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Does postoperative inflammation or sepsis generate neutrophil extracellular traps that influence colorectal cancer progression? A systematic review

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ABSTRACT

Background: Colorectal cancer is the third most common cancer worldwide. Almost half of those that have a potentially curative resection go on to develop metastatic disease. A recognized risk for recurrence is perioperative systemic inflammation and sepsis. Neutrophil extracellular traps have been implicated as promotors of tumor progression. We aimed to examine the evidence in the literature for an association between neutrophil extracellular traps and postoperative metastasis in colorectal cancer.

Materials and methods: Studies published between 2000 and December 2018 that examined the role of neutrophil extracellular traps in sepsis and inflammation in colorectal cancer and in relation to tumor-related outcomes were identified through a database search of Cochrane, CINAHL, and MEDLINE. Quality and bias assessment was carried out by 2 reviewers.

Results: Of 8,940 screened and of the 30 studies included, 21 were observational, 5 were in vivo experimental, 1 was in vitro, and 3 used a combination of these approaches.

Conclusion: There is clear evidence from the literature that presence of a preoperative systemic inflammatory response predicts cancer recurrence following potentially curative resection, but the evidence for association of sepsis and progression is lacking. There is robust experimental evidence in murine models showing that neutrophil extracellular traps are present in sepsis and are associated with cancer progression. Some human observational studies corroborate the prognostic significance of neutrophil extracellular traps in progression of colorectal cancer. Further human studies are needed to translate the experimental evidence and to definitively associate sepsis and neutrophil extracellular traps with poor colorectal cancer-specific outcomes.

1. INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, with the global burden of disease projected to increase to 2.2 million new cases and 1.1 million deaths per year by 2030 [1]. The mainstay of treatment is a curative resection, and major advances and innovation in surgical technique, the use of neoadjuvant treatment for rectal cancer, and widespread uptake of screening programs have benefited patients through earlier detection and improvements in oncologic outcomes. Despite these advances, almost half of those that undergo a resection with curative intent subsequently develop metastatic disease [2]. Although it has been established that circulating tumor cells are present at the time of surgical resection [3–6], the mechanisms that underlie

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the establishment of these micrometastatic viable tumor cells into distant metastases are poorly understood. A recognized risk of recurrence is perioperative systemic inflammation, including sepsis [7–13], and one suggested mechanism implicates neutrophil extracellular traps (NETs) as promotors of tumor metastasis and progression [14]. Thus, although surgical resection can cure, it has also been shown that surgical stress and complications can trigger systemic inflammation, and it can be speculated that, in doing so, surgery can induce production of cancer cell–trapping NETs.

Since the seminal studies by Brinkmann et al [15], our understanding of the integral role NETs play in innate immunity has expanded significantly [16,17], and a burgeoning body of literature implicates NETs in tumor development and cancer progression [14]. NETs are extracellular structures produced by neutrophils and consisting of a double-stranded DNA backbone and globular proteins and proteases from the neutrophil cytoplasm [17]. NET structures have been described as “beads on a string” [18], with these strings coalescing into larger threads of chromatin. The protein “beads” consist predominantly of histone proteins (particularly citrullinated histone H3 [H3Cit]), neutrophil elastase (NE), and myeloperoxidase (MPO) [18]. The phenomenon of generating NETs has been coined *NETosis*, for which 3 mechanisms have been observed [14,19]. The first is via slow lytic neutrophil death whereby cytoplasmic and nuclear contents leak and combine to form NETs in the extracellular space. The second is a rapid vesicular secretion of preformed NETs from the neutrophil. The third mechanism is the formation of NETs from mitochondrial DNA (mtDNA). Interestingly, mtDNA NETs lack the histone “beads” that are found on nuclear NETs and overall contribute very little to the circulating NET levels. Studies have found that the mtDNA NET levels are less than 100,000 times those of nuclear NETs [19,20].

The biological role of NETs has been widely studied at a fundamental level. NETs are known to bind to a range of pathogens to form a physical barrier and degrade virulence factors, acting as a “last line” of defense. Although this biological role is beneficial, NETs have also been shown to cause inflammatory damage to tissues, and their dysregulation is implicated in the development of autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus [21]. To date, most investigations of mechanisms and consequences of NETosis have been confined to preclinical studies, and only recent years have seen a slow emergence in NETs-focused human research. As such, the role of NETs in tumor development is currently unclear [22]. Although NETs in the local tumor environment are associated with a more favorable prognosis [22], intravascular NETs appear to have negative prognostic implications, and the presence of circulating NETs may actually promote metastasis [23–27], although this finding is predominantly from animal experimental studies. Parallel to this, it is long established that a systemic inflammatory response (SIR) and episodes of perioperative sepsis are associated with poorer cancer-specific survival (CSS) [7–13,28].

The contribution of NETs to the progression of CRC is an area of study that is in its infancy. Given the evidence that sepsis and systemic inflammation are associated with recurrence in patients who have undergone a potentially curative resection of their CRC, and the evolving knowledge of NETs in the immune response and their potential as promoters of metastasis, we postulate that NETs generated by surgical inflammation and its infectious complications may be integral in facilitating growth of circulating tumor cells into established metastasis and influencing prognosis in CRC. This process is represented in Fig. 1. The aim of this scoping systematic review is to evaluate the evidence that NETs are present in states of surgical inflammatory stress and sepsis and to examine the evidence for an association between NETs or other surrogate markers of inflammation with cancer-related outcomes in CRC. In evaluating the evidence for these questions, we aim to encompass disparate aspects of NETs biology and current understanding of the mechanisms of cancer progression in the surgical patients to understand the interplay that could ultimately be driving metastatic disease and subsequent death in CRC. (See Fig. 1.)

2. MATERIALS AND METHODS

We have adhered to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [29]. A completed PRISMA checklist can be found in Appendix 1.

2.1. Identification of studies. An electronic literature search was carried out using the registered search protocol (Appendix 2) available through Prospero CRD42017068935 [30]. Databases searched were MedLine, Cochrane Library, and CINAHL. The latest date of the literature search was 11 December 2018. Duplicates were removed. Two authors (GMC and GB) independently screened titles and abstracts against inclusion and exclusion criteria. All articles that were included for full-text screening were retrieved and evaluated for inclusion by GMC and GB. Reference lists were hand-searched for relevant articles which were then included in full-text screening until no further relevant publications meeting inclusion and exclusion criteria for full-text inclusion remained. This is represented in Fig. 2.

2.2. Study inclusion and data extraction. Studies were included if they addressed the topic of NETs in association with surgery, sepsis, and cancer or if the article addressed associations between systemic inflammation and oncologic outcomes in CRC. All articles published prior to 2000 (as the identification of NETs was published in 2004), case reports, commentary articles, and review articles were excluded, as were articles with the main topic of NETs in autoimmune diseases and articles dealing with short-term survival in CRC unless specifically focused on NETs. Full inclusion and exclusion criteria are detailed in Table 1. No data extraction for meta-analysis was performed given the scoping nature of this review.

