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RESEARCH

Transversalis fascia collagen content and the risk of surgical complications: results of a prospective study

Marcin Morawski[1] · Maciej Krasnodębski[1] · Jakub Rochoń[1] · Hubert Kubiszewski[1] · Mikołaj Staszewski[1] · Mikołaj Kuncewicz[1] · Piotr Krawczyk[1] · Paweł Rykowski[1] · Adam Bołtuć[1] · Zbigniew Lewandowski[2] · Wojciech Figiel[1] · Marek Krawczyk[1] · Michał Grąt[1]

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Abstract

Background Collagen is the major protein of the extracellular matrix that provides mechanical strength to the tissues. The relationship between the development of complications and the quality and quantity of collagen fibres has not been investigated in the literature, yet.

Methods This was a prospective study of 392 patients who underwent subcostal laparotomy for confirmed or suspected gastrointestinal malignancy. Prior to abdominal closure a sample of transversalis fascia was collected. The area covered by collagen (ACC) was measured as the mean area covered by Picosirius stained fibres in three areas of the fascia. The primary endpoint of the study was the occurrence of complications, graded according to the Clavien-Dindo over a 90-day follow-up period.

Results 392 patients were included in the study. A transversalis fascia sample was obtained in 354 patients (90.3%) and image assessment yielded a group of 259 specimens that were included in the analysis (66.1%). Predicting the development of complications of at least CD III based on ACC was associated with an AUC of 0.606 ($p = 0.027$) and an optimal threshold of 0.771. There were significantly fewer complications of at least CD III in the group of patients with ACC ≥ 0.771 (6/125) than in the group below the threshold (25/134) ($p < 0.01$).

Conclusions Collagen content may serve as an adjunct predictor of surgical risk, although its clinical utility requires further validation. There is a need for further studies on the causal nature of this relationship and modifiable risk factors related to body collagen quality.

Keywords Surgical complications · Collagen · General surgery

Introduction

Collagen is the main protein of the extracellular matrix (ECM) that provides mechanical strength to the tissues and accounts for as much as 30% of total body protein [1]. It is particularly abundant in the fascial layers of the abdomen, forming a dense array of fibrils that support the viscera. It has been shown that the quantity, quality, and composition of the fascial collagen may play an important role in the development of abdominal wall hernias [2–5]. In addition, analysis of samples from tissues rich in the protein such as fascia or skin can reflect the quality and structure of collagen throughout the body [2, 5].

Data on postoperative mortality and morbidity are important measures of the quality of patient care in surger y [6]. Methods to identify individuals at risk can contribute to improvements in patient safety and changes in the standard practice. The Clavien-Dindo (CD) classification of surgical complications is a validated outcome measure that allows for assessment of the severity of postoperative failure [7, 8]. Complications classified as CD III or higher (e.g. anastomotic failure, bleeding, biliary leakage requiring endoscopy or revision surgery) are of particular importance given their intuitive association with tissue quality and strength. The

✉ Marcin Morawski
marcin.morawski@wum.edu.pl

[1] Department of General, Transplant, and Liver Surgery, Medical University of Warsaw, Banacha 1 A, 02 - 097 Warsaw, Poland

[2] Department of Epidemiology and Biostatistics, Medical University of Warsaw, Warsaw, Poland

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relationship between the development of complications and the quality and quantity of collagen fibres is not well established in the literature. The aim of this study was to investigate the relationship between major complications after surgery and overall collagen quality.

Materials and methods

This was a prospective study involving 392 patients who underwent subcostal laparotomy for confirmed or suspected gastrointestinal malignancy at the Department of General, Transplant, and Liver Surgery at the Medical University of Warsaw between September 2018 and March 2023 (clinicaltrials.gov: NCT03561727). The trial was primarily designed to compare two abdominal closure techniques (layered and mass closure) in terms of burst abdomen or incisional hernia as a primary combined endpoint. Details of sample size calculation are described elsewhere [9]. The primary endpoint of this study was the occurrence of complications graded according to the Clavien-Dindo classification over a 90-day follow-up period [10].

Prior to abdominal closure, a sample of 5 × 5 mm was taken from the transversialis fascia and fixed in 4% buffered formalin. Tissue fragments were then rinsed in water and processed automatically through 70%, 96% and absolute alcohol solutions, alcohol and xylene solutions, and xylene series. Dehydrated tissues were embedded in paraffin at 60℃, formed into tissue blocks and cut into 3—4 µm thick pieces using a microtome. After transfer to the slides, the specimens were stained with haematoxylin and eosin (H&E) and with Picosirius Red (PSR) stain (ab150681, abcam, Cambridge, UK) according to the manufacturer's instructions. Digital images of H&E and PSR-stained specimens were obtained using NanoZoomer C9600 - 12 device (Hamamatsu Photonics K. K., Hamamatsu, Japan). Both H&E and PSR specimens were first assessed in brightfield microscopy for adequacy of procurement and to identify areas of fascial collagen. Subsequently,.ndpi images of PSR-stained tissues were obtained under fluorescence microscopy using the Texas Red filter. The images were analyzed using QuPath software (version 0.5.1) [11] to determine the area covered by collagen fibres and their morphology.

