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ORIGINAL ARTICLE

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ABOUT COVER

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ORIGINAL ARTICLE

Basic Study

Influence of anastomoses on intestine ischemia and cefuroxime concentrations: Evaluated in the ileum and colon in a porcine model

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Abstract

BACKGROUND

Anastomotic leakage is a serious complication following gastrointestinal surgery and is associated with increased morbidity and mortality. The incidence of anastomotic leakage is determined by anatomy and is reported to be between 4%-33% for colon anastomosis and 1%-3% for small intestine anastomosis. The etiology of anastomotic leakage of the intestine has been divided into three main factors: healing disturbances, communication between intra- and extra-luminal compartments, and infection. All three factors interact, and one factor will inevitably lead to the other two factors resulting in tissue ischemia, tissue necrosis, and anastomotic leakage.

AIM

To evaluate ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon in a porcine model.

METHODS

Eight healthy female pigs (Danish Landrace breed, weight 58-62 kg) were included in this study. Microdialysis catheters were placed for sampling of

was carried out according to existing laws and approved by the Danish Animal Experiments Inspectorate (license No.: 2017/15-0201-01184). All appropriate measures were taken to minimize animal pain and discomfort.

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ischemic metabolites (glucose, lactate, glycerol, and pyruvate) and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon. Cefuroxime 1.5 g was administered as an intravenous infusion over 15 min. Subsequently, dialysates and blood samples were collected over 8 h and the ischemic metabolites and cefuroxime concentrations were quantified in all samples. The concentrations of glucose, lactate, glycerol and pyruvate were determined using the CMA 600 Microdialysis Analyzer with Reagent Set A (M Dialysis AB, Sweden), and the concentrations of cefuroxime and meropenem were quantified using a validated ultra-high-performance liquid chromatography assay.

RESULTS

Only the colon anastomosis induced mean ischemic lactate/pyruvate ratios above 25 (ischemic cut-off) throughout the entire sampling interval, and simultaneously decreased glucose concentrations. The mean time for which cefuroxime concentrations were maintained above the clinical breakpoint minimal inhibitory concentration for *Escherichia coli* (8 μg/mL) ranged between 116-128 min across all the investigated compartments, and was similar between the anastomosis and non-anastomosis ileum and colon. For all pigs and in all the investigated compartments, a cefuroxime concentration of 8 μg/mL was reached within 10 min after administration. When comparing the pharmacokinetic parameters between the anastomosis and non-anastomosis sites for both ileum and colon, only colon T_{max} and half-life differed between anastomosis and non-anastomosis ($P < 0.03$). Incomplete tissue penetrations were found in all tissues except for the non-anastomosis colon.

CONCLUSION

Administering 1.5 g cefuroxime 10 min prior to intestine surgery seems sufficient, and effective concentrations are sustained for approximately 2 h. Only colon anastomosis was locally vulnerable to ischemia.

Key Words: Anastomosis; Cefuroxime; Colon; Ileum; Ischemic metabolites; Microdialysis

Core Tip: We found that only colon anastomosis was locally vulnerable to ischemia but reached similar cefuroxime concentrations to those in the remaining investigated intestine compartments. Our study suggests that administering 1.5 g cefuroxime 10 min prior to intestine surgery is sufficient, and that effective concentrations are sustained for approximately 2 h. This is the first study to investigate the influence of anastomoses on ileum and colon ischemic metabolites and cefuroxime concentrations in a simultaneous paired design.

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INTRODUCTION

Anastomotic leakage is a serious complication following gastrointestinal surgery and is associated with increased morbidity and mortality[1]. The incidence of anastomotic leakage is determined by anatomy and is reported to be between 4%-33% for colon anastomosis and 1%-3% for small intestine anastomosis[1-4]. The etiology of anastomotic leakage is multifactorial, and to some extent not fully understood[3]. Nonetheless, previous studies have suggested that the etiology is due to three main factors: healing

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disturbances, communication between intra- and extra-luminal compartments, and infection[3,5,6]. With this theory, one of these three factors can often be identified as the primary cause of the anastomotic leakage. However, it is believed that all three factors interact and one factor will lead to the two other factors resulting in tissue ischemia, tissue necrosis, and anastomotic leakage[3,7].

Gastrointestinal surgery is predisposed to infection given its vicinity to the bacterial load within the intestine. Sufficient antimicrobial prophylaxis is considered an essential preventive measure in protecting surgical anastomoses from bacterial overgrowth and relies on the achievement of therapeutic antimicrobial target site concentration[8]. While antimicrobial concentrations have been evaluated in various tissues and settings[9,10], intestine antimicrobial concentrations remain poorly investigated. Cephalosporins, *e.g.*, cefuroxime, is frequently used both prophylactically and in the treatment of infections within gastrointestinal surgery, due to its broad-spectrum efficacy against gram-positive as well as gram-negative bacteria[11].

Microdialysis is a membrane-bearing method, which allows continuous sampling of ischemic metabolites and the free antimicrobial concentrations in the interstitial space of various tissues[12,13]. It has previously been employed in various abdominal relevant sites for the study of ischemic metabolites[14-18], and for sampling cefuroxime concentrations in various extra-abdominal tissues[19,20]. We hypothesized that anastomoses of the ileum and colon would present an immediate postoperative local increase in ischemic metabolites and lower cefuroxime concentrations in comparison to the non-anastomosis intestine. To test this, we conducted a porcine study applying microdialysis for the evaluation of ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon.

MATERIALS AND METHODS

This study was conducted at the Institute of Clinical Medicine, Aarhus University Hospital, Denmark. The study was carried out according to existing laws and approved by the Danish Animal Experiments Inspectorate (license No.: 2017/15-0201-01184). All appropriate measures were taken to minimize animal pain and discomfort. Chemical analyses were performed at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark.

Ischemic metabolites

Glucose, lactate, glycerol, and pyruvate can easily and promptly be analyzed when linked to an appropriate analytical assay[12,21,22]. Under anaerobic conditions, glucose levels decrease due to a combination of increased glucose consumption, which is required in order to maintain adenosine triphosphate (ATP) production, and a decreased organ or tissue supply due to reduced perfusion[21]. Lactate is produced from pyruvate under anaerobic conditions resulting in increased lactate concentrations, decreased pyruvate concentrations, and ultimately increased lactate/pyruvate ratios[21]. A lactate/pyruvate ratio above 25 is considered to signify ischemia[22]. Glycerol is a basic component of the cell membrane. When the cell membrane is damaged, glycerol is released, and is therefore used as marker of cell damage[21].

Study procedures

Microdialysis: Microdialysis is a catheter-based technique with a semipermeable membrane at the tip of the catheter, which allows for continuous and simultaneous sampling of interstitial fluid from multiple sites[23]. Due to continuous perfusion of the semipermeable membrane, equilibrium never occurs, and the dialysate concentration only represents a fraction of the actual concentration. This fraction is referred to as the relative recovery, which can be determined by various calibration methods[23]. In this study, meropenem was used as an internal calibrator for cefuroxime[13]. Relative recovery was not determined for the ischemic metabolites. Changes in the concentration ratios between interventions or compartments, for comparison between anastomosis and non-anastomosis tissue and for ratios between metabolites (lactate/pyruvate) are quantitative measures and independent of relative recovery[24].

Equipment from M Dialysis AB (Stockholm, Sweden) was used. The microdialysis catheters consisted of CMA 70 membranes (membrane length: 20 mm, 20 kDa molecule cut-off), and CMA 107 precision pumps produced a flow rate of 2 μL/min.

Animals, anesthetic, and surgical procedure: Eight healthy female pigs (Danish

Landrace breed, weighing 58-62 kg) were included in the study. The pigs received general anesthesia during the study with the combination of propofol (500-600 mg/h, continuous infusion) and fentanyl (0.60-0.75 mg/h, continuous infusion). Temperature and pH where monitored for each pig and were kept within the range of 36.4-38.5°C and 7.40-7.50, respectively.

After induction of anesthesia, surgery was initiated. The intestines were presented *via* a midline abdominal incision. A 5 cm ileum resection, approximately 50 cm orally from the ileocaecal valve, was performed. The ileum was anastomosed end-to-end with a continuous (Monocryl^{tm} 4-0) suture using the extramucosal technique ad modum Davos (hand-sewn end-to-end extramucosal running suture). Good blood supply to the intestine ends was visualized by brisk bleeding from the arcade artery prior to suturing. One microdialysis catheter was placed in the ileum wall parallel to and approximately 0.5 cm from the anastomosis. An adjacent microdialysis catheter was placed approximately 50 cm orally from the ileum anastomosis. Subsequently, a 5 cm colon resection was performed approximately 10 cm anally from the ileocecal valve. Good blood supply to the colon ends was visualized by brisk bleeding from the arcade artery. The colon was similarly anastomosed end-to-end with a continuous (Monocryl^{tm} 4-0) suture. One microdialysis catheter was placed in the colon wall parallel to and approximately 0.5 cm from the anastomosis. An adjacent microdialysis catheter was placed approximately 30 cm anally from the colon anastomosis. All catheters were placed using splitable introducers. After placement of all catheters, the abdominal wall was carefully closed.

Following placement of the microdialysis catheters, all catheters were perfused with 0.9% NaCl containing 5 μg/mL meropenem, allowing for continuous calibration, and 30 min tissue equilibration was allowed for.

Sampling procedures: Cefuroxime 1.5 g was administered intravenously over 15 min, marking time zero. Dialysates were collected at 20 min intervals from time 0-60 min, at 30 min intervals from time 60-180 min, and at 60 min intervals from time 180-360 min and from time 420-480 min, giving a total of 11 samples during 8 h. Blood samples were collected from a central venous catheter at the midpoint of the sampling intervals.

Dialysate samples were instantly stored at -80°C until analysis. The venous blood samples were stored at 5°C for a maximum of 6 h before being centrifuged at 3000 rpm for 10 min. Plasma aliquots were then stored at -80°C until analysis.

Endpoints

For the ischemic metabolites, the primary endpoint was evaluation of lactate/pyruvate ratios. For cefuroxime concentrations, the primary endpoint was assessment of the time for which the free cefuroxime was maintained above the clinical breakpoint minimal inhibitory concentration (T>MIC) for *Escherichia coli* (8 μg/mL)[25].

Quantification techniques

Cefuroxime and meropenem concentrations: The concentrations of cefuroxime and meropenem were quantified using a validated ultra-high-performance liquid chromatography assay[26]. Inter-run imprecisions (percent coefficients of variation) were 4.7% at 2.5 μg/mL for quantification of cefuroxime and 3.0% at 2.0 μg/mL for quantification of meropenem. The lower limits of quantification were 0.06 μg/mL for cefuroxime and 0.5 μg/mL for meropenem.

Assessment of ischemic metabolites: The concentrations of glucose, lactate, glycerol and pyruvate were determined using the CMA 600 Microdialysis Analyzer with Reagent Set A (M Dialysis AB, Sweden).

Pharmacokinetic analysis and statistics

Pharmacokinetic parameters were determined for each compartment in all animals using noncompartmental analysis in Stata (v. 15.1, StataCorp LLC, College Station, TX, United States). The area under the concentration-time curves (AUC) were calculated using the trapezoidal rule. The maximum of all the recorded concentrations was defined as peak drug concentration (C_{max}), enabling calculation of the time to C_{max} (T_{max}). Half-life ($T_{1/2}$) was calculated as $\ln(2)/\lambda_{eq}$, where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration on time. The $AUC_{tissue}/AUC_{plasma}$ ratio was calculated as a measure of tissue penetration. Microsoft Excel was used to estimate the T>MIC using linear interpolation. A general comparison of the pharmacokinetic parameters and T>MIC was conducted using a repeated

measurements analysis of variance followed by pairwise comparisons made by linear regression. The Kenward-Roger approximation method was used for correction of degrees of freedom due to the small sample size. The model assumptions were tested using visual diagnosis of residuals, fitted values, and estimates of random effects. A significance level of 5% was used. Microsoft Excel was used to calculate the mean concentration difference in percentage for the ischemic markers between the anastomosis and non-anastomosis (anastomosis/non-anastomosis) ileum and colon. The measured cefuroxime and ischemic marker concentrations in the dialysate were attributed to the midpoint of the sampling intervals.

RESULTS

All pigs completed the study. The relative recovery (SD) was 24% (5) for non-anastomosis ileum, 18% (3) for ileum anastomosis, 28% (9) for non-anastomosis colon, and 27% (7) for colon anastomosis.

Ischemic metabolites

The lactate/pyruvate ratio for each compartment is depicted in Figure 1. Only the mean lactate/pyruvate ratio for the colon anastomosis was above the ischemic cut-off level of 25, and remained above 25 throughout the entire sampling interval.

The mean concentration differences (%) for glucose, lactate, glycerol, pyruvate, and lactate/pyruvate ratios between both anastomosis and non-anastomosis (anastomosis/non-anastomosis) ileum and colon are depicted in Figure 2 and Table 1. For the colon, the lactate/pyruvate ratio between anastomosis and non-anastomosis was increased in the first 75 min after placement of the microdialysis catheters, which was primarily driven by increased lactate concentrations. The lactate/pyruvate ratio then normalized. No differences were observed for the lactate/pyruvate ratio between anastomosis and non-anastomosis ileum. The glucose ratio between anastomosis and non-anastomosis colon was decreased throughout the 8 h sampling interval with a mean ratio range of 28%-49%. For the ileum, the glucose ratio (anastomosis/non-anastomosis) was only decreased in the first 135 min after placement of the microdialysis catheters and then normalized. While glycerol concentrations were similar in anastomosis and non-anastomosis colon, decreased glycerol concentrations were found in anastomosis compared to non-anastomosis ileum. The mean concentration of the ischemic metabolites for both non-anastomosis and anastomosis ileum and colon are shown in Table 2 and 3.

T>MIC

The T>MIC (8 μg/mL) results for each compartment are shown in Table 4. The mean T>MIC (8 μg/mL) ranged between 116-128 min across all investigated compartments. A similar T>MIC (8 μg/mL) was found between both anastomosis and non-anastomosis ileum and colon ($P > 0.6$). For all pigs and in all intestine compartments, a cefuroxime concentration of 8 μg/mL was reached within 10 min after administration.

Pharmacokinetic parameters

The resulting pharmacokinetic parameters are shown in Table 5 and individual concentration time profiles are depicted in Figure 3. When comparing the pharmacokinetic parameters between the anastomosis and non-anastomosis sites for both ileum and colon, only colon T_{max} and half-life differed between anastomosis and non-anastomosis ($P < 0.03$). Incomplete tissue penetrations were found in all tissues except for the non-anastomosis colon with a mean penetration of 0.90 (95% confidence interval 0.73; 1.06). When comparing plasma to the intestine compartments, plasma AUC and C_{max} were higher and T_{max} was shorter ($P < 0.02$). Only non-anastomosis colon AUC was similar to plasma AUC ($P = 0.10$).

DISCUSSION

This is the first study to investigate the influence of anastomoses on ileum and colon ischemic metabolites and cefuroxime concentrations in a simultaneous paired design. The main findings were increased lactate/pyruvate ratios in the colon anastomosis and similar T>MIC (8 μg/mL) for cefuroxime in all the investigated intestine compartments.

Table 1 The mean concentration difference (%) of ischemic metabolites between the anastomosis and non-anastomosis (anastomosis/non-anastomosis) ileum and colon

	Glucose		Lactate		Glycerol		Pyruvate		Lactate/pyruvate	
Time	Ileum (%)	Colon (%)	Ileum (%)	Colon (%)	Ileum (%)	Colon (%)	Ileum (%)	Colon (%)	Ileum (%)	Colon (%)
10	68 (47; 89)	28 (13; 44)	119 (88; 149)	186 (79; 293)	77 (65; 88)	110 (68; 151)	90 (74; 106)	78 (30; 126)	134 (99; 170)	327 (129; 526)
30	66 (46; 85)	35 (19; 51)	110 (63;157)	183 (72; 294)	75 (64; 87)	120 (67; 173)	84 (73; 95)	86 (39; 133)	135 (76; 194)	252 (148; 356)
50	69 (48; 90)	38 (21; 55)	101 (59; 143)	173 (79; 266)	71 (63; 79)	122 (80; 163)	83 (69; 97)	88 (44; 132)	133 (60; 205)	241 (133; 348)
75	69 (52; 86)	40 (24; 56)	92 (61; 123)	162 (92; 232)	70 (62; 78)	98 (56; 140)	77 (67; 87)	94 (58; 131)	123 (76; 171)	199 (120; 277)
105	67 (46; 89)	42 (19; 64)	78 (54; 101)	170 (108; 232)	80 (63; 98)	91 (62; 121)	77 (61; 93)	105 (65; 146)	108 (71; 145)	205 (90; 320)
135	72 (46; 98)	37 (12; 61)	77 (58; 96)	167 (127; 206)	73 (61; 86)	102 (67; 137)	82 (64; 100)	119 (68; 170)	98 (77; 118)	202 (71; 334)
165	72 (44; 101)	33 (12; 53)	74 (56; 92)	166 (121; 212)	78 (63; 94)	98 (65; 130)	85 (73; 98)	121 (73; 168)	89 (66; 112)	178 (78; 278)
210	66 (26; 106)	30 (11; 49)	86 (63; 108)	141 (118; 165)	74 (59; 88)	111 (71; 152)	78 (59; 96)	111 (61; 160)	114 (93; 135)	176 (86; 266)
270	89 (39; 139)	49 (23; 76)	75 (57; 94)	126 (105; 147)	78 (64; 92)	131 (73; 189)	85 (73; 96)	115 (57; 173)	96 (61; 130)	165 (75; 255)
330	98 (63; 133)	42 (22; 61)	95 (65; 126)	131 (79; 184)	81 (63; 99)	130 (75; 185)	89 (78; 100)	90 (56; 125)	107 (79; 135)	152 (112; 193)
450	65 (10; 119)	29 (17; 40)	99 (44; 155)	120 (58; 182)	87 (67; 107)	148 (87; 209)	99 (76; 122)	98 (59; 137)	100 (57; 144)	178 (89; 268)

Values are shown as means (95% confidence interval).

Microdialysis is a well-known sampling tool for the study of ischemic metabolites and have been applied in various abdominal relevant sites, *e.g.*, intraperitoneal, mediastinal, intrahepatic, and in intestine walls[14-18]. A systemic review investigated whether intraperitoneal placed microdialysis could be used for early detection of colon and rectal anastomotic leakage[27]. The study concluded that increasing intraperitoneal lactate concentrations could be associated with anastomotic leakage, but with low predictive values[27]. No studies have previously investigated ischemic metabolites in anastomotic intestine tissue. The present study does not investigate the ischemic changes related to an anastomotic leakage, but only the ischemic conditions related to anastomoses of ileum and colon. Interestingly, our data suggest that colon anastomosis is more vulnerable to ischemia, depicted by an increased lactate/pyruvate ratio and decreased glucose concentrations. This may indirectly correlate with the inherent higher risk of colon anastomosis leakage than that of the small intestine[1-4]. Although these findings may not be surprising, it may lead to a better future understanding of anastomotic leakage.

Despite a predisposed risk of infections in gastrointestinal surgery, antimicrobial tissue concentrations in the intestines remain poorly investigated. For cefuroxime, it is generally recommended that the antimicrobial tissue concentrations exceed MIC values of relevant bacteria throughout surgery in order to be efficient in a prophylactic setting[8,11]. In gastrointestinal surgery, the most commonly encountered bacterium is *Escherichia coli*, which exhibits a clinical breakpoint MIC for cefuroxime of 8 μg/mL[25]. In the present study, cefuroxime concentrations of 8 μg/mL were reached within 10 min in all the investigated compartments and were maintained above 8 μg/mL for approximately 2 h. Thus, cefuroxime displayed prompt penetration into the intestines and similar elimination rates compared to that of plasma. This indicates that administering 1.5 g cefuroxime 10 min prior to surgery is sufficient, and that effective concentrations are sustained for approximately 2 h. For gastrointestinal procedures lasting longer than 2 h, and in cases with a need for postoperative concentrations above relevant MIC or to accommodate higher MIC targets, increasing or alternative dosing regimens, *e.g.*, continuous infusion, should be considered.

There is an interesting discrepancy between the ischemic metabolite findings and cefuroxime concentrations. We found an increased vulnerability to ischemia in the colon anastomosis but almost identical pharmacokinetic cefuroxime endpoints in all intestine compartments. This may imply that cefuroxime penetration, to some extent, is independent of the local ischemic conditions. However, it is unknown whether a threshold exists, in which cefuroxime penetration decreases with increasing intestine ischemia. This calls for further investigation.

Surgery and sampling were performed on healthy juvenile pigs (aged 5 mo). Although pigs have been shown to parallel human physiology and anatomy to a large

Table 2 The mean concentration of ischemic metabolites for both non-anastomosis and anastomosis ileum

	Glucose		Lactate		Glycerol		Pyruvate		Lactate/pyruvate	
Time	Ileum	Ileum anastomosis	Ileum	Ileum anastomosis	Ileum	Ileum anastomosis	Ileum	Ileum anastomosis	Ileum	Ileum anastomosis
10	1.63 (1.11; 2.15)	1.03 (0.70; 1.35)	0.86 (0.65; 1.06)	0.99 (0.68; 1.31)	62.29 (52.23; 72.34)	47.00 (38.06; 55.94)	65.00 (57.58; 72.42)	57.86 (47.66; 68.05)	13.00 (10.90; 15.11)	17.94 (10.50; 25.37)
30	1.45 (0.99; 1.90)	0.85 (0.65; 1.05)	0.82 (0.61; 1.03)	0.87 (0.52; 1.22)	63.29 (56.37; 70.20)	47.00 (40.42; 53.58)	62.00 (54.03; 69.97)	51.14 (44.50; 57.79)	13.20 (10.43; 15.96)	17.05 (9.83; 24.28)
50	1.41 (0.85; 1.96)	0.83 (0.64; 1.03)	0.81 (0.61; 1.01)	0.79 (0.50; 1.08)	63.57 (55.13; 72.02)	44.86 (37.85; 51.86)	60.29 (50.19; 70.38)	48.43 (42.28; 54.58)	13.52 (11.36; 15.69)	16.68 (9.45; 23.93)
75	1.28 (0.78; 1.79)	0.80 (0.64; 0.96)	0.84 (0.70; 0.99)	0.77 (0.51; 1.03)	59.29 (52.26; 66.31)	41.14 (35.48; 46.81)	57.14 (49.82; 64.45)	43.43 (37.29; 49.57)	14.71 (13.25; 16.18)	17.55 (12.25; 22.85)
105	1.01 (0.82; 1.21)	0.64 (0.47; 0.81)	0.97 (0.71; 1.23)	0.71 (0.51; 0.91)	57.71 (49.13; 66.30)	45.57 (34.14; 57.00)	57.43 (47.06; 67.80)	43.14 (34.92; 51.36)	16.87 (13.03; 20.71)	17.12 (12.20; 22.04)
135	0.82 (0.69; 0.96)	0.58 (0.37; 0.79)	1.06 (0.74; 1.37)	0.77 (0.56; 0.98)	63.00 (53.30; 72.70)	46.14 (34.93; 57.36)	58.29 (44.04; 72.53)	45.86 (35.97; 55.74)	17.99 (15.12; 20.86)	16.98 (13.88; 20.08)
165	0.96 (0.62; 1.30)	0.69 (0.38; 1.00)	1.09 (0.82; 1.36)	0.78 (0.57; 0.99)	64.42 (51.05; 77.81)	48.29 (38.94; 57.63)	57.43 (45.68; 69.18)	47.86 (39.49; 56.22)	19.31 (15.34; 23.28)	16.14 (12.64; 19.64)
210	0.98 (0.55; 1.41)	0.55 (0.23; 0.87)	1.13 (0.83; 1.42)	0.93 (0.67; 1.18)	71.00 (48.40; 93.60)	51.86 (33.18; 70.54)	62.57 (51.12; 74.02)	47.71 (36.57; 58.86)	18.04 (14.11; 21.97)	19.54 (16.66; 22.42)
270	0.88 (0.34; 1.43)	0.59 (0.25; 0.92)	1.41 (0.95; 1.88)	0.99 (0.71; 1.23)	79.29 (51.38; 107.19)	57.86 (42.24; 73.47)	69.86 (57.67; 82.04)	58.14 (48.69; 67.60)	20.24 (14.07; 26.40)	17.12 (13.21; 21.03)
330	1.43 (0.34; 2.52)	0.96 (0.39; 1.53)	1.44 (0.95; 1.93)	1.27 (0.82; 1.72)	77.57 (54.66; 100.48)	58.14 (46.42; 69.87)	74.71 (57.03; 92.13)	65.86 (49.75; 81.96)	19.68 (13.31; 26.05)	18.58 (15.32; 21.83)
450	0.67 (0.41; 0.93)	0.52 (0.22; 0.82)	1.36 (1.02; 1.69)	1.14 (0.73; 1.55)	67.14 (48.62; 85.67)	55.14 (43.26; 67.03)	63.67 (50.17; 77.17)	58.86 (45.97; 71.74)	21.83 (16.66; 27.01)	18.74 (15.13; 22.35)

Values are given as means (95% confidence interval). Glucose and lactate concentrations are given as mmol/L. Glycerol and pyruvate concentrations are given as μmol/L.

extent[28], more data are needed to firmly evaluate the translational potential of these findings. Infection and inflammation have previously been correlated with decreased antimicrobial tissue concentrations in other settings[9,10]. However, all pigs in the present study had a presumed good intestinal blood supply without any influence of fibrotic or inflamed intestine tissue. Therefore, future studies assessing the effect of influenced blood flow, inflammation, fibrosis, atherosclerosis *etc.* on the ischemic metabolites and antimicrobial concentrations in larger animal studies are warranted. Finally, we investigated the ischemic and cefuroxime properties in relation to a sutured anastomosis. The use of stapled anastomoses has increased over the past years and results from the present study cannot directly be extrapolated to stapled anastomoses.

CONCLUSION

In conclusion, we found that only colon anastomosis induced increased lactate/pyruvate ratios and decreased glucose concentrations, suggesting that colon anastomoses are more vulnerable to ischemia. Moreover, we found a similar T>MIC (8 μg/mL) in all the investigated compartments. Sufficient cefuroxime intestine concentrations were reached within 10 min after administration and were maintained

Table 3 The mean concentration of ischemic metabolites for both non-anastomosis and anastomosis colon

	Glucose		Lactate		Glycerol		Pyruvate		Lactate/pyruvate	
Time	Colon	Colon anastomosis	Colon	Colon anastomosis	Colon	Colon anastomosis	Colon	Colon anastomosis	Colon	Colon anastomosis
10	2.63 (1.06; 4.21)	0.58 (0.23; 0.93)	1.65 (1.36; 1.94)	3.22 (1.21; 5.23)	99.86 (72.69; 127.02)	118.00 (55.70; 180.30)	115.71(79.45; 151.98)	85.83 (31.36; 140.31)	16.02 (11.05; 21.00)	61.18 (11.78; 110.57)
30	2.21 (1.15; 3.27)	0.62 (0.19; 1.05)	1.60 (1.38; 1.82)	2.85 (0.98; 4.72)	96.29 (70.78; 121.79)	114.50 (56.00; 173.00)	104.29 (81.34; 126.83)	86.83 (40.02; 133.64)	16.56 (12.16; 20.95)	43.94 (18.28; 69.59)
50	2.13 (1.12; 3.14)	0.75 (0.07; 1.42)	1.48 (1.21; 1.74)	2.43 (0.95; 3.91)	91.29 (65.75; 116.82)	110.50 (55.85; 165.15)	91.57 (75.05; 108.10)	78.00 (37.62; 118.38)	17.21 (11.91; 22.52)	44.01 (14.62; 73.41)
75	2.09 (1.02; 3.16)	0.92 (-0.01; 1.86)	1.36 (1.07; 1.66)	2.07 (1.01; 3.13)	89.00 (69.11; 108.89)	92.33 (42.55; 142.11)	81.71 (70.21; 93.22)	76.33 (41.95; 110.72)	17.14 (12.41; 21.88)	35.87 (13.91; 57.84)
105	1.73 (1.14; 2.31)	0.77 (0.08; 1.45)	1.29 (1.06; 1.51)	2.06 (1.23; 2.88)	93.71 (69.64; 117.79)	91.17 (47.73; 134.60)	75.00 (62.82; 87.18)	76.17 (43.34; 109.00)	17.96 (13.26; 22.67)	37.02 (14.28; 59.75)
135	1.55 (1.10; 1.99)	0.65 (0.12; 1.18)	1.35 (1.08; 1.62)	2.14 (1.46; 2.81)	99.00 (68.25; 129.75)	99.17 (54.54; 143.80)	71.86 (57.66; 86.06)	81.17 (41.82; 120.50)	20.51 (13.46; 27.55)	40.10 (12.97; 67.24)
165	1.54 (1.04; 2.05)	0.53 (0.22; 0.84)	1.52 (1.21; 1.84)	2.40 (1.47; 3.32)	95.00 (65.60; 124.40)	94.67 (51.95; 137.39)	75.14 (57.85; 92.44)	84.33 (47.06; 121.61)	22.10 (14.74; 29.46)	39.03 (15.89; 62.17)
210	1.53 (0.65; 2.41)	0.43 (0.15; 0.70)	1.89 (1.50; 2.29)	2.46 (1.91; 3.00)	103.43 (64.19;142.67)	103.83 (62.12; 145.55)	88.00 (63.70; 112.30)	87.50 (43.41; 131.59)	23.57 (16.49; 30.65)	41.06 (18.57; 63.54)
270	1.00 (0.56; 1.43)	0.48 (0.09; 0.88)	2.07 (1.75; 2.39)	2.51 (1.97; 3.06)	100.00 (66.94; 133.06)	114.50 (69.08; 159.92)	94.86 (60.15; 129.57)	88.67 (45.00; 132.34)	21.90 (16.58; 27.21)	41.39 (17.54; 65.25)
330	1.51 (0.35; 2.68)	0.70 (-0.36; 1.75)	2.59 (1.92; 3.27)	2.90 (1.85; 3.96)	94.57 (74.34; 114.80)	109.83 (69.68; 149.98)	104.43 (70.89; 137.97)	82.5 (49.76; 115.24)	22.34 (15.05; 29.63)	39.43 (23.56; 55.30)
450	0.62 (0.28; 0.96)	0.15 (0.11; 0.20)	2.49 (2.19; 2.78)	2.89 (1.54; 4.23)	80.43 (59.32; 101.53)	110.67 (63.49; 157.84)	94.00 (52.20; 135.80)	75.17 (43.75; 106.59)	22.44 (18.23; 26.65)	40.55 (28.83; 52.27)

Values are given as means (95% confidence interval). Glucose and lactate concentrations are given as mmol/L. Glycerol and pyruvate concentrations are given as μmol/L.

Table 4 The time with concentrations above the minimal inhibitory concentration (8 μg/mL) in min for plasma and for both anastomosis and non-anastomosis ileum and colon

Compartment	Non-anastomosis	Anastomosis	*P* values
Plasma	116 (96; 135)	-	-
Ileum	120 (101; 140)	116 (97; 136)	0.61
Colon	126 (106; 145)	128 (108; 148)	0.77

Time shown as means (95% confidence interval).

for approximately 2 h.

Table 5 Pharmacokinetic parameters for plasma and for both non-anastomosis and anastomosis ileum and colon

Compartment	Non-anastomosis	Anastomosis	*P* value
Plasma AUC (min μg/mL)	4849 (4003; 5786)[a]	-	-
Ileum AUC (min μg/mL)	3678 (2786; 4570)	3327 (2436; 4219)	0.28
Colon AUC (min μg/mL)	4219 (3327; 5110)	3542 (2622; 4462)	0.61
Plasma C_{max} (μg/mL)	147 (131; 163)[b]	-	-
Ileum C_{max} (μg/mL)	51 (35; 66)	46 (30; 62)	0.65
Colon C_{max} (μg/mL)	58 (42; 74)	39 (22; 56)	0.08
Plasma T_{max} (min)	10 (5; 15)[b]	-	-
Ileum T_{max} (min)	28 (23; 32)	28 (23; 32)	1.00
Colon T_{max} (min)	25 (20; 30)	33 (27; 38)	0.03
Plasma $T_{1/2}$ (min)	58 (44; 73)	-	-
Ileum $T_{1/2}$ (min)	54 (39; 68)	52 (37; 66)	0.70
Colon $T_{1/2}$ (min)	53 (38; 67)	66 (52; 81)	0.02
Ileum $AUC_{tissue}/AUC_{plasma}$	0.74 (0.57; 0.91)	0.68 (0.50; 0.85)	0.56
Colon $AUC_{tissue}/AUC_{plasma}$	0.90 (73; 1.07)	0.72 (0.53; 0.90)	0.12

AUC, Cmax, Tmax, and T1/2 are given as means (95% confidence interval).

[a]$P = 0.01$ for comparison with all compartments but not non-anastomosis colon.

[b]$P < 0.01$ for comparison with all compartments.

AUC: Area under the concentration–time curve from; C_{max}: Peak drug concentration; T_{max}: Time to C_{max}; $T_{1/2}$: Half-life; $AUC_{tissue}/AUC_{plasma}$: Area under the concentration-time curve ratio of tissue/plasma.

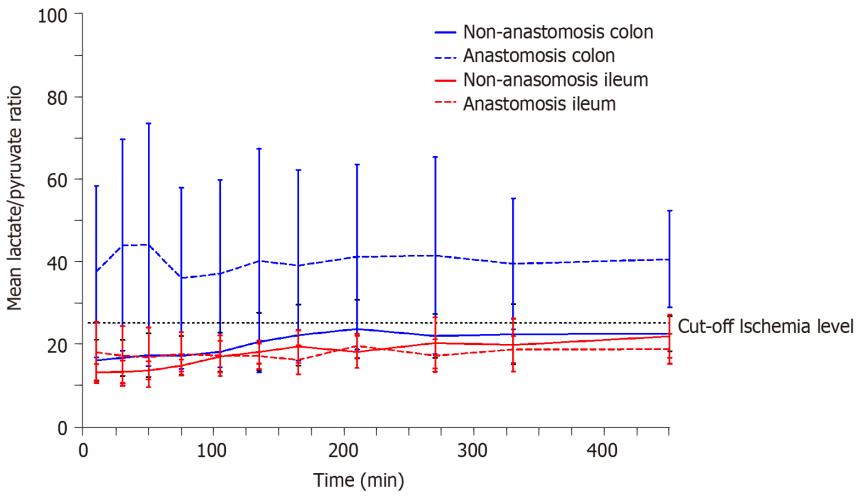

Figure 1 The mean lactate/pyruvate ratios for anastomosis and non-anastomosis ileum and colon. The ischemic cut-off of 25 is marked with a dotted line. Bars represent 95% confidence interval.

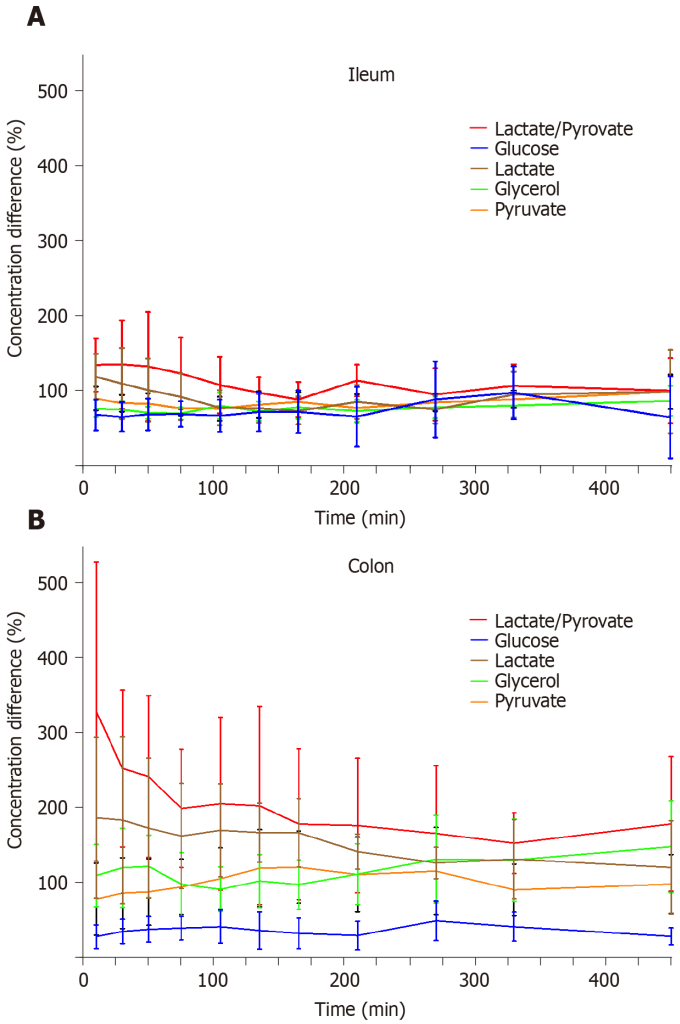

Figure 2 The mean ischemic metabolite concentration differences (%) between anastomosis and non-anastomosis (anastomosis/non-anastomosis) ileum and colon. Bars represent 95% confidence interval. MIC: Minimal inhibitory concentration.

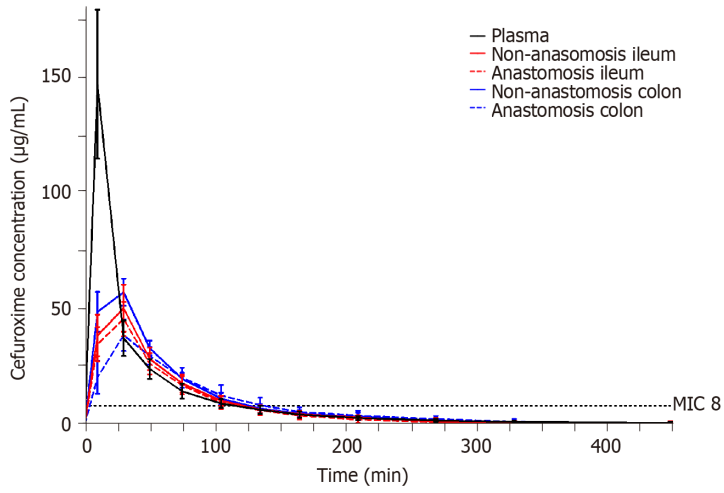

Figure 3 Mean cefuroxime concentration-time profiles for anastomosis and non-anastomosis ileum and colon. The dotted line represents the cefuroxime clinical breakpoint minimal inhibitory concentration for *Escherichia coli* (8 μg/mL). Bars represent 95% confidence interval.

ARTICLE HIGHLIGHTS

Research background

Anastomotic leakage is a serious complication following gastrointestinal surgery and is associated with increased morbidity and mortality. The etiology of anastomotic leakage is multifactorial, and to some extent, is not fully understood.

Research motivation

Previous studies have suggested that the etiology is due to three main factors: healing disturbances, communication between intra- and extra-luminal compartments, and infection. However, no studies have previously investigated ischemic metabolites in anastomotic intestine tissue and the intestine antimicrobial concentrations.

Research objectives

To evaluate ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon in a porcine model.

Research methods

Eight healthy female pigs were included. Microdialysis catheters were placed for sampling ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis ileum and colon. Cefuroxime 1.5 g was administered as an intravenous infusion over 15 min.

Research results

Only the colon anastomosis induced mean ischemic lactate/pyruvate ratios above 25 (ischemic cut-off) throughout the entire sampling interval, and simultaneously decreased glucose concentrations. The mean time for which cefuroxime concentrations were maintained above the clinical breakpoint minimal inhibitory concentration for *Escherichia coli* (8 μg/mL) ranged between 116-128 min across all the investigated compartments, and was similar between the anastomosis and non-anastomosis ileum and colon. For all pigs and in all the investigated compartments, a cefuroxime concentration of 8 μg/mL was reached within 10 min after administration.

Research conclusions

Administering 1.5 g cefuroxime 10 min prior to intestine surgery seems sufficient, and effective concentrations are sustained for approximately 2 h. Only colon anastomosis was locally vulnerable to ischemia.

Research perspectives

The present study demonstrates that microdialysis can be used to investigate ischemic metabolites and cefuroxime concentrations in both anastomosis and non-anastomosis intestines. This method may therefore have the potential to result in a better future understanding of anastomotic leakage.

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REFERENCES

1 **Snijders HS**, Wouters MW, van Leersum NJ, Kolfschoten NE, Henneman D, de Vries AC, Tollenaar RA, Bonsing BA. Meta-analysis of the risk for anastomotic leakage, the postoperative mortality caused by leakage in relation to the overall postoperative mortality. *Eur J Surg Oncol* 2012; **38**: 1013-1019 [PMID: 22954525 DOI: 10.1016/j.ejso.2012.07.111]

2 **Hyman N**, Manchester TL, Osler T, Burns B, Cataldo PA. Anastomotic leaks after intestinal anastomosis: it's later than you think. *Ann Surg* 2007; **245**: 254-258 [PMID: 17245179 DOI: 10.1097/01.sla.0000225083.27182.85]

3 **Sparreboom CL**, Wu ZQ, Ji JF, Lange JF. Integrated approach to colorectal anastomotic leakage:

Communication, infection and healing disturbances. *World J Gastroenterol* 2016; **22**: 7226-7235 [PMID: 27621570 DOI: 10.3748/wjg.v22.i32.7226]
4 **Zafar SN**, Khan MR, Raza R, Khan MN, Kasi M, Rafiq A, Jamy OH. Early complications after biliary enteric anastomosis for benign diseases: a retrospective analysis. *BMC Surg* 2011; **11**: 19 [PMID: 21864413 DOI: 10.1186/1471-2482-11-19]
5 **Meyer J**, Naiken S, Christou N, Liot E, Toso C, Buchs NC, Ris F. Reducing anastomotic leak in colorectal surgery: The old dogmas and the new challenges. *World J Gastroenterol* 2019; **25**: 5017-5025 [PMID: 31558854 DOI: 10.3748/wjg.v25.i34.5017]
6 **Sciuto A**, Merola G, De Palma GD, Sodo M, Pirozzi F, Bracale UM, Bracale U. Predictive factors for anastomotic leakage after laparoscopic colorectal surgery. *World J Gastroenterol* 2018; **24**: 2247-2260 [PMID: 29881234 DOI: 10.3748/wjg.v24.i21.2247]
7 **Oikonomakis I**, Jansson D, Hörer TM, Skoog P, Nilsson KF, Jansson K. Results of postoperative microdialysis intraperitoneal and at the anastomosis in patients developing anastomotic leakage after rectal cancer surgery. *Scand J Gastroenterol* 2019; **54**: 1261-1268 [PMID: 31630578 DOI: 10.1080/00365521.2019.1673476]
8 **Uçkay I**, Harbarth S, Peter R, Lew D, Hoffmeyer P, Pittet D. Preventing surgical site infections. *Expert Rev Anti Infect Ther* 2010; **8**: 657-670 [PMID: 20521894 DOI: 10.1586/eri.10.41]
9 **Bue M**, Hanberg P, Koch J, Jensen LK, Lundorff M, Aalbaek B, Jensen HE, Søballe K, Tøttrup M. Single-dose bone pharmacokinetics of vancomycin in a porcine implant-associated osteomyelitis model. *J Orthop Res* 2018; **36**: 1093-1098 [PMID: 29058823 DOI: 10.1002/jor.23776]
10 **Tøttrup M**, Bue M, Koch J, Jensen LK, Hanberg P, Aalbæk B, Fuursted K, Jensen HE, Søballe K. Effects of Implant-Associated Osteomyelitis on Cefuroxime Bone Pharmacokinetics: Assessment in a Porcine Model. *J Bone Joint Surg Am* 2016; **98**: 363-369 [PMID: 26935458 DOI: 10.2106/JBJS.O.00550]
11 **Mangram AJ**, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control* 1999; **27**: 97-132; quiz 133 [PMID: 10196487]
12 **Bue M**, Hanberg P, Thomassen MB, Tøttrup M, Thillemann TM, Søballe K, Birke-Sørensen H. Microdialysis for the Assessment of Intervertebral Disc and Vertebral Cancellous Bone Metabolism in a Large Porcine Model. *In Vivo* 2020; **34**: 527-532 [PMID: 32111750 DOI: 10.21873/invivo.11804]
13 **Hanberg P**, Bue M, Öbrink-Hansen K, Kabel J, Thomassen M, Tøttrup M, Søballe K, Stilling M. Simultaneous Retrodialysis by Drug for Cefuroxime Using Meropenem as an Internal Standard-A Microdialysis Validation Study. *J Pharm Sci* 2020; **109**: 1373-1379 [PMID: 31756324 DOI: 10.1016/j.xphs.2019.11.014]
14 **Emmertsen KJ**, Wara P, Soerensen FB, Stolle LB. Intestinal microdialysis--applicability, reproducibility and local tissue response in a pig model. *Scand J Surg* 2005; **94**: 246-251 [PMID: 16259177 DOI: 10.1177/145749690509400314]
15 **Sommer T**, Larsen JF. Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischaemia. *Br J Surg* 2004; **91**: 855-861 [PMID: 15227691 DOI: 10.1002/bjs.4586]
16 **Ansorge C**, Regner S, Segersvärd R, Strömmer L. Early intraperitoneal metabolic changes and protease activation as indicators of pancreatic fistula after pancreaticoduodenectomy. *Br J Surg* 2012; **99**: 104-111 [PMID: 22052299 DOI: 10.1002/bjs.7730]
17 **Ellebæk M**, Qvist N, Fristrup C, Mortensen MB. Mediastinal microdialysis in the diagnosis of early anastomotic leakage after resection for cancer of the esophagus and gastroesophageal junction. *Am J Surg* 2014; **208**: 397-405 [PMID: 24656920 DOI: 10.1016/j.amjsurg.2013.09.026]
18 **Nowak G**, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon BG. Clinical experience in continuous graft monitoring with microdialysis early after liver transplantation. *Br J Surg* 2002; **89**: 1169-1175 [PMID: 12190684 DOI: 10.1046/j.1365-2168.2002.02187.x]
19 **Tøttrup M**, Søballe K, Bibby BM, Hardlei TF, Hansen P, Fuursted K, Birke-Sørensen H, Bue M. Bone, subcutaneous tissue and plasma pharmacokinetics of cefuroxime in total knee replacement patients - a randomized controlled trial comparing continuous and short-term infusion. *APMIS* 2019; **127**: 779-788 [PMID: 31515843 DOI: 10.1111/apm.12996]
20 **Hanberg P**, Bue M, Öbrink-Hansen K, Thomassen M, Søballe K, Stilling M. Timing of Antimicrobial Prophylaxis and Tourniquet Inflation: A Randomized Controlled Microdialysis Study. *J Bone Joint Surg Am* 2020; **102**: 1857-1864 [PMID: 32769808 DOI: 10.2106/JBJS.20.00076]
21 **Korth U**, Merkel G, Fernandez FF, Jandewerth O, Dogan G, Koch T, van Ackern K, Weichel O, Klein J. Tourniquet-induced changes of energy metabolism in human skeletal muscle monitored by microdialysis. *Anesthesiology* 2000; **93**: 1407-1412 [PMID: 11149434 DOI: 10.1097/00000542-200012000-00011]
22 **Ungerstedt U**, Rostami E. Microdialysis in neurointensive care. *Curr Pharm Des* 2004; **10**: 2145-2152 [PMID: 15281890 DOI: 10.2174/1381612043384105]
23 **Kho CM**, Enche Ab Rahim SK, Ahmad ZA, Abdullah NS. A Review on Microdialysis Calibration Methods: the Theory and Current Related Efforts. *Mol Neurobiol* 2017; **54**: 3506-3527 [PMID: 27189617 DOI: 10.1007/s12035-016-9929-8]
24 **Chaurasia CS**, Müller M, Bashaw ED, Benfeldt E, Bolinder J, Bullock R, Bungay PM, DeLange EC, Derendorf H, Elmquist WF, Hammarlund-Udenaes M, Joukhadar C, Kellogg DL Jr, Lunte CE, Nordstrom CH, Rollema H, Sawchuk RJ, Cheung BW, Shah VP, Stahle L, Ungerstedt U, Welty DF, Yeo H. AAPS-FDA workshop white paper: microdialysis principles, application and regulatory

perspectives. *Pharm Res* 2007; **24**: 1014-1025 [PMID: 17458685 DOI: 10.1007/s11095-006-9206-z]

25 **European Society of Clinical Microbiology and Infectious Diseases**. Available from: https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=performSearch&BeginIndex=0&Micdif=mic&NumberIndex=50&Antib=46&Specium=-1,2020

26 **Hanberg P**, Öbrink-Hansen K, Thorsted A, Bue M, Tøttrup M, Friberg LE, Hardlei TF, Søballe K, Gjedsted J. Population Pharmacokinetics of Meropenem in Plasma and Subcutis from Patients on Extracorporeal Membrane Oxygenation Treatment. *Antimicrob Agents Chemother* 2018; **62** [PMID: 29530848 DOI: 10.1128/aac.02390-17]

27 **Ellebæk MB**, Daams F, Jansson K, Matthiessen P, Cosse C, Fristrup C, Ellebæk SB, Sabroe JE, Qvist N. Peritoneal microdialysis as a tool for detecting anastomotic leakage in patients after left-side colon and rectal resection. A systematic review. *Scand J Gastroenterol* 2018; **53**: 1625-1632 [PMID: 30457391 DOI: 10.1080/00365521.2018.1533033]

28 **Swindle MM**, Makin A, Herron AJ, Clubb FJ Jr, Frazier KS. Swine as models in biomedical research and toxicology testing. *Vet Pathol* 2012; **49**: 344-356 [PMID: 21441112 DOI: 10.1177/0300985811402846]

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ORIGINAL ARTICLE

Retrospective Study

Association of non-alcoholic fatty liver disease with gallstone disease in the United States hospitalized patient population

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Abstract

BACKGROUND

Gallstones and cholecystectomy have been proposed as risk factors for non-alcoholic fatty liver disease (NAFLD). The reason for this may be that both gallstones, as well as NAFLD share several risk factors with regards to their development. Currently, there is a lack of sufficient evidence showing an association between these clinical conditions.

AIM

To determine whether there is a meaningful association between gallstones and cholecystectomy with NAFLD.

METHODS

We queried the National Inpatient Sample database from the years 2016 and 2017 using International Classification of Diseases, 10th revision, Clinical Modification diagnosis codes to identify hospitalizations with a diagnosis of gallstone disease (GSD) (includes calculus of gallbladder without cholecystitis without obstruction and acquired absence of gallbladder) as well as NAFLD (includes simple fatty liver and non-alcoholic steatohepatitis). Odds ratios (ORs) measuring the association between GSD (includes gallstones and cholecystectomy) and NAFLD were calculated using logistic regression after adjusting for confounding variables.

RESULTS

Out of 14294784 hospitalizations in 2016-2017, 159259 were found to have NAFLD. The prevalence of NAFLD was 3.3% in patients with GSD and 1% in those without. NAFLD was prevalent in 64.3% of women with GSD as compared to 35.7% of men with GSD. After controlling for various confounders associated with NAFLD and GSD, multivariate-adjusted analysis showed that there was an association between NAFLD with gallstones [OR = 6.32; 95% confidence interval (CI): 6.15-6.48] as well as cholecystectomy (OR = 1.97; 95%CI: 1.93-2.01). The association between NAFLD and gallstones was stronger in men (OR = 6.67; 95%CI: 6.42-6.93) than women (OR = 6.05; 95%CI: 5.83-6.27). The association between NAFLD and cholecystectomy was stronger in women (OR = 2.01; 95%CI: 1.96-2.06) than men (OR = 1.85; 95%CI: 1.79-1.92). *P* value was less than 0.001 for all comparisons.

CONCLUSION

NAFLD is more prevalent in women with GSD than men. The association between NAFLD and cholecystectomy/gallstones indicates that they may be risk factors for NAFLD.

Key Words: Gallstones; Non-alcoholic fatty liver disease; Gastroenterology; Hepatology; Non-alcoholic steatohepatitis; Cholecystectomy

Core Tip: We have identified a significant association between gallstone disease and non-alcoholic fatty liver disease. This association is stronger in women with gallstone disease than men. Further, this association is strongest in the Caucasian population. It is believed that this association is due to both physiologic changes post-cholecystectomy as well as the presence of metabolic derangement common to the development of both disorders. Lifestyle modification, including weight loss, dietary alterations, exercise, decreasing alcohol intake, and screening for the development of hepatic malignancy are important in preventing the development/progression of non-alcoholic fatty liver disease.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of hepatic steatosis in the absence of other etiologies responsible for secondary fatty deposition in the liver. NAFLD is a spectrum of disease characterized initially by hepatic steatosis with gradual progression to liver fibrosis and ultimately, end stage liver disease. It is

interpretation of the data, revision of critically important intellectual content, final approval of the version to be published, and agreement of accountability for all aspects of the work; Singh J, Wani F, Albosta M and Aljadah M are credited with interpretation of the data, literature review of all sections, revision of important intellectual content, final approval of the version published, and agreement of accountability of all aspects of the work; Shah H, Khan H and Jafri SM are credited with interpretation of data, literature review, specifically for the discussion section, revision of the work for critically important intellectual content, final approval of the version published, and agreement of accountability for all aspects of the work.

Institutional review board statement: The study presented in the current manuscript, which utilizes data from the Health Cost and Utilization Project, meets all relevant ethical and regulatory standards. These data, which were received by the investigators completely deidentified, required a local data custodian, and all investigators who accessed the data completed HCUP appropriate data use training and signed data use agreements. As such, our use of the HCUP data met the Central Michigan University IRB policy that such data use is not human subjects research, and does not require their review or approval.

Informed consent statement: Consent was not obtained but the presented data are anonymized and risk of identification is low.

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one of the most frequent causes of liver disease in the United States (US), with recent studies reporting a prevalence as high as 20%-30%. Additionally, the prevalence of NAFLD continues to rise globally in line with the obesity epidemic[1,2]. Although believed to be a slowly progressive disease, it is currently the third most common reason for liver transplant in the US[3].

Multiple co-morbidities closely associated with NAFLD have been identified, including visceral obesity, type 2 diabetes mellitus (T2DM), dyslipidemia and the metabolic syndrome. Other potential risk factors that may contribute to the development of NAFLD including gallstone disease (GSD), cholecystectomy, sleep deprivation, polycystic ovarian syndrome, hypertension and pituitary disorders are not well understood[1]. However, it has been well established that some degree of metabolic derangement and insulin resistance are involved in the core pathophysiology of the development of NAFLD. Our study evaluates GSD, which includes both a diagnosis of cholelithiasis as well as sequalae associated with this diagnosis, including cholecystectomy, and its' association with the development of NAFLD. Cholesterol gallstones are the most common type of gallstones and the risk factors for their formation are largely similar to those associated with NAFLD[4]. Hepatic insulin resistance is implicated in supersaturation and excessive production of bile salts. It is not clear whether the presence of gallstones are merely a reflection of the presence of risk factors for the metabolic syndrome, which accelerate NAFLD progression, or whether NAFLD leads to gallstone formation[5]. On the other hand, cholecystectomy is believed to alter the metabolism of the enterohepatic circulation of bile acids leading to an increased risk for the development of NAFLD. In 2013, Ruhl *et al*[6] used the US National Health and Nutrition Examination Survey (NHANES) from 1988 to 1994 to report that having had a cholecystectomy may be a risk factor for the development of NAFLD. More recently, Kakati *et al*[7] found an increased prevalence of cholecystectomy among NAFLD patients than non-NAFLD patients at a tertiary care center in the US. However, the sample size of this study was small (379 patients) and it was confined to one center. Hence, a direct correlation between cholecystectomy and NAFLD has yet to be proven.

There is a paucity of literature and data in terms of large-scale multicenter retrospective studies investigating an association between GSD and NAFLD. The purpose of our study is to determine whether an association between GSD and NAFLD exists, identify the prevalence of multiple co-morbidities associated with NAFLD, and discuss risk factor modification for the prevention of the development of NAFLD in addition to halting its progression to end stage liver disease.

MATERIALS AND METHODS

Data source

The National Inpatient Sample (NIS) is a publicly available all-payer Healthcare Cost and Utilization Project (HCUP) database designed to produce US regional and national estimates[8,9]. HCUP is a family of healthcare databases and related software tools and products released by the Agency for Healthcare Research and Quality. The NIS contains data from more than 7 million hospital stays every year and approximates a 20% stratified sample of discharges from US community hospitals[9]. HCUP databases are limited data sets. Under the Health Insurance Portability and Accountability Act, review by an Institutional Review Board (IRB) is not required for the use of limited data sets[10]. Therefore, our study was exempt from IRB review.

Study design

This is a retrospective study utilizing the NIS dataset from 2016 and 2017 using International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) diagnosis codes. GSD was identified using the codes K80.20 (calculus of gallbladder without cholecystitis without obstruction) and Z90.49 (acquired absence of gallbladder). The NAFLD cohort was then generated using the codes K76.0 (simple fatty liver) and K75.81 (non-alcoholic steatohepatitis, NASH) as depicted in Supplementary Table 1. Co-morbidities and known risk factors were identified using the ICD-10 codes depicted in Supplementary Table 1.

Statistical analysis

SAS 9.3 (SAS Institute, Cary, NC, United States) was used for data analysis. Statistical review was performed by a biomedical statistician. We used NIS designated weights to produce nationally representative estimates of disease prevalence and demographic

variations. A two-tailed *P* value of less than 0.05 was considered statistically significant. Age and sex standardized prevalence of NAFLD and GSD were generated. The unadjusted prevalence of NAFLD by GSD status was compared using the chi-square (χ^2) test. The differences in demographics and co-morbidities by GSD status were also compared using the χ^2 test. We used multivariate logistic regression analysis to determine odds ratios (ORs) for NAFLD comparing GSD to those without GSD after adjusting for age, gender, race, alcohol abuse, diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, nicotine dependence, and obesity. Owing to the gender differences in the prevalence of NAFLD and GSD, ORs for men and women were also calculated separately.

RESULTS

The total sample size of the study population for the years 2016-2017 was *n* = 14294784. The total number of hospitalizations with GSD as a primary diagnosis was *n* = 534015 (87769 gallstone diagnoses and 448932 cholecystectomy diagnoses). The sample size of the NAFLD cohort was *n* = 159259, with simple fatty liver accounting for 123549 hospitalizations and NASH accounting for 36440 hospitalizations.

The prevalence of NAFLD was 3.3% in GSD hospitalizations and 1.0% in non-GSD hospitalizations. Patients with GSD and NAFLD were more likely to be older, and more likely to be women (Table 1). In white patients, GSD hospitalizations (72.1%) were more common than non-GSD hospitalizations (64.9%). For other racial groups, the percentage of non-GSD hospitalizations was higher than GSD hospitalizations. Medicare was the primary payor for 58% of hospitalizations with GSD in contrast to 39.4% of non-GSD hospitalizations. Alcohol abuse, diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, and nicotine dependence were seen in a higher proportion of GSD hospitalizations than non-GSD hospitalizations. In contrast, obesity was more common in non-GSD hospitalizations than GSD hospitalizations (Table 1). All the differences between the two groups were significant at $P < 0.001$.

After adjusting for age (Table 2), patients with gallstones were 6.85 times more likely to have NAFLD [OR = 6.85; 95% confidence interval (CI): 6.67-7.03, $P < 0.001$] whereas those with cholecystectomy were 2.14 times more likely to have NAFLD. In addition, after adjusting for age, men with gallstones were more likely to have NAFLD than women, whereas women with cholecystectomy were more likely to have NAFLD than men.

In multivariate-adjusted analysis (Table 2), patients with gallstones were 6.32 times more likely to have NALFD (OR = 6.32; 95%CI: 6.15-6.48, $P < 0.001$) and patients with cholecystectomy were 1.97 times more likely to have NAFLD (OR = 1.97; 95%CI: 1.93-2.01, $P < 0.001$). Also, in the sex-adjusted analysis, the association of NAFLD with gallstones was found to be stronger in men (OR = 6.67; 95%CI: 6.42-6.93, $P < 0.001$) than in women (OR = 6.05; 95%CI: 5.83-6.27, $P < 0.001$). The association of NAFLD with cholecystectomy was found to be stronger in women (OR = 2.01; 95%CI: 1.96-2.06, $P < 0.001$) than in men (OR = 1.85; 95%CI: 1.79-1.92, $P < 0.001$).

DISCUSSION

It has previously been established that gallstones and cholecystectomy are independently associated with NAFLD after adjustment for metabolic risk factors, especially in Asian populations[11,12]. In our study we report that the prevalence of NAFLD is 3.3% in patients with GSD, which includes both the presence of gallstones and history of cholecystectomy, and 1% in those without GSD. After controlling for various confounders associated with NAFLD and GSD, a multivariate-adjusted analysis showed that there was significant association between NAFLD with gallstones as well as cholecystectomy. The exact pathophysiology behind the presence of gallstones leading to NAFLD is not well understood, however, the association between gallstones and NAFLD might stem from the common pathogenic factors shared by both gallstone formation and NAFLD, given that the risk for gallstones is high in patients with central obesity, type 2 diabetes and insulin resistance[13]. The removal of the gallbladder has a metabolic impact on NAFLD initiation and progression. Once the gallbladder is removed, bile is continuously secreted into the small intestine. This leads to quicker enterohepatic circulation of bile acids, consequently leading to a greater influx of bile acids into the liver[6]. Also, the gallbladder is the main site of fibroblast growth factor 19 (FGF19) expression in the

Table 1 Age- and sex-standardized characteristics of hospitalizations

	No gallstone disease (*n* = 13760769)	All gallstone disease (*n* = 534015)	*P* value
NAFLD (%)	1.0	3.3	< 0.001
Age, yr (mean ± SD)	48.8 ± 27.6	63.2 ± 18.1	< 0.001
Gender (%)			
Male	43.7	35.7	< 0.001
Female	56.3	64.3	< 0.001
Race (%)			
White	64.9	72.1	< 0.001
Black	15.4	11.8	< 0.001
Hispanic	12.4	11.2	< 0.001
Others	7.3	4.9	< 0.001
Payment (%)			
Medicare	39.4	58.0	< 0.001
Medicaid	23.5	13.6	< 0.001
Private insurance	29.9	22.8	< 0.001
Others (includes self-pay)	7.1	5.5	< 0.001
Co-morbidities (%)			
Alcohol abuse	1.6	2.3	< 0.001
Diabetes mellitus	13.2	18.9	< 0.001
Dyslipidemia	22.6	33.4	< 0.001
Hypertension	29.8	43.0	< 0.001
Metabolic syndrome	0.1	0.2	< 0.001
Nicotine dependence	29.6	40.1	< 0.001
Obesity	7.5	5.4	< 0.001

NAFLD: Non-alcoholic fatty liver disease.

enterohepatobiliary system[14]. FGF19 suppresses the ability of insulin to promote synthesis of hepatic fatty acid[15]. Barrera *et al*[16] found that cholecystectomy leads to reduced serum FGF19 levels and increased bile acid synthesis. Animal studies have shown that cholecystectomy leads to increased serum triglycerides and very low-density lipoprotein, which may contribute to increased triglyceride accumulation in the liver, and ultimately, NAFLD[11]. Because of this, we postulate that cholecystectomy may contribute to NAFLD initiation or progression.

Gender differences in NAFLD continues to be a debated topic, however, it is well known that NAFLD is a sexually dysmorphic condition[17]. After analysis of the NHANES data, most studies reported a higher prevalence of NAFLD in men than women[18]. However, the study by Younossi *et al*[19], which divides individuals into lean or obese-overweight subgroups, reported that the lean NAFLD cohort was more commonly female. Our study also focused on these gender differences. We report that NAFLD was prevalent in 64.3% of women with GSD as compared to 35.7% of men with GSD. The association between NAFLD with gallstones was found to be stronger in men (OR = 6.67; 95%CI: 6.42-6.93) than in women (OR = 6.05; 95%CI: 5.83-6.27) and the association between NAFLD with cholecystectomy was found to be stronger in women (OR = 2.01; 95%CI: 1.96-2.06) than in men (OR = 1.85; 95%CI: 1.79-1.92). The pathophysiology behind this difference is attributed to natural changes in female physiology, especially in the post-menopausal years, such as increased rates of insulin resistance, central obesity, and alterations in adipose tissue distribution as a result of fluctuations in estrogen levels[20]. It has also been noted that early menarche may predispose women to an increased risk of NAFLD in adulthood, due to the association between obesity and early onset of menses[17]. Animal studies in over nourished

Table 2 Logistic regression odds ratios for the association of non-alcoholic fatty liver disease with gallstone disease

	Age-adjusted			Multivariate-adjusted[1]		
	OR	95%CI	*P* value	OR	95%CI	*P* value
All						
Gallstone disease[2]	2.99	2.94-3.03	< 0.001	2.75	2.70-2.79	< 0.001
Gallstones	6.85	6.67-7.03	< 0.001	6.32	6.15-6.48	< 0.001
Cholecystectomy	2.14	2.10-2.18	< 0.001	1.97	1.93-2.01	< 0.001
Men						
Gallstone disease	3.09	3.01-3.17	< 0.001	2.90	2.82-2.97	< 0.001
Gallstones	7.32	7.05-7.59	< 0.001	6.67	6.42-6.93	< 0.001
Cholecystectomy	1.95	1.88-2.02	< 0.001	1.85	1.79-1.92	< 0.001
Women						
Gallstone disease	2.93	2.88-3.00	< 0.001	2.63	2.58-2.69	< 0.001
Gallstones	6.48	6.26-6.72	< 0.001	6.05	5.83-6.27	< 0.001
Cholecystectomy	2.25	2.20-2.30	< 0.001	2.01	1.96-2.06	< 0.001

[1]Adjusted for age, gender, race, alcohol abuse, diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, nicotine dependence, and obesity.
[2]Gallstone disease includes both gallstones and cholecystectomy. OR: Odds ratio; CI: Confidence interval.

zebrafish models have shown that ovarian senescence causing hypoestrogenemia facilitates the development of hepatic steatosis and the fibrotic progression of liver disease[21]. Understanding gender differences in NAFLD is crucial as it will allow us to target specific groups to improve primary prevention and health promotion, as well as provide treatment strategies which may help reduce morbidity and mortality associated with NAFLD and its associated pathologies.

Significant racial differences exist with regards to the prevalence of NAFLD. Our study reports that the prevalence of NAFLD with GSD was 72.1% in the Caucasian population, followed by 11.8% in African Americans, 11.2% in Hispanics and 4.9% in other races ($P < 0.001$). This is most likely attributed to complex interactions between environmental, behavioral, and genetic factors[3,18]. One explanation for the racial variance may be a higher average BMI and visceral adiposity in the Caucasian population as compared to their African American counterparts. The most recent literature suggests that the East Asian Indian population may be at the highest risk of NAFLD[22].

Our study reports a higher prevalence of NAFLD in the GSD group. Numerous co-morbidities have been identified in the GSD disease group which could possibly be linked to the development of NAFLD, with most having some form of metabolic derangement or insulin resistance as the core pathophysiology. We analyzed some of the common co-morbidities associated with GSD, as follows:

T2DM or insulin resistance

NAFLD and T2DM often co-exist, leading to adverse outcomes[23]. The presence of NAFLD is also associated with an increased incidence of the microvascular complications of T2DM. We found that T2DM had a prevalence of 18.9% in the GSD group as compared to 13.2% in the group without GSD ($P < 0.001$). Therefore, patients with T2DM should be screened for NAFLD. Elastography, a technique used to measure tissue stiffness, can be used as a screening tool for NAFLD.

Metabolic syndrome

This is defined as a cluster of conditions that occur in conjunction leading to an increased risk of developing cardiovascular disease, diabetes, and stroke. There is a strong association between the metabolic syndrome and NAFLD[24]. Metabolic syndrome is characterized by the presence of 3 out of the following 5 criteria: (1) Abdominal obesity; waist circumference ≥ 102 cm in men and ≥ 88 cm in women; (2) Serum triglycerides ≥ 150 mg/dL or drug treatment for elevated triglycerides; (3) Serum high-density lipoprotein (HDL) cholesterol < 40 mg/dL in men and < 50

mg/dL in women or drug treatment for low HDL cholesterol; (4) Blood pressure ≥ 130/85 mmHg or drug treatment for elevated blood pressure; and (5) Fasting plasma glucose ≥ 100 mg/dL or drug treatment for elevated blood glucose.

We found that there was a higher prevalence of patients having metabolic syndrome in the GSD group (0.2%) *vs* the group without GSD (0.1%) ($P < 0.001$). Therefore, Metabolic syndrome may be closely associated with NAFLD.

Dyslipidaemia

Elevated levels of free fatty acids promote insulin resistance leading to NAFLD[25]. We found that dyslipidaemia was more common in patients in the GSD group (33.4%) compared to the non-GSD group (22.6%) ($P < 0.001$). Hypertriglyceridemia is known to be an independent risk factor for NAFLD and therefore requires appropriate screening. The Framingham Heart study also revealed that patients with fatty liver had a high prevalence of hypertriglyceridemia and low HDL levels[26].

Obesity

Weight gain is a modifiable risk factor believed to be strongly associated with the development of NAFLD. The distribution of the adipose tissue, rather than the amount, is more clearly associated with NAFLD. However, our study found a higher prevalence of obesity (7.5%) in the group without GSD compared to the GSD group (5.4%) ($P < 0.001$). Nevertheless, it has been well established that visceral adipose tissue predisposes patients to the development of NAFLD even at lower body mass index[27].

Hypertension

Essential hypertension has been known to be associated with metabolic syndrome which in-turn is associated with the development of NAFLD. Other mechanisms for hypertension leading to NAFLD development are poorly understood[28]. Our study reported a 43% prevalence of hypertension in the GSD group and a 29.8% prevalence in the group without GSD ($P < 0.001$). Hence, we advocate for a more aggressive approach to screening and treatment for hypertension.

Nicotine dependence

Some studies on humans and animal models indicate that smoking has some association with NAFLD. However, the clinical correlation of these findings remains controversial[29]. We looked at nicotine dependence and found that the prevalence of NAFLD is 40.1% in nicotine dependent patients in the GSD group *vs* 29.6% in the group without GSD ($P < 0.001$). More studies are needed to establish a significant relationship between the use of nicotine and NAFLD.

Patients with NAFLD tend to have a decreased survival rate when compared to the general population. Cardiovascular disease is a major cause of death in these patients, followed by malignancies such as hepatocellular carcinoma, as well as increased morbidity and mortality due to the sequela of chronic liver disease itself[30]. Despite a thorough investigation into available clinical trials, no effective treatment therapy or protocol currently exists for NAFLD. Therefore, a non-pharmacological approach for controlling the co-morbidities leading to not only NAFLD but also cardiovascular risk and overall mortality becomes crucial in the management of the NAFLD population. Correcting the core pathophysiology (often underlying metabolic derangement or insulin resistance) is the basis of the management of NAFLD. It can be achieved through a patient-tailored approach of risk factor modification through lifestyle changes, which consists of:

Weight loss

Weight loss of 3%-5% in patients with steatosis and 7%-10% in patients with NASH is recommended, with the long-term goal of achieving a normal BMI[31]. A 5% weight loss is associated with about a 75% rate of remission of NAFLD[32].

Diet

Patients with dyslipidaemia benefit from a low-fat diet, whereas patients with insulin resistance or Diabetes Mellitus should be advised to follow a low carbohydrate diet. Patients with NAFLD and NASH eat a lower polyunsaturated *vs* saturated Fatty Acid ratio, a lower omega-3 *vs* omega-6 ratio, and a higher amount of cholesterol, as compared to the general population[3]. Therefore, it is recommended to modify the diet to include more polyunsaturated fatty acids, omega-3 fatty acids, and decrease cholesterol consumption to less than 200 mg a day.

Exercise

The literature suggests that exercise alone has a beneficial effect in NAFLD. Exercise along with dietary modification may play a synergistic role in the management of NAFLD. Aerobic exercise, about 3-4 times a week with > 400 kcal per session is currently recommended.

Alcohol intake

The literature suggests a U-relationship between alcohol consumption, with lower intake associated with decreased overall mortality, decreased rates of cardiovascular events, lower risk of Diabetes Mellitus, and decreased incidence of the metabolic syndrome[3]. Studies have also shown a possible beneficial effect of mild alcohol consumption in NAFLD. However, we do not actively recommend alcohol consumption in NAFLD patients especially in those suffering from progressive NAFLD.

Coffee consumption

Animal and epidemiological studies reveal a possible beneficial effect of coffee consumption in terms of metabolic control and development of NAFLD[33]. Because of this, coffee consumption should not be restricted.

Cancer screening

Patients with NAFLD are at increased risk for developing malignancies. The literature does report the incidence of liver cancer in patients with NAFLD without fibrosis but not enough evidence exists to recommend a screening protocol[3]. Patients with NAFLD should also be monitored in regular screening programs for breast, prostate, colorectal and cervical cancer.

In addition to lifestyle modification, pharmacological therapies such as orlistat may be considered for assistance with weight reduction. Bariatric surgery may be considered in moderately to severely obese patients. In fact, a systematic review by Bower *et al*[34] found that among 16 studies evaluating steatosis before and after bariatric surgery, the weighted mean decrease in the incidence of steatosis was 50.2%.

There are limitations to the current study, in particular with regards to the utilization of the Healthcare Utilization Project database, including errors in relation to the ICD9 and ICD10 coding system. In order to prevent this, we have utilized codes that have been validated in previous studies. We have performed a retrospective analysis and given insight into an association between these conditions and the studied outcomes, however the nature of observational studies does not allow for the determination of causation between the variables being studied. An additional limitation is that the ICD coding system is unable to identify when patients are readmitted with the same condition. Because of this, every admission is considered a separate case and therefore a new patient encounter. A final limitation of the study is that it was not performed on the general population.

CONCLUSION

We conclude that NAFLD is more prevalent in women with GSD than men. The association of NAFLD with cholecystectomy and GSD indicates that they may be risk factors for NAFLD. Lifestyle modification through physical exercise, diet, and weight reduction can prevent gallstone formation and the subsequent need for cholecystectomy. We know that gallstones are a common disease process and cholecystectomy is a commonly performed procedure. Their impact on NAFLD should be further evaluated through prospective studies and randomized clinical trials.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is one of the most frequent causes of liver disease in the United States. The prevalence of NAFLD is rising globally in line with the obesity epidemic. The pathophysiology of the development of NAFLD is rooted in metabolic derangement and insulin resistance. In addition, the development of gallstones shares several common risk factors with that of NAFLD. Cholecystectomy, a

sequela of gallstone disease (GSD), may alter the metabolism of the enterohepatic circulation of bile acids and contribute to an increased risk of NAFLD.

Research motivation

There is a paucity of literature and data in terms of large-scale multicenter retrospective studies that have investigated an association between GSD and NAFLD.

Research objectives

To determine whether an association between GSD and NAFLD exists, identify the prevalence of multiple co-morbidities associated with NAFLD, and discuss risk factor modification for the prevention of the development of NAFLD in addition to halting its progression to end stage liver disease.

Research methods

We queried the National Inpatient Sample database from the years 2016 and 2017 using International Classification of Diseases, 10th revision, Clinical Modification diagnosis codes to identify hospitalizations with a diagnosis of GSD as well as NAFLD. Odds ratios (ORs) measuring the association between GSD and NAFLD were calculated using logistic regression after adjusting for confounding variables.

Research results

The prevalence of NAFLD was 3.3% in patients with GSD and 1% in those without. NAFLD was prevalent in 64.3% of women with GSD as compared to 35.7% of men with GSD. After controlling for confounders, multivariate-adjusted analysis showed that there was an association between NAFLD with gallstones [OR = 6.32; 95% confidence interval (CI): 6.15-6.48] as well as cholecystectomy (OR = 1.97; 95%CI: 1.93-2.01). The association between NAFLD and gallstones was stronger in men (OR = 6.67; 95%CI: 6.42-6.93) than women (OR = 6.05; 95%CI: 5.83-6.27). The association between NAFLD with cholecystectomy was stronger in women (OR = 2.01; 95%CI: 1.96-2.06) than men (OR = 1.85; 95%CI: 1.79-1.92).