2.3. Assessment of methodological quality and validity. The articles that met final inclusion criteria underwent quality assessment by GMC and JP. Articles were compared against the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [31] and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) statement [32] for human observational and animal experimental studies, respectively. Scores were designated based on these criteria. Disagreements on scores were discussed and consensus reached. Of the studies based wholly or partly in vitro, quality assessment was based on experimental robustness and external validation with replication of findings in other studies. Quality assessment of the included studies is detailed in Appendix 3.

3. RESULTS

Hand-search and database search identified 8,940 articles after deduplication. Titles and abstracts were screened. At this stage, review articles and articles dealing with SIR and solid organ malignancies were included for the purpose of reference extraction. Of these, 87 articles proceeded to full-text screening. The systematic literature search yielded a total of 30 studies after full-text screening. This process is presented in Fig. 1. Of the 30 studies included, 21 were observational, 5 were in vivo experimental, 1 was in vitro, and 3 used a combination of these approaches. The study characteristics and findings are summarized in Table 2.

3.1. The association of the SIR and sepsis with CRC outcomes. Eighteen of the 30 included studies investigated the associations between the SIR and outcomes in CRC. Surrogate markers such as C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) were used across these studies as

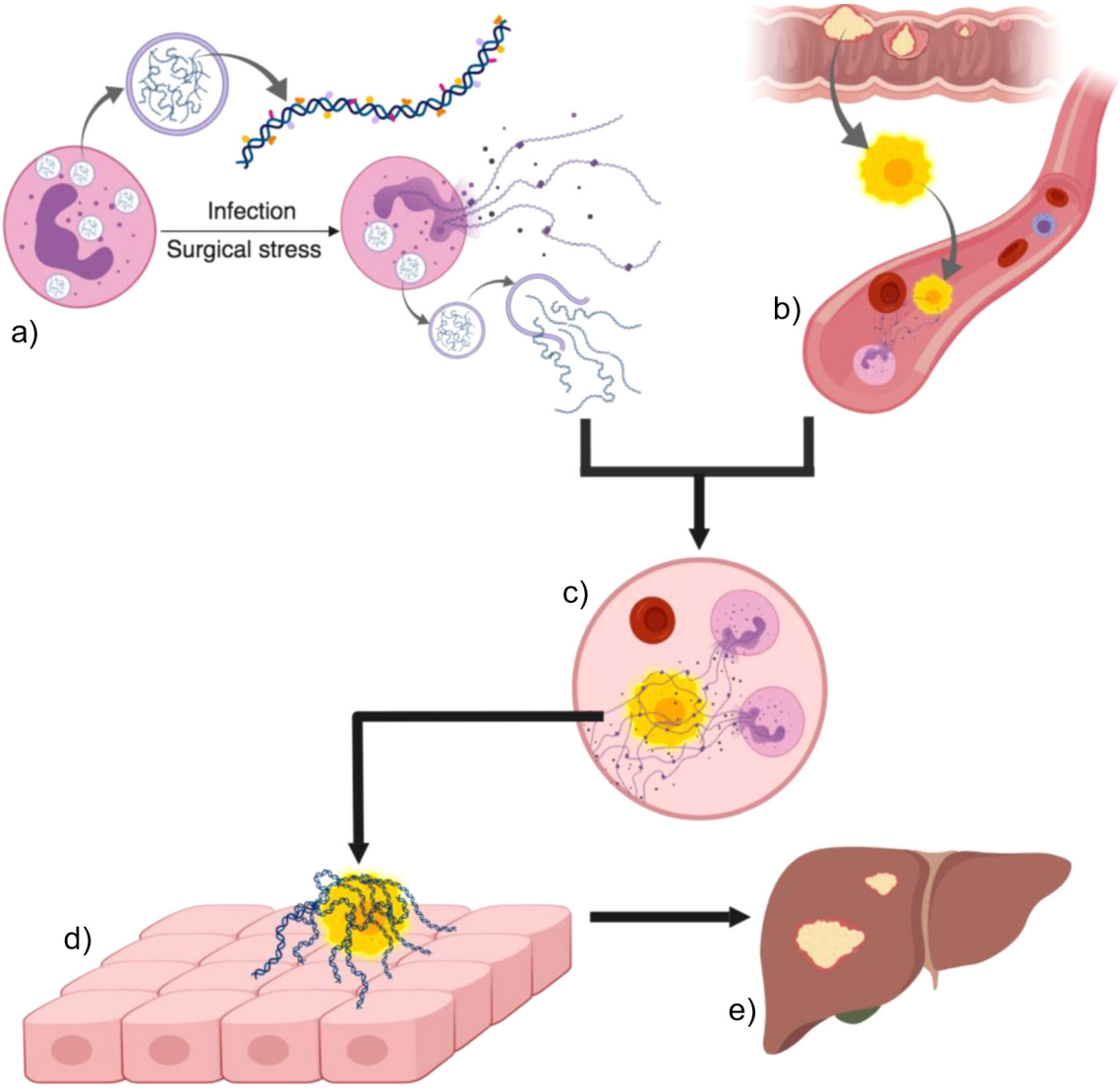

Fig. 1. *A*, NETosis is induced by surgical stress and postoperative infection, and NETs are released into systemic circulation; (*B*) concurrently, there are circulating viable tumor cells being shed by the primary tumor, (*C*) circulating tumor cells and circulating NETs interact, (*D*) tumor cells are trapped by NETs and embedded on endothelium in sites distant to the original tumor, and (*E*) metastatic deposits develop.

indicators of systemic inflammation rather than specifically investigating pathogen-induced sepsis. Seven of these 30 studies include patients with CRC undergoing cancer resection at the Glasgow Royal Infirmary, UK, and overlap in patient groups is likely in these studies [10,12,33–37]. This is summarized in Table 3. Overall, the studies from the Glasgow Royal Infirmary show that preoperative systemic inflammation confers poorer prognosis in CRC [12,34,35,37], regardless of the tool used to measure inflammation. The earliest study of 174 patients showed that preoperative but not postoperative CRP taken at 4-month follow-up was associated with CSS on multivariate analysis [10]. Crozier et al replicated these results in 2007 using postoperative day (POD) 2 CRP [34] and in 2009 substituting CRP for the modified Glasgow Prognostic Score (mGPS) which combines albumin and CRP [35]. The mGPS was later used to demonstrate the relationships between the preoperative SIR, local inflammatory response, corresponding tumor characteristics and CSS [12] as well as the local response in terms of tumor necrosis, and the association of SIR with local responses and prognosis [37]. Carruthers et al also studied CRC patients in Glasgow, although this cohort differed from the aforementioned because it included rectal cancer patients undergoing preoperative chemoradiotherapy for T3 or T4 borderline resectable or unresectable disease. Findings were consistent those above, showing that NLR over 5 and the resection margin status were significantly associated with decreased overall survival (OS), CSS, and time to local recurrence on multivariate analysis [38]. The study of Proctor et al used the Scottish Cancer Registry to identify 27,031 patients with any malignancy and to compare the prognostic value of the various inflammatory scores, including CRP, albumin, leukocyte count, neutrophil count, lymphocyte-monocyte ratio (LMR), and PLR. These values were taken from the first of any set of blood samples taken within 2 years following patient cancer diagnosis. In this study, it was found that CRP, higher mGPS, NLR, and PLR were all predictors of poorer CSS [36].