The collagen fiber morphology and organization were classified according to the modified Movin and Bonar score proposed by Peeters et al. [2]. Grade 0 is defined as separated fibres lacking orientation and the formation of bundles. Grade 1 is characterized by a dense lattice of thick fibres, which occasionally form bundles. In grade 2, the collagen fibres are well-aligned and densely packed, although irregular waving may be observed. Grade 3 was defined as a dense array of tightly packed and homogenous collagen fibres.

To estimate the area covered by collagen (ACC), a thresholder was defined based on the colour histogram analysis (full resolution of 0.23 µm/px, red channel, threshold of 30.0, sigma = 1). After applying a 250 × 250 µm grid overlay to the images, three areas of transversialis fascia were identified (62,500 μm^2 each) and the mean proportion of collagen was calculated for each image (Fig. 1). Sample assessment was blinded to patient outcomes.

Receiver operating characteristic (ROC) analyses were performed to assess the predictive value of ACC with respect to the complications of at least CD III. Optimal cut-offs were selected based on the highest Youden index. Areas under the curve (AUC) was presented with 95% confidence intervals (95% CI). Logistic regression was used to estimate odds ratios (OR) with 95% CI. Potential confounding was examined in a series of two-factor analyses, including fascial collagen content and each of the associated covariates. Multivariate analyses were performed by stepwise regression using the `MASS` package in R (*stepAIC()* function). The Akaike Information Criterion (AIC) was used to

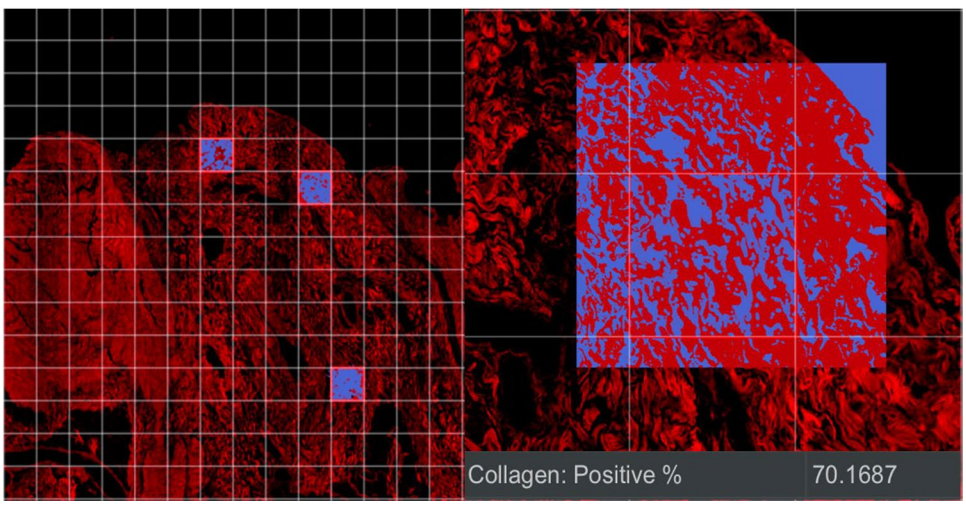

Fig. 1 Calculation of Area Covered by Collagen on Picosirius Red-stained fascia specimens

guide model selection. The final multivariable model was constructed by backward selection of the initial set of concomitant factors significant in bivariable analyses and other factors selected on the basis of between-group differences and clinical relevance. Potential confounding of the remaining known predictors of postoperative complications was assessed in a second step, based on forward selection of variables. The Fisher test, Mann–Whitney U test and Kruskall-Wallis test were used for subgroup comparisons. The level of significance was set at 0.05. Statistical analyses were performed using RStudio version 2024.4.1.748 software (RStudio, Boston, MA, USA).

Results

A total of 392 patients were included in the study. A transversialis fascia sample was obtained in 354 patients (90.3%) and image assessment yielded a group of 259 specimens that were included in the analysis (66.1%) (Supplementary Digital Content 1). Baseline characteristics of the study group are summarized in the Table 1. Patients excluded from the analysis did not differ from the study group in terms of baseline characteristics. Reasons for specimen loss included insufficient fascial area for quantitative analysis or damage to the sample during processing. An example of a specimen excluded from the analysis is shown in the Fig. 2. Thirty-one patients developed one or more complications graded by Clavien-Dindo classification of at least CD III (12.0%).

