Monocytic HLA-DR Expression for Prediction of Anastomotic Leak after Colorectal Surgery

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BACKGROUND: Earlier detection of anastomotic leakage (AL) after colorectal procedures could minimize the detrimental clinical impact of AL and thereby reduce morbidity and mortality.

STUDY DESIGN: We conducted a prospective study with assessment of the diagnostic accuracy of monocytic HLA-DR (mHLA-DR) expression compared with WBCs, C-reactive protein (CRP), and procalcitonin (PCT) in predicting AL in patients undergoing elective colorectal operation with anastomosis.

RESULTS: Comparison of the blood marker values on postoperative day (POD) 4 revealed significant differences for all markers, but the difference for mHLA-DR was highly significant (15% expression of monocytes in AL patients vs 34% in patients without AL; $p = 0.001$). Together with WBC ($p = 0.026$), mHLA-DR expression was the only test to show significance on day 3 (14% vs 31%; $p < 0.001$). Receiver operating characteristic analysis revealed that mHLA-DR expression had superior diagnostic accuracy compared with all other diagnostic markers both on POD 3 (mHLA-DR area under the curve [AUC] 0.928; WBC AUC 0.734; CRP AUC 0.707; PCT AUC 0.672) and POD 4 (mHLA-DR AUC 0.887; WBC AUC 0.738; CRP AUC 0.709; PCT AUC 0.696). Monocytic HLA-DR had a negative predictive value of at least 94% on PODs 3 and 4, as well as specificity and positive predictive values of 100% at a threshold of 23% on POD 3 and 24% on POD 4, respectively.

CONCLUSIONS: Expression of mHLA-DR appears to be a more accurate predictor for AL after colorectal operation compared with WBC, CRP, and PCT. It represents a promising test to precisely monitor the perioperative course of high-risk patients and contribute to safer discharge. (J Am Coll Surg 2019;229:200–209. © 2019 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

INTRODUCTION

Postoperative intra-abdominal septic complications after elective colorectal procedures are most often caused by anastomotic leakage (AL) and represent the most severe surgical postoperative morbidity in these patients.[1] Such complications are the main cause of prolonged hospitalization, postoperative mortality, adverse oncologic outcomes, and elevated costs.[1-6] Anastomotic leakage is often diagnosed late, often not until after discharge of the patient.[7] Early diagnosis is difficult because clinical symptoms of AL are inaccurate predictors due to varying clinical presentations.[8,9] In addition, when facilitating early hospital discharge, the concept of fast-track colorectal procedures and enhanced recovery protocols may increase the risk of missing AL and delay treatment. Precise biomarkers for the detection of this complication at an early stage can provide the best chance to minimize its severe sequelae.[3,10] A multitude of blood biomarkers for preclinical detection have been assessed so far.[1] Among them, biomarkers of ischemia as well as inflammatory and

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Abbreviations and Acronyms

AL = anastomotic leakage
AUC = area under the curve
CCI = Charlson Comorbidity Index
CRP = C-reactive protein
FACS = fluorescence-activated cell sorting
IL = interleukin
LAR = low anterior resection
mHLA-DR = monocytic HLA-DR
NPV = negative predictive value
PCT = procalcitonin
POD = postoperative day
ROC = receiver operating characteristic

microbiologic markers have been evaluated.[1] In a recent systematic review by Su'a and colleagues,[1] 36 studies evaluating 51 markers, both systemic and peritoneal, were assessed. Among the systemic markers, the most commonly assessed were C-reactive protein (CRP), procalcitonin (PCT), and WBCs.[11] However, these markers were found to perform poorly overall. In the large IMACORS study, Facy and colleagues[8] found that CRP level was more accurate than PCT, and that CRP level was a good marker on postoperative day (POD) 4. The authors recommended systematic measurement of CRP level on POD 4 to ensure safe discharge of the patient after colorectal operation. There is still an ongoing need to identify more precise and earlier predictors due to the limitations of the markers used currently. In this respect, monocytic HLA-DR (mHLA-DR) expression has not been assessed for suitability as a biomarker for the detection of AL after colorectal operation. However, decreased expression has been shown to correlate with the development of sepsis.[12-14] Therefore, measuring mHLA-DR might represent a promising diagnostic approach when extended to the early diagnosis of AL.

In the present prospective pilot study, the diagnostic accuracy of mHLA-DR expression in predicting AL after elective colorectal operation was determined in comparison with the levels of standard markers, such as WBC, CRP, and PCT.

METHODS

Study design

The current study was designed as a prospective observational pilot study. Written informed consent was obtained from all patients before enrollment. The study was approved by the Ethics Committee, Faculty of Medicine, Ludwig-Maximilians-University, Munich, Germany. Design, data acquisition, statistical methods, and manuscript preparation were carried out according to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[15]

Inclusion and exclusion criteria

Patients were considered eligible for the study if they met the following criteria: aged 18 years or older and scheduled for elective colorectal procedure with either 1 colon or 1 rectum anastomosis performed. Exclusion criteria were pregnancy, ongoing infection at the time of operation, or immune-compromised state.