Other included studies included in this review demonstrated results largely consistent with the results of the studies from Glasgow and the Scottish Cancer Registry, with only 2 studies demonstrating conflicting findings in respect to CSS [39,40]. Mallappa et al found that higher preoperative NLR was associated with poorer CSS in patients undergoing elective resection of CRC [41],

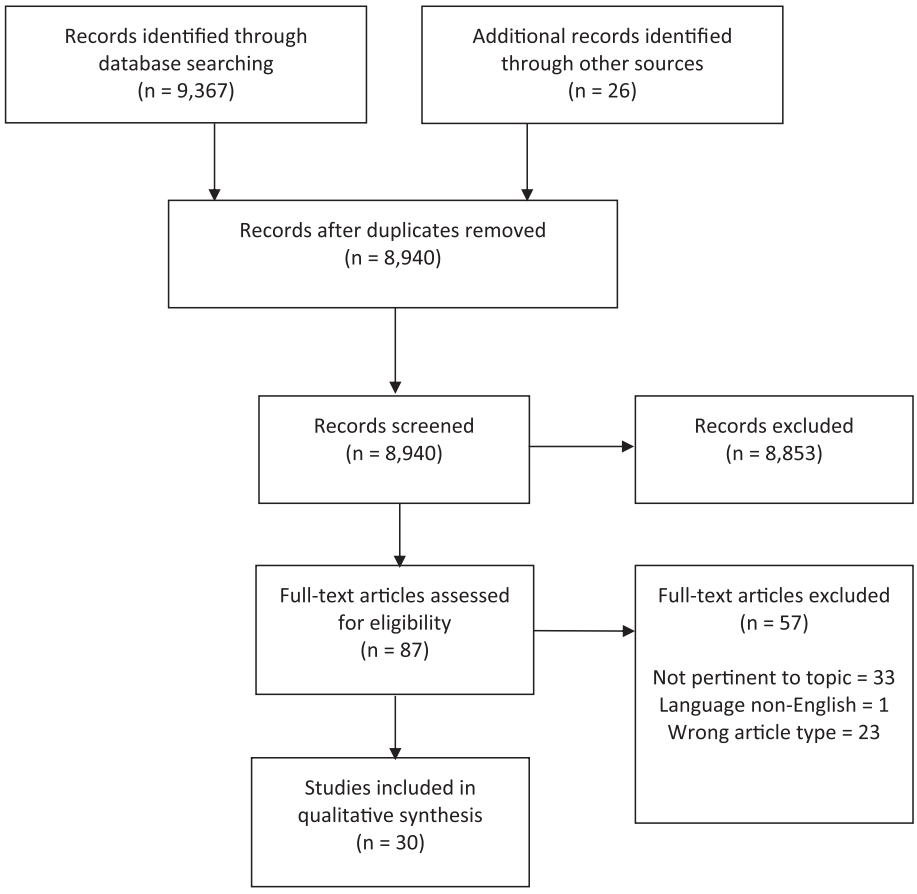

Fig. 2. PRISMA flow diagram.

and Neal et al mirrored this association in resection of potentially curative resection of colorectal liver metastases [42]. An association was again found with CSS when substituting preoperative CRP for NLR in studies by Kersten et al [43] and Mori et al [44]. However, the study by Kersten et al did exclude all patients with infection and emergency presentation, a group which would conventionally have higher levels of inflammatory markers, and in excluding these patients removed a group of interest to this review. Turner et al studied the interval change between high and low levels of inflammatory indices in patients with metastatic CRC that underwent resection of their primary tumor, and found that high NLR over 5 either preoperatively or postoperatively was predictive of OS but not CSS compared to those that maintained a consistently low NLR [40]. The 2017 study by Chan et al of 3,281 patients undergoing resection of their primary CRC also looked at interval change using LMR preoperatively and within 21–56 days postoperatively. Consistently low LMR was associated with vastly improved median survival compared to those with a high LMR at either interval [45]. NLR as an indicator of prognosis was again reported by Song et al, who found NLR to be superior to LMR, PLR, and prognostic nutritional index as an independent prognostic factor for OS and CSS in a cohort of 1,744 patients undergoing curative resection of their CRC [46]. Cutoff scores for high or low levels of each inflammatory marker were calculated based on association with overall survival using *C* index. Contradicting the results of the studies summarized thus far, Portale et al reported that, in their cohort

Table 1
Inclusion and exclusion criteria

Inclusion	Exclusion
Main topic NETs in sepsis, infection, bacteremia, and surgery	Neutropenic sepsis
Main topic NETs and cancer progression	Main topic is not cancer-free survival or cancer progression, eg, short-term survival from infection
Main topic NETs and cancer-specific prognosis	or sepsis or days spent in hospital due to infective complications of surgery
Main topic sepsis and CRC outcomes specific to cancer-specific survival and prognosis	Main topic is NETs in autoimmune inflammatory conditions
Main topic systemic inflammation and CRC specific survival	Case reports
	Commentary articles
	Letters or responses to an article
	Review articles
	Published prior to 2000
	Non–English-language article