In the general population, predicting the development of complications of at least CD III based on ACC was associated with an AUC of 0.606 (95% CI: 0.516—0.696; $p = 0.027$), 80.6% sensitivity, 52.2% specificity, 18.7% positive predictive value, and 95.2% negative predictive value, and an optimal threshold of 0.771 (Fig. 3A). There were significantly fewer complications of at least CD3 in the group of patients with ACC ≥ 0.771 (6/125) than in the group below the threshold (25/134) ($p < 0.01$). Mean ACC differed significantly between the Collagen Score groups—Score 0: 0.56 ± 0.11, Score 1: 0.72 ± 0.9, Score 2: 0.88 ± 0.06, Score 3: 0.96 ± 0.03 ($p < 0.01$) (Fig. 4). Baseline characteristic of patients with ACC ≥ 0.771 and ACC < 0.771 are presented in Table 1. The two groups differed significantly with respect to serum albumin, C-reactive protein (CRP), coronary artery disease, and haemoglobin concentrations as well as Comprehensive Complication Index (CCI) [10]. One hundred and eighty-three patients underwent liver resection (70.7%). Prediction of complications of at least CD3 based on ACC was associated with an AUC of 0.528 (95% CI: 0.410—0.646; $p = 0.355$), 75.0% sensitivity, 52.1% specificity, 13.0% positive predictive value, and 95.6% negative predictive value, and an optimal threshold of 0.770 (Fig. 3B). Similarly to the general study population, there were significantly less complications of at least CD3 in the group of patients with ACC ≥ 0.770 (4/87) than in the group below the threshold (12/80) ($p = 0.038$). We then evaluated the predictive value of ACC in relation to development of biliary fistula in the liver resection subgroup. It was associated with AUC of 0.521 (95% CI: 0.284—0.758; $p = 0.420$), 50.0% sensitivity, 67.4% specificity, 6.6% positive predictive value, and 96.7% negative predictive value, and an optimal threshold of 0.682. There was no significant difference in occurrence of biliary complications between high (4/118) and low ACC (4/57) groups ($p = 0.307$).

The crude OR for ACC ≥ 0.771 for complication of at least CD III was 0.22 (95% CI: 0.09–0.56; $p = 0.001$). The effect of high collagen content on the primary endpoint was tested against the potential effects of covariates in bivariate analyses, all of which confirmed that ACC ≥ 0.771 was a significant protective factor (Table 2). Preoperative serum albumin ($p = 0.040$) and C-reactive protein ($p = 0.002$) concentrations were significantly associated with the occurrence of at least CD III complications, independent of the fascial collagen content. Patient's age, sex, body mass index, preoperative chemotherapy, diabetes, hypertension, smoking history, malignancy, serum albumin and C-reactive protein concentrations were initially included in the multivariate analysis. The final model, consisting of independent predictors, showed that high collagen content was a significant protective factor with respect to postoperative complications of CD III or higher (OR 0.15 [95% CI: 0.05–0.46]; $p < 0.001$), adding preoperative C-reactive protein concentration ($p = 0.003$), platelets ($p = 0.07$), and patient's age ($p = 0.11$) to the model. The remaining variables were tested for extension of the final model using forward selection and none entered the model. ACC, analyzed as a continuous variable, was a significant non-linear predictor of complications $\geq$ CD III (OR 0.18 [95% CI 0.03—0.87]; $p = 0.039$) (Fig. 5). In addition, ACC did not correlate with patient age. (R =—0.049; $p = 0.43$) (Fig. 6).

In *post-hoc* analysis, ACC was also a significant predictor of surgical complications of any grade in the general study population, comprising surgical site infections (SSI), blood transfusions, need for endoscopic or surgical revision due to bleeding, intestinal or biliary fistulas, or intraabdominal collections. Prediction was associated with an AUC of 0.630 (95% CI: 0.546—0.715; $p < 0.01$), 79.5% sensitivity, 54.0% specificity, 26.1% positive predictive value, and 92.8% negative predictive value, and an optimal threshold of 0.771 (Fig. 7). There were significantly fewer surgical complications of any grade in the group of patients with ACC ≥ 0.771 (9/116) than in the group below the threshold (35/99) ($p < 0.01$).

Table 1 Baseline characteristics of the general study group and patients excluded from analyses and comparison of two groups of patients with area covered by collagen of greater than or equal to 0.771 and less than 0.771