Research conclusions

NAFLD is more prevalent in women with GSD than men. The association between NAFLD and cholecystectomy/gallstones indicates that they may be risk factors for NAFLD.

Research perspectives

There is a need for further prospective studies and randomized clinical trials to evaluate the impact of gallstones and cholecystectomy on the development of NAFLD.

REFERENCES

1 **Perumpail BJ**, Khan MA, Yoo ER, Cholankeril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017; **23**: 8263-8276 [PMID: 29307986 DOI: 10.3748/wjg.v23.i47.8263]

2 **Le MH**, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, Nguyen MH. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. *PLoS One* 2017; **12**: e0173499 [PMID: 28346543 DOI: 10.1371/journal.pone.0173499]

3 **Machado MV**, Cortez-Pinto H. Non-alcoholic fatty liver disease: what the clinician needs to know. *World J Gastroenterol* 2014; **20**: 12956-12980 [PMID: 25278691 DOI: 10.3748/wjg.v20.i36.12956]

4 **Shabanzadeh DM**. Incidence of gallstone disease and complications. *Curr Opin Gastroenterol* 2018; **34**: 81-89 [PMID: 29256915 DOI: 10.1097/MOG.0000000000000418]

5 **Ahmed MH**, Ali A. Nonalcoholic fatty liver disease and cholesterol gallstones: which comes first? *Scand J Gastroenterol* 2014; **49**: 521-527 [PMID: 24646400 DOI: 10.3109/00365521.2014.894119]

6 **Ruhl CE**, Everhart JE. Relationship of non-alcoholic fatty liver disease with cholecystectomy in the US population. *Am J Gastroenterol* 2013; **108**: 952-958 [PMID: 23545713 DOI: 10.1038/ajg.2013.70]

7 **Kakati D**, Kumar U, Russ K, Shoreibah M, Kuo YF, Jackson B, Singal AK. Cholecystectomy does not worsen progression or outcomes in non-alcoholic fatty liver disease. *Transl Gastroenterol Hepatol* 2020; **5**: 3 [PMID: 32190771 DOI: 10.21037/tgh.2019.09.03]

8 **Healthcare cost and utilization Project**. NIS Database Documentation. (cited March 31 2020). Available from: https://www.hcup-us.ahrq.gov/db/nation/nis/nisdbdocumentation.jsp

9 **Healthcare Cost and Utilization Project**. Overview of the National (Nationwide) Inpatient Sample (NIS). (cited March 31 2020). Available from: https://www.hcup-us.ahrq.gov/nisoverview.jsp

10 **Healthcare Cost and Utilization Project**. Healthcare Cost and Utilization Project Data Use Agreement Course. (cited March 25 2020). Available from: https://www.hcup-us.ahrq.gov/DUA/dua_508/DUA508version.jsp

11 **Amigo L**, Husche C, Zanlungo S, Lütjohann D, Arrese M, Miquel JF, Rigotti A, Nervi F. Cholecystectomy increases hepatic triglyceride content and very-low-density lipoproteins production in mice. *Liver Int* 2011; **31**: 52-64 [PMID: 21040411 DOI: 10.1111/j.1478-3231.2010.02361.x]

12 **Liu J**, Lin H, Zhang C, Wang L, Wu S, Zhang D, Tang F, Xue F, Liu Y. Non-alcoholic fatty liver disease associated with gallstones in females rather than males: a longitudinal cohort study in Chinese urban population. *BMC Gastroenterol* 2014; **14**: 213 [PMID: 25496394 DOI: 10.1186/s12876-014-0213-y]

13 **Fracanzani AL**, Valenti L, Russello M, Miele L, Bertelli C, Bellia A, Masetti C, Cefalo C, Grieco A, Marchesini G, Fargion S. Gallstone disease is associated with more severe liver damage in patients with non-alcoholic fatty liver disease. *PLoS One* 2012; **7**: e41183 [PMID: 22848440 DOI: 10.1371/journal.pone.0041183]

14 **Zweers SJ**, Booij KA, Komuta M, Roskams T, Gouma DJ, Jansen PL, Schaap FG. The human gallbladder secretes fibroblast growth factor 19 into bile: towards defining the role of fibroblast growth factor 19 in the enterobiliary tract. *Hepatology* 2012; **55**: 575-583 [PMID: 21953282 DOI: 10.1002/hep.24702]

15 **Bhatnagar S**, Damron HA, Hillgartner FB. Fibroblast growth factor-19, a novel factor that inhibits hepatic fatty acid synthesis. *J Biol Chem* 2009; **284**: 10023-10033 [PMID: 19233843 DOI: 10.1074/jbc.M808818200]

16 **Barrera F**, Azócar L, Molina H, Schalper KA, Ocares M, Liberona J, Villarroel L, Pimentel F, Pérez-Ayuso RM, Nervi F, Groen AK, Miquel JF. Effect of cholecystectomy on bile acid synthesis and circulating levels of fibroblast growth factor 19. *Ann Hepatol* 2015; **14**: 710-721 [PMID: 26256900 DOI: 10.1016/S1665-2681(19)30766-5]

17 **Ballestri S**, Nascimbeni F, Baldelli E, Marrazzo A, Romagnoli D, Lonardo A. NAFLD as a Sexual Dimorphic Disease: Role of Gender and Reproductive Status in the Development and Progression of Nonalcoholic Fatty Liver Disease and Inherent Cardiovascular Risk. *Adv Ther* 2017; **34**: 1291-1326 [PMID: 28526997 DOI: 10.1007/s12325-017-0556-1]

18 **Pan JJ**, Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol* 2014; **6**: 274-283 [PMID: 24868321 DOI: 10.4254/wjh.v6.i5.274]

19 **Younossi ZM**, Stepanova M, Negro F, Hallaji S, Younossi Y, Lam B, Srishord M. Nonalcoholic fatty liver disease in lean individuals in the United States. *Medicine (Baltimore)* 2012; **91**: 319-327 [PMID: 23117851 DOI: 10.1097/MD.0b013e3182779d49]

20 **Summart U**, Thinkhamrop B, Chamadol N, Khuntikeo N, Songthamwat M, Kim CS. Gender differences in the prevalence of nonalcoholic fatty liver disease in the Northeast of Thailand: A population-based cross-sectional study. *F1000Res* 2017; **6**: 1630 [PMID: 29093809 DOI: 10.12688/f1000research.12417.2]

21 **Turola E**, Petta S, Vanni E, Milosa F, Valenti L, Critelli R, Miele L, Maccio L, Calvaruso V, Fracanzani AL, Bianchini M, Raos N, Bugianesi E, Mercorella S, Di Giovanni M, Craxì A, Fargion S, Grieco A, Cammà C, Cotelli F, Villa E. Ovarian senescence increases liver fibrosis in humans and zebrafish with steatosis. *Dis Model Mech* 2015; **8**: 1037-1046 [PMID: 26183212 DOI: 10.1242/dmm.019950]

22 **Singh S**, Kuftinec GN, Sarkar S. Non-alcoholic Fatty Liver Disease in South Asians: A Review of the Literature. *J Clin Transl Hepatol* 2017; **5**: 76-81 [PMID: 28507930 DOI: 10.14218/JCTH.2016.00045]

23 **Dharmalingam M**, Yamasandhi PG. Nonalcoholic Fatty Liver Disease and Type 2 Diabetes Mellitus. *Indian J Endocrinol Metab* 2018; **22**: 421-428 [PMID: 30090738 DOI: 10.4103/ijem.IJEM_585_17]

24 **Yki-Järvinen H**. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014; **2**: 901-910 [PMID: 24731669 DOI: 10.1016/S2213-8587(14)70032-4]

25 **Mendez-Sanchez N**, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP, Barranco-Fragoso B, Cordova-Gallardo J. New Aspects of Lipotoxicity in Nonalcoholic Steatohepatitis. *Int J Mol Sci* 2018; **19** [PMID: 30011790 DOI: 10.3390/ijms19072034]

26 **Speliotes EK**, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, Hirschhorn JN, O'Donnell CJ, Fox CS. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology* 2010; **51**: 1979-1987 [PMID: 20336705 DOI: 10.1002/hep.23593]

27 **Wong RJ**, Ahmed A. Obesity and non-alcoholic fatty liver disease: Disparate associations among Asian populations. *World J Hepatol* 2014; **6**: 263-273 [PMID: 24868320 DOI: 10.4254/wjh.v6.i5.263]

28 **Oikonomou D**, Georgiopoulos G, Katsi V, Kourek C, Tsioufis C, Alexopoulou A, Koutli E, Tousoulis D. Non-alcoholic fatty liver disease and hypertension: coprevalent or correlated? *Eur J Gastroenterol Hepatol* 2018; **30**: 979-985 [PMID: 30048367 DOI: 10.1097/MEG.0000000000001191]

29 **Akhavan Rezayat A**, Dadgar Moghadam M, Ghasemi Nour M, Shirazinia M, Ghodsi H, Rouhbakhsh Zahmatkesh MR, Tavakolizadeh Noghabi M, Hoseini B, Akhavan Rezayat K. Association between smoking and non-alcoholic fatty liver disease: A systematic review and meta-analysis. *SAGE Open Med* 2018; **6**: 2050312117745223 [PMID: 29399359 DOI: 10.1177/2050312117745223]

30 **Byrne CD**, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; **62**: S47-S64 [PMID: 25920090 DOI: 10.1016/j.jhep.2014.12.012]
31 **Chalasani N**, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018; **67**: 328-357 [PMID: 28714183 DOI: 10.1002/hep.29367]
32 **Promrat K**, Kleiner DE, Niemeier HM, Jackvony E, Kearns M, Wands JR, Fava JL, Wing RR. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 121-129 [PMID: 19827166 DOI: 10.1002/hep.23276]
33 **Marventano S**, Salomone F, Godos J, Pluchinotta F, Del Rio D, Mistretta A, Grosso G. Coffee and tea consumption in relation with non-alcoholic fatty liver and metabolic syndrome: A systematic review and meta-analysis of observational studies. *Clin Nutr* 2016; **35**: 1269-1281 [PMID: 27060021 DOI: 10.1016/j.clnu.2016.03.012]
34 **Bower G**, Toma T, Harling L, Jiao LR, Efthimiou E, Darzi A, Athanasiou T, Ashrafian H. Bariatric Surgery and Non-Alcoholic Fatty Liver Disease: a Systematic Review of Liver Biochemistry and Histology. *Obes Surg* 2015; **25**: 2280-2289 [PMID: 25917981 DOI: 10.1007/s11695-015-1691-x]

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MINIREVIEWS

Neuroimmunomodulation by gut bacteria: Focus on inflammatory bowel diseases

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Abstract

Microbes colonize the gastrointestinal tract are considered as highest complex ecosystem because of having diverse bacterial species and 150 times more genes as compared to the human genome. Imbalance or dysbiosis in gut bacteria can cause dysregulation in gut homeostasis that subsequently activates the immune system, which leads to the development of inflammatory bowel disease (IBD). Neuromediators, including both neurotransmitters and neuropeptides, may contribute to the development of aberrant immune response. They are emerging as a regulator of inflammatory processes and play a key role in various auto-immune and inflammatory diseases. Neuromediators may influence immune cell's function *via* the receptors present on these cells. The cytokines secreted by the immune cells, in turn, regulate the neuronal functions by binding with their receptors present on sensory neurons. This bidirectional communication of the enteric nervous system and the enteric immune system is involved in regulating the magnitude of inflammatory pathways. Alterations in gut bacteria influence the level of neuromediators in the colon, which may affect the gastrointestinal inflammation in a disease condition. Changed neuromediators concentration *via* dysbiosis in gut microbiota is one of the novel approaches to understand the pathogenesis of IBD. In this article, we reviewed the existing knowledge on the role of neuromediators governing the pathogenesis of IBD, focusing on the reciprocal relationship among the gut microbiota, neuromediators, and host immunity. Understanding the neuromediators and host-microbiota interactions would give a better insight in to the disease pathophysiology and help in developing the new therapeutic approaches for the disease.

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Core Tip: Dysbiosis in gut bacteria is a well-established factor, and the abnormality in the enteric nervous system is an emerging aspect that influences the gut inflammation. Both of them contribute to inflammatory bowel disease (IBD) pathogenesis by modulating the host immune response. Through this review, we linked the two pathological mechanisms and explained how neuroimmunomodulation by gut bacteria play a crucial role in IBD. We elaborated all the known neuromediators produced by gut bacteria and the role of each neuromediator as well as the respective gut bacteria in inflammatory signaling pathways especially in IBD.

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INTRODUCTION

The gastrointestinal tract (GIT) is equipped with the most extensive immune system, and the largest network of neurons outside the central nervous system (CNS) called the enteric nervous system (ENS). Sometimes, ENS also referred to as "brain in gut" because it does not require any intermediate input from the brain for its functioning. The structure of ENS is organised into two Plexi, myenteric plexus and submucosal (Meissner's) plexus. Myenteric plexus is located between the longitudinal and circular muscle of muscularis propria and regulates the intestinal motility. Submucosal plexus is located in the submucosa of the intestine and regulates secretion, absorption, and blood flow[1]. Neurons of these Plexi releases various neurotransmitters that regulate the secretory and motor functions of GIT. During inflammatory bowel disease (IBD), there are morphological, histological, and immunohistochemical abnormalities in the ENS which causes neuronal hyperplasia, necrosis, ganglion, and axonal degeneration, alteration in synthesis and release of neurotransmitters. It leads to a defect in the secretory and motor functions of GIT[2].

The neurotransmitters and neuropeptides released from ENS can alter various immune cell functions. Immune cells residing in colon express various receptors for neurotransmitters, and once neurotransmitter binds to these receptors, there would be an initiation of signal transduction pathways of cytokine production[3]. These cytokines, in turn, bind to their specific receptors, expressed on sensory nerve fibers to trigger neuronal response, thus establishing a bidirectional communication. This bidirectional cross-talk between ENS and the enteric immune system is crucial to maintain visceral homeostasis. This cross-talk regulates the magnitude of inflammatory response *via* the production of cytokines, disruption of epithelial tight junctions, neutrophil recruitment, phagocytosis, modification in lymphocyte differentiation, and ultimately cell death ensues[4].

During the early postnatal life, ENS undergoes extensive development in parallel to the colonisation of gut microbiota and maturation of mucosal immune system in GIT. In germ-free mice, structural and functional abnormalities of the ENS have been observed, which suggests the role of gut microbiota in ENS development. Microbiota interacts with the nervous system through modulation of neurotransmitters production. Indeed, bacteria have been found to have the capability to produce a range of significant neurotransmitters in the gut. Therefore, gut microbiota fine-tunes the interaction between enteric nervous and immune system by altering the level of neuromediators (Figure 1).

A more thorough understanding of the interactions among neuromediators, inflammation, and neuromediators producing gut microbiota is required to ensure the effectiveness of neuromediators as a treatment option for IBD. Herein, we review the current knowledge of the role of neuromediators and bacteria that produce neurome-

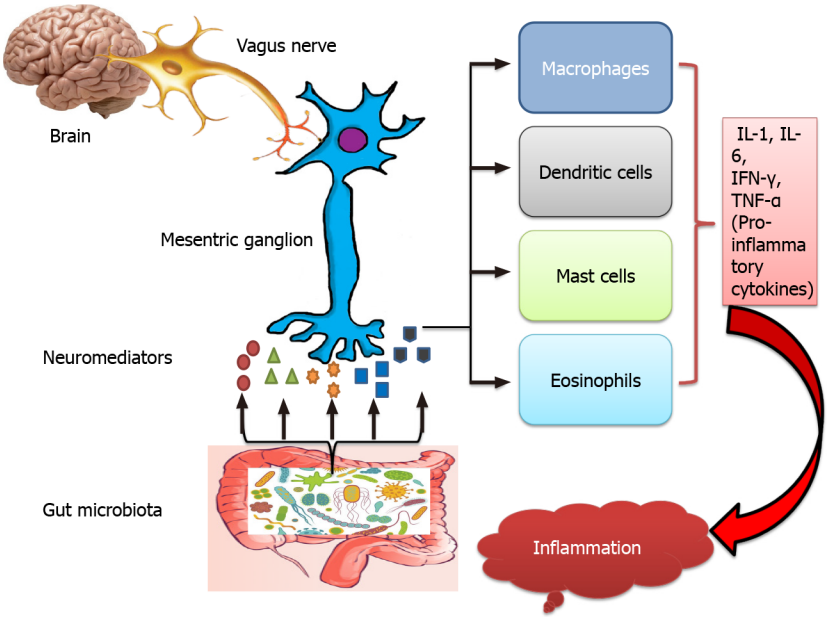

Figure 1 Modulation of cross-talk between the enteric nervous system and the enteric immune system *via* gut bacteria. Gut microbiota and vagus nerve stimulate mesenteric ganglion (enteric neuron) to produce neuromediators. Neuromediators act on various immune cells and influence their ability to release pro-inflammatory cytokines. During inflammatory bowel disease, dysbiosis in gut microbiota and abnormality in the enteric nervous system affect the level of neuromediators that results in overproduction of pro-inflammatory cytokines and promote inflammation. IL: Interleukin; TNF-α: Tumour necrosis factor-α; IFN-γ: Interferon-γ.

diators which might be a potential option in the treatment of IBD.

NEUROMEDIATORS AND IBD

A variety of neuropeptides and neurotransmitters are known to involve in the pathogenesis of IBD. Neuropeptides such as substance P (SP), neurotensin (NT), vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), corticotrophin-releasing hormone (CRH), galanin (GAL) and calcitonin gene-related peptides (CGRP) and neurotransmitters like serotonin, nitric oxide (NO), acetylcholine, noradrenaline (NA) and γ-aminobutyric acid (GABA) regulates inflammatory processes by employing immunomodulatory pathways. Role of each of these neuromediators are briefly summarized in Table 1.

SP

SP is released from neurons and also from inflammatory cells like lymphocytes, macrophages, and dendritic cells. It acts by binding to the neurokinin-1 receptor (NK-1R). It plays a vital role in the amplification of inflammatory response by inducing the release of cytokines, reactive oxygen species, and stimulates leukocyte recruitment. Increased level of SP has been observed in the colon of IBD patients and, in the synovial fluid and serum of rheumatoid arthritis (RA) patients. Also, the enhanced expression of NK-1R was reported in the colon of IBD and synoviocytes of RA patients. SP has pro-inflammatory effects in epithelial and immune cells and contributes to many inflammatory diseases, including sarcoidosis, asthma, chronic bronchitis, RA, and IBD[5]. However, in murine models of colitis, SP plays a regulatory action[6]. In a recent study, SP was observed as an accelerator for healing the dextran sodium sulfate (DSS)-induced damaged intestine *via* inhibiting inflammatory responses through the modulation of cytokine expression[7].

NT

NT is a tridecapeptide, a pro-inflammatory neuropeptide widely distributed in the

Table 1 List of neuromediators and their role in gut inflammation

Neuromediator	Distribution	Binding receptor	Function
SP	Neurons and inflammatory cells like lymphocytes, macrophages, and dendritic cells	NK-1R	Exerts pro-inflammatory effects in epithelial and immune cells and contributes to inflammatory diseases. In murine model of colitis, it plays regulatory role
NT	Nervous system and intestine	NTR1	Recognized as an immunomodulator. By interacting with immune cells, it enhances the chemotaxis and induces the cytokine release to modulate the immune response. In IBD, it exerts its pro-inflammatory effects by promoting the expression of miR-210 in intestinal epithelial cells
NPY	Central and peripheral nervous system and immune cells	Out of five receptors of NPY, NPYY1 is known to play a crucial role in immunomodulation	Regulates various immune cell functions such as T helper cell differentiation, neutrophil chemotaxis, natural killer cell activity, and granulocyte oxidative burst and NO production. In the gut, NPY is known to exert pro-inflammatory effects
VIP	Neuronal and lymphoid cells	VIPR1 and VIPR2	Identified as an anti-inflammatory molecule. administration of VIP nanomedicine in the form of VIP-SSM are capable of alleviating the symptoms of DSS- induced mice model of colitis
GAL	Vasculature, immune cells and colonic epithelial cells	GAL (1-3) receptor	Exerts anti-inflammatory effects in TNBS induced colitis model by reducing the expression and activity of iNOS
CRH	Immune cells	CRH-R1 and CRH-R2	It acts as a pro-inflammatory peptide. The expression pattern of CRH 1 and CRH 2 varies in ulcerative colitis. Inhibition of CRH1 and overexpression of CRH2 may have the therapeutic potential in IBD
CGRP	Sensory nerves projecting to the lymphoid organs, airways, and pulmonary neuroendocrine cells	CGRP receptors	CGRP negatively regulates innate immune responses and thus has potential anti-inflammatory effects. Its expression reduced in the colon of an animal model of colitis
NA	Nerves innervating the peripheral lymphoid organs	Adrenergic α and β receptors	immunomodulatory effect of NA is administered *via* cAMP. Activation of NA receptors that stimulate cAMP resulting in a shift toward Th2 responses which are anti-inflammatory and neuroprotective whereas decreased cAMP stimulates Th1 responses resulting in cell destruction and inflammation
Acetylcholine	Central and peripheral nervous system, immune cells, keratinocytes, endothelial cells, urothelial cells of the urinary bladder, airways and epithelial cells of the placenta	Nicotinic and muscarinic receptors	Muscarinic receptors mediate pro-inflammatory responses and nicotinic receptors enhance anti-inflammatory responses. Treatment of UC *via* nicotine suggests the role of the cholinergic pathway in colonic inflammation
NO	Neuron synapses and immune cells	NO does not act *via* receptors. its specificity for target cell depends on its concentration, its activity and response, and territory of target cells	NO is oxidised to reactive nitrogen oxide species which mediate most of the immunological effects. It regulates the growth, functional activity, and death of immune cells. It acts as a biomarker for monitoring disease activity due to its increased serum concentration during the active phase of both UC and CD and reduced concentration during the inactive phase of the disease
Serotonin or 5-HT	Central nervous system and EC cells of GIT	5-HT receptor	It promotes activation of lymphocytes and secretion of pro-inflammatory cytokines. It activates the signalling molecules of the NF-kB pathway during gut inflammation
GABA	Nervous system and immune system	GABA- AR and GABA-BR	GABA has several effects on immune cells, including modulation of cytokine secretion, regulation of cell proliferation, and migration. Activation of GABA-A receptor aggravates DSS induced mice model of colitis

SP: Substance P; GABA: γ-aminobutyric acid; 5-HT: 5-hydroxytryptamine; EC: Enterochromaffin; NF-kB: Nuclear factor kB; NO: Nitric oxide; NA: Noradrenaline; CD: Crohn's disease; UC: Ulcerative colitis; cAMP: Cyclic adenosine monophosphate; CGRP: Calcitonin gene-related peptide; IBD: Inflammatory bowel disease; CRH: Corticotropin-releasing hormone; iNOS: Inducible nitric oxide synthase; GAL: Galanin; TNBS: 2,4,6-trinitrobenzenesulfonic acid; VIP: Vasoactive intestinal peptide; SSM: Sterically stabilised micelles; NPY: Neuropeptide Y; NK-1R: Neurokinin-1 receptor; NTR1: Neurotensi receptor 1; DSS: Dextran sodium sulfate.

nervous system and intestine. It binds to NT receptor 1 (NTR1) which is a high-affinity receptor and expressed in neurons, immune cells, colonic epithelial cells and colon cancer cell lines. It regulates various peripheral processes including gut motility, intestinal epithelial cell proliferation, secretion, and vascular smooth muscle activity, but recently it is recognized as an immunomodulator. NT interacts with leukocytes, dendritic cells and peritoneal mast cells, inducing the release of cytokines and enhancing chemotaxis in order to modulate the immune response. The elevated level

of NT and increased expression of NTR1 have been reported in the colonic mucosa of the experimental model of colitis and ulcerative colitis (UC) patients. NT is implicated in various acute and chronic inflammatory diseases, including lung and intestinal inflammation[8-11]. In IBD, NT exerts its pro-inflammatory effects by promoting the expression of miR-210 in intestinal epithelial cells[12].

NPY

NPY is a peptide of 36 amino acids and produced abundantly by the central and peripheral nervous system and also by immune cells. Neuronal functions of NPY include modulation of blood pressure, nociception, anxiety, and appetite. It also has diverse effects on innate and adaptive immunity, including immune cell migration, cytokine release from macrophages and T helper cells, and antibody production. Out of five receptors of NPY, NPYY1 is known to play a crucial role in immunomodulation. To modulate inflammation, NPY regulates various immune cell functions such as T helper cell differentiation, neutrophil chemotaxis, natural killer cell activity, and granulocyte oxidative burst and NO production. In the gut, NPY is known to exert pro-inflammatory effects. Several clinical studies reported the role of NPY in immune or inflammatory disorders such as arthritis, asthma, and IBD[4,13-15].

VIP

VIP is a 28 amino acid neuropeptide, produced by neuronal cells and lymphoid cells. It controls the homeostasis of the immune system by carrying out a wide range of immunological functions. Recently, it has been identified as an anti-inflammatory molecule. It is reported to inhibit pro-inflammatory cytokines and chemokines production from macrophages, dendritic cells, and microglial cells. Furthermore, VIP reduces the expression of costimulatory molecules on antigen-presenting cells, resulting in the promotion of Th2 type responses and reduction in Th1 type responses. VIP has been considered as a promising target for the treatment of autoimmune as well as acute and chronic inflammatory diseases such as multiple sclerosis, RA, Crohn's disease (CD), septic shock, or autoimmune diabetes[4,16-18]. Recombinant VIP analogue protects the intestinal mucosal barrier function effectively in rats. This analogue of VIP ameliorates 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colonic injury and inflammation through downregulating the expression of tumour necrosis factor-α and upregulating the interleukin (IL)-10 expression[15,19]. Though the administration of VIP shown anti-inflammatory effect but its therapeutic use is restricted due to its rapid degradation and continuous infusion. Recently, the administration of VIP nanomedicine in the form of sterically stabilized micelles has been observed to overcome the barriers and are capable of alleviating the symptoms of DSS-induced mice model of colitis[20].

GAL

GAL is a 30 amino acid long sensory neuropeptide known to attenuate neurogenic inflammation. Among the receptors (GAL1-3), GAL-3 is most abundantly expressed on the vasculature, and immune cells and GAL-1 is the only receptor expressed in colonic epithelial cells. Various studies indicate the role of GAL-3 in inflammatory disease conditions. GAL-1 has multiple recognition sites for nuclear factor kB (NF-kB), and its expression is increased in colonic tissues of IBD patients. NF-kB is a significant player in IBD; thus, specific antagonists of GAL-1 may be used in the treatment of IBD. Administration of GAL in the TNBS-induced colitis model exerts anti-inflammatory effects by reducing the expression and activity of inducible NO synthase (iNOS)[21]. GAL may act as an immunomodulatory peptide because of its ability to sensitize natural killer cells and polymorphonuclear neutrophils towards pro-inflammatory cytokines. In neutrophil-dominated autoimmune arthritis, activation of GAL-3 can be considered as a substantial anti-inflammatory pathway. In multiple sclerosis, GAL-2 agonist has been reported to be a promising therapeutic target[22-26].

CRH

CRH is 41 amino acid neuropeptide, produced by various immune cells to regulate immune/inflammatory responses. This locally produced CRH in the peripheral organs, also called peripheral CRH. Peripheral CRH is expressed in various inflamed sites where it acts as a pro-inflammatory peptide. It is also found in the testes, adrenal medulla, ovaries, GIT, cardiovascular system, spinal cord, pancreas, lung, endometrium, and placenta. It has also shown pro-inflammatory effects in the female reproductive system. CRH exerts its biological effects by CRH-Receptor R1 and CRH-R2. CRH and CRH-Rs are known to be expressed in several components of the immune system and regulates various inflammatory phenomena. Due to its pro-inflammatory properties, the antagonist of CRH has been proposed as a potential therapeutic target in the treatment of allergic conditions (asthma, eczema, urticaria) and also in the treatment of lower gastrointestinal inflammatory diseases (chronic inflammatory bowel syndromes, irritable bowel disease, and UC)[23,27]. The expression pattern of CRH-1 and CRH-2 is found to be altered in UC. Based on their differential expression, their therapeutic role is advocated in IBD. Inhibition of CRH-1 and overexpression of CRH-2 may have the therapeutic potential[28]. Activation of CRH-1 signaling upregulates the production of vascular endothelial growth factor-A *via* cyclic adenosine monophosphate (cAMP) response-element binding protein (CREB) transcriptional activity, which results in inflammatory angiogenesis in the gut. Therefore by targeting CREB inactivation, symptoms of colitis may be ameliorated[29]. CRH is also reported to enhance gut permeability by activating mast cells that worsen the IBD pathogenesis. Thus blocking CRH receptors with appropriate antagonists can inhibit mast cell activation and may be considered as a promising therapeutic target for chronic gastrointestinal inflammatory diseases, including IBD[30,31].

CGRP

CGRP is a 37 amino acid peptide that is expressed by sensory nerves projecting to the lymphoid organs, airways, and by pulmonary neuroendocrine cells. Peripheral CGRP is a vasodilator and responsible for acute neurogenic inflammation. It upregulates the expression of IL-10 and inhibits activation of NF-kB by acting on innate immune cells. It also inhibits the production of pro-inflammatory cytokines and presentation of antigens to T cells by directly acting on dendritic cells and macrophages. CGRP negatively regulates innate immune responses and thus has potential anti-inflammatory effects. Available pieces of evidence suggest CGRP contributes to limiting tissue damage in liver inflammation, joint inflammation, and also in chronic obstructive pulmonary disease. Decreased level of CGRP was observed in the colon of an animal model of colitis which suggests its role in intestinal inflammation[32-36].

NA

NA is a primary neurotransmitter of the sympathetic nervous system, released from nerves innervating the peripheral lymphoid organs. Some evidence suggests that the immunomodulatory effect of NA is administered *via* cAMP. NA influences immune response directly by alteration in expression of adrenergic β receptors on macrophages or indirectly by alteration in level of endogenous NA. Activation of α2 adrenoceptors located on sympathetic nerve terminals results in decreased extracellular NA concentration by a negative feedback effect. Activation of NA receptors that stimulate adenylate cyclase to produce cAMP resulting in a shift toward Th2 responses which are anti-inflammatory and neuroprotective whereas decreased cAMP stimulates Th1 responses resulting in cell destruction and inflammation[3,37]. The use of the α2-adrenoceptor antagonist might be a novel therapeutic approach for the management of colitis[38].

ACETYLCHOLINE

Previously it was thought that acetylcholine is synthesised by only neurons of the parasympathetic and sympathetic nervous system, but now it is established that

acetylcholine is also synthesized by immune cells, keratinocytes, endothelial cells, urothelial cells of the urinary bladder, airways and epithelial cells of the placenta. Acetylcholine released from these cells has been reported to modulate local inflammatory processes. Muscarinic and nicotinic are the two receptor subtypes of acetylcholine. T-cells express both subtypes and activation of each subtype exhibit differential effect. Muscarinic receptors mediate pro-inflammatory responses and nicotinic receptors enhance anti-inflammatory responses. Acetylcholine binds to α7 nicotinic receptors thus inhibits the release of pro-inflammatory cytokines from macrophages, and it is referred to as "cholinergic anti-inflammatory pathway". Acetylcholinesterase is an enzyme that catabolizes acetylcholine; thus, inhibitors of acetylcholinesterase may be considered for attenuating inflammation. In the murine model of sepsis, levels of pro-inflammatory cytokines can be brought down by injecting acetylcholinesterase inhibitors intraperitoneally. Reduced level of acetylcholine has been observed in multiple sclerosis, which is characterized by heightened inflammation. In mice, lacking the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR-/-), the severity of colitis was found to be enhanced[39]. Treatment of UC *via* nicotine also suggests the role of the cholinergic pathway in colonic inflammation. Acetylcholine is well evident to play an essential role in acute or chronic inflammation or autoimmune diseases, including RA[40,41].

NO

NO is a major non-adrenergic non-cholinergic potent neurotransmitter at the neuron synapses. It is involved in the regulation of apoptosis. NO is a gaseous signaling molecule, synthesized by many cells that are involved in immunity and inflammation. However, low levels of NO gives an anti-inflammatory effect and maintain homeostasis but overproduction of NO induces inflammation and causes tissue destruction. The key enzyme involved in NO synthesis is iNOS-2. At high concentrations, NO is oxidized to reactive nitrogen oxide species which mediate most of the immunological effects. NO does not act *via* receptors, its specificity for target cell depends on its concentration, its activity and response, and territory of target cells. In the cardiovascular system, it induces vasodilation. It also regulates the growth, functional activity, and death of various cells including T lymphocytes, atrial premature complexes, neutrophils, mast cells, NK cells, and most importantly macrophages, which release NO in high concentration. Available information suggests that it contributes to the pathogenesis of inflammatory diseases of joint, gut and lungs[37,42,43]. NO may act as a biomarker for monitoring disease activity due to its increased serum concentration during the active phase of both UC and CD and reduced concentration during the inactive phase of the disease[44].

SEROTONIN OR 5-HYDROXYTRYPTAMINE

Five-hydroxytryptamine (5-HT) is a monoamine neurotransmitter and hormone which is traditionally recognized by its functions in the CNS where it is known to regulate sleep, appetite, mood, body temperature, metabolism, and sexuality. The majority of 5-HT is localized to the intestine and tryptophan hydroxylase (TPH1) enzyme catalysis the synthesis of serotonin in enterochromaffin (EC) cells of GIT. EC cells produce 5-HT more than all neuronal and other sources combined. 5-HT is reported to promote activation of lymphocytes and secretion of pro-inflammatory cytokines[45]. 5-HT is considered a potent immunomodulator and it can affect various immune cells including dendritic cells, macrophages, lymphocytes, enteric epithelial cells, and endothelial cells through 5-HT receptors and also *via* a process of serotonylation. During intestinal inflammation, 5-HT is known to mediate activation of signaling molecules of the NF-kB pathway[46]. Upregulated TPH1 and downregulated serotonin transporter (5-HT) expression leads to increased 5-HT availability resulting in enhanced 5-HT signalling, which is associated with inflammation in CD[47]. The role of 5-HT is not only limited to intestinal inflammation, but the alteration in its levels has also been observed in patients with RA and allergic airway inflammation[37,48].

GABA

GABA is an amino acid that is synthesised by decarboxylation of the glutamate with the help of enzyme glutamic acid decarboxylase. It is a classical neurotransmitter and best studied in CNS where it acts as an inhibitory neurotransmitter. Recently it has been found that the immune system is capable of synthesising GABA. GABA has several effects on immune cells, including modulation of cytokine secretion, regulation of cell proliferation, and migration. It can regulate immune responses in various autoimmune and inflammatory diseases such as multiple sclerosis, RA, psoriasis, and type 1 diabetes[49]. Reduced GABAergic signaling is reported to contribute in the pathogenesis of IBD[50]. However, a recent study demonstrated the aggravation of DSS-induced colitis through activation of GABA-A receptor[51].

NEUROMEDIATORS PRODUCING GUT MICROBIOTA AND IBD

Several commensal gut bacteria have emerged as the producers of a variety of neuromediators. These neuromediators are the result of the metabolism of indigestible fibres by gut bacteria. Many bacteria genera are recognised to produce different neuromediators. *Bacillus* family is reported to contribute to the synthesis of dopamine, various species of *Bacteroides*, *Parabacteroides*, *Lactobacillus* and *Bifidobacteria* are known to produce GABA. Similarly, serotonin is produced by *Enterococcus*, *Streptococcus*, and *Escherichia* families. Some species of *Lactobacilli* are involved in acetylcholine synthesis. Some species of *Bacillus* and *Escherichia* also produce noradrenaline[52].

Bacillus

Despite the low abundance of *Bacillus* species in the human gut, it has many beneficial effects, including probiotic features in GIT. Administration of *Bacillus subtilis* in DSS-induced mice model of colitis attenuated the gut inflammation and dysbiosis of gut microbiota[53]. It balances the pro and anti-inflammatory cytokines during disease conditions. It has also shown it's protective effects in IBD patients[54]. Bacillus is reported to produce bioactive metabolites, including neurotransmitters, that further affect the host inflammatory responses[55].

Bacteroidetes

Bacteroidetes is one of the most dominant genera of gut microbiota. It is comprised of *Bacteroides*, *Parabacteroides*, and *Alistipes*. In IBD patients, a low abundance of *Bacteroidetes* has been observed. *Bacteroidetes* confer protection against colitis by expressing polysaccharide A, which can induce the growth of regulatory T cell[56]. Various species of *Bacteroidetes* including *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides ovatus*, *Bacteroides thetaiotamicron*, *Parabacteriodes*, *Alistipes indistinctus*, *Alistipes finegoldiiand*, *Alistipes putredinis* are evident to produce GABA[57,58]. Administration of these species in LPS induced intestinal epithelial cells and animal model of colitis ameliorated colonic inflammation[59,60]. Significant reduction in the severity of gut inflammation in DSS induced mice model of colitis have been observed after oral administration of *Parabacteroides distasonis*[61].

Bifidobacterium

Bifidobacteria is considered the early colonisers of human GIT. The beneficial effects of this genus are very well established[62]. It is widely used in the preparation of probiotics and reported to exert anti-inflammatory effects. Many species such as *Bifidobacterium dentium*, *Bifidobacterium breve*, *Bifidobacterium bifidum* are found to produce GABA[58]. These species, together with some other species like *Bifidobacterium longum*, *Bifidobacterium adolescentis* are known to confer beneficial effects to IBD patients by inhibiting the NF-kB activation, blocking pro-inflammatory cytokines expression and ultimately attenuating the inflammation[63,64].

Enterococcus

Enterococcus primarily resides in the small and large intestine of human GIT. The strains of *Enterococcus* represent approximately 1% of human faecal flora. *Enterococcus faecalis* and *Enterococcus faecium* are the two dominant species found in the human gut[65]. *Enterococcus* is comprised of both commensals as well as nosocomial pathogens. However, commensals have shown several beneficial effects including antimicrobial properties, by releasing bacteriocins and genetically they are very distinct from pathogenic but still, they are not considered safe due to its pathogenic strains. *Entero-*

coccus is found to be actively involved in the biosynthesis of serotonin[66]. Increased abundance of *Enterococcus faecalis* has been observed in IBD patients where it contributes toward pathogenesis[67]. In IL-10 knockout mice, *Enterococcus faecalis* can also induce IBD[68]. Daily administration of probiotic strain of *Enterococcus faecium* in combination with *Lactobacillus helveticus* 416 and *Bifidobacterium longum* ATCC 15707 is known to relieve the symptoms in DSS-induced colitis in rats[69].

Escherichia

Escherichia coli is the regular inhabitant of human GIT. It is the most diverse member of gut microbiota which can act like commensal, probiotic, and pathogenic as well. Increased abundance of *Escherichia* is evident in several mouse models of colitis[70]. A newly identified pro-inflammatory strain of *Escherichia coli* (*E. coli*), adherent-invasive *E. coli* is detected in UC, CD and colorectal cancer. It is highly prevalent and associated with CD pathogenesis as compared to UC[71,72]. *E. coli* Nissle 1917 (EcN) is reported to produce serotonin and also enhance its bioavailability by interacting with the host. Clinical trials demonstrated the beneficial role of EcN in maintaining the UC in remission phase[73-75]. Serotonin signalling was reported to be altered in IBD patients[76]. Some strains of *E. coli* are found to exacerbate the gut inflammation, which suggested the strain-specific effects of *E. coli*[77].

Lactobacillus

Despite having a low abundance, this genus is well known for its probiotic effects[74]. The population of *Lactobacillus* is either positively or negatively associated with many diseases, including IBD[78]. Significant reduction in the *Lactobacillus* population has been observed in UC patients, and there are reports suggested the improvement in clinical symptoms of UC patients after consuming food containing *Lactobacillus*. It showed a beneficial effect in intestinal inflammation by modulating Treg cells which maintain intestinal homeostasis by secreting anti-inflammatory cytokines[79]. Various species of *Lactobacillus* like *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus* are reported to produce GABA[57].

Additionally, acetylcholine is also produced by various strains of *Lactobacillus*, especially *Lactobacillus plantarum*[80]. In a recent study, the effect of dietary probiotics is investigated in IBD induced murine model where *Lactobacillus rhamnosus* is observed as a significant producer of IL-10 and interferon-γ[81]. Group of animal studies, human trials, and in vitro studies revealed that these species of Lactobacillus are involved in controlling inflammation either by inhibiting the NF-κB induced release of pro-inflammatory cytokines or by maintaining the intestinal barrier integrity[82-88].

Streptococcus

Streptococcus is a luminal microbial genus, dominant in the distal oesophagus, duodenum, and jejunum. The most common species are *Streptococcus salivarius*, *Streptococcus thermophilus*, and *Streptococcus parasanguinis*[89]. *Streptococcus* species, including *Streptococcus thermophilus* is reported to produce serotonin[90]. Increased abundance of streptococcus has been observed in IBD patients that indicated the involvement of this genus in the severity of IBD. *Streptococcus bovis* is found to be associated with colon cancer and IBD. Streptococcus is known to interact with immune cells and modulate the secretion of pro-inflammatory cytokines that could initiate the inflammatory response in different organs[91]. Recently, immunoglobulin enriched streptococcus is reported in IBD patients that implicate a prominent role of oropharyngeal bacteria in IBD pathogenesis by triggering host immune response[92].

SIGNIFICANCE OF NEUROIMMUNOMODULATION BY GUT BACTERIA

Gut bacteria have been known to be crucial for human health. It deliberate number of benefits to the host, including digestion of indigestible carbohydrates that leads to the production of short-chain fatty acids (SCFA) and prevent the colonisation of pathogenic bacteria by producing antimicrobial peptides. SCFAs are involved in various functions like protection from epithelial injury, synthesise vitamins (vitamin B12, vitamin K and folic acid) and essential amino acids, regulate fat metabolism, boost intestinal angiogenesis, cause intestinal motility and promote proper development of immune system[93-95]. Studies conducted in IBD patients and mice models have indicated the central role of gut bacteria in the gut inflammation[96]. The new research in the field opens up new avenues to understand the IBD pathogenesis. Through

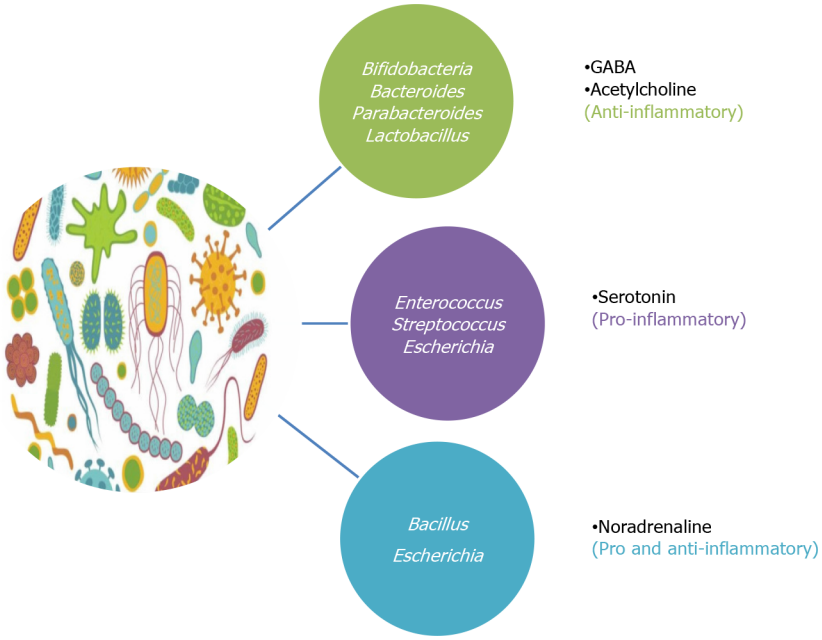

Figure 2 Inter-relation of diverse gut microbiota and their respective neuromediators with gut inflammation. Bacteria used as probiotics in inflammatory bowel disease (IBD) (green box) produces anti-inflammatory neuromediators (γ-aminobutyric acid, acetylcholine), bacteria having a detrimental role in IBD (purple box) releases pro-inflammatory neuromediator (serotonin) and bacteria having a debatable role in IBD (blue box) secrete neuromediator (noradrenaline) having both pro and anti-inflammatory properties. GABA: γ-aminobutyric acid.

numerous mechanisms, bacteria execute their part in disease pathogenesis. The revelation of secretion of neuromediators from gut microbes introduced a new area for research and a unique way of looking at the pathophysiology of IBD.

Neuromediators, apart from their classical neuronal functions, are currently being recognised as a pillar in maintaining the gut homeostasis. There are different sources of neuromediators in GIT, including enteric neurons, gut microbiota, immune cells and gut epithelial cells. Out of all the sources, microbial content is the only factor which can be extrinsically varied. Altering the neuromediators *via* gut bacteria can affect the gut physiology, signalling and immune cells secretions and function in GIT. The available literature on the signalling pathways of a variety of neuromediators and their respective gut bacteria in IBD indicated that the neuromediators released by bacteria being used as probiotic are having anti-inflammatory properties and bacteria which were reported to increase disease severity produce neuromediators with pro-inflammatory properties. For instance, GABA and acetylcholine are the anti-inflammatory neuromediators, produced by those bacteria which are very well established to attenuate gut inflammation in both DSS-induced mice model of colitis and IBD patients. Serotonin which is a pro-inflammatory neuromediator is produced by bacteria that are involved in the severity of IBD. Besides, noradrenaline, having both anti and pro-inflammatory properties, produced by two different types of bacteria, one having the beneficial role and other having the debatable role in IBD (Figure 2). This interrelation suggests that bacteria impart their effects in gut inflammation through releasing neuromediators as one of the mechanism.

CONCLUSION

Neuromediators are emerging as essential players in IBD pathogenesis. These are influenced by the complex interaction of gut microbiota, host immunity, and intestinal epithelium. During gut inflammation or IBD, dysbiosis in gut microbiota and alteration in neuromediators complicate the mechanism of gut homeostasis resulting in perturbed equilibrium (Figure 3). In-depth mechanism of neuroimmunomodulation due to gut bacteria needs to be explored more, to settle the gut homeostasis during disease. These neuromediators may prove to be a great tool to clinicians in treating inflammatory diseases. Through this review, we summarized various neuromediators produced by different gut microbiota and their significance as an immunomodulatory entity in the colon. Using gut bacteria that can produce neuromediators having anti-

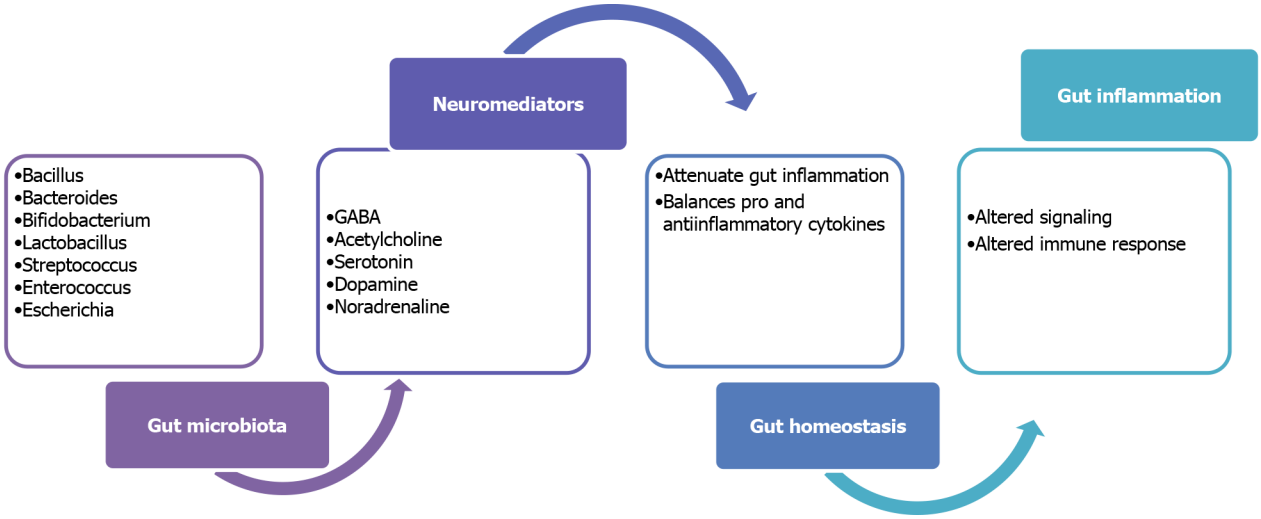

Figure 3 Role of neuromediators producing gut microbiota during gut inflammation. Gut microbiota produces various neuromediators that attenuate the gut inflammation by balancing the pro and anti-inflammatory cytokines to maintain gut homeostasis. During inflammation, dysbiosis in gut microbiota leads to alteration in respective neuromediators which may lead to altered the host immune response. GABA: γ-aminobutyric acid.

inflammatory properties for treating IBD patients may be a novel therapeutic approach and also the fertile area for future research.

REFERENCES

1 **Brookes SJ**. Classes of enteric nerve cells in the guinea-pig small intestine. *Anat Rec* 2001; **262**: 58-70 [PMID: 11146429 DOI: 10.1002/1097-0185(20010101)262:1<58::AID-AR1011>3.0.CO;2-V]
2 **Vasina V**, Barbara G, Talamonti L, Stanghellini V, Corinaldesi R, Tonini M, De Ponti F, De Giorgio R. Enteric neuroplasticity evoked by inflammation. *Auton Neurosci* 2006; **126-127**: 264-272 [PMID: 16624634 DOI: 10.1016/j.autneu.2006.02.025]
3 **Szelényi J**, Vizi ES. The catecholamine cytokine balance: interaction between the brain and the immune system. *Ann N Y Acad Sci* 2007; **1113**: 311-324 [PMID: 17584982 DOI: 10.1196/annals.1391.026]
4 **Chandrasekharan B**, Nezami BG, Srinivasan S. Emerging neuropeptide targets in inflammation: NPY and VIP. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G949-G957 [PMID: 23538492 DOI: 10.1152/ajpgi.00493.2012]
5 **O'Connor TM**, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol* 2004; **201**: 167-180 [PMID: 15334652 DOI: 10.1002/jcp.20061]
6 **Weinstock JV**. Substance P and the regulation of inflammation in infections and inflammatory bowel disease. *Acta Physiol (Oxf)* 2015; **213**: 453-461 [PMID: 25424746 DOI: 10.1111/apha.12428]
7 **Hong HS**, Hwang DY, Park JH, Kim S, Seo EJ, Son Y. Substance-P alleviates dextran sulfate sodium-induced intestinal damage by suppressing inflammation through enrichment of M2 macrophages and regulatory T cells. *Cytokine* 2017; **90**: 21-30 [PMID: 27750083 DOI: 10.1016/j.cyto.2016.10.002]
8 **Brun P**, Mastrotto C, Beggiao E, Stefani A, Barzon L, Sturniolo GC, Palù G, Castagliuolo I. Neuropeptide neurotensin stimulates intestinal wound healing following chronic intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G621-G629 [PMID: 15764810 DOI: 10.1152/ajpgi.00140.2004]
9 **Law IK**, Pothoulakis C. MicroRNA-133α regulates neurotensin-associated colonic inflammation in colonic epithelial cells and experimental colitis. *RNA Dis* 2015; **2** [PMID: 26005712 DOI: 10.14800/rd.472]
10 **Moura LI**, Silva L, Leal EC, Tellechea A, Cruz MT, Carvalho E. Neurotensin modulates the migratory and inflammatory response of macrophages under hyperglycemic conditions. *Biomed Res Int* 2013; **2013**: 941764 [PMID: 24000330 DOI: 10.1155/2013/941764]
11 **Seufferlein T**, Rozengurt E. Galanin, neurotensin, and phorbol esters rapidly stimulate activation of mitogen-activated protein kinase in small cell lung cancer cells. *Cancer Res* 1996; **56**: 5758-5764 [PMID: 8971188]
12 **Bakirtzi K**, Law IK, Xue X, Iliopoulos D, Shah YM, Pothoulakis C. Neurotensin Promotes the Development of Colitis and Intestinal Angiogenesis *via* Hif-1α-miR-210 Signaling. *J Immunol* 2016; **196**: 4311-4321 [PMID: 27076683 DOI: 10.4049/jimmunol.1501443]
13 **El-Salhy M**, Hausken T. The role of the neuropeptide Y (NPY) family in the pathophysiology of

inflammatory bowel disease (IBD). *Neuropeptides* 2016; **55**: 137-144 [PMID: 26431932 DOI: 10.1016/j.npep.2015.09.005]

14 **Wheway J**, Herzog H, Mackay F. NPY and receptors in immune and inflammatory diseases. *Curr Top Med Chem* 2007; **7**: 1743-1752 [PMID: 17979783 DOI: 10.2174/156802607782341046]

15 **El-Salhy M**, Solomon T, Hausken T, Gilja OH, Hatlebakk JG. Gastrointestinal neuroendocrine peptides/amines in inflammatory bowel disease. *World J Gastroenterol* 2017; **23**: 5068-5085 [PMID: 28811704 DOI: 10.3748/wjg.v23.i28.5068]

16 **Abad C**, Gomariz R, Waschek J, Leceta J, Martinez C, Juarranz Y, Arranz A. VIP in inflammatory bowel disease: state of the art. *Endocr Metab Immune Disord Drug Targets* 2012; **12**: 316-322 [PMID: 23094828 DOI: 10.2174/187153012803832576]

17 **Delgado M**, Abad C, Martinez C, Juarranz MG, Arranz A, Gomariz RP, Leceta J. Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. *J Mol Med (Berl)* 2002; **80**: 16-24 [PMID: 11862320 DOI: 10.1007/s00109-001-0291-5]

18 **Gonzalez-Rey E**, Delgado M. Role of vasoactive intestinal peptide in inflammation and autoimmunity. *Curr Opin Investig Drugs* 2005; **6**: 1116-1123 [PMID: 16312132]

19 **Xu CL**, Guo Y, Qiao L, Ma L, Cheng YY. Recombinant expressed vasoactive intestinal peptide analogue ameliorates TNBS-induced colitis in rats. *World J Gastroenterol* 2018; **24**: 706-715 [PMID: 29456409 DOI: 10.3748/wjg.v24.i6.706]

20 **Jayawardena D**, Anbazhagan AN, Guzman G, Dudeja PK, Onyuksel H. Vasoactive Intestinal Peptide Nanomedicine for the Management of Inflammatory Bowel Disease. *Mol Pharm* 2017; **14**: 3698-3708 [PMID: 28991483 DOI: 10.1021/acs.molpharmaceut.7b00452]

21 **Talero E**, Sánchez-Fidalgo S, Calvo JR, Motilva V. Chronic administration of galanin attenuates the TNBS-induced colitis in rats. *Regul Pept* 2007; **141**: 96-104 [PMID: 17331599 DOI: 10.1016/j.regpep.2006.12.029]

22 **Botz B**, Kemény Á, Brunner SM, Locker F, Csepregi J, Mócsai A, Pintér E, McDougall JJ, Kofler B, Helyes Z. Lack of Galanin 3 Receptor Aggravates Murine Autoimmune Arthritis. *J Mol Neurosci* 2016; **59**: 260-269 [PMID: 26941032 DOI: 10.1007/s12031-016-0732-9]

23 **Gross KJ**, Pothoulakis C. Role of neuropeptides in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 918-932 [PMID: 17343284 DOI: 10.1002/ibd.20129]

24 **Kofler B**, Brunner S, Koller A, Wiesmayr S, Locker F, Lang R, Botz B, Kemény À, Helyes Z. Contribution of the galanin system to inflammation. *Springerplus* 2015; **4**: L57 [PMID: 27386220 DOI: 10.1186/2193-1801-4-S1-L57]

25 **Lang R**, Kofler B. The galanin peptide family in inflammation. *Neuropeptides* 2011; **45**: 1-8 [PMID: 21087790 DOI: 10.1016/j.npep.2010.10.005]

26 **Wraith DC**, Pope R, Butzkueven H, Holder H, Vanderplank P, Lowrey P, Day MJ, Gundlach AL, Kilpatrick TJ, Scolding N, Wynick D. A role for galanin in human and experimental inflammatory demyelination. *Proc Natl Acad Sci USA* 2009; **106**: 15466-15471 [PMID: 19717462 DOI: 10.1073/pnas.0903360106]

27 **Nezi M**, Mastorakos G, Mouslech Z. Corticotropin Releasing Hormone And The Immune/Inflammatory Response. 2015 Jul 30. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, Grossman A, Hershman JM, Hofland J, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Purnell J, Singer F, Stratakis CA, Trence DL, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc., 2000 [PMID: 25905246]

28 **Chatzaki E**, Anton PA, Million M, Lambropoulou M, Constantinidis T, Kolios G, Taché Y, Grigoriadis DE. Corticotropin-releasing factor receptor subtype 2 in human colonic mucosa: down-regulation in ulcerative colitis. *World J Gastroenterol* 2013; **19**: 1416-1423 [PMID: 23539366 DOI: 10.3748/wjg.v19.i9.1416]

29 **Rhee SH**, Ma EL, Lee Y, Taché Y, Pothoulakis C, Im E. Corticotropin Releasing Hormone and Urocortin 3 Stimulate Vascular Endothelial Growth Factor Expression through the cAMP/CREB Pathway. *J Biol Chem* 2015; **290**: 26194-26203 [PMID: 26350463 DOI: 10.1074/jbc.M115.678979]

30 **González-Moret R**, Cebolla A, Cortés X, Baños RM, Navarrete J, de la Rubia JE, Lisón JF, Soria JM. The effect of a mindfulness-based therapy on different biomarkers among patients with inflammatory bowel disease: a randomised controlled trial. *Sci Rep* 2020; **10**: 6071 [PMID: 32269278 DOI: 10.1038/s41598-020-63168-4]

31 **Overman EL**, Rivier JE, Moeser AJ. CRF induces intestinal epithelial barrier injury *via* the release of mast cell proteases and TNF-α. *PLoS One* 2012; **7**: e39935 [PMID: 22768175 DOI: 10.1371/journal.pone.0039935]

32 **Eysselein VE**, Reinshagen M, Patel A, Davis W, Nast C, Sternini C. Calcitonin gene-related peptide in inflammatory bowel disease and experimentally induced colitis. *Ann N Y Acad Sci* 1992; **657**: 319-327 [PMID: 1637091 DOI: 10.1111/j.1749-6632.1992.tb22779.x]

33 **Holzmann B**. Antiinflammatory activities of CGRP modulating innate immune responses in health and disease. *Curr Protein Pept Sci* 2013; **14**: 268-274 [PMID: 23745695 DOI: 10.2174/13892037113149990046]

34 **Kroeger I**, Erhardt A, Abt D, Fischer M, Biburger M, Rau T, Neuhuber WL, Tiegs G. The neuropeptide calcitonin gene-related peptide (CGRP) prevents inflammatory liver injury in mice. *J Hepatol* 2009; **51**: 342-353 [PMID: 19464067 DOI: 10.1016/j.jhep.2009.03.022]

35 **Springer J**, Geppetti P, Fischer A, Groneberg DA. Calcitonin gene-related peptide as inflammatory mediator. *Pulm Pharmacol Ther* 2003; **16**: 121-130 [PMID: 12749828 DOI:

10.1016/S1094-5539(03)00049-X]
36 **Walsh DA**, Mapp PI, Kelly S. Calcitonin gene-related peptide in the joint: contributions to pain and inflammation. *Br J Clin Pharmacol* 2015; **80**: 965-978 [PMID: 25923821 DOI: 10.1111/bcp.12669]
37 **Skobowiat C**. Contribution of Neuropeptides and Neurotransmitters in colitis. *Vet Sci Tech* 2011 [DOI: 10.4172/2157-7579.S5-001]
38 **Bai A**, Lu N, Guo Y, Chen J, Liu Z. Modulation of inflammatory response *via* alpha2-adrenoceptor blockade in acute murine colitis. *Clin Exp Immunol* 2009; **156**: 353-362 [PMID: 19250273 DOI: 10.1111/j.1365-2249.2009.03894.x]
39 **Ghia JE**, Blennerhassett P, Deng Y, Verdu EF, Khan WI, Collins SM. Reactivation of inflammatory bowel disease in a mouse model of depression. *Gastroenterology* 2009; **136**: 2280-2288.e1-4 [PMID: 19272381 DOI: 10.1053/j.gastro.2009.02.069]
40 **Reale M**, de Angelis F, di Nicola M, Capello E, di Ioia M, Luca Gd, Lugaresi A, Tata AM. Relation between pro-inflammatory cytokines and acetylcholine levels in relapsing-remitting multiple sclerosis patients. *Int J Mol Sci* 2012; **13**: 12656-12664 [PMID: 23202919 DOI: 10.3390/ijms131012656]
41 **Rosas-Ballina M**, Tracey KJ. Cholinergic control of inflammation. *J Intern Med* 2009; **265**: 663-679 [PMID: 19493060 DOI: 10.1111/j.1365-2796.2009.02098.x]
42 **Coleman JW**. Nitric oxide in immunity and inflammation. *Int Immunopharmacol* 2001; **1**: 1397-1406 [PMID: 11515807 DOI: 10.1016/s1567-5769(01)00086-8]
43 **Sharma JN**, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology* 2007; **15**: 252-259 [PMID: 18236016 DOI: 10.1007/s10787-007-0013-x]
44 **Avdagić N**, Zaćiragić A, Babić N, Hukić M, Seremet M, Lepara O, Nakaš-Ićindić E. Nitric oxide as a potential biomarker in inflammatory bowel disease. *Bosn J Basic Med Sci* 2013; **13**: 5-9 [PMID: 23448603 DOI: 10.17305/bjbms.2013.2402]
45 **Bertrand PP**, Barajas-Espinosa A, Neshat S, Bertrand RL, Lomax AE. Analysis of real-time serotonin (5-HT) availability during experimental colitis in mouse. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G446-G455 [PMID: 20019165 DOI: 10.1152/ajpgi.00318.2009]
46 **Ghia JE**, Li N, Wang H, Collins M, Deng Y, El-Sharkawy RT, Côté F, Mallet J, Khan WI. Serotonin has a key role in pathogenesis of experimental colitis. *Gastroenterology* 2009; **137**: 1649-1660 [PMID: 19706294 DOI: 10.1053/j.gastro.2009.08.041]
47 **Shajib MS**, Chauhan U, Adeeb S, Chetty Y, Armstrong D, Halder SLS, Marshall JK, Khan WI. Characterization of Serotonin Signaling Components in Patients with Inflammatory Bowel Disease. *J Can Assoc Gastroenterol* 2019; **2**: 132-140 [PMID: 31294376 DOI: 10.1093/jcag/gwy039]
48 **Shajib MS**, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol (Oxf)* 2015; **213**: 561-574 [PMID: 25439045 DOI: 10.1111/apha.12430]
49 **Jin Z**, Mendu SK, Birnir B. GABA is an effective immunomodulatory molecule. *Amino Acids* 2013; **45**: 87-94 [PMID: 22160261 DOI: 10.1007/s00726-011-1193-7]
50 **Aggarwal S**, Ahuja V, Paul J. Attenuated GABAergic Signaling in Intestinal Epithelium Contributes to Pathogenesis of Ulcerative Colitis. *Dig Dis Sci* 2017; **62**: 2768-2779 [PMID: 28667430 DOI: 10.1007/s10620-017-4662-3]
51 **Ma X**, Sun Q, Sun X, Chen D, Wei C, Yu X, Liu C, Li Y, Li J. Activation of $GABA_A$ Receptors in Colon Epithelium Exacerbates Acute Colitis. *Front Immunol* 2018; **9**: 987 [PMID: 29867964 DOI: 10.3389/fimmu.2018.00987]
52 **Sarkar A**, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the Manipulation of Bacteria-Gut-Brain Signals. *Trends Neurosci* 2016; **39**: 763-781 [PMID: 27793434 DOI: 10.1016/j.tins.2016.09.002]
53 **Li Y**, Zhang T, Guo C, Geng M, Gai S, Qi W, Li Z, Song Y, Luo X, Wang N. Bacillus subtilis RZ001 improves intestinal integrity and alleviates colitis by inhibiting the Notch signalling pathway and activating ATOH-1. *Pathog Dis* 2020; **78** [PMID: 32166323 DOI: 10.1093/femspd/ftaa016]
54 **Zhang HL**, Li WS, Xu DN, Zheng WW, Liu Y, Chen J, Qiu ZB, Dorfman RG, Zhang J, Liu J. Mucosa-reparing and microbiota-balancing therapeutic effect of *Bacillus subtilis* alleviates dextrate sulfate sodium-induced ulcerative colitis in mice. *Exp Ther Med* 2016; **12**: 2554-2562 [PMID: 27698758 DOI: 10.3892/etm.2016.3686]
55 **Ilinskaya ON**, Ulyanova VV, Yarullina DR, Gataullin IG. Secretome of Intestinal *Bacilli:* A Natural Guard against Pathologies. *Front Microbiol* 2017; **8**: 1666 [PMID: 28919884 DOI: 10.3389/fmicb.2017.01666]
56 **Zhou Y**, Zhi F. Lower Level of *Bacteroides* in the Gut Microbiota Is Associated with Inflammatory Bowel Disease: A Meta-Analysis. *Biomed Res Int* 2016; **2016**: 5828959 [PMID: 27999802 DOI: 10.1155/2016/5828959]
57 **Pokusaeva K**, Johnson C, Luk B, Uribe G, Fu Y, Oezguen N, Matsunami RK, Lugo M, Major A, Mori-Akiyama Y, Hollister EB, Dann SM, Shi XZ, Engler DA, Savidge T, Versalovic J. GABA-producing Bifidobacterium dentium modulates visceral sensitivity in the intestine. *Neurogastroenterol Motil* 2017; **29** [PMID: 27458085 DOI: 10.1111/nmo.12904]
58 **Strandwitz P**, Kim KH, Terekhova D, Liu JK, Sharma A, Levering J, McDonald D, Dietrich D, Ramadhar TR, Lekbua A, Mroue N, Liston C, Stewart EJ, Dubin MJ, Zengler K, Knight R, Gilbert JA, Clardy J, Lewis K. GABA-modulating bacteria of the human gut microbiota. *Nat Microbiol* 2019; **4**: 396-403 [PMID: 30531975 DOI: 10.1038/s41564-018-0307-3]
59 **Delday M**, Mulder I, Logan ET, Grant G. Bacteroides thetaiotaomicron Ameliorates Colon Inflammation in Preclinical Models of Crohn's Disease. *Inflamm Bowel Dis* 2019; **25**: 85-96 [PMID: 30215718 DOI: 10.1093/ibd/izy281]

60 **Tan H**, Zhao J, Zhang H, Zhai Q, Chen W. Novel strains of Bacteroides fragilis and Bacteroides ovatus alleviate the LPS-induced inflammation in mice. *Appl Microbiol Biotechnol* 2019; **103**: 2353-2365 [PMID: 30666361 DOI: 10.1007/s00253-019-09617-1]

61 **Kverka M**, Zakostelska Z, Klimesova K, Sokol D, Hudcovic T, Hrncir T, Rossmann P, Mrazek J, Kopecny J, Verdu EF, Tlaskalova-Hogenova H. Oral administration of Parabacteroides distasonis antigens attenuates experimental murine colitis through modulation of immunity and microbiota composition. *Clin Exp Immunol* 2011; **163**: 250-259 [PMID: 21087444 DOI: 10.1111/j.1365-2249.2010.04286.x]

62 **O'Callaghan A**, van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Front Microbiol* 2016; **7**: 925 [PMID: 27379055 DOI: 10.3389/fmicb.2016.00925]

63 **Riedel CU**, Foata F, Philippe D, Adolfsson O, Eikmanns BJ, Blum S. Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-kappaB activation. *World J Gastroenterol* 2006; **12**: 3729-3735 [PMID: 16773690 DOI: 10.3748/wjg.v12.i23.3729]

64 **Preising J**, Philippe D, Gleinser M, Wei H, Blum S, Eikmanns BJ, Niess JH, Riedel CU. Selection of bifidobacteria based on adhesion and anti-inflammatory capacity in vitro for amelioration of murine colitis. *Appl Environ Microbiol* 2010; **76**: 3048-3051 [PMID: 20228095 DOI: 10.1128/AEM.03127-09]

65 **Dubin K**, Pamer EG. Enterococci and Their Interactions with the Intestinal Microbiome. *Microbiol Spectr* 2014; **5** [PMID: 29125098 DOI: 10.1128/microbiolspec.BAD-0014-2016]

66 **Lyte M**. Probiotics function mechanistically as delivery vehicles for neuroactive compounds: Microbial endocrinology in the design and use of probiotics. *Bioessays* 2011; **33**: 574-581 [PMID: 21732396 DOI: 10.1002/bies.201100024]

67 **Zhou Y**, Chen H, He H, Du Y, Hu J, Li Y, Zhou Y, Wang H, Chen Y, Nie Y. Increased Enterococcus faecalis infection is associated with clinically active Crohn disease. *Medicine (Baltimore)* 2016; **95**: e5019 [PMID: 27684872 DOI: 10.1097/MD.0000000000005019]

68 **Balish E**, Warner T. Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. *Am J Pathol* 2002; **160**: 2253-2257 [PMID: 12057927 DOI: 10.1016/S0002-9440(10)61172-8]

69 **Celiberto LS**, Bedani R, Dejani NN, Ivo de Medeiros A, Sampaio Zuanon JA, Spolidorio LC, Tallarico Adorno MA, Amâncio Varesche MB, Carrilho Galvão F, Valentini SR, Font de Valdez G, Rossi EA, Cavallini DCU. Effect of a probiotic beverage consumption (Enterococcus faecium CRL 183 and Bifidobacterium longum ATCC 15707) in rats with chemically induced colitis. *PLoS One* 2017; **12**: e0175935 [PMID: 28437455 DOI: 10.1371/journal.pone.0175935]

70 **Escribano-Vazquez U**, Verstraeten S, Martin R, Chain F, Langella P, Thomas M, Cherbuy C. The commensal Escherichia coli CEC15 reinforces intestinal defences in gnotobiotic mice and is protective in a chronic colitis mouse model. *Sci Rep* 2019; **9**: 11431 [PMID: 31391483 DOI: 10.1038/s41598-019-47611-9]

71 **Palmela C**, Chevarin C, Xu Z, Torres J, Sevrin G, Hirten R, Barnich N, Ng SC, Colombel JF. Adherent-invasive *Escherichia coli* in inflammatory bowel disease. *Gut* 2018; **67**: 574-587 [PMID: 29141957 DOI: 10.1136/gutjnl-2017-314903]

72 **Abdelhalim KA**, Uzel A, Gülşen Ünal N. Virulence determinants and genetic diversity of adherent-invasive Escherichia coli (AIEC) strains isolated from patients with Crohn's disease. *Microb Pathog* 2020; **145**: 104233 [PMID: 32360521 DOI: 10.1016/j.micpath.2020.104233]

73 **Nzakizwanayo J**, Dedi C, Standen G, Macfarlane WM, Patel BA, Jones BV. Escherichia coli Nissle 1917 enhances bioavailability of serotonin in gut tissues through modulation of synthesis and clearance. *Sci Rep* 2015; **5**: 17324 [PMID: 26616662 DOI: 10.1038/srep17324]

74 **Naseer M**, Poola S, Ali S, Samiullah S, Tahan V. Prebiotics and Probiotics in Inflammatory Bowel Disease: Where are we now and where are we going? *Curr Clin Pharmacol* 2020; **15**: 216-233 [PMID: 32164516 DOI: 10.2174/1574884715666200312100237]

75 **Behrouzi A**, Mazaheri H, Falsafi S, Tavassol ZH, Moshiri A, Siadat SD. Intestinal effect of the probiotic *Escherichia coli* strain Nissle 1917 and its OMV. *J Diabetes Metab Disord* 2020; **19**: 597-604 [PMID: 32550212 DOI: 10.1007/s40200-020-00511-6]

76 **Manocha M**, Khan WI. Serotonin and GI Disorders: An Update on Clinical and Experimental Studies. *Clin Transl Gastroenterol* 2012; **3**: e13 [PMID: 23238212 DOI: 10.1038/ctg.2012.8]

77 **Kittana H**, Gomes-Neto JC, Heck K, Geis AL, Segura Muñoz RR, Cody LA, Schmaltz RJ, Bindels LB, Sinha R, Hostetter JM, Benson AK, Ramer-Tait AE. Commensal *Escherichia coli* Strains Can Promote Intestinal Inflammation *via* Differential Interleukin-6 Production. *Front Immunol* 2018; **9**: 2318 [PMID: 30356663 DOI: 10.3389/fimmu.2018.02318]

78 **Heeney DD**, Gareau MG, Marco ML. Intestinal Lactobacillus in health and disease, a driver or just along for the ride? *Curr Opin Biotechnol* 2018; **49**: 140-147 [PMID: 28866243 DOI: 10.1016/j.copbio.2017.08.004]

79 **Azad MAK**, Sarker M, Li T, Yin J. Probiotic Species in the Modulation of Gut Microbiota: An Overview. *Biomed Res Int* 2018; **2018**: 9478630 [PMID: 29854813 DOI: 10.1155/2018/9478630]

80 **Nimgampalle M**, Kuna Y. Anti-Alzheimer Properties of Probiotic, *Lactobacillus plantarum* MTCC 1325 in Alzheimer's Disease induced Albino Rats. *J Clin Diagn Res* 2017; **11**: KC01-KC05 [PMID: 28969160 DOI: 10.7860/JCDR/2017/26106.10428]

81 **Ahn SI**, Cho S, Choi NJ. Effect of dietary probiotics on colon length in an inflammatory bowel disease-induced murine model: A meta-analysis. *J Dairy Sci* 2020; **103**: 1807-1819 [PMID: 31785874 DOI: 10.3168/jds.2019-17356]

82 **Le B**, Yang SH. Efficacy of *Lactobacillus plantarum* in prevention of inflammatory bowel disease. *Toxicol Rep* 2018; **5**: 314-317 [PMID: 29854599 DOI: 10.1016/j.toxrep.2018.02.007]

83 **Oh NS**, Joung JY, Lee JY, Kim Y. Probiotic and anti-inflammatory potential of Lactobacillus rhamnosus 4B15 and Lactobacillus gasseri 4M13 isolated from infant feces. *PLoS One* 2018; **13**: e0192021 [PMID: 29444150 DOI: 10.1371/journal.pone.0192021]

84 **Mu Q**, Tavella VJ, Luo XM. Role of *Lactobacillus reuteri* in Human Health and Diseases. *Front Microbiol* 2018; **9**: 757 [PMID: 29725324 DOI: 10.3389/fmicb.2018.00757]

85 **Sokovic Bajic S**, Djokic J, Dinic M, Veljovic K, Golic N, Mihajlovic S, Tolinacki M. GABA-Producing Natural Dairy Isolate From Artisanal Zlatar Cheese Attenuates Gut Inflammation and Strengthens Gut Epithelial Barrier *in vitro*. *Front Microbiol* 2019; **10**: 527 [PMID: 30936860 DOI: 10.3389/fmicb.2019.00527]

86 **Pagnini C**, Corleto VD, Martorelli M, Lanini C, D'Ambra G, Di Giulio E, Delle Fave G. Mucosal adhesion and anti-inflammatory effects of *Lactobacillus rhamnosus* GG in the human colonic mucosa: A proof-of-concept study. *World J Gastroenterol* 2018; **24**: 4652-4662 [PMID: 30416313 DOI: 10.3748/wjg.v24.i41.4652]

87 **Zhang F**, Li Y, Wang X, Wang S, Bi D. The Impact of *Lactobacillus plantarum* on the Gut Microbiota of Mice with DSS-Induced Colitis. *Biomed Res Int* 2019; **2019**: 3921315 [PMID: 30915354 DOI: 10.1155/2019/3921315]

88 **Wang J**, Ji H, Wang S, Liu H, Zhang W, Zhang D, Wang Y. Probiotic *Lactobacillus plantarum* Promotes Intestinal Barrier Function by Strengthening the Epithelium and Modulating Gut Microbiota. *Front Microbiol* 2018; **9**: 1953 [PMID: 30197632 DOI: 10.3389/fmicb.2018.01953]

89 **Jandhyala SM**, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015; **21**: 8787-8803 [PMID: 26269668 DOI: 10.3748/wjg.v21.i29.8787]

90 **Scheri GC**, Fard SN, Schietroma I, Mastrangelo A, Pinacchio C, Giustini N, Serafino S, De Girolamo G, Cavallari EN, Statzu M, Laghi L, Vullo A, Ceccarelli G, Vullo V, d'Ettorre G. Modulation of Tryptophan/Serotonin Pathway by Probiotic Supplementation in Human Immunodeficiency Virus-Positive Patients: Preliminary Results of a New Study Approach. *Int J Tryptophan Res* 2017; **10**: 1178646917710668 [PMID: 28607543 DOI: 10.1177/1178646917710668]

91 **Heidarian F**, Noormohammadi Z, Asadzadeh Aghdaei H, Alebouyeh M. Relative Abundance of Streptococcus spp. and its Association with Disease Activity in Inflammatory Bowel Disease Patients Compared with Controls. *Arch Clin Infect Dis* 2017; **12** [DOI: 10.5812/archcid.57291]

92 **Rengarajan S**, Vivio EE, Parkes M, Peterson DA, Roberson EDO, Newberry RD, Ciorba MA, Hsieh CS. Dynamic immunoglobulin responses to gut bacteria during inflammatory bowel disease. *Gut Microbes* 2020; **11**: 405-420 [PMID: 31203722 DOI: 10.1080/19490976.2019.1626683]

93 **Hooper LV**. Bacterial contributions to mammalian gut development. *Trends Microbiol* 2004; **12**: 129-134 [PMID: 15001189 DOI: 10.1016/j.tim.2004.01.001]

94 **Hooper LV**, Gordon JI. Commensal host-bacterial relationships in the gut. *Science* 2001; **292**: 1115-1118 [PMID: 11352068 DOI: 10.1126/science.1058709]

95 **Hamer HM**, Jonkers DM, Bast A, Vanhoutvin SA, Fischer MA, Kodde A, Troost FJ, Venema K, Brummer RJ. Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. *Clin Nutr* 2009; **28**: 88-93 [PMID: 19108937 DOI: 10.1016/j.clnu.2008.11.002]

96 **Taurog JD**, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernández-Sueiro JL, Balish E, Hammer RE. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med* 1994; **180**: 2359-2364 [PMID: 7964509 DOI: 10.1084/jem.180.6.2359]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Platelet count as a screening tool for compensated cirrhosis in chronic viral hepatitis

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Abstract

BACKGROUND

Simple tools for clinicians to identify cirrhosis in patients with chronic viral hepatitis are medically necessary for treatment initiation, hepatocellular cancer screening and additional medical management.