Clinical data assessment

For prospective, standardized data assessment, case report forms were used. Patient-specific, perioperative, and histopathologic parameters were systematically assessed for each participant.

Perioperative clinical follow-up and blood marker measurement

Pre- and postoperatively, all patients were clinically examined by the attending surgeon of the colorectal surgery unit of the department. There was daily documentation of abdominal symptoms, signs of postoperative ileus or gastroparesia, quality and quantity of drain fluid, fever (defined as a core body temperature >38.0°C), diuresis, and other clinical signs of sepsis, such as tachycardia, increased respiratory rate, or agitation/lethargy. The attending surgeon made the decision to perform endoscopy or carry out imaging (contrast-enhanced CT) to detect AL according to her or his criteria. The diagnosis of AL was made by contrast-enhanced CT, enteric content in the drain, endoscopy, or operation (intraoperative diagnosis). All ALs were considered irrespective of their clinical implication and significance,[8] however, they were stratified into major and minor leaks, as reported previously.[16] On day 1 before operation and within the postoperative course until POD 8, peripheral venous blood was taken every day in the morning (between 8:30 AM and 09:30 AM) at constant time intervals. Blood levels of WBC, CRP, PCT, and mHLA-DR were measured. Blood WBC, CRP, and PCT were measured through certified routine diagnostic procedures in the Department of Laboratory Medicine, University Hospital of Munich, using standardized commercial kits according to manufacturer's recommendations. Fluorescence-activated cell sorting (FACS) was used to gate monocytes based on their CD14 expression and to measure mHLA-DR expression. Antibodies were used according to manufacturer's recommendation. First, 25.0 μL ethylenediaminetetraacetic acid blood was incubated with immunoglobulin G to minimize unspecific antibody binding. For surface staining, fluorochrome-conjugated

anti-human monoclonal antibody was incubated with blood for 30 minutes at room temperature. Next, an erythrocyte lysis step was done. For characterization of monocytes, anti-CD14 antibodies (anti-CD14-ECD, clone RMO52; Beckman Coulter) and anti-HLA-DR antibody (anti-HLA-DR-PC5, clone Immu357; Beckman Coulter) were applied. Cells were washed with phosphate-buffered saline and samples were analyzed in a FACS flow cytometer COULTER EPICS XL (Beckman Coulter). The monocyte population was selected using side and forward scatter, CD14, and CD45 gating. The obtained FACS data were displayed and analyzed using WinMDI 2.9e software. Quantitative results were then given as the percentage of mHLA-DR-positive monocytes.

End point

The primary end point of the current study was the diagnostic accuracy of mHLA-DR expression on PODs 3 and 4 for predicting AL compared with WBC concentration, CRP level, and PCT level.

Statistical analysis

Descriptive results were given as numbers and percentages, means with SD, or medians and (interquartile) ranges, where appropriate. For comparison of frequencies, chi-square test or Fisher's exact test (low frequency) were used, depending on the variable. For comparison of continuous variables, the Mann-Whitney U test for non-parametric analysis was applied. To avoid multiple testing, statistical comparisons of blood markers were only performed to compare baseline levels and at time points that had been hypothesized as primary end points (PODs 3 and 4). The diagnostic accuracy of tests was quantified using the area (AUC) under the receiver operating characteristic (ROC) curve. The Youden index was used to estimate marker-specific cutoff values. The optimal cutoff value was determined by calculating the maximum value of the Youden index, which is equivalent to the maximum difference between sensitivity and (1 - specificity). A diagnostic test was considered excellent with an AUC of 0.90 to 0.99, good with an AUC of 0.80 to 0.89, fair with an AUC of 0.70 to 0.79, and poor with an AUC <0.70.[17] To assess the independent predictive value of mHLA-DR and the standard marker CRP, multivariate modeling was performed. Therefore, a logistic model with AL as the target variable and baseline variables that were significantly associated with AL as influential variables was estimated. The fitted values of this model were subsequently used as predictor for the calculation of the ROC/AUC. To evaluate the additional predictive accuracy of mHLA-DR expression and CRP over these baseline covariates, a second model was estimated adding mHLA-DR and CRP to the logistic regression model mentioned. Calculation of AUC/ROC was performed as described. Finally, DeLong's test for 2 correlated ROC curves was used to compare the 2 resulting ROC/AUC. As a prospect for a future validation study entailing a superiority design, sample size was calculated using the observed AUC values of mHLA-DR expression and CRP on POD 2, 3, and 4. The α was set at 0.05 at a power of 0.80 ($1 - \beta$). The calculated sample size is the minimum required sample size for the observed difference in AUCs to be significantly different from 0 (meeting the required significance and power). For statistical analyses and creation of the figures, SPSS, version 23.0 (IBM Corp) and *R* packages (*R*-studio, haven, ggplot2,[18] ggparallel,[19] RColorBrewer, plyr,[20] dplyr, MASS,[21] and pROC[22]) were used.