Variables	Study population ($n = 259$)	Excluded population ($n = 133$)	p	ACC ≥ 0.771 ($n = 125$)	ACC < 0.771 ($n = 134$)	p
Sex			0.89			0.49
male	129 (49.8%)	68 (51.1%)		59 (47.2%)	70 (52.2%)	
female	130 (50.2%)	65 (48.9%)		66 (52.8%)	64 (47.8%)	
Age (years)	60 (49—66)	62 (53–66)	0.21	59 (47—65)	61 (50—67)	0.13
Body mass index (kg/m^2)	26.3 (23.4—29.4)	25.6 (23.0–26.6)	0.63	26.0 (23.8—29.4)	26.6 (23.1—29.5)	0.95
Primary diagnosis			0.79			0.14
Malignant disease						
Colorectal liver metastases	91 (35.1%)	44 (33.1%)	0.74	44 (35.2%)	47 (35.1%)	> 0.99
Cholangiocarcinoma	43 (16.6%)	17 (12.8%)	0.38	20 (16.0%)	23 (17.2%)	0.87
Hepatocellular carcinoma	25 (9.7%)	10 (7.5%)	0.58	9 (7.2%)	16 (11.9%)	0.21
Gallbladder carcinoma	20 (7.7%)	14 (10.5%)	0.35	14 (11.2%)	6 (4.5%)	0.06
Pancreatic carcinoma	6 (2.3%)	4 (3%)	0.74	2 (1.6%)	4 (3.0%)	0.69
Neuroendocrine tumors	4 (1.5%)	5 (3.5%)	0.17	1 (0.8%)	3 (2.2%)	0.62
Other malignant	32 (12.4%)	19 (14.3%)	0.64	19 (15.2%)	13 (9.7%)	0.19
Benign disease						
Echinococcosis	5 (1.9%)	1 (0.8%)	0.67	4 (3.2%)	1 (0.7%)	0.20
Focal nodular hyperplasia	3 (1.2%)	2 (1.5%)	> 0.99	2 (1.6%)	1 (0.7%)	0.61
Other non-malignant	30 (11.6%)	17 (12.8%)	0.74	10 (8.0%)	20 (14.9%)	0.12
Comorbidities						
Diabetes	36 (13.9%)	23 (17.3%)	0.37	18 (14.4%)	18 (13.4%)	0.86
Hypertension	83 (32.0%)	54 (40.6%)	0.11	35 (28%)	48 (35.8%)	0.14
COPD	2 (0.8%)	4 (3.0%)	0.19	1 (0.8%)	1 (0.7%)	> 0.99
Thyroid disease	25 (9.7%)	10 (7.5%)	0.58	11 (8.8%)	14 (10.4%)	0.67
Coronary artery disease	17 (6.6%)	5 (3.8%)	0.35	4 (3.2%)	13 (9.7%)	0.042
Preoperative laboratory tests						
white blood cells (10^3/mm^3)	6.4 (5.4—8.2)	6.9 (5.5–8.1)	0.16	6.5 (5.4—8.1)	6.3 (5.2—8.2)	0.40
haemoglobin (g/dL)	13.3 (12.2—14.3)	13.5 (12.4–14.2)	0.54	13.5 (12.5—14.5)	13.1 (12.0—14.0)	0.022
platelets (10^3/mm^3)	230 (189—272)	239 (179–287)	0.78	238 (200—278)	224 (180—270)	0.09
creatinine (mg/dL)	0.8 (0.7—1.0)	0.8 (0.7–0.9)	0.49	0.8 (0.7—0.9)	0.8 (0.7—1.0)	0.05
bilirubin (mg/dL)	0.5 (0.4—0.8)	0.5 (0.4–0.7)	0.59	0.5 (0.3—0.7)	0.6 (0.4—0.9)	0.26
albumin (g/dL)	4.3 (4.0—4.6)	4.3 (4.0–4.6)	0.89	4.4 (4.0—4.7)	4.2 (3.9—4.5)	< 0.001
INR	1.0 (1.0—1.1)	1.0 (1.0–1.1)	0.23	1.0 (1.0—1.1)	1.0 (1.0—1.1)	0.77
CRP (mg/L)	3.1 (1.3—7.6)	3.7 (1.2–8.6)	0.86	2.6 (1.1—6.0)	3.5 (1.4—10.1)	0.049
CCI	0.0 (0.0—8.7)	0.0 (0.0–8.7)	> 0.99	0.0 (0.0—8.7)	0.0 (0.0—8.7)	0.011
Area covered by collagen	0.8 (0.6—0.9)	-		0.9 (0.8—0.9)	0.6 (0.6—0.7)	< 0.001

Discussion

Collagen structure and density are responsible for the strength and resilience of ECM. It seems intuitive that the mechanical properties of tissues throughout the viscera may be related to the development of some surgical complications. This notion is supported by the existing studies of hernia development in the context of collagen quality [3, 12–14]. There is a paucity of data on the importance of collagen with regard to the outcomes of the surgical treatment. In our study, we demonstrated the predictive value of the collagen content in the transversialis fascia in relation to surgical complications. It appears that low collagen density and structural disorganisation may play a role in the development of surgical complications. This has been shown by a higher risk of occurrence of complications of at least CD III, incorporating adverse events most likely related to mechanical tissue strength, in patients with lower collagen content. This effect persisted after controlling for study group heterogeneity in the subgroup analysis of patients who underwent liver resection—this group that was the most numerous in the cohort. The clinical significance of the ACC beyond 0.771

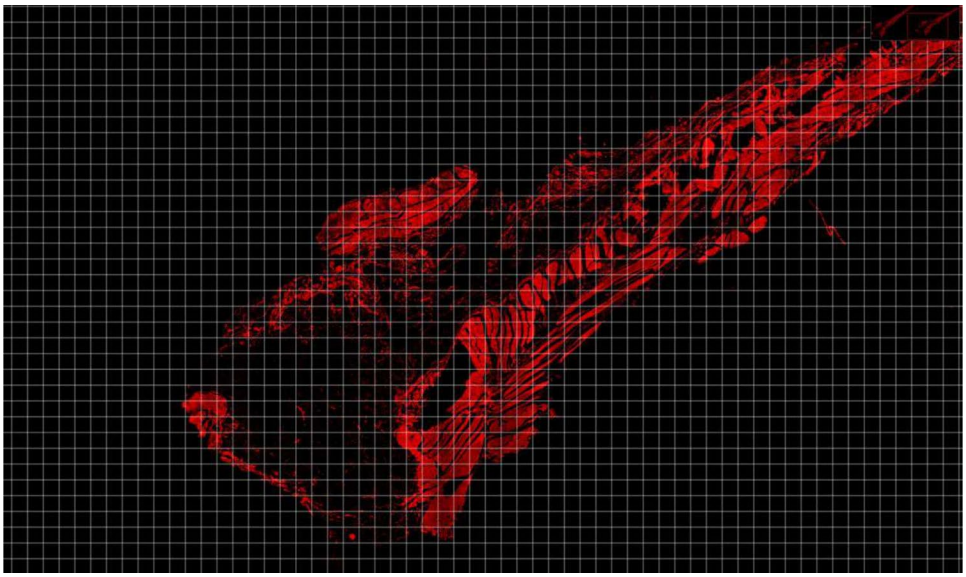

Fig. 2 An example of a specimen excluded from analysis due to insufficient fascial area. The sample contains predominantly skeletal muscle and adipose tissue with little fascial area