Table 2
Summary of included studies

Study	Study design	Findings
Albrengues et al [27]	In vivo (mouse)	Transition of murine breast cancer cells to G1/S phase of cell cycle is neutrophil dependent, and NETs inhibition or DNase I prevented or decreased LPS-induced "awakening" in dormant murine and human breast cancer cells.
Canna et al [33]	Observational (human)	Elevated CRP and low percentage tumor volume of CD4 + T-lymphocytes both predict poor cancer-specific survival in patients undergoing potentially curative resection for CRC.
Carruthers et al [38]	Observational (human)	R status and NLR are associated with overall and disease-free survival and time to local recurrence in patients having preoperative chemoradiotherapy for T3 or T4 rectal cancer. Neutrophil count, lymphocyte count, PLR, CEA, and albumin did not show associations with any outcomes.
Chan et al [45]	Observational (human)	Low preoperative and postoperative NLR predicts better median survival than high preoperative ratio, or change from preoperative low levels to postoperative high ratio in patients undergoing curative resection for CRC.
Cools-Lartigue et al [23]	In vivo (mouse)	Intravascular NETs are generated and are associated with trapping of circulating tumor cells. NET trapping is associated with increased metastatic disease. This is decreased by NET inhibitors (DNAse or neutrophil elastase inhibitor).
Crozier et al [34]	Observational (human)	Preoperative CRP, but not CRP on POD2, predicts poor cancer-specific survival in patients undergoing potentially curative resection for CRC.
Crozier et al [35]	Observational (human)	Emergency presentation and elevated mGPS were predictive of poor cancer-specific survival in patients undergoing potentially curative resection for CRC.
Inoue et al [25]	Observational (human), in vivo (mouse), in vitro	Albumin can modulate intravascular NETosis, and mice either deficient in albumin or treated with iodocetamine (inhibitor of albumin free thiols) had increased NETosis, which promoted lung predominant metastases after injection of head and neck cancer cells.
Kersten et al [43]	Observational (human)	High preoperative CRP correlates with poorer cancer-specific survival in all stages of CRC in patients undergoing any surgery for CRC. Excludes emergency presentation and any patients with infection.
Kressner et al [47]	Observational (human)	There is association between perineal infection and local recurrence but not abdominal sepsis and recurrence in patients undergoing potentially curative resection for rectal cancer.
Laurent et al [8]	Observational (human)	Postoperative morbidity is associated with increased recurrence in patients undergoing potentially curative liver resection for CRC liver metastases.
Mallappa et al [41]	Observational (human)	Preoperative NLR >5 is associated with CRC recurrence in patients undergoing potentially curative resection for CRC.
McDonald et al [54]	In vivo (mouse)	NETs are released during endotoxemia and sepsis. NETs ensnare bacteria. Bacterial trapping is increased by 4-fold in the presence of NETs.
McMillan et al [10]	Observational (human)	Increased cancer stage and preoperative and postoperative CRP were associated with overall and cancer-specific survival in patients undergoing potentially curative resection for CRC.
Mori et al [44]	Observational (human)	Higher preoperative CRP was associated with poorer cancer-specific survival in patients undergoing potentially curative resection for CRC, but NLR and PLR were not predictive on multivariate analysis. Low levels of infiltrating CD8 + T-cells in CRC tissue were a predictor of poorer cancer-specific survival.
Najmeh et al [57]	In vivo (mouse)	In a murine model of intra-abdominal sepsis, beta-1 integrin expression on cancer cells and NETs facilitates adhesion. This is partially diminished when treated with DNAse 1.
Neal et al [42]	Observational (human)	High preoperative NLR and derived NLR, but not PLR or LMR, are predictors of shortened overall and cancer-specific survival in patients undergoing potentially curative liver resection for CRC metastases.
Park et al [26]	Observational (human), in vivo (mouse), in vitro	Breast cancer cells can promote NETosis in the absence of infection in mice. GCSF primes neutrophils for NETosis. NETs deposition in human primary and metastatic breast cancer tissue is associated with aggressive tumor subtypes. Treatment with DNase I–coated nanoparticles decreases metastatic tumor burden in mice.
Pilsczek et al [19]	Observational (human)	CEA but not PLR or NLR is an independent predictor of 5-y overall and disease-free survival in patients undergoing laparoscopic resection of stage I–III rectal cancer.
Portale et al [39]	In vitro	A new mechanism of NET formation was observed. Neutrophils produce NETs in response to *S aureus* through a process of rapid (5–60 min) vesicular secretion. Mitochondrial DNA contributed minimally to NETs.
Proctor et al [36]	Observational (human)	Elevated preoperative mGPS, NLR, PLR, prognostic index, and prognostic nutrition index were predictive of reduced cancer-specific survival in cancer patients with a range of malignancies. mGPS and prognostic index were predictive of reduced cancer-specific survival in CRC.
Richards et al [37]	Observational (human)	Tumor necrosis, high preoperative mGPS, low inflammatory infiltrate in CRC tissue, and cancer stage were associated with reduced cancer-specific survival in patients undergoing potentially curative resection of CRC. Tumor necrosis was associated with an increase in mGPS and reduced inflammatory infiltrate.
Richardson et al [55]	Observational (human) with ex vivo analysis	Neutrophils isolated from CRC patients having surgery and subsequently stimulated by fMLP, LPS and IL-8 have reduced NETs formation, inhibition of apoptosis, and an increase in phagocytosis in response to surgery.
Richardson et al [56]	Observational (human) with ex vivo analysis	NETs levels from neutrophils isolated and stimulated from aforementioned CRC patient cohort and from a cohort of healthy controls are higher from CRC patients, and NETs levels from neutrophils isolated preoperatively may be associated with adverse patient outcomes.
Roxburgh et al [12]	Observational (human)	High preoperative mGPS and low peritumoral inflammatory infiltrate are associated with poor cancer-specific survival in patients undergoing potentially curative resection for CRC.
Song et al [46]	Observational (human)	NLR is superior to LMR, PLR, and prognostic nutritional index as independent predictor of overall survival and cancer-specific survival in 1,744 patients having curative resection of CRC.
Thalin et al [58]	Observational (human)	In a cohort of patients with advanced incurable cancer, NETs were significantly increased in cancer patients compared to groups of severely ill patients and healthy controls
Tohme et al [24]	Mixed in vivo (mouse) and observational (human)	Increased postoperative NETosis was associated with >4-fold reduction in disease-free survival in patients undergoing potentially curative liver resection for CRC liver metastases. In a murine model of liver I/R injury, increased NETosis correlated with increased metastatic disease. This was reduced on treatment with NET inhibitors.
Turner et al [40]	Observational (human)	Reversal of a preoperatively high NLR following resection of primary tumor was associated with increased overall survival in patients with metastatic CRC.
Yipp et al [53]	In vivo (mouse)	In a murine model of superficial bacterial skin infection, NETosis (via a non–cell death pathway) was confined to the local environment. The same was shown in humans.

Table 3
Summary of Glasgow Royal Infirmary studies

Study	Year	Patient no.	Years incl.	Additional incl. & excl. criteria	Inflammatory marker	Measurement intervals	Minimum follow-up
McMillan et al [10]	2003	174	1993–1998	Curative resection of CRC, staged by Dukes	CRP	Preoperative 4 mo postoperative	Unclear
Canna et al [33]	2005	147	1997–2001	Curative resection of Dukes B or C CRC	CRP	Preoperative	30 mo
Carruthers et al [38]				T3 and T4 borderline or unresectable rectal cancer having preoperative chemoradiotherapy.	NLR, PLR, albumin, CEA	Preradiotherapy	0.5 mo
Crozier et al [34]	2007	180	1999–2004	Curative resection of CRC. Excludes any emergency cases, preoperative radiotherapy, clinical infection, and inflammatory conditions	CRP	Preoperative Day 2 postoperative	22 mo
Crozier et al [35]	2009	188	1999–2006	Curative resection of CRC, preoperative CRP and albumin available	mGPS	Preoperative	12 mo
Roxburgh et al [12]	2009	287	1997–2004	Curative resection of CRC. Excludes emergency presentation, infection, chronic inflammatory conditions, preoperative radiotherapy	GPS	Preoperative	34 mo
Proctor et al [36]	2011	27,031	2000–2007	Patients with any cancer, as identified in the Scottish Cancer Registry that had blood tests recorded any time before diagnosis	CRP, albumin, white cell count (WCC), neutrophils, LMR, PLR, mGPS, NLR, PI	Variable	Unclear
Richards et al [37]	2012	343	1997–2007	Stage I–III CRC	mGPS	Preoperative	45 mo

of 150 patients, both PLR and NLR showed poor discriminative performance in predicting both 5-year overall and disease-free survival (DFS). The only independent variable that was found to be associated with OS and DFS was carcinoembryonic antigen (CEA) level, with the area under the curve for DFS being 0.48 and 0.47 for PLR and NLR, respectively [39].

With regard to how sepsis specifically as a driver of the SIR is associated with CRC outcomes, 2 studies extracted are of relevance. Laurent et al conducted a retrospective study of 311 patients undergoing liver resection of colorectal metastases, in which 51% of postoperative morbidity was due to sepsis. Multivariate analysis showed that postoperative morbidity was an independent predictor of recurrence, although sepsis itself was not analyzed as an independent variable separate to postoperative morbidity in general [8]. Upon examining the influence of abdominoperineal septic complication on recurrence in rectal cancer, Kresser et al found no association between abdominal sepsis and prognosis, but perineal infection increased the incidence of local recurrence. Infection at either site did not have any significant influence in respect to CSS [47].

