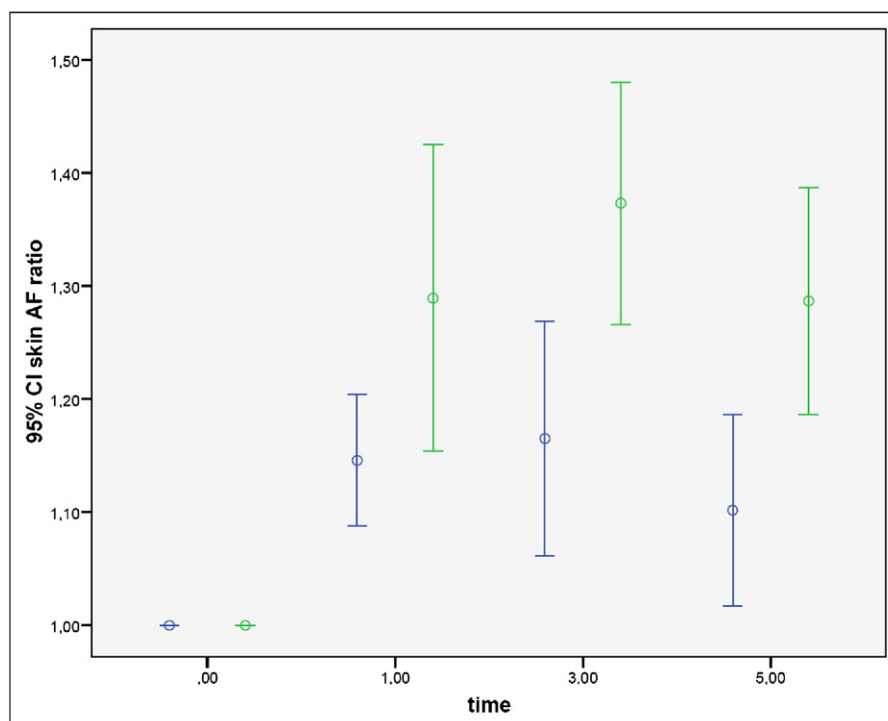


Figure 4 Error bar (95% CI) for ratio in skin AF on days 1, 3, and 5 after colorectal operation in the group of patients with (green-light) versus without (blue-dark) complications.

reflects surgical stress and postoperative outcome must be determined from larger studies. AF levels may also be influenced by metabolic burden (eg, hyperglycemia), although skin AF is a validated measure of oxidative stress in many diseases. In the present study, we did not measure specific oxidation end products or other markers for oxidative stress. Skin AF correlated strongly with serum markers of oxidative stress and advanced oxidation products in a recent study in patients with sepsis (unpublished data). To determine the independent prognostic value of skin AF, a larger study is warranted, including analyses of both metabolic and oxidative stress. Also, the effect of, for example, parenteral feeding on AF levels will be analyzed. Finally, laparoscopic techniques were not used in the present study. To what extent laparoscopy may reduce oxidative stress and influence AF values will be analyzed in a subsequent study. All patients were treated according an enhanced recovery program. Whether this influences AF values will also be analyzed in that study.

Conclusions

To the best of our knowledge, this is the first study to demonstrate an association between a noninvasive marker of oxidative stress and surgical stress and outcomes. This pilot study clearly shows that skin AF increases after colorectal surgery. Comparable studies with larger numbers of patients are in progress to analyze whether skin AF really

reflects patients' condition after surgery and the risk for postoperative complications.

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