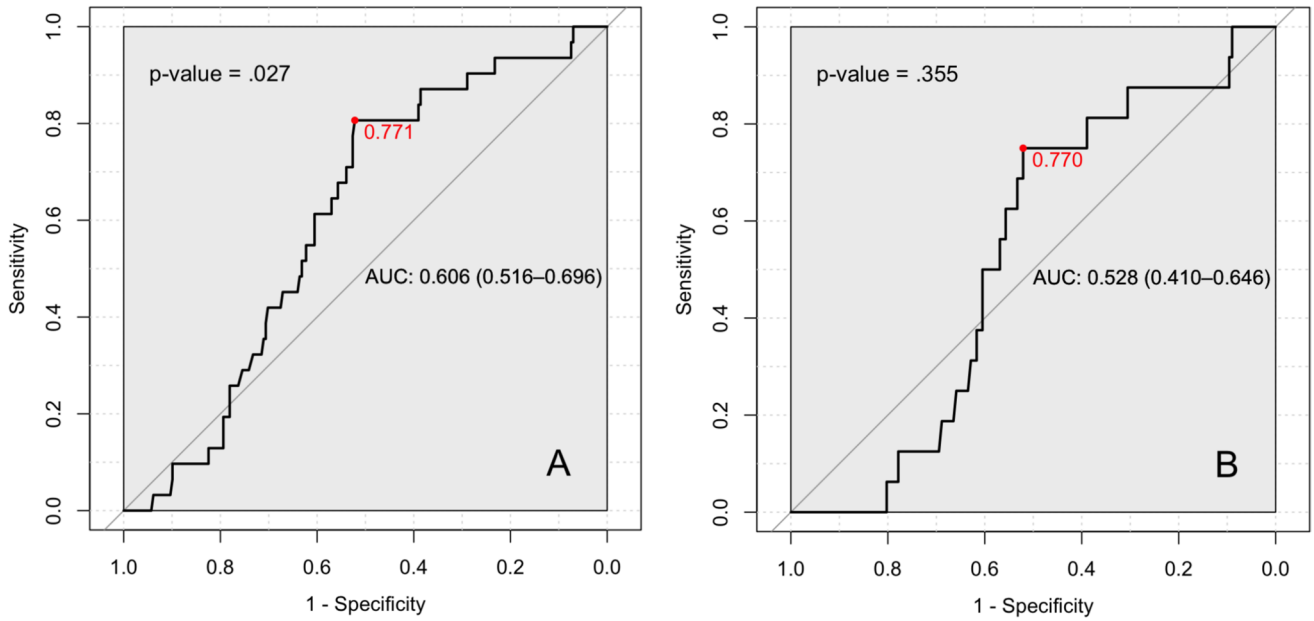

Fig. 3 Receiver operating characteristic curves for prediction of complications of more than Clavien-Dindo III in **A** the general study cohort and in **B** the subgroup of patients undergoing liver resection

in the general group and in the liver resection group seems limited as the AUC is 0.606 and 0.528, respectively. This cut-off point may be overfitting to this particular dataset, but it provides a reasonable cut-off point for the analyses performed and for further external validation. *Post-hoc* analysis showed that purely surgical complications were significantly more common in the patients with lower collagen content. However, this effect was not observed with regard to biliary fistulas in the patients who underwent liver resection. This may be due to both the aggressive chemical nature of the bile and the small number of events observed in the study. In addition, the results suggest that fascial collagen content may be a valuable marker of collagen quantity and quality throughout the body [15]. Nutritional status, expressed as serum albumin concentration, was also a significant predictor of postoperative complications irrespective of the collagen content that is consistent with the existing knowledge [16, 17].

As has been shown by Chen et al., the collagen score assessed in the submucosal region of the gastric wall was a significant predictor of the anastomotic leakage in patients undergoing surgery for oesophageal cancer [18]. There is