The aforementioned studies when considered together show evidence that presence of a SIR is associated with poor oncologic outcomes in CRC. Of note is that few have examined infection and sepsis independently, which is of interest given that rapid NETosis is known to occur in response to bacteria. It must also be noted that there is significant heterogeneity in the markers of the SIR used (NLR, PLR, LMR, mGPS, CRP), the time points and intervals at which inflammatory markers were measured, and the cutoff levels used to deem levels "high" or "low" when used for dichotomous rather than continuous analysis. Several factors limit the potential to synthesize the results of these studies. McMillan et al, by using CRP at 4 months postoperation, cannot necessarily capture potential association between immediate postoperative SIR, and a substantial number of patients did not have a CRP measured at one of either the preoperative or postoperative stage [10]. The use of CRP on POD2 by Crozier et al likely reflects the SIR to surgery per se rather than any subsequent sepsis that ensues from surgical interventions [34]. Proctor et al have significant heterogeneity of the time points used for measurement of inflammatory markers, and many intercurrent diseases and interventions may have occurred. These factors are not controlled for, nor are these included as factors in their analysis. The study of Laurent et al is of importance in that sepsis was examined as a variable under the umbrella of postoperative morbidity. However, drawing conclusions about the prognostic implication of postoperative sepsis specifically is difficult, as sepsis was not analyzed as an independent variable separate to postoperative morbidity. Ultimately, this collection of evidence, although broadly showing that presence of a SIR is associated with prognosis, also presents contradicting and conflicting findings regarding the validity of any single measurement of the SIR as an independent predictor of CSS.

3.2. The presence of NETs in SIR, surgery, and sepsis. The evidence demonstrating that NETs are generated by and present in the SIR, surgery, and sepsis is sourced primarily from experimental in vitro and animal studies, although more recent human studies in surgical patients have furthered the field and made early steps to translate early experimental findings to the clinical sphere. In addition to NETs-specific evidence, NETs have been implicated to be present in sepsis by a number of human clinical observational studies that measured circulating cell-free DNA (cfDNA), of which some component is thought to be comprised of NETs [48–50]. NETs, as components of cfDNA, have also been studied in trauma patients as markers of sepsis and predictors of "second hit" insults [50,51]. In the studies examining NETs specifically, the methods used for NET quantification were reasonably consistent, using a combination of fluorescing antibodies against various components of NETs (such as NE, histone H2A, citrullinated histone H3) and SytoxGreen (a molecule that is fluorescent when intercalated into DNA strands but impermeable to live cells and hence will only fluoresce when bound to cfDNA) [52].

Pilsczek et al isolated human neutrophils and exposed them to a variety of gram-negative and gram-positive bacteria which resulted in a 2- to 3-fold increase in extracellular DNA within 1 hour. Interestingly, this response was exaggerated to a 10-fold increase with exposure to both *Staphylococcus aureus* and *S pyogenes*, as well as *Pseudomonas aeruginosa*, although it is worth noting that *P aeruginosa* is the only bacteria tested that extrudes any significant amount of intrinsic DNA into the extracellular environment. Overall, this study suggests that in vitro exposure to bacteria triggers neutrophils to undergo NETosis, more so through a rapid vesicular secretion rather than lytic cell death [19]. At the time of the study of Pilsczek et al, much of the evidence for the biology and role of

NETs was from in vitro models, and there was skepticism from some regarding the physiological relevance of NETs. This has to some degree been addressed using animal models. Yipp et al used a murine model of *S aureus* superficial wound infection to explore the physiological behavior of NETs in vivo [53]. NETs were visualized as thin sheets of DNA in the wound but were not present in the blood vessels of the infected skin or underlying muscle. This suggests that a confined infection alone, in the absence of systemic sepsis, is not sufficient to trigger intravascular disseminated NETosis. The study went on to explore a murine sepsis model by intravascular injection of human *S aureus* and *S pyogenes*. These dead, washed bacteria can be recognized by the innate immune system but cannot release endotoxins. Significant release of histones and NE occurred within minutes, providing further evidence of bacterial recognition by neutrophils leading to NETosis. Intraperitoneal sepsis models have been used to demonstrate NETosis in the acute phase of sepsis [54]. Intraperitoneal injection of *Escherichia coli* after lipopolysaccharide (LPS) challenge led to intravascular NET accumulation in the hepatic sinusoids after just 4 hours. This study was the first to show evidence of in vivo NETosis in response to bacterial insult (albeit through exposure to only 1 pathogen) and that NETs are generated in the acute phase of *E coli* sepsis. NET responses to polymicrobial infection have been demonstrated using the cecal ligation and puncture (CLP) models of intra-abdominal sepsis. This was generated with the animals' own microbiome, invariably leading to intra-abdominal sepsis. Significantly more NETs were observed in the hepatic sinusoids and pulmonary capillaries in the CLP group compared to sham groups, demonstrating that endogenously generated sepsis has results consistent with those observed in the aforementioned exogenously induced models [23].

Richardson et al published 2 studies in 2017 that examined NETosis in the context of CRC surgery. NETosis was examined in an ex vivo fashion in which neutrophils were isolated from 44 patients undergoing curative resection for CRC and then stimulated with interleukin (IL)-8, LPS, and N-formyl-methionyl-leucyl-phenylalanine (fMLP). In the first study, results suggested that adverse patient outcomes are associated with increased preoperative NETs production (in response to all stimuli), and the authors concluded that this is indicative of increased neutrophil activation, possibly as a result of cancer-associated inflammation [55]. In the subsequent study, the authors elaborated on systemic neutrophil function more broadly in the same group of patients. Neutrophils were isolated preoperatively and on POD1 and POD3. Measures of viability and apoptosis were performed, and NET generation in response to various sterile stimuli was performed. Statistically significant findings included differences in NETs formation over the perioperative period (in the absence of stimulation and with all stimuli used), that increased NET production was found in those that had significant complications (Clavien-Dindo grade 3 or more), and that those whose length of stay exceeded 5 days. These findings only reached significance when the trigger for NETosis was fMLP, whereas the other inflammatory stimuli failed to result in significant NETosis. There were no statistically significant differences in NETs production when comparing operative technique, colonic or rectal cancer, or Dukes stage [56].

3.3. NETs interact with cancer cells and are promotors of cancer progression. Several studies have used in vitro and animal models to demonstrate interactions between NETs and cancer cells and have begun to investigate translational potential in cancer patients. Cools Lartigue et al investigated the influence of NETs on cancer progression in septic mice, showing that mice subjected to a CLP before intrasplenic injection of lung cancer cells or intravenous melanoma cells have a significantly greater burden of hepatic metastatic disease for both tumor types compared to mice that had sham surgery. Supported by this finding, the authors inferred that the CLP group had widespread NET deposition in the liver and lungs and that NETs are integral for trapping CTCs in these septic mice. Mice treated with systemic DNase or neutrophil elastase inhibitor (which degrades and inhibits NET activity, respectively) prior to tumor cell challenge had decreased development of hepatic metastases compared to the nontreated mice. One final novel element to this study was real-time video acquisition demonstrating arrest of circulating lung cancer cells in liver sinusoids, which stained densely for histone. This was not due to sinusoidal plugging, as neutrophils continued to migrate freely. Hence, it was concluded that NETs were directly responsible for trapping CTCs [23]. Further work by this group used the same animal models of sepsis and CTCs to study the mechanisms behind NET-CTC interactions and found that there was increased expression of β_1 integrin on both CTCs and NETs. However, blockade of β_1 integrin did not completely inhibit the NET-CTC adhesion and interaction, and the authors suggest that this is likely to be just one of many NET-CTC interactions that cause trapping of tumor cells [57].