RESULTS

Population characteristics and clinical course

Overall, 69 consecutive patients undergoing colorectal procedures with anastomosis were considered eligible and were included in the study. Data from 8 patients had to be excluded from the analysis due to missing blood measurements. Data from 61 patients were retained for the final analysis.

The clinical characteristics of the entire study population are displayed in Table 1. There were no re-admissions and no in-hospital mortalities. Most of the surgical indications were cancer (67%); sigmoid or rectal resections with rectal anastomosis (73%) were the most common surgical procedures in this population. Half of the patients showed an American Society of Anesthesiologists classification of >2, with a mean Charlson Comorbidity Index (CCI) of 3.9. The total complication rate was 29%. Thirteen (21%) patients had intra-abdominal septic complications, 9 (14%) patients were finally diagnosed with AL and the diagnoses were made 5.2 ± 2.1 days after the operation. Median POD when AL was diagnosed was POD 6 (interquartile range 3 to 7 days). Eight of 9 (89%) patients with AL had colorectal cancer and 7 of those cancer patients (88%) showed advanced tumor stages and relevant comorbidities. Five of the 9 ALs (8% of the entire cohort) were classified as major AL, and underwent percutaneous drainage or re-operation. The other 4 patients were treated conservatively. The AL rate was not representative for the calendar year in which the study was conducted. The corresponding annual overall AL rate was 8%.

When patients with and without AL were compared, the CCI ($p = 0.007$) and the type of resection (low anterior resection [LAR]; $p = 0.022$) showed significant

Table 1. Patient Characteristics and Clinical Course

Parameter	All patients	No AL	AL	p Value
Total, n (%)	61 (100)	52 (85)	9 (14)	NA
Age, y, median (range)	63 (23–84)	63 (23–84)	63 (30–78)	0.887
Sex, n (%)				0.729
Male	37 (61)	32 (62)	5 (56)	
Female	24 (39)	20 (38)	4 (44)	
BMI, kg/m^2, median (range)	26 (16–41)	25 (16–41)	23 (18–36)	0.470
Charlson Comorbidity Index, mean ± SD	3.9 ± 3.2	3.4 ± 3.1	6.8 ± 3.2	0.007
American Society of Anesthesiologists class >2, n (%)	30 (49)	23 (44)	7 (78)	0.081
Type of surgical resection, n (%)				
Low anterior rectal resection	24 (39)	17 (33)	7 (78)	0.022
Anterior rectal resection/sigmoid resection	21 (34)	20 (38)	1 (11)	0.146
Right hemicolectomy	8 (13)	7 (13)	1 (11)	1.000
Proctocolectomy with ileoanal pouch anastomosis	3 (5)	3 (6)	0	1.000
Other	5 (8)	5 (10)	0	1.000
Surgical indication/underlying disease, n (%)				
Cancer	41 (67)	33 (63)	8 (89)	0.249
Diverticular disease	16 (26)	15 (29)	1 (11)	0.423
Chronic inflammatory bowel disease	4 (7)	4 (8)	0	1.000
Length of operation, min, mean ± SD	215 ± 86	210 ± 82	240 ± 104	—
Preoperative chemoradiotherapy, n (%)	12 (20)	9 (17)	3 (33)	0.361
Clinical symptom, POD 3				
Fever, n (%)	8 (13)	6 (12)	2 (22)	0.336
Tachycardia, n (%)	5 (8)	3 (6)	2 (22)	0.154
Arterial hypotension, n (%)	5 (8)	5 (10)	0	1.000
Glasgow Coma Scale score, mean ± SD	14.9 ± 0.4	14.9 ± 0.4	14.9 ± 0.3	0.385
Clinical symptom, POD 4				
Fever, n (%)	11 (18)	8 (15)	3 (33)	0.343
Tachycardia, n (%)	5 (8)	3 (6)	2 (22)	0.154
Arterial hypotension, n (%)	5 (8)	5 (10)	0	1.000
Glasgow Coma Scale score, mean ± SD	14.9 ± 0.4	14.9 ± 0.3	14.7 ± 0.7	0.156
Total complication, n (%)	18 (29)	9 (17)	9 (100)	<0.001
Intra-abdominal septic complication, n (%)	13 (21)	4 (8)	9 (100)	<0.001
Total AL, n (%)	9 (14)	N/A	9 (100)	NA
Major AL, n (%)	5 (8)	N/A	5 (56)	NA
Time of diagnosis AL, POD, mean ± SD	5.2 ± 2.1	N/A	5.2 ± 2.1	NA

AL, anastomotic leak; NA, not applicable; POD, postoperative day.

differences (Table 1). There were no differences in other baseline variables. Of note, the prevalence of clinical signs of systemic inflammation or sepsis was comparable between patients with and without AL on POD 3 and 4, respectively (Table 1).