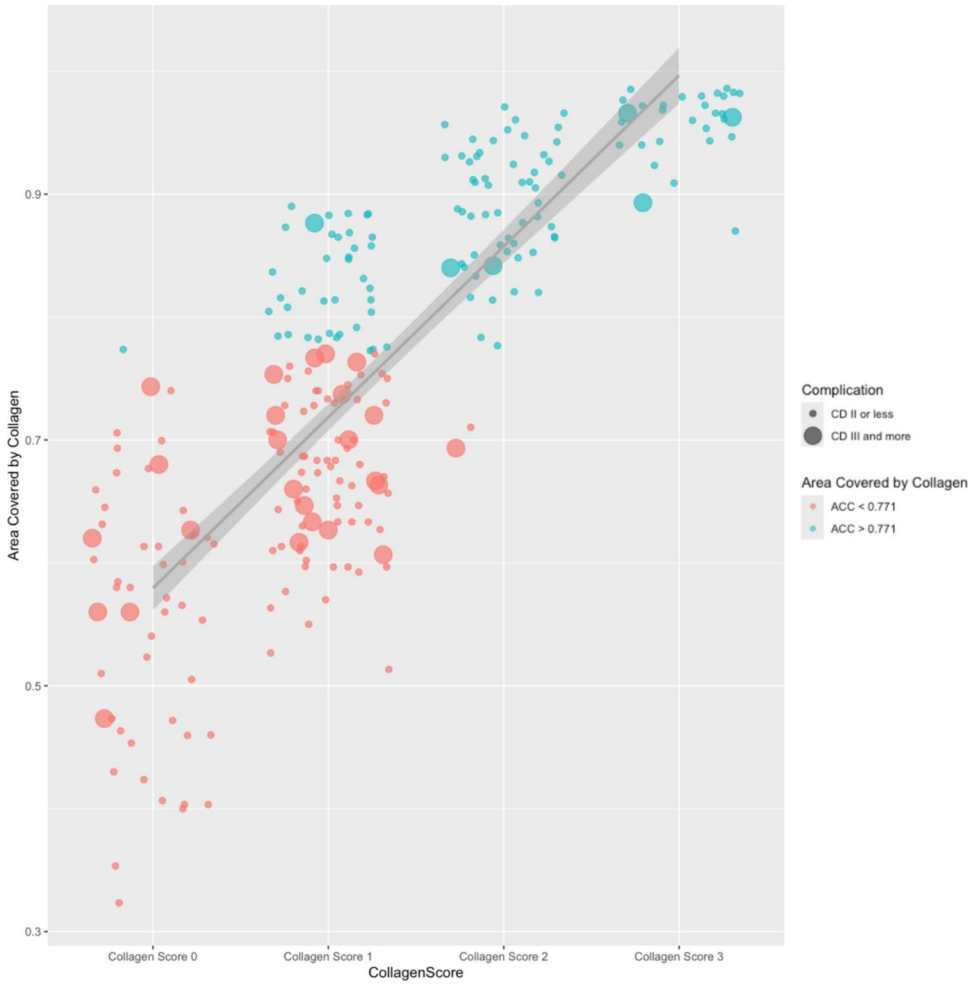

Fig. 4 The scatter plot displaying the distribution of area covered by collagen against the collagen scores; each point representing an individual observation, with its size indicating the complication severity defined as CD III or more (bigger dot) and CD II or less (smaller dot). Points are further colored by thresholds of area covered by collagen: red for ACC < 0.771 and blue for ACC > 0.771

also data to support the idea that anastomosis reinforcement with collagen patches may prevent leakage and failure [19, 20]. The effectiveness of vessel sealing with electrical devices also appeared to depend on the collagen and elastin content of the wall of the vessels [21–23]. All these results establish a link between the collagen quality and the development of surgical complications such as bleeding, anastomotic failure or fistulas.

In our group, collagen quantity did not correlate with patient age, suggesting that factors other than ageing may be involved in the variability of collagen quality between patients. It has been suggested that impaired collagen turnover may be responsible for hernia development [12, 24]. This may be due to genetic polymorphism of proteins involved in collagen remodelling such as metalloproteinases involved in collagen degradation [15, 25, 26]. As has been shown by Henriksen, impaired local and systemic collagen turnover may be responsible for hernia formation [27]. Numerous mechanisms may be responsible for the systemic degradation of collagen, weakening its structure and leading to reduced mechanical properties. Increased systemic activity of matrix metalloproteinase 2 (MMP- 2) has been shown in younger patients with inguinal hernias [28–30]. The activity of MMPs and tissue inhibitor of MMPs (TIMP- 1) can be measured in patient serum, that provides a less invasive marker of collagen quality and turnover and may be helpful in identifying patients at risk in relation to surgical morbidity and mortality. In addition, neo-epitopes for collagen formation (e.g. N-terminal pro-peptide of collagen I [P1 NP] or C-terminal pro-peptide of collagen III [Pro-C3]) and degradation (e.g. MMP- 2, MMP- 9, and MMP- 13 generated fragments of collagen I [C1M]) can be measured in patient serum and may be used as markers of impaired collagen turnover [31]. Identification of individuals at risk may also be based on a less invasive approach of collagen quantification based on skin biopsies, that have been shown to represent overall body collagen [2]. The influence of the collagen mechanical properties has also been widely studied in orthopaedic research. Collagen quality can be affected by systemic diseases (e.g. diabetes mellitus), smoking, and physical inactivity [32–34]. Nutritional interventions such as hydrolyzed collagen or vitamin C supplementation and exercise have been shown to be effective in increasing collagen synthesis [35–38]. This is in line with what is known

Table 2 Effect of high collagen content on the primary endpoint (postoperative complications classified as Clavien-Dindo III or more) adjusted for covariates of interest in a series of bivariate and multivariate analyses