Tohme et al used a murine model of sterile rather than sepsis-induced NETosis and murine CRC to show that NETosis results in increased tumor burden. Mice underwent hepatic ischemia-reperfusion (I/R) injury, mimicking the physiological process that occurs in hepatic resection for colorectal liver metastases. The mice that had I/R were found to have widespread intrahepatic NET deposition compared to the control group. These mice were then challenged with mouse-derived CRC cells via intrasplenic injection with or without daily DNase treatment. DNase is proposed to reduce NETs levels through the degradation of the DNA backbone that is a key component of NETs. Treatment groups had 68% reduction in hepatic tumor burden compared to controls [24]. These results demonstrate that the same pathological outcome occurs in sterile inflammation and surgical insults as in sepsis.

Murine models were again used by Park et al in an extensive study using a combination of in vitro, murine in vivo, and human observational approaches. This study ultimately found that breast cancer cells can promote NETosis in the absence of infection, that granulocyte colony-stimulating factor (GCSF) primes neutrophils for NETosis, that NETs deposition in primary and metastatic breast cancer deposits is associated with aggressive tumor subtypes, and that treatment with DNase I–coated nanoparticles decreased metastatic tumor burden. Mice injected with murine breast cancer cells had pulmonary NETs deposits seen with immunofluorescent staining, suggesting a phenomenon of cancer-induced NETosis. This mechanism was independent of direct neutrophil-cancer cell interaction, demonstrated via Transwell chamber assays. In a cohort of human patients with metastatic breast cancer, immunofluorescent staining revealed the presence of NETs in 16 of 20 primary tumors and 13 of 19 metastatic lung lesions and, furthermore, that the number of NETs was highest in more aggressive triple-negative tumors. Finally, mice injected with breast cancer cells were treated with intraperitoneal injection of DNase I–coated nanoparticles or control nanoparticles. One third of the treated mice had no detectable histological metastases compared to all 10 of the control group who had microscopic or macroscopic metastatic deposits. This suggests that NETs are critical for metastatic colonization [26].

In studies conducted to examine the mechanisms by which cancer drives NETs and vice versa, Albrengues et al [27] examined the associations between tobacco smoke exposure and LPS on NETosis and dormant cancer cell awakening, whereas Inoue et al [25] examined interactions between plasma-free thiols, albumin, and NETs. In their 2018 study, Albrengues et al demonstrated neutrophil-dependent transitioning of murine breast cancer cells to the G1/S phase of the cell cycle and furthermore demonstrated that NETs are a powerful external stimulus for cancer cell “awakening” after PAD4 inhibitors or DNase I administered free or on coated nanoparticles prevented or decreased LPS-induced awakening in dormant murine and human breast cancer cells. The authors hypothesized

that this awakening effect of NETs on cancer cells may be due to NETs remodeling of extracellular matrix laminin to produce an integrin $\alpha_3\beta_1$ activating epitope, which in turn may signal multiple intracellular awakening pathways in cancer cells [27]. Inoue et al used in vitro and murine models to identify that albumin, as a pool of plasma-free thiols and a physiological regulator of plasma redox balance, can modulate intravascular NETosis. Additionally, mice either genetically deficient of albumin or treated with iodocetamine (a pharmacologic inhibitor of albumin free thiols) demonstrated increased NETosis with a predominant pulmonary NETs deposition, which also promoted lung-predominant metastases after injection of head and neck squamous cell carcinoma cells. These findings were tested in a group of 22 human nonmetastatic head and neck squamous cell carcinomas. In the patients that developed lung metastases, their midtreatment levels of plasma-free thiols were significantly lower when compared to those that did not develop metastases, and a significant increase in NETs levels (measured by CitH3 levels) was present in those with low levels of nonoxidized albumin. Ultimately, the authors postulate from these results that plasma redox imbalance through a decreased level of albumin derived plasma free thiols could be a mechanism leading to elevated circulating NETs levels and subsequent metastatic progression [25].

Thalin et al examined the relationships between NETs and cancer in a very different clinical context to most other studies investigating this topic. Rather than examining NET levels in patients undergoing surgery or investigating potential biological processes and interactions, NETs were observed in a cohort of 60 patients with advanced incurable cancer in a palliative care facility and compared to healthy controls and noncancer patients with severe illness. Plasma samples were collected and levels of MPO-DNA complexes, H3Cit (a marker of NETs), cfDNA, NE, MPO, GCSF, IL-8, IL-6, TNFα, and IL-1β were quantified. MPO-DNA complexes were significantly increased in cancer patients compared to severely ill patients and healthy controls (and were also higher in the severely ill patients than healthy controls). Positive correlations were found between plasma levels of MPO-DNA (ie, NETs) complexes, H3Cit, cfDNA, NE, and MPO, suggesting that all these markers are present in neutrophil activation and NETosis [58].

3.4. The influence of NETs on CRC outcomes and prognosis. In the same article which used liver I/R in mice to induce NETosis, Tohme et al also examined the association between NETs level and cancer recurrence in a group of 50 patients that underwent partial hepatectomy for metastatic CRC (which is considered a potentially curative procedure). Circulating NETs levels were measured using MPO-DNA complex assay on POD 1 and then fold-change was determined comparing to healthy controls. Patients were grouped into "high" or "low" categories based on median fold-change. The risk of recurrence was 4.22 times higher in patients with "high" MPO-DNA complex levels [24]. This was the first study to definitively measure circulating NETs in a human population and to associate NETs with CRC specific prognosis. As Richardson et al in their the 2 previously mentioned studies examined NETs production from neutrophils ex vivo [55,56], we note that Tohme et al is the only human study to date that examines the relationship between active circulating NETs levels and CRC outcomes and as such is the lone bridge between animal experimental results and clinical evidence in CRC.

4. DISCUSSION

This review has identified that there is substantial experimental evidence that intravascular NETs are generated in the context of both pharmacologically or surgically induced sterile systemic inflammation and in sepsis [19,23,27,54], and further that the presence of intravascular NETs produces a deleterious oncologic outcome [23,24,58]. Coupled with the body of clinical evidence that heightened SIR confers worsened cancer-specific outcomes [10,12,34,35,37,38,40–46], it can be postulated that NETs are potential targets that can be inhibited or degraded and that this intervention could decrease the negative oncological impact of perioperative SIR and sepsis to improve cancer-specific survival. This could be achieved with medications already in widespread clinical use, such as recombinant DNase I. This is of major significance in the potential future treatment matrix for CRC, but a major limiting factor for the application of this postulate is the relative lack of direct clinical evidence that intravascular circulating NETs are present in surgical CRC patients and that these levels are associated with outcomes. However, the observational experimental evidence is robust and paves the way for further investigation.

