Int J Colorectal Dis (2017) 32:935–945
DOI 10.1007/s00384-017-2799-3

REVIEW

Peritoneal fluid biomarkers in the detection of colorectal anastomotic leaks: a systematic review

Emma C. Wright[1] · **Patricia Connolly**[2] · **Mark Vella**[1] · **Susan Moug**[1]

Accepted: 2 March 2017 / Published online: 12 April 2017

Abstract
Purpose Anastomotic leak (AL) in colorectal surgery leads to significant morbidity, mortality and poorer oncological outcomes. Diagnosis of AL is frequently delayed as current methods of detection are not 100% sensitive or specific. 'Biomarkers', such as cytokines and markers of ischaemia, from the milieu of the anastomosis may aid early detection. This paper aims to review the evidence for their role in AL detection, allowing identification of targets for future research.
Methods A systematic review was performed using PubMed, MEDLINE and Cochrane Library databases. Papers concerning detection or prediction of AL with biomarkers were identified. References within the papers were used to identify further relevant articles.
Results Research has taken place in small cohorts with varying definitions of AL. Lactate has consistently been shown to be elevated in patients with intra-abdominal complications and ALs. pH on post-operative day 3 showed excellent specificity. Despite mixed results, a meta-analysis found that the cytokines tumour necrosis factor-α and interleukin-6 were elevated early in AL. Detection of bacteria in drain fluid by RT-PCR has good specificity but a high rate of false positives.
Conclusions Peritoneal cytokines, lactate and pH have the potential to identify AL early. The consistency of the results for lactate and pH, alongside the fact that they are easy, quick and inexpensive to test, makes them the most attractive targets. Studies in larger cohorts with standardized definitions of AL are required to clarify their usefulness. Emerging biosensor technology may facilitate the development of small, low-cost and degradable intra-abdominal devices to measure peritoneal fluid biomarkers.

Keywords Anastomotic leak · Colorectal surgery · Lactate

Introduction

Anastomotic leak (AL) in colorectal surgery potentially leads to significant morbidity, mortality and suboptimal oncological outcomes. The incidence of AL is variable ranging from 1 to 30% [1] depending on the anatomical location of the anastomosis, and low extraperitoneal anastomoses have a higher risk of AL [2]. Early detection is crucial as instigation of prompt treatment leads to reductions in morbidity and mortality and consequently improved patient outcomes.

Currently, there is no reliable test to accurately detect AL. Serial measurement of clinical observations combined with blood markers, such as C-reactive protein, has low specificity as many patients who make an uncomplicated recovery have abnormalities of these parameters at some stage during their recovery [3]. If AL is suspected, commonly, a contrast-enhanced computed tomographic (CT) scan is performed which can again be inconclusive. First, in the early stages, a leak may be too subtle to be identified. Second, a leak can be difficult to distinguish from early post-operative changes relying upon the experience and interpretation of the radiologist.

Given these limitations, new strategies to detect AL are required. One such strategy is the measurement of biomarkers (e.g. cytokines, matrix metalloproteinases (MMPs), lipopolysaccharides (LPS, a marker of bacteria) and markers of ischaemia such as lactate) in the immediate environment of the

✉ Emma C. Wright
Emmawright8@nhs.net

[1] Department of General Surgery, Royal Alexandra Hospital, Ward 26 Day Room, Corsebar Road, Paisley PA2 9PN, UK

[2] Department of Biomedical Engineering, Wolfson Centre, University of Strathclyde, Glasgow G4 0NW, UK

anastomosis by sampling peritoneal fluid. These local biomarkers reflect conditions in the milieu of the anastomosis and have the potential to detect AL earlier and with greater specificity than current methods. In 2008, Komen et al. suggested the criteria for biomarkers of AL in peritoneal fluid [4] (Table 1).

This review aims to appraise the current evidence base into local biomarkers of AL allowing the identification of the most promising emerging biomarkers and discussion of their limitations and future potential clinical role.

Methods

A systematic review of the literature was carried out in accordance with the PRISMA statement [5]. A literature search was performed on the 7th of January 2016 in the following databases: Embase (1947–7th January 2016), PubMed/MEDLINE (1st January 1948–7th January 2016) and Cochrane Library (1st January 2005–7th January 2016). The search terms used were as follows: (colorectal OR gastrointestinal) AND (anastomotic OR anastomosis) AND (biomarker OR marker OR indicator OR predict) AND (leak OR leakage OR integrity). The bibliographies of the articles retrieved were reviewed to identify additional relevant studies not retrieved in the initial search.

To be included, studies had to meet the following criteria: written in the English language, involving human studies, submitted as full papers and focusing on the creation of colorectal anastomoses. Studies were excluded if they focused on risk factor identification, prevention, management or outcomes of AL.

After removal of duplicates, 257 English language articles were initially retrieved. After initial screening, 20 articles remained for full text review, following which 7 studies were excluded. This left 13 studies for inclusion in the descriptive analysis (Fig. 1).

Results

The 13 biomarker studies were grouped into four categories: ischaemia, bacterial infection, inflammation (cytokines) and wound repair (MMPs).