AIM

To determine whether platelets or other laboratory markers can be used as a simple method to identify the development of cirrhosis.

METHODS

Clinical, biochemical and histologic laboratory data from treatment naive chronic viral hepatitis B (HBV), C (HCV), and D (HDV) patients at the NIH Clinical Center from 1985-2019 were collected and subjects were randomly divided into training and validation cohorts. Laboratory markers were tested for their ability to identify cirrhosis (Ishak ≥ 5) using receiver operating characteristic curves and an optimal cut-off was calculated within the training cohort. The final cut-off was tested within the validation cohort.

RESULTS

Overall, 1027 subjects (HCV = 701, HBV = 240 and HDV = 86), 66% male, with mean (standard deviation) age of 45 (11) years were evaluated. Within the training cohort (n = 715), platelets performed the best at identifying cirrhosis compared to other laboratory markers [Area Under the Receiver Operating Characteristics curve (AUROC) = 0.86 (0.82-0.90)] and sensitivity 77%, specificity 83%, positive predictive value 44%, and negative predictive value 95%. All other

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tested markers had AUROCs ≤ 0.77. The optimal platelet cut-off for detecting cirrhosis in the training cohort was 143 × 10^9/L and it performed equally well in the validation cohort (n = 312) [AUROC = 0.85 (0.76-0.94)].

CONCLUSION
The use of platelet counts should be considered to identify cirrhosis and ensure optimal care and management of patients with chronic viral hepatitis.

Key Words: Chronic hepatitis B; Chronic hepatitis C; Chronic hepatitis D; Platelets; Cirrhosis; Non-invasive assessment

Core Tip: Platelet count is a well-recognized surrogate marker for progression of liver disease, however a specific cut-off for cirrhosis has not been established. In this study, platelet counts can accurately stratify chronic viral hepatitis patients with cirrhosis; and a platelet count > 143 × 10^9/L appears to have the most clinical utility in ruling out cirrhosis across all chronic viral hepatitis. This widely available laboratory value may be useful in decision making for the management of patients with chronic viral hepatitis and represents a finding which may be of particular value in a primary care setting.

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INTRODUCTION

Globally, chronic hepatitis B, C, and D virus (HBV, HCV and HDV respectively) affect about 325 million people[1]. Progression of these viral infections is associated with serious complications including cirrhosis, hepatic decompensation, hepatocellular carcinoma, and death. With effective treatments for hepatitis B and C, the Centers for Disease Control and Prevention have advocated for widespread screening for viral hepatitis in adults[2,3]. There has also been a paradigm shift where primary care physicians are increasingly tasked with managing and treating these patients[4], and various programs have allowed for expanded care in areas with poor access to viral hepatitis care[5]. In addition, numerous efforts worldwide have aimed to increase the number of providers with the ability to manage chronic viral hepatitis, including the national viral hepatitis action plan 2017-2020 by the U.S. Department of Health and Human Services[6] and the Mukh-Mantri Punjab Hepatitis C Relief Fund program in India[7].

The decision of when and whom to treat in chronic viral hepatitis infections is often dependent upon the stage of liver disease[8,9]. Currently, liver biopsy is the gold standard for staging disease severity in patients with liver disease. However, liver biopsies are invasive, performed by a specialist and access may be limited in resource-poor regions. To date, no single routinely measured laboratory marker has been explored for the identification of cirrhosis. Although expert consensus suggests that thrombocytopenia, with a laboratory cutoff value of < 150 × 10^9/L, is a surrogate marker for cirrhosis, this has mostly been demonstrated in patients with chronic HCV[10,11]. More recently, platelet counts have been used in conjunction with other markers. Current hepatology guidelines state that clinically significant portal hypertension can be identified by "liver stiffness > 20-25 kPa, alone or combined with platelet count and spleen size"[12]. Unfortunately, ultrasound-based techniques [such as Vibration Controlled Transient Elastography (VCTE)] providing an assessment of liver stiffness and cirrhosis are not widely available in all regions and to all healthcare providers.

Common serum laboratory tests, including platelet counts, have been included in

non-invasive markers of liver fibrosis or cirrhosis and have demonstrated clinical utility in the management of hepatitis C[9,13]. However, these non-invasive markers have not been shown to be as useful in chronic HBV due to its complex natural history[14,15]. Nonetheless, these tools have provided a cost-effective method to identify disease progression in patients with chronic viral hepatitis. Unfortunately, these tests require an on-line calculator as well as interpretation of various cutoff values and although often used by hepatologists and gastroenterologists, they remain unknown to primary care providers. Additionally, while their use for diagnosis of advanced fibrosis is widespread, they are not as powerful in determining cirrhosis as ultrasound-based methods[16,17].

With the increasing role of primary care providers in the management of chronic viral hepatitis, the development of a widely available and versatile tool in identifying patients with cirrhosis is clinically necessary. In this group of patients, additional management and treatment considerations may be required, as well as a referral to a specialist. In this study, we explore whether platelets or other commonly measured laboratory markers, alone, can be used as a simple and effective way to characterize the progression of viral hepatitis and whether a threshold can be identified for the development of cirrhosis.

MATERIALS AND METHODS

Study population

This retrospective, cross-sectional study consisted of patients infected with HBV, HCV or HDV and who underwent liver biopsy at the National Institutes of Health Clinical Center between 1985 and June 2019. Chronic viral hepatitis infection was established if patients demonstrated viral positivity for at least six months and/or histology consistent with the respective chronic infection. Chronic hepatitis B infection was established with the presence of hepatitis B surface antigen (HBsAg) in serum and positive HBsAg or hepatitis B core antigen staining on histology. Chronic hepatitis D co-infection was established with the presence of anti-HDV antibodies and HDV RNA in serum or positive hepatitis D antigen staining on histology in patients with chronic HBV. In patients who underwent biopsy after 1991, chronic hepatitis C was established using the presence of HCV RNA in serum for six months. In those who underwent biopsy prior to 1991, patients with presence of clinical and histologic features of non-A non-B hepatitis were later confirmed to have HCV infection by testing for HCV RNA using stored serum.

Patients with concomitant chronic non-viral liver diseases, multiple viral hepatitis (besides HBV/HDV co-infection), or HIV co-infection were excluded. In addition, patients were judged to be in adequate overall health to undergo liver biopsy and had no severe systemic diseases. All patients were enrolled in clinical research protocols approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board and gave written, informed consent for participation. Pre-treatment liver biopsies were reviewed, and concurrent laboratory values were also collected using the NIH Biomedical Translational Research Information System. Laboratory results within two months prior to the liver biopsy and initiation of any treatment were utilized for analysis.

Liver histopathology

All liver biopsies were scored and analyzed by a single hepatopathologist (DEK). Ishak fibrosis scores were used to score hepatic fibrosis, ranging from 0 (no fibrosis) to 6 (cirrhosis)[18]. Cirrhosis was defined as a score ≥ 5. Inflammation was scored using the modified histologic activity index (HAI), ranging from 0-18[19]. The total HAI score comprised of the summation of periportal inflammation, lobular inflammation, and portal inflammation.

Statistical methods

Training and validation cohorts: The entire cohort was randomly divided into training and validation cohorts using simple random sampling and a sample rate of 0.3. Selection was stratified by gender and virus type. Univariate comparisons of the two cohorts were conducted using student *t*-tests and chi-square tests where appropriate. Based on this analysis the training and validation cohorts were similar.

Biomarker selection: The training cohort was used to single out the best performing biomarker to identify cirrhosis status. Spearman's correlations were calculated in the

training cohort to determine the association between fibrosis and selected laboratory markers. Of the significantly correlated laboratory parameters, those with an absolute value of Spearman's R greater than 0.3 (moderate correlation) were selected for further analysis within the training cohort[20]. Logistic regression was used to create receiver operating curves and calculate the area under the curve (AUROC) of each selected laboratory parameter within the training cohort. Laboratory markers were log transformed to assure normality of the data. Sensitivity, specificity, positive predictive value, and negative predictive value were also used to measure performance. Delong Test was used to compare ROC curves for different laboratory parameters within the same sample group. Youden's index, as well as sensitivity, specificity, positive predictive value, and negative predictive value were all used to determine the optimal platelet cut-off point to predict cirrhosis. Once this analysis was completed in the training cohort, the most significant factor in the training cohort was tested in the validation cohort and by virus within the validation cohort through AUROC values, sensitivity, specificity, positive predictive value, and negative predictive value. Fibrosis-4 index (Fib-4) and AST (aspartate aminotransferase) to Platelet Ratio Index (APRI) were calculated using the established formulas[9,13]. All analysis was conducted using SAS 9.4 (Cary, NC, United States).

RESULTS

Study demographics

A total of 1027 untreated subjects with viral hepatitis were evaluated (HCV = 701, HBV = 240, HDV = 86). The mean age of the cohort was 45 years (SD: 11) and 66% of subjects were male. Baseline demographics for the training and validations cohort are displayed in Table 1. In the training cohort, the mean Ishak fibrosis score was 2.4 (SD: 1.8) and 15% of patients were cirrhotic.

Mean platelet count in the training cohort was 187 × 10^9/L (SD: 64). Mean alanine aminotransferase (ALT) and AST values were elevated within the training cohort [104 IU/mL (SD: 88); 70 IU/mL (SD: 55) respectively]. Mean albumin, prothrombin time, total bilirubin, and alkaline phosphatase values were within normal limits.

Using a single laboratory marker to identify cirrhosis

Laboratory markers commonly used to characterize liver disease were tested for their ability to identify cirrhosis within the training cohort (Table 2). These markers included transaminases, platelet count, total bilirubin, prothrombin time, albumin, and alkaline phosphatase. On Spearman's correlation of the training cohort, all tested laboratory markers appeared to be significantly correlated with Ishak fibrosis stage; however, only platelets, ALT, AST, alkaline phosphatase, and prothrombin time had Spearman correlations > 0.3 (Table 2).

Out of all of these laboratory markers, platelets performed the best at identifying cirrhosis compared to other laboratory markers (AUROC = 0.86, 95%CI 0.82-0.90), with all other markers with AUROCs ≤ 0.77 (Table 3). Prothrombin time had the next highest AUROC in the entire cohort (0.76, 95%CI 0.71-0.82). When comparing the ROC curves by the Delong test, platelets performed significantly better than all other tested laboratory markers in the training cohort (P < 0.002). Platelet counts compared favorably to both APRI [AUROC 0.84 (95%CI 0.80-0.88)] and Fib-4 [AUROC 0.88 (95%CI 0.85-0.91)].

Calculating a platelet cut-off for cirrhosis

The optimized platelet cut-off for detecting cirrhosis in the training cohort was 143 × 10^9/L (sensitivity: 77%, specificity: 83%, positive predictive value: 44%, negative predictive value: 95%). Figure 1 shows an overall decrease in the distribution of platelet count by Ishak fibrosis in the training and validation cohorts. Additionally, the demarcated, calculated platelet cut-off of 143 × 10^9/L appears to separate a majority of subjects with Ishak fibrosis ≥ 5 (Figure 1).

Platelet performance in validation cohort

The cutoff calculated in the training cohort was applied to the entire validation cohort and was also evaluated for each viral hepatitis. The performance of platelets to identify cirrhosis is demonstrated in Figure 2; platelets performed adequately in each virus (AUROC ≥ 0.81) and performed best in the HDV/HBV co-infection subset of the validation cohort (AUROC = 0.87). In the entire validation cohort, platelets performed

Table 1 Baseline demographics

	Training (*n* = 715)	Validation (*n* = 312)	*P* value
Age (yr)	45.6 (10.7)	44.5 (11.1)	0.1
Male/female (%)	66/34	66/34	1.0
Platelets ($\times 10^9$/L)	186.7 (64.4)	190.6 (68.2)	0.4
Alanine aminotransferase (IU/L)	103.8 (88.1)	105.1 (89.1)	0.8
Aspartate aminotransferase (IU/L)	69.9 (55.0)	68.0 (53.3)	0.6
Albumin (g/dL)	3.9 (0.46)	3.9 (0.39)	0.2
Alkaline phosphatase (IU/L)	82.3 (39.2)	79.0 (29.2)	0.1
Prothrombin time (s)	13.0 (1.3)	12.9 (1.1)	0.3
Total bilirubin (mg/dL)	0.81 (0.48)	0.77 (0.45)	0.2
Ishak fibrosis	2.4 (1.8)	2.3 (1.7)	0.3
HAI inflammation	8.0 (3.0)	7.9 (3.1)	0.5
HBV/HCV/HDV (%)	23/68/8	23/68/9	1.0

Values presented as mean (SD) unless otherwise noted.

Table 2 Spearman correlations between Ishak fibrosis and liver tests within training cohort

	R	*P* value
Platelets	-0.49	< 0.0001
AST	0.51	< 0.0001
ALT	0.37	< 0.0001
Alkaline phosphatase	0.35	< 0.0001
Prothrombin time	0.33	< 0.0001
Albumin	-0.30	< 0.0001
Total bilirubin	0.18	< 0.0001

Table 2 shows the calculated Spearman *R* and *P* value for the correlations between Ishak fibrosis and the indicated laboratory value. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 3 Area under the curve using selected liver tests within the training cohort

Platelets	ALT	AST	Alkaline phosphatase	Prothrombin time
0.86 (0.82, 0.90)	0.65 (0.59, 0.71)	0.76 (0.71, 0.81)	0.76 (0.71, 0.81)	0.77 (0.71, 0.82)

Values presented as Area Under the Receiver Operating Characteristics curve (AUROC) (95% Wald confidence interval). Table 3 displays the calculated AUROC and 95% Wald confidence interval for each selected laboratory marker in identifying cirrhosis (Ishak ≥ 5) in the training cohort and the entire cohort. Overall, when compared by Delong test, platelets have a significantly greater AUROC value than each of the other laboratory values ($P > 0.002$). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

with an AUROC of 0.85 (95%CI 0.76-0.94) and performed as well as APRI [AUROC 0.82 (95%CI 0.74-0.90)] and Fib-4 [AUROC 0.86 (95%CI 0.80-0.93)]. In general, the optimal platelet cut-off had a higher negative predictive value than positive predictive values (Table 4).

For simplicity, it may be suggested that a platelet cut-off of 143 × 10^9/L be rounded to 140 × 10^9/L instead. The sensitivity, specificity, positive predicative value, and negative predictive values were not greatly altered in the validation cohort (73%, 86%, 48%, 95% respectively) (Table 5).

Table 4 Performance of optimal platelet cut-offs in validation cohort

	Platelet cut-off (× 10^9/L)	AUROC	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Entire validation cohort	143	0.85 (0.76-0.93)	79	84	33	98
HBV	143	0.81 (0.53-1.00)	83	82	29	98
HCV	143	0.83 (0.72-0.94)	75	86	31	98
HDV	143	0.87 (0.74-1.00)	100	60	47	100

Table 4 displays the calculated cut-offs and sensitivity, specificity, positive predictive values, and negative predictive values for each the calculated optimal cut-off within the validation cohort. AUROC: Area Under the Receiver Operating Characteristics curve. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus.

Table 5 Performance of platelet cut-offs in training cohort

Platelet counts (× 10^9/L)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
130	67	91	57	94
140	73	86	48	95
143	74	83	44	94
150	78	78	38	95

Table 5 displays the calculated sensitivity, specificity, positive predictive values, and negative predictive values for four cut-off platelet counts within the training cohort.

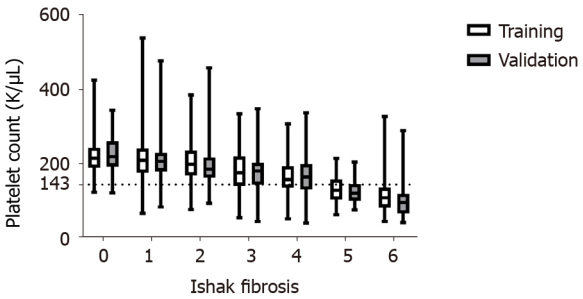

Figure 1 Platelet count distribution by Ishak fibrosis. This figure displays the distribution of platelets in the training and validation cohorts by Ishak fibrosis. The dotted line indicates the calculated optimal platelet cut-off (143 × 10^9/L).

DISCUSSION

In the largest reported cross-sectional retrospective study of patients with chronic viral hepatitis evaluating routinely measured laboratory tests, platelet counts were identified as a surrogate marker for the development of cirrhosis. In comparison to other commonly performed clinical tests in a primary care setting, platelet counts performed the best and had the highest AUROC in identifying patients with cirrhosis. An optimized platelet cut-off value of 143 × 10^9/L across all chronic viral hepatitis infections suggesting cirrhosis was validated. A rounded platelet count of 140 × 10^9/L appears to show similar performance in identifying cirrhosis as well. Given that primary care providers are uniquely positioned in managing patients with chronic viral hepatitis, these results offer a simple and effective method to determine severity of liver disease in a primary care setting without additional testing. The ability to rule out cirrhosis through a simple surrogate marker may provide a simplified approach to connecting patients to treatment and optimal medical management.

Thrombocytopenia is often recognized as a complication of liver disease and has been used as a surrogate marker for varices, portal hypertension, and increased risk of hepatocellular carcinoma; typical complications of cirrhosis[21-23]. Mechanistically,

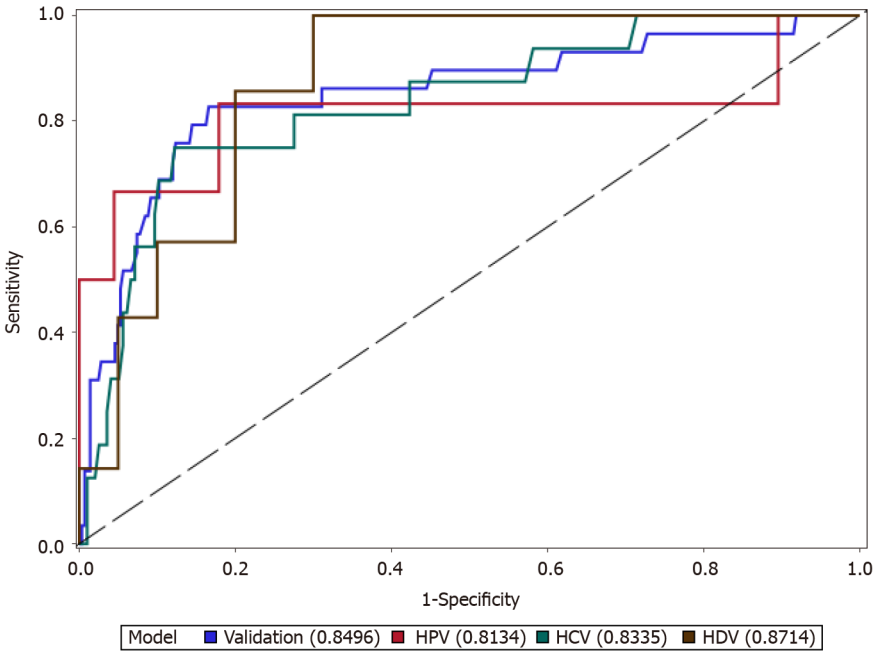

Figure 2 Receiver operating characteristic curves for platelet performance. Receiver operating characteristic curves testing the performance of platelets in identifying cirrhosis in chronic viral hepatitis patients. Area Under the Receiver Operating Characteristics curves (AUROC) were calculated for the entire validation cohort and by virus subgroups within the validation cohort. AUROC values are displayed in the figure key. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus.

there are several possible explanations for the thrombocytopenia in chronic liver disease; such as, splenic sequestration of platelets, decreased platelet production, and decreased thrombopoietin activity[22,24]. Historically, only thrombocytopenia below 50 × 10^9/L has demonstrated clinical relevance[25]. Recently, various scores incorporating platelet counts have been proposed as a surrogate screening tool for complications of portal hypertension, most notably high-risk varices, including the Baveno VI criteria, the expanded Baveno VI criteria, and the albumin, bilirubin and platelet criteria (ABP criteria)[21,26,27]. In these scores, the suggested platelet count cut-offs range from 110 × 10^9/L to 150 × 10^9/L. Nonetheless, these models are restricted to patients with an established diagnosis of cirrhosis.

Additionally, platelets have been incorporated into non-invasive biomarkers of fibrosis such as Fib-4 and APRI, formulas typically utilized by sub-specia-lists[9,13]. Non-invasive biomarkers have been gaining interest as a useful tool in risk stratification in liver disease. However, transaminases, including AST are required for their calculation. This represents a significant drawback in the primary care setting due to increased evidence in certain regions of the world advocating for limiting hepatic screening panels to ALT and alkaline phosphatase[14,28]. Likewise, the cost-effectiveness of this strategy has also been described[29]. Over time, this approach has become an integral part of guidelines, including from the British Society of Gastroenterology[30]. Additionally, these indexes do not perform as well as patented biomarkers (FibroTest, FibroSure, Enhanced Liver Fibrosis) which are not widely available and are costly[31,32]. Therefore, in this context the use of a simple tool, such as platelet counts alone, can be a valuable tool for following patients with viral hepatitis prior to developing cirrhosis. In our cohort, platelet counts alone performed similarly to calculated non-invasive markers. This study demonstrates that thrombocytopenia below 143 × 10^9/L on its own is of clinical importance in viral hepatitis and is a useful single laboratory test to rule out cirrhosis.

According to the World Health Organization, health equity has still not been achieved by countries of all socioeconomic levels. In order to breach this gap in care, an increasing number of primary care physicians are being trained to care for patients with chronic liver disease through programs and resources such as Project ECHO, HepCCaTT (offering care for HCV), and the HBV Primary Care Workgroup[5,33-35] (all in the United States) or the Mukh-Mantri Punjab Hepatitis C Relief Fund in India[7]. However, chronic liver disease is just one of many chronic illnesses that primary care physicians are called upon to manage in these settings. The utility of other non-invasive markers may be limited in resource poor-settings. Both Fib-4 and APRI require multiple laboratory marker measurements, calculations, and knowledge

of validated cut-offs for correct interpretation[9,13,32]. VCTE, while simple and useful technology, is expensive and may not be available at all centers of care. In addition, complex algorithms including a sequential use of non-invasive markers to improve their accuracy have also been suggested[36,37]. These non-invasive markers are useful in specialist care settings, but might not be optimal in resource limited settings where primary-care physicians are the main point of care.

While platelet count has been proven to be an important indicator of liver disease progression, it is important to note that the platelet counts represented in this retrospective single center study's cohort may differ from those seen in a typical primary care setting. Given the specialized setting of the National Institutes of Health, this population may have a higher prevalence of cirrhosis than the typical primary care setting, and this may enhance the performance of platelet count as a marker of cirrhosis within this study. This study proposes the use of a single, commonly measured laboratory marker to monitor the progression of chronic viral hepatitis and identifies a clinically relevant cut-off for clinical decision making and to rule-out cirrhosis. Further studies would provide more information about the clinical outcomes of these patients, on what the degree of thrombocytopenia may imply for these patients and how platelet counts should be included in non-invasive monitoring algorithms. The strength of this study lies in the large cohort of chronically infected patients with histology and three etiologies of viral hepatitis with the inclusion of patients with chronic delta hepatitis.

CONCLUSION

While platelet count has been established as a surrogate marker for disease progression, a specific cut-off for cirrhosis has not been established. Platelet counts can accurately stratify chronic viral hepatitis patients with cirrhosis, a finding which may be of particular value in a primary care setting. As a potential non-invasive biomarker, a platelet count > 143 × 10^9/L or the rounded value 140 × 10^9/L appear to have the most clinical utility in ruling out cirrhosis across all chronic viral hepatitis. This routine and widely available laboratory value may be useful in the identification of patients with cirrhosis from chronic viral hepatitis which has downstream consequences related to their treatment and management and should be further explored for these purposes.

ARTICLE HIGHLIGHTS

Research background

The diagnosis of cirrhosis in patients with chronic viral hepatitis has both treatment and management implications. Identifying these patients is crucial in order to ensure proper care, prevent complications of cirrhosis and for judicious allocation of resources.

Research motivation

With an increasing reliance on primary care in management of chronic viral hepatitis, reliable simple non-invasive assessments of cirrhosis are needed in order to identify cirrhosis and to determine requirement of referral to specialized care.

Research objectives

To evaluate the performance of single laboratory markers, with an emphasis on platelet counts, to identify development of cirrhosis in patients with chronic hepatitis B virus, hepatitis C virus, and hepatitis D virus infection.

Research methods

Retrospective study comparing the accuracy of single laboratory markers in determining cirrhosis (defined as Ishak fibrosis score ≥ 5). Area Under the Receiver Operating Characteristics curve (AUROC), sensitivity, specificity, positive predictive value and negative predictive value were measured first in a training cohort and then in a validation cohort.

Research results

In a cohort of 1027 subjects, compared to other single laboratory markers, platelet counts performed the best at identifying cirrhosis [AUROC 0.86 (0.82-0.90)] and sensitivity 77%, specificity 83%, positive predictive value 44%, and negative predictive value 95%. The optimal cut-off point was 143 × 10^9/L. This performed equally well in a validation cohort.

Research conclusions

Platelet counts are the most reliable single serological marker in ruling out cirrhosis in patients with chronic viral hepatitis. Thrombocytopenia can potentially be used in the primary care setting for management of patients with viral hepatitis.

Research perspectives

Future research directions include validation of this cut-off value of platelet counts in other cohorts of patients with liver disease and evaluation of longitudinal trends of thrombocytopenia.

REFERENCES

1 **World Health Organization**. Global Hepatitis Report, 2017. [cited 21 January 2021]. In: World Health Organization [Internet]. Available from: https://apps.who.int/iris/bitstream/handle/10665/255016/9789241565455-eng.pdf;jsessionid=8B2E8C7D9287FF00B5C9E04740D157C9?sequence=1

2 **Schillie S**, Wester C, Osborne M, Wesolowski L, Ryerson AB. CDC Recommendations for Hepatitis C Screening Among Adults - United States, 2020. *MMWR Recomm Rep* 2020; **69**: 1-17 [PMID: 32271723 DOI: 10.15585/mmwr.rr6902a1]

3 **Weinbaum CM**, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW; Centers for Disease Control and Prevention (CDC). Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]

4 **Wallace J**, Hajarizadeh B, Richmond J, McNally S, Pitts M. Managing chronic hepatitis B - the role of the GP. *Aust Fam Physician* 2012; **41**: 893-898 [PMID: 23145424]

5 **Lewiecki EM**, Rochelle R. Project ECHO: Telehealth to Expand Capacity to Deliver Best Practice Medical Care. *Rheum Dis Clin North Am* 2019; **45**: 303-314 [PMID: 30952400 DOI: 10.1016/j.rdc.2019.01.003]

6 **U.S. Department of Health and Human Services**. National Viral Hepatitis Action Plan 2017-2020. [cited 21 January 2021]. In: U.S. Department of Health and Human Services [Internet]. Available from: https://www.hhs.gov/sites/default/files/National%20Viral%20Hepatitis%20Action%20Plan%202017-2020.pdf

7 **Dhiman RK**, Grover GS, Premkumar M, Taneja S, Duseja A, Arora S, Rathi S, Satsangi S, Roy A; MMPHCRF Investigators. Decentralized care with generic direct-acting antivirals in the management of chronic hepatitis C in a public health care setting. *J Hepatol* 2019; **71**: 1076-1085 [PMID: 31325468 DOI: 10.1016/j.jhep.2019.07.006]

8 **AASLD-IDSA HCV Guidance Panel**. Hepatitis C Guidance 2018 Update: AASLD-IDSA Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. *Clin Infect Dis* 2018; **67**: 1477-1492 [PMID: 30215672 DOI: 10.1093/cid/ciy585]

9 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

10 **Renou C**, Muller P, Jouve E, Bertrand JJ, Raoult A, Benderriter T, Halfon P. Revelance of moderate isolated thrombopenia as a strong predictive marker of cirrhosis in patients with chronic hepatitis C virus. *Am J Gastroenterol* 2001; **96**: 1657-1659 [PMID: 11374731 DOI: 10.1111/j.1572-0241.2001.03830.x]

11 **Cheung RC**, Currie S, Shen H, Bini EJ, Ho SB, Anand BS, Hu KQ, Wright TL, Morgan TR; VA HCV-001 Study Group. Can we predict the degree of fibrosis in chronic hepatitis C patients using routine blood tests in our daily practice? *J Clin Gastroenterol* 2008; **42**: 827-834 [PMID: 18285716 DOI: 10.1097/MCG.0b013e318046ea9a]

12 **Garcia-Tsao G**, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. *Hepatology* 2017; **65**: 310-335 [PMID: 27786365 DOI: 10.1002/hep.28906]

13 **Sterling RK**, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**: 1317-1325 [PMID: 16729309 DOI: 10.1002/hep.21178]

14 **Lilford RJ**, Bentham LM, Armstrong MJ, Neuberger J, Girling AJ. What is the best strategy for investigating abnormal liver function tests in primary care? *BMJ Open* 2013; **3** [PMID: 23794594 DOI: 10.1136/bmjopen-2013-003099]

15 **Kim WR**, Berg T, Asselah T, Flisiak R, Fung S, Gordon SC, Janssen HL, Lampertico P, Lau D, Bornstein JD, Schall RE, Dinh P, Yee LJ, Martins EB, Lim SG, Loomba R, Petersen J, Buti M, Marcellin P. Evaluation of APRI and FIB-4 scoring systems for non-invasive assessment of hepatic fibrosis in chronic hepatitis B patients. *J Hepatol* 2016; **64**: 773-780 [PMID: 26626497 DOI: 10.1016/j.jhep.2015.11.012]

16 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P; FIBROSTIC study group. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**: 1013-1021 [PMID: 20850886 DOI: 10.1016/j.jhep.2010.05.035]

17 **Castera L**. Noninvasive methods to assess liver disease in patients with hepatitis B or C. *Gastroenterology* 2012; **142**: 1293-1302.e4 [PMID: 22537436 DOI: 10.1053/j.gastro.2012.02.017]

18 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]

19 **Knodell RG**, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981; **1**: 431-435 [PMID: 7308988 DOI: 10.1002/hep.1840010511]

20 **Hinkle DE WW**, Jurs SG. Applied Statistics for the Behavioral Sciences. 5th ed. Boston: Houghton Mifflin, 2003

21 **de Franchis R**; Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

22 **Afdhal N**, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, Esteban R. Thrombocytopenia associated with chronic liver disease. *J Hepatol* 2008; **48**: 1000-1007 [PMID: 18433919 DOI: 10.1016/j.jhep.2008.03.009]

23 **Lu SN**, Wang JH, Liu SL, Hung CH, Chen CH, Tung HD, Chen TM, Huang WS, Lee CM, Chen CC, Changchien CS. Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. *Cancer* 2006; **107**: 2212-2222 [PMID: 17019738 DOI: 10.1002/cncr.22242]

24 **Tana MM**, Zhao X, Bradshaw A, Moon MS, Page S, Turner T, Rivera E, Kleiner DE, Heller T. Factors associated with the platelet count in patients with chronic hepatitis C. *Thromb Res* 2015; **135**: 823-828 [PMID: 25728497 DOI: 10.1016/j.thromres.2015.02.010]

25 **Giannini EG**. Review article: thrombocytopenia in chronic liver disease and pharmacologic treatment options. *Aliment Pharmacol Ther* 2006; **23**: 1055-1065 [PMID: 16611265 DOI: 10.1111/j.1365-2036.2006.02889.x]

26 **Augustin S**, Pons M, Maurice JB, Bureau C, Stefanescu H, Ney M, Blasco H, Procopet B, Tsochatzis E, Westbrook RH, Bosch J, Berzigotti A, Abraldes JG, Genescà J. Expanding the Baveno VI criteria for the screening of varices in patients with compensated advanced chronic liver disease. *Hepatology* 2017; **66**: 1980-1988 [PMID: 28696510 DOI: 10.1002/hep.29363]

27 **Kew GS**, Chen ZJ, Yip AW, Huang YWC, Tan LY, Dan YY, Gowans M, Huang DQ, Lee GH, Lee YM, Lim SG, Low HC, Muthiah MD, Tai BC, Tan PS. Identifying Patients With Cirrhosis Who Might Avoid Screening Endoscopy Based on Serum Albumin and Bilirubin and Platelet Counts. *Clin Gastroenterol Hepatol* 2021; **19**: 199-201.e2 [PMID: 31712081 DOI: 10.1016/j.cgh.2019.11.015]

28 **Lilford RJ**, Bentham L, Girling A, Litchfield I, Lancashire R, Armstrong D, Jones R, Marteau T, Neuberger J, Gill P, Cramb R, Olliff S, Arnold D, Khan K, Armstrong MJ, Houlihan DD, Newsome PN, Chilton PJ, Moons K, Altman D. Birmingham and Lambeth Liver Evaluation Testing Strategies (BALLETS): a prospective cohort study. *Health Technol Assess* 2013; **17**: i-xiv, 1-307 [PMID: 23834998 DOI: 10.3310/hta17280]

29 **Xu Q**, Higgins T, Cembrowski GS. Limiting the testing of AST: a diagnostically nonspecific enzyme. *Am J Clin Pathol* 2015; **144**: 423-426 [PMID: 26276772 DOI: 10.1309/AJCPO47VAWYRIDHG]

30 **Newsome PN**, Cramb R, Davison SM, Dillon JF, Foulerton M, Godfrey EM, Hall R, Harrower U, Hudson M, Langford A, Mackie A, Mitchell-Thain R, Sennett K, Sheron NC, Verne J, Walmsley M, Yeoman A. Guidelines on the management of abnormal liver blood tests. *Gut* 2018; **67**: 6-19 [PMID: 29122851 DOI: 10.1136/gutjnl-2017-314924]

31 **Chou R**, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection: a systematic review. *Ann Intern Med* 2013; **158**: 807-820 [PMID: 23732714 DOI: 10.7326/0003-4819-158-11-201306040-00005]

32 **Patel K**, Sebastiani G. Limitations of non-invasive tests for assessment of liver fibrosis. *JHEP Rep* 2020; **2**: 100067 [PMID: 32118201 DOI: 10.1016/j.jhepr.2020.100067]

33 **Marciano S**, Haddad L, Plazzotta F, Mauro E, Terraza S, Arora S, Thornton K, Ríos B, García Dans C, Ratusnu N, Calanni L, Allevato J, Sirotinsky ME, Pedrosa M, Gadano A. Implementation of the ECHO® telementoring model for the treatment of patients with hepatitis C. *J Med Virol* 2017; **89**: 660-664 [PMID: 27551942 DOI: 10.1002/jmv.24668]

34 **Boodram B**, Kaufmann M, Aronsohn A, Hamlish T, Peregrine Antalis E, Kim K, Wolf J, Rodriguez I, Millman AJ, Johnson D. Case Management and Capacity Building to Enhance Hepatitis C

Treatment Uptake at Community Health Centers in a Large Urban Setting. *Fam Community Health* 2020; **43**: 150-160 [PMID: 32079971 DOI: 10.1097/FCH.0000000000000253]

35 **Hepatitis B Primary Care Workgroup**. Hepatitis B Management: Guidance for the Primary Care Provider. [cited 21 January 2021]. In: Hepatitis B Online [Internet]. Available from: https://www.hepatitisb.uw.edu/page/primary-care-workgroup/guidance

36 **Boursier J**, de Ledinghen V, Zarski JP, Fouchard-Hubert I, Gallois Y, Oberti F, Calès P; multicentric groups from SNIFF 32, VINDIAG 7, and ANRS/HC/EP23 FIBROSTAR studies. Comparison of eight diagnostic algorithms for liver fibrosis in hepatitis C: new algorithms are more precise and entirely noninvasive. *Hepatology* 2012; **55**: 58-67 [PMID: 21898504 DOI: 10.1002/hep.24654]

37 **Sebastiani G**, Halfon P, Castera L, Pol S, Thomas DL, Mangia A, Di Marco V, Pirisi M, Voiculescu M, Guido M, Bourliere M, Noventa F, Alberti A. SAFE biopsy: a validated method for large-scale staging of liver fibrosis in chronic hepatitis C. *Hepatology* 2009; **49**: 1821-1827 [PMID: 19291784 DOI: 10.1002/hep.22859]

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ORIGINAL ARTICLE

Prospective Study

Impact of cytomegalovirus reactivation just before liver transplantation: A prospective cohort study

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Conflict-of-interest statement: The

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Abstract

BACKGROUND

Cytomegalovirus (CMV) is the most common viral pathogen after liver transplantation (LT). Although reactivation of CMV infection is generally described in the context of immunosuppression, it has also been described in critically ill immunocompetent patients including cirrhotic patients.

AIM

To determine the incidence of reactivated CMV prior to LT.

METHODS

This was a prospective cohort study evaluating adult patients who underwent LT between 2014 and 2016. A plasma sample was obtained from all patients for CMV quantitative real-time PCR testing right before transplantation. Patients were followed for at least 1 year to assess the following outcomes: Incidence of CMV infection, organ rejection and overall mortality.

authors declare no conflict of interest.

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RESULTS

A total of 72 patients were enrolled. Four patients died before transplantation, thus 68 patients were followed up for a median of 44 mo (20-50 mo). In 23/72 patients (31.9%) CMV was reactivated before transplantation. Post-transplantation, 16/68 (23.5%) patients had CMV infection and that was significantly associated with the recipient being CMV negative and a CMV-positive donor. Pre-transplant CMV reactivation was not associated with overall mortality (log rank: 0.9).

CONCLUSION

This study shows that CMV infection is common in patients with chronic liver disease just before LT, but the clinical impact of this infection seems to be negligible.

Key Words: Liver transplantation; Cytomegalovirus infection; Quantitative real-time PCR; Risk factors; Liver cirrhosis; Molecular biology

Core Tip: Cytomegalovirus (CMV) commonly reactivates before liver transplantation in patients with chronic liver conditions. This prospective cohort study demonstrates for the first time that although frequent, CMV reactivation has limited clinical impact when occurring just before liver transplantation.

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INTRODUCTION

Cytomegalovirus (CMV) is the most common viral pathogen after liver transplantation (LT). Most infections occur between the 3rd and the 12th postoperative week, reaching the highest incidence around the 5th post-transplant week. The overall incidence of CMV infection is between 50%-60% in liver transplant recipients, with 20%-30% of patients demonstrating symptomatic infection[1]. The incidence of post-transplant CMV infection depends mainly on the recipient and donor serological profile. Accordingly, it is more frequent in the context of positive immunoglobulin G (IgG) CMV serology in donors, and negative recipients (*i.e.*, D+/R- status), with more than half of these patients developing visceral disease, in the absence of antiviral prophylaxis[2]. The lowest-risk groups include positive serology for both donors and recipients (D+/R+ status) and a negative status for both donors and recipients (D-/R-). The incidence of CMV infection in such low-risk groups ranges between 5%-40%[3]. Intense immunosuppression and fulminant hepatitis transplantation are also important risk factors for infection.

Although reactivation of CMV infection is mostly described in the context of overt immunosuppression, reactivation may also occur in critically ill immunocompetent patients[4-7] associated with increased mortality[8,9]. A subgroup of particular interest is patients with chronic liver diseases[10,11]. Whether CMV reactivation in these individuals that are listed for LT has any impact on post-transplant outcomes has not been determined[12]. Therefore, here we investigate the frequency and impact of CMV reactivation in patients with chronic liver disease on the waiting list for LT. In particular, we were interested to study the impact of plasma circulating CMV DNA in terms of organ rejection, reactivation of CMV post-transplantation and overall mortality.

MATERIALS AND METHODS

This was a prospective cohort study that evaluated adult (≥ 18 years of age) patients with chronic liver disease listed to undergo LT at Santa Casa de Misericórdia de Porto Alegre. Santa Casa is a referral hospital for organ transplantation in Latin America, and performs approximately 60 liver transplant procedures every year. Patients were non-consecutively enrolled between the years 2014 and 2016.

Clinical and demographic data obtained in this study included age, gender, presence of comorbidities, Model for End-Stage Liver Disease (MELD) score, donor and recipient IgG serostatus for CMV infection, presence of hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, renal insufficiency, hepatocarcinoma, fulminant hepatitis and re-transplantation.

Patients were followed for a minimum of 1 year after LT. During this period, all episodes of CMV reactivation [detected by either quantitative real-time PCR (qRT-PCR) and/or pp65 antigenemia] were documented, as well as events of CMV disease, organ rejection and overall mortality. Screening for CMV reactivation was performed monthly for the first three months after transplantation or whenever the patient presented with clinical symptoms such as fever, fatigue, organ rejection or in the case of diagnostic uncertainty (according to the institutional protocol of low resource countries). Antiviral prophylaxis was not used, instead preemptive treatment against CMV was applied to all patients, including sero-discordant patients.

Molecular tests

At the time the enrolled participants were called in for LT, 4 mL of plasma was collected in an ethylene diamine tetraacetic acid tube centrifuged at 1300 g for 15 min and frozen at -80°C until nucleic acid extraction for analysis of CMV qRT-PCR.

DNA was extracted using the Qiagen DNA Mini Kit (Qiagen Inc., Valencia, United States) following the manufacturer's instructions. qRT-PCR reactions were performed using an in-house assay calibrated with the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques NIBSC code: 09/162 that targets the genes UL 34 and UL 80.5. Primers and probes used in this study were described by Ho and Barry and the sequences are shown in the supplementary material with some modifications in the probe design[13]. The reagents and concentration of the qRT-PCR reaction are shown in the supplementary material. Amplification was performed in an 7500 real-time PCR system (Thermo Scientific, United States), the thermocycling conditions for the qRT-PCR reaction were: 1 cycle of 2 min at 50°C; 2 min at 95°C; followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. The results are reported in International Units (IU/mL) according to CMV World Health Organization standards[14]. The limit of detection and quantification of the test was 60.26 IU/mL, and the results were considered positive only above this cut-off value.

Statistical analysis

Statistical calculations were performed using SPSS 20.0 software. The Chi-square test or Fisher's exact test compared categorical variables, as appropriate. For continuous variables, we used the Student *t*-test or Mann-Whitney test, as appropriate. Multivariate analysis with a logistic regression model was used to estimate the probability of an association between active CMV infection immediately before the procedure and post-transplant reactivation. All variables demonstrating $P < 0.20$ at univariate analysis were considered for multivariate analysis, in addition to the variables of known biological significance. Kaplan Meier and Cox regression tests were used to evaluate predictors of mortality. For all statistical tests used, a value of $P < 0.05$ was considered statistically significant.

Sample size calculation

Considering the primary endpoint of the study and based on studies showing that approximately 50% of cirrhotic patients have detectable plasma CMV DNA[15], 64 patients would need to be studied, considering an alpha error of 0.05 and 80% of power. Thus, respecting a confidence interval of 95%, and to account for possible losses (10%), we estimated to include 70 patients.

Ethical aspects

This study was approved by the Research Ethics Committee at Santa Casa de Misericordia of Porto Alegre, No. 294/2010. All patients signed an informed consent form and agreed to participate in the study.

RESULTS

A total of 72 patients were enrolled in the study. Four patients died before transplantation; thus, 68 patients were followed up for a median of 44 mo (25%-75% percentile: 20-50 mo). Clinical and demographic characteristics of the patients are shown in Table 1. The majority of patients were female (70.8%) had active chronic hepatitis C infection (63.9%) and hepatocellular carcinoma (58.3%). Only 5 patients (6.9%) were CMV sero-discordant (D+/R-).

CMV reactivation was demonstrated in 31.9% (23/72) of patients before transplantation. Median plasma CMV DNA concentration in these patients was 1.212 IU/mL (25%-75% percentile: 560-4.197 IU/mL). In addition, two IgG negative patients had CMV reactivation but none received treatment at that time (7.486 and 7.917 UI/mL). Following LT, CMV infection occurred in 16/67 patients (23.8%) including two patients with IgG negative/PCR positive. At univariate analysis, the only statistically significant factor associated with post-transplant CMV infection was a CMV negative recipient with a positive CMV donor (Table 2). Multivariate analysis confirmed this as the only statistically significant factor for the prediction of post-transplant CMV infection [Odds ratio (OR): 11.5; 95% confidence interval (CI): 1.1-120; $P = 0.04$].

The crude mortality rate was 20/68 (29.4%), median 7.7 mo (perc 25-75: 1-12), and 7/22 (31.8%) in patients with pre-transplant CMV reactivation ($P = 0.763$). In Kaplan-Meier analyses, pre-transplant CMV reactivation had no impact on mortality following LT (log rank: 0.92) (Figure 1). Cox regression analysis also identified no statistically significant factor for mortality in this cohort.

DISCUSSION

This is the first study to document the frequency of CMV infection just before LT in patients with chronic liver disease, using a very sensitive diagnostic tool (qPCR). We observed a high frequency of CMV infection in these patients (31.9%), even though it had no impact on clinically significant variables in the post-transplant period, including CMV infection/disease, organ rejection and mortality. CMV viremic patients usually had a low CMV viral load (median: 1212 IU/mL).

Our results were probably influenced by the profile of patients being transplanted in our institution, which follows the modified Milan criteria[16], together with the proportion of patients with hepatocellular carcinoma (58.3%), as these patients usually have better performance with a lower MELD, which could induce a lower CMV reactivation rate. Nevertheless, in a similar study, a pre-LT reactivation incidence of 0.7% was found, much lower than that in our study[12]. Our findings were similar to the incidence of reactivation in intensive care patients (31%; 95%CI: 24%-39%) as shown in a recent meta-analysis[9].

When comparing with the findings in the literature, Lapiński *et al*[17] evaluated 123 patients with chronic HCV hepatitis for the presence of CMV infection, also determined by qPCR. CMV DNAemia, predominantly at low levels, was detected in 18 (14.6%) patients. Similar to our study, there was no correlation with HCV viral load, and detection of CMV DNA did not result in clinical and laboratory changes[17]. Bayram *et al*[15] quantitatively evaluated the presence of CMV infection in liver biopsy samples from 44 patients with chronic HBV and 25 patients with chronic HCV infection. CMV infection was demonstrated by qPCR in 52.3% of patients with HBV and in 36% of patients with HCV. Histological activity scores (necroinflammation and fibrosis) were worse in patients who were infected with CMV[15].

We observed that CMV was reactivated in 23% of patients in the post-transplant period, which is comparable to other studies[1-3] as most of them were low or moderate risk for infection (CMV receptor positive in 93%). Moreover, we did not find any association between reactivation before transplantation and reactivation after transplantation in both univariate and multivariate analyses. According to the literature, only a high risk for CMV infection (D+/R-) was statistically associated with CMV reactivation following LT (OR:11.5, 95%CI: 1.1-120, $P = 0.04$). We also did not identify pre-transplant CMV reactivation as a risk factor for organ rejection or overall mortality when both 30 d and 1-year mortality were considered.

This investigation has several limitations, including being a single-center study. In addition, patient selection occurred by convenience (sampling was not consecutive), which may have added some selection bias. Given that the reactivation rate was lower than initially expected (32% *vs* 50%), despite the sample calculation, we had small

Table 1 Patient characteristics and frequency of cytomegalovirus reactivation before liver transplantation

	Total (%)	Reactivation (%)	RR (95%CI)	*P* value
Number of patients (%)	72 (100)	23 (32)		
Gender (male)	21 (29.2)	7 (33.3)	1.09 (0.37-3.23)	0.871
Mean age, years (SD)	56.3 (9.6)	57.3 (9.2)	NA	0.900
MELD, median (IqR)	12 (14)	12 (12)	NA	0.712
Lymphocyte count, median (IqR)	929 (808)	929 (770)	NA	0.471
CMV receptor IgG-negative	5 (8.7)	2 (40)	0.68 (0.11-4.40)	0.652
HCV	46 (63.9)	15 (32.6)	1.09 (0.39-3.1)	0.872
HBV	5 (6.9)	1 (20)	0.51 (0.05-4.9)	1.000
Hepatocarcinoma	42 (58.3)	14 (33.3)	1.17 (0.42-3.2)	0.765
Fulminant hepatitis	2 (3)	0	NA	NA
Diabetes mellitus	24 (33.3)	8 (33.3)	1.1 (0.38-3.13)	0.858
Renal failure	8 (11.1)	5 (62.5)	4.26 (0.92-19.7)	0.100
Re-transplant	2 (3)	0	NA	NA

CI: Confidence interval; IqR: Interquartile range; MELD: Model for end-stage liver disease; NA: Not applicable; RR: Relative risk; SD: Standard deviation; CMV: Cytomegalovirus; HCV: Hepatitis C virus; HBV: Hepatitis B virus; IgG: Immunoglobulin G.

Table 2 Predictors of cytomegalovirus infection after liver transplantation

	CMV (%)	No CMV (%)	RR (95%CI)	*P* value
Number of patients (%)	16/68 (23.5)	52/68 (76.5%)		
CMV reactivation before transplantation	7/16 (43.8)	15/52 (28.8)	1.91 (0.6-6.1)	0.265
Quantitative PCR pre-transplant (IU/mL), mean (SD)	2862 (5696)	868 (2756)	NA	0.154
Gender (male)	4/16 (25)	16/52 (30.8)	0.75 (0.2-2.7)	0.762
Mean age, years (SD)	55 (10.3)	57.3 (8)	NA	0.373
MELD score, median (IqR)	11 (4)	12 (11)	NA	0.254
Lymphocyte count, median (IqR)	1101 (1109)	918 (754)	NA	0.580
Organ rejection	3/16 (18.7)	8/52 (15.3)	1.27 (0.3-5.5)	0.716
CMV-negative receptor	3/16 (18.7)	1/52 (1.9)	11.7 (1.1-122.6)	0.038
Hepatitis C infection	9/16 (56.2)	34/52 (65.4)	0.7 (0.2-2.1)	0.508
Hepatitis B infection	1/16 (6.2)	4/52 (7.7)	0.8 (0.1-7.1)	0.100
Hepatocarcinoma	9/16 (56.2)	30/52 (57.7)	0.9 (0.3-2.9)	0.919
Fulminant hepatitis	0	1/52 (1.9)	NA	NA
Diabetes mellitus	6/16 (37.5)	16/52 (30.8)	1.3 (0.4-4.3)	0.615
Renal failure	2/7 (12.5)	5/52 (9.6)	1.3 (0.2-7.7)	0.664
Re-transplantation	1/16 (6.2)	1/52 (1.9)	3.4 (0.2-57.7)	0.418

CI: Confidence interval; IqR: Interquartile range; IU: International units; MELD: Model for end-stage liver disease; NA: Not applicable; RR: Relative risk; SD: Standard deviation; CMV: Cytomegalovirus.

numbers of some of the events, which may have mainly affected the multivariate analysis.

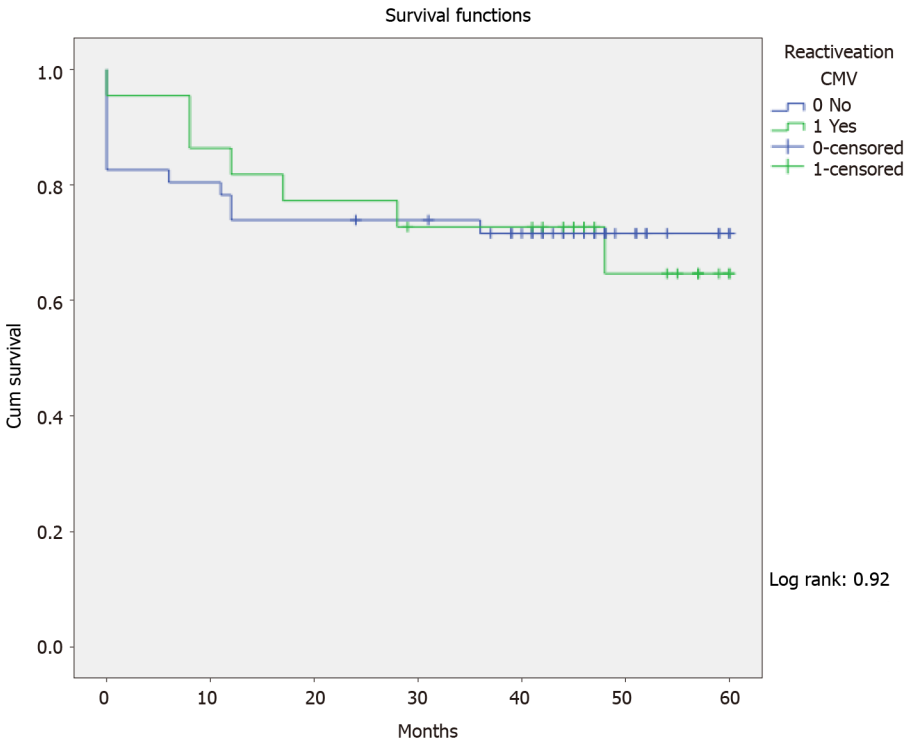

Figure 1 In Kaplan-Meier analyses pre-transplant cytomegalovirus reactivation had no impact on mortality following liver transplantation (log rank: 0.92). CMV: Cytomegalovirus.

CONCLUSION

The findings of this study suggest that pre-transplant CMV reactivation has no influence on LT results, and has no impact on post-transplant CMV reactivation or overall mortality. Based on this study, screening for CMV DNAemia before LT does not seem justified. A larger sample size, better quality and multicenter studies are required to fully elucidate this issue.

ARTICLE HIGHLIGHTS

Research background

The overall incidence of cytomegalovirus (CMV) infection is between 50%-60% in liver transplant recipients, with 20%-30% of patients demonstrating a symptomatic infection[1]. The incidence of post-transplant CMV infection depends mainly on the recipient and donor serological profile. The lowest-risk groups include positive serology for both donors and recipients (D+/R+ status) and a negative status for both donors and recipients (D-/R-). Although reactivation of CMV infection is mostly described in the context of overt immunosuppression, reactivation may also occur in critically ill immunocompetent patients[4-7] associated with increased mortality[8,9].

Research motivation

A subgroup of particular interest is patients with chronic liver diseases[10,11]. Whether CMV reactivation in these individuals that are listed for liver transplantation has any impact on post-transplant outcomes has not been determined[12].

Research objectives

To determine the incidence of reactivated CMV prior to liver transplantation.

Research methods

This was a prospective cohort study that evaluated adult (≥ 18 years of age) patients with chronic liver disease listed to undergo liver transplantation at a referral hospital for organ transplantation in Latin America. Patients were followed for a minimum of 1 year after liver transplantation. During this period, all episodes of CMV reactivation

[detected by either quantitative real-time PCR (qRT-PCR) and/or pp65 antigenemia] were documented, as well as events of CMV disease, organ rejection and overall mortality. Screening for CMV reactivation was performed monthly for the first three months after transplantation or whenever the patient presented with clinical symptoms. At the time the enrolled participants were called in for liver transplantation, plasma was collected for analysis of CMV qRT-PCR.

Research results

A total of 72 patients were enrolled in the study. Four patients died before transplantation, thus 68 patients were followed up for a median of 44 mo (25%-75% percentile: 20-50 mo). CMV reactivation was demonstrated in 31.9% (23/72) of patients before transplantation. Median plasma CMV DNA concentration in these patients was 1.212 IU/mL (25%-75% percentile: 560-4.197 IU/mL). Following liver transplantation, CMV infection occurred in 16/67 patients (23.8%).

The crude mortality rate was 20/68 (29.4%), median 7.7 mo (perc 25-75: 1-12), and 7/22 (31.8%) in patients with pre-transplant CMV reactivation (P = 0.763). In Kaplan-Meier analyses, pre-transplant CMV reactivation had no impact on mortality following liver transplantation (log rank: 0.92) (Figure 1). Cox regression analysis also identified no statistically significant factor for mortality in this cohort.

Research conclusions

The findings of this study suggest that pre-transplant CMV reactivation has no influence on liver transplantation results, and has no impact on post-transplant CMV reactivation or overall mortality.

Research perspectives

Based on this study, screening for CMV DNAemia before liver transplantation does not seem justified. A larger sample size, better quality and multicenter studies are required to fully elucidate this issue.

REFERENCES

1 **Kotton CN**, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, Humar A; The Transplantation Society International CMV Consensus Group. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation. *Transplantation* 2018; **102**: 900-931 [PMID: 29596116 DOI: 10.1097/TP.0000000000002191]

2 **Brasil IRC**, Custodio-Lima J, Sampaio RL, Pierre AMM, Esmeraldo TM, Lima RVC, Lima LFA, Esmeraldo RM. Pre-emptive Therapy for Cytomegalovirus in Post-transplantation Liver Patients With Donor-Positive/Recipient-Negative Serostatus. *Transplant Proc* 2017; **49**: 871-873 [PMID: 28457414 DOI: 10.1016/j.transproceed.2017.03.003]

3 **Mengelle C**, Rostaing L, Weclawiak H, Rossignol C, Kamar N, Izopet J. Prophylaxis versus pre-emptive treatment for prevention of cytomegalovirus infection in CMV-seropositive orthotopic liver-transplant recipients. *J Med Virol* 2015; **87**: 836-844 [PMID: 25655981 DOI: 10.1002/jmv.23964]

4 **Jain M**, Duggal S, Chugh TD. Cytomegalovirus infection in non-immunosuppressed critically ill patients. *J Infect Dev Ctries* 2011; **5**: 571-579 [PMID: 21841300 DOI: 10.3855/jidc.1487]

5 **von Müller L**, Klemm A, Weiss M, Schneider M, Suger-Wiedeck H, Durmus N, Hampl W, Mertens T. Active cytomegalovirus infection in patients with septic shock. *Emerg Infect Dis* 2006; **12**: 1517-1522 [PMID: 17176565 DOI: 10.3201/eid1210.060411]

6 **Kutza AS**, Muhl E, Hackstein H, Kirchner H, Bein G. High incidence of active cytomegalovirus infection among septic patients. *Clin Infect Dis* 1998; **26**: 1076-1082 [PMID: 9597229 DOI: 10.1086/520307]

7 **Jaber S**, Chanques G, Borry J, Souche B, Verdier R, Perrigault PF, Eledjam JJ. Cytomegalovirus infection in critically ill patients: associated factors and consequences. *Chest* 2005; **127**: 233-241 [PMID: 15653989 DOI: 10.1378/chest.127.1.233]

8 **Ziemann M**, Sedemund-Adib B, Reiland P, Schmucker P, Hennig H. Increased mortality in long-term intensive care patients with active cytomegalovirus infection. *Crit Care Med* 2008; **36**: 3145-3150 [PMID: 18936696 DOI: 10.1097/CCM.0b013e31818f3fc4]

9 **Li X**, Huang Y, Xu Z, Zhang R, Liu X, Li Y, Mao P. Cytomegalovirus infection and outcome in immunocompetent patients in the intensive care unit: a systematic review and meta-analysis. *BMC Infect Dis* 2018; **18**: 289 [PMID: 29954328 DOI: 10.1186/s12879-018-3195-5]

10 **Varani S**, Lazzarotto T, Margotti M, Masi L, Gramantieri L, Bolondi L, Landini MP. Laboratory signs of acute or recent cytomegalovirus infection are common in cirrhosis of the liver. *J Med Virol* 2000; **62**: 25-28 [PMID: 10935984 DOI: 10.1002/1096-9071(200009)62:1<25::aid-jmv4>3.0.co;2-e]

11 **Brindley SM**, Lanham AM, Karrer FM, Tucker RM, Fontenot AP, Mack CL. Cytomegalovirus-specific T-cell reactivity in biliary atresia at the time of diagnosis is associated with deficits in

regulatory T cells. *Hepatology* 2012; **55**: 1130-1138 [PMID: 22105891 DOI: 10.1002/hep.24807]

12 **Bruminhent J**, Razonable RR. Outcomes of patients with cytomegalovirus viremia at the time of liver transplantation. *Liver Transpl* 2014; **20**: 1029-1033 [PMID: 24838342 DOI: 10.1002/lt.23915]

13 **Jones S**, Webb EM, Barry CP, Choi WS, Abravaya KB, Schneider GJ, Ho SY. Commutability of Cytomegalovirus WHO International Standard in Different Matrices. *J Clin Microbiol* 2016; **54**: 1512-1519 [PMID: 27030491 DOI: 10.1128/JCM.03292-15]

14 **Fryer JF**, Heath AB, Minor PD; Collaborative Study Group. A collaborative study to establish the 1st WHO International Standard for human cytomegalovirus for nucleic acid amplification technology. *Biologicals* 2016; **44**: 242-251 [PMID: 27179913 DOI: 10.1016/j.biologicals.2016.04.005]

15 **Bayram A**, Ozkur A, Erkilic S. Prevalence of human cytomegalovirus co-infection in patients with chronic viral hepatitis B and C: a comparison of clinical and histological aspects. *J Clin Virol* 2009; **45**: 212-217 [PMID: 19497785 DOI: 10.1016/j.jcv.2009.05.009]

16 **Firl DJ**, Sasaki K, Agopian VG, Gorgen A, Kimura S, Dumronggittigule W, McVey JC, Iesari S, Mennini G, Vitale A, Finkenstedt A, Onali S, Hoppe-Lotichius M, Vennarecci G, Manzia TM, Nicolini D, Avolio AW, Agnes S, Vivarelli M, Tisone G, Ettorre GM, Otto G, Tsochatzis E, Rossi M, Viveiros A, Cillo U, Markmann JF, Ikegami T, Kaido T, Lai Q, Sapisochin G, Lerut J; European Hepatocellular Cancer Liver Transplant Study Group, Aucejo FN. Charting the Path Forward for Risk Prediction in Liver Transplant for Hepatocellular Carcinoma: International Validation of HALTHCC Among 4,089 Patients. *Hepatology* 2020; **71**: 569-582 [PMID: 31243778 DOI: 10.1002/hep.30838]

17 **Lapiński TW**, Kovalchuk O, Parfieniuk A, Flisiak R. [Prevalence, clinical and therapeutical implications of active CMV infection in patients with chronic hepatitis C]. *Przegl Epidemiol* 2009; **63**: 305-309 [PMID: 19799266]

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REVIEW

Potential role of micro ribonucleic acids in screening for anal cancer in human papilloma virus and human immunodeficiency virus related malignancies

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Abstract

Despite advances in antiretroviral treatment (ART), human immunodeficiency virus (HIV) continues to be a major global public health issue owing to the increased mortality rates related to the prevalent oncogenic viruses among people living with HIV (PLWH). Human papillomavirus (HPV) is the most common sexually transmitted viral disease in both men and women worldwide. High-risk or oncogenic HPV types are associated with the development of HPV-related malignancies, including cervical, penile, and anal cancer, in addition to oral cancers. The incidence of anal squamous cell cancers is increasing among PLWH, necessitating the need for reliable screening methods in this population at risk. In fact, the currently used screening methods, including the Pap smear, are invasive and are neither sensitive nor specific. Investigators are interested in circulatory and tissue micro ribonucleic acids (miRNAs), as these small non-coding RNAs are ideal biomarkers for early detection and prognosis of cancer. Multiple miRNAs are deregulated during HIV and HPV infection and their deregulation contributes to the pathogenesis of disease. Here, we will review the molecular basis of HIV and HPV co-infections and focus on the pathogenesis and epidemiology of anal cancer in PLWH. The limitations of screening for anal cancer and the need for a reliable screening program that involves specific miRNAs with diagnostic and therapeutic values is also discussed.

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Core Tip: Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. People living with human immunodeficiency virus (HIV) are at high risk of acquiring HPV infection and developing HPV-associated malignancies, including anal cancer, independent of acquired immune deficiency syndrome. This high risk is associated with several factors including the dysregulation of cellular micro ribonucleic acids (miRNAs) and the direct interaction between HIV and HPV. Dysregulated miRNAs are known to play a role in HIV, HPV infections, and HPV-related cancers. Here, we discuss the role of HIV in HPV-associated pathogenesis and important implications of miRNAs on current screening for and early detection of anal cancer.

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INTRODUCTION

At the end of 2019, approximately 36900000 people were estimated to be living with human immunodeficiency virus (HIV)[1]. Despite the advances in antiretroviral treatment (ART) and the increase in number of patients accessing ART since 2010, cancer mortality in people living with HIV (PLWH) remains high[2]. Mortality from HIV associated illnesses decreased substantially since its peak in 2004 mainly due to a decrease in the incidence of opportunistic infections. With the introduction of highly active ART in 1996, there has been a substantial improvement of clinical outcomes in PLWH[3-5]. This has brought an increase in life expectancy and a change in the age distribution of PLWH[6,7]. The risk of developing cancer increases with age; and as PLWH are now aging, the burden of cancer has substantially increased in this population. Since the beginning of the epidemic, HIV was associated with Kaposi's sarcoma, aggressive B-cell lymphomas, and invasive cervical cancer. Diagnosis of these cancers in PLWH confers the diagnosis of acquired immune deficiency syndrome (AIDS) and are thus termed as AIDS-defining cancers. Other types of cancers are non-AIDS defining, such as anal carcinoma, Hodgkin lymphoma, hepatocellular carcinoma, and lung cancer. These have been increasingly recognized to occur in PLWH and have become a leading cause of death[8-11]. One reason behind the increase in the rate of non-AIDS defining cancers in PLWH is increased prevalence of oncogenic viruses in this population, one of which is human papilloma virus (HPV)[12].

HPV is the most common sexually transmitted viral disease in both men and women worldwide[13]. HPV targets epithelial cells and includes more than 200 types that exist with genomic differences. About 40 types specifically infect the anogenital epithelium and upper digestive tract, among which 15-20 types are considered as high-risk HPV (HR-HPV), including HPV16 and HPV18[14]. Oncogenic or HR-HPV types are associated with the development of high-grade intraepithelial lesions and consequently, cancers of the anogenital region and oropharynx. About 99.9% of cervical cancers and 80%-90% of anal squamous cell cancers (ASCC) are associated with infection with HR-HPV[15]. While the incidence of cervical cancer has remained stable over the years, the incidence of ASCC has increased, particularly in PLWH[16]. With these increasing trends, it is imperative to screen for anal cancer in this high-risk population. However, many of the currently used screening methods, including the Pap smear, are invasive and require specialized equipment. In addition, the Pap smear is neither specific (specificity is approximately 75%) nor sensitive (approximately 55%)

[17]. Thus, identification of non-invasive and more effective methods is crucial.

Micro ribonucleic acids (miRNAs) have emerged as clinically useful molecular biomarkers for better management and treatment of many types of cancers. In HPV-associated cancers, miRNAs have been shown to be deregulated and involved in the pathogenesis of the disease. Given that the molecular mechanisms involved in anal cancer development during HIV infection are still unclear, characterization of miRNA expression in the context of HIV infection and anal cancer and the identification of relevant biomarkers could help elucidate the potential role of HIV and HPV in the progression of ASCC, as well as help prevent and treat anal cancer.

In this review, we will focus on the mechanisms and pathogenesis underlying HIV and HPV infections and the epidemiology and risk factors of anal cancer. We will also discuss the need for anal cancer screening, especially in HIV-infected individuals and the potential implementation of miRNAs as screening and therapeutic tools in high-risk populations.

HIV INFECTION

HIV-1 is the causative agent of AIDS. HIV-1 is a retrovirus whose genome is composed of 2 copies of single-stranded RNA molecules. HIV genome has 9 open reading frames and encodes for precursor proteins that give rise to 15 viral proteins. These proteins can be classified into structural and regulatory. The structural proteins include Gag, Env, and Pol. The matrix, capsid (CA), nucleocapsid, and p6 proteins are generated from Gag precursor and make up the core of the virus particle. The Env polyprotein is subsequently processed to generate the envelope proteins, gp120 and gp41. The *pol* gene encodes viral enzymes: Protease (PR), reverse transcriptase, and integrase. The HIV genome also encodes essential regulatory elements, Tat and Rev, and accessory regulatory proteins: Vif, Vpr and Nef[18].

HIV envelope glycoprotein mediates HIV cell entry by binding to its primary receptor, CD4 molecule, expressed on target cells, such as CD4+ T cells, monocytes, and macrophages. HIV entry also requires binding a chemokine coreceptor, CCR5 or CXCR4[19]. Viral entry is followed by reverse transcription of the viral RNA genome, integration of the provirus into cellular genome, synthesis of viral genome, and assembly and budding of the newly formed virions. When no new viral proteins are produced, infected cells can revert to latency[20].

HIV targets and kills CD4+ T cells, monocytes, macrophages, and microglial cells, however the main targets of HIV infection and subsequent destruction are the CD4+ T cells[21,22].

The mechanisms underlying CD4+ T cell death are still not well defined. The permissivity status of CD4+ T cells during HIV infection determines the pathway by which these cells die (Figure 1). Abortively-infected[23,24], productively infected[25-28], and HIV-uninfected (bystander) CD4+ T cells undergo cell death through different mechanisms[27].

HIV INFECTION AND CD8+ T CELL RESPONSES

In addition to progressive CD4 lymphopenia, HIV infection is also associated with impaired HIV-specific CD8+ T cell responses. CD8+ T cells play an important role in eliminating viruses. Recognition of infected cells occurs through T cell receptor that binds processed viral antigen expressed by major histocompatibility complex (MHC) I molecules on the surface of infected cells. Recognition is followed by a cascade of activation events leading to the release of granzymes and perforin and killing of infected cell. Activated CD8+ T cells also release anti-viral cytokines that act to control viral replication[29]. Despite the over activation of the immune system during HIV infection, it seems that HIV-specific CD8+ T cell responses fail to clear viral infection [30-32] and this can be attributed to several factors. HIV-infected cells sometimes revert to latency and are known to act as viral reservoirs. In this case, the absence of HIV protein expression on the surface of infected cells hinders recognition by CD8+ T cells[33]. Interestingly, several studies have shown that HIV proteins are capable of escaping CD8+ T cell recognition by modulating the expression of MHC I on surface of infected cells (Figure 2)[34]. Andrieu *et al*[35] showed that the Nef protein can down-regulate surface MHC I expression on DC, thereby impairing CD8+ T-cell maturation. In addition, HIV viruses are prone to rapid mutations which enables them to escape immune surveillance[36,37]. Chronic immune stimulation can have adverse effects on

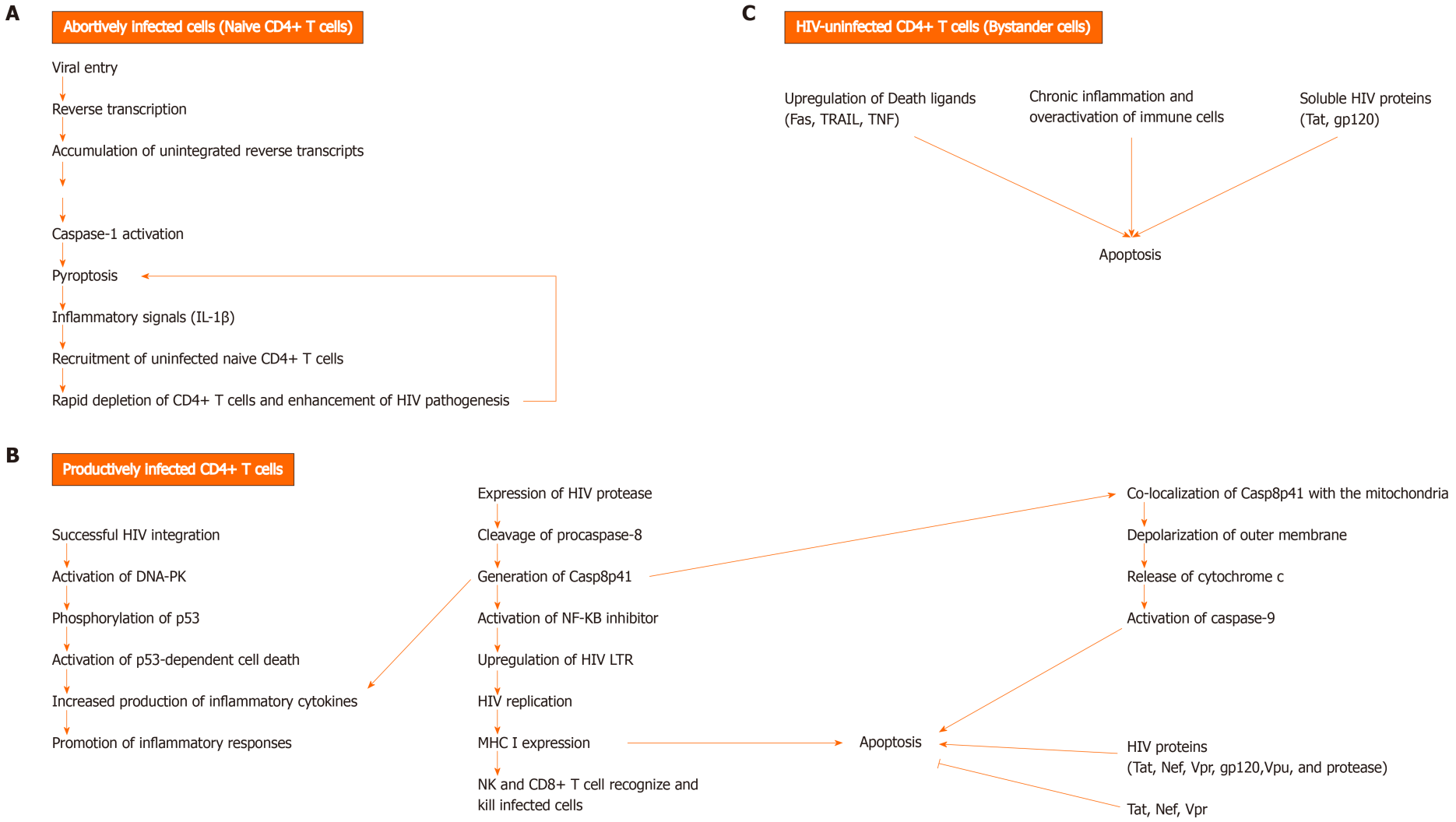

Figure 1 Mechanisms of CD4+ T cell death during human immunodeficiency virus infection. A: Unsuccessful human immunodeficiency virus (HIV) infection can lead to HIV-infected CD4+ T cell death by pyroptosis, an inflammatory programmed cell death that occurs *via* caspase-1 activation. As a result, inflammatory signals, such as Interleukin-1β are released. The accumulation of unintegrated reverse transcripts, following viral entry and reverse transcription, can indirectly activate caspase-1 and induce pyroptosis in resting CD4+ T cells with abortive viral infection. Pyroptosis is thought to greatly contribute to the rapid depletion of CD4+ T cells and development of chronic inflammation, as a result of proinflammatory cytokine release from dying CD4+ T cells, which in turn causes the recruitment of uninfected and naïve CD4+ T cells into the lymphoid tissues. These cytokines trigger pyroptosis in the recruited cells, leading to a vicious cycle of inflammation, thereby enhancing HIV pathogenesis by creating an overactive immune environment and further cell death; B: Cell death can occur in productively infected CD4+ T cells, following successful HIV integration and expression of HIV protease (PR). HIV PR can cleave cellular

procaspase-8 and generate Casp8p41. This fragment activates the transcription factor NF-kappaB inhibitor and thus, induces HIV replication by upregulating HIV long terminal repeats (LTRs). Casp8p41 expression promotes inflammatory responses by enhancing the production of pro-inflammatory cytokines. Besides, Casp8p41 induces apoptosis by directly co-localizing with the mitochondria and depolarizing its outer membrane. The subsequent release of cytochrome c from mitochondria leads to activation of caspase 9 and cell death. Another mechanism by which viral integration triggers cell death is through the activation of deoxyribonucleic acid-dependent protein kinase, resulting in phosphorylation of p53 and activation of p53-dependent cell death program. HIV Tat, Nef, and Vpr can have both pro- and anti- apoptotic effects. On the other hand, gp120, Vpu, and protease have pro-apoptotic effects. HIV-infected cells can be killed by cytotoxic lymphocytes, natural killer cells and CD8+ T cells, which become highly active during infection; C: HIV-uninfected cells, known as bystander cells, usually die by apoptosis during the course of infection, due to either: upregulation of death ligands (Fas, TRAIL, TNF), activation-induced cell death due to chronic inflammation and over activation of immune cells, or direct cytotoxic effects of soluble HIV proteins (Tat, gp120). DNA-PK: DNA-dependent protein kinase.