There are many limitations that must be considered in the evidence that examines the SIR and CRC outcomes. An issue in the interpretation of this body of evidence as a whole is the heterogeneity in the particular specific inflammatory markers tested, the wide variation in the time points and intervals at which they were measuring these markers, and the large number of studies with significant patient cohort overlap (demonstrated in Table 3). Several large meta-analyses have found that anastomotic leak is associated with recurrence and oncologic outcomes, yet this is not apparent in the included studies [11,13,59]. All but one of the included studies consistently show an association between preoperative SIR and poor oncologic prognosis in CRC, but the evidence for a similar association with postoperative SIR is lacking due to the variation surrounding how and when postoperative inflammatory markers were collected and complications were measured. A caveat when interpreting the evidence that adverse oncologic outcomes are associated with postoperative complications is that these complications may delay or disrupt commencement of adjuvant chemotherapy. Delay to adjuvant chemotherapy has been shown to be associated with worse survival in resected CRC [60].

There is a small but increasingly robust body of evidence demonstrating that NETs are generated in sepsis. Mouse models have provided definitive evidence of this. The methods used for NET quantification have been consistent and well reasoned, although as knowledge of NETs biology has evolved, much more specific and reliable methods of detecting NETs rather than nonspecific cell-free DNA or identification of proteins that are complexed with DNA in NETs (and also found in abundance elsewhere) have become commonplace [61]. For example, many early studies used PicoGreen to detect DNA presumed to be NETs in tissue and plasma samples. This can detect any extracellular DNA, which can be from NETs but also from many other sources. More recent studies have instead stained tissue for colocalization of DNA with MPO, NE, or CitH3, or used a sandwich enzyme-linked immunosorbent assay for plasma MPO- or NE-DNA complexes in a now much utilised method first detailed by Kessenbrock et al [62]. These are much more specific methods for NETs detection, as these complexes are not found in non-NETs extracellular DNA.

Several studies of septic patients and trauma patients have proposed the conclusion that these conditions promote NETosis, although, similarly to how PicoGreen has been used, their methods of NET detection rely predominantly on identification of total serum cfDNA, to which NETs contribute but are not the sole component in the absolute level of cfDNA. cfDNA also contains cellular nuclear debris, bacterial DNA, and tumor DNA. Dwivedi et al examined 80 septic intensive care unit patients and found that levels of cfDNA were better predictors of intensive care unit mortality than conventional scoring systems [48], whereas Meng et al found that cfDNA levels in 39 trauma patients were significantly increased in those that developed sepsis compared to the nonsepsis trauma patients [50]. Margraf et al also examined cfDNA in trauma patients and found in their cohort of 37 that higher cfDNA levels were associated with development of the theoretical "second hit"

inflammatory insult, organ failure, and sepsis [51]. NETs have been shown to be significantly increased in the synovial fluid of patients with septic arthritis compared to noninfectious joint inflammation in a study of 42 patients by Logters et al, with a more specific polymerase chain reaction–based approach for NET quantification rather than total cfDNA levels alone [49]. The validity of the overall conclusion from these studies, that NETs are present in sepsis in human cohorts, must be considered cautiously. Measurement of NETs can occur through more specific assays such as MPO-DNA complex enzyme-linked immunosorbent assay, especially in the context of sepsis where there may be significant contribution from cellular debris and bacterial DNA.

Translation of the experimental animal evidence in NETs, and the promising effect of NETs inhibitors on tumor establishment, is sorely lacking. Although Cools-Lartigue et al and Najmeh et al have clearly demonstrated negative oncologic implications of NET-CTC interactions to suggest that NETs promote cancer progression, their clinical relevance and translation to human studies are limited by the fact that the murine models subvert the natural course of cancer and sepsis in humans. In this regard, the step between animal experimental studies and clinical evidence may be better bridged by orthotopic models of CRC that mimic the natural course of a primary colorectal adenocarcinoma and its surgical treatment. However, the pilot study of Tohme et al in colorectal liver metastases resection patients showed promising results consistent with the findings in animal studies [24], and the work of Thalin et al supports a hypothesis that NETs are inherently involved in the process of human cancer growth and progression [58].

The clinical evidence of if and how surgery stimulates NETosis is in a germinal stage. The studies of Richardson et al are among the first clinical studies to address this, although as their findings are based on neutrophil function and stimulated NETosis ex vivo [55,56], evidence of whether the same processes are occurring at the time in surgical patients remains scant with the exception of the human study arm of Tohme et al [24] and some studies examining local NETosis in the surgical field. Kanamaru et al found that NETs were present on peritoneum at the conclusion of surgery in 27 patients undergoing radical gastrectomy for gastric cancer, but whether NETosis extended beyond the surgical field is unknown because no circulating NETs levels were measured and no analysis of association to oncologic outcomes was performed.

Multiple studies in this review have suggested that inhibiting or degrading NETs may be advantageous to oncologic outcomes, but as yet, no human studies have been conducted that use NETs inhibitors such as DNase to identify any modification in oncologic outcomes. DNase itself has been used for decades in the treatment of cystic fibrosis and empyema in the form of recombinant human DNase through the inhaled and intrapleural administration, respectively [63–66]. Studies of intravenous DNase treatment are less common, although from the available evidence with limited patient numbers, no adverse events have been noted when used for the treatment of systemic lupus erythematosus (SLE). Several animal experimental studies have shown positive oncologic effects with systemic administration of DNase, and Mai et al demonstrated that DNase administered 6-hourly "rescued" septic mice from death following a CLP, with decreased levels of cfDNA and IL-6 and suppression of organ damage.

The relationship between circulating NETs and primary CRC is yet to be examined. Most studies that examine the relationship between the SIR and CRC outcomes use surrogate serum markers, none of which have been proven to correlate with intravascular NETs. Most studies have not aimed to capture these surrogate markers across consecutive days in the postoperative period; hence, they cannot be used to separate systemic inflammation from pre-existing oncologic processes, surgical stress, or infective complications. Although the novel studies of Richardson et al linked NETosis in surgical CRC patients, the findings of decreased ability for NETosis in isolated neutrophils may not reflect the in vivo circulating behavior of the neutrophils and circulating NETs levels. An alternate hypothesis is that decreased ex vivo NETosis could be due to prior high activity NETosis and a level of "burn out" in these neutrophils. The behavior and patterns of in vivo NETosis over consecutive days in the dynamic perioperative period are yet to be studied; hence, it is unknown if intravascular NETosis is triggered by surgical stress, if it is amplified when septic complications occur, and ultimately if circulating, targetable NETs play a role in promoting propagation of metastatic disease in CRC.

In conclusion, although there is a paucity of high-level evidence directly linking NETs to CRC progression, this review shows that further investigations and human observational studies are warranted to characterize the levels of and trends in circulating NETs and identify any interplay with postoperative events, ultimately to determine if the presence of circulating NETs is associated with oncologic outcomes. Understanding the physiological and pathological mechanisms influencing this process could illuminate potential therapeutic targets to influence this process. There may be significant potential to modulate the relationship between NETs and CRC and ultimately alter the course of disease, providing a putative benefit for CRC patients and an increasing breadth of tools to augment the arsenal for clinicians treating them. Future directions may be aimed at improving individualized treatment based on patient systemic inflammatory factors and at therapeutic targeting of NETs to prevent NET-CTC-endothelial interaction to ultimately decrease death from metastatic CRC.