There is a wide heterogeneity in the study populations, end points analysed and definitions of AL. Several studies looked only at anterior resections for colorectal cancer [6–9], whereas others included benign colorectal conditions [10–13] and even patients undergoing abdominal aortic aneurysm (AAA) repair [11] and cholecystectomy [12]. Most studies were in the elective setting but some encompassed emergency trauma cases [13]. The majority used AL as the end point, but others, such as Horer et al. [11] and Alonso et al. [14], included AL in a broader category of 'major or infective complications'. The definition and diagnosis of AL varied widely (see Tables 2, 3, 4 and 5). No two studies used the same definition and some did not provide a definition (e.g. [11, 12, 16, 19, 22]). Most relied upon a clinical suspicion to direct further investigations such as endoscopy, radiological imaging or rectal examination to confirm a leak. In contrast, one study routinely imaged on post-operative day (POD 6) and 'clinical' and 'subclinical' leaks (detected only on imaging and not needing specific treatment) were analysed together [8]. One study specifically stated that fistulas were not considered to represent AL [7]. The wide variability in the definition is not unique to this group of studies. A 2001 review of 97 studies identified 56 different definitions of AL [25]. In the 13 papers, the severity of the leaks was not always clear, and neither the type of intervention used was required nor whether those requiring operative versus non-operative management were analysed together.

Table 1 Komen et al. criteria for a biomarker of AL [4]

Suggested criteria for a biomarker of AL in peritoneal fluid:
Significant change in biomarker concentration in anastomotic leakage
Stability of the biomarker in the peritoneal environment and drain fluid
Biomarker level not influenced by the primary disease
Biomarker with sufficient sensitivity and specificity for anastomotic leakage
Biomarker allows for easy, fast and cheap real-time testing

Biomarkers of ischaemia

Seven studies have looked at biomarkers of ischaemia measured either by microdialysis or in peritoneal drain fluid (Table 2). The principle of this approach is that ischaemia at the anastomosis or in nearby bowel increases the risk of a leak [26, 27]. In the presence of adequate oxygen, glycolysis proceeds via the Krebs cycle and glucose is converted to pyruvate with a high output of adenosine triphosphate (ATP). If oxygen supply is poor, ATP is generated via the less efficient anaerobic pathway whereby pyruvate is reduced to lactate. Consequently, in an ischaemic environment, lactate rises and pyruvate falls accompanied by accumulation of carbon dioxide so pH decreases. The balance of aerobic/anaerobic metabolism can be measured by the lactate/pyruvate ratio (LP ratio) with an increased ratio indicating an ischaemic environment. Lactate and other markers of ischaemia can be measured in peripheral blood but they lack specificity in detecting AL [28].

Several studies used microdialysis [6, 7, 10, 11]. This technique has been previously used to study tissue ischaemia [29]. It involves inserting very small probes with dialysis membranes into the operative field at the end of a procedure. Fluid flows through the circuit constantly allowing measurement of analytes including lactate and pyruvate. Trials in animal models of intestinal