Postoperative blood marker levels

Figure 1 shows the postoperative dynamics of WBC concentration (Fig. 1A), CRP level (Fig. 1B), PCT level (Fig. 1C), and mHLA-DR expression (Fig. 1D). In Figure 2, the boxplots of these markers are displayed for PODs 3 and 4 and the median values with interquartile ranges are given in Table 2 for preoperative values and those of PODs 1 to 7. Preoperatively, none of the blood markers showed significant differences between patients with and without AL. The inflammatory markers, WBC, CRP, and PCT, were found to be increased in all patients' blood within the first postoperative days (Figs. 1A to 1C), and mHLA-DR decreased (Fig. 1D) as a result of the surgical trauma. The WBC count peaked on POD 1 (Fig. 1A), and levels of CRP and PCT had their peak on POD 2 (Figs. 1B, 1C). After peak values had been reached, WBC concentrations, especially those of CRP, persisted at higher levels in patients with AL

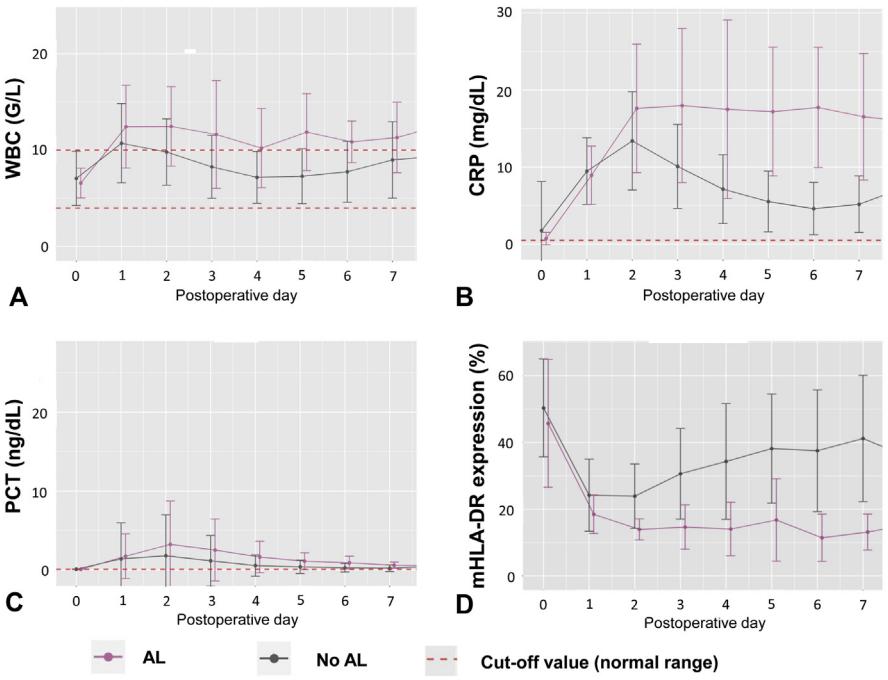

Figure 1. Postoperative dynamics of concentrations of (A) WBC; (B) C-reactive protein (CRP); (C) procalcitonin (PCT); and (D) the proportion of HLA-DR-expressing monocytes (mHLA-DR). AL, anastomotic leak.

compared with those without AL (Fig. 1B). For PCT, this was insignificant. Inversely, mHLA-DR dropped postoperatively and values began to increase on POD 2 in patients without AL (Fig. 1D). Comparison of the blood marker values on POD 4 revealed significant differences for all markers, and the difference for mHLA-DR were highly significant (15% expression of monocytes in AL patients vs 34% in patients without AL; $p = 0.001$) (Table 2). Together with WBC ($p = 0.026$), mHLA-DR expression was the only test to show significance on day 3 (14% vs 31%; $p < 0.001$).

Diagnostic accuracy of monocytic HLA-DR expression

Results of the ROC analysis are shown in Table 3 and Figure 3. In 2 patients, AL was diagnosed on POD 3 and they had to be excluded from the ROC analyses. These 2 patients both had minor AL with conservative treatment. Data from all patients with major AL were used for the ROC calculations. The HLA-DR expression proved to be an excellent diagnostic test on POD 3 and a good test on POD 4, with the highest AUCs of 0.928 and 0.887 measured compared with the other markers. WBC concentrations and CRP levels could be considered fair diagnostic tests based on results on PODs 3 and 4, and measurements of PCT were observed to be of poor diagnostic quality with the lowest AUC of all markers. In addition, with a good negative predictive value (NPV) of ≥94% and sensitivity of ≥73%, mHLA-DR testing showed high specificity rates and positive predictive value of 100% at a cutoff of 23% on POD 3 and 24% on POD 4, respectively. A better sensitivity was only obtained from CRP testing on POD 3 with 82% at a threshold of 16.6 mg/dL (Table 3).