CD8+ T cell function. Several inhibitory molecules (Figure 2) are expressed by CD8+ T cells during chronic inflammation, and therefore impair the function of HIV-specific CD8+ T cell response[38]. Importantly, a small fraction of CD8+ T cells become infected with HIV and are susceptible to the direct cytotoxic effects of the virus[39,40]. It has also been shown that CD8+ T cell counts begin to decline during late stages of infection[41]. The pro-apoptotic properties of HIV gp120 protein may contribute to this decline[42,43]. Several studies showed that CD4+ T cell loss also impacts the function of CD8+ T cell, whereby CD4+ T cells are required to maintain cell-mediated immune responses against HIV[44,45]. Tregs, a subpopulation of CD4+ T cells that have a regulatory and suppressive role in autoimmune diseases and cancer, have been shown to contribute to the progression of AIDS disease by inhibiting HIV-specific CD4+ and CD8+ T cell responses[46].

HPV GENOME

HPV can deregulate cellular proteins, including p53 and Retinoblastoma protein (pRb), thus mediating epithelial transformation and malignancy. HPV genome consists of a circular DNA that encodes the early proteins E1, E2, E4, E5, E6, and E7, and the late proteins L1 and L2 (Figure 3). E1 and E2 play an important role during HPV replication by binding to the viral replication origin, whereas E4 proteins are involved in virion release. E5, E6, and E7 are viral oncoproteins whose increased expression and activity is associated with enhanced proliferation of HPV-infected epithelial cells. L1 and L2 are structural proteins that form the viral capsid[47].

HPV INFECTION AND CD8+ T CELL RESPONSES

CD8+ T cells play a key role in the immune responses against HPV. *In vivo* studies using mouse models have shown that cells expressing HPV-16 E6 and E7 antigens are recognized and killed by cytotoxic T lymphocyte (CTL) cells[48,49]. In fact, E7-specific CTLs were detected in lesions containing tumor cells[50]. CD8+ T cells recognize viral antigens presented by MHC I/peptide complexes expressed on the surface of infected cells. However, this interaction is not sufficient to induce the killing of the infected cell.

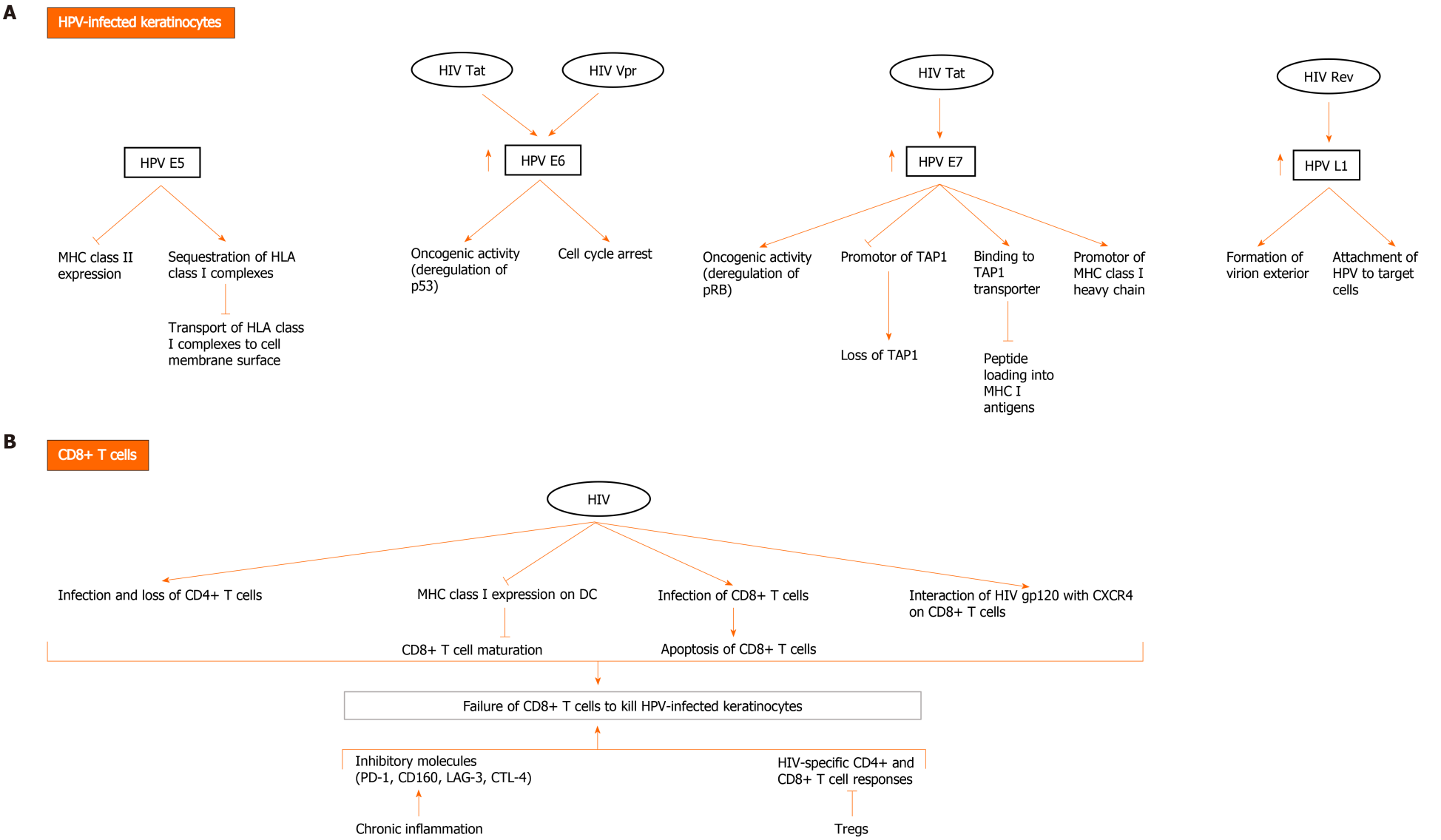

Figure 2 Possible mechanisms of direct and indirect interactions between human papillomavirus and human immunodeficiency virus to evade the immune system and mediate human papillomavirus carcinogenesis. A: Human immunodeficiency virus (HIV) and human papillomavirus (HPV) contribute to HPV-related carcinogenesis and evasion of immune cells through several mechanisms involving direct interaction between HIV and HPV proteins

in HPV-infected keratinocytes. HPV E5 oncoprotein downregulates major histocompatibility complex (MHC) II expression and sequesters human leukocyte antigen (HLA) class I complexes in keratinocytes, thereby blocking transport of HLA class I complexes to cell membrane surface. HPV E6 exerts oncogenic effects, mainly through deregulation of p53 and induction of cell cycle arrest. HPV E7 exerts oncogenic activity through deregulating pRB. It promotes downregulation of MHC class I expression through downregulating peptide transporter 1 associated with antigen processing (TAP1) and binding to TAP transporter, and thus inhibiting peptide loading into MHC I antigens. It also downregulates promotor of MHC I heavy chain. Importantly, HIV Tat upregulates the expression of HPV16 E6 and E7, enhancing their oncogenic effects. It also increases the expression of HPV L1. HIV Rev indirectly upregulates HPV L1 expression. HIV Vpr interacts with HPV E6 protein to induce cell cycle arrest and oncogenesis; B: HIV infection diminishes immune response to HPV infection, resulting in HPV persistence and pathogenesis. Failure of CD8+ T cells to kill HPV-infected keratinocytes is a major event in HIV and HPV co-infection and occurs through multiple mechanisms. In addition to loss of CD4+ T cells due to infection with HIV, downregulation of MHC I expression on dendritic cells (DC) by HIV inhibits CD8+ T cell maturation. A small fraction of CD8+ T cells become infected with HIV and are susceptible to the direct cytotoxic effects of the virus. Moreover, HIV gp120 interacts with CXCR4 on CD8+ T cells and affects their function. Other factors also contribute to inhibition of HIV-specific CD4+ and CD8+ T cell responses and include activation of Tregs and the expression of inhibitory molecules (programmed death-1 [PD-1], CD160, lymphocyte activation gene 3, cytotoxic T lymphocyte antigen-4 [CTLA-4]) by CD8+ T cells during chronic inflammation induced by HIV.

Signaling from activated dendritic cells and virus specific CD4+ T cells is highly important for stimulating and maintaining an efficient CTL activation[51]. It has been reported that peripheral blood mononuclear cell (PBMC) cultures from healthy individuals showed HPV16-specific CD4+ T-cell and CTL responses directed against HPV16 E2, E6 and/or E7[52-55]. Activated circulatory CD4+ and CD8+ T cells migrate from peripheral blood to infected tissues in healthy individuals[56]. Interestingly, these responses are mostly detected in women without cervical intraepithelial neoplasia (CIN)[57], and less commonly in women with CIN[54]. Nakagawa *et al*[55] also showed that the absence of CTL response to E6 proteins is associated with persistence of HPV16 infection in HPV-infected women without squamous intraepithelial lesions. HPV deregulates MHC I expression during infection (Figure 2). Multiple studies have reported the down regulation of MHC I expression in cervical cancer cells[57] and laryngeal papilloma[58]. This may be due to the loss of the peptide transporter 1 associated with antigen processing (TAP1), whose promotor appears to be downregulated by HPV 16 and 18 E7. The latter proteins also downregulate the promotor of MHC class I heavy chain[59]. A study has documented that HPV 11 E7 binds to the TAP transporter protein, thereby blocking peptide loading into MHC I antigens[60]. Other studies have reported that HPV 16 E5 plays a role in sequestering human leukocyte antigen class I complexes in the Golgi apparatus, which thus prevents their transport to the cell membrane surface[61]. The expression of MHC II is also modulated during HPV infection and carcinogenesis. MHC II are usually expressed by antigen presenting cells only however, it has been shown that keratinocytes, in cervical premalignant lesions and cancer, upregulate the expression of MHC II, because of the production of pro-inflammatory cytokines. On the other hand, HPV 16 E5 can block the expression of these molecules[62].

Nevertheless, the induction of a systemic T cell-mediated response against HPV proteins (E6, E7, and others) results in successful viral clearance in healthy individuals. In contrast, HIV infection leads to a progressive loss of CD4+ T cells[63]. Thus, even though antigen presenting cells express and present HPV peptides on their cell surface, in the absence of CD4+ T cell, CD8+ T cells fail to maintain their activity and thus, fail to kill HPV-infected cells.

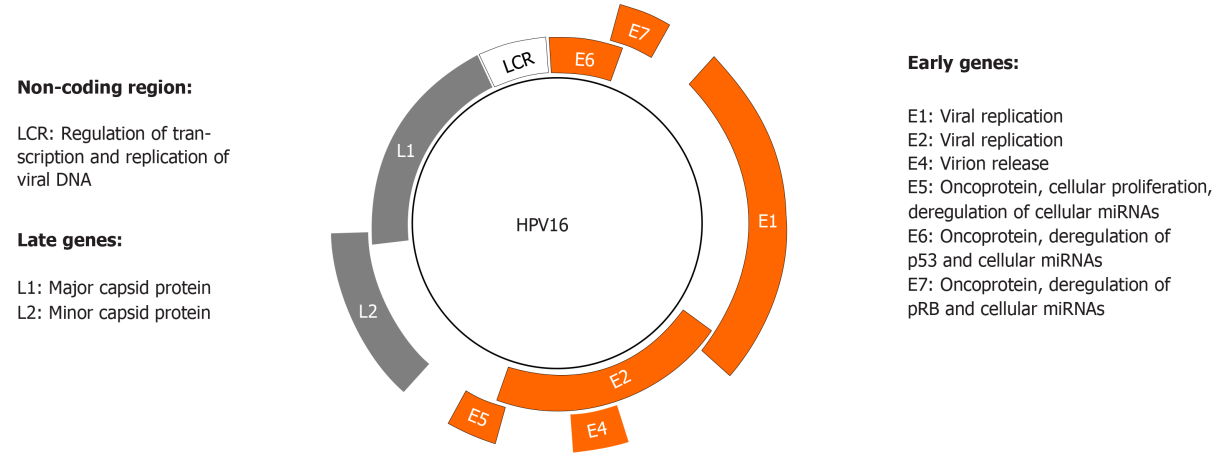

Figure 3 Human papillomavirus genome organization and function. Human papillomavirus (HPV) has a circular double-stranded genome, which is divided into three regions: early, late, and non-coding long control region. The latter regulates transcription and replication of viral deoxyribonucleic acid. The early and late regions encode eight proteins whose major functions are shown.

HPV and HIV have developed a wide spectrum of mechanisms to evade immune responses. Given the ability of both viruses to modulate cellular pathways in infected and uninfected cells, and thus immune surveillance and responses[64], many mechanisms of immune evasion may be possible (Figure 2). HIV infection may directly or indirectly result in protecting HPV-infected keratinocytes from CTL-mediated killing. Therefore, HIV may affect both keratinocytes and CD8+ T cells, and thus favor HPV pathogenesis. Importantly, HIV proteins have been shown to interact with HPV proteins directly and indirectly by enhancing their expression and/or activation, promoting cancer[65-68]. HIV Tat increases the expression of HPV16 E6 and E7, enhancing their oncogenic effects. It also increases the expression of HPV L1, which forms the exterior of the virion and mediates initial attachment to target cells[65,66]. Rev indirectly upregulates HPV L1 expression[67]. Vpr interacts with HPV E6 protein to induce cell cycle arrest in cervical cancer cells[68]. However, evidence of interaction between the two viruses remains scarce and needs further investigation.

ANAL SQUAMOUS CELL CANCER PATHOGENESIS

ASCC are cancers that arise in the transitional or squamous zone of the anal canal and are mostly caused by HPV16 and 18. It is believed that the basal layer cells in the epithelium of this transitional zone can become infected with HPV after the occurrence of micro-abrasions. Most individuals who acquire HPV mount the appropriate immune response and clear HPV infection within a year. However, HPV may persist in others and could lead to either low-grade or high-grad (HSIL) squamous intraepithelial lesions , and can be further classified into anal intraepithelial neoplasia (AIN) 1, 2 or 3[69,70].

ANAL SQUAMOUS CELL CANCER EPIDEMIOLOGY AND RISK FACTORS

Anal cancer is uncommon with 48541 new cases reported worldwide in 2018 as by the GLOBOCAN estimates[71]. However, its epidemiology has changed over the past 2 decades. A steady increase in the incidence and prevalence rates of ASCC has been reported. In the United Kingdom, a 70% increase in its incidence rates has been noted since the early 1990s[72]. The United States has reported similar trends with a 2.9% increase in incident rates each year since 1975[73]. In 2021, there will be an estimated 9090 new anal cancer cases and 1430 new anal cancer deaths[74]. The increase in incidence has been associated with multiple factors that include lifetime number of sexual partners, smoking, receptive anal intercourse, genital warts, and infection with HIV[75-77]. More than 90% of ASCCs have been found to be related to HPV, mainly HPV 16 and 18. Among men, the highest proportion of HPV is in men who have sex with men (MSM) and ranges between 50%-60%[76,78]. This proportion is even higher in HIV-infected MSM and reaches 90% in some studies[79,80]. Additionally, this

population is infected with multiple HR-HPV types[81,82]. Not surprisingly, the prevalence of HSIL and anal cancer mirrors that of anal HPV in these populations where the incidence of neoplasia is higher than that of the general population. Compared to heterosexual men, MSMs have a 20 times increased risk of developing ASCC. HIV-positive MSMs have an even greater risk[76].

An obvious relationship between HIV, HPV, and anal cancer was illustrated in a population study in the United States between 1980-2005[73]. Authors found that HIV infection had a strong impact on the trends of anal cancer among males where incidence rate increased by 3.4% annually overall and by 1.7% in those without HIV-infection. A meta-analysis of 53 studies by Machalek *et al*[76] assessed the prevalence and incidence of HPV, AIN, and anal cancer in MSM and reported a substantial difference between HIV-positive and HIV-negative men for prevalence of any type of HPV (P = 0.005), including any HR-HPV (P = 0.01), prevalence of any anal cytological abnormality (P = 0.005), and low-grade anal lesions (P = 0.01). Analysis of recent studies reporting on histological abnormalities, high-grade AIN, and anal cancer revealed a significant difference between HIV-positive MSM *vs* HIV-negative MSM [76]. In another study, the incident rate for anal cancer was reported to be 69 per 100,000 person-years (PY) in HIV-positive MSMs *vs* 14 per 100,000 PY in HIV-negative MSMs[83]. Contrary to AIDS defining cancers, whose rates have decreased after the introduction of ART, the incidence rates of ASCC have shown an increase by 3%[8,76, 84]. This may be attributable to a longer lifespan of PLWH allowing them to live longer with oncogenic HPV giving time for the development of HSIL and ASCC. In addition, PLWH have been found to have multiple types of HPV with Müller *et al*[82] reporting PLWH having a 7 times higher risk of having multiple types of HPV as compared to HIV-negative individuals.

The link between a lower rate of HR-HPV clearance and development of ASCC in PLWH is still being investigated. Studies have shown that in HIV-positive individuals, HR-HPV infection is cleared at a slower rate than HIV-negative individuals. Geskus *et al*[85] observed that HPV16 had the lowest clearance for both prevalent positive and incident positive infection. Additionally, authors reported a decreasing clearance rate with increasing HIV viral load. Results from a recently updated meta-analysis showed that clearance rate of HPV infection among PLWH was approximately half compared to that of HIV-negative individuals, with similar findings reported for HR-HPV[86]. Whether CD4 count affects the clearance rate or not is not well established. In the same meta-analysis above, Looker *et al*[86] reported a possible, but non-significant, reduction in clearance of HPV with lower CD4 counts. In a nested case-control study from the Swiss HIV Cohort Study, lower CD4 counts in PLWH were correlated with the development of ASCC. Authors reported that the best predictor was a CD4 count 6-7 years prior to ASCC diagnosis. Beyond that point, authors found that the ASCC risk was less sensitive to CD4 counts, highlighting the importance of starting ART early before the establishment of precancerous lesions[87].

ANAL CANCER AND SCREENING

There are no formal guidelines on anal cancer screening due to the lack of trials assessing the effectiveness of such screening practices. However, with the accumulating evidence of an increasing incidence of anal cancer in PLWH, there is increased advocacy for screening in these high-risk populations, drawing on the proven value of cervical cytology in reducing cervical cancer. Additionally, cost-effective models of screening MSM for AIN every 2-3 years have shown possible gains in life-expectancy and quality of life[88,89].

Screening consists of detection and treatment of anal HSIL. Detection can be done through anal cytology, digital rectal examination, high resolution anoscopy (HRA), and/or biopsy. While some experts have advocated the use of HRA for initial screening because of the high prevalence of AIN in PLWH and MSM, anal cytology remains a preferred initial method due to limited availability of HRA especially in developing countries[64]. Yet, all the previously mentioned tools have several limitations and disadvantages. For example, HRA is invasive, and cytology is neither sensitive nor specific. Therefore, a non-invasive method with high sensitivity and specificity for detection of precancerous and cancerous anal lesions is needed.

ROLE OF MIRNAS AS POTENTIAL BIOMARKERS FOR ANAL CANCER SCREENING IN PLWH

Circulatory and tissue miRNAs have become of interest to investigators, as these small non-coding RNAs possess distinctive properties that make them ideal biomarkers for detection and prognosis of cancer. They play an important role in gene regulation by inducing the degradation and inhibiting the translation of the corresponding mRNAs [90,91]. They can also activate the expression of genes by targeting their promotors[92, 93]. They are well known for their pleiotropic effects in many important cellular processes, such as apoptosis, proliferation, and differentiation[94]. They are significantly stable in the circulation, as well as in plasma and serum[95]. miRNAs are dysregulated in many cancers, including HPV-related cancers and their deregulation contributes to pathogenesis of disease[96-98]. Although several miRNAs were identified in different types of cancer, they have not been used in clinical practice, possibly due to the lack of standardized methods, often leading to contradicting data [98,99]. Exosome-encapsulated miRNAs are currently investigated to overcome the challenges associated with free-circulating miRNAs[100,101].

Globular profiling of miRNAs in cancer and normal tissues has been established in different types of cancers, including breast[102], lung[103], colon, liver, and pancreatic [104] cancers, which have allowed for the identification of a series of miRNAs that are deregulated in these cancers. However, an invasive method, such as surgery and biopsy collection, is needed to analyze the tissues. Thus, researchers are investigating the use of plasma and serum miRNAs as potential circulatory biomarkers for different purposes. This would allow for non-invasive quantification of these biomarkers and potentially for detection of premalignant lesions and screening of early tumorigenesis. In the context of HPV-associated cancer, miRNAs have been studied and documented as mediators or suppressors of pathogenesis[96,97,105]. Some of these miRNAs have been shown to be deregulated by HPV E5, E6, and E7 oncoproteins in different cells and tissues (Table 1). By downregulating p53, E6 alters the expression of many miRNAs that are transcribed by p53. On the other hand, E7 releases E2F transcription factor from pRB-E2F complex by degrading pRB. As a result, E2F becomes free to activate the transcription of many miRNAs. The mechanism by which E5 deregulates cellular miRNAs is still unclear[106]. The deregulation of many of these miRNAs was shown to affect several hallmarks of cancer, including enhanced proliferation, inhibition of apoptosis, invasion, and metastasis. A recent study showed that miR-129 was significantly upregulated in the serum and cervical cancer tissues collected from 72 patients, suggesting the possibility of using this miRNA as a biomarker for the detection of cervical cancer. Interestingly, HPV typing detected HPV16 in all cancer samples studied[107]. Another study identified a miRNA signature panel consisting of 9 miRNAs (miR-9, miR-15b, miR-20a, miR-31, miR-93, miR-183, miR-184, miR-222, and let-7b) with a combined area under the curve of 0.89 for CIN3 detection in HPV-positive self-samples of women with CIN3[108]. Recently, Shi *et al*[109] identified an optimal subset of 7 signature miRNAs, including miR144, miR147b, miR2182, miR425, miR451, miR483, and miR486 in cervical cancer. Functional enrichment analysis showed that the latter miRNAs are involved in carcinogenic pathways, such as Wnt signaling pathway and transforming growth factor-β signaling pathway. Importantly, altered miRNAs have been investigated mainly in cervical cancer cell lines[96] and cervical carcinoma samples[110]. However, miRNAs have been less studied in anal cancer and a single study showed that HPV16-E7 protein is capable of inducing miR-15b in anal carcinoma biopsies[111].

HIV infection also dysregulates cellular miRNA biogenesis and expression profiles [112-114]. For example, HIV Tat and Vpr affect miRNA biogenesis by binding Dicer or Drosha[115-117], while trans-activation response modulates TRBP, an important component of the miRNA generation complex. HIV infection is known to both upregulate and downregulate several cellular miRNAs in HIV-infected human PBMC, T cells, monocyte-derived macrophages (MDMs), latently infected CD4+ T cells, plasma samples, HUT78 cells, and CD4+ T cells from either acute or chronic HIV-infected individuals. Few studies determined the expression of cellular miRNAs in HIV-infected cell lines (Table 2). Recently, Biswas *et al*[118] established a comparative global miRNA expression profile in human PBMC and MDMs infected with HIV-1/HIV-2. Differentially expressed miRNAs were identified in these cells. Pathway analysis using Kyoto Encyclopedia of Genes and Genomes database showed that the deregulated miRNAs are likely to be involved in p53 signaling pathway, PI3K-Akt signaling pathways, Mitogen-activated protein kinase signaling pathways, FoxO signaling pathway, and NF-kappaB inhibitor signaling pathway, all of which play a

Table 1 Cellular micro ribonucleic acids deregulated by human papillomavirus proteins

HPV protein	miRNA target	miRNA expression level	Sample type	Biological effect(s)	Ref.
E5	miR-146a	+	E5-expressing HaCat cells	Promoted cell proliferation	[106]
	miR-203	-	E5-expressing HaCat cells	Increased expression of p63	[106]
	miR-324-5p	-	E5-expressing HaCat cells	Contributed to cervical carcinogenesis	[106]
E6	miR-20a	+	CaSki and SiHa (HPV16+) human cervical cell lines	Promoted cell growth through downregulating PDCD6 and activating Akt and p38	[156]
	miR-20b	+	HeLa (HPV18+), SiHa and Caski human cervical cancer cell lines; Cervical carcinoma tissues	Reduced TIMP2 expression and induced EMT, migration, and invasion	[157]
	miR-23b	-	SiHa and CaSki cell lines	Increased expression of uPA and induction of migration in human cervical cancer cells	[158]
	miR-30c-2*	-	HPV-infected NSCLC; TL1 cell line	Correlated with tumor stage and lymph node metastasis	[159]
	miR-34a	-	CaSki and SiHa cell lines, HPV18-positive cell lines HeLa and C411, HPV68-positive cell line ME180Cervical cancer tissues	Inhibited cell proliferation; Increased LDHA expression levels, inhibited Warburg effect and reprogrammed glycolysis through targeting LDHA	[160,161]
	miR-145	-	Hela, SiHa, and CaSki cell lines; Cervical cancer tissues	Modulated invasion and therapy resistance of cervical cancer cells	[162]
	miR-195	-	HeLa and SiHa cell line cervical cancer tissue samples	Promoted cell proliferation, invasion, and metastasis	[163]
	miR-218	-	HPV16 positive cervical cell lines and tissues; Cervical cancer tissues	Increased expression of LAMB3, SFMFBT1, and DCUN1D1, promoted EMT, migration, and invasion in cervical cancer associated with clinicopathological characteristics of patients	[164,165]
	miR-375	-	SiHa and CaSki cell lines; Cervical tissue samples	Modulated EMT in cervical cancer; Enhanced invasion and metastasis of cervical carcinoma cells through targeting SP1	[166,167]
	miR-2861	-	SiHa and CaSki cell lines; Cervical cancer tissues	Enhanced cell proliferation and invasion, and inhibited apoptosis in cervical cancer cells; Negatively associated with advanced tumor stage and lymph node metastasis	[168]
E7	miR-15b	+	HPV16 E7-expressing tumors from anal carcinoma patients; CaSki cell line	Downregulated cyclin E1; Increased expression of several E2F-regulated genes	[111]
	miR-20a	+	OSCC tissues	Inhibited cell proliferation, invasion, and migration	[169]
	miR-21	+	HPV16 E7-transfected Hela cells; Cervical cancer tissue	Enhanced cervical carcinoma cell proliferation, growth, and invasion; Involved in cervicitis and cervical cancer progression	[170,171]
	miR-25	+	HVK-derived raft tissues infected with either HPV16 or HPV18	Increased expression correlated with the progression of the cervical lesions, making it a potential biomarker for CINs and cervical cancer	[96]
	miR-27b	+	HPV 16-positive human cervical carcinoma tissues; SiHa and CaSki cell lines	Reduced PLK2 expression; Promoted cell proliferation and inhibited paclitaxel-induced cell apoptosis; Inhibited PPARγ expression and promoted proliferation and invasion	[172,173]
	miR-205	+	HPV-positive keratinocytes	Activated Akt pathway and upregulated cyclin D1 levels, resulting in increased proliferation	[174]
	miR-323	+	Cervical cancer cell lines transfected with HPV 16 E7 and SiHa cervical cancer cells	---	[175]
E6/E7	miR-16	+	HFK-derived raft cultures with HPV16	---	[96]

		infection; HVK-derived raft tissues infected with either HPV16 or HPV18; CIN3 and Cervical carcinoma tissues with HR HPV infection		
miR-22	-	HVK-derived raft tissues infected with either HPV16 or HPV18	Suppressed tumor growth and metastasis	[96,176]
miR-24	+	HPV-positive keratinocytes	Reduced p27 expression level and enhanced proliferation	[174]
miR-29a	-	HVK-derived raft tissues infected with either HPV16 or HPV18	---	[96]
miR-92a	+	CIN and cervical carcinoma tissues with HR HPV infection, and raft tissues with HPV16 or HPV18 infection	Increased expression correlated with the progression of the cervical lesions, and may serve as a biomarker for CINs and cervical cancer	[96]
miR-100	-	HFK-derived raft cultures with HPV18 infection	---	[96]
miR-125a	-	Cervical carcinoma tissuesSiHa and HeLa cell lines	Increased STAT3 expression and enhanced tumorigenesis and metastasis	[177]
miR-146a-5p	-	HPV16 E6/E7-positive keratinocytes; Hela, SiHa, and CaSki cell lines	Enhanced expression of KDM2B; Promoted proliferation and migration	[178]
miR-203	-	NHKs and NFKs expressing E6, E7, or combination	Increased expression of p63 and promotion of cell proliferation	[179,180]
miR-378	+	CIN3 and cervical carcinoma tissues with HR HPV infection, and raft tissues with HPV16 or HPV18 infection	---	[96]

"+": Upregulated; "-": Downregulated; miRNAs: Micro ribonucleic acid; HPV: Human papillomavirus; PDCD6: Programmed cell death 6; TIMP-2: Tissue inhibitor of metalloproteinase 2; EMT: Epithelial to mesenchymal transition; NSCLC: Non-small cell lung cancer; LDHA: Lactate dehydrogenase A; LAMB3: Laminin 5 β3; SFMFBT1: Scm-like with four MBT domains 1; DCUN1D1: Defective in cullin neddylation 1; Domain containing 1; OSCC: Oral squamous cell carcinoma; HVK: Human vaginal keratinocytes; PLK2: Polo-like kinase2; CIN: Cervical intraepithelial neoplasia; HFK: Human foreskin keratinocytes; CIN3: Cervical intraepithelial neoplasia 3; HR HPV: High risk HPV.

role in carcinogenesis[118,119]. HIV Tat, Nef, and Vpr have been reported to alter the expression levels of many miRNAs and contribute to HIV pathogenesis (Table 3). On the other hand, cellular miRNAs also target HIV genome, but it is still unclear whether these miRNAs are effective during HIV infection[112].

Overall, molecular mechanisms that contribute to anal cancer pathogenesis and progression are still elusive. One of the reasons that little progress has been made in understanding the mechanisms of carcinogenesis in this type of cancer is the scarcity of *in vitro* and *in vivo* model systems for investigating anal cancer. Thus, further studies are required to gain insight into the mechanisms involved in anal caner. This is particularly important as these mechanisms may involve miRNAs, which may be further investigated as potential targets for cancer therapy. The use of miRNA-based therapeutics has been investigated in clinical trials in several countries. MicroRNA mimics and anti-miRNAs (antagomirs) are now under investigation as potential therapeutic agents for multiple cancers. miRNA mimics may be administered to replace downregulated miRNAs, which usually act as tumor suppressors in cancers. On the other hand, many miRNAs have been targeted for inhibition in the treatment of several cancers. These miRNAs are referred to as oncomiRs and their overexpression in cancer contributes to pathogenesis. In the context of cervical cancer, Lee *et al*[110] showed that treatment with anti-miR-199a suppressed cervical cell growth *in vitro*. Additionally, a study has shown a promising role for the tumor suppressor miR-34a, which is downregulated in HPV-positive cancers, in repressing oncogenic transformations. Both miR-34a and miR-125 are downregulated in cervical cancer samples and correlate with cervical cancer invasiveness[120]. Interestingly, a recent phase 1 study of MRX34, a liposomal miR-34a mimic, was conducted with patients having advanced solid tumors[121]. Thereby, this miRNA may be a good candidate for treatment of HPV-related cancers, including anal cancer[122]. In addition, anti-miRs targeted at miR-122, which has been shown to be upregulated in HIV-1 infected Jurkat cells, reached clinical phase II trials and were investigated for treating hepatitis C infection [123]. Other candidate miRNAs are being tested in clinical trials, paving the way for developing miRNA-based drugs for treating several illnesses and cancer diseases[122, 124].

LIMITATIONS AND CONSIDERATIONS FOR THE USE OF MIRNAS AS BIOMARKERS FOR ANAL CANCER SCREENING

In the case of anal cancer, where HIV and HPV pathogenesis play a role in the development of the disease in PLWH, a major challenge is to distinguish HIV-specific miRNAs, HPV-specific miRNAs, and HIV and HPV co-infection-specific miRNAs. Major limitations include the absence of studies implementing computational models to identify these miRNAs, technical issues associated with conventional miRNA extraction and detection tools, and scarcity of anal cancer *in vitro* and *in vivo* models. Ongoing studies are still being conducted to study miRNA profiles during HIV[125-127] and HPV[128-130] infections. With the appropriate application of advanced bioinformatic analysis tools and computational models, the identification of the most predictive miRNAs, even from complex datasets would be possible. These tools are becoming widespread and have already been used to identify potential miRNA biomarkers for Ebola[131] and severe acute respiratory syndrome coronavirus 2[132], in addition, these tools have been used to decipher potential miRNA biomarkers in a wide variety of cancers, including melanoma[133,134], breast[135], colon[136], and lung cancer[137].

In addition to the conventional miRNA detection platforms which include Northern blotting, in situ hybridization, next generation sequencing, reverse transcription qPCR, and microarrays[138], new miRNA extraction and detection platforms have emerged to compensate for the limitations of conventional assays[139]. These technologies are referred to as point-of-care (PoC) technologies and include isothermal amplification-based assays[140], lateral flow assay-based systems[141], nanobead-based[142], electro-chemical-based[143], and microfluidic chip-based[144] strategies. The latter, which is also known as Lab-on-a-chip or microchip, is highly specific, cost-effective, and a quick approach for the multiplexed detection of miRNAs[139]. It has been used to test miRNAs in several biological samples, including blood of breast cancer patients [145]. Importantly, this system has been also used to quantify miRNAs in plasma extracellular vesicles (EVs), including exosomes. EVs are secreted by body cells and are found in body fluids including plasma, urine, and synovial fluid[146]. They have been shown to carry and stabilize miRNAs in the blood[147]. A unique feature of exosomes is the presence of cell-specific proteins[148], which enables identification of exosomes released from cancer cells. Examining specific miRNAs released from tumors and tumor niche, instead of whole blood miRNA profiling would provide a more accurate way of distinguishing HIV-specific and HPV-specific miRNAs, given the unique viral tropism of each. Exosomal miRNAs would enable the identification of the cell origin and might be a better source when compared to non-exosomal, cell-free miRNAs. Recently, studies that profiled and analyzed miRNAs from different sources were reviewed[149]. Authors concluded that 71% of the studies stated that exosomes are the best source of miRNAs as biomarkers. Detecting EVs miRNA signature has already been proven to be a good prognostic tool in several cancers including colorectal[150] and pancreatic cancer[151].

Interestingly, organ-on-chip and organoids are being used to study infectious diseases and cancer. These models can be used to assess HPV virus-Langerhans cells interactions[152] and HPV-oral mucosa epithelia interactions[153]. Cell-to-cell communication can be also studied by co-culturing cancer cells with immune cells, and thus allows the study of cancer-immune interaction. Organoids can be used to model tumor-derived EVs, also known as oncosomes, in addition to EVs released by stromal cells in tumor microenvironment[154]. Very recently, researchers established organoid cultures from human ecto-and endocervix. Cells collected using Pap brush method were used to derive organoids from cervical tissue. The established patient-derived model system resembled causative HPV infection[155], and thus could be used for modeling HPV-related pathogenesis, in addition to exploring the role of HPV and HIV in deregulating miRNAs. The same derivation method can be used to derive organoids from healthy or tumor anal tissue to assess miRNA deregulation by HIV and/or HPV. These model systems could be used to test the efficacy of engineered miRNA-loaded EVs in targeting anal cancer cells to deliver potential miRNA therapeutic molecules[156-180].

It is important to note that although extensive research has been conducted to identify candidate miRNA biomarkers for cancer screening, the development of new techniques, such as PoC for miRNA detection is still at the very early stage and a work on progress. Further progress is required to achieve the desired goal of using PoC testing for detecting and distinguishing miRNAs deregulated by oncogenic viral infections, including HPV. Therefore, the identification of miRNAs deregulated by

Table 2 Cellular micro ribonucleic acids deregulated during human immunodeficiency virus infection

Sample type	miRNAs deregulated	Ref.
PBMCs from HIV-infected patients	↑ miR-9; ↓ miR-29c, miR-31, miR-125b, miR-146b-5p, miR-150, Let-7g	[181]
T cells from HIV-infected individuals	↓ miR-16, miR-146b, miR-150, miR-223	[182]
HIV-1 infected PBMCs	↑ miR-223; ↓ miR-21, miR-26a, miR-29a, miR-29b, miR-29c, miR-155	[183]
HIV-1 infected PBMCs	↑ miR-3195, miR-3656, miR-4492, and miR-6087; ↓ miR-1273h-3p, miR-1273h-5p, miR-671-5p, and miR-7-5p	[118]
HIV-2-infected PBMCs	↑ miR-18a-3p and hsa-miR-320b	[118]
HIV-2-infected MDMs	↑ miR-542-3p, miR-375, miR-195-5p, miR-30c-2-3p, miR-4802-3p, and miR-26b-5p	[118]
HIV-1- and HIV-2-infected MDMs	↓ miR-148b-5p, hsa-miR-26a-2-3p, miR-199a-1, miR-199a-2, and miR-874-5p	[118]
HIV-1-transfected HeLa cells	↓ miR-16, miR-93, miR-148b, miR-221	[184]
HIV-1 infected Jurkat cells.	↑ miR-122, miR-297, miR-370, and miR-373; ↓ miR-17-5p and miR-20a	[185]
latently infected CD4+ T cells	↑ miR-196b and miR-1290	[186]
PBMCs obtained from HIV-1 positive individuals with high viral load	↑ miR19b, miR-34a, miR-144, miR-146a, miR-155, miR-382, miR-615-3p	[187]
Plasma obtained from patients with HIV infection	↓ miR-3162-3p	[188]
HIV-1-infected HUT78 cells and CD4+ T cells from chronic HIV-1 infected individuals	↓ Let-7 miRs	[189]
HIV-1 positive plasma samples in the acute stage infection	↑ miR-16-5p, miR-20b-5p, miR-24-3p, miR-142-5p, miR-195-5p, miR-206, miR-223-3p, miR-885-5p, and let-7 g-3p; ↓ miR-34c-3p, miR-181c-3p, miR-202-3p, and miR-409-3p	[190]
HIV-1 infected CD4+ T cells	↓ miR-20a and miR-106b	[191]

↑: Upregulation; ↓: Downregulation; miRNAs: Micro ribonucleic acid; HIV: Human Immunodeficiency virus; PBMC: Peripheral blood mononuclear cells; MDM: Monocyte-derived macrophages.

HIV, HPV, and HIV-HPV co-infection warrants further research. More accurate and standardized methods are required for implementation of miRNAs as biomarkers for anal cancer diagnosis[181-198]. Importantly, the widespread use of high-throughput sequencing, PoC technologies, and advanced computational analysis tools may facilitate discovering and distinguishing these miRNAs.

CONCLUSION

HPV is the most common sexually transmitted infection worldwide. PLWH are at high risk of acquiring HPV infection and developing HPV-associated malignancies, independent of AIDS. Anal cancer incidence, though rare in the general population, has been rising significantly in PLWH. The lack of standard screening programs contributes to the increased incidence of anal cancer, and thus, there is a need for anal dysplasia screening and treatment in PLWH. The discovery of highly sensitive and specific biomarkers would enable the early detection of anal cancer and the improved survival of HIV-infected patients. There is a need for relevant biomarkers that could be integrated into clinical practice and thus, aid in the detection, diagnosis, and treatment of high-risk patients. miRNAs have become valuable tools for detection and treatment of many types of cancer. Given their deregulation and potentially significant role in HPV-related pathogenesis and in HIV infections, miRNAs may serve as diagnostic and prognostic biomarkers that can enhance HIV patients' outcomes and provide better management of the disease. Genome-wide profiling of miRNAs and validation of miRNA targets in tissue and blood samples of people infected with HIV and HR-HPV is important to establish miRNA expression signatures in this population and would help develop non-invasive miRNA therapeutic strategies for treatment of anal cancer.

Table 3 Cellular micro ribonucleic acids deregulated by human Immunodeficiency virus proteins

HIV protein	miRNA target	Effect	Sample type	Biological effect	Ref.
Tat	miR-21, miR-29a, miR-222, miR-1290	+	Tat101-expressing Jurkat cells; Resting PBMCs from healthy donors were transiently transfected with Tat101-expressing vector	Targeted mRNAs of genes involved in apoptosis, T cell migration, and proliferation	[192]
	miR-128a, and miR-3182	-	Tat101-expressing Jurkat cells	----	[192]
	miR-132	+	Tat-transfected astrocytes and neurons, astrocytes from Tat-transgenic mice, and HIV-infected astrocytes	Involved in the direct neurotoxicity of Tat	[193]
	miR-129, miR-135a, miR-181a, miR-495, miR-523, miR-524, miR-539, let-7	-	U-87MG (astrocyte cell line), HEK 293T, and HeLa cells transfected with wild-type Tat	Downregulation of β-catenin activity	[194]
	miR-101	+	BMVECs exposed to Tat C	Decreased the expression of VE-cadherin	[195]
	miR-34a and miR-138	+	Astrocytoma cell line A172 and rat primary astrocytes exposed to Tat	Upregulated NF-κB and promoted activation of astrocytes	[196]
Nef	miR-573 and miR-638	+	Human monocytic U937 cells that stably expressed HIV-1 Nef	Altered several pathways involved in HIV pathogenesis	[197]
	miR16-1, miR-18, miR-19a, miR-20a, miR-21, miR-27a, miR-29b, miR-125b, miR-146a, miR-146b-3p, miR-181a, miR-223, miR-570, miR-610 and miR-624	-	Human monocytic U937 cells that stably expressed HIV-1 Nef	Altered several pathways involved in HIV pathogenesis	[197]
	miR-17, miR-19a, miR-19b, miR-20a, miR26a, miR-28, miR-29a, miR-29b, miR-29c, miR-92a, miR-125b, miR-149, miR-150, miR-223, miR-324-5p, miR-378 and miR-382	+	Nef exosomes	Inhibited HIV replication	[197]
Vpr	miR-942-5p	+	PEL cells	Targeted IκBα and activation of NF-κB signalling	[198]
	miR-711	+	PEL cells	Directly targeted Notch1 and reduced levels of IκBα transcript	[198]

"+": Upregulation; "-": Downregulation; miRNA: Micro ribonucleic acid; HIV: Human Immunodeficiency virus; HEK: Human embryonic kidney cells; BMVEC: Human brain microvascular endothelial cells; PEL: Primary effusion lymphoma; NF-κB: NF-kappaB inhibitor.

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REFERENCES

1 **GBD 2017 Disease and Injury Incidence and Prevalence Collaborators**. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; **392**: 1789-1858 [PMID: 30496104 DOI: 10.1016/S0140-6736(18)32279-7]

2 **Agace WW**, Roberts AI, Wu L, Greineder C, Ebert EC, Parker CM. Human intestinal lamina propria and intraepithelial lymphocytes express receptors specific for chemokines induced by inflammation. *Eur J Immunol* 2000; **30**: 819-826 [PMID: 10741397]

3 **May MT**, Sterne JA, Costagliola D, Sabin CA, Phillips AN, Justice AC, Dabis F, Gill J, Lundgren J, Hogg RS, de Wolf F, Fätkenheuer G, Staszewski S, d'Arminio Monforte A, Egger M; Antiretroviral Therapy (ART) Cohort Collaboration. HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy: a collaborative analysis. *Lancet* 2006; **368**: 451-458 [PMID: 16890831 DOI: 10.1016/S0140-6736(06)69152-6]

4 **Mocroft A**, Brettle R, Kirk O, Blaxhult A, Parkin JM, Antunes F, Francioli P, D'Arminio Monforte A, Fox Z, Lundgren JD; EuroSIDA study group. Changes in the cause of death among HIV positive subjects across Europe: results from the EuroSIDA study. *AIDS* 2002; **16**: 1663-1671 [PMID: 12172088 DOI: 10.1097/00002030-200208160-00012]

5 **Palella FJ Jr**, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD; HIV Outpatient Study Investigators. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J Acquir Immune Defic Syndr* 2006; **43**: 27-34 [PMID: 16878047 DOI: 10.1097/01.qai.0000233310.90484.16]

6 **Gueler A**, Moser A, Calmy A, Günthard HF, Bernasconi E, Furrer H, Fux CA, Battegay M, Cavassini M, Vernazza P, Zwahlen M, Egger M; Swiss HIV Cohort Study, Swiss National Cohort. Life expectancy in HIV-positive persons in Switzerland: matched comparison with general population. *AIDS* 2017; **31**: 427-436 [PMID: 27831953 DOI: 10.1097/QAD.0000000000001335]

7 **Samji H**, Cescon A, Hogg RS, Modur SP, Althoff KN, Buchacz K, Burchell AN, Cohen M, Gebo KA, Gill MJ, Justice A, Kirk G, Klein MB, Korthuis PT, Martin J, Napravnik S, Rourke SB, Sterling TR, Silverberg MJ, Deeks S, Jacobson LP, Bosch RJ, Kitahata MM, Goedert JJ, Moore R, Gange SJ; North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of IeDEA. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. *PLoS One* 2013; **8**: e81355 [PMID: 24367482 DOI: 10.1371/journal.pone.0081355]

8 **Shiels MS**, Engels EA. Evolving epidemiology of HIV-associated malignancies. *Curr Opin HIV AIDS* 2017; **12**: 6-11 [PMID: 27749369 DOI: 10.1097/COH.0000000000000327]

9 **Smith CJ**, Ryom L, Weber R, Morlat P, Pradier C, Reiss P, Kowalska JD, de Wit S, Law M, el Sadr W, Kirk O, Friis-Moller N, Monforte Ad, Phillips AN, Sabin CA, Lundgren JD; D:A:D Study Group. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 2014; **384**: 241-248 [PMID: 25042234 DOI: 10.1016/S0140-6736(14)60604-8]

10 **Yarchoan R**, Uldrick TS. HIV-Associated Cancers and Related Diseases. *N Engl J Med* 2018; **378**: 2145 [PMID: 29847760 DOI: 10.1056/NEJMc1804812]

11 **Abbar B**, Veyri M, Solas C, Poizot-Martin I, Spano JP. [HIV and cancer: Update 2020]. *Bull Cancer* 2020; **107**: 21-29 [PMID: 31980144 DOI: 10.1016/j.bulcan.2020.01.001]

12 **de Martel C**, Shiels MS, Franceschi S, Simard EP, Vignat J, Hall HI, Engels EA, Plummer M. Cancers attributable to infections among adults with HIV in the United States. *AIDS* 2015; **29**: 2173-2181 [PMID: 26182198 DOI: 10.1097/QAD.0000000000000808]

13 **Giuliano AR**, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjose S, Kjaer SK, Muñoz N, Schiffman M, Bosch FX. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vaccine* 2008; **26** Suppl 10: K17-K28 [PMID: 18847554 DOI: 10.1016/j.vaccine.2008.06.021]

14 **Schiffman M**, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007; **370**: 890-907 [PMID: 17826171 DOI: 10.1016/S0140-6736(07)61416-0]

15 **Centers for Disease Control and Prevention (CDC)**. Human papillomavirus-associated cancers - United States, 2004-2008. *MMWR Morb Mortal Wkly Rep* 2012; **61**: 258-261 [PMID: 22513527]

16 **Shiels MS**, Pfeiffer RM, Gail MH, Hall HI, Li J, Chaturvedi AK, Bhatia K, Uldrick TS, Yarchoan R, Goedert JJ, Engels EA. Cancer burden in the HIV-infected population in the United States. *J Natl Cancer Inst* 2011; **103**: 753-762 [PMID: 21483021 DOI: 10.1093/jnci/djr076]

17 **Nkwabong E**, Laure Bessi Badjan I, Sando Z. Pap smear accuracy for the diagnosis of cervical precancerous lesions. *Trop Doct* 2019; **49**: 34-39 [PMID: 30222058 DOI: 10.1177/0049475518798532]

18 **Frankel AD**, Young JA. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem* 1998; **67**: 1-25 [PMID: 9759480 DOI: 10.1146/annurev.biochem.67.1.1]

19 **Dragic T**. An overview of the determinants of CCR5 and CXCR4 co-receptor function. *J Gen Virol* 2001; **82**: 1807-1814 [PMID: 11457985 DOI: 10.1099/0022-1317-82-8-1807]

20 **Craigie R**, Bushman FD. HIV DNA integration. *Cold Spring Harb Perspect Med* 2012; **2**: a006890 [PMID: 22762018 DOI: 10.1101/cshperspect.a006890]

21 **Dalgleish AG**, Beverley PC, Clapham PR, Crawford DH, Greaves MF, Weiss RA. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 1984; **312**: 763-767 [PMID: 6096719 DOI: 10.1038/312763a0]

22 **Lifson JD**, Reyes GR, McGrath MS, Stein BS, Engleman EG. AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. *Science* 1986; **232**: 1123-1127 [PMID: 3010463 DOI: 10.1126/science.3010463]

23 **Doitsh G**, Cavrois M, Lassen KG, Zepeda O, Yang Z, Santiago ML, Hebbeler AM, Greene WC. Abortive HIV infection mediates CD4 T cell depletion and inflammation in human lymphoid tissue. *Cell* 2010; **143**: 789-801 [PMID: 21111238 DOI: 10.1016/j.cell.2010.11.001]

24 **Doitsh G**, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, Hunt PW, Hatano H, Sowinski S, Muñoz-Arias I, Greene WC. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; **505**: 509-514 [PMID: 24356306 DOI: 10.1038/nature12940]

25 **Cooper A**, García M, Petrovas C, Yamamoto T, Koup RA, Nabel GJ. HIV-1 causes CD4 cell death through DNA-dependent protein kinase during viral integration. *Nature* 2013; **498**: 376-379 [PMID: 23739328 DOI: 10.1038/nature12274]

26 **Cossarizza A**. Apoptosis and HIV infection: about molecules and genes. *Curr Pharm Des* 2008; **14**: 237-244 [PMID: 18220834 DOI: 10.2174/138161208783413293]

27 **Cummins NW**, Badley AD. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. *Cell Death Dis* 2010; **1**: e99 [PMID: 21368875 DOI: 10.1038/cddis.2010.77]

28 **Chávez-Galán L**, Arenas-Del Angel MC, Zenteno E, Chávez R, Lascurain R. Cell death mechanisms induced by cytotoxic lymphocytes. *Cell Mol Immunol* 2009; **6**: 15-25 [PMID: 19254476 DOI: 10.1038/cmi.2009.3]

29 **Yang OO**, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, Walker BD. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 1997; **71**: 3120-3128 [PMID: 9060675 DOI: 10.1128/JVI.71.4.3120-3128.1997]

30 **Borrow P**, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 1994; **68**: 6103-6110 [PMID: 8057491 DOI: 10.1128/JVI.68.9.6103-6110.1994]

31 **Ndhlovu ZM**, Kamya P, Mewalal N, Kløverpris HN, Nkosi T, Pretorius K, Laher F, Ogunshola F, Chopera D, Shekhar K, Ghebremichael M, Ismail N, Moodley A, Malik A, Leslie A, Goulder PJ, Buus S, Chakraborty A, Dong K, Ndung'u T, Walker BD. Magnitude and Kinetics of CD8+ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. *Immunity* 2015; **43**: 591-604 [PMID: 26362266 DOI: 10.1016/j.immuni.2015.08.012]

32 **Dalod M**, Dupuis M, Deschemin JC, Goujard C, Deveau C, Meyer L, Ngo N, Rouzioux C, Guillet JG, Delfraissy JF, Sinet M, Venet A. Weak anti-HIV CD8(+) T-cell effector activity in HIV primary infection. *J Clin Invest* 1999; **104**: 1431-1439 [PMID: 10562305 DOI: 10.1172/JCI7162]

33 **Huang SH**, Ren Y, Thomas AS, Chan D, Mueller S, Ward AR, Patel S, Bollard CM, Cruz CR, Karandish S, Truong R, Macedo AB, Bosque A, Kovacs C, Benko E, Piechocka-Trocha A, Wong H, Jeng E, Nixon DF, Ho YC, Siliciano RF, Walker BD, Jones RB. Latent HIV reservoirs exhibit inherent resistance to elimination by CD8+ T cells. *J Clin Invest* 2018; **128**: 876-889 [PMID: 29355843 DOI: 10.1172/JCI97555]

34 **Collins KL**, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature* 1998; **391**: 397-401 [PMID: 9450757 DOI: 10.1038/34929]

35 **Andrieu M**, Chassin D, Desoutter JF, Bouchaert I, Baillet M, Hanau D, Guillet JG, Hosmalin A. Downregulation of major histocompatibility class I on human dendritic cells by HIV Nef impairs antigen presentation to HIV-specific CD8+ T lymphocytes. *AIDS Res Hum Retroviruses* 2001; **17**: 1365-1370 [PMID: 11602047 DOI: 10.1089/08892220152596623]

36 **Phillips RE**, Rowland-Jones S, Nixon DF, Gotch FM, Edwards JP, Ogunlesi AO, Elvin JG, Rothbard JA, Bangham CR, Rizza CR. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 1991; **354**: 453-459 [PMID: 1721107 DOI: 10.1038/354453a0]

37 **Koup RA**. Virus escape from CTL recognition. *J Exp Med* 1994; **180**: 779-782 [PMID: 7520471 DOI: 10.1084/jem.180.3.779]

38 **Blackburn SD**, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009; **10**: 29-37 [PMID: 19043418 DOI: 10.1038/ni.1679]

39 **De Maria A**, Pantaleo G, Schnittman SM, Greenhouse JJ, Baseler M, Orenstein JM, Fauci AS. Infection of CD8+ T lymphocytes with HIV. Requirement for interaction with infected CD4+ cells and induction of infectious virus from chronically infected CD8+ cells. *J Immunol* 1991; **146**: 2220-2226 [PMID: 1706390]

40 **Brenchley JM**, Hill BJ, Ambrozak DR, Price DA, Guenaga FJ, Casazza JP, Kuruppu J, Yazdani J, Migueles SA, Connors M, Roederer M, Douek DC, Koup RA. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. *J Virol* 2004; **78**: 1160-1168 [PMID: 14722271 DOI: 10.1128/jvi.78.3.1160-1168.2004]

41 **Margolick JB**, Muñoz A, Donnenberg AD, Park LP, Galai N, Giorgi JV, O'Gorman MR, Ferbas J. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. The Multicenter AIDS Cohort Study. *Nat Med* 1995; **1**: 674-680 [PMID: 7585150 DOI: 10.1038/nm0795-674]

42 **Herbein G**, Mahlknecht U, Batliwalla F, Gregersen P, Pappas T, Butler J, O'Brien WA, Verdin E. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* 1998; **395**: 189-194 [PMID: 9744279 DOI: 10.1038/26026]

43 **Blanco J**, Barretina J, Cabrera C, Gutiérrez A, Clotet B, Esté JA. CD4(+) and CD8(+) T cell death during human immunodeficiency virus infection in vitro. *Virology* 2001; **285**: 356-365 [PMID: 11437669 DOI: 10.1006/viro.2001.0969]

44 **Kalams SA**, Buchbinder SP, Rosenberg ES, Billingsley JM, Colbert DS, Jones NG, Shea AK, Trocha AK, Walker BD. Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. *J Virol* 1999; **73**: 6715-6720 [PMID: 10400769 DOI: 10.1128/JVI.73.8.6715-6720.1999]

45 **Matloubian M**, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol* 1994; **68**: 8056-8063 [PMID: 7966595 DOI: 10.1128/JVI.68.12.8056-8063.1994]

46 **Chevalier MF**, Weiss L. The split personality of regulatory T cells in HIV infection. *Blood* 2013; **121**: 29-37 [PMID: 23043072 DOI: 10.1182/blood-2012-07-409755]

47 **Alp Avcı G**. [Genomic organization and proteins of human papillomavirus]. *Mikrobiyol Bul* 2012; **46**: 507-515 [PMID: 22951665]

48 **Chen LP**, Thomas EK, Hu SL, Hellström I, Hellström KE. Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection antigen. *Proc Natl Acad Sci USA* 1991; **88**: 110-114 [PMID: 1846033 DOI: 10.1073/pnas.88.1.110]

49 **Feltkamp MC**, Smits HL, Vierboom MP, Minnaar RP, de Jongh BM, Drijfhout JW, ter Schegget J, Melief CJ, Kast WM. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. *Eur J Immunol* 1993; **23**: 2242-2249 [PMID: 7690326 DOI: 10.1002/eji.1830230929]

50 **Evans EM**, Man S, Evans AS, Borysiewicz LK. Infiltration of cervical cancer tissue with human papillomavirus-specific cytotoxic T-lymphocytes. *Cancer Res* 1997; **57**: 2943-2950 [PMID: 9230206]

51 **Melief CJ**, Van Der Burg SH, Toes RE, Ossendorp F, Offringa R. Effective therapeutic anticancer vaccines based on precision guiding of cytolytic T lymphocytes. *Immunol Rev* 2002; **188**: 177-182 [PMID: 12445291 DOI: 10.1034/j.1600-065x.2002.18816.x]

52 **de Jong A**, van der Burg SH, Kwappenberg KM, van der Hulst JM, Franken KL, Geluk A, van Meijgaarden KE, Drijfhout JW, Kenter G, Vermeij P, Melief CJ, Offringa R. Frequent detection of human papillomavirus 16 E2-specific T-helper immunity in healthy subjects. *Cancer Res* 2002; **62**: 472-479 [PMID: 11809698]

53 **de Jong A**, van Poelgeest MI, van der Hulst JM, Drijfhout JW, Fleuren GJ, Melief CJ, Kenter G, Offringa R, van der Burg SH. Human papillomavirus type 16-positive cervical cancer is associated with impaired CD4+ T-cell immunity against early antigens E2 and E6. *Cancer Res* 2004; **64**: 5449-5455 [PMID: 15289354 DOI: 10.1158/0008-5472.CAN-04-0831]

54 **Nakagawa M**, Stites DP, Farhat S, Sisler JR, Moss B, Kong F, Moscicki AB, Palefsky JM. Cytotoxic T lymphocyte responses to E6 and E7 proteins of human papillomavirus type 16: relationship to cervical intraepithelial neoplasia. *J Infect Dis* 1997; **175**: 927-931 [PMID: 9086151 DOI: 10.1086/513992]

55 **Nakagawa M**, Stites DP, Patel S, Farhat S, Scott M, Hills NK, Palefsky JM, Moscicki AB. Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. *J Infect Dis* 2000; **182**: 595-598 [PMID: 10915094 DOI: 10.1086/315706]

56 **van den Hende M**, van Poelgeest MI, van der Hulst JM, de Jong J, Drijfhout JW, Fleuren GJ, Valentijn AR, Wafelman AR, Slappendel GM, Melief CJ, Offringa R, van der Burg SH, Kenter GG. Skin reactions to human papillomavirus (HPV) 16 specific antigens intradermally injected in healthy subjects and patients with cervical neoplasia. *Int J Cancer* 2008; **123**: 146-152 [PMID: 18404684 DOI: 10.1002/ijc.23502]

57 **IARC Working Group on the Evaluation of Carcinogenic Risks to Humans**. Human papillomaviruses. *IARC Monogr Eval Carcinog Risks Hum* 2007; **90**: 1-636 [PMID: 18354839]

58 **Vambutas A**, Bonagura VR, Steinberg BM. Altered expression of TAP-1 and major histocompatibility complex class I in laryngeal papillomatosis: correlation of TAP-1 with disease. *Clin Diagn Lab Immunol* 2000; **7**: 79-85 [PMID: 10618282 DOI: 10.1128/cdli.7.1.79-85.2000]

59 **Georgopoulos NT**, Proffitt JL, Blair GE. Transcriptional regulation of the major histocompatibility complex (MHC) class I heavy chain, TAP1 and LMP2 genes by the human papillomavirus (HPV) type 6b, 16 and 18 E7 oncoproteins. *Oncogene* 2000; **19**: 4930-4935 [PMID: 11039910 DOI: 10.1038/sj.onc.1203860]

60 **Vambutas A**, DeVoti J, Pinn W, Steinberg BM, Bonagura VR. Interaction of human papillomavirus type 11 E7 protein with TAP-1 results in the reduction of ATP-dependent peptide transport. *Clin Immunol* 2001; **101**: 94-99 [PMID: 11580231 DOI: 10.1006/clim.2001.5094]

61 **Ashrafi GH**, Haghshenas MR, Marchetti B, O'Brien PM, Campo MS. E5 protein of human papillomavirus type 16 selectively downregulates surface HLA class I. *Int J Cancer* 2005; **113**: 276-283 [PMID: 15386416 DOI: 10.1002/ijc.20558]

62 **Zhang B**, Li P, Wang E, Brahmi Z, Dunn KW, Blum JS, Roman A. The E5 protein of human papillomavirus type 16 perturbs MHC class II antigen maturation in human foreskin keratinocytes treated with interferon-gamma. *Virology* 2003; **310**: 100-108 [PMID: 12788634 DOI: 10.1016/s0042-6822(03)00103-x]

63 **Ahdieh L**, Klein RS, Burk R, Cu-Uvin S, Schuman P, Duerr A, Safaeian M, Astemborski J, Daniel R, Shah K. Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. *J Infect Dis* 2001; **184**: 682-690 [PMID: 11517428 DOI: 10.1086/323081]

64 **Brickman C**, Palefsky JM. Human papillomavirus in the HIV-infected host: epidemiology and pathogenesis in the antiretroviral era. *Curr HIV/AIDS Rep* 2015; **12**: 6-15 [PMID: 25644977 DOI: 10.1007/s11904-014-0254-4]

65 **Kim RH**, Yochim JM, Kang MK, Shin KH, Christensen R, Park NH. HIV-1 Tat enhances replicative potential of human oral keratinocytes harboring HPV-16 genome. *Int J Oncol* 2008; **33**: 777-782 [PMID: 18813791]

66 **Tornesello ML**, Buonaguro FM, Beth-Giraldo E, Giraldo G. Human immunodeficiency virus type 1 tat gene enhances human papillomavirus early gene expression. *Intervirology* 1993; **36**: 57-64 [PMID: 8294182 DOI: 10.1159/000150322]

67 **Tan W**, Felber BK, Zolotukhin AS, Pavlakis GN, Schwartz S. Efficient expression of the human papillomavirus type 16 L1 protein in epithelial cells by using Rev and the Rev-responsive element of human immunodeficiency virus or the cis-acting transactivation element of simian retrovirus type 1.

J Virol 1995; **69**: 5607-5620 [PMID: 7637007 DOI: 10.1128/JVI.69.9.5607-5620.1995]

68 **Toy EP**, Rodríguez-Rodríguez L, McCance D, Ludlow J, Planelles V. Induction of cell-cycle arrest in cervical cancer cells by the human immunodeficiency virus type 1 viral protein R. *Obstet Gynecol* 2000; **95**: 141-146 [PMID: 10636517 DOI: 10.1016/s0029-7844(99)00464-0]

69 **Darragh TM**, Colgan TJ, Thomas Cox J, Heller DS, Henry MR, Luff RD, McCalmont T, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC; Members of the LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol* 2013; **32**: 76-115 [PMID: 23202792 DOI: 10.1097/PGP.0b013e31826916c7]

70 **Leeds IL**, Fang SH. Anal cancer and intraepithelial neoplasia screening: A review. *World J Gastrointest Surg* 2016; **8**: 41-51 [PMID: 26843912 DOI: 10.4240/wjgs.v8.i1.41]

71 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

72 **Wilkinson JR**, Morris EJ, Downing A, Finan PJ, Aravani A, Thomas JD, Sebag-Montefiore D. The rising incidence of anal cancer in England 1990-2010: a population-based study. *Colorectal Dis* 2014; **16**: O234-O239 [PMID: 24410872 DOI: 10.1111/codi.12553]

73 **Shiels MS**, Kreimer AR, Coghill AE, Darragh TM, Devesa SS. Anal Cancer Incidence in the United States, 1977-2011: Distinct Patterns by Histology and Behavior. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 1548-1556 [PMID: 26224796 DOI: 10.1158/1055-9965.EPI-15-0044]

74 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021; **71**: 7-33 [PMID: 33433946 DOI: 10.3322/caac.21654]

75 **Daling JR**, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, Carter JJ, Porter PL, Galloway DA, McDougall JK. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer* 2004; **101**: 270-280 [PMID: 15241823 DOI: 10.1002/cncr.20365]

76 **Machalek DA**, Poynten M, Jin F, Fairley CK, Farnsworth A, Garland SM, Hillman RJ, Petoumenos K, Roberts J, Tabrizi SN, Templeton DJ, Grulich AE. Anal human papillomavirus infection and associated neoplastic lesions in men who have sex with men: a systematic review and meta-analysis. *Lancet Oncol* 2012; **13**: 487-500 [PMID: 22445259 DOI: 10.1016/S1470-2045(12)70080-3]

77 **Frisch M**, Glimelius B, van den Brule AJ, Wohlfahrt J, Meijer CJ, Walboomers JM, Goldman S, Svensson C, Adami HO, Melbye M. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med* 1997; **337**: 1350-1358 [PMID: 9358129 DOI: 10.1056/NEJM199711063371904]

78 **Chin-Hong PV**, Husnik M, Cranston RD, Colfax G, Buchbinder S, Da Costa M, Darragh T, Jones D, Judson F, Koblin B, Mayer KH, Palefsky JM. Anal human papillomavirus infection is associated with HIV acquisition in men who have sex with men. *AIDS* 2009; **23**: 1135-1142 [PMID: 19390418 DOI: 10.1097/QAD.0b013e32832b4449]

79 **Darwich L**, Cañadas MP, Videla S, Coll J, Molina-López RA, Sirera G, Clotet B; Can Ruti HIV-HPV Team. Prevalence, clearance, and incidence of human papillomavirus type-specific infection at the anal and penile site of HIV-infected men. *Sex Transm Dis* 2013; **40**: 611-618 [PMID: 23859907 DOI: 10.1097/01.OLQ.0000430798.61475.08]

80 **Vajdic CM**, van Leeuwen MT, Jin F, Prestage G, Medley G, Hillman RJ, Stevens MP, Botes LP, Zablotska I, Tabrizi SN, Grulich AE. Anal human papillomavirus genotype diversity and co-infection in a community-based sample of homosexual men. *Sex Transm Infect* 2009; **85**: 330-335 [PMID: 19342375 DOI: 10.1136/sti.2008.034744]

81 **Hernandez AL**, Efird JT, Holly EA, Berry JM, Jay N, Palefsky JM. Incidence of and risk factors for type-specific anal human papillomavirus infection among HIV-positive MSM. *AIDS* 2014; **28**: 1341-1349 [PMID: 24959962 DOI: 10.1097/QAD.0000000000000254]

82 **Müller EE**, Rebe K, Chirwa TF, Struthers H, McIntyre J, Lewis DA. The prevalence of human papillomavirus infections and associated risk factors in men-who-have-sex-with-men in Cape Town, South Africa. *BMC Infect Dis* 2016; **16**: 440 [PMID: 27549219 DOI: 10.1186/s12879-016-1706-9]

83 **D'Souza G**, Wiley DJ, Li X, Chmiel JS, Margolick JB, Cranston RD, Jacobson LP. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *J Acquir Immune Defic Syndr* 2008; **48**: 491-499 [PMID: 18614927 DOI: 10.1097/QAI.0b013e31817aebfe]

84 **Robbins HA**, Shiels MS, Pfeiffer RM, Engels EA. Epidemiologic contributions to recent cancer trends among HIV-infected people in the United States. *AIDS* 2014; **28**: 881-890 [PMID: 24300545 DOI: 10.1097/QAD.0000000000000163]

85 **Geskus RB**, González C, Torres M, Del Romero J, Viciana P, Masiá M, Blanco JR, Iribarren M, De Sanjosé S, Hernández-Novoa B, Ortiz M, Del Amo J; CoRIS-HPV Study Group. Incidence and clearance of anal high-risk human papillomavirus in HIV-positive men who have sex with men: estimates and risk factors. *AIDS* 2016; **30**: 37-44 [PMID: 26355673 DOI: 10.1097/QAD.0000000000000874]

86 **Looker KJ**, Rönn MM, Brock PM, Brisson M, Drolet M, Mayaud P, Boily MC. Evidence of synergistic relationships between HIV and Human Papillomavirus (HPV): systematic reviews and meta-analyses of longitudinal studies of HPV acquisition and clearance by HIV status, and of HIV acquisition by HPV status. *J Int AIDS Soc* 2018; **21**: e25110 [PMID: 29873885 DOI: 10.1002/jia2.25110]

87 **Bertisch B**, Franceschi S, Lise M, Vernazza P, Keiser O, Schöni-Affolter F, Bouchardy C, Dehler S, Levi F, Jundt G, Ess S, Pawlita M, Kovari H, Wandeler G, Calmy A, Cavassini M, Stöckle M,

Clifford G; Swiss HIV Cohort Study Investigators. Risk factors for anal cancer in persons infected with HIV: a nested case-control study in the Swiss HIV Cohort Study. *Am J Epidemiol* 2013; **178**: 877-884 [PMID: 23900553 DOI: 10.1093/aje/kwt153]

88 **Goldie SJ**, Kuntz KM, Weinstein MC, Freedberg KA, Palefsky JM. Cost-effectiveness of screening for anal squamous intraepithelial lesions and anal cancer in human immunodeficiency virus-negative homosexual and bisexual men. *Am J Med* 2000; **108**: 634-641 [PMID: 10856411 DOI: 10.1016/s0002-9343(00)00349-1]

89 **Goldie SJ**, Kuntz KM, Weinstein MC, Freedberg KA, Welton ML, Palefsky JM. The clinical effectiveness and cost-effectiveness of screening for anal squamous intraepithelial lesions in homosexual and bisexual HIV-positive men. *JAMA* 1999; **281**: 1822-1829 [PMID: 10340370 DOI: 10.1001/jama.281.19.1822]

90 **Guo H**, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature* 2010; **466**: 835-840 [PMID: 20703300 DOI: 10.1038/nature09267]

91 **Olsen PH**, Ambros V. The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation. *Dev Biol* 1999; **216**: 671-680 [PMID: 10642801 DOI: 10.1006/dbio.1999.9523]

92 **Place RF**, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci USA* 2008; **105**: 1608-1613 [PMID: 18227514 DOI: 10.1073/pnas.0707594105]

93 **Huang V**, Place RF, Portnoy V, Wang J, Qi Z, Jia Z, Yu A, Shuman M, Yu J, Li LC. Upregulation of Cyclin B1 by miRNA and its implications in cancer. *Nucleic Acids Res* 2012; **40**: 1695-1707 [PMID: 22053081 DOI: 10.1093/nar/gkr934]

94 **Bartel DP**. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]

95 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Zen K, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170 DOI: 10.1038/cr.2008.282]

96 **Wang X**, Wang HK, Li Y, Hafner M, Banerjee NS, Tang S, Briskin D, Meyers C, Chow LT, Xie X, Tuschl T, Zheng ZM. microRNAs are biomarkers of oncogenic human papillomavirus infections. *Proc Natl Acad Sci USA* 2014; **111**: 4262-4267 [PMID: 24591631 DOI: 10.1073/pnas.1401430111]

97 **Lajer CB**, Garnæs E, Friis-Hansen L, Norrild B, Therkildsen MH, Glud M, Rossing M, Lajer H, Svane D, Skotte L, Specht L, Buchwald C, Nielsen FC. The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. *Br J Cancer* 2012; **106**: 1526-1534 [PMID: 22472886 DOI: 10.1038/bjc.2012.109]

98 **Harden ME**, Prasad N, Griffiths A, Munger K. Modulation of microRNA-mRNA Target Pairs by Human Papillomavirus 16 Oncoproteins. *mBio* 2017; **8**: e02170-16 [PMID: 28049151 DOI: 10.1128/mBio.02170-16]

99 **Mar-Aguilar F**, Mendoza-Ramírez JA, Malagón-Santiago I, Espino-Silva PK, Santuario-Facio SK, Ruiz-Flores P, Rodríguez-Padilla C, Reséndez-Pérez D. Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. *Dis Markers* 2013; **34**: 163-169 [PMID: 23334650 DOI: 10.3233/DMA-120957]

100 **Gallo A**, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012; **7**: e30679 [PMID: 22427800 DOI: 10.1371/journal.pone.0030679]

101 **Nedaeinia R**, Manian M, Jazayeri MH, Ranjbar M, Salehi R, Sharifi M, Mohaghegh F, Goli M, Jahednia SH, Avan A, Ghayour-Mobarhan M. Circulating exosomes and exosomal microRNAs as biomarkers in gastrointestinal cancer. *Cancer Gene Ther* 2017; **24**: 48-56 [PMID: 27982021 DOI: 10.1038/cgt.2016.77]

102 **Iorio MV**, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 2005; **65**: 7065-7070 [PMID: 16103053 DOI: 10.1158/0008-5472.CAN-05-1783]

103 **Tian F**, Li R, Chen Z, Shen Y, Lu J, Xie X, Ge Q. Differentially Expressed miRNAs in Tumor, Adjacent, and Normal Tissues of Lung Adenocarcinoma. *Biomed Res Int* 2016; **2016**: 1428271 [PMID: 27247934 DOI: 10.1155/2016/1428271]

104 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

105 **Gocze K**, Gombos K, Kovacs K, Juhasz K, Gocze P, Kiss I. MicroRNA expressions in HPV-induced cervical dysplasia and cancer. *Anticancer Res* 2015; **35**: 523-530 [PMID: 25550598]

106 **Louten J**, Beach M, Palermino K, Weeks M, Holenstein G. MicroRNAs Expressed during Viral Infection: Biomarker Potential and Therapeutic Considerations. *Biomark Insights* 2015; **10**: 25-52 [PMID: 26819546 DOI: 10.4137/BMI.S29512]

107 **Farzanehpour M**, Mozhgani SH, Jalilvand S, Faghihloo E, Akhavan S, Salimi V, Azad TM. Serum and tissue miRNAs: potential biomarkers for the diagnosis of cervical cancer. *Virol J* 2019; **16**: 116 [PMID: 31590680 DOI: 10.1186/s12985-019-1220-y]

108 **Snoek BC**, Verlaat W, Babion I, Novianti PW, van de Wiel MA, Wilting SM, van Trommel NE, Bleeker MCG, Massuger LFAG, Melchers WJG, Sie D, Heideman DAM, Snijders PJF, Meijer CJLM, Steenbergen RDM. Genome-wide microRNA analysis of HPV-positive self-samples yields novel triage markers for early detection of cervical cancer. *Int J Cancer* 2019; **144**: 372-379 [PMID: 30192375 DOI: 10.1002/ijc.31855]

109 **Shi C**, Yang Y, Zhang L, Zhang T, Yu J, Qin S, Gao Y. Optimal subset of signature miRNAs consisting of 7 miRNAs that can serve as a novel diagnostic and prognostic predictor for the progression of cervical cancer. *Oncol Rep* 2019; **41**: 3167-3178 [PMID: 30942460 DOI: 10.3892/or.2019.7097]

110 **Lee JW**, Choi CH, Choi JJ, Park YA, Kim SJ, Hwang SY, Kim WY, Kim TJ, Lee JH, Kim BG, Bae DS. Altered MicroRNA expression in cervical carcinomas. *Clin Cancer Res* 2008; **14**: 2535-2542 [PMID: 18451214 DOI: 10.1158/1078-0432.CCR-07-1231]

111 **Myklebust MP**, Bruland O, Fluge Ø, Skarstein A, Balteskard L, Dahl O. MicroRNA-15b is induced with E2F-controlled genes in HPV-related cancer. *Br J Cancer* 2011; **105**: 1719-1725 [PMID: 22045185 DOI: 10.1038/bjc.2011.457]

112 **Sun G**, Rossi JJ. MicroRNAs and their potential involvement in HIV infection. *Trends Pharmacol Sci* 2011; **32**: 675-681 [PMID: 21862142 DOI: 10.1016/j.tips.2011.07.003]

113 **Tan Gana NH**, Onuki T, Victoriano AF, Okamoto T. MicroRNAs in HIV-1 infection: an integration of viral and cellular interaction at the genomic level. *Front Microbiol* 2012; **3**: 306 [PMID: 22936931 DOI: 10.3389/fmicb.2012.00306]

114 **Swaminathan G**, Navas-Martín S, Martín-García J. MicroRNAs and HIV-1 infection: antiviral activities and beyond. *J Mol Biol* 2014; **426**: 1178-1197 [PMID: 24370931 DOI: 10.1016/j.jmb.2013.12.017]

115 **Bennasser Y**, Le SY, Benkirane M, Jeang KT. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. *Immunity* 2005; **22**: 607-619 [PMID: 15894278 DOI: 10.1016/j.immuni.2005.03.010]

116 **Bennasser Y**, Yeung ML, Jeang KT. HIV-1 TAR RNA subverts RNA interference in transfected cells through sequestration of TAR RNA-binding protein, TRBP. *J Biol Chem* 2006; **281**: 27674-27678 [PMID: 16887810 DOI: 10.1074/jbc.C600072200]

117 **Casey Klockow L**, Sharifi HJ, Wen X, Flagg M, Furuya AK, Nekorchuk M, de Noronha CM. The HIV-1 protein Vpr targets the endoribonuclease Dicer for proteasomal degradation to boost macrophage infection. *Virology* 2013; **444**: 191-202 [PMID: 23849790 DOI: 10.1016/j.virol.2013.06.010]

118 **Biswas S**, Chen E, Haleyurgirisetty M, Lee S, Hewlett I, Devadas K. Comparison of miRNA Expression Profiles between HIV-1 and HIV-2 Infected Monocyte-Derived Macrophages (MDMs) and Peripheral Blood Mononuclear Cells (PBMCs). *Int J Mol Sci* 2020; **21**: 6970 [PMID: 32971935 DOI: 10.3390/ijms21186970]

119 **Barbu MG**, Condrat CE, Thompson DC, Bugnar OL, Cretoiu D, Toader OD, Suciu N, Voinea SC. MicroRNA Involvement in Signaling Pathways During Viral Infection. *Front Cell Dev Biol* 2020; **8**: 143 [PMID: 32211411 DOI: 10.3389/fcell.2020.00143]

120 **Ribeiro J**, Marinho-Dias J, Monteiro P, Loureiro J, Baldaque I, Medeiros R, Sousa H. miR-34a and miR-125b Expression in HPV Infection and Cervical Cancer Development. *Biomed Res Int* 2015; **2015**: 304584 [PMID: 26180794 DOI: 10.1155/2015/304584]

121 **Hong DS**, Kang YK, Borad M, Sachdev J, Ejadi S, Lim HY, Brenner AJ, Park K, Lee JL, Kim TY, Shin S, Becerra CR, Falchook G, Stoudemire J, Martin D, Kelnar K, Peltier H, Bonato V, Bader AG, Smith S, Kim S, O'Neill V, Beg MS. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. *Br J Cancer* 2020; **122**: 1630-1637 [PMID: 32238921 DOI: 10.1038/s41416-020-0802-1]

122 **Chakraborty C**, Sharma AR, Sharma G, Lee SS. Therapeutic advances of miRNAs: A preclinical and clinical update. *J Adv Res* 2021; **28**: 127-138 [PMID: 33364050 DOI: 10.1016/j.jare.2020.08.012]

123 **Ottosen S**, Parsley TB, Yang L, Zeh K, van Doorn LJ, van der Veer E, Raney AK, Hodges MR, Patick AK. In vitro antiviral activity and preclinical and clinical resistance profile of miravirsen, a novel anti-hepatitis C virus therapeutic targeting the human factor miR-122. *Antimicrob Agents Chemother* 2015; **59**: 599-608 [PMID: 25385103 DOI: 10.1128/AAC.04220-14]

124 **Hanna J**, Hossain GS, Kocerha J. The Potential for microRNA Therapeutics and Clinical Research. *Front Genet* 2019; **10**: 478 [PMID: 31156715 DOI: 10.3389/fgene.2019.00478]

125 **Moghoofei M**, Najafipour S, Mostafaei S, Tavakoli A, Bokharaei-Salim F, Ghorbani S, Javanmard D, Ghaffari H, Monavari SH. MicroRNAs Profiling in HIV, HCV, and HIV/HCV Co-Infected Patients. *Curr HIV Res* 2021; **19**: 27-34 [PMID: 32900348 DOI: 10.2174/1570162X18666200908112113]

126 **Ma L**, Zhang H, Zhang Y, Li H, An M, Zhao B, Ding H, Xu J, Shang H, Han X. Integrated analysis of lncRNA, miRNA and mRNA profiles reveals potential lncRNA functions during early HIV infection. *J Transl Med* 2021; **19**: 135 [PMID: 33794921 DOI: 10.1186/s12967-021-02802-9]

127 **Heinson AI**, Woo J, Mukim A, White CH, Moesker B, Bosque A, Spina CA, Woelk CH, Macarthur BD, Beliakova-Bethell N. Micro RNA Targets in HIV Latency: Insights into Novel Layers of Latency Control. *AIDS Res Hum Retroviruses* 2021; **37**: 109-121 [PMID: 33045840 DOI: 10.1089/AID.2020.0150]

128 **Shah UJ**, Nasiruddin M, Dar SA, Khan MKA, Akhter MR, Singh N, Rabaan AA, Haque S.