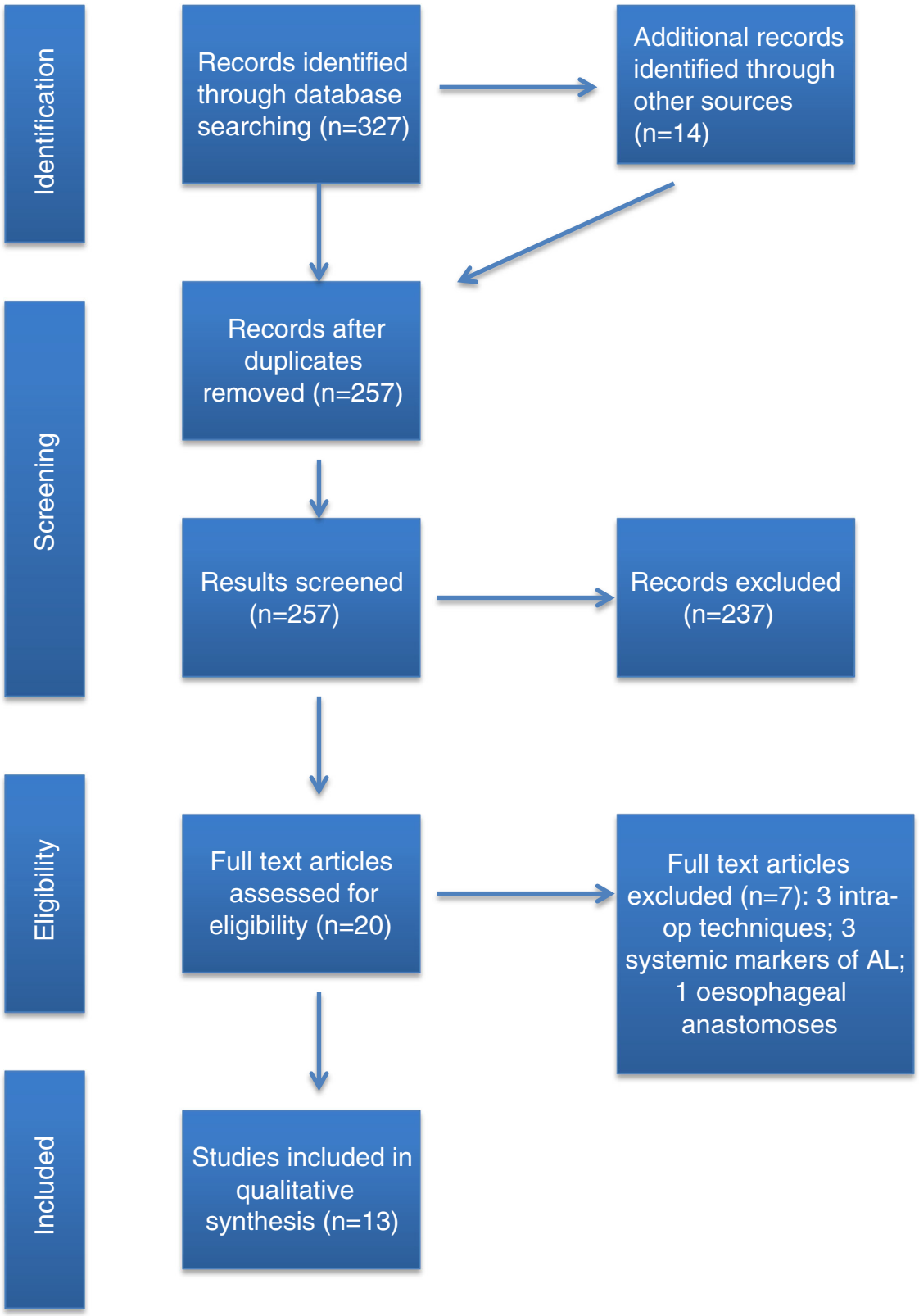

Fig. 1 PRISMA flow diagram

ischaemia showed that increased lactate and LP ratios were reliable markers of local ischaemia [30, 31].

Matthiessen et al. [6] measured LP ratio and glucose for 6 days following anterior resections in 23 patients. Four 'early

Table 2 Summary of studies looking at biomarkers of ischaemia

Authors [ref]	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of biomarker measurement	Frequency and duration of biomarker measurement	Main results
Millan et al. [8]	pH	Prospective	All patients had CT or contrast imaging on POD 6. Divided into subclinical and clinical but analysed together	90	10	Anterior resections for colorectal cancer	Tonometry catheter placed just superior to the anastomosis	Samples taken at 24 and 48 h after surgery	pH at 24 h, but not 48 h, significantly lower in those with AL
Matthiessen et al. [6]	LP ratio, glucose	Prospective	Peritonitis caused by leakage, pelvic abscess or discharge of faeces from drain or a fistula. Confirmed by imaging or DRE	23	7	Anterior resections for colorectal cancer	Measurement via microdialysis	Samples every 2 h until POD 2 and then every 6 h until POD 6	LP ratio significantly increased on PODs 5 and 6 in those with AL. Glucose not significant
Pedersen et al. [7]	Glucose, lactate, pyruvate, glycerol, LP ratio	Prospective	Peritonitis or abscess with 'proven dehiscence'—no mention of how it was proven. Fistulas not counted as AL	50	4	Low anterior resections for cancer	Measurement via microdialysis	Samples every 4 h until discharge or POD 10	3 patients had a late leak and lactate levels and LP ratio was significantly increased prior to onset of symptoms. In 1 case of early leak, increased lactate coincided with onset of symptoms
Horer et al. [11]	Glycerol, lactate, pyruvate, glucose, LP ratio	Prospective	No definition given. AL included in 'major complication' category	60	16[a]	Various gastrointestinal and intra-abdominal vascular surgeries	Measurement via microdialysis	Every 2 h until 48 h post-surgery	LP ratio was significantly elevated and glycerol levels significantly lower in those with AL Lactate alone—no significant difference
Yang et al. [9]	pH	Prospective	Pelvic abscess, faecal/purulent discharge from drain, fistulas, peritonitis confirmed radiologically and all needed additional surgical treatment	753	57	Anterior resections for cancer	Peritoneal drain sample	Daily until POD 12	pH <6.978 on POD 3 significantly associated with AL, sensitivity 98.7% and specificity 94.7%
Bini et al. [12]	Lactate	Prospective	No definition given. AL included in group requiring 'reintervention'	88	31[a]	Various gastrointestinal operations	Measurement from peritoneal drain on POD 4 in those meeting the criteria: late passage of flatus, pain, pyrexia and raised white cell count	Once on POD 4	Peritoneal lactate level >9.1 mmol and peritoneal/serum lactate level ratio >4.5 significantly associated with required reintervention
Daams et al. [10]	Lactate, pyruvate, glucose, glycerol	Prospective	Clinical suspicion of intra-abdominal complication was investigated by CT to diagnose AL. All AL were confirmed at reoperation or endoscopy	24	3	Left-sided colonic resections for cancer or diverticular disease	Microdialysis measurements every 4 h until POD 5		Mean intra-peritoneal lactate levels, but not LP ratio, was significantly higher in those with AL

[a] End point was 'patient requiring reintervention'; of the 31 in this group, 11 had an AL

leaks' on PODs 2–14 and three 'late leaks' on PODs 18–22 were analysed together as a 'leak group'. LP ratios on PODs 5 and 6 were significantly higher in those with AL, and glucose was not significantly different. However, as the numbers were low, it was not possible to determine cut-off levels for LP ratio to indicate the likelihood of a leak. Pedersen et al. [7] looked at 45 low anterior resections. In one AL on POD 5, lactate levels were significantly increased 18 h before the onset of symptoms, and LP ratio became significantly elevated at the onset of symptoms. Three other patients developed a leak at day 10 or later. LP ratio and lactate levels were significantly increased compared to controls throughout the first 5 days following surgery [7]. Daams et al. [10] measured lactate, pyruvate, glycerol and glucose every 4 h for 96 h in 24 patients undergoing left-sided colorectal anastomoses. In three patients with ALs, median intra-peritoneal lactate levels but not LP ratio were significantly higher. There appeared to be spikes in lactate prior to POD 3 in the leak group but cut-off values were not described. It is important to note that two of the leaks occurred early at PODs 4 and 5, and the third was said to have occurred at POD 97 but the three were grouped together. Horer et al. [11] had similar findings, and they noted that increased LP ratio and decreased glycerol levels were associated with 'major intra-abdominal complications'. However, this was in a varied cohort including colorectal anastomosis, AAA repair, gastric procedures and cholecystectomy.