Multivariate analysis of the diagnostic accuracy of mHLA-DR expression and CRP values was performed entering CCI and the type of resection (LAR) into the final models for PODs 3 and 4, respectively. Due to the high rate of AL patients in the LAR group, the combined AUC for CCI and LAR was high (POD 3: 0.90; POD 4: 0.92). The combined AUC of baseline variables and mHLA-DR expression was 0.98 ($p = 0.057$) and 0.97 ($p = 0.205$) for POD 3 and POD 4, respectively. Regarding CRP, combined AUCs were lower with 0.94 ($p = 0.241$) and 0.93 ($p = 0.573$) for PODs 3 and 4, respectively. When only CCI was considered as baseline variable (AUC POD 3: 0.78; AUC POD 4: 0.84), the independent increase in AUC by mHLA-DR expression and CRP was higher. For POD 3, combined AUCs were 0.97 ($p = 0.023$) and 0.84 ($p = 0.371$) for mHLA-DR expression and CRP, respectively. On POD 4 combined AUCs were 0.95 ($p = 0.129$) and 0.86 ($p = 0.640$) for mHLA-DR expression and CRP, respectively.

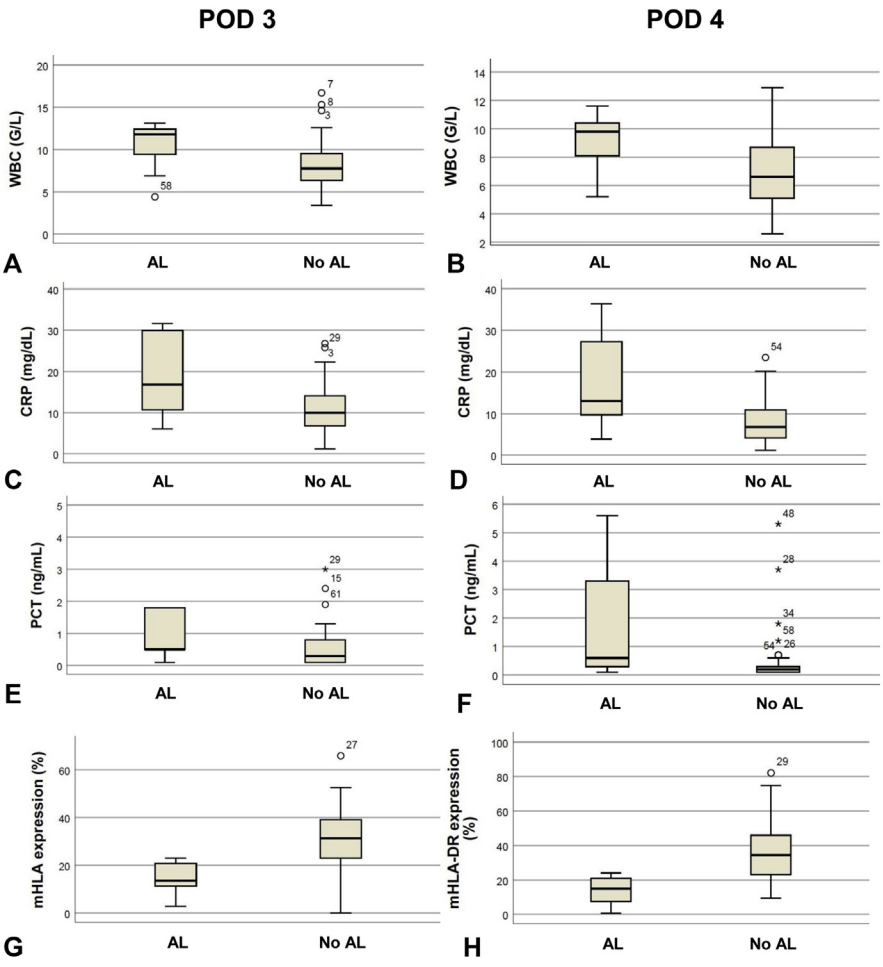

Figure 2. Boxplots of the concentrations of (A, B) WBC; (C, D) C-reactive protein (CRP); (E, F) procalcitonin (PCT); and (G, H) monocytic HLA-DR expression (mHLA-DR) for the postoperative days (POD) 3 (A, C, E, and G) and 4 (B, D, F, and H), respectively. AL, anastomotic leak.

DISCUSSION

The aim of our study was to determine the diagnostic accuracy of mHLA-DR expression in predicting the incidence of AL after colorectal operation. Compared with levels of WBC, CRP, and PCT, the mHLA-DR expression yielded the best AUC values with excellent and good test results on PODs 3 and 4, respectively. Compared with the standard tests, mHLA-DR expression in patients with or without AL was observed to show highly significant differences on PODs 3 and 4. On multivariate analysis, mHLA-DR expression was able to increase the combined AUC more compared with CRP, with statistical significance at POD 3 when adjusted for comorbidities.