Emerging biomarkers and clinical significance of HPV genotyping in prevention and management of cervical cancer. *Microb Pathog* 2020; **143**: 104131 [PMID: 32169490 DOI: 10.1016/j.micpath.2020.104131]

129 **Sadri Nahand J**, Moghoofei M, Salmaninejad A, Bahmanpour Z, Karimzadeh M, Nasiri M, Mirzaei HR, Pourhanifeh MH, Bokharaei-Salim F, Mirzaei H, Hamblin MR. Pathogenic role of exosomes and microRNAs in HPV-mediated inflammation and cervical cancer: A review. *Int J Cancer* 2020; **146**: 305-320 [PMID: 31566705 DOI: 10.1002/ijc.32688]

130 **Chen Y**, Song Y, Mi Y, Jin H, Cao J, Li H, Han L, Huang T, Zhang X, Ren S, Ma Q, Zou Z. microRNA-499a promotes the progression and chemoresistance of cervical cancer cells by targeting SOX6. *Apoptosis* 2020; **25**: 205-216 [PMID: 31938895 DOI: 10.1007/s10495-019-01588-y]

131 **Teng Y**, Wang Y, Zhang X, Liu W, Fan H, Yao H, Lin B, Zhu P, Yuan W, Tong Y, Cao W. Systematic Genome-wide Screening and Prediction of microRNAs in EBOV During the 2014 Ebolavirus Outbreak. *Sci Rep* 2015; **5**: 9912 [PMID: 26011078 DOI: 10.1038/srep09912]

132 **Saini S**, Saini A, Thakur CJ, Kumar V, Gupta RD, Sharma JK. Genome-wide computational prediction of miRNAs in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) revealed target genes involved in pulmonary vasculature and antiviral innate immunity. *Mol Biol Res Commun* 2020; **9**: 83-91 [PMID: 32802902 DOI: 10.22099/mbrc.2020.36507.1487]

133 **Mumford SL**, Towler BP, Pashler AL, Gilleard O, Martin Y, Newbury SF. Circulating MicroRNA Biomarkers in Melanoma: Tools and Challenges in Personalised Medicine. *Biomolecules* 2018; **8**: 21 [PMID: 29701682 DOI: 10.3390/biom8020021]

134 **Shams R**, Saberi S, Zali M, Sadeghi A, Ghafouri-Fard S, Aghdaei HA. Identification of potential microRNA panels for pancreatic cancer diagnosis using microarray datasets and bioinformatics methods. *Sci Rep* 2020; **10**: 7559 [PMID: 32371926 DOI: 10.1038/s41598-020-64569-1]

135 **Rehman O**, Zhuang H, Muhamed Ali A, Ibrahim A, Li Z. Validation of miRNAs as Breast Cancer Biomarkers with a Machine Learning Approach. *Cancers (Basel)* 2019; **11**: 431 [PMID: 30917548 DOI: 10.3390/cancers11030431]

136 **Zhu J**, Xu Y, Liu S, Qiao L, Sun J, Zhao Q. MicroRNAs Associated With Colon Cancer: New Potential Prognostic Markers and Targets for Therapy. *Front Bioeng Biotechnol* 2020; **8**: 176 [PMID: 32211396 DOI: 10.3389/fbioe.2020.00176]

137 **Kang HW**, Crawford M, Fabbri M, Nuovo G, Garofalo M, Nana-Sinkam SP, Friedman A. A mathematical model for microRNA in lung cancer. *PLoS One* 2013; **8**: e53663 [PMID: 23365639 DOI: 10.1371/journal.pone.0053663]

138 **Zhu CS**, Zhu L, Tan DA, Qiu XY, Liu CY, Xie SS, Zhu LY. Avenues Toward microRNA Detection *In Vitro*: A Review of Technical Advances and Challenges. *Comput Struct Biotechnol J* 2019; **17**: 904-916 [PMID: 31346383 DOI: 10.1016/j.csbj.2019.06.018]

139 **Dave VP**, Ngo TA, Pernestig AK, Tilevik D, Kant K, Nguyen T, Wolff A, Bang DD. MicroRNA amplification and detection technologies: opportunities and challenges for point of care diagnostics. *Lab Invest* 2019; **99**: 452-469 [PMID: 30542067 DOI: 10.1038/s41374-018-0143-3]

140 **Jia H**, Li Z, Liu C, Cheng Y. Ultrasensitive detection of microRNAs by exponential isothermal amplification. *Angew Chem Int Ed Engl* 2010; **49**: 5498-5501 [PMID: 20602382 DOI: 10.1002/anie.201001375]

141 **Ying N**, Ju C, Sun X, Li L, Chang H, Song G, Li Z, Wan J, Dai E. Lateral flow nucleic acid biosensor for sensitive detection of microRNAs based on the dual amplification strategy of duplex-specific nuclease and hybridization chain reaction. *PLoS One* 2017; **12**: e0185091 [PMID: 28945768 DOI: 10.1371/journal.pone.0185091]

142 **Degliangeli F**, Pompa PP, Fiammengo R. Nanotechnology-based strategies for the detection and quantification of microRNA. *Chemistry* 2014; **20**: 9476-9492 [PMID: 24989446 DOI: 10.1002/chem.201402649]

143 **Hamidi-Asl E**, Palchetti I, Hasheminejad E, Mascini M. A review on the electrochemical biosensors for determination of microRNAs. *Talanta* 2013; **115**: 74-83 [PMID: 24054564 DOI: 10.1016/j.talanta.2013.03.061]

144 **Arata H**, Komatsu H, Hosokawa K, Maeda M. Rapid and sensitive microRNA detection with laminar flow-assisted dendritic amplification on power-free microfluidic chip. *PLoS One* 2012; **7**: e48329 [PMID: 23144864 DOI: 10.1371/journal.pone.0048329]

145 **Salim B**, Athira MV, Kandaswamy A, Vijayakumar M, Saravanan T, Sairam T. Microfluidic device for novel breast cancer screening by blood test using miRNA beacon probe. *Biomed Microdevices* 2017; **19**: 89 [PMID: 28965149 DOI: 10.1007/s10544-017-0230-z]

146 **Lässer C**. Exosomal RNA as biomarkers and the therapeutic potential of exosome vectors. *Expert Opin Biol Ther* 2012; **12** Suppl 1: S189-S197 [PMID: 22506888 DOI: 10.1517/14712598.2012.680018]

147 **Mitchell PS**, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008; **105**: 10513-10518 [PMID: 18663219 DOI: 10.1073/pnas.0804549105]

148 **Subra C**, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, De Medina P, Monsarrat B, Perret B, Silvente-Poirot S, Poirot M, Record M. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. *J Lipid Res* 2010; **51**: 2105-2120 [PMID: 20424270 DOI: 10.1194/jlr.M003657]

149 **Nik Mohamed Kamal NNSB**, Shahidan WNS. Non-Exosomal and Exosomal Circulatory MicroRNAs: Which Are More Valid as Biomarkers? *Front Pharmacol* 2019; **10**: 1500 [PMID: 32038230 DOI: 10.3389/fphar.2019.01500]

150 **Fu F**, Jiang W, Zhou L, Chen Z. Circulating Exosomal miR-17-5p and miR-92a-3p Predict Pathologic Stage and Grade of Colorectal Cancer. *Transl Oncol* 2018; **11**: 221-232 [PMID: 29367070 DOI: 10.1016/j.tranon.2017.12.012]

151 **Goto T**, Fujiya M, Konishi H, Sasajima J, Fujibayashi S, Hayashi A, Utsumi T, Sato H, Iwama T, Ijiri M, Sakatani A, Tanaka K, Nomura Y, Ueno N, Kashima S, Moriichi K, Mizukami Y, Kohgo Y, Okumura T. An elevated expression of serum exosomal microRNA-191, - 21, -451a of pancreatic neoplasm is considered to be efficient diagnostic marker. *BMC Cancer* 2018; **18**: 116 [PMID: 29385987 DOI: 10.1186/s12885-018-4006-5]

152 **Jackson R**, Eade S, Zehbe I. An epithelial organoid model with Langerhans cells for assessing virus-host interactions. *Philos Trans R Soc Lond B Biol Sci* 2019; **374**: 20180288 [PMID: 30955491 DOI: 10.1098/rstb.2018.0288]

153 **Driehuis E**, Kolders S, Spelier S, Lõhmussaar K, Willems SM, Devriese LA, de Bree R, de Ruiter EJ, Korving J, Begthel H, van Es JH, Geurts V, He GW, van Jaarsveld RH, Oka R, Muraro MJ, Vivié J, Zandvliet MMJM, Hendrickx APA, Iakobachvili N, Sridevi P, Kranenburg O, van Boxtel R, Kops GJPL, Tuveson DA, Peters PJ, van Oudenaarden A, Clevers H. Oral Mucosal Organoids as a Potential Platform for Personalized Cancer Therapy. *Cancer Discov* 2019; **9**: 852-871 [PMID: 31053628 DOI: 10.1158/2159-8290.CD-18-1522]

154 **Fiorini E**, Veghini L, Corbo V. Modeling Cell Communication in Cancer With Organoids: Making the Complex Simple. *Front Cell Dev Biol* 2020; **8**: 166 [PMID: 32258040 DOI: 10.3389/fcell.2020.00166]

155 **Lõhmussaar K**, Oka R, Espejo Valle-Inclan J, Smits MHH, Wardak H, Korving J, Begthel H, Proost N, van de Ven M, Kranenburg OW, Jonges TGN, Zweemer RP, Veersema S, van Boxtel R, Clevers H. Patient-derived organoids model cervical tissue dynamics and viral oncogenesis in cervical cancer. *Cell Stem Cell* 2021 [PMID: 33852917 DOI: 10.1016/j.stem.2021.03.012]

156 **Liu X**. Up-regulation of miR-20a by HPV16 E6 exerts growth-promoting effects by targeting PDCD6 in cervical carcinoma cells. *Biomed Pharmacother* 2018; **102**: 996-1002 [PMID: 29710555 DOI: 10.1016/j.biopha.2018.03.154]

157 **Cheng Y**, Geng L, Zhao L, Zuo P, Wang J. Human papillomavirus E6-regulated microRNA-20b promotes invasion in cervical cancer by targeting tissue inhibitor of metalloproteinase 2. *Mol Med Rep* 2017; **16**: 5464-5470 [PMID: 28849054 DOI: 10.3892/mmr.2017.7231]

158 **Au Yeung CL**, Tsang TY, Yau PL, Kwok TT. Human papillomavirus type 16 E6 induces cervical cancer cell migration through the p53/microRNA-23b/urokinase-type plasminogen activator pathway. *Oncogene* 2011; **30**: 2401-2410 [PMID: 21242962 DOI: 10.1038/onc.2010.613]

159 **Wu YL**, Hsu NY, Cheau-Feng Lin F, Lee H, Cheng YW. MiR-30c-2* negative regulated MTA-1 expression involved in metastasis and drug resistance of HPV-infected non-small cell lung cancer. *Surgery* 2016; **160**: 1591-1598 [PMID: 27506865 DOI: 10.1016/j.surg.2016.06.025]

160 **Wang X**, Wang HK, McCoy JP, Banerjee NS, Rader JS, Broker TR, Meyers C, Chow LT, Zheng ZM. Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6. *RNA* 2009; **15**: 637-647 [PMID: 19258450 DOI: 10.1261/rna.1442309]

161 **Zhang R**, Su J, Xue SL, Yang H, Ju LL, Ji Y, Wu KH, Zhang YW, Zhang YX, Hu JF, Yu MM. HPV E6/p53 mediated down-regulation of miR-34a inhibits Warburg effect through targeting LDHA in cervical cancer. *Am J Cancer Res* 2016; **6**: 312-320 [PMID: 27186405]

162 **Shi M**, Du L, Liu D, Qian L, Hu M, Yu M, Yang Z, Zhao M, Chen C, Guo L, Wang L, Song L, Ma Y, Guo N. Glucocorticoid regulation of a novel HPV-E6-p53-miR-145 pathway modulates invasion and therapy resistance of cervical cancer cells. *J Pathol* 2012; **228**: 148-157 [PMID: 22287315 DOI: 10.1002/path.3997]

163 **Zhong J**, Yuan H, Xu X, Kong S. MicroRNA195 inhibits cell proliferation, migration and invasion by targeting defective in cullin neddylation 1 domain containing 1 in cervical cancer. *Int J Mol Med* 2018; **42**: 779-788 [PMID: 29750306 DOI: 10.3892/ijmm.2018.3660]

164 **Martinez I**, Gardiner AS, Board KF, Monzon FA, Edwards RP, Khan SA. Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene* 2008; **27**: 2575-2582 [PMID: 17998940 DOI: 10.1038/sj.onc.1210919]

165 **Jiang Z**, Song Q, Zeng R, Li J, Lin X, Chen X, Zhang J, Zheng Y. MicroRNA-218 inhibits EMT, migration and invasion by targeting SFMBT1 and DCUN1D1 in cervical cancer. *Oncotarget* 2016; **7**: 45622-45636 [PMID: 27285984 DOI: 10.18632/oncotarget.9850]

166 **Díaz-González Sdel M**, Deas J, Benítez-Boijseauneau O, Gómez-Cerón C, Bermúdez-Morales VH, Rodríguez-Dorantes M, Pérez-Plasencia C, Peralta-Zaragoza O. Utility of microRNAs and siRNAs in cervical carcinogenesis. *Biomed Res Int* 2015; **2015**: 374924 [PMID: 25874209 DOI: 10.1155/2015/374924]

167 **Liu S**, Song L, Yao H, Zhang L, Xu D, Gao F, Li Q. MiR-375 Is Epigenetically Downregulated by HPV-16 E6 Mediated DNMT1 Upregulation and Modulates EMT of Cervical Cancer Cells by Suppressing lncRNA MALAT1. *PLoS One* 2016; **11**: e0163460 [PMID: 27658300 DOI: 10.1371/journal.pone.0163460]

168 **Xu J**, Wan X, Chen X, Fang Y, Cheng X, Xie X, Lu W. miR-2861 acts as a tumor suppressor *via* targeting EGFR/AKT2/CCND1 pathway in cervical cancer induced by human papillomavirus virus 16 E6. *Sci Rep* 2016; **6**: 28968 [PMID: 27364926 DOI: 10.1038/srep28968]

169 **Hu J**, Ge W, Xu J. HPV 16 E7 inhibits OSCC cell proliferation, invasion, and metastasis by upregulating the expression of miR-20a. *Tumour Biol* 2016; **37**: 9433-9440 [PMID: 26781875 DOI: 10.1007/s13277-016-4817-4]

170 **Kong Q**, Wang W, Li P. Regulator role of HPV E7 protein on miR-21 expression in cervical carcinoma cells and its functional implication. *Int J Clin Exp Pathol* 2015; **8**: 15808-15813 [PMID: 26884851]

171 **Bumrungthai S**, Ekalaksananan T, Evans MF, Chopjitt P, Tangsiriwatthana T, Patarapadungkit N, Kleebkaow P, Luanratanakorn S, Kongyingyoes B, Worawichawong S, Pientong C. Up-Regulation of miR-21 Is Associated with Cervicitis and Human Papillomavirus Infection in Cervical Tissues. *PLoS One* 2015; **10**: e0127109 [PMID: 26010154 DOI: 10.1371/journal.pone.0127109]

172 **Liu F**, Zhang S, Zhao Z, Mao X, Huang J, Wu Z, Zheng L, Wang Q. MicroRNA-27b up-regulated by human papillomavirus 16 E7 promotes proliferation and suppresses apoptosis by targeting polo-like kinase2 in cervical cancer. *Oncotarget* 2016; **7**: 19666-19679 [PMID: 26910911 DOI: 10.18632/oncotarget.7531]

173 **Yao J**, Deng B, Zheng L, Dou L, Guo Y, Guo K. miR-27b is upregulated in cervical carcinogenesis and promotes cell growth and invasion by regulating CDH11 and epithelial-mesenchymal transition. *Oncol Rep* 2016; **35**: 1645-1651 [PMID: 26706910 DOI: 10.3892/or.2015.4500]

174 **McKenna DJ**, Patel D, McCance DJ. miR-24 and miR-205 expression is dependent on HPV onco-protein expression in keratinocytes. *Virology* 2014; **448**: 210-216 [PMID: 24314651 DOI: 10.1016/j.virol.2013.10.014]

175 **Mandal P**, Saha SS, Sen S, Bhattacharya A, Bhattacharya NP, Bucha S, Sinha M, Chowdhury RR, Mondal NR, Chakravarty B, Chatterjee T, Roy S, Chattapadhyay A, Sengupta S. Cervical cancer subtypes harbouring integrated and/or episomal HPV16 portray distinct molecular phenotypes based on transcriptome profiling of mRNAs and miRNAs. *Cell Death Discov* 2019; **5**: 81 [PMID: 30937183 DOI: 10.1038/s41420-019-0154-x]

176 **Xin M**, Qiao Z, Li J, Liu J, Song S, Zhao X, Miao P, Tang T, Wang L, Liu W, Yang X, Dai K, Huang G. miR-22 inhibits tumor growth and metastasis by targeting ATP citrate lyase: evidence in osteosarcoma, prostate cancer, cervical cancer and lung cancer. *Oncotarget* 2016; **7**: 44252-44265 [PMID: 27317765 DOI: 10.18632/oncotarget.10020]

177 **Fan Z**, Cui H, Xu X, Lin Z, Zhang X, Kang L, Han B, Meng J, Yan Z, Yan X, Jiao S. MiR-125a suppresses tumor growth, invasion and metastasis in cervical cancer by targeting STAT3. *Oncotarget* 2015; **6**: 25266-25280 [PMID: 26389681 DOI: 10.18632/oncotarget.4457]

178 **Peta E**, Sinigaglia A, Masi G, Di Camillo B, Grassi A, Trevisan M, Messa L, Loregian A, Manfrin E, Brunelli M, Martignoni G, Palù G, Barzon L. HPV16 E6 and E7 upregulate the histone lysine demethylase KDM2B through the c-MYC/miR-146a-5p axys. *Oncogene* 2018; **37**: 1654-1668 [PMID: 29335520 DOI: 10.1038/s41388-017-0083-1]

179 **Melar-New M**, Laimins LA. Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins. *J Virol* 2010; **84**: 5212-5221 [PMID: 20219920 DOI: 10.1128/JVI.00078-10]

180 **McKenna DJ**, McDade SS, Patel D, McCance DJ. MicroRNA 203 expression in keratinocytes is dependent on regulation of p53 Levels by E6. *J Virol* 2010; **84**: 10644-10652 [PMID: 20702634 DOI: 10.1128/JVI.00703-10]

181 **Witwer KW**, Watson AK, Blankson JN, Clements JE. Relationships of PBMC microRNA expression, plasma viral load, and CD4+ T-cell count in HIV-1-infected elite suppressors and viremic patients. *Retrovirology* 2012; **9**: 5 [PMID: 22240256 DOI: 10.1186/1742-4690-9-5]

182 **Houzet L**, Yeung ML, de Lame V, Desai D, Smith SM, Jeang KT. MicroRNA profile changes in human immunodeficiency virus type 1 (HIV-1) seropositive individuals. *Retrovirology* 2008; **5**: 118 [PMID: 19114009 DOI: 10.1186/1742-4690-5-118]

183 **Sun G**, Li H, Wu X, Covarrubias M, Scherer L, Meinking K, Luk B, Chomchan P, Alluin J, Gombart AF, Rossi JJ. Interplay between HIV-1 infection and host microRNAs. *Nucleic Acids Res* 2012; **40**: 2181-2196 [PMID: 22080513 DOI: 10.1093/nar/gkr961]

184 **Balasubramaniam M**, Pandhare J, Dash C. Are microRNAs Important Players in HIV-1 Infection? *Viruses* 2018; **10**: 110 [PMID: 29510515 DOI: 10.3390/v10030110]

185 **Triboulet R**, Mari B, Lin YL, Chable-Bessia C, Bennasser Y, Lebrigand K, Cardinaud B, Maurin T, Barbry P, Baillat V, Reynes J, Corbeau P, Jeang KT, Benkirane M. Suppression of microRNA-silencing pathway by HIV-1 during virus replication. *Science* 2007; **315**: 1579-1582 [PMID: 17322031 DOI: 10.1126/science.1136319]

186 **Wang P**, Qu X, Zhou X, Shen Y, Ji H, Fu Z, Deng J, Lu P, Yu W, Lu H, Zhu H. Two cellular microRNAs, miR-196b and miR-1290, contribute to HIV-1 Latency. *Virology* 2015; **486**: 228-238 [PMID: 26469550 DOI: 10.1016/j.virol.2015.09.016]

187 **Duskova K**, Nagilla P, Le HS, Iyer P, Thalamuthu A, Martinson J, Bar-Joseph Z, Buchanan W, Rinaldo C, Ayyavoo V. MicroRNA regulation and its effects on cellular transcriptome in human immunodeficiency virus-1 (HIV-1) infected individuals with distinct viral load and CD4 cell counts. *BMC Infect Dis* 2013; **13**: 250 [PMID: 23721325 DOI: 10.1186/1471-2334-13-250]

188 **Huang J**, Lai J, Liang B, Jiang J, Ning C, Liao Y, Zang N, Wang M, Qin F, Yu J, Wei W, Ye L, Liang H. mircoRNA-3162-3p is a potential biomarker to identify new infections in HIV-1-infected patients. *Gene* 2018; **662**: 21-27 [PMID: 29627523 DOI: 10.1016/j.gene.2018.04.002]

189 **Swaminathan S**, Suzuki K, Seddiki N, Kaplan W, Cowley MJ, Hood CL, Clancy JL, Murray DD, Méndez C, Gelgor L, Anderson B, Roth N, Cooper DA, Kelleher AD. Differential regulation of the

Let-7 family of microRNAs in CD4+ T cells alters IL-10 expression. *J Immunol* 2012; **188**: 6238-6246 [PMID: 22586040 DOI: 10.4049/jimmunol.1101196]

190 **Biswas S**, Haleyurgirisetty M, Lee S, Hewlett I, Devadas K. Development and validation of plasma miRNA biomarker signature panel for the detection of early HIV-1 infection. *EBioMedicine* 2019; **43**: 307-316 [PMID: 31005516 DOI: 10.1016/j.ebiom.2019.04.023]

191 **Guha D**, Mancini A, Sparks J, Ayyavoo V. HIV-1 Infection Dysregulates Cell Cycle Regulatory Protein p21 in CD4+ T Cells Through miR-20a and miR-106b Regulation. *J Cell Biochem* 2016; **117**: 1902-1912 [PMID: 26755399 DOI: 10.1002/jcb.25489]

192 **Sánchez-Del Cojo M**, López-Huertas MR, Díez-Fuertes F, Rodríguez-Mora S, Bermejo M, López-Campos G, Mateos E, Jiménez-Tormo L, Gómez-Esquer F, Díaz-Gil G, Alcamí J, Coiras M. Changes in the cellular microRNA profile by the intracellular expression of HIV-1 Tat regulator: A potential mechanism for resistance to apoptosis and impaired proliferation in HIV-1 infected CD4+ T cells. *PLoS One* 2017; **12**: e0185677 [PMID: 28968466 DOI: 10.1371/journal.pone.0185677]

193 **Rahimian P**, He JJ. HIV-1 Tat-shortened neurite outgrowth through regulation of microRNA-132 and its target gene expression. *J Neuroinflammation* 2016; **13**: 247 [PMID: 27634380 DOI: 10.1186/s12974-016-0716-2]

194 **Sardo L**, Vakil PR, Elbezanti W, El-Sayed A, Klase Z. The inhibition of microRNAs by HIV-1 Tat suppresses beta catenin activity in astrocytes. *Retrovirology* 2016; **13**: 25 [PMID: 27060080 DOI: 10.1186/s12977-016-0256-y]

195 **Mishra R**, Singh SK. HIV-1 Tat C modulates expression of miRNA-101 to suppress VE-cadherin in human brain microvascular endothelial cells. *J Neurosci* 2013; **33**: 5992-6000 [PMID: 23554480 DOI: 10.1523/JNEUROSCI.4796-12.2013]

196 **Hu G**, Liao K, Yang L, Pendyala G, Kook Y, Fox HS, Buch S. Tat-Mediated Induction of miRs-34a & -138 Promotes Astrocytic Activation *via* Downregulation of SIRT1: Implications for Aging in HAND. *J Neuroimmune Pharmacol* 2017; **12**: 420-432 [PMID: 28236278 DOI: 10.1007/s11481-017-9730-0]

197 **Aqil M**, Naqvi AR, Mallik S, Bandyopadhyay S, Maulik U, Jameel S. The HIV Nef protein modulates cellular and exosomal miRNA profiles in human monocytic cells. *J Extracell Vesicles* 2014; **3** [PMID: 24678387 DOI: 10.3402/jev.v3.23129]

198 **Yan Q**, Zhao R, Shen C, Wang F, Li W, Gao SJ, Lu C. Upregulation of MicroRNA 711 Mediates HIV-1 Vpr Promotion of Kaposi's Sarcoma-Associated Herpesvirus Latency and Induction of Pro-proliferation and Pro-survival Cytokines by Targeting the Notch/NF-κB-Signaling Axis. *J Virol* 2018; **92**: e00580-18 [PMID: 29976660 DOI: 10.1128/JVI.00580-18]

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WJGP mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal pathophysiology and covering a wide range of topics including disorders of the esophagus, stomach and duodenum, small intestines, pancreas, biliary system, and liver.

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REVIEW

Cholangiocarcinoma and liver transplantation: What we know so far?

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Abstract

Cholangiocarcinoma (CCA) is a type of cancer with increasing prevalence around the world that originates from cholangiocytes, the epithelial cells of the bile duct. The tumor begins insidiously and is distinguished by high grade neoplasm, poor outcome, and high risk for recurrence. Liver transplantation has become broadly accepted as a treatment option for CCA. Liver transplantation is expected to play a crucial role as palliative and curative therapy for unresectable hilar CCA and intrahepatic CCA. The purpose of this study was to determine which cases with

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CCA should be subjected to liver transplantation instead of resection, although reported post-transplant recurrence rate averages approximately 20%. This review also aims to highlight the molecular current frontiers of CCA and directions of liver transplantation for CCA.

Key Words: Cholangiocarcinoma; Liver transplantation; Primary sclerosing cholangitis; Neoadjuvant chemoradiotherapy

Core Tip: Currently, there are many controversial hypotheses concerning liver transplantation in cholangiocarcinoma (CCA) and risk factors and molecular pathogenesis of CCA, with a focus on primary sclerosing cholangitis. Here, we mainly review the current advances in classification and treatment of CCA.

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INTRODUCTION

Cholangiocarcinomas (CCA), also known as bile duct cancer, constitute a diverse group of biliary epithelial tumors affecting the intrahepatic CCA (iCCA), perihilar CCA (pCCA), and distal bile duct CCA (dCCA)[1]. CCA is the second leading cause of liver malignancy after hepatocellular carcinoma (HCC), and the overall incidence and mortality rates of CCA have increased progressively worldwide in the last 4 decades [2]. Primary sclerosing cholangitis (PSC) as a chronic liver disease can increase the risk for CCA reaching approximately 10% or 398-fold *vs* with the general population[3,4]. CCA has remained the common cause of death at the global level among PSC patients, whereby 30% of all CCAs are recognized annually after diagnosing PSC[5,6]. CCA is generally considered to be one of the contraindications in relation to liver transplantation characterized by poor prognosis. CCA patients have a median survival of 2 years following diagnosis. The only potentially curative treatment chance is surgery, depending on the stages of disease[7]. It has been shown that neoadjuvant therapy with liver transplantation as a novel treatment exhibits better survival rates with fewer recurrence in comparison with conventional resection for localized, node-negative hilar cholangiocarcinoma (hCCA)[8]. CCAs are a highly aggressive epithelial malignancy, and many patients represent advanced stages of disease[9]. Early detection of CCA still remains a challenge owing to its 'silent' clinical feature (most patients in the initial stage at the time of diagnosis are asymptomatic) and difficult to reach anatomical sites[10]. It seems that the use of liver transplantation for the treatment of CCA can influence clinical outcomes in patients around the world. This class of tumor driving from the bile duct epithelial cells is clinically malignant, and its occurrence and prognosis are mostly associated with its anatomic location within the biliary tree and its chance to achieve complete resection with negative margins[11]. This review summarizes the risk factors and molecular pathogenesis of CCA, with a focus on PSC and liver transplantation along with advances in classification and treatment.

CCA CLASSIFICATION

CCA may be originated from the different cell types of the biliary tract, including cholangiocytes, the epithelial cells lining of the biliary surface epithelium, the epithelial cells of the peribiliary glands, hepatic progenitor cells, or any other mature hepatocytes that have become malignant. In this regard, CCA could also be classified

in terms of anatomical, histological, and molecular aspects[12].

Anatomical classification of CCA

According to anatomical location of the tumor, CCA will most commonly be classified into three sub-groups: (1) iCCA; (2) pCCA; and (3) dCCA (Table 1)[12,13]. Given the tumor location, iCCA typically arises from the intrahepatic biliary tract including segmental bile ducts to smaller branches of the intrahepatic biliary system. Thus, this subtype of CCA occurred in the periphery of the second-order bile ducts[13]. Also, iCCA represents approximately 20% of all CCA reported cases[14]. pCCA arises around the hepatic ducts and their junctions[15]. Finally, dCCA refers to the malignancy that occurs in the common bile duct, *i.e.* originated from Vater's ampulla [14,16].

Histological classification of CCA

From the histological point of view, characteristics of pCCA and dCCA that can be considered as extrahepatic CCA (eCCA) subtypes are conventionally mucin-producing adenocarcinomas or papillary tumors. On the other hand, iCCAs are more heterogeneous than two other subtypes of CCA. Histological studies showed that the adenocarcinoma is formed by columnar to cuboidal epithelial cells in the tubular structures, acini formation, and micropapillary architecture with variable morphological aspects, which are the most common types of iCCA[12].

Moreover, it has been suggested that, according to the level or size of the displayed bile duct, iCCA is classified into two main histological subtypes. First, the small bile duct iCCA that presents as small-sized tubular or acinar adenocarcinoma. These tumors commonly originated from small intrahepatic bile ducts, progenitor cells, and mature hepatocytes[17]. In contrast, large bile ducts iCCA derive from large intrahepatic bile ducts and/or associated peribiliary glands. Moreover, depending on the origin of the large bile duct iCCA, the histological aspects of this subtype of iCCAs are partly similar to pCCA and dCCA. However, the gross examination is not sufficient for accurate tumor classification, and further histological, molecular, and clinical investigation is required[18].

Molecular classification of CCA

First, it needs to be explained that, due to some differences in the characteristics of the existing studies, including different molecular detection methods and diversity in the selection of populations, there is still no consensus on the molecular characteristics of CCA classification[17]. However, it is possible to establish an acceptable relationship between the anatomical and molecular aspects of CCA subtypes. Integrative molecular analyses not only provided the functional information for CCA classification but also were used to understand the pathogenesis and signaling pathways underlying the CCA carcinogenesis and progression[14].

Mutation-based classification is the main approach of CCA molecular classification. For instance, the isoforms 1 and 2 of isocitrate dehydrogenase (IDH1 and IDH2) and *NRAS* mutations are the main molecular manifestation of iCCA, whereas eCCA typically showed *TP53*, *KRAS*, and *BRAF* mutations[14,19]. Also, it has been reported that *IDH1/2* and *BAP1* mutations and fibroblast growth factor receptor 2 (FGFR2) fusions are the main molecular characteristics of iCCA, while protein kinase CAMP-activated catalytic subunit alpha (PRKACA) and AT-rich interactive domain-containing protein 1B mutations are more common in eCCA. Besides, *KRAS*, *GNAS*, and *TP53* mutations are shared between iCCA and eCCA[19]. Interestingly, FGFR2 pairs with PRKACA in iCCA, as well as PRKACB in eCCA[20]

On the other hand, previous molecular studies also have attempted to connect the morphological CCA subtypes with specific molecular-based patterns. In this regard, the large-duct type iCCAs have a specific molecular property such as high mutation frequency of oncogenes and tumor suppressor genes and lack other gene mutations that are typically seen in small-duct iCCA. It has been reported that *KRAS* and *TP53* are two prominent genes with high mutation frequency in the large-duct type iCCAs as well as lack of *IDH1/2* mutations and *FGFR2*-fusions, which are molecular characteristics of small-duct iCCA[21].

In addition to mutation and sequence alterations, epigenetic study based on the methylation profiles of CCA subtypes can be used for CCA classification. For example, CCA has been related to hypermethylation at the promoter of tumor suppressor genes, such as *DAPK*, *P14* (ARF), and *ASC*[22]. Moreover, despite the different patterns of methylation in GC-rich regions (CpG islands) in the CCA subtypes-related genes, it has been revealed that there is an alteration in CpG methylation that belonged to WNT, transforming growth factor-β, phosphatidylinositol 3 kinase, mitogen-activated

Table 1 Summary of anatomical, histomorphological, and molecular characteristics of cholangiocarcinoma subtypes

Anatomical classification		Histomorphological classification	Molecular specification (gene alterations)
iCCA	Small intrahepatic bile ducts iCCA	Mass forming tumors[17]	IDH1/2, FGFR2, EPHA2, BAP1[14,19]
	Large intrahepatic bile ducts iCCA	Mass forming, periductal, or intraductal mucinous tumors[17,18]	EPHA2, BAP1, KRAS, TP53, GNAS, NRAS, MRAS, SMAD4[12,14,21]
eCCA	Perihilar CCA	Intraductal mucinous tumors[12,17]	KRAS, TP53, GNAS, NRAS, MRAS, SMAD4, ARID1B, PRKACA, BRAF[14,19,24]
	Distal CCA	Periductal mucinous tumors[12,17]	KRAS, TP53, GNAS, NRAS, MRAS, SMAD4, ARID1B, PRKACB, BRAF[14,19,24]

CCA: Cholangiocarcinomas; iCCA: Intrahepatic CCA; eCCA: Extra-hepatic CCA; EPHA2: Ephrin type-A receptor 2 precursor; FGFR2: Fibroblast growth factor receptor 2; BAP1: BRCA1 associated protein-1; NRAS: Neuroblastoma RAS viral [v-ras] oncogene homolog; KRAS: Kirsten rat sarcoma virus; TP53: Tumor protein; PRKACA: Protein kinase cAMP-activated catalytic subunit alpha.

protein kinase, and NOTCH signaling pathways[14]. Furthermore, the results of various studies showed that molecular characteristics of CCA subtypes consisting of sequence and copy number alterations, gene expression, and DNA methylation can be categorized into different clusters, but the details of this issue are beyond the scope of this article[23].

In addition to the mentioned above, another recent large cohort of CCA suggested that according to whole-gene expression data, chromosomal aberrations, and signaling pathway activation, CCA can be divided into two molecular subgroups: (1) inflammation class; and (2) proliferation class, which accounts for 38% and 62% of CCA cases, respectively[24]. The inflammation class of CCA has been characterized by the activation of inflammatory response and overexpression of T helper 2)-related cytokines and down-regulation of Th1-related cytokines. Moreover, it has been reported that several oncogenic pathways were enriched in the proliferation class that is accompanied by activation of receptor tyrosine kinase pathways (*i.e.* epidermal growth factor, RAS, AKT, MET, angiogenesis-related vascular endothelial growth factor, and platelet-derived growth factor) and Kirsten rat sarcoma viral oncogene homolog mutations[14,24].

Despite all of the before-mentioned data about molecular CCA classification, many other studies provide more useful information about molecular characteristics of CCA subtypes, such as the information derived from the noncoding RNA alteration, proteomics, and radiogenomic studies, which should be discussed in a separate article focusing on molecular classification of CCA[24].

Molecular pathogenesis of CCA

Cholangiocarcinogenesis is linked not only with genetic and epigenetic alterations but also with major changes in the microenvironment of the tumor. These modifications contribute to the triggering of different signaling pathways that are able to drive the initiation and progression of tumors[25]. Chronic inflammation contributes to increased exposure of cholangiocytes to Wnt inflammatory mediators, interleukin-6, cyclo-oxygenase-2, and tumor necrosis factor-alpha, leading to progressive mutations in some critical cancer-related genes including tumor suppressors, proto-oncogenes, and DNA mismatch-repair[26]. Increased apoptosis, decreased pH, and activation of extracellular signal-regulated kinase 1/2, Akt, and nuclear factor-kappa B signaling pathways following the accumulation of bile acids from cholestasis lead to promotion of survival, cell proliferation, and migration. Vascular endothelial growth factor, transforming growth factor-β, hepatocyte growth factor, and other microRNAs (miRNAs) are other mediators that are upregulated in CCA. Tumor development, angiogenesis, and migration are triggered by increased expression of the glucose transporter protein type 1, the cell surface receptor c-Met, and the sodium iodide symporter. The composition of the extracellular matrix and macrophage/fibroblast recruitment result in stromal shifts that establish a microenvironment to promotes cell survival, invasion, and metastasis[25,27-29]. The major signaling pathways involved in CCA are illustrated in Figure 1.

Genetic factors in the pathogenesis of CCA

Few studies have described chromosomal abnormalities in CCA, and, due to the limited number of samples and large genetic variation between the population groups

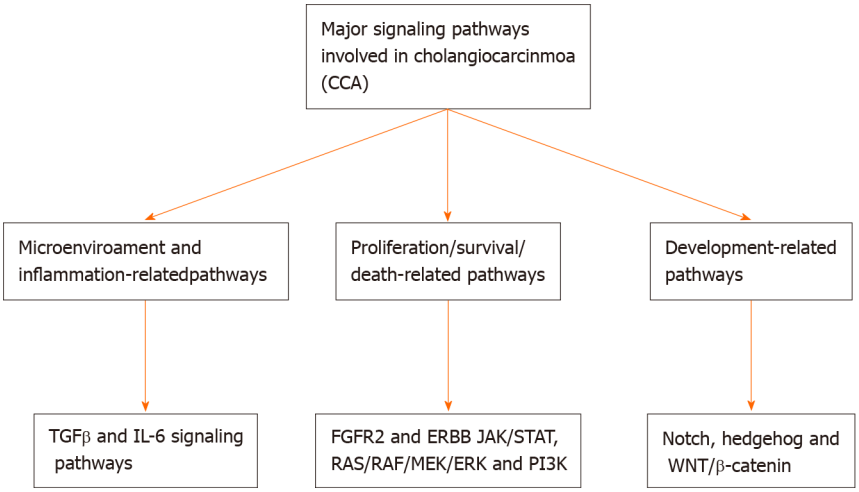

Figure 1 Major signalling pathways involved in cholangiocarcinoma. The major signaling pathways involved in cholangiocarcinomas could be categorized into three main class: (1) Microenvironment and inflammation-related pathways; (2) Pathways related to proliferation/survival/death; and (3) Development-related pathways. TGF-β: Transforming growth factor-β; CCA: Cholangiocarcinoma; IL: Interleukin; FGFR2: Fibroblast growth factor receptor 2; PI3K: Phosphatidylinositol 3 kinase; ERK: Extracellular signal-regulated kinase; JAK/STAT: Janus kinase and signal transducer and activator of transcription.

examined, the findings have been difficult to interpret. Data have revealed gains at 1q, 7p, 8q, 17q, and/or 20q and losses at 1p, 3p, 4q, 6q, 8p, 9pq, 13q, 14q, 17p, 18q, and/or 21q[24,30]. Curiously, genetic heterogeneity may be correlated with CCA in cells other than cholangiocytes. Natural killer cells and T-lymphocytes, for instance, express the natural killer group 2D receptor that plays an important role in cytotoxicity and tumor surveillance regulated by cells. One study indicated that the risk of experiencing CCA ranged significantly in patients with PSC, according to the patient's natural killer group 2D alleles; some were protective and others more than doubled the risk[31]. As potential risk factors for CCA, host genetic factors, alone or combined with environmental factors, have been investigated. For polymorphic variants that may be correlated with greater vulnerability to CCA, genes coding for xenobiotic detoxification, multidrug resistance, enzymes responsible for carcinogen metabolism, DNA repair, folate metabolism, and inflammation have been investigated. However, due to the inclusion of gallbladder and ampullary cancers in their evaluation in some of these reports and the lack of replication in separate cohorts, no conclusive conclusions can be taken. Multiple gene polymorphisms have been correlated with greater and reduced danger of experiencing CCA in many hospital-based, case-control studies. Due to the different populations of the sample and the lack of replication of the study in separate cohorts, it is hard to draw definite conclusions about these results. Table 2 summarizes genetic mutations and polymorphisms associated with CCA.

Epigenetic alterations in CCA

By the advent of array-based and deep sequencing techniques, technological advances have taken epigenetics into the omics-age, emphasizing the role of the epigenome in the human carcinogenesis process, including DNA CpG methylation, histone modifications, and non-coding RNA organisms. Only few systematic CCA epigenomic reports have been conducted, and data on abnormal CpG promoter methylation have mainly focused on individual genes in the CCA regulation[32]. In various important cancer-associated genes in CCA, abnormal epigenetic modulation such as promoter hypermethylation, was reported[32,33]. Studies examining these modifications to existing prognostic and predictive gene signatures have not yet been investigated in CCA to predict the therapeutic benefits of agents targeting the cancer epigenome. In CCA, the well-studied epigenetic process is DNA methylation. The promoter regions of tumor suppressor genes are highly methylated (promoter hypermethylation) in CCA tumorigenesis, which contributes to gene silencing. The promoter hypermethylation of genes involved in the repair of DNA, cell cycle, apoptosis, metabolism of carcinogen/drugs, and cell adhesion has been documented in CCA[33,34]. Some of the most frequent epigenetic events reported in CCA by methylation is summarized in Table 3.

Table 2 Genetic mutations and polymorphisms associated with cholangiocarcinoma

Gene (Full name)	Protein (Full name)	Normal function(s)	Ref.
ATP8B1 (ATPase Phospholipid Transporting 8B1)	FIC1 (Familial Intrahepatic Cholestasis type 1)	Transmembrane phospholipid transfer	Wadsworth *et al*[88], 2011
ABCB11 (ATP Binding Cassette Subfamily B Member 11)	BSEP (Bile Salt Exporter Pump)	Transport of cholate conjugates from hepatocytes to bile	Wadsworth *et al*[88], 2011
ABCC2 (ATP Binding Cassette Subfamily C Member 2)	MRP2 (Multidrug resistance-associated protein 2)	Transport of endogenous and xenobiotic compounds from hepatocytes to bile	Hoblinger *et al* [89], 2009
ABCB4 (ATP Binding Cassette Subfamily B Member 4)	MDR3 (MHC class I polypeptide-related sequence A)	Transport of lipids from hepatocytes to bile	Khabou *et al* [90], 2019
COX-2 (Cyclooxygenase 2)	COX-2 (Cyclooxygenase 2)	Inflammatory cytokine	Kim *et al*[91], 2002
CYP1A2 (Cytochrome P450 1A2)	CYP1A2 (Cytochrome P450 1A2)	Xenobiotic metabolism	Prawan *et al* [92], 2005
KLRK1 (Killer Cell Lectin Like Receptor K1)	NKG2D (NKG2-D type II integral membrane protein)	Tumor surveillance	Melum *et al* [93], 2008
MTHFR (Methylenetetrahydrofolate Reductase)	MTHFR (5,10-Methylenetetrahydrofolate reductase)	DNA methylation	Ko *et al*[94], 2006
NAT2 (N-Acetyltransferase 2)	ARY2(Arylamine N-acetyltransferase 2)	Drug and carcinogen metabolism	Prawan *et al* [92], 2005
PTGS2 (Prostaglandin-endoperoxide synthase 2)	PTGS2 (Prostaglandin G/H synthase 2)	The key enzyme in prostaglandin biosynthesis, and acts both as a dioxygenase and as a peroxidase	Sakoda *et al* [95], 2006
XRCC1 (X-ray repair cross complementing 1)	XRCC1 (DNA repair protein XRCC1)	Involved in DNA single-strand break repair by mediating the assembly of DNA break repair protein complexes	Huang *et al* [96], 2008
GSTO1(Glutathione S-transferase omega-1)	GST01 (Glutathione S-transferase omega-1)	Detoxification of endogenous and xenobiotic compounds	Marahatta *et al* [97], 2006
MICA (MICA PERB11.1)	MICA (MHC class I polypeptide-related sequence A)	Stress-induced self-antigen and Ligand for the KLRK1/NKG2D receptor	Melum *et al* [93], 2008
NR1H4(Nuclear Receptor Subfamily 1 Group H Member 4)	BAR (FXR) (Bile acid receptor (Farnesoid X receptor)	Negative feedback inhibitor of bile acid synthesis	Wadsworth *et al*[88], 2011
TYMS (Thymidylate Synthetase)	TYMS (Thymidylate synthase)	DNA repair	Razumilava *et al*[61], 2014
XRCC1 (X-Ray Repair Complementing Defective Repair in Chinese Hamster Cells 1)	XRCC1 (DNA repair protein XRCC1)	DNA repair	Gong *et al*[98], 2015
APC (Adenomatous polyposis coli)	APC (Adenomatous polyposis coli)	Tumor suppressor	Kang *et al*[99], 1999
ARID1A (AT-Rich Interaction Domain 1A)	ARID1a (AT-rich interactive domain-containing protein 1A)	Transcription factor	Razumilava *et al*[61], 2014
BAP1 (BRCA1 Associated Protein 1)	BAP1 (Ubiquitin carboxyl-terminal hydrolase BAP1)	Regulates cell growth	Yoshino *et al* [100], 2020
BCL-2 (B cell Lymphoma-2)	Bcl-2 (B-cell lymphoma 2)	Regulates apoptosis	Fingas *et al* [101], 2010
BRAF (B Rapidly Accelerated Fibrosarcoma)	B-Raf (B-Rapidly Accelerated Fibrosarcoma)	Proto-oncogene	Sia *et al*[24], 2013
BRCA1 (Breast Cancer 1)	BRCA1 (Breast cancer type 1 susceptibility protein)	Tumor suppressor and DNA repair	Paradiso *et al* [102], 2020
BRCA2 (Breast Cancer 2)	BRCA2 (Breast cancer type 2 susceptibility protein)	DNA repair	
CCND1(Cyclin D1)	CCND1 (G1/S-specific cyclin-D1)	Regulates cell growth	Yoshino *et al* [100], 2020
CDH1(Cadherin 1)	E-cadherin (Epithelial cadherin)	Tumor suppressor, cell adhesion	Ross *et al*[103], 2014
CDK6 (Cyclin-Dependent Kinase 6)	CDK6 (Cyclin-Dependent Kinase 6)	Controls cell cycle and differentiation	

CTNNB1 (Catenin Beta 1)	B-catenin	Proto-oncogene	O'Dell *et al* [104], 2012
EGFR (ERBB1) (Epidermal Growth Factor Receptor)	EGFR (ErbB-1) (Epidermal Growth Factor Receptor)	Proto-oncogene	
ERBB2 (HER2) (Avian Erythroblastosis oncogene B2)	ErbB-2 (HER2) (Receptor tyrosine-protein kinase erbB-2)	Proto-oncogene	
FBXW7 (F-Box and WD Repeat Domain Containing 7)	FBXW7 (F-box/WD repeat-containing protein 7)	Component of proteasomal protein degradation pathway	Ross *et al*[103], 2014
FGF19 (Fibroblast Growth Factor 19)	FGF19 (Fibroblast Growth Factor 19)	Regulation of bile salt synthesis	
FGFR2 (Fibroblast Growth Factor Receptor 2)	FGFR2 (Fibroblast Growth Factor Receptor 2)	Cell surface receptor regulating cell proliferation, differentiation, migration and apoptosis	
IDH1 (Isocitrate dehydrogenase 1)	Isocitrate de-hydrogenase 1 (Isocitrate dehydrogenase (cytoplasmic))	Glucose metabolism, indirectly mitigates oxidative stress	Nabeshima *et al*[105], 2020
IDH2 (Isocitrate dehydrogenase 2)	Isocitrate de-hydrogenase 2 (Isocitrate dehydrogenase (mitochondrial))	Glucose metabolism, indirectly mitigates oxidative stress	
Keap1 (Kelch-like ECH-associated protein 1)	KEAP1 (Kelch-like ECH-associated protein 1)	Prevents Nrf2-driven transcription	Ma *et al*[106], 2020
KRAS (Kirsten Rat Sarcoma)	K-Ras (Kirsten Rat Sarcoma)	Proto-oncogene	Tannapfel *et al* [107], 2000
MDM2 (Mouse Double Minute 2)	Mdm2 (E3 ubiquitin-protein ligase Mdm2)	Proto-oncogene, p53 inhibitor	Ross *et al*[103], 2014
MYC (Avian myelocytomatosis virus oncogene cellular homolog)	Myc (Myc proto-oncogene protein)	Proto-oncogene	Zhou *et al* [108], 2019
NF1 (Neurofibromin 1)	NF1 (Neurofibromin)	Stimulates Ras activity	Ross *et al*[103], 2014
PBRM1 (Polybromo 1)	PBRM1 (Protein polybromo-1)	Negative regulator of cell proliferation	Luchini *et al* [109], 2017
PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha)	PIK3CA (Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform)	Generates PIP3 that activates signalling cascades for cell growth, survival and motility	Xu *et al*[110], 2011
PTEN (Phosphatase and Tensin Homolog)	PTEN (Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN)	Tumor suppressor	Zhu *et al*[111], 2014
RAD51AP1 (RAD51 Associated Protein 1)	RAD51AP1 (RAD51 Associated Protein 1)	DNA damage repair	Liu *et al*[112], 2021
RASSF1A (Ras association domain family 1 isoform A)	RASSF1A (Ras association domain-containing protein 1 isoform A)	Tumor suppressor	Chen *et al*[113], 2005
SMAD4 (Small Mothers Against Decapentaplegic 4)	SMAD4 (Small Mothers Against Decapentaplegic 4)	Tumor suppressor, transcription factor	Yoshino *et al* [100], 2020
SOCS3 (Suppressor of Cytokine Signaling 3)	SOCS3 (Suppressor of Cytokine Signaling 3)	Signal transduction inhibitor	Andersen *et al* [114], 2012
TP53 (Tumor Protein 53)	p53 (Protein 53)	Tumor suppressor	O'Dell *et al* [104], 2012

NKG2D: Natural killer group 2D.

Non-coding RNAs changes in CCA

MiRNAs are a type of small non-coding RNA that is involved in the post-transcriptional regulation of gene expression. The upregulation/downregulation in multiple miRNAs have been reported in CCA, wherein dysregulated miRNAs led to mitosis, increased cell survival, and metastasis[35]. However, whether the alteration in miRNA expression in CCA is part of the process of carcinogenesis or the consequence of established CCA remains to be fully understood[36]. Long non-coding RNAs (lncRNAs) widely transcribed in the genome are evolving as key cancer regulators and play crucial roles in almost every facet of cell biology, including tumorigenesis. *Via* their association with DNA, proteins, and RNA, lncRNAs control cells' malignant transformation. The molecular mechanisms of lncRNA involved in CCA tumorigenesis may therefore be promising targets for therapeutic intervention and diagnostic applications in the battle against cancer[37,38]. The majority of upregulated genes are

Table 3 DNA methylation in the genomic sequences of specific genes that are associated with the pathogenesis of cholangiocarcinoma

Gene (location)	Function	Epigenetic modification/effect	Outcome	Ref.
p16INK4A or CDKN2A (9p21)	Tumor suppressor gene Regulates cell proliferation and oncogenesis	Promoter region hypermethylation of the p16 ^{INK4A} results in gene inactivation. Common event in PSC-associated CCA	More frequent in ECC cases. More commonly observed in tumors with vascular invasion. Poor clinical outcome	Ueki *et al* [115], 2004
p14ARF (9p21)	Encoded by the β transcript of CDKN2A (p16/CDKN2A)	Methylation of p14^{ARF} MF = 38 and 25% (32.35); 40.2% liver fluke CCA (37)	Increased tumorigenesis in CCA	Kim *et al* [116], 2007
p15INK4b or p15 (9p21)	Effecter of TGF-β-mediated cell cycle arrest	Promoter hypermethylation of p15 gene	Increased tumorigenesis in CCA	Yang *et al* [117], 2005
p73 gene (1p36.3)	Tumor suppressor gene and related to the p53 gene	Promoter region hypermethylation increased tumorigenesis	Increased tumorigenesis in CCA	
TMS1/ASC (16p11.2)	Tumor suppressor gene	Aberrant methylation of the TMS1/ASC cause inactivation of gene	Associated with CCA	Liu *et al* [118], 2006
FHIT (3p14.2)	Tumor suppressor gene	Promoter hypermethylation of the FHIT gene results in epigenetic silencing of the FHIT promoter region	Development of intrahepatic CCAs	Foja *et al* [119], 2005
RASSF1A (3p21.3)	Tumor suppressor gene induces cell cycle arrest by inhibiting the accumulation of cyclin D1	Hypermethylation of its CpG island promoter region results in inactivation	Promoter methylation is more common in ECC than	Wong *et al* [120], 2002
hMLH1 (3p21.3)	DNA mismatch repair gene	Promoter methylation/hypermethylation of the hMLH1 gene	Methylation frequencies vary in sporadic CCA, biliary papillary, neoplasms, and liver fluke-related CCA. Associated with poorly differentiated subtype of CCA with vascular invasion	Yang *et al* [117], 2005
APC (5q21–q22)	Tumor suppressor gene Controls cell division, cell-cell interactions and cell migration and invasion, and conservation of chromosomal number during cell division	APC gene hypermethylation	Worse clinical outcome in CCA	Yang *et al* [117], 2005
RAR-β (or *HAP, RRB2* and *NR1B2*) (3p24)	Mediates cellular signaling in embryonic morphogenesis, cell growth and differentiation by regulating gene expression	Gene silencing by promoter region hypermethylation Results in increased tumorigenesis	Increased tumorigenesis in CCA	
Epithelial (*E*) *cadherin gene* (16q22.1)	Tumor suppressor gene	Hypermethylation of the promoter region of E gene Results in loss of function and contribute to progression of cancer by increasing proliferation, invasion and metastasis	Development of intrahepatic CCA	Lee *et al* [121], 2002
DAPK (9q34.1)	Tumor suppressor gene Positive mediator of interferon-γ (IFN-γ)-induced programmed cell death	DAPK gene hypermethylation	Associated with poorly differentiated CCAs and with a poor prognosis	Tozawa *et al*[122], 2004
CHFR gene (12q24.33)	Tumor suppressor gene Delays the entry into the metaphase	Gene silencing by promoter hypermethylation	Increased tumorigenesis in CCA	
RUNX3 gene (Ip36)	Tumor suppressor gene Regulate proliferation of the biliary tract epithelium	Methylation of RUNX3 results in gene silencing	Associated with poorer survival	
GSTP gene (1q43)	Regulate drug and xenobiotic. metabolism	Promoter region hypermethylation	Hypermethylation more frequent in ICCA than in ECC	Lee *et al* [121], 2002
MGMT gene (10q26)	Responsible for repairing alkylation. DNA damage inhibits estrogen receptor-mediated cell proliferation	Methylation of discrete regions of the MGMT CpG island, results in heterochromatinization of the MGMT transcription start site and silencing of the gene	Increased frequency of GC to AT transitions in oncogenes and tumor suppressor genes and a poor prognosis	Koga *et al* [123], 2005
BLU gene (3p21.3)	Tumor suppressor gene	Gene methylation	Increased tumorigenesis in CCA	Tischoff *et al*[124], 2005
SEMA3B (3p21.3)	Tumor suppressor gene by inducing apoptosis. Plays a critical role in the guidance of growth cones during neuronal development	Methylation of SEMA3B gene	Increased tumorigenesis in CCA	
TIMP3 gene	Plays a role in the induction of	CpG island methylation of TIMP3 gene	Associated with worse survival	Lee *et al*

(22q12.3)	apoptosis			[121], 2002
RIZ1	Tumor suppressor gene	Methylation of RIZ1 Results in chromatin compaction and gene silencing MF = 38% liver fluke CCA (47)	Increased proliferation and migration of CCA cell line	Khaenam *et al*[125], 2010
OPCML	Tumor suppressor gene	Hypermethylation of OPCML	Increased tumorigenesis in CCA	Sriraksa *et al*[126], 2011
GSTP1	Tumor suppressor gene	Methylation of GSTP1	Increased tumorigenesis in CCA	Yang *et al* [117], 2005
COX-2/PTGS2 (1q25.2–q25.3)	Acts both as a dioxygenase and as a peroxidase	Methylation of COX-2 gene	Increased tumorigenesis in CCA	Lee *et al* [121], 2002
THBS1 gene (15q15)	Mediates cell-to-cell and cell-to-matrix interactions and play roles in platelet aggregation, angiogenesis and tumorigenesis	Hypermethylation in the promoter region of THBS1 gene	Increased tumorigenesis in CCA	Tischoff *et al*[124], 2005
SOCS3	responsible for sustained IL-6/STAT-3 signaling and enhanced Mcl-1 expression in cholangiocarcinoma	Hypermethylation in the promoter region of SOCS3 gene	Increased tumorigenesis in CCA	Zhang *et al* [127], 2012

TGF-β: Transforming growth factor-β; CCA: Cholangiocarcinomas; IL: Interleukin; TIMP3: Tissue inhibitors of metalloproteinase 3; SOCS3: Suppressor of cytokine signaling 3; STAT-3: Signal transducer and activator of transcription; THBS1: Thrombospondin 1; SEMA3B: Semaphorin 3B; GSTP1: Glutathione S-transferase pi gene; OPCML: Opioid binding protein/cell adhesion molecule-like gene; MGMT: O6-methylguanine DNA methyltransferase; RIZ1: Retinoblastoma protein-interacting zinc finger gene 1.

involved in carcinogenesis, diseases of the hepatic system, and transduction of signals. The miRNAs and lncRNAs related to the promotion of the pathogenesis of CCA are indicated in Tables 4 and 5.

EPIDEMIOLOGY

Several publications have shown that PSC has an annual incidence rate of 0.77 *per* 100000 persons. PSC is more prevalent in adults between 25-years-old and 45-years-old; the median age of diagnosis of PSC is 41 years. Patients with PSC have a considerably higher risk of CCA, with an estimated incidence rate ranging from approximately 0.5% to 1.5% annually and lifetime incidence of 20%[4,39,40]. The estimated prevalence of CCA in patients with PSC ranges from 6.5% to 13.3%[4,41,42]. A recent cohort study on 7121 patients from 37 countries showed the prevalence of CCA in patients with PSC to be 8.3%[43]. In high prevalence regions, such as Scandinavian countries, PSC is the most common indication for liver transplantation[44]. Death attributed to PSC is increased nearly four-fold as compared to the general population, in part because of end-stage liver disease; however, more than 40% of deaths in PSC patients have been attributed to cancer development[4].

In Western countries, PSC is the most common known predisposing factor for CCA. The risk of CCA development *per* year among patients with PSC is 0.5% to 1.5%, with estimated lifetime prevalence of 5%–10%[45]. Several potential risk factors for CCA in PSC patients have been evaluated; smoking and alcohol consumption are increasingly recognized as risk factors for CCA[46].

Epidemiologic data studies regarding CCA mortality risk indicate that age-adjusted death rate for iCCA is increasing while trend mortality from pCCA and dCCA is expected to decrease worldwide[47]. Although the recorded rise in the incidence of CCA during the past 30 years has been observed as an increase in iCCA, it might be due to potential misclassification of perihilar tumors as iCCAs[48]. The age-adjusted incidence rate according to the United States database for iCCA enhanced from 0.59 *per* 100000 population in 1990 to 0.91 in 2001. Subsequently, the age-adjusted incidence rate decreased to 0.6 *per* 100000 population by 2007. Contrarily, the incidence rate among pCCA plus dCCA patients remained approximately 0.8 *per* 100000 population until 2001 then steadily increased to 0.97 until 2007. Perihilar CCA was identified as iCCAs before 2001 and subsequently was recognized as pCCA after releasing the 3rd edition of Classification of Tumors. This amendment plausibly affected the aforementioned alterations in specific incidence rates of both CCA subtypes[49].

Table 4 Unique microRNAs that were identified to promote the pathogenesis of cholangiocarcinoma

miRNAs	Target gene	Correlation with CCA tumorigenesis	Upregulated/downregulated	Ref.
miR-26a	GSK-3b	Tumor growth	Upregulated	Zhang *et al*[127], 2012
miR-24	MEN1(11q13)	Tumor suppressor gene	Upregulated	Ehrlich *et al*[128], 2017
miR-29b	MCL-1	Tumor suppressor gene	Downregulated	Stutes *et al*[129], 2007
let-7a	NF2	Tumor suppressor gene	Upregulated	
miR-148a	DNMT-1	Regulate methyltransferase	Downregulated	Braconi *et al*[130], 2010
miR-124	SMYD3	Migration and invasion of CCA cells	Downregulated	Zeng *et al*[131], 2012
miR-21	PTEN	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-152	DNMT-1	Regulate methyltransferase	Downregulated	Braconi *et al*[130], 2010
miR-200b	PTPN12	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-429	CDH-6	Tumor suppressor gene	Upregulated	Goeppert *et al*[133], 2016
miR-122, miR-145, miR-200c, miR-221, and miR-222	Multiple	Associated with tumorigenesis of ICCA	Downregulated	Karakatsanis *et al*[134], 2013
miR-21, miR-31, and miR-223	Multiple	No association with clinic-pathological parameters of CCA	Upregulated	
miR-370	MAP3K8	Tumor suppressor gene	Downregulated	Stutes *et al*[129], 2007
miR-141	CLOCK	Tumor suppressor gene	Upregulated	Meng *et al*[132], 2006
miR-214	Twist	Oncogene	Downregulated	Li *et al*[135], 2012

CCA: Cholangiocarcinoma; CXCR4: C-X-C chemokine receptor type 4; MAP3K8: Mitogen-Activated Protein Kinase Kinase Kinase 8; PTEN: Phosphatase and TENsin homolog deleted on chromosome 10; GSK-3b: Glycogen synthase kinase 3 beta; SMYD3: SET and MYN-domain containing 3; MCL-1: myeloid cell leukemia-1; NF2: Neurofibromatosis type 2.

iCCA is a primary carcinoma of the liver with rare entity, accounting for about 3% of global gastrointestinal cancers[50]. iCCA comprises 8%–10% of all CCA and has a distinguished disease course, incidence, and prevalence of disease from hilar and eCCA[51]. In addition, in spite of the fact that iCCA has been historically mistaken for other HCC[52], previous studies have shown that ICC accounts for 10%–20% of primary liver malignancies[53]. iCCA is uncommon in individuals under 40 years of age; it occurs primarily at an old age with the peak incidence in the 5th and 7th decade of life[54]. In the United States it is estimated a slight male predominance in iCCA cases (1.5 fold) over women[54].

Despite the low frequency of iCCA *vs* HCC, the incidence of iCCA appears to be increasing worldwide[55]. This increased risk of incidence rate is independent of tumor size and staging, and it is implausibly secondary to earlier diagnosis[55]. In the United States the incidence of iCCA during the past 30-year period enhanced 165% to 0.95 cases/100000 population[55]. A similar rise in iCCA incidence rate has also been reported in the United Kingdom, Japan, and Crete[56].

Globally, there is a certain disparity incidence of iCCA, with markedly lower rates of iCCA reported in Western nations when compared to East Asian countries[50]. This demographic variation is explained mainly by the prevalence of risk factors for iCCA in these East Asian countries[57].

In addition, Hispanic-Americans (1.22 *per* 100000 population) were considered to be significantly susceptible to high incidence of iCCA compared to other ethnic groups; for instance, African-Americans have a low rate of incidence (0.3 *per* 100000 population). The researchers have shown that this disparity may reflect genetic diversity, cultural differences, and socio-economic status in iCCA susceptibility[58].

Several risk factors implicated in iCCA pathogenesis have demographical prevalence. A previous report indicated that approximately 40% of iCCA patients will have no detectable risk factor, suggesting the need to be explored for further research in this regard[59].

Table 5 Upregulated long non-coding RNAs that are reported in cholangiocarcinomas

LncRNA	Possible mechanism	Clinical relevance	Ref.
AFAP1-AS1	(1) Decreasing the expression of c-Myc, Cyclin D1, MMP-2 and MMP-9; and (2) Decreasing the AFAP1 expression and promoting cell stress filament integrity	Unfavorable prognostic biomarker; potential therapeutic target	Lu *et al*[136], 2017
CCAT2	-	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[137], 2018
HULC	Activating CXCR4 by sponging to miR-372/miR-373 as ceRNA	Potential therapeutic target	Wang *et al*[138], 2016
ASAP1-IT1	Interacting with hedgehog signaling pathway	Unfavorable prognostic biomarker; potential therapeutic target	Guo *et al*[139], 2018
CPS1IT1	Coexpressed with host gene CPS1	Unfavorable prognostic biomarker; potential therapeutic target	Lu *et al*[136], 2017
EPIC1	Directly interacting with Mys	-	Li *et al*[140], 2018
H19	Activating IL-6 by sponging to let-7a/let-7b as ceRNA	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[141], 2017
CCAT1	Sponging to miR-152 as ceRNA	Independent prognostic factor; potential therapeutic target	Jiang *et al*[142], 2017
LINC01296	Modulating MYCN transcription by sponge miR-5095 as ceRNA	Potential therapeutic target	Jiang *et al*[142], 2017
PCAT1	Enhancing Wnt/β-catenin signaling through miR-122 repression and WNT1 expression	Potential therapeutic target	Zhang *et al*[143], 2017
SNHG1	Modulating cancer-related gene like CDKN1A by co-operating with chromatin-modifying enzymes as EZH2	Unfavorable prognostic biomarker; potential therapeutic target	Yu *et al*[144], 2018
MALAT1	(1) Activating PI3K/Akt pathway; and (2) miR-204-dependent CXCR4 regulation as ceRNA	Unfavorable prognostic biomarker; potential therapeutic target	Tan *et al*[145], 2017
PVT1	Binding to epigenetic modification complexes, adjusting the expression of ANGPTL4	Potential therapeutic target	Yang *et al*[146], 2018
UCA1	(1) Facilitating apoptosis *via* Bcl-2/caspase-3 pathway; (2) Activating AKT/GSK-3β/CCND1 axis; and (3) Upregulating MMP-9	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[147], 2017
SPRY4-IT1	Recruiting EZH2, LSD1 or DNMT1 *via* sponging to miR-101-3p	Unfavorable prognostic biomarker; potential therapeutic target	Xu *et al*[148], 2018
T-UCRs	Downstream of Wnt pathway and sponging to miR-193b	Unfavorable prognostic biomarker; potential therapeutic target	Carotenuto *et al*[149], 2017

LncRNA: Long non-coding RNAs; ceRNA: competing endogenous RNAs; MMP9: Matrix metallopeptidase 9; EZH2: Enhancer of zeste homolog 2; PI3K: Phosphoinositide 3-kinase; LSD1: lysine-specific demethylase 1; DNMT1: DNA (cytosine-5)-methyltransferase 1; CCND1: cell cycle proteins, cyclin D1; ANGPTL4: Angiopoietin-like protein 4.

It is believed that PSC is a predisposing factor for the development of iCCA. Both biliary inflammation and subsequent chronic proliferative activation of hepatic stem cells potentially predispose to iCCA formation[60]. It has been reported that PSC patients possess a lifetime incidence of CCA from approximately 5%–10%, while 50% of cases are recognized during 2 years of the course of PSC[61]. Additionally, researchers showed a predisposing risk of iCCA (odds ratio: 2.2; 95% confidence interval: 1.2–3.9) in ulcerative colitis patients[59]. The iCCA arose in PSC patients earlier, despite most individuals diagnosed between the ages of 30 and 50.

Recent results indicated that cancer risk is higher among patients with primary biliary stones and chronic biliary tract inflammation. Furthermore, incidence risk of iCCA has been found to be approximately 7% in hepaticolithiasis patients[61]. Another Asian study demonstrated that hepaticolithiasis in CCA patients followed by surgical resection is nearly 70% in Taiwan[60].

Furthermore, congenital anomalies of biliary tree, like Caroli's disease and fibrocystic hepatic disorder, reveal approximately 15% lifetime risk factors of iCCA following the 2nd decade of life[60]. Caroli's disease is a rare inherited disorder characterized by cystic widening of ducts in the liver, usually in a bilobar pattern. iCCA risk has been shown to be rising among subjects with bile stasis, cholangitis, and chronic inflammation[62].

CCA represents approximately 3% of all gastrointestinal cancers. The total incidence rate of CCA appears to have increased dramatically over the past 30 years[49]. The 5-

year overall survival rates after diagnosis remained at 10% during this span of time [46].