Bini et al. [12] looked at lactate via peritoneal drain fluid on POD 4 in patients who met all four of the following criteria: pyrexia (>38.3), raised white cell count (>12), delayed passage of flatus (>72 h) and abdominal pain. A peritoneal/serum lactate level >4.5 or a peritoneal lactate level >9.1 was significantly associated with post-operative complications requiring intervention (ALs were included in this group). However, this selection bias with no control group limits the interpretation of these findings.

Two studies investigated pH as a biomarker. In the first study, a catheter was sited intraluminally just above the anastomosis and pH measured via tonometry (a non-invasive method to measure intramucosal pH in hollow organs). All patients were imaged on POD 6 and 'clinical' and 'subclinical' leaks analysed together in a 'leak group'. The 'leak group' had a significantly lower pH. A pH <7.28 yielded a specificity of 98.3% but sensitivity was only 28.1% [8]. Yang et al. [9] measured the pH of fluid collected from peritoneal drains up to POD 12 following rectal surgery in 753 patients. There were 57 leaks (7.6%) on PODs 6–12 requiring surgical intervention, and subclinical leaks were not included. pH was significantly lower in patients who leaked. pH <6.978 on POD 3 showed excellent sensitivity (98.7%) and specificity (94.7%). However, no confidence intervals or ranges were provided so it is difficult to know how much overlap there is between groups. The authors highlight that a decline in pH was seen in all patients prior to detection of an AL.

Overall, of the biomarkers of ischaemia, lactate and pH emerge as the best candidates for further research. Most studies measured lactate by microdialysis [6, 7, 10, 11] which is expensive, labour-intensive and technically challenging (e.g. nine technical failures in [10]). In contrast, measurement of lactate from peritoneal drain fluid may provide a quick, easy and inexpensive alternative. The stability of lactate and pH in peritoneal drainage fluid needs to be addressed. The stability of these biomarkers in peritoneal fluid has not been explored, but it has been shown that lactate levels rise in blood if there is a delay in analysis [32]. It would therefore be pertinent to address this issue in peritoneal fluid.

Biomarkers of bacterial infection

This approach is based upon the principle that if the colorectal anastomosis breaks down bacteria, which are normally contained within the bowel, lumen spills into the peritoneal cavity. Four studies explored whether detection of intra-peritoneal bacteria and its quantitative assessment could aid early detection of AL (Table 3).

Fouda et al. [17] looked at intra-peritoneal bacterial colonization in peritoneal drain fluid samples from 56 low anterior resections on PODs 1, 3 and 5. Eight ALs were 'detected clinically' and confirmed on imaging. *Escherichia coli*, *Bacteroides* and *Pseudomonas* were cultured significantly more often on PODs 1, 3 and 5 and *Klebsiella* on PODs 3 and 5 in patients with AL. However, specificity was low as there were several false positives with all four bacteria detected in patients without an AL.

The time delay in growing cultures limits its applicability and usefulness as a rapidly analysable biomarker. This was addressed in a pilot study of 17 patients using real-time PCR (RT-PCR). Peritoneal drain fluid was sampled daily up to POD 5 using *Es. coli* and *Enterococcus faecalis* as indicator organisms. RT-PCR results were fully concordant with microbiological cultures. However, the technique lacked specificity as there were four false positive results [16]. The authors progressed to a multicentre study of 243 patients undergoing elective left-sided colonic anastomosis. There were 19 leaks (7.8%) on mean POD 6 (2–26 days). *Es. coli* concentration was significantly increased on PODs 4 and 5 and *En. faecalis* on PODs 2, 3 and 4 in those with AL. *En. faecalis* on POD 3 achieved the best results with a sensitivity and negative predictive value of 92.9 and 98.7%, respectively. The diagnostic odds ratio was 31.6 but specificity and positive predictive value were only 70.9 and 30.2%, respectively [18]. Despite the large number of false positives, the study demonstrated that the absence of *En. faecalis* on day 3 could potentially exclude AL.

In 1996, Junger et al. [15] looked at peritoneal drain LPS levels (a component of the outer wall of gram-negative bacteria) in 22 patients. LPS levels were significantly elevated in

Table 3 Summary of studies looking at bacterial biomarkers

Authors [ref]	Bacterial biomarker studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of biomarker measurement	Duration and frequency of measurement	Main results
Junger et al. [15]	Lipopolysaccharide (LPS)	Prospective	'Clinical signs of a leak'	22	3	'Colorectal anastomoses'	Sample from peritoneal drain fluid	Daily until POD 8	LPS significantly increased in those with AL
Komen et al. [16]	*Es. coli*, *En. faecalis*	Prospective	Not defined	9	0	Various colorectal resections from ileocaecal resections—low anterior resection; benign and malignant	Sample from peritoneal drain fluid Fluid sent for bacterial culture and for RT-PCR to detect bacteria	Morning daily sample until POD 5	RT-PCR results fully concordant with culture results but there were 4 false positives
Fouda et al. [17]	*Es. coli*, *Klebsiella*, *Pseudomonas*, *Bacteroides*	Prospective	Gas, pus or faecal discharge from the drain, faecal discharge from the operative wound, pelvic abscess, peritonitis and rectovaginal fistula. Confirmed by radiological contrast study, CT scan or digital rectal palpation	56	8	Low anterior resections for malignancy	Sample from peritoneal drain fluid.	Samples on PODs 1, 3 and 5	*Es. coli*, *Klebsiella*, *Pseudomonas* and *Bacteroides* cultured significantly more often from patients with AL
Komen et al. [18]	*Es. coli*, *En. faecalis*	Prospective	A clinical state requiring reintervention confirmed by radiological studies, operative findings or faecal discharge from the drain	243	19	Left-sided colorectal resections; benign and malignant	Sample from peritoneal drain fluid Fluid sent for RT-PCR to detect bacteria	Morning daily sample until POD 5	*Es. coli* concentration significantly increased on PODs 4 and 5 and *En. faecalis* on PODs 2, 3 an 4 in those with AL Best diagnostic odds ratio was *En. faecalis* on POD 3

three patients with AL. However, two of these patients had surgery for perforated sigmoid diverticulitis, so levels may have been elevated due to pre-existing bacterial contamination. LPS has not been explored again.