This article contains no studies with human participants or animals performed by any of the authors. Informed consent was not required for this study because it used secondary sources only.

Author contribution

Study conception: GMC, SRS, SK, PGP; generation and analysis of data: GMC, JAP, GLB; interpretation and reporting of data: GMC, MMW, AM, SRS, SK, PGP; drafting of manuscript: GMC, SK, MMW, SRS, PGP; critical editing: all authors.

Conflicts of interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Appendix 1. PRISMA 2009

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1; title page of manuscript
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background;	2 (manuscript)

(continued on next page)

(*continued*)

Section/topic	#	Checklist item	Reported on page #
		objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3–5 (manuscript) "The role of NETs in facilitating progression of CRC is an area of study that is at a very early stage. Given the evidence that sepsis and systemic inflammation are associated with recurrence in patients that have undergone a potentially curative resection of their CRC and the evolving knowledge of NETs in the immune response and their potential as promoters of metastasis, we postulated that NETs generated by sepsis are integral in facilitating metastasis and influencing prognosis in CRC."
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5 "The aim of this scoping systematic review is to evaluate the evidence that NETs are present in states of surgical inflammatory stress and sepsis, and to examine the evidence for an association between NETs or other surrogate markers of inflammation, with cancer-related outcomes in CRC."
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (eg, Web address), and, if available, provide registration information including registration number.	5 "An electronic literature search was carried out using the registered search protocol, available through Prospero CRD42017068935."
Eligibility criteria	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	6, Table 1
Information sources	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5 "An electronic literature search was carried out using the registered search protocol, available through Prospero CRD42017068935 [30]. Databases searched were MedLine, Cochrane Library, and CINAHL. The latest date of the literature search was 11 December 2018."
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5; see PROSPERO page for search strategy.
Study selection	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5–6, Table 1
Data collection process	10	Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	N/A; "No data extraction was performed for meta-analysis given the nature of this systematic review."
Data items	11	List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made.	N/A; "No data extraction was performed for meta-analysis given the nature of this systematic review."
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6, Appendix 2 "Articles were compared against the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement and the Animal Research: Reporting of In Vivo Experiments (ARRIVE) statement for observational and experimental studies respectively. Scores were designated based off these criteria."
Summary measures	13	State the principal summary measures (eg, risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, I^2) for each meta-analysis.	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	N/A given heterogeneity of study type and topic
Additional analyses	16	Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were prespecified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6, Fig. 1 (flow diagram)
Study characteristics	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	Table 2, Table 3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Appendix 2, as a component of quality assessment
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple	N/A

(*continued*)

Section/topic	#	Checklist item	Reported on page #
		summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A given heterogeneity of study type and topic
Additional analysis	23	Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression [see item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (eg, healthcare providers, users, and policy makers).	18–22
Limitations	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review-level (eg, incomplete retrieval of identified research, reporting bias).	18–22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22–23
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	Title page disclosures

From Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(7): e1000097. doi:10.1371/journal.pmed1000097
For more information, visit: www.prisma-statement.org.

Appendix 2. Search protocol

#	Searches
1	Neutrophil extracellular traps
2	Neutrophil extracellular trap
3	Sepsis
4	Bacterial infections/or intra-abdominal infections/or pelvic infection/or bacteremia/or shock, septic
5	Systemic inflammatory response syndrome
6	DNA, Mitochondrial
7	1 or 2 or 3 or 4 or 5 or 6
8	Colorectal surgery/or general surgery/or surgical oncology/or thoracic surgery/or urology
9	Neoplasms
10	Resection
11	8 and 9
12	9 and 7
13	10 and 7
14	11 or 12 or 13
	Colorectal surgery/or general surgery/or surgical oncology/or thoracic surgery/or urology AND Neoplasms
	OR
	Neoplasms AND systemic inflammatory response syndrome/or neutrophil extracellular traps/or neutrophil extracellular trap/or sepsis/or bacterial infections/or intra-abdominal infection/or pelvic infection/or bacteremia/or shock, septic/or DNA, mitochondrial
	OR
	Resection AND systemic inflammatory response syndrome/or neutrophil extracellular traps/or neutrophil extracellular trap/or sepsis/or bacterial infections/or intra-abdominal infection/or pelvic infection/or bacteremia/or shock, septic/ or DNA, mitochondrial
15	14 and 2000:2018 limit

Appendix 3

Quality assessment of included studies			
Study	Publisher (year)	Quality assessment tool	Score
Albrengues et al [27]	*Science* (2018)	ARRIVE	16/38
Canna et al [33]	*Br J Canc* (2005)	STROBE	20/30
Carruthers et al [38]	*Colorectal Dis* (2012)	STROBE	23/30
Chan et al [45]	*Ann Surg* (2017)	STROBE	23/30
Cools-Lartigue et al [23]	*J Clin Invest* (2013)	ARRIVE	18/38
Crozier et al [34]	*Br J Surg* (2007)	STROBE	19/30
Crozier et al [35]	*Am J Surg* (2009)	STROBE	20/30
Inoue et al [25]	*Nat Commun* (2018)	ARRIVE	18/38
		STROBE	20/30
Kersten et al [43]	*Acta Oncologica* (2013)	STROBE	27/30
Kressner et al [47]	*Dis Col & Rect* (2002)	STROBE	20/30
Laurent et al [8]	*Br J Surg* (2003)	STROBE	17/30
Mallappa et al [41]	*Colorectal Dis* (2013)	STROBE	22/30
McDonald et al [54]	*Cell Host & Microbe* (2012)	ARRIVE	17/38
McMillan et al [10]	*Br J Surg* (2003)	STROBE	21/30
Mori et al [44]	*Dig Dis Sci* (2015)	STROBE	22/30
Najmeh et al [57]	*Int J Canc* (2017)	STROBE	13/30
Neal et al [42]	*Arch Surg* (2011)	STROBE	23/30
Park et al [26]	*Sci Transl Med* (2016)	ARRIVE	22/38
Pilsczek et al [19]	*J Immunol* (2010)	N/A	N/A
Portale et al [39]	*J Gastrointest Surg* (2018)	STROBE	25/30
Proctor et al [36]	*Eur J Canc* (2011)	STROBE	20/30
Richards et al [37]	*Br J Surg* (2012)	STROBE	24/30
Richardson et al [55]	*Int J Inflam* (2017)		22/30
Richardson et al [56]	*J Surg Res* (2017)		23/30
Roxburgh et al [12]	*Ann Surg* (2009)	STROBE	25/30
Song et al [46]	*BMC Cancer* (2017)	STROBE	27/30
Thalin et al [58]	*PLoS One* (2017)	STROBE	23/30
Tohme et al [24]	*Cancer Res* (2016)	STROBE	19/30
		ARRIVE	15/38
Turner et al [40]	*Clin Colorectal Cancer* (2015)	STROBE	24/30
Yipp et al [53]	*Nat Med* (2012)	ARRIVE	14/38

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