LIVER TRANSPLANTATION

CCA is a highly fatal malignancy tumor due to late clinical presentation[57]. While it is generally believed that standard of care is resection, most patients who present with metastatic disease are deemed unresectable[63]. The liver transplantation outcomes alone for unresectable conditions have been disappointing[64]. A previous study examined the effectiveness of a novel modality combining neoadjuvant chemoradiotherapy followed by liver transplantation. Survival outcomes from a combination of neoadjuvant chemoradiotherapy and liver transplantation for CCA are considerably excellent in comparison with resection[65]. Thus, even if transplantation may be a useful cure for unresectable iCCA, survival output remains poor. Orthotopic liver transplantation utilization is increasing within the United States and appears promising because it may obviate complications to achieve surgical margins into the liver. Unfortunately, efforts during the past decades were poor. In addition, according to the registry between 1968 and 1997, researchers have reported a 28% 5-year survival rate with a 51% risk of tumor recurrence rate after liver transplantation[64]. Furthermore, during the first 2 years, 84% of recurrences were identified and can occur in up to 47% of candidates of liver allograft. Other surgery centers in Europe reported a similar result; the 3-year survival for 36 patients was 30%[66]. Accordingly, most liver transplant centers historically consider CCA a contraindication for liver transplantation[67].

There are many benefits for liver transplantation *vs* conventional resection to acquire complete elimination of tumor. There is some difficulty in evaluating hepatic duct tumor involvement before resection, and this is the most frequent reason for failure towards the achievement of an R0 resection. This problem is considerably obviated by liver transplantation. Liver transplantation promotes extirpation of all adjacent tissue and resection of the caudate. Liver transplantation facilitates arterial and portal venous inflow preservation to the remaining liver. Liver transplantation provides wide local excision and higher patient survival than what could be achieved with conventional resection[8].

Researchers conclude that neoadjuvant supportive treatment therapy in combination with liver transplantation presently appears to have fared far better than resection for selected patients with regional lymph node negative hCCA. Surgical staging information is essential; 23% of patients had localized lymph node metastases and concomitant extrahepatic disorder, which increased subsequent risk for transplantation. In a quarter of patients with underlying PSC, pancreatoduodenectomy may be required to obtain complete removal of the patient's tumor with biliary tract involvement at the time of transplantation. Liver transplantation in combination with neoadjuvant treatment should be considered as an alternative option to surgical resection for patients with hCCA[8].

Liver transplantation as an important therapeutic option for iCCA is still debated. It has been reported that iCCA recurs within 5 years of liver transplantation among 70% of patients[68]. Locoregional interventions, such as radiofrequency ablation and transarterial chemoembolization, have garnered attention as a therapeutic alternative for localized, unresectable iCCA patients[69]. The standard treatment for patients with advanced-stage iCCA is the most common combinations, which includes systemic chemotherapy regimen of gemcitabine and cisplatin. According to a recent clinical study, liver transplantation could be a treatment choice for patients with early detected unresectable iCCA (*i.e.* ≤ 2 cm), with better survival results compared with those of HCC[70].

iCCA remains a contraindication for liver transplantation in most clinical centers around the world because of very poor prognosis, with a 2-year overall survival rate of approximately 30%[71]. The lack of standardization due to different patients' selection and the absence of neoadjuvant treatments are expected to change outcomes[72]. The best survival was achieved in hCCA thanks to careful patient selection for neoadjuvant radiotherapy. Results from cohort studies after 2014 confirmed promising results after liver transplantation for iCCA[70]. The significance of proper patient selection criteria was first evaluated in a global multicentric report among iCCA patients who underwent liver transplantation[70]. The only curative treatments available for pCCA are surgical resection and neoadjuvant chemoradiation therapy after liver transplantation. Owing to the existence of parenchymal liver disease, PSC patients in

most cases need liver transplantation as the preferred choice when compared to surgical resection[1]. Besides this, recent studies confirm that in PSC patients, intense immunosuppression ensuing liver transplantation increased risk of disease recurrence [73]. Liver transplantation for pCCA patients following neoadjuvant chemoradiation treatment establishes a proper long-term survival rate in a group of selected candidates with unresectable early stage pCCA and patients with PSC–related pCCA. Commitment to appropriate selection criteria, heavy neoadjuvant intervention, operative staging before liver transplantation, and specified technical procedures throughout the transplant process are required for success[74].

The research evidence shows that neoadjuvant therapy for liver transplantation is an effective treatment for unresectable early stage pCCA and pCCA occurring in the setting of PSC[75]. Recently, programmed cell death protein 1 inhibitors are noticed as a promising therapeutic option for CCA. Chimeric antigen receptor T cells, oncolytic viruses cancer vaccines and bispecific antibodies, show a remarkable ability to achieve satisfactory results.

Furthermore, the combinations of immunotherapy with other immunotherapeutics such as conventional therapies display some efficacy, and various studies have provided new insights into their administration in antitumor therapy[76]. The main barrier to successful liver transplantation and effective treatment is the availability of donor organs[75]. According to data from the Mayo Clinic and several other centers, from the start of therapy, a promising survival rate between 5-10 years was reached [77]. Post-transplant survival is approaching 50% at 5 years for both pCCA–related PSC and *de novo* pCCA, and these findings rationalize the use of both deceased and living donors.

After onset of this therapy in 1993, significant increases were observed in the time elapsed between the end of neoadjuvant treatment and liver transplantation. This interval can differ widely between patients by blood type compatibility, transplant center address, and availability of living donor organs. It has been shown that longer time elapsed between neoadjuvant therapy and liver transplantation results in reducing local recurrence[78]. Selection of patients with prolonged intervals and better oncologic biography, who are less susceptible to advancing the disease following neoadjuvant treatment, are less prone to develop recurrence post-transplantation. However, patients with radiation-induced fibrosis and longer intervals can significantly complicate the staging and transplant operations. Living donor liver transplant (LDLT) may solve these problems by removing the need to waitlist for a deceased donor and help physicians for optimal timing of liver transplantation. Recent findings based on clinical study demonstrated that LDLT and deceased donor liver transplant (DDLT) outcomes for pCCA-associated PSC are similar. In addition, LDLT for *de novo* pCCA shows a recurrence tendency and slightly worse patient survival outcomes *vs* DDLT. Despite these minor differences, researchers have been looking into possible mechanisms of disease progression following neoadjuvant treatment for a period to choose those patients who are at risk due to disease progression in order to prevent post-transplant disease recurrences[79]. A previous report indicated that liver transplantation is more effective and achieved better survival and less recurrence than surgical resection, and that the indications for liver transplantation and neoadjuvant treatment should advocate for resectable pCCA patients. According to these favorable findings, physicians have advocated for this viewpoint for patients with pCCA-associated PSC and transplanted many such patients at many transplant centers.

The role of neoadjuvant chemoradiation therapy and liver transplantation remains a consideration though, especially in *de novo* pCCA patients. Earlier studies were equivocal and unable to detect whether a subset of patients with *de novo* pCCA may benefit from liver transplantation *vs* surgical resection[80]. In 2015, American Hepato-Pancreato-Biliary Association recommended that surgical resection can be standard curative treatment for patients with resectable *de novo* pCCA[81]. Recent reports have suggested that liver transplantation *vs* surgical resection for hCCA patients who may need a liver transplant had better prognosis than those found after resection[82]. Analysis of results obtained from multicenter study between 2000 to 2015 showed that patients with pCCA not associated with PSC continued to show superiority of transplant compared to resection with promising post-transplant survival outcomes at 3 and 5 years (54% *vs* 44%, $P = 0.03$; 54% *vs* 29%, $P = 0.03$)[82]. Additionally, researchers pointed out 5-year estimated overall survival of 41% for patients enrolled onto clinical trials of neoadjuvant treatment/transplant procedure *vs* 27% among those patients who underwent surgical resection[83]. This discrepancy (14%) is too minor to approve the use of a donor liver for resectable non-PSC related pCCA[83]. In France a multi-center randomized clinical trial evaluating neoadjuvant chemoradiation and liver transplantation in comparison with resection will further elucidate pivotal details

on these equivocal results.

In brief, over the past 2 decades liver transplantation has been currently considered the proven treatment of unresectable early stage pCCA and pCCA associated with PSC. Outstanding findings can be attained by stringent adherence to patient selection criteria and clinical management, application of high-dose neoadjuvant radiation therapy, and clinical staging before liver transplantation. Liver transplantation in combination with neoadjuvant treatment can obtain outcomes similar to surgical resection for unresectable early stage pCCA patients, and this is the treatment of choice administered for patients with pCCA arising in the setting of PSC[84]. Approximately 5% of all cases affected by pCCA require liver transplantation under the Mayo eligibility criteria. If the liver does not work properly, without transplantation, a median survival time is approximately 1 year. pCCA is reported as the most common malignancy and aggressive type of the biliary duct and arises from biliary lining the liver hilum[85]. The Mayo Clinic and other international centers are recently selecting the optimal subgroup to treat patients with locally advanced pCCA by neoadjuvant chemoradiation in combination with liver transplantation[65,86]. Outcome of patients treated according to this guideline, a 5-year survival rate of 53%, marginally improves the survival rate of patients after surgery for resectable type of disease[86,87].

CONCLUSION

It is most important to understand oncological suitability, donor liver organ availability, as well as ability to obtain appropriate long-term results in patients with CCA with or without PSC. In pCCA not associated with PSC, liver transplantation seems to provide promising survival. In resectable types of pCCA patients, neoadjuvant chemoradiotherapy and liver transplantation by strict selection criteria may improve the survival rate of patients compared to unresectable early stage pCCA patients. Owing to the shortage of available organs, it still remains unknown whether liver transplantation and neoadjuvant chemoradiotherapy should be increasingly considered for other classifications of CCA. Imbalance between organ supply and demand further conducts a need for stringent indications and contraindications in recognizing liver transplantation proper status. It is also essential for doctors to stay up to date with the general indications for liver transplantation and to consider when it is suitable or unsuitable to refer patients for transplant evaluation.

REFERENCES

1 **Rizvi S**, Gores GJ. Pathogenesis, diagnosis, and management of cholangiocarcinoma. *Gastroenterology* 2013; **145**: 1215-1229 [PMID: 24140396 DOI: 10.1053/j.gastro.2013.10.013]

2 **Saha SK**, Zhu AX, Fuchs CS, Brooks GA. Forty-Year Trends in Cholangiocarcinoma Incidence in the U.S.: Intrahepatic Disease on the Rise. *Oncologist* 2016; **21**: 594-599 [PMID: 27000463 DOI: 10.1634/theoncologist.2015-0446]

3 **Boberg KM**, Lind GE. Primary sclerosing cholangitis and malignancy. *Best Pract Res Clin Gastroenterol* 2011; **25**: 753-764 [PMID: 22117640 DOI: 10.1016/j.bpg.2011.10.002]

4 **Boonstra K**, Weersma RK, van Erpecum KJ, Rauws EA, Spanier BW, Poen AC, van Nieuwkerk KM, Drenth JP, Witteman BJ, Tuynman HA, Naber AH, Kingma PJ, van Buuren HR, van Hoek B, Vleggaar FP, van Geloven N, Beuers U, Ponsioen CY; EpiPSCPBC Study Group. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology* 2013; **58**: 2045-2055 [PMID: 23775876 DOI: 10.1002/hep.26565]

5 **Boberg KM**, Bergquist A, Mitchell S, Pares A, Rosina F, Broomé U, Chapman R, Fausa O, Egeland T, Rocca G, Schrumpf E. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. *Scand J Gastroenterol* 2002; **37**: 1205-1211 [PMID: 12408527 DOI: 10.1080/003655202760373434]

6 **Chapman MH**, Webster GJ, Bannoo S, Johnson GJ, Wittmann J, Pereira SP. Cholangiocarcinoma and dominant strictures in patients with primary sclerosing cholangitis: a 25-year single-centre experience. *Eur J Gastroenterol Hepatol* 2012; **24**: 1051-1058 [PMID: 22653260 DOI: 10.1097/MEG.0b013e3283554bbf]

7 **Welzel TM**, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 2006; **98**: 873-875 [PMID: 16788161 DOI: 10.1093/jnci/djj234]

8 **Rea DJ**, Heimbach JK, Rosen CB, Haddock MG, Alberts SR, Kremers WK, Gores GJ, Nagorney DM. Liver transplantation with neoadjuvant chemoradiation is more effective than resection for hilar cholangiocarcinoma. *Ann Surg* 2005; **242**: 451-8; discussion 458 [PMID: 16135931 DOI:

10.1097/01.sla.0000179678.13285.fa]
9 **Jarnagin WR**, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507-17; discussion 517 [PMID: 11573044 DOI: 10.1097/00000658-200110000-00010]
10 **Barr Fritcher EG**, Voss JS, Brankley SM, Campion MB, Jenkins SM, Keeney ME, Henry MR, Kerr SM, Chaiteerakij R, Pestova EV, Clayton AC, Zhang J, Roberts LR, Gores GJ, Halling KC, Kipp BR. An Optimized Set of Fluorescence In Situ Hybridization Probes for Detection of Pancreatobiliary Tract Cancer in Cytology Brush Samples. *Gastroenterology* 2015; **149**: 1813-1824.e1 [PMID: 26327129 DOI: 10.1053/j.gastro.2015.08.046]
11 **Goldaracena N**, Gorgen A, Sapisochin G. Current status of liver transplantation for cholangiocarcinoma. *Liver Transpl* 2018; **24**: 294-303 [PMID: 29024405 DOI: 10.1002/lt.24955]
12 **Nakanuma Y**, Kakuda Y. Pathologic classification of cholangiocarcinoma: New concepts. *Best Pract Res Clin Gastroenterol* 2015; **29**: 277-293 [PMID: 25966428 DOI: 10.1016/j.bpg.2015.02.006]
13 **Krasinskas AM**. Cholangiocarcinoma. *Surg Pathol Clin* 2018; **11**: 403-429 [PMID: 29751883 DOI: 10.1016/j.path.2018.02.005]
14 **Louis C**, Papoutsoglou P, Coulouarn C. Molecular classification of cholangiocarcinoma. *Curr Opin Gastroenterol* 2020; **36**: 57-62 [PMID: 31895230 DOI: 10.1097/MOG.0000000000000611]
15 **Nakanuma Y**, Sato Y, Harada K, Sasaki M, Xu J, Ikeda H. Pathological classification of intrahepatic cholangiocarcinoma based on a new concept. *World J Hepatol* 2010; **2**: 419-427 [PMID: 21191517 DOI: 10.4254/wjh.v2.i12.419]
16 **Banales JM**, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, Lind GE, Folseraas T, Forbes SJ, Fouassier L, Geier A, Calvisi DF, Mertens JC, Trauner M, Benedetti A, Maroni L, Vaquero J, Macias RI, Raggi C, Perugorria MJ, Gaudio E, Boberg KM, Marin JJ, Alvaro D. Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol* 2016; **13**: 261-280 [PMID: 27095655 DOI: 10.1038/nrgastro.2016.51]
17 **Kendall T**, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B, Carpino G. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int* 2019; **39** Suppl 1: 7-18 [PMID: 30882996 DOI: 10.1111/liv.14093]
18 **Cardinale V**, Wang Y, Carpino G, Reid LM, Gaudio E, Alvaro D. Mucin-producing cholangiocarcinoma might derive from biliary tree stem/progenitor cells located in peribiliary glands. *Hepatology* 2012; **55**: 2041-2042 [PMID: 22262236 DOI: 10.1002/hep.25587]
19 **Nakamura H**, Arai Y, Totoki Y, Shirota T, Elzawahry A, Kato M, Hama N, Hosoda F, Urushidate T, Ohashi S, Hiraoka N, Ojima H, Shimada K, Okusaka T, Kosuge T, Miyagawa S, Shibata T. Genomic spectra of biliary tract cancer. *Nat Genet* 2015; **47**: 1003-1010 [PMID: 26258846 DOI: 10.1038/ng.3375]
20 **Valle JW**, Lamarca A, Goyal L, Barriuso J, Zhu AX. New Horizons for Precision Medicine in Biliary Tract Cancers. *Cancer Discov* 2017; **7**: 943-962 [PMID: 28818953 DOI: 10.1158/2159-8290.CD-17-0245]
21 **Borger DR**, Tanabe KK, Fan KC, Lopez HU, Fantin VR, Straley KS, Schenkein DP, Hezel AF, Ancukiewicz M, Liebman HM, Kwak EL, Clark JW, Ryan DP, Deshpande V, Dias-Santagata D, Ellisen LW, Zhu AX, Iafrate AJ. Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* 2012; **17**: 72-79 [PMID: 22180306 DOI: 10.1634/theoncologist.2011-0386]
22 **Banales JM**, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, Cardinale V, Carpino G, Andersen JB, Braconi C, Calvisi DF, Perugorria MJ, Fabris L, Boulter L, Macias RIR, Gaudio E, Alvaro D, Gradilone SA, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Rizvi S, Heimbach J, Koerkamp BG, Bruix J, Forner A, Bridgewater J, Valle JW, Gores GJ. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 557-588 [PMID: 32606456 DOI: 10.1038/s41575-020-0310-z]
23 **Jusakul A**, Cutcutache I, Yong CH, Lim JQ, Huang MN, Padmanabhan N, Nellore V, Kongpetch S, Ng AWT, Ng LM, Choo SP, Myint SS, Thanan R, Nagarajan S, Lim WK, Ng CCY, Boot A, Liu M, Ong CK, Rajasegaran V, Lie S, Lim AST, Lim TH, Tan J, Loh JL, McPherson JR, Khuntikeo N, Bhudhisawasdi V, Yongvanit P, Wongkham S, Totoki Y, Nakamura H, Arai Y, Yamasaki S, Chow PK, Chung AYF, Ooi LLPJ, Lim KH, Dima S, Duda DG, Popescu I, Broet P, Hsieh SY, Yu MC, Scarpa A, Lai J, Luo DX, Carvalho AL, Vettore AL, Rhee H, Park YN, Alexandrov LB, Gordân R, Rozen SG, Shibata T, Pairojkul C, Teh BT, Tan P. Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of Cholangiocarcinoma. *Cancer Discov* 2017; **7**: 1116-1135 [PMID: 28667006 DOI: 10.1158/2159-8290.CD-17-0368]
24 **Sia D**, Hoshida Y, Villanueva A, Roayaie S, Ferrer J, Tabak B, Peix J, Sole M, Tovar V, Alsinet C, Cornella H, Klotzle B, Fan JB, Cotsoglou C, Thung SN, Fuster J, Waxman S, Garcia-Valdecasas JC, Bruix J, Schwartz ME, Beroukhim R, Mazzaferro V, Llovet JM. Integrative molecular analysis of intrahepatic cholangiocarcinoma reveals 2 classes that have different outcomes. *Gastroenterology* 2013; **144**: 829-840 [PMID: 23295441 DOI: 10.1053/j.gastro.2013.01.001]
25 **Fouassier L**, Marzioni M, Afonso MB, Dooley S, Gaston K, Giannelli G, Rodrigues CMP, Lozano E, Mancarella S, Segatto O, Vaquero J, Marin JJG, Coulouarn C. Signalling networks in

cholangiocarcinoma: Molecular pathogenesis, targeted therapies and drug resistance. *Liver Int* 2019; **39** Suppl 1: 43-62 [PMID: 30903728 DOI: 10.1111/liv.14102]

26 **Zabron A**, Edwards RJ, Khan SA. The challenge of cholangiocarcinoma: dissecting the molecular mechanisms of an insidious cancer. *Dis Model Mech* 2013; **6**: 281-292 [PMID: 23520144 DOI: 10.1242/dmm.010561]

27 **Rizvi S**, Borad MJ, Patel T, Gores GJ. Cholangiocarcinoma: molecular pathways and therapeutic opportunities. *Semin Liver Dis* 2014; **34**: 456-464 [PMID: 25369307 DOI: 10.1055/s-0034-1394144]

28 **Kiguchi K**. Molecular aspects of cholangiocarcinoma. *J Hepatobiliary Pancreat Sci* 2014; **21**: 371-379 [PMID: 24420749 DOI: 10.1002/jhbp.66]

29 **Farshidfar F**, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, Hinoue T, Hoadley KA, Gibb EA, Roszik J, Covington KR, Wu CC, Shinbrot E, Stransky N, Hegde A, Yang JD, Reznik E, Sadeghi S, Pedamallu CS, Ojesina AI, Hess JM, Auman JT, Rhie SK, Bowlby R, Borad MJ; Cancer Genome Atlas Network, Zhu AX, Stuart JM, Sander C, Akbani R, Cherniack AD, Deshpande V, Mounajjed T, Foo WC, Torbenson MS, Kleiner DE, Laird PW, Wheeler DA, McRee AJ, Bathe OF, Andersen JB, Bardeesy N, Roberts LR, Kwong LN. Integrative Genomic Analysis of Cholangiocarcinoma Identifies Distinct IDH-Mutant Molecular Profiles. *Cell Rep* 2017; **18**: 2780-2794 [PMID: 28297679 DOI: 10.1016/j.celrep.2017.02.033]

30 **Sia D**, Tovar V, Moeini A, Llovet JM. Intrahepatic cholangiocarcinoma: pathogenesis and rationale for molecular therapies. *Oncogene* 2013; **32**: 4861-4870 [PMID: 23318457 DOI: 10.1038/onc.2012.617]

31 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

32 **Isomoto H**. Epigenetic alterations associated with cholangiocarcinoma (review). *Oncol Rep* 2009; **22**: 227-232 [PMID: 19578760]

33 **Sandhu DS**, Shire AM, Roberts LR. Epigenetic DNA hypermethylation in cholangiocarcinoma: potential roles in pathogenesis, diagnosis and identification of treatment targets. *Liver Int* 2008; **28**: 12-27 [PMID: 18031477 DOI: 10.1111/j.1478-3231.2007.01624.x]

34 **Limpaiboon T**. Epigenetic aberrations in cholangiocarcinoma: potential biomarkers and promising target for novel therapeutic strategies. *Asian Pac J Cancer Prev* 2012; **13** Suppl: 41-45 [PMID: 23480763]

35 **O'Rourke CJ**, Munoz-Garrido P, Aguayo EL, Andersen JB. Epigenome dysregulation in cholangiocarcinoma. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1423-1434 [PMID: 28645654 DOI: 10.1016/j.bbadis.2017.06.014]

36 **Wangyang Z**, Daolin J, Yi X, Zhenglong L, Lining H, Yunfu C, Xingming J. NcRNAs and Cholangiocarcinoma. *J Cancer* 2018; **9**: 100-107 [PMID: 29290774 DOI: 10.7150/jca.21785]

37 **Schmitt AM**, Chang HY. Long Noncoding RNAs in Cancer Pathways. *Cancer Cell* 2016; **29**: 452-463 [PMID: 27070700 DOI: 10.1016/j.ccell.2016.03.010]

38 **Yang W**, Li Y, Song X, Xu J, Xie J. Genome-wide analysis of long noncoding RNA and mRNA co-expression profile in intrahepatic cholangiocarcinoma tissue by RNA sequencing. *Oncotarget* 2017; **8**: 26591-26599 [PMID: 28427159 DOI: 10.18632/oncotarget.15721]

39 **Takakura WR**, Tabibian JH, Bowlus CL. The evolution of natural history of primary sclerosing cholangitis. *Curr Opin Gastroenterol* 2017; **33**: 71-77 [PMID: 28030370 DOI: 10.1097/MOG.0000000000000333]

40 **Fevery J**, Verslype C, Lai G, Aerts R, Van Steenbergen W. Incidence, diagnosis, and therapy of cholangiocarcinoma in patients with primary sclerosing cholangitis. *Dig Dis Sci* 2007; **52**: 3123-3135 [PMID: 17431781 DOI: 10.1007/s10620-006-9681-4]

41 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174 DOI: 10.1016/s0168-8278(01)00288-4]

42 **Bakhshi Z**, Hilscher MB, Gores GJ, Harmsen WS, Viehman JK, LaRusso NF, Gossard AA, Lazaridis KN, Lindor KD, Eaton JE. An update on primary sclerosing cholangitis epidemiology, outcomes and quantification of alkaline phosphatase variability in a population-based cohort. *J Gastroenterol* 2020; **55**: 523-532 [PMID: 31932891 DOI: 10.1007/s00535-020-01663-1]

43 **Weismüller TJ**, Trivedi PJ, Bergquist A, Imam M, Lenzen H, Ponsioen CY, Holm K, Gotthardt D, Färkkilä MA, Marschall HU, Thorburn D, Weersma RK, Fevery J, Mueller T, Chazouillères O, Schulze K, Lazaridis KN, Almer S, Pereira SP, Levy C, Mason A, Naess S, Bowlus CL, Floreani A, Halilbasic E, Yimam KK, Milkiewicz P, Beuers U, Huynh DK, Pares A, Manser CN, Dalekos GN, Eksteen B, Invernizzi P, Berg CP, Kirchner GI, Sarrazin C, Zimmer V, Fabris L, Braun F, Marzioni M, Juran BD, Said K, Rupp C, Jokelainen K, Benito de Valle M, Saffioti F, Cheung A, Trauner M, Schramm C, Chapman RW, Karlsen TH, Schrumpf E, Strassburg CP, Manns MP, Lindor KD, Hirschfield GM, Hansen BE, Boberg KM; International PSC Study Group. Patient Age, Sex, and Inflammatory Bowel Disease Phenotype Associate With Course of Primary Sclerosing Cholangitis. *Gastroenterology* 2017; **152**: 1975-1984.e8 [PMID: 28274849 DOI: 10.1053/j.gastro.2017.02.038]

44 **Fosby B**, Melum E, Bjøro K, Bennet W, Rasmussen A, Andersen IM, Castedal M, Olausson M, Wibeck C, Gotlieb M, Gjertsen H, Toivonen L, Foss S, Makisalo H, Nordin A, Sanengen T, Bergquist A, Larsson ME, Soderdahl G, Nowak G, Boberg KM, Isoniemi H, Keiding S, Foss A, Line PD, Friman S, Schrumpf E, Ericzon BG, Höckerstedt K, Karlsen TH. Liver transplantation in

the Nordic countries - An intention to treat and post-transplant analysis from The Nordic Liver Transplant Registry 1982-2013. *Scand J Gastroenterol* 2015; **50**: 797-808 [PMID: 25959101 DOI: 10.3109/00365521.2015.1036359]

45 **Razumilava N**, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 13-21.e1; quiz e3 [PMID: 22982100 DOI: 10.1016/j.cgh.2012.09.009]

46 **Tyson GL**, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011; **54**: 173-184 [PMID: 21488076 DOI: 10.1002/hep.24351]

47 **Patel T**. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001; **33**: 1353-1357 [PMID: 11391522 DOI: 10.1053/jhep.2001.25087]

48 **Khan SA**, Emadossadaty S, Ladep NG, Thomas HC, Elliott P, Taylor-Robinson SD, Toledano MB. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; **56**: 848-854 [PMID: 22173164 DOI: 10.1016/j.jhep.2011.11.015]

49 **Khan SA**, Davidson BR, Goldin RD, Heaton N, Karani J, Pereira SP, Rosenberg WM, Tait P, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Wasan H; British Society of Gastroenterology. Guidelines for the diagnosis and treatment of cholangiocarcinoma: an update. *Gut* 2012; **61**: 1657-1669 [PMID: 22895392 DOI: 10.1136/gutjnl-2011-301748]

50 **Maithel SK**, Gamblin TC, Kamel I, Corona-Villalobos CP, Thomas M, Pawlik TM. Multidisciplinary approaches to intrahepatic cholangiocarcinoma. *Cancer* 2013; **119**: 3929-3942 [PMID: 23963845 DOI: 10.1002/cncr.28312]

51 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. Proceedings of the Seminars in liver disease; 2004. New York: Thieme Medical Publishers, 2004: 115-125

52 **Palmer WC**, Patel T. Are common factors involved in the pathogenesis of primary liver cancers? *J Hepatol* 2012; **57**: 69-76 [PMID: 22420979 DOI: 10.1016/j.jhep.2012.02.022]

53 **Poultsides GA**, Zhu AX, Choti MA, Pawlik TM. Intrahepatic cholangiocarcinoma. *Surg Clin North Am* 2010; **90**: 817-837 [PMID: 20637950 DOI: 10.1016/j.suc.2010.04.011]

54 **Dodson RM**, Weiss MJ, Cosgrove D, Herman JM, Kamel I, Anders R, Geschwind JF, Pawlik TM. Intrahepatic cholangiocarcinoma: management options and emerging therapies. *J Am Coll Surg* 2013; **217**: 736-750.e4 [PMID: 23890842 DOI: 10.1016/j.jamcollsurg.2013.05.021]

55 **Shaib YH**, Davila JA, McGlynn K, El-Serag HB. Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? *J Hepatol* 2004; **40**: 472-477 [PMID: 15123362 DOI: 10.1016/j.jhep.2003.11.030]

56 **Mouzas IA**, Dimoulios P, Vlachonikolis IG, Skordilis P, Zoras O, Kouroumalis E. Increasing incidence of cholangiocarcinoma in Crete 1992-2000. *Anticancer Res* 2002; **22**: 3637-3641 [PMID: 12552969]

57 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125 [PMID: 15192785 DOI: 10.1055/s-2004-828889]

58 **McLean L**, Patel T. Racial and ethnic variations in the epidemiology of intrahepatic cholangiocarcinoma in the United States. *Liver Int* 2006; **26**: 1047-1053 [PMID: 17032404 DOI: 10.1111/j.1478-3231.2006.01350.x]

59 **Shaib YH**, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005; **128**: 620-626 [PMID: 15765398 DOI: 10.1053/j.gastro.2004.12.048]

60 **Portolani N**, Baiocchi GL, Coniglio A, Piardi T, Grazioli L, Benetti A, Ferrari Bravo A, Giulini SM. Intrahepatic cholangiocarcinoma and combined hepatocellular-cholangiocarcinoma: a Western experience. *Ann Surg Oncol* 2008; **15**: 1880-1890 [PMID: 18443881 DOI: 10.1245/s10434-008-9933-y]

61 **Razumilava N**, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; **383**: 2168-2179 [PMID: 24581682 DOI: 10.1016/S0140-6736(13)61903-0]

62 **Shimonishi T**, Sasaki M, Nakanuma Y. Precancerous lesions of intrahepatic cholangiocarcinoma. *J Hepatobiliary Pancreat Surg* 2000; **7**: 542-550 [PMID: 11180885 DOI: 10.1007/s005340070002]

63 **Marchan EM**, Landry JC. Neoadjuvant chemoradiation followed by orthotopic liver transplantation in cholangiocarcinomas: the emory experience. *J Gastrointest Oncol* 2016; **7**: 248-254 [PMID: 27034793 DOI: 10.3978/j.issn.2078-6891.2015.117]

64 **Meyer CG**, Penn I, James L. Liver transplantation for cholangiocarcinoma: results in 207 patients. *Transplantation* 2000; **69**: 1633-1637 [PMID: 10836374 DOI: 10.1097/00007890-200004270-00019]

65 **Darwish Murad S**, Kim WR, Harnois DM, Douglas DD, Burton J, Kulik LM, Botha JF, Mezrich JD, Chapman WC, Schwartz JJ, Hong JC, Emond JC, Jeon H, Rosen CB, Gores GJ, Heimbach JK. Efficacy of neoadjuvant chemoradiation, followed by liver transplantation, for perihilar cholangiocarcinoma at 12 US centers. *Gastroenterology* 2012; **143**: 88-98.e3; quiz e14 [PMID: 22504095 DOI: 10.1053/j.gastro.2012.04.008]

66 **Robles R**, Figueras J, Turrión VS, Margarit C, Moya A, Varo E, Calleja J, Valdivieso A, Valdecasas JC, López P, Gómez M, de Vicente E, Loinaz C, Santoyo J, Fleitas M, Bernardos A, Lladó L, Ramírez P, Bueno FS, Jaurrieta E, Parrilla P. Spanish experience in liver transplantation for hilar and peripheral cholangiocarcinoma. *Ann Surg* 2004; **239**: 265-271 [PMID: 14745336 DOI: 10.1097/01.sla.0000108702.45715.81]

67 **Goldstein RM**, Stone M, Tillery GW, Senzer N, Levy M, Husberg BS, Gonwa T, Klintmalm G. Is liver transplantation indicated for cholangiocarcinoma? *Am J Surg* 1993; **166**: 768-71; discussion

771 [PMID: 8273866 DOI: 10.1016/s0002-9610(05)80696-8]

68 **Sapisochin G**, Fidelman N, Roberts JP, Yao FY. Mixed hepatocellular cholangiocarcinoma and intrahepatic cholangiocarcinoma in patients undergoing transplantation for hepatocellular carcinoma. *Liver Transpl* 2011; **17**: 934-942 [PMID: 21438129 DOI: 10.1002/lt.22307]

69 **Kuhlmann JB**, Blum HE. Locoregional therapy for cholangiocarcinoma. *Curr Opin Gastroenterol* 2013; **29**: 324-328 [PMID: 23337933 DOI: 10.1097/MOG.0b013e32835d9dea]

70 **Mazzaferro V**, Gorgen A, Roayaie S, Droz Dit Busset M, Sapisochin G. Liver resection and transplantation for intrahepatic cholangiocarcinoma. *J Hepatol* 2020; **72**: 364-377 [PMID: 31954498 DOI: 10.1016/j.jhep.2019.11.020]

71 **Becker NS**, Rodriguez JA, Barshes NR, O'Mahony CA, Goss JA, Aloia TA. Outcomes analysis for 280 patients with cholangiocarcinoma treated with liver transplantation over an 18-year period. *J Gastrointest Surg* 2008; **12**: 117-122 [PMID: 17963015 DOI: 10.1007/s11605-007-0335-4]

72 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

73 **Fosby B**, Karlsen TH, Melum E. Recurrence and rejection in liver transplantation for primary sclerosing cholangitis. *World J Gastroenterol* 2012; **18**: 1-15 [PMID: 22228965 DOI: 10.3748/wjg.v18.i1.1]

74 **Tan EK**, Taner T, Heimbach JK, Gores GJ, Rosen CB. Liver Transplantation for Peri-hilar Cholangiocarcinoma. *J Gastrointest Surg* 2020; **24**: 2679-2685 [PMID: 32671802 DOI: 10.1007/s11605-020-04721-4]

75 **Schaefer B**, Zoller H, Schneeberger S. Con: Liver transplantation for expanded criteria malignant diseases. *Liver Transpl* 2018; **24**: 104-111 [PMID: 29125687 DOI: 10.1002/lt.24975]

76 **Guo X**, Shen W. Latest evidence on immunotherapy for cholangiocarcinoma. *Oncol Lett* 2020; **20**: 381 [PMID: 33154779 DOI: 10.3892/ol.2020.12244]

77 **Heimbach JK**, Gores GJ, Haddock MG, Alberts SR, Pedersen R, Kremers W, Nyberg SL, Ishitani MB, Rosen CB. Predictors of disease recurrence following neoadjuvant chemoradiotherapy and liver transplantation for unresectable perihilar cholangiocarcinoma. *Transplantation* 2006; **82**: 1703-1707 [PMID: 17198263 DOI: 10.1097/01.tp.0000253551.43583.d1]

78 **Duignan S**, Maguire D, Ravichand CS, Geoghegan J, Hoti E, Fennelly D, Armstrong J, Rock K, Mohan H, Traynor O. Neoadjuvant chemoradiotherapy followed by liver transplantation for unresectable cholangiocarcinoma: a single-centre national experience. *HPB (Oxford)* 2014; **16**: 91-98 [PMID: 23600750 DOI: 10.1111/hpb.12082]

79 **Croome KP**, Rosen CB, Heimbach JK, Nagorney DM. Is Liver Transplantation Appropriate for Patients with Potentially Resectable De Novo Hilar Cholangiocarcinoma? *J Am Coll Surg* 2015; **221**: 130-139 [PMID: 25872685 DOI: 10.1016/j.jamcollsurg.2015.01.064]

80 **Chen XP**, Lau WY, Huang ZY, Zhang ZW, Chen YF, Zhang WG, Qiu FZ. Extent of liver resection for hilar cholangiocarcinoma. *Br J Surg* 2009; **96**: 1167-1175 [PMID: 19705374 DOI: 10.1002/bjs.6618]

81 **Mansour JC**, Aloia TA, Crane CH, Heimbach JK, Nagino M, Vauthey JN. Hilar cholangiocarcinoma: expert consensus statement. *HPB (Oxford)* 2015; **17**: 691-699 [PMID: 26172136 DOI: 10.1111/hpb.12450]

82 **Ethun CG**, Lopez-Aguiar AG, Anderson DJ, Adams AB, Fields RC, Doyle MB, Chapman WC, Krasnick BA, Weber SM, Mezrich JD, Salem A, Pawlik TM, Poultsides G, Tran TB, Idrees K, Isom CA, Martin RCG, Scoggins CR, Shen P, Mogal HD, Schmidt C, Beal E, Hatzaras I, Shenoy R, Cardona K, Maithel SK. Transplantation Versus Resection for Hilar Cholangiocarcinoma: An Argument for Shifting Treatment Paradigms for Resectable Disease. *Ann Surg* 2018; **267**: 797-805 [PMID: 29064885 DOI: 10.1097/SLA.0000000000002574]

83 **Rosen CB**. Transplantation Versus Resection for Hilar Cholangiocarcinoma: An Argument for Shifting Paradigms for Resectable Disease in Annals of Surgery 2018. *Ann Surg* 2018; **267**: 808-809 [PMID: 29465456 DOI: 10.1097/SLA.0000000000002716]

84 **Tan EK**, Rosen CB, Heimbach JK, Gores GJ, Zamora-Valdes D, Taner T. Living Donor Liver Transplantation for Perihilar Cholangiocarcinoma: Outcomes and Complications. *J Am Coll Surg* 2020; **231**: 98-110 [PMID: 32035181 DOI: 10.1016/j.jamcollsurg.2019.12.037]

85 **Edge SB**, Byrd DR, Carducci MA, Compton CC, Fritz A, Greene F. AJCC cancer staging manual. New York: Springer, 2010

86 **Heimbach JK**, Gores GJ, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB. Liver transplantation for unresectable perihilar cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 201-207 [PMID: 15192792 DOI: 10.1055/s-2004-828896]

87 **Vugts JJA**, Gaspersz MP, Roos E, Franken LC, Olthof PB, Coelen RJS, van Vugt JLA, Labeur TA, Brouwer L, Besselink MGH, IJzermans JNM, Darwish Murad S, van Gulik TM, de Jonge J, Polak WG, Busch ORC, Erdmann JL, Groot Koerkamp B, Buettner S. Eligibility for Liver Transplantation in Patients with Perihilar Cholangiocarcinoma. *Ann Surg Oncol* 2021; **28**: 1483-1492 [PMID: 32901308 DOI: 10.1245/s10434-020-09001-8]

88 **Wadsworth CA**, Dixon PH, Wong JH, Chapman MH, McKay SC, Sharif A, Spalding DR, Pereira SP, Thomas HC, Taylor-Robinson SD, Whittaker J, Williamson C, Khan SA. Genetic factors in the pathogenesis of cholangiocarcinoma. *Dig Dis* 2011; **29**: 93-97 [PMID: 21691113 DOI: 10.1159/000324688]

89 **Hoblinger A**, Grunhage F, Sauerbruch T, Lammert F. Association of the c.3972C>T variant of the multidrug resistance-associated protein 2 Gene (MRP2/ABCC2) with susceptibility to bile duct cancer. *Digestion* 2009; **80**: 36-39 [PMID: 19451719 DOI: 10.1159/000212990]

90 **Khabou B**, Trigui A, Boudawara TS, Keskes L, Kamoun H, Barbu V, Fakhfakh F. A homozygous ABCB4 mutation causing an LPAC syndrome evolves into cholangiocarcinoma. *Clin Chim Acta* 2019; **495**: 598-605 [PMID: 31181191 DOI: 10.1016/j.cca.2019.06.007]

91 **Kim CJ**, Cox C, Dupont E, Reintgen DS. Accurate staging of women with breast cancer. *J Surg Oncol* 2002; **79**: 2-4 [PMID: 11754370 DOI: 10.1002/jso.10024]

92 **Prawan A**, Kukongviriyapan V, Tassaneeyakul W, Pairojkul C, Bhudhisawasdi V. Association between genetic polymorphisms of CYP1A2, arylamine N-acetyltransferase 1 and 2 and susceptibility to cholangiocarcinoma. *Eur J Cancer Prev* 2005; **14**: 245-250 [PMID: 15901993 DOI: 10.1097/00008469-200506000-00008]

93 **Melum E**, Karlsen TH, Schrumpf E, Bergquist A, Thorsby E, Boberg KM, Lie BA. Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. *Hepatology* 2008; **47**: 90-96 [PMID: 18023027 DOI: 10.1002/hep.21964]

94 **Ko KH**, Kim NK, Yim DJ, Hong SP, Park PW, Rim KS, Kim S, Hwang SG. Polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR C677T) and thymidylate synthase enhancer region (TSER) as a risk factor of cholangiocarcinoma in a Korean population. *Anticancer Res* 2006; **26**: 4229-4233 [PMID: 17201138]

95 **Sakoda LC**, Gao YT, Chen BE, Chen J, Rosenberg PS, Rashid A, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Cohen-Webb H, Yeager M, Welch R, Chanock S, Fraumeni JF Jr, Hsing AW. Prostaglandin-endoperoxide synthase 2 (PTGS2) gene polymorphisms and risk of biliary tract cancer and gallstones: a population-based study in Shanghai, China. *Carcinogenesis* 2006; **27**: 1251-1256 [PMID: 16361272 DOI: 10.1093/carcin/bgi314]

96 **Huang WY**, Gao YT, Rashid A, Sakoda LC, Deng J, Shen MC, Wang BS, Han TQ, Zhang BH, Chen BE, Rosenberg PS, Chanock SJ, Hsing AW. Selected base excision repair gene polymorphisms and susceptibility to biliary tract cancer and biliary stones: a population-based case-control study in China. *Carcinogenesis* 2008; **29**: 100-105 [PMID: 17984110 DOI: 10.1093/carcin/bgm247]

97 **Marahatta SB**, Punyarit P, Bhudisawasdi V, Paupairoj A, Wongkham S, Petmitr S. Polymorphism of glutathione S-transferase omega gene and risk of cancer. *Cancer Lett* 2006; **236**: 276-281 [PMID: 15992993 DOI: 10.1016/j.canlet.2005.05.020]

98 **Gong Y**, Qi M, Chen J, Fang R, Mai C, Chen T, Tang H, Tang Y. XRCC1 Arg194Trp and Arg399Gln polymorphisms and risk of extrahepatic cholangiocarcinoma: a hospital-based case-control study in China. *Int J Clin Exp Med* 2015; **8**: 19339-19345 [PMID: 26770573]

99 **Kang YK**, Kim WH, Lee HW, Lee HK, Kim YI. Mutation of p53 and K-ras, and loss of heterozygosity of APC in intrahepatic cholangiocarcinoma. *Lab Invest* 1999; **79**: 477-483 [PMID: 10212000]

100 **Yoshino J**, Akiyama Y, Shimada S, Ogura T, Ogawa K, Ono H, Mitsunori Y, Ban D, Kudo A, Yamaoka S, Tanabe M, Tanaka S. Loss of ARID1A induces a stemness gene ALDH1A1 expression with histone acetylation in the malignant subtype of cholangiocarcinoma. *Carcinogenesis* 2020; **41**: 734-742 [PMID: 31665232 DOI: 10.1093/carcin/bgz179]

101 **Fingas CD**, Katsounas A, Kahraman A, Siffert W, Jochum C, Gerken G, Nückel H, Canbay A. Prognostic assessment of three single-nucleotide polymorphisms (GNB3 825C>T, BCL2-938C>A, MCL1-386C>G) in extrahepatic cholangiocarcinoma. *Cancer Invest* 2010; **28**: 472-478 [PMID: 19968497 DOI: 10.3109/07357900903095714]

102 **Paradiso AV**, Patruno M, Digennaro M, Tommasi S, Pilato B, Argentiero A, Brunetti O, Silvestris N. Somatic BRCA Mutation in a Cholangiocarcinoma Patient for HBOC Syndrome Detection. *Front Oncol* 2020; **10**: 1292 [PMID: 32903564 DOI: 10.3389/fonc.2020.01292]

103 **Ross JS**, Wang K, Gay L, Al-Rohil R, Rand JV, Jones DM, Lee HJ, Sheehan CE, Otto GA, Palmer G, Yelensky R, Lipson D, Morosini D, Hawryluk M, Catenacci DV, Miller VA, Churi C, Ali S, Stephens PJ. New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing. *Oncologist* 2014; **19**: 235-242 [PMID: 24563076 DOI: 10.1634/theoncologist.2013-0352]

104 **O'Dell MR**, Huang JL, Whitney-Miller CL, Deshpande V, Rothberg P, Grose V, Rossi RM, Zhu AX, Land H, Bardeesy N, Hezel AF. Kras(G12D) and p53 mutation cause primary intrahepatic cholangiocarcinoma. *Cancer Res* 2012; **72**: 1557-1567 [PMID: 22266220 DOI: 10.1158/0008-5472.CAN-11-3596]

105 **Nabeshima T**, Hamada S, Taguchi K, Tanaka Y, Matsumoto R, Yamamoto M, Masamune A. *Keap1* deletion accelerates mutant *K-ras*/*p53*-driven cholangiocarcinoma. *Am J Physiol Gastrointest Liver Physiol* 2020; **318**: G419-G427 [PMID: 31961719 DOI: 10.1152/ajpgi.00296.2019]

106 **Ma B**, Meng H, Tian Y, Wang Y, Song T, Zhang T, Wu Q, Cui Y, Li H, Zhang W, Li Q. Distinct clinical and prognostic implication of IDH1/2 mutation and other most frequent mutations in large duct and small duct subtypes of intrahepatic cholangiocarcinoma. *BMC Cancer* 2020; **20**: 318 [PMID: 32293336 DOI: 10.1186/s12885-020-06804-6]

107 **Tannapfel A**, Benicke M, Katalinic A, Uhlmann D, Köckerling F, Hauss J, Wittekind C. Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 2000; **47**: 721-727 [PMID: 11034592 DOI: 10.1136/gut.47.5.721]

108 **Zhou M**, Zhu Y, Hou R, Mou X, Tan J. Identification of candidate genes for the diagnosis and treatment of cholangiocarcinoma using a bioinformatics approach. *Oncol Lett* 2019; **18**: 5459-5467

[PMID: 31612054 DOI: 10.3892/ol.2019.10904]

109 **Luchini C**, Robertson SA, Hong SM, Felsenstein M, Anders RA, Pea A, Nottegar A, Veronese N, He J, Weiss MJ, Capelli P, Scarpa A, Argani P, Kapur P, Wood LD. PBRM1 loss is a late event during the development of cholangiocarcinoma. *Histopathology* 2017; **71**: 375-382 [PMID: 28394406 DOI: 10.1111/his.13234]

110 **Xu RF**, Sun JP, Zhang SR, Zhu GS, Li LB, Liao YL, Xie JM, Liao WJ. KRAS and PIK3CA but not BRAF genes are frequently mutated in Chinese cholangiocarcinoma patients. *Biomed Pharmacother* 2011; **65**: 22-26 [PMID: 21051183 DOI: 10.1016/j.biopha.2010.06.009]

111 **Zhu AX**, Borger DR, Kim Y, Cosgrove D, Ejaz A, Alexandrescu S, Groeschl RT, Deshpande V, Lindberg JM, Ferrone C, Sempoux C, Yau T, Poon R, Popescu I, Bauer TW, Gamblin TC, Gigot JF, Anders RA, Pawlik TM. Genomic profiling of intrahepatic cholangiocarcinoma: refining prognosis and identifying therapeutic targets. *Ann Surg Oncol* 2014; **21**: 3827-3834 [PMID: 24889489 DOI: 10.1245/s10434-014-3828-x]

112 **Liu CC**, Veeraraghavan J, Tan Y, Kim JA, Wang X, Loo SK, Lee S, Hu Y, Wang XS. A Novel Neoplastic Fusion Transcript, *RAD51AP1-DYRK4*, Confers Sensitivity to the MEK Inhibitor Trametinib in Aggressive Breast Cancers. *Clin Cancer Res* 2021; **27**: 785-798 [PMID: 33172895 DOI: 10.1158/1078-0432.CCR-20-2769]

113 **Chen YJ**, Tang QB, Zou SQ. Inactivation of RASSF1A, the tumor suppressor gene at 3p21.3 in extrahepatic cholangiocarcinoma. *World J Gastroenterol* 2005; **11**: 1333-1338 [PMID: 15761971 DOI: 10.3748/wjg.v11.i9.1333]

114 **Andersen JB**, Thorgeirsson SS. Genetic profiling of intrahepatic cholangiocarcinoma. *Curr Opin Gastroenterol* 2012; **28**: 266-272 [PMID: 22395571 DOI: 10.1097/MOG.0b013e3283523c7e]

115 **Ueki T**, Hsing AW, Gao YT, Wang BS, Shen MC, Cheng J, Deng J, Fraumeni JF Jr, Rashid A. Alterations of p16 and prognosis in biliary tract cancers from a population-based study in China. *Clin Cancer Res* 2004; **10**: 1717-1725 [PMID: 15014024 DOI: 10.1158/1078-0432.ccr-1137-3]

116 **Kim BH**, Cho NY, Choi M, Lee S, Jang JJ, Kang GH. Methylation profiles of multiple CpG island loci in extrahepatic cholangiocarcinoma versus those of intrahepatic cholangiocarcinomas. *Arch Pathol Lab Med* 2007; **131**: 923-930 [PMID: 17550320 DOI: 10.5858/2007-131-923-MPOMCI]

117 **Yang B**, House MG, Guo M, Herman JG, Clark DP. Promoter methylation profiles of tumor suppressor genes in intrahepatic and extrahepatic cholangiocarcinoma. *Mod Pathol* 2005; **18**: 412-420 [PMID: 15467712 DOI: 10.1038/modpathol.3800287]

118 **Liu XF**, Zhu SG, Zhang H, Xu Z, Su HL, Li SJ, Zhou XT. The methylation status of the TMS1/ASC gene in cholangiocarcinoma and its clinical significance. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 449-453 [PMID: 16911948]

119 **Foja S**, Goldberg M, Schagdarsurengin U, Dammann R, Tannapfel A, Ballhausen WG. Promoter methylation and loss of coding exons of the fragile histidine triad (FHIT) gene in intrahepatic cholangiocarcinomas. *Liver Int* 2005; **25**: 1202-1208 [PMID: 16343073 DOI: 10.1111/j.1478-3231.2005.01174.x]

120 **Wong N**, Li L, Tsang K, Lai PB, To KF, Johnson PJ. Frequent loss of chromosome 3p and hypermethylation of RASSF1A in cholangiocarcinoma. *J Hepatol* 2002; **37**: 633-639 [PMID: 12399230 DOI: 10.1016/s0168-8278(02)00269-6]

121 **Lee S**, Kim WH, Jung HY, Yang MH, Kang GH. Aberrant CpG island methylation of multiple genes in intrahepatic cholangiocarcinoma. *Am J Pathol* 2002; **161**: 1015-1022 [PMID: 12213730 DOI: 10.1016/S0002-9440(10)64262-9]

122 **Tozawa T**, Tamura G, Honda T, Nawata S, Kimura W, Makino N, Kawata S, Sugai T, Suto T, Motoyama T. Promoter hypermethylation of DAP-kinase is associated with poor survival in primary biliary tract carcinoma patients. *Cancer Sci* 2004; **95**: 736-740 [PMID: 15471559 DOI: 10.1111/j.1349-7006.2004.tb03254.x]

123 **Koga Y**, Kitajima Y, Miyoshi A, Sato K, Kitahara K, Soejima H, Miyazaki K. Tumor progression through epigenetic gene silencing of O(6)-methylguanine-DNA methyltransferase in human biliary tract cancers. *Ann Surg Oncol* 2005; **12**: 354-363 [PMID: 15915369 DOI: 10.1245/ASO.2005.07.020]

124 **Tischoff I**, Markwarth A, Witzigmann H, Uhlmann D, Hauss J, Mirmohammadsadegh A, Wittekind C, Hengge UR, Tannapfel A. Allele loss and epigenetic inactivation of 3p21.3 in malignant liver tumors. *Int J Cancer* 2005; **115**: 684-689 [PMID: 15704097 DOI: 10.1002/ijc.20944]

125 **Khaenam P**, Jearanaikoon P, Pairojkul C, Bhudhisawasdi V, Limpaiboon T. Genetic and epigenetic alterations of RIZ1 and the correlation to clinicopathological parameters in liver fluke-related cholangiocarcinoma. *Exp Ther Med* 2010; **1**: 385-390 [PMID: 22993552 DOI: 10.3892/etm_00000060]

126 **Sriraksa R**, Zeller C, El-Bahrawy MA, Dai W, Daduang J, Jearanaikoon P, Chau-In S, Brown R, Limpaiboon T. CpG-island methylation study of liver fluke-related cholangiocarcinoma. *Br J Cancer* 2011; **104**: 1313-1318 [PMID: 21448164 DOI: 10.1038/bjc.2011.102]

127 **Zhang J**, Han C, Wu T. MicroRNA-26a promotes cholangiocarcinoma growth by activating β-catenin. *Gastroenterology* 2012; **143**: 246-56.e8 [PMID: 22484120 DOI: 10.1053/j.gastro.2012.03.045]

128 **Ehrlich L**, Hall C, Venter J, Dostal D, Bernuzzi F, Invernizzi P, Meng F, Trzeciakowski JP, Zhou T, Standeford H, Alpini G, Lairmore TC, Glaser S. miR-24 Inhibition Increases Menin Expression and Decreases Cholangiocarcinoma Proliferation. *Am J Pathol* 2017; **187**: 570-580 [PMID: 28087162 DOI: 10.1016/j.ajpath.2016.10.021]

129 **Stutes M**, Tran S, DeMorrow S. Genetic and epigenetic changes associated with cholangiocarcinoma: from DNA methylation to microRNAs. *World J Gastroenterol* 2007; **13**: 6465-6469 [PMID: 18161915 DOI: 10.3748/wjg.v13.i48.6465]

130 **Braconi C**, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* 2010; **51**: 881-890 [PMID: 20146264 DOI: 10.1002/hep.23381]

131 **Zeng B**, Li Z, Chen R, Guo N, Zhou J, Zhou Q, Lin Q, Cheng D, Liao Q, Zheng L, Gong Y. Epigenetic regulation of miR-124 by hepatitis C virus core protein promotes migration and invasion of intrahepatic cholangiocarcinoma cells by targeting SMYD3. *FEBS Lett* 2012; **586**: 3271-3278 [PMID: 22819820 DOI: 10.1016/j.febslet.2012.06.049]

132 **Meng F**, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology* 2006; **130**: 2113-2129 [PMID: 16762633 DOI: 10.1053/j.gastro.2006.02.057]

133 **Goeppert B**, Ernst C, Baer C, Roessler S, Renner M, Mehrabi A, Hafezi M, Pathil A, Warth A, Stenzinger A, Weichert W, Bähr M, Will R, Schirmacher P, Plass C, Weichenhan D. Cadherin-6 is a putative tumor suppressor and target of epigenetically dysregulated miR-429 in cholangiocarcinoma. *Epigenetics* 2016; **11**: 780-790 [PMID: 27593557 DOI: 10.1080/15592294.2016.1227899]

134 **Karakatsanis A**, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. *Mol Carcinog* 2013; **52**: 297-303 [PMID: 22213236 DOI: 10.1002/mc.21864]

135 **Li B**, Han Q, Zhu Y, Yu Y, Wang J, Jiang X. Down-regulation of miR-214 contributes to intrahepatic cholangiocarcinoma metastasis by targeting Twist. *FEBS J* 2012; **279**: 2393-2398 [PMID: 22540680 DOI: 10.1111/j.1742-4658.2012.08618.x]

136 **Lu X**, Zhou C, Li R, Deng Y, Zhao L, Zhai W. Long Noncoding RNA AFAP1-AS1 Promoted Tumor Growth and Invasion in Cholangiocarcinoma. *Cell Physiol Biochem* 2017; **42**: 222-230 [PMID: 28535506 DOI: 10.1159/000477319]

137 **Xu Y**, Yao Y, Qin W, Zhong X, Jiang X, Cui Y. Long non-coding RNA CCAT2 promotes cholangiocarcinoma cells migration and invasion by induction of epithelial-to-mesenchymal transition. *Biomed Pharmacother* 2018; **99**: 121-127 [PMID: 29329034 DOI: 10.1016/j.biopha.2018.01.061]

138 **Wang WT**, Ye H, Wei PP, Han BW, He B, Chen ZH, Chen YQ. LncRNAs H19 and HULC, activated by oxidative stress, promote cell migration and invasion in cholangiocarcinoma through a ceRNA manner. *J Hematol Oncol* 2016; **9**: 117 [PMID: 27809873 DOI: 10.1186/s13045-016-0348-0]

139 **Guo L**, Zhou Y, Chen Y, Sun H, Wang Y, Qu Y. LncRNA ASAP1-IT1 positively modulates the development of cholangiocarcinoma *via* hedgehog signaling pathway. *Biomed Pharmacother* 2018; **103**: 167-173 [PMID: 29653361 DOI: 10.1016/j.biopha.2018.04.015]

140 **Li Y**, Cai Q, Li W, Feng F, Yang L. Long non-coding RNA EPIC1 promotes cholangiocarcinoma cell growth. *Biochem Biophys Res Commun* 2018; **504**: 654-659 [PMID: 30205958 DOI: 10.1016/j.bbrc.2018.08.174]

141 **Xu Y**, Wang Z, Jiang X, Cui Y. Overexpression of long noncoding RNA H19 indicates a poor prognosis for cholangiocarcinoma and promotes cell migration and invasion by affecting epithelial-mesenchymal transition. *Biomed Pharmacother* 2017; **92**: 17-23 [PMID: 28528181 DOI: 10.1016/j.biopha.2017.05.061]

142 **Jiang XM**, Li ZL, Li JL, Zheng WY, Li XH, Cui YF, Sun DJ. LncRNA CCAT1 as the unfavorable prognostic biomarker for cholangiocarcinoma. *Eur Rev Med Pharmacol Sci* 2017; **21**: 1242-1247 [PMID: 28387907]

143 **Zhang F**, Wan M, Xu Y, Li Z, Leng K, Kang P, Cui Y, Jiang X. Long noncoding RNA PCAT1 regulates extrahepatic cholangiocarcinoma progression *via* the Wnt/β-catenin-signaling pathway. *Biomed Pharmacother* 2017; **94**: 55-62 [PMID: 28753454 DOI: 10.1016/j.biopha.2017.07.025]

144 **Yu Y**, Zhang M, Wang N, Li Q, Yang J, Yan S, He X, Ji G, Miao L. Epigenetic silencing of tumor suppressor gene CDKN1A by oncogenic long non-coding RNA SNHG1 in cholangiocarcinoma. *Cell Death Dis* 2018; **9**: 746 [PMID: 29970899 DOI: 10.1038/s41419-018-0768-6]

145 **Tan X**, Huang Z, Li X. Long Non-Coding RNA MALAT1 Interacts With miR-204 to Modulate Human Hilar Cholangiocarcinoma Proliferation, Migration, and Invasion by Targeting CXCR4. *J Cell Biochem* 2017; **118**: 3643-3653 [PMID: 28059437 DOI: 10.1002/jcb.25862]

146 **Yang Q**, Yu Y, Sun Z, Pan Y. Long non-coding RNA PVT1 promotes cell proliferation and invasion through regulating miR-133a in ovarian cancer. *Biomed Pharmacother* 2018; **106**: 61-67 [PMID: 29957467 DOI: 10.1016/j.biopha.2018.06.112]

147 **Xu Y**, Yao Y, Leng K, Li Z, Qin W, Zhong X, Kang P, Wan M, Jiang X, Cui Y. Long non-coding RNA UCA1 indicates an unfavorable prognosis and promotes tumorigenesis *via* regulating AKT/GSK-3β signaling pathway in cholangiocarcinoma. *Oncotarget* 2017; **8**: 96203-96214 [PMID: 29221199 DOI: 10.18632/oncotarget.21884]

148 **Xu Y**, Yao Y, Jiang X, Zhong X, Wang Z, Li C, Kang P, Leng K, Ji D, Li Z, Huang L, Qin W, Cui Y. SP1-induced upregulation of lncRNA SPRY4-IT1 exerts oncogenic properties by scaffolding EZH2/LSD1/DNMT1 and sponging miR-101-3p in cholangiocarcinoma. *J Exp Clin Cancer Res*

2018; **37**: 81 [PMID: 29642935 DOI: 10.1186/s13046-018-0747-x]

149 **Carotenuto P**, Fassan M, Pandolfo R, Lampis A, Vicentini C, Cascione L, Paulus-Hock V, Boulter L, Guest R, Quagliata L, Hahne JC, Ridgway R, Jamieson T, Athineos D, Veronese A, Visone R, Murgia C, Ferrari G, Guzzardo V, Evans TRJ, MacLeod M, Feng GJ, Dale T, Negrini M, Forbes SJ, Terracciano L, Scarpa A, Patel T, Valeri N, Workman P, Sansom O, Braconi C. Wnt signalling modulates transcribed-ultraconserved regions in hepatobiliary cancers. *Gut* 2017; **66**: 1268-1277 [PMID: 27618837 DOI: 10.1136/gutjnl-2016-312278]

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EDITORIAL

Bacterial translocation in patients undergoing major gastrointestinal surgery and its role in postoperative sepsis

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Abstract

Bacteria of the human intestinal microflora have a dual role. They promote digestion and are part of a defense mechanism against pathogens. These bacteria could become potential pathogens under certain circumstances. The term "bacterial translocation" describes the passage of bacteria of the gastrointestinal tract through the intestinal mucosa barrier to mesenteric lymph nodes and other organs. In some cases, the passage of bacteria and endotoxins could result in blood stream infections and in multiple organ failure. Open elective abdominal surgery more frequently results in malfunction of the intestinal barrier and subsequent bacterial translocation and blood stream infections than laparoscopic surgery. Postoperative sepsis is a common finding in patients who have undergone non-elective abdominal surgeries, including trauma patients treated with laparotomy. Postoperative sepsis is an emerging issue, as it changes the treatment plan in surgical patients and prolongs hospital stay. The association between bacterial translocation and postoperative sepsis could provide novel treatment options.

Key Words: Bacterial translocation; Major gastrointestinal surgery; Postoperative sepsis; Intestinal permeability; Microbiota

Core Tip: Increased intestinal permeability can potentially induce intestinal flora dysbiosis. Bacterial translocation, attributed to intestinal barrier impairment, may lead to systematic infection in the postoperative period. The definitive correlation between translocation and postoperative sepsis is yet to be proven, but the latter is an emerging issue for patients undergoing major gastrointestinal surgeries.

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INTRODUCTION

The incidence of postoperative sepsis has increased in the past decades, with the proportion of severe sepsis cases rising to unprecedented levels. Cases of sepsis are noted both after elective and emergency surgeries, but in the cases of elective surgeries, mortality is not respectively affected[1]. Gastrointestinal perforation is the most common surgical condition requiring immediate surgical intervention. More specifically, colonic perforation may cause peritonitis through the spread of bacteria from the intestines, and, therefore, there is a high risk for further bacterial spread *via* blood flow[2].

Gut microbiota affects the host decisively in both states of health and illness. The human gut microbiota consists of numerous bacteria that coexist and play a beneficial role in normal functions of the intestine. In normal conditions, bacteria of the gut assist in the absorption of nutrients. In illness, there are vast changes that alter the balance of these bacteria, leading to proliferation of potentially dangerous bacteria, capable of causing infections[3]. Diseases like colorectal cancer, inflammatory bowel disease, and diseases of the liver could alter the relationship between bacteria of the gut and the host.

In addition to bacterial dissemination due to mechanical disruption of the continuity of the intestinal barrier, as in the case of perforation, another potential mechanism proposed is bacterial translocation. Bacterial translocation is the movement of bacteria or their products from the intestinal lumen through the mucosa layer to a normally sterile tissue[4]. The most common routes for bacterial passage from the intestine to the systemic circulation and eventually to distant organs are the lymphatic route and the vascular route[5].

Major abdominal surgeries are procedures that promote an imbalance in intestinal bacteria. Patients undergoing major abdominal surgery are considered at high risk of developing postoperative infections as a result of bacterial translocation. Those undergoing emergency surgery are at even higher risk[6]. An increase in morbidity and mortality has been shown in cases of ascertained translocation to locoregional mesenteric lymph nodes[7,8].

ROLE OF THE INTESTINAL BARRIER

The intestinal barrier interacts with the contents of the intestinal lumen at immunological and chemical levels, besides being a physical barrier. It is composed of a single layer of columnar epithelial cells, which have diverse functions, such as absorptive, secretory and immune functions. The majority of intestinal epithelial cells are absorptive enterocytes. Other types of intestinal epithelial cells are secretory goblet cells, Paneth cells, and enteroendocrine cells. All these cells are under constant renewal by intestinal epithelial stem cells located in the bases of mucosal crypts[9].

Commensal bacteria found in the intestinal lumen prevent the proliferation of potential pathogens through regulating intestinal pH and decreasing the nutrients required by those pathogens. On the surface of the lumen, a layer of water, the glycocalyx, and the mucus layer containing immunoglobulin A (IgA) create a first defensive line, preventing adhesion of pathogenic bacteria to the epithelium and diminishing interaction between pathogen and epithelial cells. In addition, antimicrobial agents secreted by epithelial cells attract monocytes and assist in the opsonization of macrophages. Immunoglobulins and cytokines are secreted by cells of the lamina propria, as those cells are part of the innate and acquired immune system and play a vast role in immunological regulation in the intestine[10]. Besides having a role as a physical barrier, the mucus layer of the intestine contains an abundance of secretory IgA and antimicrobial proteins. There is a substantial difference in the composition of the mucus layer between the small and large intestine. This layer in the small intestine is penetrable by bacteria, while the large intestine has both a penetrable

outer mucus layer and an impenetrable inner mucus layer. Intestinal epithelial cells create a defense barrier below the layers of mucous inside the lumen of the intestine [11]. A barrier formed by mucins between the intestinal lumen and intestinal epithelial cells can regulate expression of tolerogenic and inflammatory cytokines[12].

INTESTINAL PERMEABILITY

Intestinal epithelial cells are connected to each other with tight junctions[9,10]. Tight junctions are an assembly of multiple proteins located on the apical part of neighboring epithelial cells and affect paracellular permeability, as they selectively regulate permeability. Tight junctions are fundamental in maintaining intestinal barrier function. They act as adhesive and mechanical mediators, maintaining barrier function, but do not seal the paracellular space. There are two functional protein categories, namely integral transmembrane proteins that form a network between adjacent cell membranes and peripheral membranes. Four integral transmembrane proteins are occludin, claudin, junctional adhesion molecule, and tricellulin[13,14]. In certain conditions of intestinal inflammation, it is shown that these tight junctions dysfunction, increasing permeability. The repair process of the epithelial cells affects intestinal motility and is considered an important factor in intestinal barrier function[9, 10,15].

Intestinal permeability is the condition during which soluble molecules and fluids are exchanged between the intestinal lumen and tissues. In normal conditions, intestinal barrier homeostasis acts to prevent this exchange, but both permeability and barrier function are dynamic states[16]. Dysfunction of the mucosal barrier can be found in both stress-associated conditions and in a diverse group of conditions in otherwise healthy people. It has been shown that there is increased intestinal permeability in patients with gastroenterological diseases correlated with intestinal inflammation, especially in those with inflammatory bowel disease. In addition, healthy relatives of these patients are at high risk of developing increased intestinal permeability[17,18]. Use of non-steroidal anti-inflammatory drugs may alter the structural normality of the intestinal lumen, thereby impairing the barrier and potentially increasing permeability[19]. More importantly, studies have shown that in a number of patients undergoing abdominal surgery, bacterial DNA was detected as early as a few hours postoperatively, indicating a relation between surgery and translocation[6].

INTESTINAL MICROBIOTA IN GASTROINTESTINAL DISEASES

The human intestinal microbiota plays a main role in intestinal metabolism and in immunological response of the intestines[20]. Balance of the intestinal microbiota is a prerequisite for a healthy intestinal environment. Imbalance of microbiota and of the host immune system is present in intestinal diseases. Altered concentrations of commensal intestinal bacteria depends on disease activity, and this can easily be noted when patients are compared to healthy individuals[21].

Studies in patients with colorectal cancer have shown that alterations in microbiota are also associated with tumorigenesis. These alterations are characterized by the dominance of certain bacteria species. In the spotlight are *Fusobacterium nucleatum*, *Escherichia coli*, and *Bacteroides fragilis*[22]. Analyses of intestinal microbiota are performed using 16S ribosomal RNA techniques. When the aforementioned bacteria species are increased, other bacteria are depleted. Gram-positive bacteria are vastly affected, and *Clostridia* species are also decreased in these patients[23-26]. *Bacteroides fragilis* colonizes the intestine and has a prominent place in the microbiota. Although *Escherichia coli* is considered a commensal bacteria, some of its species are potential pathogens, promoting intestinal inflammation and producing oncogenic toxins. This phenomenon, when there is an imbalance in intestinal flora, is called dysbiosis. Dysbiosis may characterize inflammatory gastrointestinal diseases and colorectal cancer but may also be explained by the changes in dietary habits that have occurred over the past decades. The importance of metabolites and their products to intestinal inflammation have led to increased concern for the impact of metabolic diseases on microbiota[23,27].

Bowel obstruction has both local and systemic effects. Fecal retention promotes bacterial overgrowth. Besides changes in bowel motility, moderate inflammation is a probable finding. This inflammatory response may lead to systemic responses, with

sepsis and septic shock being the most serious. The causative factor for these systemic responses is bacterial translocation[28].

BACTERIAL TRANSLOCATION, ABDOMINAL SURGERY, AND POSTOPERATIVE SEPSIS

Bacterial translocation, attributed to increased intestinal permeability, can be present as early as 2 h after abdominal surgery. The grade and the prognosis depend on the severity of surgical trauma and the presence of intestinal ischemia. Studies have proposed that the potential mechanism involves visceral vasoconstriction due to surgical trauma, anesthetic agents, intestinal ischemia, and blood loss. In addition, production of vasoactive agents and release of inflammatory cytokines promote visceral vasoconstriction and therefore intestinal ischemia. Postoperative bacterial translocation is associated with systematic infection and systematic inflammatory response[6] (Figure 1).

In order to assess and confirm bacterial translocation, cultures from mesenteric lymph nodes are taken. Furthermore, blood cultures are collected from patients in the postoperative period. These samples are assessed using real-time polymerase chain reaction techniques to identify bacteria. In the case of bacterial translocation, positive cultures of samples from mesenteric lymph nodes have been reported to have slightly higher specificity[29-31]. The most common isolated bacterium associated with translocation is *Escherichia coli*[32]. A feasible method proposed to assess and monitor the progress of bacterial translocation is the evaluation of levels of D-lactate. D-lactate is a product of bacteria normally found in the intestinal lumen and is not metabolized by the human body. Levels of plasma D-lactate are used as a postoperative indicator of dissemination of these bacteria from the intestinal tract to the mesenteric lymph nodes, liver, spleen, and bloodstream[33].

Infections in the postoperative period are found to be more common in patients with identified bacterial translocation. A positive result in cultures taken from mesenteric lymph nodes is a more accurate prognostic factor than cultures from surgical site, intra-abdominal fluid collection, or peripheral blood samples. In other words, mesenteric lymph nodes act as beacons for progression of the infection[34]. This fact raises concerns regarding prophylactic use of antibiotics in patients undergoing abdominal surgery. Elective surgeries are performed under better circumstances and with better precautions taken. Emergency surgeries and surgeries for trauma are considered high-risk for the development of bacterial translocation, thus requiring use of antibiotics in the perioperative period[35]. Patients with advanced colorectal and gastric cancer, potentially associated with cachexia, are also in need of prophylactic use of antibiotics due to immunological imbalance induced by the progressed disease[36,37]. Prophylaxis against bacterial translocation seems to be associated with better survival rates in cancer patients who undergo surgery[7]. However, while gastric and colon resections are correlated with augmented rates of translocation, the use of antibiotics does not seem to prevent the occurrence of translocation[38,39].

The definite significance of bacterial translocation is yet to be determined, although there is evidence suggesting a causative role for sepsis. In some cases of sepsis, the causative factor was determined to be bacteria found in the intestine. In critically ill and frail patients undergoing major abdominal surgeries, those bacteria cause sepsis and even septic morbidity[40]. Sepsis is a diverse syndrome of varying severity. Late diagnosis and treatment could lead to more severe illness, even septic shock. In some cases, it may cause multi-organ failure. Severe sepsis is characterized by the presence of hypoperfusion or hypotension and by the failure of at least one organ[41]. However, this is hard to verify in most cases, as in cases of multi-organ failure occurring early postoperatively, and it is probably due to the inflammatory response causing endothelial cell activation. In contrast, late-onset multi-organ failure may be attributed to bacterial translocation, as it creates an imbalance between proinflammatory and anti-inflammatory cytokines[42]. When the septic condition in surgical patients is so severe that it causes a state of immunosuppression, multi-organ failure is responsible, with high mortality rates (reaching 50%-80%). This fact supports the theory of gut-induced sepsis[43].

Postoperative sepsis was found to be most common among men and among older and low-income populations. Besides these characteristics, other factors regarding hospitalization are also crucial. Larger hospital bed size, urban hospital location, and non-teaching status were associated with higher postoperative sepsis rates.

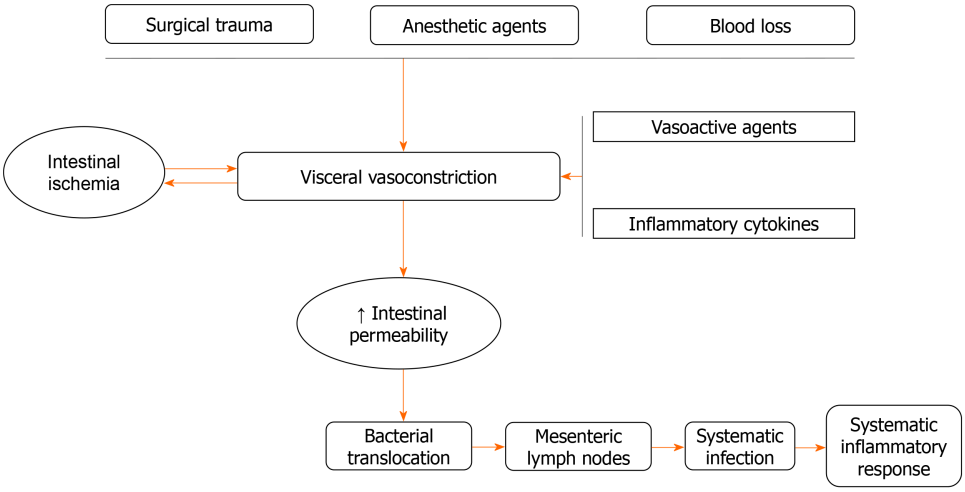

Figure 1 Mechanism of bacterial translocation after major gastrointestinal surgery.

Comorbidities like diabetes, chronic renal disease, cardiovascular disease, and hypertension increase the risk for postoperative sepsis. Patients' preoperative status and lifestyle choices contribute to modifying the relative risk. In addition, the type of surgery also has an impact, as gastric, biliary and colorectal surgeries were associated with relatively higher rates of postoperative sepsis, when at the same time esophageal surgery had the lowest risk of postoperative sepsis[44-46] (Figure 2). Although the incidence is rising, especially in elderly patients, mortality rates are decreasing[47].

As sepsis progresses, the release of proinflammatory cytokines triggers the production of toxic mediators that damage the endothelium, thus leading to increased capillary leakage. In addition, the release of agents that act as vasodilators, resulting in hypotension, indicates that evolution of sepsis to septic shock and subsequently to multi-organ failure requires vigilance. Early detection and therapeutic intervention could improve outcome and prognosis. Diagnosis is based on both clinical assessment and taking into consideration other factors, such as impaired consciousness and severe underlying diseases. Hypotension, oliguria, and acute altered mental status are indicative signs of severe sepsis[48]. As this condition continues to cause concern, efforts are being made to create a predictive score that will help physicians to assess probability of postoperative sepsis and mortality and to intervene sooner[49].