Overall, bacterial biomarkers for AL may have some utility in acting as a 'rule-out' test for AL. Whilst RT-PCR for bacteria offers fast, real-time testing, it is not commonly performed in most hospital laboratories which may limit its usefulness.

Biomarkers of inflammation (cytokines)

Seven studies have explored the measurement of peritoneal cytokines to detect AL (Table 4). Cytokines are glycoproteins involved in regulating inflammation and are thought to be the main mediators of the systemic inflammatory response to sepsis [33–36]. Peritoneal cytokine levels are elevated compared to serum cytokine levels after major abdominal surgery and in peritonitis [13, 37, 38].

There is wide heterogeneity in the type of operations performed, underlying pathology and definitions of AL. In addition, most involved small cohorts and measurements of peritoneal cytokines have been taken at varying times and frequencies from every 4 h for 2 days [6] to once daily for 7 days [19].

TNFa

Herwig et al. [13], Ugras et al. [20] and Matthiessen et al. [6] found that TNFa was significantly higher on POD 1 in patients who developed an AL. However, no cut-off value could be established to give an estimate of risk and confidence intervals overlapped in each group. Fouda et al. [17] and Yamamoto et al. [21], which contained the largest sample of patients (n = 100), found TNFa did not reach statistical significance until POD 3. Both also noted that TNFa decreased significantly from PODs 1 to 3 in patients who did not develop a leak [17, 21]. In contrast, Bertram et al. [19], who used a similar methodology, looked at TNFa levels for 7 days in 25 patients and found that TNFa was not helpful in predicting AL.

IL-6

Ugras et al. [20], Herwig et al. [13], Matthiesen et al. [6] and Fouda et al. [17] all found IL-6 to be significantly higher in patients who developed an AL from POD 1; however, Yamamoto et al. [21] demonstrated no significant difference until POD 3 and Bertram et al. [19] found no difference in IL-6 levels at anytime in the first 7 days post-op.

A more recent case-control study [14] looked at IL-6 levels on PODs 2 and 4 in 60 patients after various colorectal resections. Thirty leaks and intra-abdominal abscesses were grouped into an 'infection' category and compared to 30 controls with an uncomplicated recovery. IL-6 was significantly higher on both days in the 'infection group'. They did not state how or when the leaks were detected.

Other cytokines

Of the other cytokines studied, the pro-inflammatory IL-1b was found to be significantly elevated on POD 3 [13, 21] and the anti-inflammatory IL-10 on POD 1 in patients who developed an AL [6, 17, 20].

Overall, despite some conflicting results, a meta-analysis (which did not include Alonso et al. [14]) found that IL-6 and TNF were significantly raised from PODs 1 to 2, respectively, in those who developed a leak [39]. Studies with larger sample sizes and better standardization of definitions and sampling protocols are required to clarify their role.

Biomarkers of wound repair (MMPs)

MMPs are zinc-dependent enzymes responsible for tissue turnover by extracellular matrix degradation. Tissue inhibitors of metalloproteinase (TIMPs) are the natural inhibitors of MMPs. The balance of MMPs and TIMPs is involved in physiological and pathological processes including inflammation [40]. Three papers, with wide variation in study population, sampling frequency and end points, explored peritoneal MMPs and TIMPs (Table 5).

Pasternak et al. [23] measured MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9 and MMP-13 once at 4 h after low anterior resections in 30 patients. Five early leaks between PODs 2 and 13 and five late leaks between PODs 13 and 41 were analysed together. Only MMP-8 and MMP-9 were significantly elevated in the AL group.

Baker and Leaper [22] measured MMP-1, MMP-3, MMP-8, MMP-9, TIMP-1 and TIMP-2 for 7 days after various colorectal anastomoses in 58 patients. The end point was 'major complications' which included AL. Levels of MMP-2 (day 3), MMP-2 (day 6) and MMP-9 (days 6 and 7) were positively correlated with complications, whereas TIMP-2 (days 2 and 3) and TIMP-1 (day 7) were negatively correlated. Only MMP-9 was significantly elevated in both studies. However, Kostic et al. [24], who looked specifically at MMP-9 on PODs 1, 3, 5 and 7 in 150 patients undergoing elective left-sided colorectal resections (with 15 clinically detected ALs), found that levels were not significantly different to those without a leak. The inconsistent results for MMPs limit their usefulness in future research.