ACC ≥ 0.771 versus ACC < 0.771		Bivariate analysis			Multivariate analysis	
OR (95% CI)	p	Covariate	OR (95% CI)	p	OR (95% CI)	p
0.23 (0.09–0.59)	0.002	Age	1.31 (0.93 – 1.85)	0.13	1.39 (0.93 – 2.06)	0.11
0.22 (0.09–0.56)	0.001	Male sex	1.18 (0.55 – 2.57)	0.66		
0.22 (0.09–0.56)	0.001	BMI	1.07 (0.73 – 1.56)	0.74		
0.21 (0.08–0.53)	0.001	NRS ≥ 3 points	0.61 (0.13–2.82)	0.53		
0.21 (0.08–0.54)	0.001	Malignancy	1.90 (0.54 – 6.73)	0.32		
0.21 (0.08–0.54)	0.001	Liver cirrhosis	1.58 (0.29–8.43)	0.59		
0.21 (0.08–0.52)	< 0.001	Preoperative chemotherapy	0.77 (0.34–1.75)	0.54		
0.21 (0.08–0.53)	0.001	Smoking	0.78 (0.35–1.76)	0.55		
0.22 (0.09–0.55)	0.001	Diabetes	1.67 (0.61–4.56)	0.31		
0.21 (0.08–0.55)	0.001	Hypertension	1.36 (0.62–2.99)	0.44		
0.25 (0.08–0.52)	< 0.001	COPD	- [a]	0.99		
0.21 (0.08–0.53)	< 0.001	Thyroid disease	1.81 (0.61–5.46)	0.28		
0.20 (0.08–0.51)	< 0.001	Coronary artery disease	0.65 (0.14–3.08)	0.59		
0.21 (0.08–0.54)	0.001	White Blood Cell count	1.21 (0.83 – 1.75)	0.32		
0.22 (0.09–0.56)	0.002	Haemoglobin	0.99 (0.68 – 1.46)	0.98		
0.23 (0.09–0.58)	0.002	Platelets	0.81 (0.53 – 1.24)	0.34	0.65 (0.41 – 1.04)	0.07
0.25 (0.10–0.63)	0.004	Albumin	0.70 (0.50 – 0.98)	0.040		
0.22 (0.09–0.56)	0.001	Bilirubin	1.28 (0.97 – 1.70)	0.09		
0.23 (0.09–0.59)	0.002	Creatinine	1.34 (0.97 – 1.85)	0.08		
0.15 (0.05–0.44)	< 0.001	C-reactive protein	1.62 (1.20 – 2.19)	0.002	1.60 (1.17 – 2.19)	0.003

a – the estimate of coefficients were not presented due to the overestimation resulting from the small number of events

To identify the most parsimonious model for predicting incisional hernia or burst abdomen, we employed stepwise regression using the Akaike Information Criterion (AIC) as the selection criterion. Backward elimination was used for elimination of the following arbirtary set of variables: age, sex, body mass index, preoperative chemotherapy, diabetes, hypertension, smoking history, malignancy, serum albumin and C-reactive protein concentrations. All the remaining factors listed in Table 2 were tested for inclusion in the model using forward selection and none entered the model

BMI Body Mass Index, *NRS* Nutritional Risk Score, *COPD* Chronic obstructive pulmonary disease

OR Odds ratio, *95% CI* 95% confidence interval. Odds ratios were calculated per 10 years increase for patient age; and 1 standard deviation increase for BMI (4.02 kg/m^2); white blood cell count (2.27 × 10^3 cells/mm^3); hemoglobin (1.71 g/dL); platelets (87.51 × 10^3 cells/mm^3); albumins (0.61 g/dL); bilirubin (1.70 mg/dL); creatinine (0.26 mg/dL); C-reactive protein (27.22 mg/L)

Analyses included patients with complete data

about the effects of the Enhanced Recovery After Surgery (ERAS) protocol and prehabilitation [39].

It can also be hypothesised that gut microflora may influence the collagen quality and further increase the risk of developing surgical complications. Some strains of bacteria are able to produce collagenases, which can affect the risk of anastomotic leakage [40, 41]. Gut dysbiosis may be addressed by preoperative probiotics administration, especially in patients at risk [42]. Preoperative probiotics were shown to be effective in prevention of surgical site infection and anastomotic leaks [42–44]. Changes in the gut microbiome may also alter bone metabolism, as recently demonstrated in patients undergoing sleeve gastrectomy [45]. Patients undergoing bariatric surgery were characterized by increased P1 NP and Pro-C3 concentrations, that was associated with a greater change in gut microbial composition.

To address the limitations of this study, although the determination of random areas for collagen measurement can be considered subjective, the assessment was blinded to the occurrence of complications. Sirius staining may over-stain non-collagenous tissue, resulting in a higher estimate of collagen content. We attempted to avoid this systematic bias by first examining the tissue under bright field microscopy to confirm that the assessed areas stained by Sirius Red corresponded to the fascial areas. Although the visualisation of collagen fibres stained with PSR is dependent on their orientation, we have used a method of fluorescent imaging that is both sensitive and specific for collagen fibers and is unaffected by sample orientation