Anastomotic leakage after colorectal operation is a dreaded complication and it results in unfavorable perioperative and oncologic outcomes.[1,2,4-6] Several studies have investigated the use of blood markers as early predictors of intra-abdominal infections or AL to decrease morbidity, mortality, and costs. The aim of those investigations is to diagnose and treat complications early or to discharge the patient safely within the first 4 to 5 days postoperatively, according to the concept of fast-track or enhanced recovery procedures.[8] It was observed, however, that >40% of the incidences of AL were diagnosed after discharge.[7] In principle, this highlights the need for an excellent test with high positive predictive value and NPV. The most common systemic inflammation markers that are increased after colorectal operations are WBCs/neutrophils, CRP, PCT, and interleukin (IL)-6.[1] More rarely, IL-1, IL-1β, IL-2, IL-5, IL-8, IL-10, tumor necrosis factor−α, soluble tumor necrosis factor receptor, vascular endothelial growth factor, hemoglobin, prothrombin fragments 1 and 2, thrombin anti-thrombin

Table 2. Levels of Inflammatory Blood Markers on Postoperative Days 3 and 4 in Patients with and without Anastomotic Leak

Blood marker	All patients	No AL	AL	p Value*
Total, n (%)	61 (100)	52 (85)	9 (14)	NA
WBC, g/L, median (IQR)				
Preoperative	6.2 (2.8)	6.2 (3.2)	6.4 (1.8)	0.943
POD 1	10.5 (4.8)	10.0 (4.7)	11.6 (6.6)	NA
POD 2	9.4 (4.8)	9.0 (4.1)	13.3 (5.8)	NA
POD 3	8.2 (4.1)	7.8 (3.2)	11.8 (4.6)	0.026
POD 4	6.8 (4.6)	6.6 (3.8)	9.8 (3.1)	0.009
POD 5	7.0 (4.3)	6.3 (3.1)	11.8 (6.4)	NA
POD 6	7.8 (4.4)	6.7 (3.6)	11.7 (3.6)	NA
POD 7	8.2 (5.0)	7.8 (3.6)	11.0 (5.8)	NA
C-reactive protein, mg/dL, median (IQR)				
Preoperative	0.3 (1.0)	0.3 (1.0)	0.4 (0.9)	0.501
POD 1	8.1 (5.4)	8.1 (5.4)	8.2 (6.9)	NA
POD 2	13.7 (8.8)	13.0 (6.9)	14.6 (13.1)	NA
POD 3	10.9 (8.8)	10.0 (7.3)	16.8 (20.5)	0.501
POD 4	8.2 (8.4)	6.8 (6.9)	13.0 (20.5)	0.010
POD 5	7.1 (9.1)	4.5 (6.6)	13.9 (13.8)	NA
POD 6	5.7 (8.6)	3.8 (6.7)	17.1 (12.9)	NA
POD 7	8.3 (12.7)	5.2 (9.1)	17.1 (16.2)	NA
Procalcitonin, ng/mL, median (IQR)				
Preoperative	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.792
POD 1	0.5 (0.8)	0.5 (0.8)	0.7 (1.9)	NA
POD 2	0.4 (0.6)	0.4 (0.6)	0.5 (5.3)	NA
POD 3	0.3 (0.8)	0.3 (0.7)	0.5 (5.3)	0.098
POD 4	0.2 (0.5)	0.2 (0.2)	0.6 (3.2)	0.023
POD 5	0.2 (0.4)	0.1 (0.2)	0.7 (1.4)	NA
POD 6	0.1 (0.3)	0.1 (0.2)	0.5 (1.4)	NA
POD 7	0.2 (0.3)	0.1 (0.1)	0.5 (0.6)	NA
mHLA-DR, %, median (IQR)				
Preoperative	52.0 (21.0)	52.3 (14.7)	50.8 (16.0)	0.694
POD 1	20.8 (12.0)	21.7 (12.0)	16.8 (9.0)	NA
POD 2	20.8 (15.0)	21.8 (13.0)	14.4 (5.0)	NA
POD 3	27.4 (17.0)	31.4 (17.0)	13.6 (11.0)	<0.001
POD 4	27.4 (23.0)	34.4 (23.0)	14.9 (15.0)	0.001
POD 5	33.2 (22.0)	36.4 (19.0)	10.7 (23.0)	NA
POD 6	30.9 (26.0)	36.8 (26.0)	10.1 (7.0)	NA
POD 7	33.7 (33.0)	45.2 (29.0)	13.5 (10.0)	NA

*Comparisons of AL vs No AL, respectively.
AL, anastomotic leak; IQR, interquartile range; mHLA-DR, monocytic HLA-DR expression; NA, not applicable; POD, postoperative day.

complex, soluble fibrin, tissue plasminogen activator, plasminogen activator inhibitor-1, platelets, sodium, albumin, calprotectin, fatty acid binding proteins, and others have been investigated as more or less suitable markers (summarized by Su'a and colleagues[1]). Altogether, CRP appears to be the most widely used test, and for many surgeons it seems to be the most helpful tool in guiding postoperative care in combination with the patient's clinical course.[23] In contrast, clinical symptoms alone, such as neurologic signs (septic encephalopathy), pulmonary events, fever, absence of bowel activity, or local tenderness of the abdomen, represent unreliable predictors for AL and they can appear far later (after PODs 4 to 6).[9,24]