Potential clinical applicability

Peritoneal drainage following colorectal surgery used to be routine but this is now debated. A Cochrane review of six RCTs found no reduction in rates of AL when a drain was

Table 4 Summary of studies looking at cytokines

Authors [ref]	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Method of measurement	Frequency and duration of measurement of biomarkers	Main results
Herwig et al. [13]	IL-1, IL-6, TNFa	Prospective	Clinical diagnosis confirmed by endoscopy, contrast radiology and finally confirmed at laparotomy	24	12	Right hemicolectomy to anterior resections for malignancy, inflammatory bowel disease, diverticulosis and trauma	Peritoneal drain fluid sample taken daily within 2 h of emptying drainage bag	Daily until PODs 4–9	IL-6 and TNFa from PODs 1 and IL-1 from POD 3 significantly increased in those with AL
Bertram et al. [19]	IL-6, TNFa	Prospective	No definition provided but states AL was confirmed at relaparotomy	25	3	Right hemicolectomies to anterior resections for adenomas and cancers	Peritoneal drain fluid sample taken daily at 8 a.m.	Daily until POD 7	IL-6 and TNFa not helpful in predicting AL
Matthiessen et al. [6]	IL-6, IL-10, TNFa	Prospective	Peritonitis caused by leakage, pelvic abscess or discharge of faeces from drain or a fistula. Confirmed by imaging or DRE	23	7	Anterior resections for colorectal cancer	Peritoneal drain fluid sample	Peritoneal drain sample every 6 until 42 h after surgery	IL-6, IL-10 significantly increased from PODs 1 to 2 in those with AL TNFa only significant on POD 1
Ugras et al. [20]	IL-6, IL-10, TNFa	Prospective	'Clinical signs (gas, pus, faeces from pelvic drain; pelvic abscess, peritonitis, pus from rectum or rectovaginal fistula) along with biological tests and abdominal CT findings'	34	4	Right hemicolectomies to low anterior resections for colorectal cancer	Peritoneal drain fluid samples	Daily from peritoneal drains until POD 5	IL-6, IL-10 and TNFa all significantly increased in those with AL on all days Those without AL had decreasing cytokine levels
Fouda et al. [17]	IL-6, IL-10, TNFa	Prospective	Gas, pus or faecal discharge from the drain, faecal discharge from the wound, pelvic abscess, peritonitis and rectovaginal confirmed by CT, contrast scan or rectal palpation	56	8	Low anterior resections for rectal cancer	Peritoneal drain fluid sample	Peritoneal drain samples on PODs 1, 3 and 5	IL-6 and IL-10 significantly increased on PODs 1, 3 and 5 and TNFa significantly increased on PODs 3 and 5 in those with AL
Yamamoto et al. [21]	IL-1, IL-6, TNFa	Prospective	Post-operative peritonitis with leak thereafter confirmed by contrast study, ultrasound or CT scan	100	8	Anterior resections and AP resections for colorectal cancer	Peritoneal drain fluid sample taken within 2 h of emptying the drain	Peritoneal drain samples on PODs 1, 2 and 3	IL-1, IL-6 and TNFa significantly increased on POD 3 in those with AL Cytokine levels significantly increased over the days in those with AL and fell in those without AL
Alonso et al. [14]	IL-6	Prospective	Clinical suspicion of a leak was confirmed by radiological study or findings at reoperation. Intra-abdominal abscesses were not classified as AL but abscess and ALs were analysed together as an 'infective complications' group	60	30	Right hemicolectomies to anterior resections for colorectal cancer	Baseline IL-6 level measured by lavaging abdominal cavity immediately after laparotomy or creation of pneumoperitoneum Post-operative samples taken from peritoneal drain fluid	Peritoneal drain sample at 48 h and 4 days post-surgery	IL-6 significantly increased at both time points in those with AL

Table 5 Summary of studies looking at biomarkers of wound repair

Authors [ref]	Biomarkers studied	Study design	Definition of AL	No. of patients	No. of leaks	Operations	Methods of biomarker measurement	Frequency and duration of biomarker measurement	Main results
Baker and Leaper [22]	MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, TIMP-1, TIMP-2	Prospective	Not specified—AL grouped with 'major complications'	58	NS	Right hemicolectomies to AP resections for colorectal cancer	Sample from peritoneal drain	Daily until PODs 5–8	MMP-2 (POD 3), MMP-2 (POD 6), MMP-9 (PODs 6 and 7) positively correlated with complications TIMP-2 (PODs 2 and 3) and TIMP-1 (POD 7) negatively correlated with complications
Pasternak et al. [23]	MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13 TIMP-1, TIMP-2	Prospective	Clinical diagnosis including fistulas confirmed by CT, contrast study or DRE	29	10	Low anterior resections for colorectal cancer	Sample from peritoneal drain	Once at 4 h post-operation	MMP-8 and MMP-9 significantly increased in those with AL
Kostic et al. [24]	MMP-9	Prospective	Clinical diagnosis—finding of pus/faeces in drain, pelvic abscess, peritonitis or fistula	150	15	Left-sided colorectal resections for cancer	Sample from peritoneal drain MMP-9 measured via ELISA	On PODs 1, 3, 5 and 7	MMP-9 not significantly different in those with AL

NS not specified

placed [41]. There was some suggestion that drains may have a role in infra-peritoneal rectal anastomoses, but further trials are needed to clarify this; so, until such time, placement of a drain is at the discretion of the surgeon. This makes it unlikely that measurement of lactate and pH from drains would gain widespread acceptance as a means to detect AL. However, the future may lie in the use of biosensor technology. Screen-printed sensors can be created at a low cost and are capable of measuring samples with volumes as small as several microlitres [42]. Creation of such a sensor for lactate/pH is a realistic aim. This could be incorporated into a tiny removable piece of tubing. Wireless technology also creates the possibility of producing a biodegradable implant which could be fixed around the anastomosis and remotely signal information about the anastomotic environment. It is to be hoped that the development of this technology may ultimately allow the widespread use of a simple, acceptable and low-cost method of measuring lactate and pH in the peritoneal fluid surrounding an anastomosis and thus provide early recognition of AL leading to significant reductions in patient morbidity and mortality.