The cornerstone of treatment is fluid resuscitation to address hypovolemia, hypotension, and hypoperfusion. Hemodynamic stability could be restored using vasopressors when fluids alone are not adequate to maintain blood pressure. Furthermore, broad-spectrum intravenous antibiotics should be administered within the first hour. The choice of antibiotics should be guided by the suspected causative factors. Response of patients to treatment must be monitored closely, because in cases where there is no improvement, surgical intervention may be needed[50]. Novel treatments have been proposed for postoperative sepsis due to bacterial translocation, such as the use of probiotics and prebiotics. These are considered living microorganisms, which can be beneficial. *Lactobacillus* and *Bifidobacterium* are the most commonly used. They act through competition with pathogens for binding sites and nutrients. Probiotics also induce immunological response and reduce inflammation. Prebiotics are non-digestible food ingredients that promote the growth and the increase in activity of certain intestinal bacteria. These treatments have been studied well in patients with sepsis in intensive care units, with results being promising, as prophylactic use of probiotics has been shown to reduce infections, sepsis, and mortality. Another potential treatment is fecal microbiota transplantation. This is a technique that attempts to restore commensal bacteria in the intestinal epithelium. It also acts as an immunomodulatory tool, as it assists intestinal crypts to express immunological pathways. This being said, this technique prevents severe inflammation and dysregulation of intestinal lumen homeostasis[51].

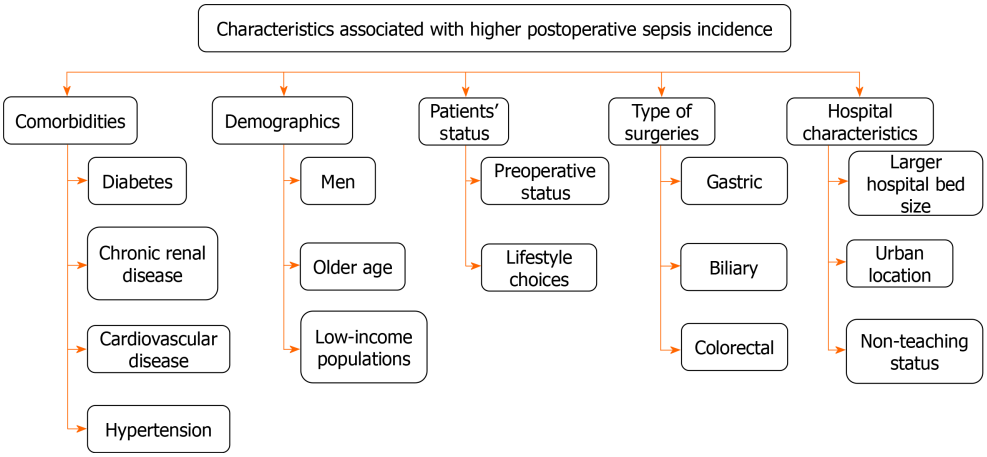

Figure 2 Characteristics associated with higher postoperative sepsis incidence.

LAPAROTOMY *VS* LAPAROSCOPIC SURGERY AND THEIR IMPACT IN BACTERIAL TRANSLOCATION

The effect of increased intra-abdominal pressure on bacterial translocation has been under investigation. Abdominal surgeries are associated with increased intra-abdominal pressure. Studies have shown that bacterial translocation usually occurs at pressure levels above 14 mmHg[52]. Patients undergoing laparoscopic surgeries should be monitored, as pneumoperitoneum significantly increases intra-abdominal pressure. Randomized control trials regarding patients with colorectal cancer have concluded that there is an increase in intra-abdominal pressure, systemic endotoxemia, and bacterial translocation during both open and laparoscopic resection but without a statistically significant difference between the two groups[53]. The effect of pneumoperitoneum in translocation was also studied in animal models. It was found to provoke alterations in the inflammatory response, with milder inflammation and quicker restoration. However, there was no evidence supporting the premise that laparoscopic surgery is related to higher incidence of bacterial translocation[54-56].

CONCLUSION

Postoperative sepsis is an emerging issue that can be present as soon as a few hours postoperatively and requires immediate treatment. It may cause severe disease and result in high mortality rates, especially in frail and elderly surgical patients. Bacterial translocation is proposed as a causative factor of postoperative sepsis. This fact suggests that intestinal microbiota combined with altered homeostasis in the intestinal barrier could create a chain of events leading to sepsis, as commensal bacteria translocate to usually sterile tissues. Bacterial translocation has been noted both in laparotomy and in laparoscopic surgeries, with no significant differences regarding incidence. Proper management and early intervention are needed, based on the fundamentals of sepsis treatment. Over the past few years, data regarding novel treatments using probiotics, which assist classic treatments, have been developed. More randomized studies will be needed to clarify the role of these treatments in postoperative sepsis in the years to come.

REFERENCES

1 **Fried E**, Weissman C, Sprung C. Postoperative sepsis. *Curr Opin Crit Care* 2011; **17**: 396-401 [PMID: 21677580 DOI: 10.1097/MCC.0b013e328348bee2]

2 **Xu X**, Dong HC, Yao Z, Zhao YZ. Risk factors for postoperative sepsis in patients with gastrointestinal perforation. *World J Clin Cases* 2020; **8**: 670-678 [PMID: 32149051 DOI: 10.12998/wjcc.v8.i4.670]

3 **Meng M**, Klingensmith NJ, Coopersmith CM. New insights into the gut as the driver of critical illness and organ failure. *Curr Opin Crit Care* 2017; **23**: 143-148 [PMID: 28092310 DOI: 10.1097/MCC.0000000000000386]

4 **Komatsu S**, Yokoyama Y, Nagino M. Gut microbiota and bacterial translocation in digestive surgery: the impact of probiotics. *Langenbecks Arch Surg* 2017; **402**: 401-416 [PMID: 28321508 DOI: 10.1007/s00423-017-1577-6]

5 **Deitch EA**. Gut-origin sepsis: evolution of a concept. *Surgeon* 2012; **10**: 350-356 [PMID: 22534256 DOI: 10.1016/j.surge.2012.03.003]

6 **Qiao Z**, Li Z, Li J, Lu L, Lv Y. Bacterial translocation and change in intestinal permeability in patients after abdominal surgery. *J Huazhong Univ Sci Technolog Med Sci* 2009; **29**: 486-491 [PMID: 19662368 DOI: 10.1007/s11596-009-0419-3]

7 **Chin KF**, Kallam R, O'Boyle C, MacFie J. Bacterial translocation may influence the long-term survival in colorectal cancer patients. *Dis Colon Rectum* 2007; **50**: 323-330 [PMID: 17237910 DOI: 10.1007/s10350-006-0827-4]

8 **Sedman PC**, Macfie J, Sagar P, Mitchell CJ, May J, Mancey-Jones B, Johnstone D. The prevalence of gut translocation in humans. *Gastroenterology* 1994; **107**: 643-649 [PMID: 8076751 DOI: 10.1016/0016-5085(94)90110-4]

9 **Peterson LW**, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol* 2014; **14**: 141-153 [PMID: 24566914 DOI: 10.1038/nri3608]

10 **Keita AV**, Söderholm JD. The intestinal barrier and its regulation by neuroimmune factors. *Neurogastroenterol Motil* 2010; **22**: 718-733 [PMID: 20377785 DOI: 10.1111/j.1365-2982.2010.01498.x]

11 **Coates M**, Lee MJ, Norton D, MacLeod AS. The Skin and Intestinal Microbiota and Their Specific Innate Immune Systems. *Front Immunol* 2019; **10**: 2950 [PMID: 31921196 DOI: 10.3389/fimmu.2019.02950]

12 **Shan M**, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K, He B, Cassis L, Bigas A, Cols M, Comerma L, Huang B, Blander JM, Xiong H, Mayer L, Berin C, Augenlicht LH, Velcich A, Cerutti A. Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals. *Science* 2013; **342**: 447-453 [PMID: 24072822 DOI: 10.1126/science.1237910]

13 **Buckley A**, Turner JR. Cell Biology of Tight Junction Barrier Regulation and Mucosal Disease. *Cold Spring Harb Perspect Biol* 2018; **10** [PMID: 28507021 DOI: 10.1101/cshperspect.a029314]

14 **Lee B**, Moon KM, Kim CY. Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. *J Immunol Res* 2018; **2018**: 2645465 [PMID: 30648119 DOI: 10.1155/2018/2645465]

15 **Camilleri M**, Madsen K, Spiller R, Greenwood-Van Meerveld B, Verne GN. Intestinal barrier function in health and gastrointestinal disease. *Neurogastroenterol Motil* 2012; **24**: 503-512 [PMID: 22583600 DOI: 10.1111/j.1365-2982.2012.01921.x]

16 **Odenwald MA**, Turner JR. Intestinal permeability defects: is it time to treat? *Clin Gastroenterol Hepatol* 2013; **11**: 1075-1083 [PMID: 23851019 DOI: 10.1016/j.cgh.2013.07.001]

17 **Katzka DA**, Geno DM, Blair HE, Lamsam JL, Alexander JA, Camilleri M. Small intestinal permeability in patients with eosinophilic oesophagitis during active phase and remission. *Gut* 2015; **64**: 538-543 [PMID: 24957264 DOI: 10.1136/gutjnl-2013-305882]

18 **Hollander D**, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives. A possible etiologic factor. *Ann Intern Med* 1986; **105**: 883-885 [PMID: 3777713 DOI: 10.7326/0003-4819-105-6-883]

19 **Camilleri M**. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut* 2019; **68**: 1516-1526 [PMID: 31076401 DOI: 10.1136/gutjnl-2019-318427]

20 **Zhu B**, Wang X, Li L. Human gut microbiome: the second genome of human body. *Protein Cell* 2010; **1**: 718-725 [PMID: 21203913 DOI: 10.1007/s13238-010-0093-z]

21 **Kataoka K**. The intestinal microbiota and its role in human health and disease. *J Med Invest* 2016; **63**: 27-37 [PMID: 27040049 DOI: 10.2152/jmi.63.27]

22 **Tilg H**, Adolph TE, Gerner RR, Moschen AR. The Intestinal Microbiota in Colorectal Cancer. *Cancer Cell* 2018; **33**: 954-964 [PMID: 29657127 DOI: 10.1016/j.ccell.2018.03.004]

23 **Sittipo P**, Lobionda S, Lee YK, Maynard CL. Intestinal microbiota and the immune system in metabolic diseases. *J Microbiol* 2018; **56**: 154-162 [PMID: 29492872 DOI: 10.1007/s12275-018-7548-y]

24 **Lucas C**, Barnich N, Nguyen HTT. Microbiota, Inflammation and Colorectal Cancer. *Int J Mol Sci* 2017; **18** [PMID: 28632155 DOI: 10.3390/ijms18061310]

25 **Mizutani S**, Yamada T, Yachida S. Significance of the gut microbiome in multistep colorectal carcinogenesis. *Cancer Sci* 2020; **111**: 766-773 [PMID: 31910311 DOI: 10.1111/cas.14298]

26 **Kim M**, Vogtmann E, Ahlquist DA, Devens ME, Kisiel JB, Taylor WR, White BA, Hale VL, Sung J, Chia N, Sinha R, Chen J. Fecal Metabolomic Signatures in Colorectal Adenoma Patients Are Associated with Gut Microbiota and Early Events of Colorectal Cancer Pathogenesis. *mBio* 2020; **11** [PMID: 32071266 DOI: 10.1128/mBio.03186-19]

27 **Ramakrishna BS**. Role of the gut microbiota in human nutrition and metabolism. *J Gastroenterol Hepatol* 2013; **28** Suppl 4: 9-17 [PMID: 24251697 DOI: 10.1111/jgh.12294]

28 **Hegde S**, Lin YM, Golovko G, Khanipov K, Cong Y, Savidge T, Fofanov Y, Shi XZ. Microbiota dysbiosis and its pathophysiological significance in bowel obstruction. *Sci Rep* 2018; **8**: 13044 [PMID: 30177854 DOI: 10.1038/s41598-018-31033-0]

29 **Akashi K**, Yokoyama Y, Mizuno T, Abe T, Fukaya M, Asahara T, Nagino M. Association Between Preoperative Muscle Mass and Intraoperative Bacterial Translocation in Patients Undergoing Hepatectomy, Pancreatoduodenectomy, and Esophagectomy. *Ann Surg Oncol* 2019; **26**: 4805-4813

[PMID: 31407185 DOI: 10.1245/s10434-019-07707-y]
30 **Yokoyama Y**, Fukaya M, Mizuno T, Ebata T, Asahara T, Nagino M. Clinical importance of "occult-bacterial translocation" in patients undergoing highly invasive gastrointestinal surgery: A review. *Surg Today* 2021; **51**: 485-492 [PMID: 32857253 DOI: 10.1007/s00595-020-02126-z]
31 **Schietroma M**, Pessia B, Colozzi S, Carlei F, Amicucci G. Does bacterial translocation influence the postoperative infections in splenectomized patients after abdominal trauma? *Surgeon* 2018; **16**: 94-100 [PMID: 28162908 DOI: 10.1016/j.surge.2016.09.009]
32 **O'Boyle CJ**, MacFie J, Mitchell CJ, Johnstone D, Sagar PM, Sedman PC. Microbiology of bacterial translocation in humans. *Gut* 1998; **42**: 29-35 [PMID: 9505882 DOI: 10.1136/gut.42.1.29]
33 **Murray MJ**, Gonze MD, Nowak LR, Cobb CF. Serum D(-)-lactate levels as an aid to diagnosing acute intestinal ischemia. *Am J Surg* 1994; **167**: 575-578 [PMID: 8209931 DOI: 10.1016/0002-9610(94)90101-5]
34 **Nieves E**, Tobón LF, Ríos DI, Isaza A, Ramírez M, Beltrán JA, Garzón-Ospina D, Patarroyo MA, Gómez A. Bacterial translocation in abdominal trauma and postoperative infections. *J Trauma* 2011; **71**: 1258-1261 [PMID: 21502873 DOI: 10.1097/TA.0b013e318215140e]
35 **Saadia R**. Trauma and bacterial translocation. *Br J Surg* 1995; **82**: 1243-1244 [PMID: 7552007 DOI: 10.1002/bjs.1800820928]
36 **Mi L**, Lin J, Zheng H, Xu X, Zhang J, Zhang D. Bacterial translocation contributes to cachexia from locally advanced gastric cancer. *Hepatogastroenterology* 2012; **59**: 2348-2351 [PMID: 22709824 DOI: 10.5754/hge11810]
37 **Jiang Y**, Lin J, Zhang D, Yu Z, Li Q, Jiang J, Li J. Bacterial translocation contributes to cachexia and its possible pathway in patients with colon cancer. *J Clin Gastroenterol* 2014; **48**: 131-137 [PMID: 24162171 DOI: 10.1097/01.mcg.0000436437.83015.17]
38 **Koratzanis G**, Giamarellos-Bourboulis EJ, Papalambros E, Giamarellou H. Bacterial translocation following intrabdominal surgery. Any influence of antimicrobial prophylaxis? *Int J Antimicrob Agents* 2002; **20**: 457-460 [PMID: 12458141 DOI: 10.1016/s0924-8579(02)00237-6]
39 **Diepenhorst GM**, van Ruler O, Besselink MG, van Santvoort HC, Wijnandts PR, Renooij W, Gouma DJ, Gooszen HG, Boermeester MA. Influence of prophylactic probiotics and selective decontamination on bacterial translocation in patients undergoing pancreatic surgery: a randomized controlled trial. *Shock* 2011; **35**: 9-16 [PMID: 20577144 DOI: 10.1097/SHK.0b013e3181ed8f17]
40 **MacFie J**, O'Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P. Gut origin of sepsis: a prospective study investigating associations between bacterial translocation, gastric microflora, and septic morbidity. *Gut* 1999; **45**: 223-228 [PMID: 10403734 DOI: 10.1136/gut.45.2.223]
41 **Levy MM**, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G; SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; **31**: 1250-1256 [PMID: 12682500 DOI: 10.1097/01.CCM.0000050454.01978.3B]
42 **Lemaire LC**, van Lanschot JJ, Stoutenbeek CP, van Deventer SJ, Wells CL, Gouma DJ. Bacterial translocation in multiple organ failure: cause or epiphenomenon still unproven. *Br J Surg* 1997; **84**: 1340-1350 [PMID: 9361586]
43 **Vaishnavi C**. Translocation of gut flora and its role in sepsis. *Indian J Med Microbiol* 2013; **31**: 334-342 [PMID: 24064638 DOI: 10.4103/0255-0857.118870]
44 **Chen PY**, Luo CW, Chen MH, Yang ML, Kuan YH. Epidemiological Characteristics of Postoperative Sepsis. *Open Med (Wars)* 2019; **14**: 928-938 [PMID: 31989043 DOI: 10.1515/med-2019-0110]
45 **Vogel TR**, Dombrovskiy VY, Carson JL, Graham AM, Lowry SF. Postoperative sepsis in the United States. *Ann Surg* 2010; **252**: 1065-1071 [PMID: 20571363 DOI: 10.1097/SLA.0b013e3181dcf36e]
46 **Blair LJ**, Huntington CR, Cox TC, Prasad T, Lincourt AE, Gersin KS, Heniford BT, Augenstein VA. Risk factors for postoperative sepsis in laparoscopic gastric bypass. *Surg Endosc* 2016; **30**: 1287-1293 [PMID: 26130133 DOI: 10.1007/s00464-015-4349-9]
47 **Bouza C**, López-Cuadrado T, Amate-Blanco JM. Characteristics, incidence and temporal trends of sepsis in elderly patients undergoing surgery. *Br J Surg* 2016; **103**: e73-e82 [PMID: 26670423 DOI: 10.1002/bjs.10065]
48 **Sartelli M**, Catena F, Di Saverio S, Ansaloni L, Malangoni M, Moore EE, Moore FA, Ivatury R, Coimbra R, Leppaniemi A, Biffl W, Kluger Y, Fraga GP, Ordonez CA, Marwah S, Gerych I, Lee JG, Tranà C, Coccolini F, Corradetti F, Kirkby-Bott J. Current concept of abdominal sepsis: WSES position paper. *World J Emerg Surg* 2014; **9**: 22 [PMID: 24674057 DOI: 10.1186/1749-7922-9-22]
49 **Gabriel RA**, Trivedi S, Schmidt UH. A Point-Based Risk Calculator Predicting Mortality in Patients That Developed Postoperative Sepsis. *J Intensive Care Med* 2020; 885066620960991 [PMID: 33043770 DOI: 10.1177/0885066620960991]
50 **Dellinger RP**, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R; Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; **39**: 165-228 [PMID: 23361625 DOI: 10.1007/s00134-012-2769-8]
51 **Bassetti M**, Bandera A, Gori A. Therapeutic Potential of the Gut Microbiota in the Management of Sepsis. *Crit Care* 2020; **24**: 105 [PMID: 32204720 DOI: 10.1186/s13054-020-2780-3]
52 **Polat C**, Aktepe OC, Akbulut G, Yilmaz S, Arikan Y, Dilek ON, Gokce O. The effects of increased

intra-abdominal pressure on bacterial translocation. *Yonsei Med J* 2003; **44**: 259-264 [PMID: 12728466 DOI: 10.3349/ymj.2003.44.2.259]

53 **Schietroma M**, Pessia B, Carlei F, Cecilia EM, Amicucci G. Intestinal permeability, systemic endotoxemia, and bacterial translocation after open or laparoscopic resection for colon cancer: a prospective randomized study. *Int J Colorectal Dis* 2013; **28**: 1651-1660 [PMID: 23917392 DOI: 10.1007/s00384-013-1751-4]

54 **Horattas MC**, Haller N, Ricchiuti D. Increased transperitoneal bacterial translocation in laparoscopic surgery. *Surg Endosc* 2003; **17**: 1464-1467 [PMID: 12802657 DOI: 10.1007/s00464-001-8289-1]

55 **Wang G**, Wu R, Guo F, Liu W, Chen X, Yu Q. Effects of carbon dioxide pneumoperitoneum on the inflammatory response and bacterial translocation in intraabdominal infection. *J Laparoendosc Adv Surg Tech A* 2014; **24**: 199-204 [PMID: 24568561 DOI: 10.1089/lap.2013.0129]

56 **Shan CX**, Ni C, Qiu M, Jiang DZ, Li M. Influence of laparoscopy vs. laparotomy on bacterial translocation and systemic inflammatory responses in a porcine model with peritonitis. *J Invest Surg* 2014; **27**: 73-80 [PMID: 24665843 DOI: 10.3109/08941939.2013.837564]

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ORIGINAL ARTICLE

Clinical and Translational Research

Chemokine receptor 8 expression may be linked to disease severity and elevated interleukin 6 secretion in acute pancreatitis

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Abstract

BACKGROUND

Acute pancreatitis (AP) is an inflammatory disease, which presents with epigastric pain and is clinically diagnosed by amylase and lipase three times the upper limit of normal. The 2012 Atlanta classification stratifies the severity of AP as one of three risk categories namely, mild AP (MAP), moderately severe AP (MSAP), and severe AP (SAP). Challenges in stratifying AP upon diagnosis suggest that a better understanding of the underlying complex pathophysiology may be beneficial.

AIM

To identify the role of the chemokine receptor 8 (*CCR8*), expressed by T-helper type-2 Lymphocytes and peritoneal macrophages, and its possible association to Interleukin (IL)-6 and AP stratification.

METHODS

This study was a prospective case-control study. A total of 40 patients were recruited from the Chris Hani Baragwanath Academic Hospital and the Charlotte Maxeke Johannesburg Academic Hospital. Bioassays were performed on 29

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patients (14 MAP, 11 MSAP, and 4 SAP) and 6 healthy controls as part of a preliminary study. A total of 12 mL of blood samples were collected at Day (D) 1, 3, 5, and 7 post epigastric pain. Using multiplex immunoassay panels, real-time polymerase chain reaction (qRT-PCR) arrays, and multicolour flow cytometry analysis, immune response-related proteins, genes, and cells were profiled respectively. GraphPad Prism™ software and fold change (FC) analysis was used to determine differences between the groups. $P<0.05$ was considered significant.

RESULTS

The concentration of IL-6 was significantly different at D3 post epigastric pain in both the MAP group and MSAP group with $P = 0.001$ and $P = 0.013$ respectively, in a multiplex assay. When a FC of 2 was applied to identify differentially expressed genes using RT^{2}Profiler, *CCR8* was shown to increase steadily with disease severity from MAP (1.33), MSAP (38.28) to SAP (1172.45) median FC. Further verification studies using RT-PCR showed fold change increases of *CCR8* in MSAP and SAP ranging from 1000 to 1000000 times when represented as Log_{10}, compared to healthy control respectively at D3. The findings also showed differing lymphocyte and monocyte cell frequency between the groups. With monocyte population frequency as high as 70% in MSAP at D3.

CONCLUSION

The higher levels of *CCR8* and IL-6 in the severe patients and immune cell differences compared to MAP and controls provide an avenue for exploring AP stratification to improve management.

Key Words: Acute Pancreatitis; Severity; Stratification, Interleukin-6; Chemokine Receptor 8; Lymphocytes; Monocytes

Core Tip: Chemokine receptor 8 (*CCR8*) is a chemokine receptor that is highly expressed on monocytes and cells of T helper type-2 (Th2) lineage including innate lymphoid cells group 2 and 3 (ILC2 and 3). This study shows possible linkages between increasing *CCR8* expression and severity in mainly moderately severe acute pancreatitis (MSAP) patients when compared to mild acute pancreatitis (MAP). Differing lymphocyte and monocyte cell frequencies suggest that in MAP, interleukin (IL)-6 was highly expressed in lymphocytes, and in the severe patients [MSAP and severe acute pancreatitis (SAP)] were highly expressed by monocytes. The findings open doors for future work, which could include an in-depth look at IL-6 producing cells such as Th2 Lymphocytes, monocytes, and innate ILC2 to determine cell-associated cytokine as a novel approach in prognosticating AP disease severity.

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease that presents with epigastric pain and is clinically diagnosed by amylase and lipase levels three times the upper limit of normal[1]. The disease is localized to the pancreas and is triggered by the premature release of digestive enzymes resulting from damaged pancreatic acinar cells[2-3]. Through activation of the immune system, patients develop a systemic inflammatory response syndrome (SIRS) and subsequently, single or multiple organ failure leading to high mortality[4]. This disease is one of the most common cause of hospital admissions and has an annual incidence of 80 in 100000 people worldwide[5-7].

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The severity of acute pancreatitis is classified as mild, moderate, or severe[1]. Mild AP (MAP) presents with no organ failure and no local complications. Moderately severe acute pancreatitis (MSAP) only differs from severe AP (SAP) in that the patients have transient organ failure (OF) within 48 h and possibly pancreatic necrosis[1,8-9]. If OF persists for more than 48 h the patient is classified as severe[1]. MSAP is further defined by specified local complications or exacerbation of the co-morbid disease. Local complications include pancreatic fluid collections, pancreatic and peripancreatic necrosis (sterile or infected), pseudocyst, and walled-off necrosis (sterile or infected) [1]. Due to the complications and subsequent high mortality observed with increasing disease severity, the need arises for early stratification of the disease through the understanding of the pathogenesis of the disease and its systemic inflammatory response[4,7,10].

It is generally accepted that the premature release or activation by trypsin of proenzymes (including trypsinogen) is the initial trigger of pancreatitis[11]. Under normal conditions, trypsin and other proteolytic enzymes are blocked from activation by serine protease inhibitor, Kazal type 1, which is secreted by acinar cells[11]. AP is characterized by the activation of trypsin and other events such as obstruction and passage of gallstones in the bile duct (in the case of acute biliary pancreatitis), which in turn blocks the transport of trypsin to the small intestine[7,12]. This leads to premature activation of lipase and elastase causing intracellular damage of cells and subsequently inflammation and thrombosis. Damaged acinar cells are unable to regulate trypsin activity leading to further inflammation and eventual tissue damage through excessive amounts of activated enzymes within the pancreas. Lipase in particular, induces necrosis in fat cells within the pancreas leading to local recruitment of proinflammatory markers including cytokines[7,13].

Identifying prognostic markers of AP would ensure early patient stratification. Markers such as C-reactive protein (CRP), nuclear factor kappa B (NF-κB), and IL-6 have been identified as potential prognostic markers in AP. CRP, an acute-phase reactant produced by the liver and induced by IL-6, is well described as an inflammatory marker for the disease. It has been demonstrated as an effective prognostic marker of AP severity at 48 h after admission, although other studies found that its strength as a prognostic marker is prominent only at 72 h after admission[11,14-15]. NF-κB, on the other hand, is a transcription factor involved in cell proliferation[13]. This molecule is responsible for cellular responses to free radicals such as reactive oxygen species, production of inflammatory cytokines (IL-2, IL-6, TNF-α, IL-1β, and IL-8), and excess production of calcium within acinar cells, which results in premature activation of trypsinogen[13]. NF-κB is also responsible for activating the cytokine cascade that manifests as SIRS[13,6-17].

Considering that AP is an inflammatory disease, continuous efforts to fully understand its immunopathogenesis are critical to potentially improve management. This is due to the underlying complex pathophysiology associated with the disease [18]. For autoimmune diseases, the excessive recruitment of inflammatory mediators and subsequent increase in the production of cytokines and chemokines after an insult is responsible for inflammation[19]. This condition is further aggravated by the continued recruitment and infiltration of macrophages, neutrophils, and lymphocytes to the site of injury[19-21]. The resulting inflammation from the tissue injury, as a result of damage to the pancreas due to either obstruction or passage of gallstones, in biliary AP, can be attributed to damage-associated molecular patterns, which may result in necrosis of the pancreas in more severe forms of AP[20]. These inflammatory molecules are then recognized by pattern recognition receptors of the innate immune system. This process mobilizes the recruitment of neutrophils, macrophages, dendritic cells, and mast cells in the peripheral blood and at the site of injury, which in turn produces cytokines including IL-1, IL-6, and TNF-α[20-21]. This results in inflammation at the site of injury and phagocytosis by macrophages and neutrophils[21]. Phagocytosis activates antigen-presenting cells (APCs), which include macrophages, dendritic cells, and B cells[21]. Another cell type involved in innate immunity is natural killer (NK) cells, which help activate the adaptive immune system (AIS) by increasing the production of interferon-gamma (IFN-γ), a recognized initiator of the AIS[21-22]. This presentation process of the AIS activates T cell proliferation[22]. Naïve T cells will differentiate into cytotoxic T cells (CD8+) or T helper (Th) cells (CD4+ cells). CD8+ cells eliminate the threat of infected cells and tumorous cells[21]. Once the threat is eliminated, another group of T cells, T regulatory cells, suppress the immune response to achieve homeostasis[19].

Natural Killer cells belong to a group of cells known as innate lymphoid cells (ILCs). These cells are responsible for regulating immune responses and are mainly found within the tissues[21-22]. The ILCs have been described as mirrors of the T helper cells

but within the innate immunity[23]. Three groups of ILCs produce the same cytokines as T helper cells, *i.e.*, ILC group 1 (ILC1) produces Th1 cytokines; ILC2 produces Th2 cytokines and ILC3 produces Th17 cytokines[21-22]. As detailed in Supplementary Table 1, the group 1 ILCs produce IFN-γ and require T-box transcription factor for their proliferation; group 2 ILCs require transcription factor *GATA-3* and *ROR-α* to develop and will produce Th2 cytokines, such as IL-4, IL-5, and IL-13[24]. Group 3, ILCs depend on the transcription factor, *ROR-γt*, for their development and produce IL-17 and IL-22 and granulocyte-macrophage colony-stimulating factor (GM-CSF)[22]. ILCs have also been reported to act as antagonists of both innate and adaptive immune cells[25], by mimicking the activity of T regulatory cells in achieving immune homeostasis[22].

CCR8, a chemokine receptor, is highly expressed on monocytes and cells of Th2 Lineage including ILC2 and ILC3[26]. This chemokine is also expressed on peritoneal macrophages in tissue and lymphocytes of Th2 Lineage[27]. Studies demonstrate that NF-κB is suppressed in *CCR8* deficient mice and that macrophage chemotaxis in the peritoneal cavity, which includes the pancreas, is Chemokine (C-C motif) ligand 1 (CCL1), which is the ligand of *CCR8* is dependent[27]. *CCR8* and its ligand, CCL1, are known to recruit and activate macrophages in type 1 diabetes[28-29]. This study is the first to describe *CCR8* in AP and its possible linkages to lymphocyte and monocyte cell frequencies.

This study utilized patients' samples at different severities (MAP, MSAP, and SAP) to profile inflammatory genes, and proteins (including *CCR8* and IL-6) and identified those that were distinctly upregulated or downregulated. White blood cell populations were characterised and assessed and linkages to gene and protein expression are proposed as potential prognostic markers for AP. The findings also provide insights that are more recent and contribute to the scarce literature on the prevalence, demographics, and etiology of AP in an African setting.

MATERIALS AND METHODS

Patient recruitment and sample collection

Ethics approval for this study was obtained from the Human Research Ethics Committee Medical of the University of the Witwatersrand (Ethics No. M180133). All patients included in the study were duly informed and written consent was received before blood samples were taken. Using the Revised Atlanta Classification (RAC) for AP[1], patients were recruited from the Hepatopancreatobiliary Unit of the Chris Hani Baragwanath Academic Hospital (CHBAH) and the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa, from August 2018 to September 2019. The total number of patients recruited was 40 (21 MAP, 14 MSAP, and 5 SAP) and 6 healthy volunteers were recruited as controls after being age and sex-matched to recruited patients. Blood samples were collected on Day 1, 3, 5, and 7 post epigastric pain using three BD vacutainer® purple blood collection tubes (BD Biosciences, New Jersey, United States) with 4 mL of blood each. Patients on average presented at the hospital approximately after 72 h of pain (day 3 of post epigastric pain). Clinicians within the Gastrointestinal Unit of the respective hospitals diagnosed patients and classified them into the three groups (MAP, MSAP, and SAP). The stratification of severity was determined using the RAC guidelines.

Sampling and processing: Overview

The different aspects of the study included different numbers of patients as illustrated (Figure 1). From the 40 patients, plasma and cell samples were processed in the laboratory within 4 h of phlebotomy. Plasma from a total of 31 out of 40 patients was analysed using the Th1/Th2/Th17 cytometric bead array (CBA) kit in an initial exploratory study. Based on this analysis, plasma samples from 23 patients were randomly selected for analysis of selected Th17 related cytokines including IL-6 using the MILLIPLEX® MAP Human Th17 Magnetic Bead Panel kit (Millipore™, Massachusetts, United States).

RNA was extracted from peripheral blood mononuclear cells (PBMCs) using the TriReagent® (Sigma Aldrich, Missouri, United States) method from 13 patients for screening of genes with the human innate and adaptive RT² Profiler 96-well PCR array plates (QIAGEN, Hilden, Germany). Findings showed dose-dependent expression of the *CCR8* gene with disease severity, prompting further analysis in 29 patients using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) to verify its roles. To characterize cell types into monocytes, lymphocytes, and

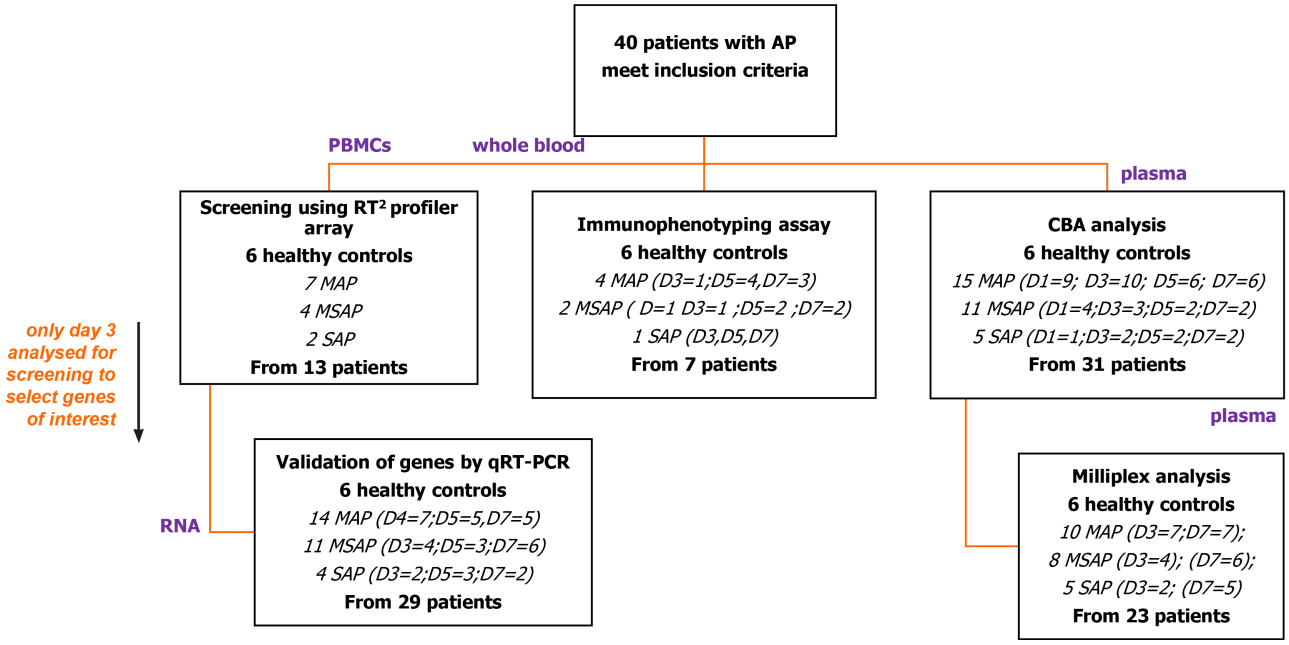

Figure 1 Flow diagram of patient recruitment. From the 40 patients and 6 healthy controls recruited over the study period, peripheral blood mononuclear cells, whole blood and plasma were used for the various study assays as shown. Peripheral blood mononuclear cells (PBMCs) from 13 patients with Day 3 data were used to do a screening study of innate and adaptive immune cell genes using RT^2 Profiler Array (Qiagen, Hilden, Germany). *CCR8* was selected as a target gene and further verification studies done in 29 patients as depicted. For immunophenotyping, 12 antibodies were selected to discriminate monocytes, lymphocytes, and granulocytes and their subpopulations from blood samples of seven patients. An exploratory study of seven Th1/Th2/Th17 cytokines was done on 31 patient samples and 23 of these randomly selected for further analysis using the MILLIPLEX® assay. PBMCs: Peripheral blood mononuclear cells; RT^2: Reverse transcriptase square; D: Day; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis; *CCR8*: Chemokine receptor 8; Th1/Th2/Th17: T helper type 1/2/17.

granulocytes, seven patients were included in an antibody specific multicolour immunophenotyping flow cytometry experiment.

Blood processing

From the blood samples, plasma was isolated by gravity separation for 45 min at room temperature followed by centrifugation at 1500 r/min for 30 min. Plasma samples were aliquoted (200 μL) in single use vials and stored at -80 °C until needed.

Using Ficoll-Paque™ (GE Healthcare, Illinois, United States) separation method, as per the manufacturer's instructions, PBMCs were separated and stored in single use aliquots in liquid nitrogen in a freezing medium (10% dimethyl sulphoxide in fetal bovine serum, Sigma Aldrich, Missouri, United States) until required. Samples were only thawed once to preserve integrity.

Cytokine expression analysis

Protein expression analysis was performed using two methods as depicted in Figure 1. The first was a BD BioSciences cytometric bead array Th1/Th2/Th17 kit that served as an exploratory step to determine the concentration of interleukin (IL-2), IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A cytokines. The assay was done on 31 AP patients (15 MAP, 11 MSAP, and 5 SAP) and 6 healthy control donor samples on days 1, 3, 5, and 7 post epigastric pain (see the supplementary section for detailed protocol). The second analysis was done using a MILLIPLEX® MAP Human Th17 Magnetic Bead Panel kit (Millipore™, Massachusetts, United States).

Using the MILLIPLEX® MAP Human Th17 Magnetic Bead Panel kit

In the MILLIPLEX® assay, preselected cytokines, based on the performance of the CBA analysis and based on literature and previous work from the research group were used [30-32]. These cytokines were; IL-17A, IL-21, and IL-6, IFN-γ, IL-23, IL-28λ, and TNF-β measured from 23 randomly selected AP patient samples (10 MAP, 8 MSAP, and 5 SAP) from the pool of 31 patient samples tested in the CBA assay on days 3 and 7 post epigastric pain. Six healthy controls were included.

A solid 96 well plate was prepared using the manufacturer's instructions. Plates were run on BioPlex® 2200 system (BioRAD, California, United States) and data were

collected and analysed using BioPlex® Manager 5.0 software (BioRad, California, United States). All samples and controls were measured in duplicate to minimize errors. Controls included quality control (QC) 1 samples (low level) and QC2 samples (high level) as well as standards with the lowest dilution at 4:1. The observed concentration of cytokines was determined by excluding outliers and values extrapolated beyond the standard range. Values designated by an asterisk as per the BioPlex® Manager 5.0 software, were inputted as zero while values labeled as Out of Range were not considered in the analysis.

Total RNA extraction

Total RNA was extracted using the TriReagent® (Sigma Aldrich, Missouri, United States) protocol, according to the manufacturer's instructions, from the isolated PBMCs on Day 3, 5, and 7 samples. However, initial screening was performed on 13 (MAP, n = 7; MSAP, n = 4; SAP, n = 2) Day 3 samples only. The quality of RNA was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific, Massachusetts, United States), and samples with an A260/280 ratio > 1.8 were observed across all samples[33-34].

Complementary DNA synthesis and PCR array analysis

Complementary DNA synthesis (cDNA) was performed from 250 ng/μL of total RNA using the RT^2 First Strand Kit (QIAGEN, Hilden, Germany), according to the manufacturers' instructions. A genomic DNA elimination mix was first prepared and incubated for 5 min at 42 °C in a SimpliAmp™ thermocycler (ThermoFischer Scientific, Massachusetts, United States), which was subsequently placed on ice for 1 min. Following this, a 20 μL cDNA synthesis reaction was prepared and run at 42°C for 15 min followed by incubation at 95 °C for 5 min. From the cDNA, 102 μL was added to the PCR mixture and loaded onto the human innate and adaptive RT^2 Profiler 96-well PCR array plates (QIAGEN, Hilden, Germany). The mixture was amplified on Quant Studio 1 Real-Time System (Thermo Fischer Scientific, Massachusetts, United States) the PCR reaction was run for 40 cycles including a 10 min hot start at 95 °C for 1 cycle; 95 °C for 15 s and 60 °C for 1 min. The human innate and adaptive RT^2 Profiler array includes 96 genes, 5 of which are reference genes and 3 reverse-transcription controls, 3 positive PCR controls, and 1 human genomic DNA control. Using the QIAGEN GeneGlobe online tool (https://geneglobe.qiagen.com/za/analyze/), a fold-change of 2 was applied as the cut-off for differential analysis comparing the expression level of genes in the 3 severity groups to healthy control.

Verification of selected gene targets using Real-time PCR

After screening of Day 3 samples for early immune markers with the RT^2 Profiler PCR Array Human Innate and Adaptive Immune Responses (QIAGEN, Hilden, Germany) the *CCR8* gene was selected for further analysis. Twenty-nine patients (MAP = 14, MSAP = 11, SAP = 4) were included in this assay as stated in Figure 1 and Table 1. The TaqMan® Fast Advanced Master Mix (Thermo Fischer, Massachusetts, United States) was used to perform duplex qRT-PCR. The PCR reaction was run for 40 cycles including a 2 min hold at 95°C for 1 cycle; 95°C for 1 s and 60 °C for 20 s. Normalisation was done using RPL13A on VIC (assay ID *Hs04194366_g1*, Thermo Fischer Scientific, Massachusetts, United States) as the reference gene. This gene is well established in AP disease models as a reference gene[35]. The target gene was *CCR8* on FAM (assay ID: *Hs00174764_m1,* Thermo Fischer Scientific, Massachusetts, United States). The Quant Studio™ 1 Real-Time System (Thermo Fischer Scientific, Massachusetts, United States) was used to run the RT-qPCR reactions. The $2^{-\Delta\Delta CT}$ method was used to calculate relative changes in gene expression[36].

Immunophenotyping using multicolor flow cytometry analysis

Selected blood samples from days 3, 5, and 7 of onset of AP symptoms were analysed using multicolour flow cytometry to determine immune cell frequency levels to make a correlation to protein production or expression. The sampled patients included 4 in the MAP group, 2 patients from the MSAP group; 1 patient from the SAP group. Six healthy participant samples were used as controls. While the numbers here are small, given the well characterized levels of monocytes, lymphocytes, and granulocytes in AP patients from the literature[18,20,37-38], inferences from this preliminary data will be discussed with reference to the literature.

A 12-colour panel was established to characterize heterogeneous cell populations in the three risk categories of AP. Using the lyse/wash method, whole blood was used to isolate white blood cells from 100 μL of blood from an EDTA blood tube within 6 h of

Table 1 Demographic characteristics of the acute pancreatitis patients included in the gene expression analysis study

Parameter	Value [*n*, %]
AP patient demographics	*n* = 29
MAP	14 (48)
MSAP	11 (38)
SAP	4 (14)
Age (yr), [median (IQR)]	41 (23, 76)
Male (*n*, %)	17 (49)
Female (*n*, %)	12 (51)
AP etiology/risk factor	
Biliary (*n*, %)	13 (45)
Alcohol (*n*, %)	13 (45)
ERCP (*n*, %)	1 (3)
Antiretroviral (*n*, %)	2 (7)
Healthy control Demographics	
Age (yr), [median (IQR)]	36.5 (23, 55)
Male (*n*, %)	3 (50)
Female (*n*, %)	3 (50)

AP: Acute pancreatitis; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; IQR: Interquartile range; ERCP: Endoscopic retrograde cholangiopancreatography.

phlebotomy. Antibodies were optimized by titration to optimally stain lymphocytes populations and subpopulations using CD3 BD Horizon Brilliant™ Ultraviolet (BUV); CD4 Alexa flour; CD8 Brilliant Violet™ 605; CD56 PE Phycoerythrin Cyanine 7 (PECy7), CD16 PECy5) and monocyte populations using CD16PECy5 and CD14 Peridinin-Chlorophyll-protein cyanine 5.5 (PerCPCy5.5), and CD14PerCP Cy5.5 and human leukocyte D related (HLA-DR BV650). Other antibodies that were included in the 12 colour panel but not reported in the study are listed in Supplementary Table 2. All antibodies were from BD Biosciences, (New Jersey, United States).

Cells were prepared both as fully stained samples and as unstained samples. Fully stained samples were suspended in BD Horizon brilliant buffer (BD Biosciences, New Jersey, United States) and stained with selected antibodies (see Supplementary Table 2). The cells were then incubated in the dark for 20 min and thereafter fixed with 2 mL of diluted BD FACS Lyse (BD Biosciences, New Jersey, United States) and incubated for 12 min with intermittent mixing with a pipette. The cells were then washed with diluted Dulbecco's Phosphate Buffered Saline (Sigma Aldrich, Missouri, United States) at 150 x *g* for 5 min. Approximately 100000 cells were acquired on BD LSRFortessa™ II flow cytometer (BD Biosciences, New Jersey, United States) for each sample at a threshold of 5,000 after the necessary quality controls using FACSDiva™ software version 5 (BD, Biosciences, New Jersey, United States). The controls included voltages optimization using single stains, compensation for spillover was done using CompBeads (Anti-Mouse Ig, κ/Negative Control Compensation Particles Set; BD Biosciences, New Jersey, United States) and 8 peak beads (BD Biosciences, New Jersey, United States) were used to determine linearity in fluorescence detection channels on every sample run.

Data was further analysed using FlowJo LLC version 10 (BD, Biosciences, New Jersey, United States) with previously linked compensation controls from FACSDiva™ software. Cells were gated as singlets, then further as granulocytes, lymphocytes, and monocytes using forward scatter and side scatter properties as well as fluorescent antibody stains for specific subsets. Doublets were excluded using Forward scatter height (FSC-H) and FSC area (FSC-A), then FSC and side scatter (SSC) were used to discriminate white blood cells namely lymphocytes, granulocytes, and monocytes. All populations were represented as percentages of parent populations. Of the 12 antibodies used for cell differentiation, analysis was done for *CCR8* associated cell

populations. These include lymphocytes and monocytes[39]. These populations were lymphocyte subpopulations ($CD3^{-}CD16^{+}$ $CD56^{+/-}$, $CD3^{-}CD16^{+}CD57^{+/-}$) and monocyte populations and subpopulations ($CD14^{+/-}$ $CD16^{+/-}$ and $CD14^{+}HLA\text{-}DR^{+/-}$).

Statistical and Data analysis

The cytokine data and qRT-PCR data were analysed using GraphPad Prism™ software version 8 (GraphPad Software Inc, California, United States). A Shapiro-Wilk test was used to test for normality. Once data was determined to be non-parametric, a Kruskal Wallis test was used to determine significant differences between the healthy control groups and between the MAP, MSAP, and SAP groups. The *P* values were considered significant at $P < 0.05$. A Dunn's Multiple Comparison Test was used to perform a post hoc analysis to eliminate type 1 errors. Immunophenotyping data focused on lymphocytes and monocytes as they relate to *CCR8* expression[39] and were presented as percentages and ratios. The statistical methods of this study were reviewed by Mr. Glory Chidumwa from the Division of Epidemiology and Biostatistics, School of Public Health, Faculty of Health Sciences, University of the Witwatersrand.

RESULTS

Patient demographics

A total of 40 patients were included in the overall study using prescribed inclusion criteria from 1 August 2018 to 22 August 2019 from CHBAH and CMJAH in Johannesburg, South Africa. Of these 40 patients, 29 were reported in the gene expression studies (Figure 1). The gender distribution of the 29 patients was 41% females and 59% males. The most common etiologies of AP were alcohol and biliary-related with each category consisting of 45% of the recruited patients (Table 1). The median age of the patients was 36.5 years. The MSAP group age range was between 26 to 76 years and that of the SAP group was between 40 and 69 years old.

Secreted IL-6 expression differentiates severity groups in early acute pancreatitis

In the exploratory CBA assay, data were expressed as Mean Fluorescent Intensity (MFI) as shown in Supplementary Figure 1. In the analysis of the data, only the MFI of IL-6 revealed changes between patient plasma samples at Day 3. On Day 1, the MAP group had a high expression of IL-6 at above 5000 MFI, which was significantly different from healthy controls ($P = 0.015$). At Day 3 in the MAP a significant difference was reported with $P = 0.004$ when compared to the healthy control. In the MSAP group, there was a significant difference on Day 3 ($P = 0.004$) and 7 ($P = 0.029$). IL-6 MFI was in the region of 5000 for the SAP patient.

The results from the MILLIPLEX® data showed visible trends between severities over time as well as between groups. The mean concentration of IL-6 in the MAP group was 20 ± 4.9pg/mL on Day 3 and dropped to 2.9 ± 1.7pg/mL on Day 7. A similar trend was seen in the MSAP group with a drop in mean concentration from 13 ± 4 pg/mL on Day 3 to 10 ± 7.7pg/mL on Day 7. The IL-6 concentration was significantly different at D3 for MAP ($n = 7$) and MSAP ($n = 4$) compared to healthy controls with $P = 0.001$ and $P = 0.013$ respectively (Figure 2). The concentration of the SAP group was not significantly different at both Days 3 ($n = 2$) and 7 ($n = 5$) compared to healthy controls with $P = 0.094$ and $P = 0.186$ respectively. However, the mean concentration of IL-6 in the SAP group was higher compared to the MAP and MSAP groups. The concentration at Day 3 was 50 pg/mL (this included two patients with individual IL-6 concentrations of 0.13 pg/mL and 100 pg/mL). The mean concentration at Day 7 was 65 ± 62pg/mL as shown in Figure 2.

Differential gene expression in the different acute pancreatitis severity groups

A fold change (FC) of 2 was applied to identify differentially expressed genes in the patient groups (7 MAP, 4 MSAP, and 2 SAP) at Day 3 compared to healthy controls from the RT^{2}First Strand Kit (QIAGEN, Hilden, Germany) assay as summarized in Table 2. Of the 96 genes analysed (represented by the heat map in Figure 3), a total of 31 genes were downregulated while 9 genes were upregulated in the MAP group with *CXCL8* (fold change = -45.26) and *CD14* (FC = -21.58) being the most downregulated compared to the healthy control samples. The chemokine receptor *CCR6* was also downregulated in the MAP group (FC = -21.05). In the MSAP patients, 68 genes were upregulated and 4 were downregulated. The downregulated genes included *CCL5* (FC

Table 2 List of selected genes and their fold changes in mild, mild acute pancreatitis, moderate, moderately severe acute pancreatitis, and severe acute pancreatitis patients when compared to healthy controls

Gene symbol	MAP (*n* = 7)	MSAP (*n* = 4)	SAP (*n* = 2)
CCL5	-2.97	-3.76	-15.22
CCR8	1.33	38.28	1172.45
IL10	-1.30	58.62	-1.47
FOXP3	3.90	137.02	96.27
IL13	-1.92	83.66	19.53
IL17A	1.72	116.93	2.56
IL23A	-5.60	18.07	6.57
IL4	-1.13	108.64	36.83
IL5	1.33	192.59	1.21
NOD1	-8.93	-14.62	64.21
MPO	1.33	91.77	11.8

MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis; *CCL5:* Chemokine (C-C Motif) Ligand 5; *CCR8*: Chemokine receptor 8; *IL:* Interleukin-(4; 5; 10; 13; 17A; 23A); FOXP3: Forkhead box P3; *NOD1*: Nucleotide-binding oligomerization domain-containing protein 1; *MPO*: Myeloperoxidase.

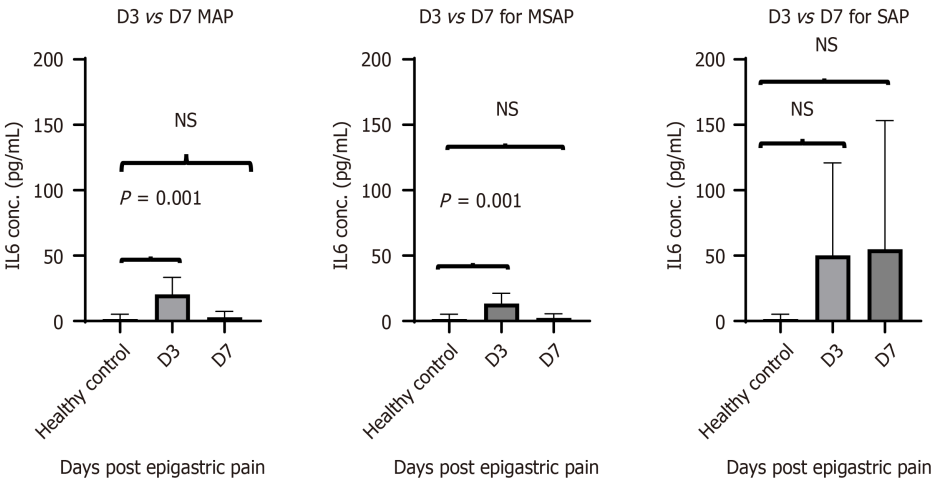

Figure 2 IL-6 secretion levels acquired using the MILLIPLEX® MAP Human Th17 Magnetic Bead Panel kit. Analysis was done on 23 patients sampled on Day 3 and Day 7 and 6 healthy controls were included. The concentration of interleukin (IL)-6 was highest in the SAP group 50 ± 50 pg/mL and 65 ± 61 pg/ml on D3 (*n* = 2) and D7 (*n* = 5) respectively. The MAP group IL-6 levels were 13 ± 8 pg/mL (*n* = 7) and MSAP 20 ± 13 pg/mL groups (*n* = 4) on D3. Significant differences were observed between the healthy controls (*n* = 6) and MSAP at D3 (*n* = 4) with *P* = 0.014 and *P* = 0.013 respectively. A Dunn's multiple comparison test was used as a post hoc to adjust *P* values. D: Day of the specific severity group; *n*: number; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis; IL-6: Interleukin-6.

= -3.76) and the upregulated genes included *FOXP3* (FC = 137.02) and *APCS* (FC = 262.91) being the most downregulated and overexpressed, respectively. Importantly, moderately severe patients had the highest number of upregulated genes, specifically those involved in inflammation such as *IL4* (FC = 108.64), *IL5* (FC = *192.59)*, *IL23A* (FC = 18.07), *GATA-3* (FC = 11.58), and *CRP* (FC = 177.42), as shown in the heat map in Figure 3. A total of 34 genes were upregulated in the SAP patients while 25 were downregulated. *CCR8* (FC = 1172.45) and *CD8A* (FC = -74.26) were the top upregulated and downregulated genes, respectively in the SAP group. Notably, *CCR8* increased steadily with disease severity producing the highest fold change across all groups. Other genes that increased with severity were *GAPDH, NOD1, TRL 1* and *TICAM 1, TBX21,* and *CASP1,* which are all genes closely associated with *CCR8* (Figure 3).

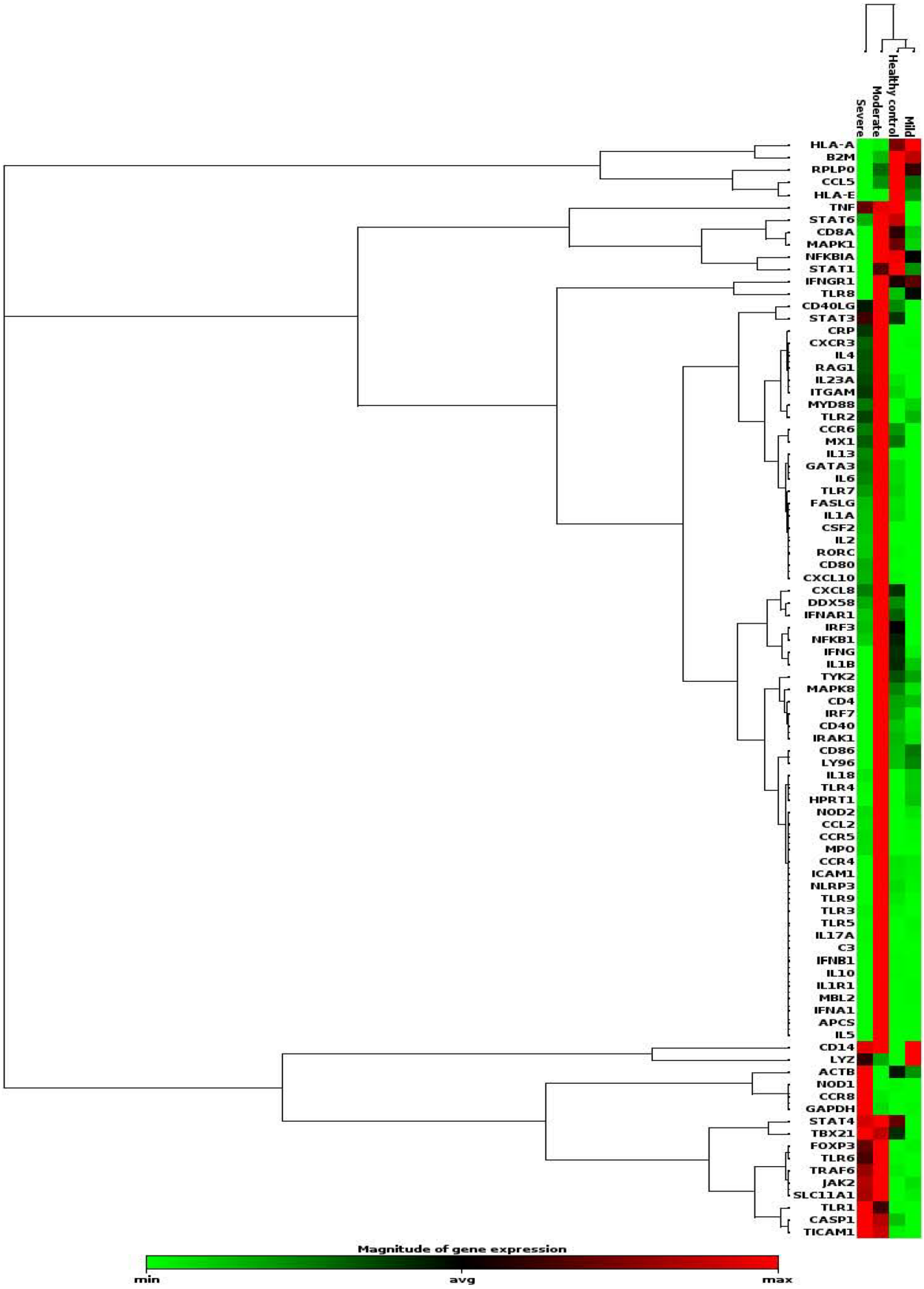

Figure 3 A heat map showing gene dysregulation in mild moderate and severe acute pancreatitis patients compared to healthy controls. Hierarchical cluster of all the genes across patient severities are shown. Red colour represents upregulated genes, green is downregulated and black is unchanged. Chemokine receptor 8 *(CCR8)* was shown to increase with severity and so were *GAPDH, NOD1, TRL 1, TICAM 1, TBX21,* and *CASP1,* which are associated with *CCR8* expression. *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase; *NOD1*: Nucleotide-binding oligomerization domain-containing protein 1; *TRL 1*: Toll like

receptor 1*; TICAM 1:* Toll Like Receptor Adaptor Molecule 1*; TBX21*: T-Box Transcription Factor 21*; CASP1:* Caspase 1; apoptosis-related cysteine peptidase; *CCR8:* Chemokine receptor 8; MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis.

CCR8 expression and the severity of AP by Real-time PCR

The real-time PCR verification findings were plotted as Log10 of fold change ($2^{-\Delta\Delta CT}$), shown in Figure 4. The results show that at Day 3 post epigastric pain the fold change of *CCR8* for the MAP group compared with the healthy control group was almost 1 to 1 (Figure 4A). Whereas the MSAP is 1000 times more than the healthy control for the same day (Figure 4B). The SAP group was 10000000 times that of the healthy control at Day 3 (Figure 4C). This was due to an individual sample that can be considered as an outlier. This group had an FC of a 1090632 ± 1090631 (Figure 4C). On Day 5 and Day 7 the fold change dropped to almost 1:1 ratio with the healthy control in the MAP and SAP group. In the MSAP group, the FC on Day 5 was consistent with Day 3 Levels and dropped slightly to 800 ± 846 on Day 7 as observed in the comparisons of the FC of *CCR8* within groups on different days (Table 3).

NK subsets in an MSAP patient and immune suppression

In one of the sampled patients from the MSAP group, NK cell frequencies of $CD3^-CD16^+CD56^-$ doubled from 12% to 27%, and those from the $CD3^-CD16^+CD56^-$ subsets increased in percentage from 19.8% to 49.6% from Day 3 to Day 5. NK cell subsets, which were $CD57^+$ increased by over 30% for MSAP patients from Day 3 to Day 5 (Supplementary Figure 2).

Monocyte cell populations and severity

In the immunophenotyping analysis by flow cytometry, 7 patients were recruited, 4 MAP, 2 MSAP, and 1 SAP as shown in Figure 1. Cells known to express *CCR8*, namely lymphocytes (including NK cells belonging to ILC1) and monocytes were assessed as part of a multicolour panel using flow cytometry[39]. Classical monocyte subpopulations (CD14+CD16-) were higher in more severe patients with the MSAP patient having as much as 71.6% of the parent population on day 3, dropping to undetectable levels on day 5 (Figure 5A and B). In the SAP patients, the classical monocyte population consistently increased by more than 7% from Day 3 to 5 (Figure 6A and B). In the MSAP patient, the percentage of HLA-DR+ monocyte increased by 43% from Day 3 to Day 5 (Figure 5C and D). Whereas the percentage of HLA-DR+ monocytes increased from 4.2% on Day 3 to 13.5% on Day 5 in the SAP patient (Figure 6C and D).

DISCUSSION

In this preliminary study, the demographics of 29 AP patients, the role of *CCR8*, IL-6, and the frequency of cells expressing these biomolecules were explored. Patient demographics were as expected with older patients falling into more severe groups[1, 40]. The study demonstrated that the increase in IL-6 Levels maintained an upward trend in the SAP group up to Day 7, compared to the healthy control group, the MAP, and MSAP group (Figure 2). The consistency in the concentration of IL-6 protein levels in the SAP group in the peripheral blood is likely as a result of the observed activated monocytes and hence *CCR8* expression on these cells. CRP is a well-defined severity marker in acute pancreatitis and is initiated by elevation of IL-6[14,38]. Elevated levels of *CRP*, an acute phase reactant, in the pooled sample of the MSAP and SAP group (Table 2, Supplementary Table 3) may be due to increased monocyte cell populations [37]. Although, IL-6 was not shown as a useful independent marker to distinguish different risk categories of AP in this study, cells producing IL-6 such as monocytes (Figure 5A and B, Figure 6A and B) and NK cells (Supplementary Figure 2), which are part of group 1 ILCs, increased in frequency at Day 3 and 5 in the MSAP and SAP group[24,39]. A possibility exists in exploring the potential prognostic value of a lymphocyte to monocyte ratio based on the resulting difference in frequency in MAP compared to MSAP and SAP.

Our findings further show the presence of HLA-DR dim to negative monocyte subsets in an SAP patient suggesting downregulation (Figure 6C and D). This supports findings from a study that found that the presence of monocytes that do not express HLA-DR correlates with organ dysfunction in AP[37]. An important observation was that in the MSAP patient at Day 3 (Figure 5C), HLA-DR was downregulated but upregulated by Day 5 (Figure 5D) showing resolve in organ failure, supportive of the

Table 3 Gene expression levels (fold change $2^{\wedge-\Delta\Delta CT}$) for chemokine receptor 8 gene in acute pancreatitis patients.

Severity	Mean fold change ($2^{\wedge-\Delta\Delta CT}$)
MAP D3	0.8 ± 0.22
MAP D5	0.9 ± 0.25
MAP D7	1.0 ± 0.26
MSAP D3	1386 ± 1372
MSAP D5	68.0 ± 67
MSAP D7	848 ± 846
SAP D3	1090632 ± 1090631
SAP D5	1.1, NA
SAP D7	1.3 ± 0.46

MAP: Mild acute pancreatitis; MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis. D: Day; D3: Day 3 post epigastric pain; *CCR8*: Chemokine receptor 8; $2^{\wedge-\Delta\Delta CT}$: Fold change, is used to measure change in the expression level of a gene[32]; NA: Not available due to single data points.

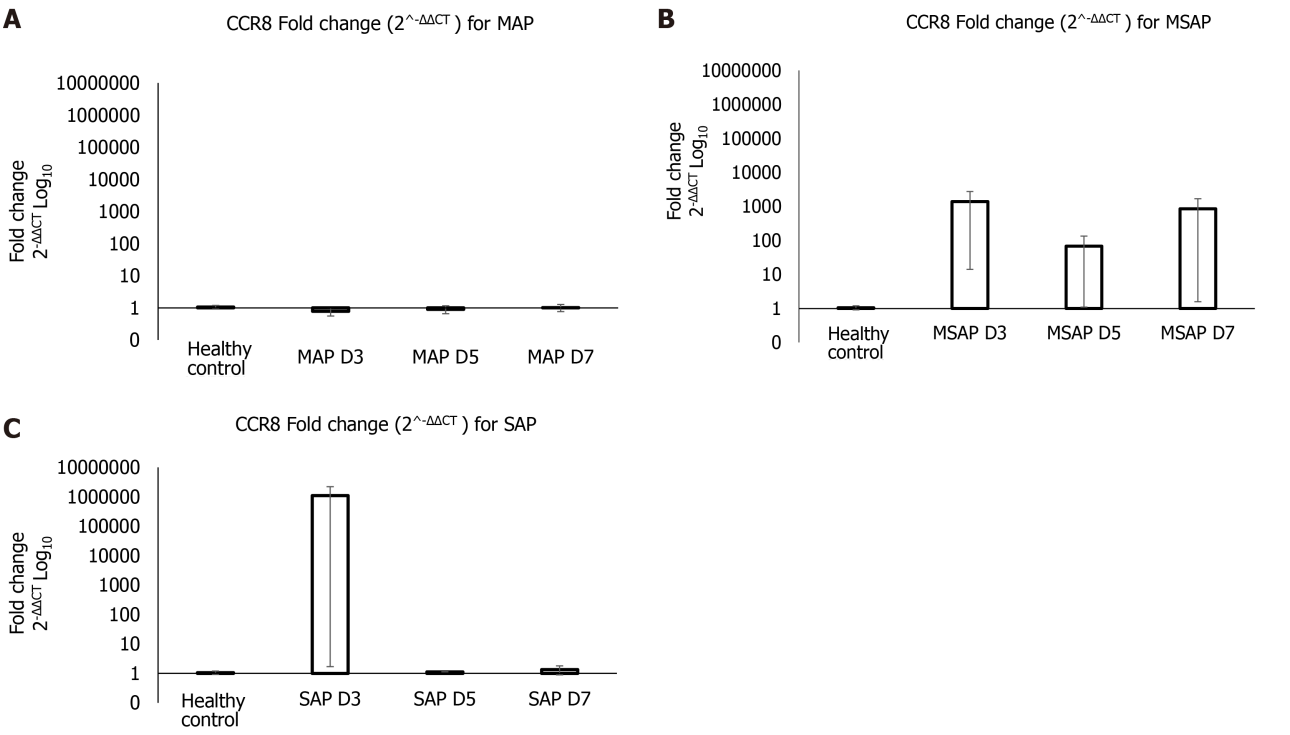

Figure 4 Gene expression analysis for chemokine receptor 8 in different severities at Day 3, 5 and 7 post epigastric pain due to acute pancreatitis for mild acute pancreatitis, moderately severe acute pancreatitis, and severe acute pancreatitis. The data is shown on a Log10 transformed scale of the fold change ($2^{-\Delta\Delta CT}$) normalized using *RPL13A* gene on VIC fluorescent dye (*Hs04194366_g1*, Thermo Fischer Scientific) as reference gene. A: The FC of *CCR8* was less than 1 at D3 and D5, then increased slightly to 1 at D7 for MAP group compared to healthy controls. B: At D3 and D7 the FC for the MSAP group was 1000 times more than in the healthy controls. C: In the SAP group the FC at D3 was 1000000 times more than in the healthy controls and the D5 and D7 was 1 time more. FC: fold change. D: D3 of the specific severity group; MAP: Mild acute pancreatitis: MSAP: Moderately severe acute pancreatitis; SAP: Severe acute pancreatitis; *CCR8*: Chemokine receptor 8.

MSAP classification[1]. The presence of immunosuppressive NK cell subsets, which are $CD57^+$ (Supplementary Figure 2) may also play an important role in this[41]. $CD3^-CD16^-CD57^+$ cell subsets have a protective function in autoimmune disease[41]. This further supports the hypothesis of a possible linkage between monocyte and lymphocyte frequencies to severity based on the observed decrease of classical monocytes from Day 3 to 5 in the MSAP patient who experienced transient organ failure. These preliminary results may indicate possible links between monocytes and NK cells in the stratification of the MSAP group of patients.

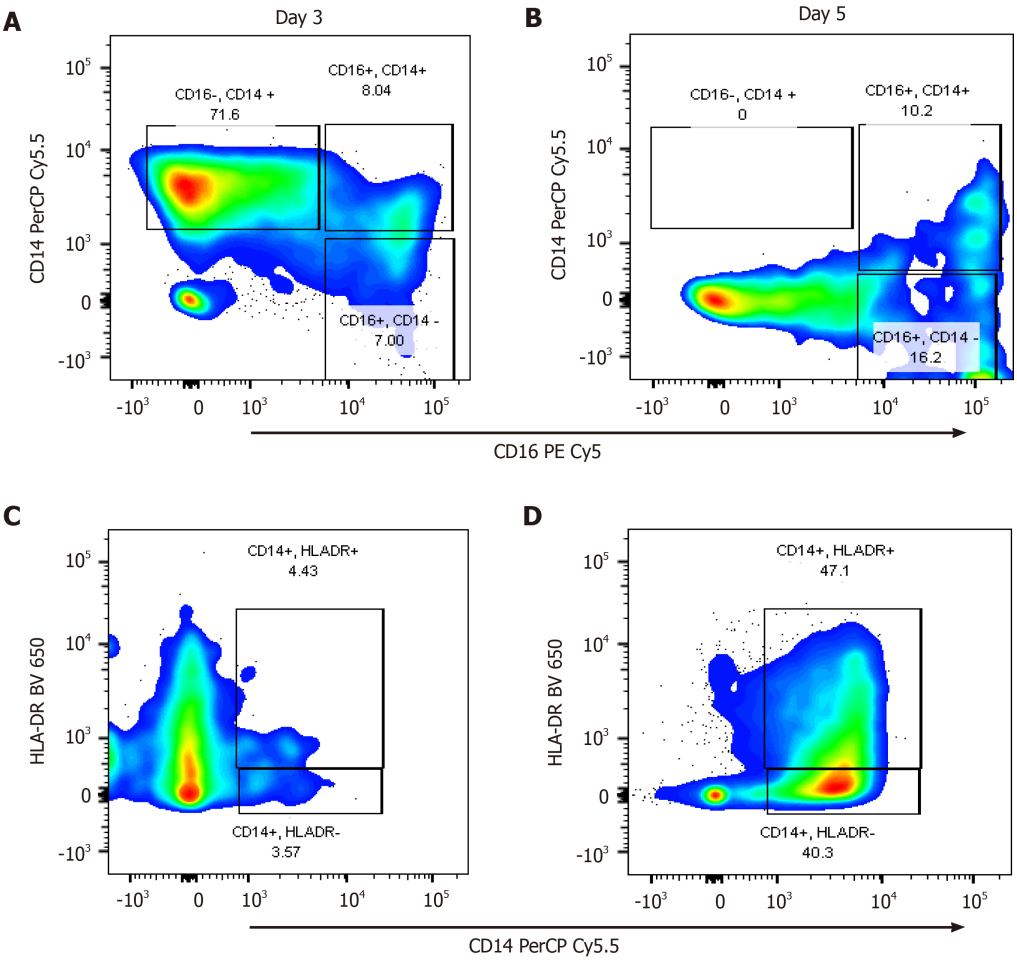

Figure 5 Representative moderately severe acute pancreatitis profile of the monocyte subpopulation. Cells were gated into intermediate (CD14+CD16+), classical (CD14+CD16-), non-classical monocytes (CD16+CD14-) and HLA-DR+/- monocytes. A and B: They showed CD14 PerCP Cy5.5 and CD16 PECy5 plot for Day 3 and Day 5 respectively. Classical monocyte subpopulations (CD14+CD16-) were higher in more severe patients with the MSAP patient having as much as 70% of the parent population on Day 3, which subsequently dropped to undetectable levels on Day 5. C and D: They showed a plot of CD14 PerCP Cy5.5 and HLA-DR BV650 for Day 3 and 5 respectively. The percentage of HLA-DR+ monocytes increased from 4% on Day 3 to 47% on Day 5. BUV: BD Horizon Brilliant™ Ultraviolet; Cy: Cyanine; BV: Brilliant Violet™; HLA DR: Human leukocyte D related; PE: Phycoerythrin; PerCP: Peridinin-Chlorophyll-protein; CD: Cluster of differentiation.

This study investigated expression patterns of several inflammatory and immune response-related molecules at the early stages of AP. We further describe a hypothetical model, which is deduced from this preliminary study and literature (Figure 7).

CCR8, a chemokine receptor, is known to be highly expressed on monocytes and cells of Th2 lineage including innate lymphoid cells group 2 (ILC2) and ILC3 cells[26,42]. Cells of the ILC1 population that are $CD56^-$ are found abundantly in peripheral blood in the disease state[17,18]. These cells are known to suppress autoimmune diseases[41]. This may explain the reason why organ failure is resolved in the MSAP patient compared to the NK cell-deficient SAP patient[1,36]. Acinar cell injury and elevation of trypsin in pancreatic tissue are followed by excessive recruitment of monocytes, neutrophils, and ILCs, to the local site of injury[42-44]. These ILCs include NK cells (ILC1), ILC2, and ILC3 cells. Once the pancreatic tissue is damaged due to AP, monocytes, and macrophages are responsible for the maintenance of inflammation [37,45]. Thus, the upregulation of *CCR8* observed in this study may be due to increased levels of activated monocytes in peripheral blood. The main agonist of *CCR8* is its own ligand *CCL1*[46]. *CCL1* in the peripheral blood is highly expressed on classical, non-classical, and intermediate monocytes[39]. In other autoimmune diseases such as cancers of the renal system, *CCR8* positive cells, namely monocytes and granulocytes were the most abundant in the bloodstream and contributed to prolonged inflammation within patients[47]. *CCR8* is also expressed on peritoneal macrophages in tissue and lymphocytes of Th2 Lineage[27]. Oshio *et al*[27] demonstrated that NF-κB is suppressed in *CCR8* deficient mice and that macrophage chemotaxis in the peritoneal cavity, which includes the pancreas, is *CCL1* dependent.