Su'a and colleagues[1] reviewed 8 studies for the diagnostic performance of WBCs. Half of them found that WBC concentration predicts AL with AUCs from 0.63 to 0.77. In this recent review, CRP was able to predict

Table 3. Results of the Receiver Operating Characteristic Analysis Displaying the Area under the Curve, the Youden Index, the Specific Cutoff Values, Sensitivity, Specificity, and Positive and Negative Predictive Value on Postoperative Days 3 and 4

Blood marker	AUC	Youden index	Cutoff	Sensitivity, %	Specificity, %	PPV, %	NPV, %
WBC							
POD 3	0.734	0.503	9.25 g/L	72.6	77.8	36.1	94.3
POD 4	0.738	0.510	7.70 g/L	65.3	85.7	44.2	93.5
C-reactive protein							
POD 3	0.707	0.376	16.6 mg/dL	82.0	55.6	26.3	94.5
POD 4	0.709	0.531	9.4 mg/dL	67.4	85.7	48.2	93.6
Procalcitonin							
POD 3	0.672	0.418	0.45 ng/mL	64.0	77.8	26.3	92.6
POD 4	0.696	0.381	0.25 ng/mL	66.7	71.4	28.8	92.5
mHLA-DR							
POD 3	0.928	0.771	23.1%	77.1	100	100	94.6
POD 4	0.887	0.739	24.1%	73.9	100	100	94.0

AUC, area under the curve; mHLA-DR, monocytic HLA-DR expression; NPV, negative predictive value; POD, postoperative day; PPV, positive predictive value.

AL in 11 of 18 studies analyzed.[1] For PODs 3 and 4, the AUCs ranged from 0.72 to 0.88.[1] Procalcitonin was examined in 6 studies, with 5 of them reporting it to be predictive for AL, and with AUCs from 0.68 to 0.88.[1] Overall, our results for concentrations of WBCs, CRP, and PCT levels are in line with these findings, although the AUCs of CRP and PCT in the current study tended to be located near the lower margin of these reported ranges.

Detection of surface mHLA-DR was shown to be an excellent diagnostic test in the current pilot study, with an AUC of 0.928 already on POD 3 and 100% specificity on both PODs 3 and 4 with excellent positive predictive value (100%) and NPV (94.6% and 94.0% for PODs 3 and 4, respectively). To our knowledge, mHLA-DR has never been analyzed with regard to its diagnostic accuracy for AL after colorectal procedures. We find that mHLA-DR appears to have far better accuracy than the standard tests examined here and in other investigations. It seems comparable with the few excellent results of serum calprotectin or combination of markers.[25,26] Diminished expression of mHLA-DR has been reported as a reliable indicator of acquired immunosuppression, especially in ICU patients.[12,27] During sepsis and after severe trauma, a large number of alterations of the cellular immune functions have been reported.[28-30] Monocytes thereby undergo functional reprogramming from a pro-inflammatory to an immunosuppressive state.[13] Sepsis-related monocytosis is accompanied by loss of surface protein and gene expression of HLA-DR, and this results in reduced antigen presentation by these cells (monocyte anergy).[13,31] As such, mHLA-DR expression has been shown to improve the diagnosis of septic complications and the prediction of mortality.[12,31] Winkler and colleagues[13] speculated that it might be suitable to differentiate between postoperative and sepsis patients. Our data emphasize this predictive potential and show that mHLA-DR expression might represent a promising marker for the prediction of AL after colorectal operations because of the possible insight into the actual state and capacity of the innate immune response that is gained.[13]

Our study was limited by several factors, including the monocentric design and number of patients. The effort of this explorative pilot study was high due to daily non-automated FACS analyses of mHLA-DR expression. As there are no data available on mHLA-DR expression in colorectal patient populations, daily measurements were performed until POD 8. In addition, no valid sample size calculation was possible in advance. To show the statistically significant diagnostic superiority of mHLA-DR compared with CRP within ROC analysis on PODs 2, 3, and 4, there would have to be 115, 129, and 196 patients enrolled in a validation study, respectively. In addition, future studies could focus on measurements on PODs 2, 3, and 4 only. By doing this, costs and workload could be reduced. The promising results of the current pilot study should be further investigated and this could include potential intervention strategies that could lead to earlier and effective treatment of (incipient) AL.[25] Leak rates have been reported to vary between 1% and 40%, depending on the study population and the exact AL definition.[32] In the current study, 9 of 61 patients were diagnosed with AL, irrespective of its clinical significance just as in other large trials, such as the IMACORS study.[8] Ortega-Deballon and colleagues[32] reported a dehiscence rate of 15.5%, as they included all forms of leaks, just as we did. In line with that, the definition of AL in our study was strict, including radiologic leaks as well.