Conclusions

Peritoneal cytokines, lactate and pH have the potential to identify AL early. The consistency of the results for lactate and pH, alongside the fact that they are easy, quick and inexpensive to test, makes them the most attractive targets. In addition to clarifying their stability in peritoneal fluid, future studies should aim to use a homogenous study population with a standardized AL definition.

Acknowledgements We gratefully acknowledge the financial assistance from grants from The Aileen Lynn Bequest Fund, Royal College of Physicians and Surgeons of Glasgow and from the Ileostomy Association, West of Scotland and UK.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kingham TP, Pachter HL (2009) Colonic anastomotic leak: risk factors, diagnosis, and treatment. J Am Coll Surg 208(2):269–278
2. Platell C, Barwood N, Dorfmann G, Makin G (2007) The incidence of anastomotic leaks in patients undergoing colorectal surgery. Color Dis 9(1):71–79
3. Erb L, Hyman NH, Osler T (2014) Abnormal vital signs are common after bowel resection and do not predict anastomotic leak. J Am Coll Surg 218(6):1195–1199. doi:10.1016/j.jamcollsurg.2013.12.059
4. Komen N, de Bruin RW, Kleinrensink GJ, Jeekel J, Lange JF (2008) Anastomotic leakage, the search for a reliable biomarker.