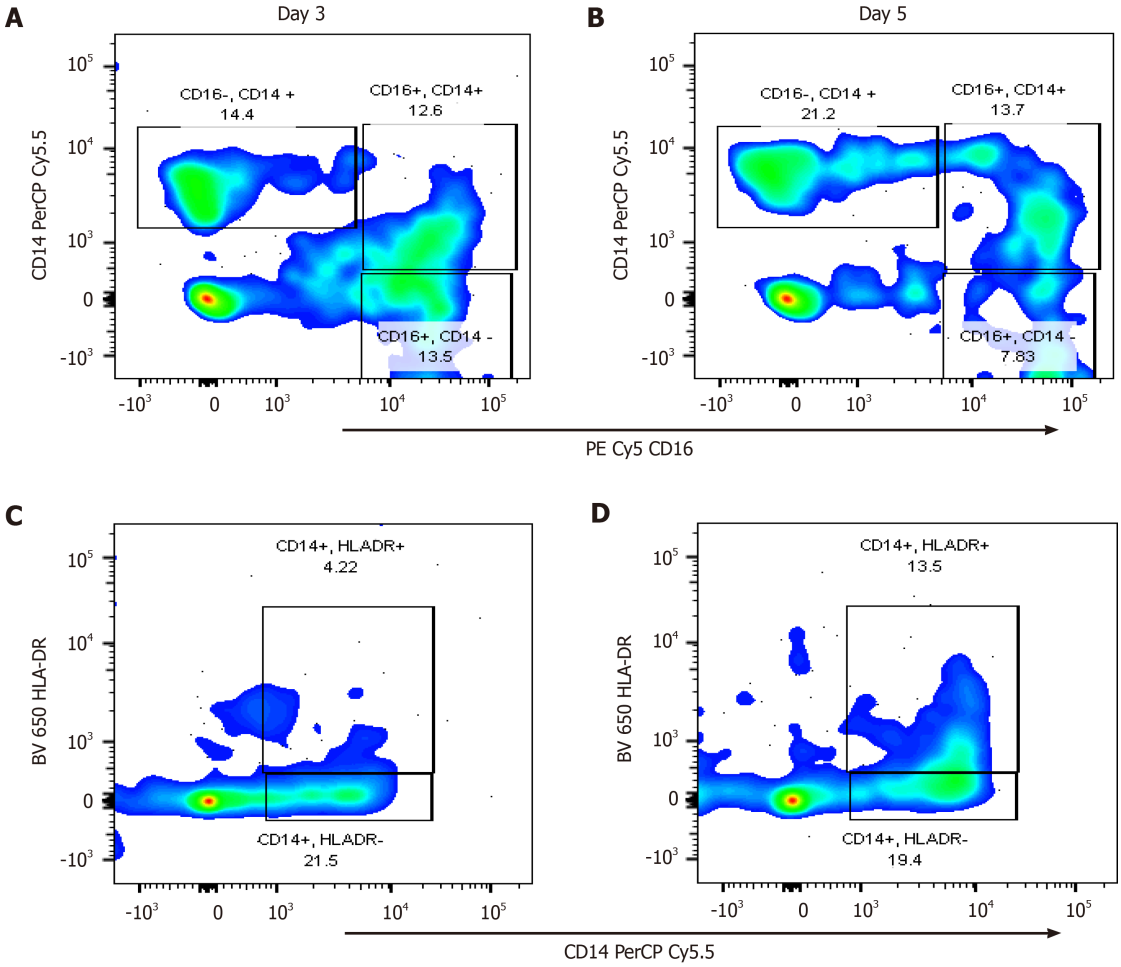

Figure 6 A plot generated from FlowJo™ version 10 (Oregon, United States) of an severe acute pancreatitis patient for the monocyte subpopulations. Cells were gated into intermediate ($CD16^+CD14^+$), classical ($CD16^-CD14^+$) and non-classical monocytes ($CD16^+CD14^-$). A and B: They showed CD14CD16 plot for Day 3 and Day 5 respectively. C and D: They showed a plot of CD14HLA-DR for Day 3 and 5 respectively. The percentage of $HLA\text{-}DR^+$ monocytes increased from 4% on Day 3 to 13% on Day 5. BUV: BD Horizon Brilliant™ Ultraviolet; CD: Cluster of differentiation; Cy: Cyanine; BV: Brilliant Violet™; HLA DR: Human leukocyte D related; PE: Phycoerythrin; PerCP: Peridinin-Chlorophyll-protein; CD: Cluster of differentiation.

This provides a possible link between monocyte expression and upregulation of *CCR8* in more severe patients observed in this study.

CCR8 gene was concomitantly upregulated with *TLR1*, *NOD1*, *CASP1*, and *GAPDH* (Table 2, Supplementary Table 3). These genes are all expressed on activated monocytes[39] and were observed in the analysis of the pooled samples (Table 2 and Supplementary Figure 3). Studies looking at inflammation in pancreatic injury have shown that continued release of proinflammatory cytokines by macrophages, increased number of neutrophils, and excess levels of nitric oxide impaired tissue regeneration and contributed to organ tissue damage[48]. This suggests that the observed increase of *CCR8* levels in MSAP patients, and to an extent the SAP patients, could be associated with macrophages and monocytes levels. The 1090631 fold upregulation of *CCR8* in the SAP group was due to one sample and was observed in the results of the pooled sample in the RT^2 profiler analysis (Figure 4C). This means that the *CCR8* expression levels in the SAP group may not necessarily be representative due to the limited number in this group suggesting the need for further research.

Several genes associated with Th2 Lymphoid cells were upregulated in the MSAP group. The upregulation of the transcription factor, *GATA-3*, and the *IL4, IL5, and IL13* genes in Supplementary Table 3 may indicate a stronger type-2 response in MSAP patients compared to the SAP group, which is a result of excessive recruitment of macrophages and monocytes in pancreatic tissues and the bloodstream respectively [22,49]. On the other hand, upregulation of proinflammatory genes such as *IL6*, *CRP*, and *FOXP3* (Supplementary Table 3) associated with *CCR8/CCL1* in the MSAP group may be attributed to ILC3 and or Th17 cells. Overexpression of *FOXP3 via* the *STAT3* pathway was directly proportional to the observed increase in fold changes for *IL-17A*

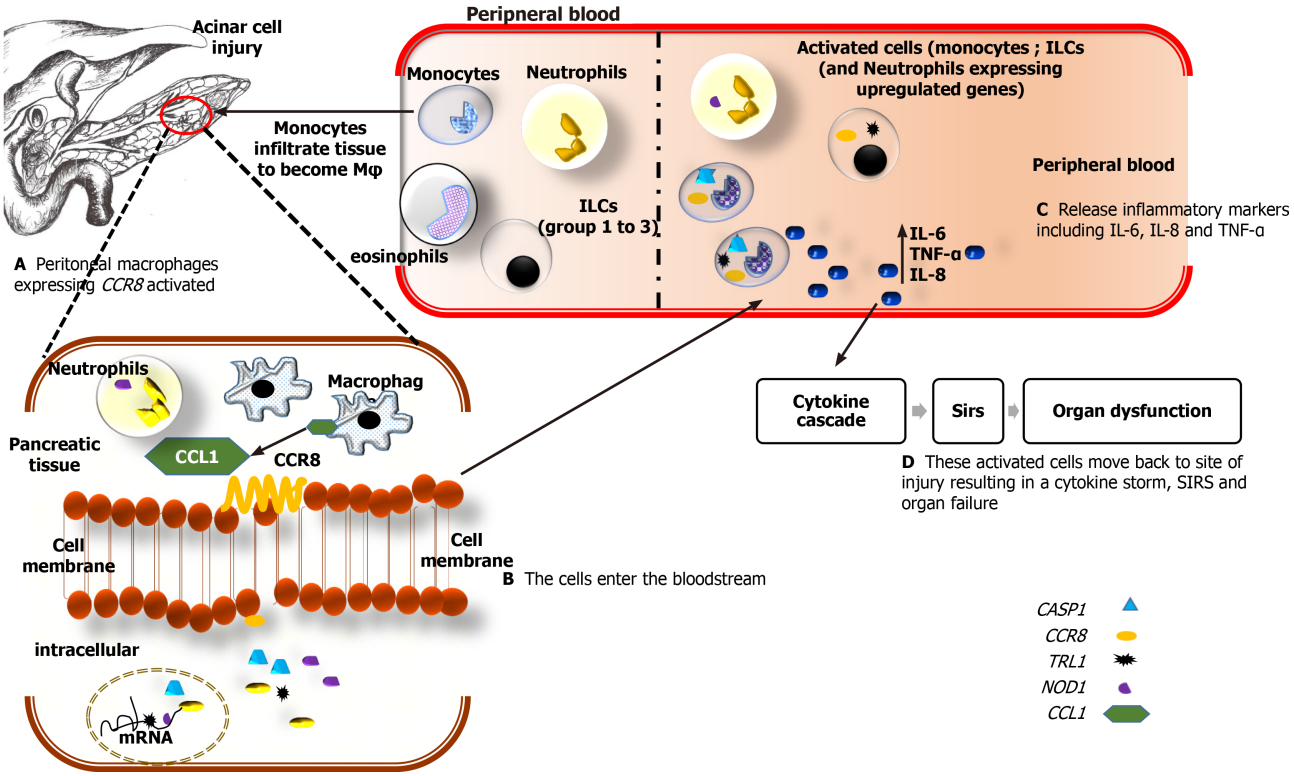

Figure 7 The schematic shows the possible mechanism by which chemokine receptor 8 is upregulated in peripheral blood. A: Upon elevation of trypsin in the pancreas due to acinar cell injury in patients with acute pancreatitis, monocytes, lymphoid cell groups (ILCs), neutrophils and eosinophils migrate to the site of injury. Once in the pancreatic tissue, the monocytes become activated to macrophages (φ) which then express the chemokine (C-C motif) ligand 1 (*CCL1*) gene. The *CCL1* binds in turn binds to the chemokine receptor 8 (CCR8) receptor on the surface of macrophages, ILC2 cells, and neutrophils. In patients with MSAP, ILC2 related cytokines, interleukin-4 (IL-4), IL-5, and IL-13 are upregulated while downregulation of these cytokines was observed in SAP patients (Supplementary Table 3). The observed increases in *CCR8* in the SAP patients may be due to excessive activation of macrophages and monocytes. The activated cells (ILCs, neutrophils, monocytes) may be releasing IL-1-β, tumor necrosis factor alpha (TNF-α), IL-6 at local sites, which send signals that activate and recruit inflammatory cells which include macrophages, neutrophils, ILC2, and ILC3; B-D: These cells migrate into the bloodstream and will express Toll-like receptor 1, Nucleotide-binding oligomerization domain, caspase 1 genes. These genes are mainly expressed on monocytes that will release proinflammatory cytokines such as IL-6, IL-8, and TNF-α and, depending on their levels in the peripheral blood, will cause dysregulation that leads to a systemic inflammatory response and consequently single or multiple organ failure. IL: Interleukin; *CASP1*: Caspase 1, apoptosis-related cysteine peptidase; *CCR8*: Chemokine receptor 8; *TRL1*: Toll-like receptor-1; *NOD1*: Nucleotide-binding oligomerization domain-containing protein 1; *CCL1*: Chemokine ligand 1; TNF-α: Tumor necrosis factor alpha.

and *IL 23A* genes[19]. This is expected since *STAT3* is responsible for differentiation in Th17 Lineage and has been implicated in autoimmune diseases[46]. Therefore, it is likely that an elevation of these Th17 cytokines may be due to the ILC3 group[22].

This preliminary study has its limitations. Like many clinical studies, obtaining the ideal sample size, which is adequate (not too small or too big) for the interpretation of the results is important in how the results are extrapolated. Here, we sampled 40 patients overall with MAP, MSAP, and SAP at three different time points (D3, 5, and 7) and tested samples from 29 as shown in Figure 1. Due to this being a time study, we noted a trend where patients dropped out after consenting or were too weak or too sick to participate, especially from the SAP group. Presentation at the hospital was also usually delayed and this could be attributed to the socio-economic state of the patients who tend to delay seeking treatment. To circumvent this challenge, where applicable (especially for the SAP group), the results presented here have been discussed with inferences to supporting literature and further work with expanded numbers is planned.

Possible concerns about treatment affecting the expression of *CCR8* and IL-6 are valid. However, the general treatment guideline for AP in the hospital unit is based on supportive care where all patients are treated according to the same protocol, none of which can influence immune responses. In mild AP, only analgesia and fluids are prescribed and nutrition is maintained with a combination of enteral and/or parenteral feeding. In the Moderate and severe group, organ support is implemented depending on the organ dysfunction. Antibiotics nor steroids are used routinely in the first phase of the inflammatory response in any of the patients and as such, we do not think that the treatment will influence the expression of IL-6 or CCR8 up to and including day 7.

CONCLUSION

This study proposes possible linkages between the upregulation of *CCR8* and IL6 elevation with AP disease severity. Simultaneously, monocytes, ILCs, and Th2 Lymphocyte frequencies, found to differentiate MAP, may differentiate MSAP and SAP groups. These findings may be beneficial as prognostic parameters in early AP stratification. Despite the limitation in sample sizes, these preliminary findings are supported by the literature. The data indicate that *CCR8*, IL-6 Levels, and associated immune molecules and cell types may be promising parameters to improve or complement existing ones for patient risk stratification in AP. The data further contributes to the scarce literature in AP from an African setting.

ARTICLE HIGHLIGHTS

Research background

Chemokine receptor 8 (*CCR8*) is a chemokine receptor that is highly expressed on monocytes and cells of T helper type-2 Lineage including innate lymphoid cells group 2 and 3 (ILC2 and 3). Upregulation in more severe cases of acute pancreatitis (AP) may be linked to elevated levels of interleukin (IL)-6 and upregulation of *CCR8*.

Research motivation

There is currently no known treatment for AP and no clear early immune markers to effectively distinguish between moderately severe AP and severe AP. The complex underlying pathophysiology further complicates this, necessitating studies to better understand the ensuing immune responses for improved stratification.

Research objectives

To identify the role of the CCR8, expressed by Th2 Lymphocytes and peritoneal macrophages, and its possible association to IL-6 as early markers to assist with AP stratification.

Research methods

A total of 40 patients were recruited from the Chris Hani Baragwanath Hospital and the Charlotte Maxeke Johannesburg Academic Hospital in Johannesburg, South Africa. Bioassays were performed on 29 patients consisting of 14 mild AP (MAP), 11 moderately severe AP (MSAP), and 4 severe AP (SAP) and 6 healthy controls as part of a preliminary study. A total of 12 mL of blood samples were collected at Day (D) 1, 3, 5, and 7 post epigastric pain. Using multiplex immunoassay panels, real-time polymerase chain reaction (RT-PCR) arrays, and multicolour flow cytometry analysis, immune response-related proteins, genes, and cells were profiled respectively. The fold change (FC) analysis was used to determine differences between the groups.

Research results

This study shows possible linkages between increasing *CCR8* expression and severity in mainly MSAP patients when compared to MAP. The concentration of IL-6 was significantly different at D3 post epigastric pain in both MAP group and MSAP group with $P = 0.001$ and $P = 0.013$ respectively, in a multiplex assay. *CCR8* was shown to increase with severity with the following FC for MAP (1.33), MSAP (38.28) to SAP (1172.45). Further verification studies using RT-PCR showed fold change increases of *CCR8* in MSAP and SAP ranging from 1000 to 1000000 times when represented as Log_{10}, compared to healthy controls respectively at Day 3 post epigastric pain.

Research conclusions

Notable increases in *CCR8* and IL-6 in severe patients were observed. Lymphocyte and monocyte cell frequencies suggest that in MAP, IL-6 was highly expressed in lymphocytes, and the severe patients (MSAP and SAP) were highly expressed by monocytes. This provides an avenue for exploring AP stratification to improve management.

Research perspectives

There is an opportunity to further investigate IL-6 producing cells such as T helper 2 lymphocytes, monocytes, and innate lymphoid cells group 2 and associated *CCR8*

increases, to determine cell-associated cytokine as a novel approach for AP risk stratification.

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REFERENCES

1 **Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]

2 **Leung PS**, Ip SP. Pancreatic acinar cell: its role in acute pancreatitis. *Int J Biochem Cell Biol* 2006; **38**: 1024-1030 [PMID: 16423553 DOI: 10.1016/j.biocel.2005.12.001]

3 **Zheng L**, Xue J, Jaffee EM, Habtezion A. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. *Gastroenterology* 2013; **144**: 1230-1240 [PMID: 23622132 DOI: 10.1053/j.gastro.2012.12.042]

4 **Kylänpää L**, Rakonczay Z Jr, O'Reilly DA. The clinical course of acute pancreatitis and the inflammatory mediators that drive it. *Int J Inflam* 2012; **2012**: 360685 [PMID: 23304633 DOI: 10.1155/2012/360685]

5 **Nesvaderani M**, Eslick GD, Vagg D, Faraj S, Cox MR. Epidemiology, aetiology and outcomes of acute pancreatitis: A retrospective cohort study. *Int J Surg* 2015; **23**: 68-74 [PMID: 26384834 DOI: 10.1016/j.ijsu.2015.07.701]

6 **Anderson F**, Thomson SR. Pancreatitis in a high HIV prevalence environment. *S Afr Med J* 2017; **107**: 706-709 [PMID: 28809620 DOI: 10.7196/SAMJ.2017.v107i8.10296]

7 **Derrick D**, Frandy F, Wirawan AD. Acute Pancreatitis-Etiology, Pathogenesis, Pathophysiology and the current trend in its management and prevention. *Indones J Gastroenterol Hepatol Dig Endosc (Online)* 2019; **20**: 27-38 [DOI: 10.24871/201201927-37]

8 **De Campos T**, Parreira JG, Assef JC, Rizoli S, Nascimento B, Fraga GP. Classification of acute pancreatitis. *Rev Col Bras Cir* 2013; **40**: 164-168 [PMID: 23752646 DOI: 10.1590/s0100-69912013000200015]

9 **Dellinger EP**, Forsmark CE, Layer P, Lévy P, Maraví-Poma E, Petrov MS, Shimosegawa T, Siriwardena AK, Uomo G, Whitcomb DC, Windsor JA; Pancreatitis Across Nations Clinical Research and Education Alliance (PANCREA). Determinant-based classification of acute pancreatitis severity: an international multidisciplinary consultation. *Ann Surg* 2012; **256**: 875-880 [PMID: 22735715 DOI: 10.1097/SLA.0b013e318256f778]

10 **Singh P**, Garg PK. Pathophysiological mechanisms in acute pancreatitis: Current understanding. *Indian J Gastroenterol* 2016; **35**: 153-166 [PMID: 27206712 DOI: 10.1007/s12664-016-0647-y]

11 **Leal C**, Almeida N. Predicting Severity in Acute Pancreatitis: A Never-Ending Quest. *GE Port J Gastroenterol* 2019; **26**: 232-234 [PMID: 31328136 DOI: 10.1159/000499680]

12 Do JH. Mechanism of Severe Acute Pancreatitis: Focusing on Development and Progression. *Korean J Pancreas Biliary Tract* 2015; **20**: 115-123 [DOI: 10.15279/kpba.2015.20.3.115]

13 **Lerch MM**, Gorelick FS. Models of acute and chronic pancreatitis. *Gastroenterology* 2013; **144**: 1180-1193 [PMID: 23622127 DOI: 10.1053/j.gastro.2012.12.043]

14 **Stirling AD**, Moran NR, Kelly ME, Ridgway PF, Conlon KC. The predictive value of C-reactive protein (CRP) in acute pancreatitis - is interval change in CRP an additional indicator of severity? *HPB (Oxford)* 2017; **19**: 874-880 [PMID: 28693979 DOI: 10.1016/j.hpb.2017.06.001]

15 **Meher S**, Mishra TS, Sasmal PK, Rath S, Sharma R, Rout B, Sahu MK. Role of Biomarkers in Diagnosis and Prognostic Evaluation of Acute Pancreatitis. *J Biomark* 2015; **2015**: 519534 [PMID: 26345247 DOI: 10.1155/2015/519534]

16 **Jakkampudi A**, Jangala R, Reddy BR, Mitnala S, Nageshwar Reddy D, Talukdar R. NF-κB in acute pancreatitis: Mechanisms and therapeutic potential. *Pancreatology* 2016; **16**: 477-488 [PMID: 27282980 DOI: 10.1016/j.pan.2016.05.001]

17 **Sharma D**, Jakkampudi A, Reddy R, Reddy PB, Patil A, Murthy HVV, Rao GV, Reddy DN, Talukdar R. Association of Systemic Inflammatory and Anti-inflammatory Responses with Adverse Outcomes in Acute Pancreatitis: Preliminary Results of an Ongoing Study. *Dig Dis Sci* 2017; **62**: 3468-3478 [PMID: 29080144 DOI: 10.1007/s10620-017-4813-6]

18 **Peng C**, Li Z, Yu X. The Role of Pancreatic Infiltrating Innate Immune Cells in Acute Pancreatitis.

Int J Med Sci 2021; **18**: 534-545 [PMID: 33390823 DOI: 10.7150/ijms.51618]
19 **Zambrano-Zaragoza JF**, Romo-Martínez EJ, Durán-Avelar Mde J, García-Magallanes N, Vibanco-Pérez N. Th17 cells in autoimmune and infectious diseases. *Int J Inflam* 2014; **2014**: 651503 [PMID: 25152827 DOI: 10.1155/2014/651503]
20 **Fonteh P**, Smith M, Brand M. Adaptive Immune Cell Dysregulation and Role in Acute Pancreatitis Disease Progression and Treatment. *Arch Immunol Ther Exp (Warsz)* 2018; **66**: 199-209 [PMID: 29189884 DOI: 10.1007/s00005-017-0495-1]
21 **Marshall JS**, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol* 2018; **14**: 49 [PMID: 30263032 DOI: 10.1186/s13223-018-0278-1]
22 **Vivier E**, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie ANJ, Mebius RE, Powrie F, Spits H. Innate Lymphoid Cells: 10 Years On. *Cell* 2018; **174**: 1054-1066 [PMID: 30142344 DOI: 10.1016/j.cell.2018.07.017]
23 **Panda SK**, Colonna M. Innate Lymphoid Cells in Mucosal Immunity. *Front Immunol* 2019; **10**: 861 [PMID: 31134050 DOI: 10.3389/fimmu.2019.00861]
24 **Willinger T**. Metabolic Control of Innate Lymphoid Cell Migration. *Front Immunol* 2019; **10**: 2010 [PMID: 31507605 DOI: 10.3389/fimmu.2019.02010]
25 **Crome SQ**, Ohashi PS. Immunoregulatory functions of innate lymphoid cells. *J Immunother Cancer* 2018; **6**: 121 [PMID: 30454038 DOI: 10.1186/s40425-018-0433-8]
26 **Bielecki B**, Mazurek A, Wolinski P, Glabinski A. Expression of chemokine receptors CCR7 and CCR8 in the CNS during ChREAE. *Scand J Immunol* 2007; **66**: 383-392 [PMID: 17850582 DOI: 10.1111/j.1365-3083.2007.01954.x]
27 **Oshio T**, Kawashima R, Kawamura YI, Hagiwara T, Mizutani N, Okada T, Otsubo T, Inagaki-Ohara K, Matsukawa A, Haga T, Kakuta S, Iwakura Y, Hosokawa S, Dohi T. Chemokine receptor CCR8 is required for lipopolysaccharide-triggered cytokine production in mouse peritoneal macrophages. *PLoS One* 2014; **9**: e94445 [PMID: 24714157 DOI: 10.1371/journal.pone.0094445]
28 **Cantor J**, Haskins K. Recruitment and activation of macrophages by pathogenic CD4 T cells in type 1 diabetes: evidence for involvement of CCR8 and CCL1. *J Immunol* 2007; **179**: 5760-5767 [PMID: 17947648 DOI: 10.4049/jimmunol.179.9.5760]
29 **Zychowska M**, Rojewska E, Piotrowska A, Kreiner G, Nalepa I, Mika J. Spinal CCL1/CCR8 signaling interplay as a potential therapeutic target - Evidence from a mouse diabetic neuropathy model. *Int Immunopharmacol* 2017; **52**: 261-271 [PMID: 28961489 DOI: 10.1016/j.intimp.2017.09.021]
30 **Thomson JE**, Brand M, Fonteh P. The immune imbalance in the second hit of pancreatitis is independent of IL-17A. *Pancreatology* 2018; **18**: 246-252 [PMID: 29422392 DOI: 10.1016/j.pan.2018.01.007]
31 **Kay PS**, Smith M, Brand M. The Initiating Immune Response of Acute Pancreatitis May be Mediated by the T-Helper 17 Pathway. *Pancreas* 2017; **5**
32 **Thomson JE**, Nweke EE, Brand M, Nel M, Candy GP, Fonteh PN. Transient Expression of Interleukin-21 in the Second Hit of Acute Pancreatitis May Potentiate Immune Paresis in Severe Acute Pancreatitis. *Pancreas* 2019; **48**: 107-112 [PMID: 30451792 DOI: 10.1097/MPA.0000000000001207]
33 **Huggett JF**, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, Bustin SA. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. *Clin Chem* 2013; **59**: 892-902 [PMID: 23570709 DOI: 10.1373/clinchem.2013.206375]
34 **Bustin SA**, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009; **55**: 611-622 [PMID: 19246619 DOI: 10.1373/clinchem.2008.112797]
35 **Yan Z**, Gao J, Lv X, Yang W, Wen S, Tong H, Tang C. Quantitative Evaluation and Selection of Reference Genes for Quantitative RT-PCR in Mouse Acute Pancreatitis. *Biomed Res Int* 2016; **2016**: 8367063 [PMID: 27069927 DOI: 10.1155/2016/8367063]
36 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]
37 **Cesar Machado MC**, Mendonca Coelho AM. Role of Peritoneal Macrophages on Local and Systemic Inflammatory Response in Acute Pancreatitis. *IntechOpen* 2012
38 **Mayerle J**, Dummer A, Sendler M, Malla SR, van den Brandt C, Teller S, Aghdassi A, Nitsche C, Lerch MM. Differential roles of inflammatory cells in pancreatitis. *J Gastroenterol Hepatol* 2012; **27** Suppl 2: 47-51 [PMID: 22320916 DOI: 10.1111/j.1440-1746.2011.07011.x]
39 **Thul PJ**, Lindskog C. The human protein atlas: A spatial map of the human proteome. *Protein Sci* 2018; **27**: 233-244 [PMID: 28940711 DOI: 10.1002/pro.3307]
40 **Wu BU**, Banks PA. Clinical management of patients with acute pancreatitis. *Gastroenterology* 2013; **144**: 1272-1281 [PMID: 23622137 DOI: 10.1053/j.gastro.2013.01.075]
41 **Nielsen CM**, White MJ, Goodier MR, Riley EM. Functional Significance of CD57 Expression on Human NK Cells and Relevance to Disease. *Front Immunol* 2013; **4**: 422 [PMID: 24367364 DOI: 10.3389/fimmu.2013.00422]
42 **Shrivastava P**, Bhatia M. Essential role of monocytes and macrophages in the progression of acute

pancreatitis. *World J Gastroenterol* 2010; **16**: 3995-4002 [PMID: 20731012 DOI: 10.3748/wjg.v16.i32.3995]

43 **Gukovsky I**, Li N, Todoric J, Gukovskaya A, Karin M. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1199-209.e4 [PMID: 23622129 DOI: 10.1053/j.gastro.2013.02.007]

44 **Endo Y**, Hirahara K, Yagi R, Tumes DJ, Nakayama T. Pathogenic memory type Th2 cells in allergic inflammation. *Trends Immunol* 2014; **35**: 69-78 [PMID: 24332592 DOI: 10.1016/j.it.2013.11.003]

45 **Barsheshet Y**, Wildbaum G, Levy E, Vitenshtein A, Akinseye C, Griggs J, Lira SA, Karin N. $CCR8^{+}$ $FOXp3^{+}$ T_{reg} cells as master drivers of immune regulation. *Proc Natl Acad Sci U S A* 2017; **114**: 6086-6091 [PMID: 28533380 DOI: 10.1073/pnas.1621280114]

46 **Eruslanov E**, Stoffs T, Kim WJ, Daurkin I, Gilbert SM, Su LM, Vieweg J, Daaka Y, Kusmartsev S. Expansion of CCR8(+) inflammatory myeloid cells in cancer patients with urothelial and renal carcinomas. *Clin Cancer Res* 2013; **19**: 1670-1680 [PMID: 23363815 DOI: 10.1158/1078-0432.CCR-12-2091]

47 **Folias AE**, Penaranda C, Su AL, Bluestone JA, Hebrok M. Aberrant innate immune activation following tissue injury impairs pancreatic regeneration. *PLoS One* 2014; **9**: e102125 [PMID: 25010227 DOI: 10.1371/journal.pone.0102125]

48 **Davis BP**, Rothenberg ME. Inflammatory and Effector Cells/Cell Migration. Third Edition. Pediatric Allergy: Principles and Practice: Third Edition. Elsevier Inc, 2016: 41-53.e4 [DOI: 10.1016/B978-0-323-29875-9.00005-7]

49 **Dabrowski A**, Osada J, Dabrowska MI, Wereszczynska-Siemiatkowska U. Monocyte subsets and natural killer cells in acute pancreatitis. *Pancreatology* 2008; **8**: 126-134 [PMID: 18382098 DOI: 10.1159/000123605]

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ORIGINAL ARTICLE

Observational Study

Prediction of hereditary nonpolyposis colorectal cancer using mRNA *MSH2* quantitative and the correlation with nonmodifiable factor

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Institutional review board statement: The study follows the international review ethical board with approval from the Hasanuddin University Ethics Committee (Indonesia).

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Abstract

BACKGROUND

Hereditary non-polyposis colon cancer is a dominantly inherited syndrome of

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colorectal cancer (CRC), with heightened risk for younger population. Previous studies link its susceptibility to the DNA sequence polymorphism along with Amsterdam and Bethesda criteria. However, those fail in term of applicability.

AIM

To determine a clear cut-off of *MSH2* gene expression for CRC heredity grouping factor. Further, the study also aims to examine the association of risk factors to the CRC heredity.

METHODS

The cross-sectional study observed 71 respondents from May 2018 to December 2019 in determining the CRC hereditary status through *MSH2* mRNA expression using reverse transcription-polymerase chain reaction and the disease's risk factors. Data were analyzed through Chi-Square, Fischer exact, t-test, Mann-Whitney, and multiple logistics.

RESULTS

There are significant differences of *MSH2* within CRC group among tissue and blood; yet, negative for significance between groups. Through the blood gene expression fifth percentile, the hereditary CRC cut-off is 11059 fc, dividing the 40 CRC respondents to 32.5% with hereditary CRC. Significant risk factors include age, family history, and staging. Nonetheless, after multivariate control, age is just a confounder. Further, the study develops a probability equation with area under the curve 82.2%.

CONCLUSION

Numerous factors have significant relations to heredity of CRC patients. However, true important factors are staging and family history, while age and others are confounders. The study also established a definite cut-off point for heredity CRC based on mRNA *MSH2* expression, 11059 fc. These findings shall act as concrete foundations on further risk factors and/or genetical CRC future studies.

Key Words: Colorectal cancer; *MSH2* gene; Non-modifiable factors; Risk probability

Core Tip: The study has determined a definitive cut-off for grouping colorectal cancer (CRC) with its heredity using the *MSH2* mRNA gene expression, which amounts to 11059 fc. The gene expressions may differ between blood and tissue sample of the CRC group, yet none between CRC and control group. Nevertheless, subsequent risk factors of family history and staging are found to be significant toward the heredity. The after-mentioned risk factors act as urgent reminder for highly risky people with family history of CRC and/or high CRC staging to have themselves and their immediate family members to undergo routine examinations as well as strict preventions.

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INTRODUCTION

Colorectal cancer (CRC) or also known as colorectal adenocarcinoma is a group of cancer that manifest in the colon and/or rectum. The cancer first arises as polyps that comprised of extraneous cells from the uncontrolled proliferation because of genetic

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mutations. These benign masses of flesh will then achieve even greater hyper-replication, survival, and angiogenesis, leading to a malignant carcinoma (CRC) which then can metastasize[1].

In fact, CRC holds the fourth place in the top ten of the most diagnosed global cancer with around 2000000 incidence cases, while taking third place for worldwide cancer mortality with approximately 1000000 deaths[2]. Within very high human development index countries, Hungary and Norway have the highest age-standardized rates of CRC over 100000 populations for male and female respectively (70.6 and 39.3)[3]. Meanwhile, the top age-standardized death rates are taken both for male and female by Hungary with 31.2 and 14.8 over 100000 population[3].

Specifically, CRC can be further divided according to its differentiation, epidemiology, and hereditary. Interesting potential lies dormant in CRC hereditary status, where as far as the author's known, there has been no gold standard measure to classify one's CRC into hereditary [*i.e.*, hereditary non-polyposis colon cancer (HNPCC)] or sporadic.

HNPCC or sometimes known to the general citizens as lynch syndrome (LS) is a hereditary mutation of the *MLH1*, *MSH2*, *MSH6*, *EpCAM*, and *PMS2* genes which contribute to the development of CRC yet also the passing of the autosomal dominant mutated genes and thus the heightened susceptibility to the offspring[4]. LS can be found in younger people compared to overall CRC as these mutations provide grounds for CRC rapid development. Within his or her lifetime, the risk of developing LS is around 4.1%-4.4%[5].

The incidence of LS can be said to mainly comes from the mutation of *MSH2* gene on chromosome 2. The protein translated by that subsequent gene encode MutS Homolog 2 protein which functions as a DNA mismatch repair protein. When doing its intended functions, it bonds with MSH6 or MSH3 to procure MutSα or MutSb complex according to specifications of the DNA damage, namely: Transcription repair, base excision repair, homologous recombination, *etc*[6-8].

Identification of those who carry the mutated genes of *MSH2* or the groups would benefit the patients as early detection and adequate prevention can reduce morbidity, mortality, and recurrence risk of LS[9,10]. Several studies have tried to implement the Bethesda and Amsterdam criteria to solve this dynamic screening of LS, yet the effort failed due to its complexity and arduousness especially in small family and late age of onset settings[11-15]. Consequently, when trying to address the problem from its roots of the mutated genes, establishment of the definite gene expression may act as a well cut-off point to categorize CRC into LS or non-LS with high potential of becoming a gold standard measure. The subsequent practices utilize specific enzymes that pinpoint the post-transcription mRNA strategically[16].

These techniques of separating the CRC intro groups, enable risk factor assessments toward hereditary and sporadic CRC types. Modifiable risk factors for LS and CRC are body mass index (BMI), physical activity, diet, lifestyle (*i.e.*, smoking, alcohol, and narcotics), routine medications, and diabetes mellitus (DM). Insulin resistance and the hyperglycemic state of the body can predispose a person to CRC. The excess of blood sugar trigger Warburg effect (carcinogenic glycolysis) through modulation of glucose metabolism[17,18]. Studies on DM relations to CRC prove a 1.17-1.42 hazard ratio along with 11536/559375 DM patients have CRC[19,20]. On the other hand, non-modifiable risk factors for LS and CRC involve the race, age, gender, heredity, radiations, and some diseases (*i.e.*, inflammatory bowel disease or cystic fibrosis).

Above all, the hazardous nature of CRC and LS, scarce information on CRC risk factors identifications, and the lack of gold standard for categorizing hereditary measurements, thus the present study urgently aimed to compute suitable *MSH2* gene expression for appropriate cut-off and certify the associations from the risk factors.

MATERIALS AND METHODS

Study population and subject enrollment

The present study was conducted using cross-sectional design with 71 respondents divided into 31 respondents in the control group, those who have been sequentially matched (*i.e.*, age, sex, and BMI) with the case group. The study involves tumor and malignancy sector which are a sensitive section of health as it rapidly deteriorates health while integrated to other bodily system. Henceforth, strict exclusion criteria were adapted in the current study, namely: (1) The presence or history of other cancer; (2) The presence or history of inflammatory bowel disease; (3) Chemotherapy or radiotherapy in progress or history; (4) Refusal of participation; and (5) Missing or

incomplete data.

From May 2018 to December 2019, the respondents are taken from the internal medicine outpatient and inpatient department of Tarakan General Hospital and Siloam Hospitals Lippo Village through consecutive sampling. Sample size calculated using 5% alpha and power 80%.

Research operatives

Three major steps are contained within this study. Initially, the study collects respondents and their clinical data of demographics and malignancy characteristics. Then, the respective mRNA gene expression of *MSH2* was quantified using polymerase chain reaction (PCR) and studied in assessing within groups and between groups differences as well as its hereditary significance. Whereas the last component of the study involves risk factor analysis toward hereditary of CRC and its probability model.

Sample collection and data measurement

Biopsy tissues of suspected CRC tissues and venous blood are the key samples of this study. Then, the samples are placed in a L6 buffer and have their RNA extracted. The L6 buffer are concocted earlier according to the RNA Boom extraction method of the Hasanuddin laboratory. Next, RT-PCR targeting the *MSH2* mRNA were done to measure the gene expression.

The PCR are conducted through the DNA multiplication, denaturation, primer attachment, and amplification stage. Specific Korean primers are supplied to specifically target the *MSH2* gene: CAT-CCA-GGC-ATG-CTT-GTG-TTG-A (forward) and GCA-GTC-CAC-AAT-GGA-CAC-TTC (reverse). The mechanics and PCR analysis follow the Bio-Rad protocols from Unites States of America using the power SYBR green master mix kit[21-23].

Statistical analysis

Data tabulation was done through Microsoft Excel 365, while SPSS v26 is the software of choice for the statistical analysis. Missing data is excluded from the study. The respondents' demographics are characterized using descriptive statistics; yet, Chi-Square or Fischer test are applied for categorical factors, while t-test or Mann-Whitney for numerical factors. Significance obtained if P value < 0.05.

RESULTS

The present study employs 71 respondents among the 19-mo study period, which comprised of 56.34% in the case group and 43.66% in the control group. Respondents within the control group are adequately matched according to the case group characteristics, proven with no significant difference (P > 0.05) in the demographic characteristics between the groups as depicted in Table 1.

Among the respondents on the CRC group, histopathological samples are taken and observed. Specifically, 90.00% respondents have adenocarcinoma while 7.50% have adenocarcinoma with Signet ring cell and 2.50% have neuroendocrine carcinoma. Nevertheless, specifications on the histopathological profile can also be seen from the level of differentiation. Well-differentiated biopsies are found in 26 (66.7%) respondents, intermediately differentiated in 6 (15.4%) respondents, and poorly differentiated in 7 (17.9%).

Subsequently, the study utilizes PCR analysis to measure the *MSH2* mRNA gene expression in blood and tissue between the groups. Significance is observed when comparing the gene expression within the CRC group between blood and tissue (12554.50 *vs* 7485.00). However, as pictured in Table 2, there is no significant difference of MSH2 mRNA expression between CRC and control groups (P = 0.116 and 0.465).

Moreover, the group with CRC were then subdivided based on each respondent hereditary status. One is considered having hereditary condition if his or her blood mRNA *MSH2* gene expression less than the cut-off from the fifth percentile, 11059 fc. It was established that 67.50% of the CRC group respondents have non-hereditary status, even including one-third of those with positive family history of CRC. Likewise, Table 3 portrayed the relationship of risk factors to hereditary status.

CRC has numerous substantial risk factors in theory. However, within the 40 CRC group respondents, only three factors are deemed essential hereditarily: Age, tumor staging, and family history. Uniquely, among all locations and proximity potential for CRC, hereditary does not hold any significance (P = 0.595 and 0.476). There is also no

Table 1 Baseline characteristic and *MSH2* gene expression

Characteristic	CRC group	Control group	*P* value
Age (yr)	56.8 ± 8.4	51.6 ± 13.4	> 0.05
Sex			
Male	21 (52.5)	13 (41.9)	
Female	19 (47.5)	18 (58.1)	
Body mass index (kg/m^2)	22.4 ± 3.3	23.6 ± 3.4	

CRC: Colorectal cancer.

Table 2 *MSH2* gene expression

MSH2 expression	CRC group	Control group	*P* value
Blood			
Median (range)	12554.50 (4230.00-14559.00)	12146.00 (11029.00-13633.00)	0.116
mean ± SD	11411.05 ± 2912.45	12219.87 ± 756.87	0.465
Tissue	7 485.00 (4174.00-14218.00)		

CRC: Colorectal cancer; SD: Standard deviation.

difference on biopsies differentiation between the groups (P = 0.287 and 0.999).

The study found 5.60 times increase in risk of CRC between those < 50 years old and over, in which a 9.05-year difference is found between the subsequent groups. Similarly, respondents with hereditary CRC are mostly within the C stage (54.85%) while the non-group mostly in B (37.04%). This pattern holds true even when the stages are divided into C-D and A-B clusters, where the CRC group dominate the former cluster while the latter cluster for the rest. Yet, the clustering of stages is insignificant (P = 0.116).

Family history of CRC and its hereditary follow a significant linear relationship (P = 0.008). Those who has history of CRC in his/her family majorly belong to the hereditary group (61.54%) and vice versa. There is also a notable risk increase for those who has CRC history amounting to 9.20 times than those who don't.

Bringing further to multivariate perspective, the current study applies multiple logistic analysis to find the truly significant risk factors toward CRC hereditary and its subsequent probability. Table 4 explain the regression where staging and family history are truly significant (P = 0.034 and 0.006), while age is just confounder. The unstandardized coefficients can be morphed to a LOGIT and probability functions of CRC hereditary as follows (Eq. 1 and 2).

$$\text{LOGIT} = -3.165 + 2.395 \times \text{staging} + 3.126 \times \text{history} \quad \text{(Eq. 1)}$$

$$\text{Probability} = \frac{1}{1 + e^{-\text{LOGIT}}} = \frac{1}{1 + e^{-(-3.165 + 2.395 \times \text{staging} + 3.126 \times \text{history})}} \quad \text{(Eq. 2)}$$

The variables of staging and history hold the value of either one or zero. Representatively, score of one amount to C or D in the staging component and presence of family history. Furthermore, the equations and probabilities are having good fit and not due to chance by having significant Hosmer and Lameshow statistics as well as 82.2% area under the curve (AUC) for receiving operator curve (ROC) (Figure 1).

DISCUSSION

From May 2018 to December 2019, 71 respondents were collected with 56.34% prevalence of CRC among the pre-elderly population (45-59 years old). The CRC group is predominantly male (52.5%) and classified with normal BMI. The discrepancy also found on similar studies in the Asia region (*i.e.*, Japan, China, Korea, and Hong Kong) where CRC has 20.7-64.8 incidence rate over 100 thousand populations, in which differ by 6.3-28.1 compared to the female incidence rate[24-27]. The theory on hormonal difference between gender is suspected to be the leading cause of the CRC

Table 3 Colorectal cancer hereditary risk factors

Factor	CRC hereditary, yes (n: 13)	CRC hereditary, no (n: 27)	OR (95%CI)	*P* value
Age	50.69 ± 14.99	59.74 ± 11.68		0.043
< 50	8 (61.54)	6 (22.22)	5.60 (1.33-23.62)	0.031
> 50	5 (38.46)	21 (77.78)		
Gender			0.69 (0.18-2.59)	0.826
Male	6 (46.15)	15 (55.56)		
Female	7 (53.85)	12 (44.44)		
Location			-	0.595
Caecum	1 (7.69)	1 (3.70)		
Ascending colon	2 (15.38)	4 (14.81)		
Transverse colon	2 (15.38)	2 (7.41)		
Descending colon	0 (0.00)	1 (3.70)		
Sigmoid	3 (23.08)	7 (25.93)		
Rectum	5 (38.46)	12 (44.44)		
Proximity			1.79 (0.44-7.32)	0.476
Proximal colon	5 (38.46)	7 (25.93)		
Distal colon	8 (61.54)	20 (74.07)		
Staging			-	0.020
A	0 (0.00)	7 (25.93)		
B	4 (30.77)	10 (37.04)		
C	7 (54.85)	9 (33.33)		
D	2 (15.38)	1 (3.70)		
Group staging			3.83 (0.93-15.72)	0.116
C-D	9 (69.23)	10 (37.04)		
A-B	4 (30.77)	17 (62.96)		
Family history			9.20 (1.97-42.97)	0.008
Yes	8 (61.54)	4 (14.81)		
No	5 (38.46)	23 (85.19)		
Differentiation[1]				
Poor	4 (30.77)	3 (11.54)	2.52 (0.46-13.80)	0.287
Intermediate	0 (0.00)	6 (23.08)	-	0.999
Well	9 (69.23)	17 (65.38)		

[1]Loss of differentiation data in one sample. CRC: Colorectal cancer; OR: Odds ratio; CI: Confidence interval.

dominancy in male.

CRC higher prevalence in the male gender is the result of female protectiveness from the disease. The sex hormone of estradiol and progesterone acts as protective mechanism for CRC development in the body. The hormonal clinical trial in 2019 observe that introduction of estradiol and progesterone combination treatment provide increased apoptosis of tumor cells ($P < 0.05$) while lowering tumor cell proliferations ($P < 0.01$)[28]. Aside from its function as a sexual hormone, estrogen or estradiol play an important role in cell turnover. It acts as a bridge for ion transport that regulates cells' pH, intracellular ions, and several protein activations[29]. Estrogen also exudes anti-inflammatory properties due to its ability to bond and modulate leukocytes including natural killer cell, neutrophils, dendritic cells, *etc*[30].

Table 4 Multivariate regression of colorectal cancer hereditary risk factor

Factor	B	SE	*P* value	OR (95%CI)
Staging	2.395	1.130	0.034	10.970 (1.199-100.382)
History	3.126	1.143	0.006	22.784 (2.423-214.273)
Constant	-3.165	1.094	0.004	0.042

SE: Standard error; OR: Odds ratio; CI: Confidence interval.

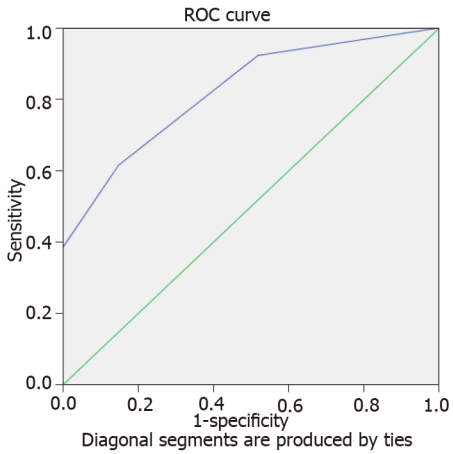

Figure 1 *MSH2* colorectal cancer hereditary probability receiving operator curve. ROC: Receiving operator curve.

Moreover, associations are observed of body anthropometry to CRC within many global and Asian studies. For instance, a Japanese prospective study finds significant difference of BMI to colon cancer respondents (P = 0.004-0.007)[31]. Akin notion is discovered in a Korean study where every five cm increment in height increase the risk for CRC in men by 1.04-1.06 and in women by 1.00-1.08[32]. Both after multivariate control with other factors. Another Korean study also view significant association of BMI to CRC especially the distal colon and rectal cancer (P < 0.001 and 0.016)[33]. These trends are not restricted only to the Asian region. A global meta-analysis obtains a relative risk of 1.25 (1.18-1.32) for CRC with the highest *vs* lowest height[34]. Likewise, a prospective systematic review corroborates the idea where CRC's risk multiply by 1.06 for every 5 kg/m^2 increase in BMI[35].

BMI has indirect relations to CRC. Higher BMI equates to higher number of cells and tissues in the body, giving rise to higher chance of genetic mutations and malignancy. People with higher stature also found to have longer intestine length than others (r: 0.827), leading to more proliferation rate and chance of cancer[36]. Further, CRC can be influenced by other diseases. Acromegaly and insulin-like growth factor abnormality for example procure modulation in body height, BMI, and thus CRC[37-39].

With the advancement of technology in healthcare and information, genetic studies for diseases' prevention, detection, and treatment have developed in a rapid pace. Specimens with DNA or RNA materials of the patients or family can be analyzed to account for the disease. This methodology has been implemented for several diseases [9]. Representatively, in assessing the hereditary status of a given CRC patient, his or her mRNA gene expression can be compared to a defined cut-off, where ≥ equates to positive status. Henceforth, the study uses 5th percentile cut-off points which are often used in M, C, D, and A statistic of circular data in a wrapped Cauchy distribution[40]. Although significant dissonance is present between blood and tissue samples in the CRC group, there are no significant difference between the first, third, and fifth percentile of *MSH2* gene expression which leads to the acceptance of utilizing 5th percentile cut-off amounting to 11059 fc. Consequently, 32.5% of CRC patients are categorized to the hereditary group. The rate supports the discovery of 22% hereditary CRC by Chang *et al*[41].

Table 3 provide risk factors assessment between the hereditary and sporadic CRC groups. Significant risk factors fall on the age, staging, and family history. The sporadic CRC has older patients than the counterpart with Δ: 9.05 years and 77.78% proportion for those > 50 years old. Generally, age of 40 years old and over has significantly higher incidence of CRC[42]. A 2018 study paints that there is a sharp increase in CRC age-specific incidence as early as 35, then the pre-elderly age of 35-64 [Δ: 60.2 (male) and ± 35 (female)], and over 65 years old [Δ: 237.5 (male) and 131.4 (female)][24]. Nonetheless, Yurgelun *et al*[43] describe that LS mutation carriers have significantly younger age at CRC diagnosis with Δ: 11.1 years ($P < 0.001$)[43]. HNPCC specifically has only ± 20% probability to develop around the age of 50 and 50% for 70 years old or above[44].

Age is an unfortunate risk for malignancy. The older a person is getting, he or she accumulate a lot of endogenous factors (*i.e.*, diet, chronic inflammation, metabolism, waning immune system, *etc.*) and exogenous factors (*i.e.*, genotoxins, mutations, medications, environmental triggers, *etc.*) which stimulate oxidative stress and reactive oxygen species that initiate DNA damage, mutations, and uncontrolled cell growth [45]. The body proinflammatory state as time passes also become a progressive breeding ground for malignancy[46].

Insignificant relations are found in gender, tumor location, and histological differentiation. The hereditary CRC are predominated by female (53.85%) while the sporadic by male (55.56%) yet the difference is negligible ($P = 0.826$). This is consistent with earlier studies where Dominguez-Valentin *et al*[47] ascertain that in earlier years of elderly age, *MSH2* carries similar risk of CRC in terms of gender[47]. Further, this ascertain that even though female is protected from overall CRC due to its hormonal effect, there is no importance to the heredity status of the CRC.

Both hereditary and sporadic CRCs tend to be in the distal colon (61.54% *vs* 74.07%), especially the rectum (38.46% *vs* 44.44%). Supremacy of CRCs in the rectum also seen in a 2020 general hospital study where rectal CRC amounts to 61.8% prevalence[48]. Yet the locations are inessential to the heredity status ($P = 0.476$). Theory upon this predicament include the intrinsic and extrinsic factors within everyone. Carethers[49] in his 2018 study disclose that the overall risk of CRC and the CRC risk of different regions of the colon are affiliated to one's physical activity, gender, height, BMI, smoking status, alcohol intake, diabetes, medications, and hormonal therapy[49]. For example, physical activity reduces the overall CRC risk and the proximal colon CRC risk; while increase in height do not affect the rectal specific location while profoundly heightening risk of overall, proximal, and distal locations.

Likewise, no significance can be seen between histopathological differentiation and CRC heredity status. Current study observes higher poorly differentiated CRC in the hereditary compared to the sporadic group (30.77% *vs* 11.54%), which is akin to the study by Sun dictating that HNPCC features prominent lymphocyte infiltrations and RER+ status, which easily translates to poor differentiation and resulting in more within the HNPCC compared to the sporadic group[50]. On the contrary, the heredity group also has more well differentiated specimens (Δ: 3.85%). The contrast may be due to amounts of proteins and cytokines within the tissue. The tissue staining with chromogranin A produce significant difference of 13.6% between hereditary and sporadic group[50]. The positive staining with after-mentioned stain has high correlation with tumor's grade and stage[51,52]. Meanwhile, the dissonance may also happen due to defects on the sample when taken by colonoscopy biopsy as opposed to a surgery.

Independent staging of the CRC produces significant results between hereditary and sporadic groups ($P = 0.020$), with the former mostly in stage C (54.85%) and the latter in stage B (37.04%). The findings contradict data by Yurgelun *et al*[43], where most LS mutation carriers are in stage II (45.5%)[43]. Difference may occur due to the health system flaw in detecting cancer and the pathophysiology of the CRC.

HNPCC or LS is very hard to be detected as a cancer diagnosis must precede the genetic diagnosis. Even in the developed country of United States, only < 1% of the Americans with LS know about the disease presence[53,54]. The rate may lessen in Indonesia where technology is not as advanced in the United States, genetic testing is not a routine test and very expensive, as well as Indonesians' tradition to not seek the healthcare center if there are no symptoms or still bearable.

Incidentally, family history has linear relationship to the hereditary *vs* sporadic type of CRC ($P = 0.008$). Hereditary CRC majorly has positive history patients (61.54%) while the sporadic group doesn't (14.81%). The conditions amount to 9.20 (1.97-42.97) times increase of risk in developing hereditary CRC when one has family history of CRC. HNPCC is an autosomal dominant disease leading to its presence in every generation of the familial generation, as a dominant trait will always be expressed

Table 5 Scenarios of colorectal cancer hereditary probability

Scenario	Family history[1]	Staging[2]	LOGIT[3]	Probability[4]
1	1	1	2.356	0.913
2	1	0	-0.039	0.490
3	0	1	-0.770	0.316
4	0	0	-3.165	0.041

[1]1: Yes, 0: No.
[2]1: C or D, 0: A or B.
[3]LOGIT: -3.165 + 2.395 × staging + 3.126 × history.
[4]Probability: $1/(1 + e^{-LOGIT})$.

according to the mendelian law of inheritance. Simultaneously, similar relationship also observed in a 2017 LS study where LS mutation carriers have $P < 0.001$ for all first- and second-degree family history of CRC[43].

Multiple regression control of the factors demonstrates that staging and family history is truly significant ($P = 0.034$ and 0.006) while age just a confounder. The analysis then developed applicative equations (Eq. 1 and 2) to predict the heredity of CRC, where examples of their usage are listed in Table 5. The analysis has a satisfactory fit criteria with significant Lemeshow and adequate AUC.

Subsequently, the probability prediction model of the current study can be one of the prospective tools to overcome the weaknesses of the Amsterdam and Bethesda criteria. Personal family history and Mendelian family genogram are important for the diagnosis of Hereditary CRC, with the Amsterdam and Bethesda criteria being the standard diagnosis tools for LS. However, those tools often face difficulties, especially for smaller families and late age of disease onset[11-15,55,56]. In addition, individual specific genotype and environmental traits assessment may be utilized to overcome the hurdle of empirical recurrence risk removal because of its impracticality in incomplete penetrance and late onset[11-15]. Meanwhile, aside from the practical probability prediction model of the present study, mRNA *MSH2* gene expression can be used through Bayesian theorem with prior pedigree risk modifications and conditional information.

Nevertheless, the limitation of the present study includes the un-generalization of the study sample**.** Participants are taken from the hospitals which indicates the possibility of selection bias and unrepresentativeness of the public. Future studies should determine whole genome sequencing to validate these findings and establish a gold standard for Hereditary CRC.

CONCLUSION

Numerous factors have significant relations to heredity of LS CRC patients. However, true important factors are staging and family history, while others (age) are confounders. The study also established a definite cut-off point for heredity LS CRC/HNPCC based on mRNA *MSH2* expression, 11059 fc. These findings shall act as concrete foundations on further risk factors and/or genetical LS CRC future studies.

ARTICLE HIGHLIGHTS

Research background

The lack of golden standard for categorizing hereditary status of colorectal cancer (CRC) poses diagnostic and management problems. Identifying proper techniques is urgent to procure the best care, prevention, risk factors management, and treatment of CRC be it hereditary or sporadic, along with judicious resource consumption.

Research motivation

The lack of golden standard leaves a gaping hole in the LS CRC healthcare system. Previous guideline of Bethesda and Amsterdam have tried yet fail in the applicability

area especially with later age onset and smaller family. These coupled with the hazardous nature of CRC or lynch syndrome and scarce information on CRC risk factors identifications motivate the authors to commence the present study.

Research objectives

To determine the gold standard cut-off of *MSH2* gene expression for hereditary cluster as well as to identify and examine the relationship of hereditary non-polyposis colon cancer (HNPCC) with its non-modifiable risk factors.

Research methods

Consecutive sampling of the hospital internal medicine patients with CRC provides the case group. Then, the control group was concocted by matching the characteristics of the case group. *MSH2* mRNA was then analyzed through blood and tissue collection and reverse transcription-polymerase chain reaction. Further, the gene expression cut-off determined using percentile technique akin to Cauchy distribution of M, C, A, and D circular data statistics. CRC groups then clustered into hereditary and sporadic according to the *MSH2* gene expression against the cut-off. Lastly, risk factors are contrasted between each cluster and developed into a prediction model.

Research results

In a group of 40 CRCs differentiated into 13 hereditary and 27 sporadic through MSH2 mRNA cut-off in 11059 fc, significant risk factors for the hereditary CRC are family history and staging with (OR: 22.784, 95%CI: 2.423-214.273, P = 0.006; OR: 10.970, 95%CI: 1.199-100.382, P = 0.034). Moreover, a prediction model is concocted with area under the curve 82.2%.

Research conclusions

The cut-off of *MSH2* mRNA 5th percentile provided rough clustering of hereditary and sporadic CRC groups. Significant risk factors toward HNPCC are family history and staging, while age is just a confounder.

Research perspectives

Future research directions include validation of the determined cut-off and reliability testing of the risk factors in a bigger sample size and/or with the general population. Further, a longitudinal study on the risk factors effects should be evaluated.

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REFERENCES

1 **Huck MB**, Bohl JL. Colonic Polyps: Diagnosis and Surveillance. *Clin Colon Rectal Surg* 2016; **29**: 296-305 [PMID: 31777460 DOI: 10.1055/s-0036-1584091]
2 **Ewing I**, Hurley JJ, Josephides E, Millar A. The molecular genetics of colorectal cancer. *Frontline Gastroenterol* 2014; **5**: 26-30 [PMID: 24416503 DOI: 10.1136/flgastro-2013-100329]
3 **World Health Organization**. The Global Cancer Observatory. Very High HDI 2021. [cited 10 January 2021]. Available from: https://www.who.int/
4 **Sobocińska J**, Kolenda T, Teresiak A, Badziąg-Leśniak N, Kopczyńska M, Guglas K, Przybyła A, Filas V, Bogajewska-Ryłko E, Lamperska K, Mackiewicz A. Diagnostics of Mutations in MMR/*EPCAM* Genes and Their Role in the Treatment and Care of Patients with Lynch Syndrome. *Diagnostics (Basel)* 2020; **10** [PMID: 33027913 DOI: 10.3390/diagnostics10100786]
5 **Siegel RL**, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 145-164 [PMID: 32133645 DOI: 10.3322/caac.21601]
6 **Mellon I**, Rajpal DK, Koi M, Boland CR, Champe GN. Transcription-coupled repair deficiency and mutations in human mismatch repair genes. *Science* 1996; **272**: 557-560 [PMID: 8614807 DOI: 10.1126/science.272.5261.557]
7 **de Wind N**, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 1995; **82**: 321-330 [PMID: 7628020 DOI: 10.1016/0092-8674(95)90319-4]
8 **Pitsikas P**, Lee D, Rainbow AJ. Reduced host cell reactivation of oxidative DNA damage in human

cells deficient in the mismatch repair gene hMSH2. *Mutagenesis* 2007; **22**: 235-243 [PMID: 17351251 DOI: 10.1093/mutage/gem008]

9 **Turnpenny PD**, Elllard S. The Genetics of Cancer and Cancer Genetics. In: Emery's Elements of Medical Genetics. 15th ed. United Kingdom: Elsevier, 2016: 177–199

10 **Wagner A**, Tops C, Wijnen JT, Zwinderman K, van der Meer C, Kets M, Niermeijer MF, Klijn JG, Tibben A, Vasen HF, Meijers-Heijboer H. Genetic testing in hereditary non-polyposis colorectal cancer families with a MSH2, MLH1, or MSH6 mutation. *J Med Genet* 2002; **39**: 833-837 [PMID: 12414824 DOI: 10.1136/jmg.39.11.833]

11 **Turnpenny PD**, Elllard S. Pattern of Inheritance. In: Emery's Elements of Medical Genetics. 15th ed. United Kingdom: Elsevier, 2016

12 **Nussbaum R**, McInnes R, Willard H. Lynch Syndrome (DNA Mismatch Repair Gene Mutations, MM 120435). In: Thompson & Thompson Genetics in Medicine. 8th ed. Philadelphia: Elsevier, 2015

13 **Yang Y**, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, Braxton A, Beuten J, Xia F, Niu Z, Hardison M, Person R, Bekheirnia MR, Leduc MS, Kirby A, Pham P, Scull J, Wang M, Ding Y, Plon SE, Lupski JR, Beaudet AL, Gibbs RA, Eng CM. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med* 2013; **369**: 1502-1511 [PMID: 24088041 DOI: 10.1056/NEJMoa1306555]

14 **Biesecker LG**, Green RC. Diagnostic clinical genome and exome sequencing. *N Engl J Med* 2014; **370**: 2418-2425 [PMID: 24941179 DOI: 10.1056/NEJMra1312543]

15 **Kilpivaara O**, Aaltonen LA. Diagnostic cancer genome sequencing and the contribution of germline variants. *Science* 2013; **339**: 1559-1562 [PMID: 23539595 DOI: 10.1126/science.1233899]

16 **Li M**, Wang IX, Li Y, Bruzel A, Richards AL, Toung JM, Cheung VG. Widespread RNA and DNA sequence differences in the human transcriptome. *Science* 2011; **333**: 53-58 [PMID: 21596952 DOI: 10.1126/science.1207018]

17 to: *Personality and Problematic Internet Use Among Chinese College Students: The Mediating Role of Maladaptive Cognitions Over Internet Use* by Zhou N, Geng X, Du H, Wu L, Xu J, Ma S, Zhang J, Yu C, Liang Y, Meng J, Yuan X, Cao H, and Fang X. Cyberpsychol Behav Soc Netw. 2018; 21(11):719-726. DOI: 10.1089/cyber.2018.0279. *Cyberpsychol Behav Soc Netw* 2019; **22**: 617 [PMID: 31526294 DOI: 10.1089/cyber.2018.0279.correx]

18 **Kitazawa M**, Hatta T, Sasaki Y, Fukui K, Ogawa K, Fukuda E, Goshima N, Okita N, Yamada Y, Nakagama H, Natsume T, Horimoto K. Promotion of the Warburg effect is associated with poor benefit from adjuvant chemotherapy in colorectal cancer. *Cancer Sci* 2020; **111**: 658-666 [PMID: 31823471 DOI: 10.1111/cas.14275]

19 **Ma Y**, Yang W, Song M, Smith-Warner SA, Yang J, Li Y, Ma W, Hu Y, Ogino S, Hu FB, Wen D, Chan AT, Giovannucci EL, Zhang X. Type 2 diabetes and risk of colorectal cancer in two large U.S. prospective cohorts. *Br J Cancer* 2018; **119**: 1436-1442 [PMID: 30401889 DOI: 10.1038/s41416-018-0314-4]

20 **Ali Khan U**, Fallah M, Sundquist K, Sundquist J, Brenner H, Kharazmi E. Risk of colorectal cancer in patients with diabetes mellitus: A Swedish nationwide cohort study. *PLoS Med* 2020; **17**: e1003431 [PMID: 33186354 DOI: 10.1371/journal.pmed.1003431]

21 **Zhou Z**, Liu H, Wang C, Lu Q, Huang Q, Zheng C, Lei Y. Long non-coding RNAs as novel expression signatures modulate DNA damage and repair in cadmium toxicology. *Sci Rep* 2015; **5**: 15293 [PMID: 26472689 DOI: 10.1038/srep15293]

22 **Hatta M**, Surachmanto EE, Islam AA, Wahid S. Expression of mRNA IL-17F and sIL-17F in atopic asthma patients. *BMC Res Notes* 2017; **10**: 202 [PMID: 28606156 DOI: 10.1186/s13104-017-2517-9]

23 **Sirait RH**, Hatta M, Ramli M, Islam AA, Arief SK. Systemic lidocaine inhibits high-mobility group box 1 messenger ribonucleic acid expression and protein in BALB/c mice after closed fracture musculoskeletal injury. *Saudi J Anaesth* 2018; **12**: 395-398 [PMID: 30100837 DOI: 10.4103/sja.SJA_685_17]

24 **Jung KW**, Won YJ, Kong HJ, Lee ES; Community of Population-Based Regional Cancer Registries. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2015. *Cancer Res Treat* 2018; **50**: 303-316 [PMID: 29566481 DOI: 10.4143/crt.2018.143]

25 **Chen W**, Sun K, Zheng R, Zeng H, Zhang S, Xia C, Yang Z, Li H, Zou X, He J. Cancer incidence and mortality in China. *Zhongguo Aizheng Yanjiu Zazhi* 2018; **30**: 1-12 [DOI: 10.21147/j.issn.1000-9604.2018.01.01]

26 **Hong Kong Cancer Registry (HKCaR)**. Colorectal Cancer in 2016. [cited 10 January 2021]. Available from: https://www3.ha.org.hk/cancereg/

27 **National Cancer Registry (Ministry of Health L and W)**. Cancer Statistics in Japan. [cited 10 January 2021]. Available from: https://www.facs.org/quality-programs/cancer/ncdb/

28 **Sasso CV**, Santiano FE, Campo Verde Arboccó F, Zyla LE, Semino SN, Guerrero-Gimenez ME, Pistone Creydt V, López Fontana CM, Carón RW. Estradiol and progesterone regulate proliferation and apoptosis in colon cancer. *Endocr Connect* 2019; **8**: 217-229 [PMID: 30738018 DOI: 10.1530/EC-18-0374]

29 **Maingi JW**, Tang S, Liu S, Ngenya W, Bao E. Targeting estrogen receptors in colorectal cancer. *Mol Biol Rep* 2020; **47**: 4087-4091 [PMID: 32246248 DOI: 10.1007/s11033-020-05414-6]

30 **McCarthy M**, Raval AP. The peri-menopause in a woman's life: a systemic inflammatory phase that enables later neurodegenerative disease. *J Neuroinflammation* 2020; **17**: 317 [PMID: 33097048 DOI: 10.1186/s12974-020-01998-9]

31 **Shimizu N**, Nagata C, Shimizu H, Kametani M, Takeyama N, Ohnuma T, Matsushita S. Height,

weight, and alcohol consumption in relation to the risk of colorectal cancer in Japan: a prospective study. *Br J Cancer* 2003; **88**: 1038-1043 [PMID: 12671701 DOI: 10.1038/sj.bjc.6600845]

32 **Sung J**, Song YM, Lawlor DA, Smith GD, Ebrahim S. Height and site-specific cancer risk: A cohort study of a korean adult population. *Am J Epidemiol* 2009; **170**: 53-64 [PMID: 19403842 DOI: 10.1093/aje/kwp088]

33 **Shin A**, Joo J, Bak J, Yang HR, Kim J, Park S, Nam BH. Site-specific risk factors for colorectal cancer in a Korean population. *PLoS One* 2011; **6**: e23196 [PMID: 21853085 DOI: 10.1371/journal.pone.0023196]

34 **Song X**, Gong X, Zhang T, Jiang W. Height and risk of colorectal cancer: a meta-analysis. *Eur J Cancer Prev* 2018; **27**: 521-529 [PMID: 28683006 DOI: 10.1097/CEJ.0000000000000390]

35 **Abar L**, Vieira AR, Aune D, Sobiecki JG, Vingeliene S, Polemiti E, Stevens C, Greenwood DC, Chan DSM, Schlesinger S, Norat T. Height and body fatness and colorectal cancer risk: an update of the WCRF-AICR systematic review of published prospective studies. *Eur J Nutr* 2018; **57**: 1701-1720 [PMID: 29080978 DOI: 10.1007/s00394-017-1557-1]

36 **Minko E**, Pagano A, Caceres N, Adar T, Márquez S. Human intestinal tract length and relationship with body height. *FASEB J* 2014; **28** [DOI: 10.1096/fasebj.28.1_supplement.916.4]

37 **Dworakowska D**, Grossman AB. Colonic Cancer and Acromegaly. *Front Endocrinol (Lausanne)* 2019; **10**: 390 [PMID: 31293513 DOI: 10.3389/fendo.2019.00390]

38 **Murphy N**, Carreras-Torres R, Song M, Chan AT, Martin RM, Papadimitriou N, Dimou N, Tsilidis KK, Banbury B, Bradbury KE, Besevic J, Rinaldi S, Riboli E, Cross AJ, Travis RC, Agnoli C, Albanes D, Berndt SI, Bézieau S, Bishop DT, Brenner H, Buchanan DD, Onland-Moret NC, Burnett-Hartman A, Campbell PT, Casey G, Castellví-Bel S, Chang-Claude J, Chirlaque MD, de la Chapelle A, English D, Figueiredo JC, Gallinger SJ, Giles GG, Gruber SB, Gsur A, Hampe J, Hampel H, Harrison TA, Hoffmeister M, Hsu L, Huang WY, Huyghe JR, Jenkins MA, Keku TO, Kühn T, Kweon SS, Le Marchand L, Li CI, Li L, Lindblom A, Martín V, Milne RL, Moreno V, Newcomb PA, Offit K, Ogino S, Ose J, Perduca V, Phipps AI, Platz EA, Potter JD, Qu C, Rennert G, Sakoda LC, Schafmayer C, Schoen RE, Slattery ML, Tangen CM, Ulrich CM, van Duijnhoven FJB, Van Guelpen B, Visvanathan K, Vodicka P, Vodickova L, Vymetalkova V, Wang H, White E, Wolk A, Woods MO, Wu AH, Zheng W, Peters U, Gunter MJ. Circulating Levels of Insulin-like Growth Factor 1 and Insulin-like Growth Factor Binding Protein 3 Associate With Risk of Colorectal Cancer Based on Serologic and Mendelian Randomization Analyses. *Gastroenterology* 2020; **158**: 1300-1312.e20 [PMID: 31884074 DOI: 10.1053/j.gastro.2019.12.020]

39 **Kasprzak A**. Insulin-Like Growth Factor 1 (IGF-1) Signaling in Glucose Metabolism in Colorectal Cancer. *Int J Mol Sci* 2021; **22** [PMID: 34208601 DOI: 10.3390/ijms22126434]

40 **Abuzaid AH**, El-hanjouri MM, Kulab MM. On Discordance Tests for the Wrapped Cau-chy Distribution. *Open J Stat* 2015; **5**: 245-253 [DOI: 10.4236/ojs.2015.54026]

41 **Chang DT**, Pai RK, Rybicki LA, Dimaio MA, Limaye M, Jayachandran P, Koong AC, Kunz PA, Fisher GA, Ford JM, Welton M, Shelton A, Ma L, Arber DA. Clinicopathologic and molecular features of sporadic early-onset colorectal adenocarcinoma: an adenocarcinoma with frequent signet ring cell differentiation, rectal and sigmoid involvement, and adverse morphologic features. *Mod Pathol* 2012; **25**: 1128-1139 [PMID: 22481281 DOI: 10.1038/modpathol.2012.61]

42 **Noone A**, Howlader N, Krapcho M, Miller D, Brest A, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA. SEER Cancer Statistics Review 1975-2015. National Cancer Institute; 2018. [cited 10 January 2021]. Available from: https://www.researchgate.net/publication/285863025_SEER_Cancer_statistics_review_1975-2004

43 **Yurgelun MB**, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, Ukaegbu CI, Brais LK, McNamara PG, Mayer RJ, Schrag D, Meyerhardt JA, Ng K, Kidd J, Singh N, Hartman AR, Wenstrup RJ, Syngal S. Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer. *J Clin Oncol* 2017; **35**: 1086-1095 [PMID: 28135145 DOI: 10.1200/JCO.2016.71.0012]

44 **Bonadona V**, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C, Noguès C, Lejeune-Dumoulin S, Olivier-Faivre L, Polycarpe-Osaer F, Nguyen TD, Desseigne F, Saurin JC, Berthet P, Leroux D, Duffour J, Manouvrier S, Frébourg T, Sobol H, Lasset C, Bonaïti-Pellié C; French Cancer Genetics Network. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA* 2011; **305**: 2304-2310 [PMID: 21642682 DOI: 10.1001/jama.2011.743]

45 **Ahmad S**, Akhter F, Shahab U, Rafi Z, Khan MS, Nabi R, Ahmad K, Ashraf JM, Moinuddin. Do all roads lead to the Rome? *Semin Cancer Biol* 2018; **49**: 9-19 [PMID: 29113952 DOI: 10.1016/j.semcancer.2017.10.012]

46 **Rea IM**, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA. Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines. *Front Immunol* 2018; **9**: 586 [PMID: 29686666 DOI: 10.3389/fimmu.2018.00586]

47 **Dominguez-Valentin M**, Sampson JR, Seppälä TT, ten Broeke SW, Plazzer J-P, Nakken S, Engel C, Aretz S, Jenkins MA, Sunde L, Bernstein I, Capella G, Balaguer F, Thomas H, Evans DG, Burn J, Greenblatt M, Hovig E, Cappel WHDVTN, Sijmons RH, Bertario L, Tibiletti MG, Cavestro GM, Lindblom A, Valle AD, Lopez-Köstner F, Gluck N, Katz LH, Heinimann K, Vaccaro CA, Büttner R, Görgens H, Holinski-Feder E, Morak M, Holzapfel S, Hüneburg R, Doeberitz MVK, Loeffler M, Rahner N, Schackert HK, Steinke-Lange V, Schmiegel W, Vangala D, Pylvänäinen K, Renkonen-Sinisalo L, Hopper JL, Win AK, Haile RW, Lindor NM, Gallinger S, Marchand LL, Newcomb PA, Figueiredo JC, Thibodeau SN, Wadt K, Therkildsen C, Okkels H, Ketabi Z, Moreira L, Sánchez A,

Serra-Burriel M, Pineda M, Navarro M, Blanco I, Green K, Lalloo F, Crosbie EJ, Hill J, Denton OG, Frayling IM, Rødland EA, Vasen H, Mints M, Neffa F, Esperon P, Alvarez K, Kariv R, Rosner G, Pinero TA, Gonzalez ML, Kalfayan P, Tjandra D, Winship IM, Macrae F, Möslein G, Mecklin J, Nielsen M, Møller P. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med* 2020; **22**: 15-25 [DOI: 10.1038/s41436-020-0892-4]

48 **Anthonysamy MA**, Maker LPLI, Gotra IM, Saputra H. Prevalence of colorectal carcinoma based on microscopic type, sex, age and anatomical location in Sanglah General Hospital. *Intisari Sains Medis* 2020; **11**: 272-276 [DOI: 10.15562/ism.v10i2.171]

49 **Carethers JM**. Risk factors for colon location of cancer. *Transl Gastroenterol Hepatol* 2018; **3**: 76 [PMID: 30505963 DOI: 10.21037/tgh.2018.09.15]

50 **Sun MH**. Neuroendocrine differentiation in sporadic CRC and hereditary nonpolyosis colorectal cancer. *Dis Markers* 2004; **20**: 283-288 [PMID: 15528794 DOI: 10.1155/2004/379053]

51 **Jesinghaus M**, Konukiewitz B, Keller G, Kloor M, Steiger K, Reiche M, Penzel R, Endris V, Arsenic R, Hermann G, Stenzinger A, Weichert W, Pfarr N, Klöppel G. Colorectal mixed adenoneuroendocrine carcinomas and neuroendocrine carcinomas are genetically closely related to colorectal adenocarcinomas. *Mod Pathol* 2017; **30**: 610-619 [PMID: 28059096 DOI: 10.1038/modpathol.2016.220]

52 **Herold Z**, Dank M, Herold M, Nagy P, Rosta K, Somogyi A. Histopathological Chromogranin A-Positivity Is Associated with Right-Sided Colorectal Cancers and Worse Prognosis. *Cancers (Basel)* 2020; **13** [PMID: 33383764 DOI: 10.3390/cancers13010067]

53 **Butterworth AS**, Higgins JP, Pharoah P. Relative and absolute risk of colorectal cancer for individuals with a family history: a meta-analysis. *Eur J Cancer* 2006; **42**: 216-227 [PMID: 16338133 DOI: 10.1016/j.ejca.2005.09.023]

54 **Hampel H**, de la Chapelle A. The search for unaffected individuals with Lynch syndrome: do the ends justify the means? *Cancer Prev Res (Phila)* 2011; **4**: 1-5 [PMID: 21205737 DOI: 10.1158/1940-6207.CAPR-10-0345]

55 **Guttmacher AE**, Collins FS, Carmona RH. The family history--more important than ever. *N Engl J Med* 2004; **351**: 2333-2336 [PMID: 15564550 DOI: 10.1056/NEJMsb042979]

56 **Goecke T**, Schulmann K, Engel C, Holinski-Feder E, Pagenstecher C, Schackert HK, Kloor M, Kunstmann E, Vogelsang H, Keller G, Dietmaier W, Mangold E, Friedrichs N, Propping P, Krüger S, Gebert J, Schmiegel W, Rueschoff J, Loeffler M, Moeslein G; German HNPCC Consortium. Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC Consortium. *J Clin Oncol* 2006; **24**: 4285-4292 [PMID: 16908935 DOI: 10.1200/JCO.2005.03.7333]

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