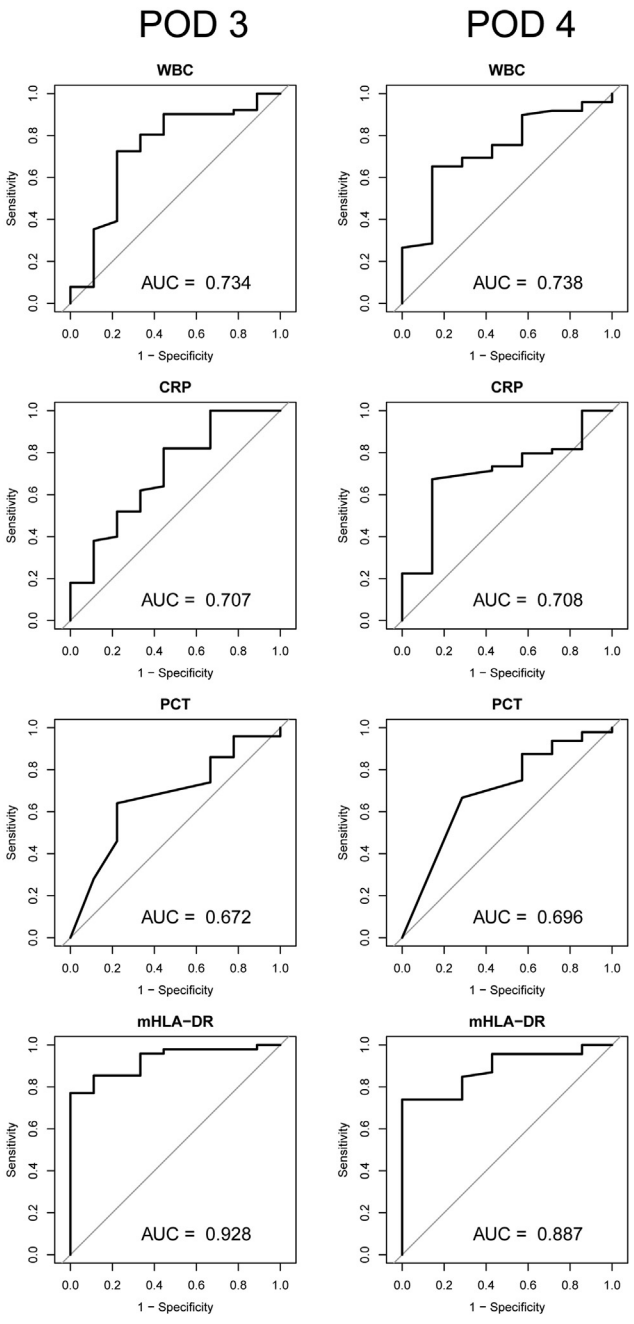

Figure 3. Receiver operating characteristic curves for the WBC, C-reactive protein (CRP), procalcitonin (PCT), and the proportion of HLA-DR-expressing monocytes (mHLA-DR) for the postoperative days (PODs) 3 and 4, respectively. The measurements of the proportion of HLA-DR–expressing monocytes (mHLA-DR) did show the highest values of the area under the curve (AUC) with 0.928 and 0.887 on PODs 3 and 4, respectively.

In addition, CT scan and endoscopy were performed unstintingly. This could have increased the detection rate, just as the policy to place a drain in every patient. The high rate of minor leaks in anterior resection and LAR patients might be explained by this fact as well. Overall, these differences in study design and postoperative monitoring limit the comparison of available studies because the incidence of AL depends on its detection rate.[32] In addition, very early AL occurrence (ie PODs 2 and 3) might not be adequately predicted in the future, as tests with high accuracy on POD 1 will be difficult to establish. For 2 patients having minor AL diagnosed on POD 3, sepsis marker assessment on PODs 3 and 4 was inherently unhelpful. Strengths of the study include its prospective design avoiding decreased sensitivity due to missing measurements and bias caused by retrospective analysis, such as sensitivity and timing of the onset or detection of AL.[33]

CONCLUSIONS

In the current pilot study, mHLA-DR expression was observed to be far more accurate than WBC concentration, CRP levels, and PCT level in diagnosing AL after colorectal operation. It indicated AL considerably earlier than the other tests and had more independent impact in a multivariate model, especially on day 3 after operation. Although mHLA-DR measurement requires higher methodologic effort than standard markers, it might represent a promising test in high-risk patients undergoing colorectal operations with anastomosis, such as those with low resection and a significant risk profile or those refusing diverting ostomy. Earlier detection of AL in these patients might enable effective treatment and reduce morbidity and mortality. With its high specificity and NPV, mHLA-DR expression can also contribute to safer discharge.

Author Contributions

Study conception and design: Sint, Lutz, Faist, Schiergens
Acquisition of data: Sint, Lutz, Faist, Bazhin, Rentsch, Schiergens
Analysis and interpretation of data: Sint, Lutz, Assenmacher, Küchenhoff, Faist, Bazhin, Rentsch, Werner, Schiergens
Drafting of manuscript: Sint, Schiergens
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