A review of the literature. Color Dis 10(2):109–115 . doi:10.1111/j.1463-1318.2007.01430.xdiscussion 115-107
5. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 151(4):264–269
6. Matthiessen P, Strand I, Jansson K, Tornquist C, Andersson M, Rutegard J, Norgren L (2007) Is early detection of anastomotic leakage possible by intraperitoneal microdialysis and intraperitoneal cytokines after anterior resection of the rectum for cancer? Dis Colon Rectum 50(11):1918–1927. doi:10.1007/s10350-007-9023-4
7. Pedersen ME, Qvist N, Bisgaard C, Kelly U, Pedersen S (2009) Peritoneal microdialysis. Early diagnosis of anastomotic leakage after LAR for rectal cancer. Scand J Surg 98(3):148–153
8. Millan M, Garcia-Granero E, Flor B, Garcia-Botello S, Lledo S (2006) Early prediction of anastomotic leak in colorectal cancer surgery by intramucosal pH. Dis Colon Rectum 49(5):595–601. doi:10.1007/s10350-006-0504-7
9. Yang L, Huang X-E, Xu L, Zhou X, Zhou J-N, Yu D-S, Li D-Z, Guan X (2013) Acidic pelvic drainage as a predictive factor for anastomotic leakage after surgery for patients with rectal cancer. Asian Pac J Cancer Prev 14(9):5441–5447. doi:10.7314/apjcp.2013.14.9.5441
10. Daams F, Wu Z, Cakir H, Karsten TM, Lange JF (2014) Identification of anastomotic leakage after colorectal surgery using microdialysis of the peritoneal cavity. Tech Coloproctol 18(1):65–71. doi:10.1007/s10151-013-1020-0
11. Horer TM, Norgren L, Jansson K (2011) Intraperitoneal glycerol levels and lactate/pyruvate ratio: early markers of postoperative complications. Scand J Gastroenterol 46(7–8):913–919. doi:10.3109/00365521.2011.568519
12. Bini R, Ferrari G, Apra F, Viora T, Leli R, Cotogni P (2014) Peritoneal lactate as a potential biomarker for predicting the need for reintervention after abdominal surgery. J Trauma Acute Care Surg 77(2):376–380. doi:10.1097/TA.0000000000000302
13. Herwig R, Glodny B, Kühle C, Schlüter B, Brinkmann OA, Strasser H, Senninger N, Winde G (2002) Early identification of peritonitis by peritoneal cytokine measurement. Dis Colon Rectum 45(4):514–521
14. Alonso S, Pascual M, Salvans S, Mayol X, Mojal S, Gil MJ, Grande L, Pera M (2015) Postoperative intra-abdominal infection and colorectal cancer recurrence: a prospective matched cohort study of inflammatory and angiogenic responses as mechanisms involved in this association. Eur J Surg Oncol 41(2):208–214. doi:10.1016/j.ejso.2014.10.052
15. Junger W, Junger W, Miller K, Bahrami S, Redl H, Schlag G, Moritz E (1995) Early detection of anastomotic leaks after colorectal surgery by measuring endotoxin in the drainage fluid. Hepato-Gastroenterology 43(12):1523–1529
16. Komen N, Morsink MC, Beiboer S, Miggelbrink A, Willemsen P, van der Harst E, Lange JF, van Leeuwen WB (2009) Detection of colon flora in peritoneal drain fluid after colorectal surgery: can RT-PCR play a role in diagnosing anastomotic leakage? J Microbiol Methods 79(1):67–70. doi:10.1016/j.mimet.2009.08.004
17. Fouda E, El Nakeeb A, Magdy A, Hammad EA, Othman G, Farid M (2011) Early detection of anastomotic leakage after elective low anterior resection. J Gastrointest Surg 15(1):137–144. doi:10.1007/s11605-010-1364-y
18. Komen N, Slieker J, Willemsen P, Mannaerts G, Pattyn P, Karsten T, de Wilt H, van der Harst E, van Leeuwen W, Decaestecker C, Jeekel H, Lange JF (2014) Polymerase chain reaction for Enterococcus faecalis in drain fluid: the first screening test for symptomatic colorectal anastomotic leakage. The Appeal-study: analysis of parameters predictive for evident anastomotic leakage. Int J Color Dis 29(1):15–21. doi:10.1007/s00384-013-1776-8
19. Bertram P, Junge K, Schachtrupp A, Götze C, Kunz D, Schumpelick V (2009) Peritoneal release of TNFα and IL-6 after elective colorectal surgery and anastomotic leakage. J Investig Surg 16(2):65–69. doi:10.1080/08941930390194398
20. Ugras B, Giris M, Erbil Y, Gokpinar M, Citlak G, Issever H, Bozbora A, Oztezcan S (2008) Early prediction of anastomotic leakage after colorectal surgery by measuring peritoneal cytokines: prospective study. Int J Surg 6(1):28–35. doi:10.1016/j.ijsu.2007.10.001
21. Yamamoto T, Umegae S, Matsumoto K, Saniabadi AR (2011) Peritoneal cytokines as early markers of peritonitis following surgery for colorectal carcinoma: a prospective study. Cytokine 53(2):239–242. doi:10.1016/j.cyto.2010.10.006
22. Baker EA, Leaper DJ (2003) Profiles of matrix metalloproteinases and their tissue inhibitors in intraperitoneal drainage fluid: relationship to wound healing. Wound Repair Regen 11(4):268–274
23. Pasternak B, Matthiessen P, Jansson K, Andersson M, Aspenberg P (2010) Elevated intraperitoneal matrix metalloproteinases-8 and -9 in patients who develop anastomotic leakage after rectal cancer surgery: a pilot study. Color Dis 12(7 Online):e93–e98. doi:10.1111/j.1463-1318.2009.01908.x
24. Kostic Z, Panisic M, Milev B, Mijuskovic Z, Slavkovic D, Ignjatovic M (2015) Diagnostic value of serial measurement of C-reactive protein in serum and matrix metalloproteinase-9 in drainage fluid in the detection of infectious complications and anastomotic leakage in patients with colorectal resection. Vojnosanit Pregl 72(10):889–898. doi:10.2298/vsp140723011k
25. Bruce J, Krukowski Z, Al-Khairy G, Russell E, Park K (2001) Systematic review of the definition and measurement of anastomotic leak after gastrointestinal surgery. Br J Surg 88(9):1157–1168
26. Thornton FJ, Barbul A (1997) Healing in the gastrointestinal tract. Surg Clin N Am 77(3):549–573
27. Locke R, Hauser CJ, Shoemaker WC (1984) The use of surface oximetry to assess bowel viability. Arch Surg 119(11):1252
28. Corke C, Glenister K (2001) Monitoring intestinal ischaemia. Crit Care Resusc 3(3):176–180
29. Kanthan R, Shuaib A, Griebel R, Miyashita H (1995) Intracerebral human microdialysis in vivo study of an acute focal ischemic model of the human brain. Stroke 26(5):870–873
30. Klaus S, Heringlake M, Gliemroth J, Bruch H-P, Bahlmann L (2002) Intraperitoneal microdialysis for detection of splanchnic metabolic disorders. Langenbeck's Arch Surg 387(7–8):276–280
31. Sommer T, Larsen J (2004) Intraperitoneal and intraluminal microdialysis in the detection of experimental regional intestinal ischaemia. Br J Surg 91(7):855–861
32. Calatayud O, Tenias J (2003) Effects of time, temperature and blood cell counts on levels of lactate in heparinized whole blood gas samples. Scand J Clin Lab Invest 63(4):311–314
33. Dinarello CA (1984) Interleukin-1. Rev Infect Dis 6(1):51–95
34. Hesse D, Tracey K, Fong Y, Manogue K, Palladino M Jr, Cerami A, Shires G, Lowry S (1988) Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 166(2):147–153
35. Wong GG, Clark SC (1988) Multiple actions of interleukin 6 within a cytokine network. Immunol Today 9(5):137–139
36. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC (1989) Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 242(2):237–239
37. Jansson K, Redler B, Truedsson L, Magnuson A, Matthiessen P, Andersson M, Norgren L (2004) Intraperitoneal cytokine response after major surgery: higher postoperative intraperitoneal versus systemic cytokine levels suggest the gastrointestinal tract as the major source of the postoperative inflammatory reaction. Am J Surg 187(3):372–377

38. Wiik H, Karttunen R, Haukipuro K, Syrjälä H (2001) Maximal local and minimal systemic cytokine response to colorectal surgery: the influence of perioperative filgrastim. Cytokine 14(3):188–192
39. Cini C, Wolthuis A, D'Hoore A (2013) Peritoneal fluid cytokines and matrix metalloproteinases as early markers of anastomotic leakage in colorectal anastomosis: a literature review and meta-analysis. Color Dis 15(9):1070–1077. doi:10.1111/codi.12192
40. Verma RP, Hansch C (2007) Matrix metalloproteinases (MMPs): chemical–biological functions and (Q) SARs. Bioorg Med Chem 15(6):2223–2268
41. de Jesus EC, Karliczek A, Matos D, Castro AA, Atallah ÁN (2004) Prophylactic anastomotic drainage for colorectal surgery. Cochrane Library Syst Rev
42. Ahmed MU, Hossain MM, Safavieh M, Wong YL, Rahman IA, Zourob M, Tamiya E (2016) Toward the development of smart and low cost point-of-care biosensors based on screen printed electrodes. Crit Rev Biotechnol 36(3):495–505