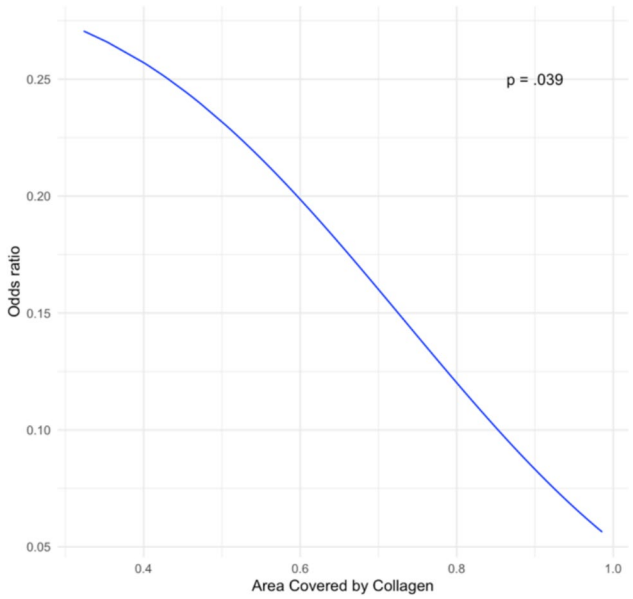

Fig. 5 Odds ratio of developing complication graded as CD III or more as a function of Area Covered by Collagen in the general study population

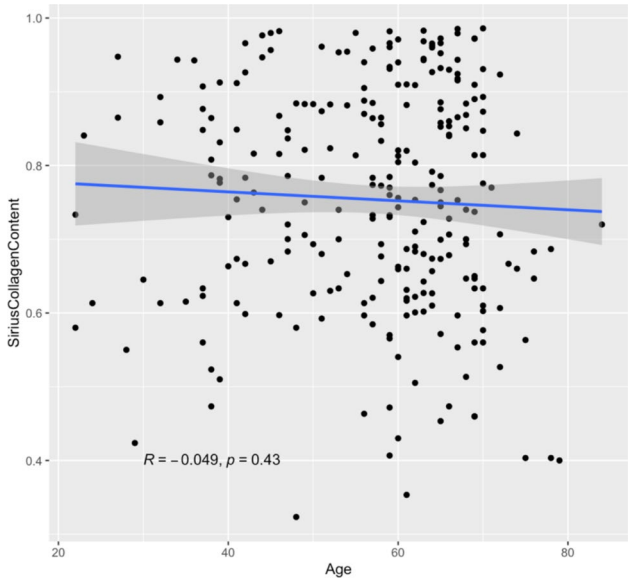

Fig. 6 Scatter plot illustrating the relationship between age and Sirius collagen content. Each dot represents an individual observation. The fitted blue line represents the linear regression trend, with the shaded region depicting the 95% confidence interval

[46–48]. Thus, this method reduces concerns regarding sample processing and evaluation. Although the Bonar score was originally designed to assess the patellar tendon, it has also been used for other tendons and fascia [2, 49]. In addition, the heterogeneity of the general population

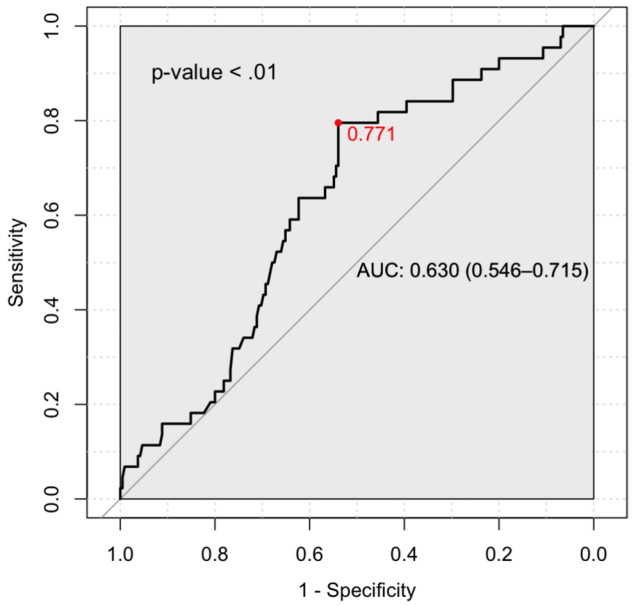

Fig. 7 Receiver operating characteristic curves for prediction of surgical complications of any grade in the general study cohort

with respect to the extent of surgery leads to different individual risks of developing complications. However, a subgroup analysis of patients who underwent liver resection showed a similar result to the general group. The study is also susceptible to bias as some patients were not included in the analyses due to a lack of fascial samples or insufficient facial area for measurements to be taken. However, we showed that the two groups did not differ in terms of baseline characteristics. The study also lacks external validity, that constitutes a drawback for the results may be overfitted to these data. Further studies are warranted to clarify on the relationship between body collagen content and surgical complications, including external multicenter validation. Despite limitations of the study, we have shown that high fascial collagen content was a significant protective factor independent of the known risk factors for postoperative morbidity and mortality in a series of bivariate and multivariate analyses.

Conclusion

In conclusion, collagen content may serve as an adjunct predictor of surgical risk, although its clinical utility requires further validation. There is a need for further studies on the causal nature of this relationship and specific modifiable risk factors related to body collagen quality with particular emphasis on interventional trials in high-risk groups.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval The study was in accordance with the ethical standards of the Helsinki Declaration of 1975 and was approved by the institutional review board (KB/2/2018).

Consent All patients provided informed consent before inclusion.

Competing interests The authors declare no competing interests.

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