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Mini-Review

Prognostic circulating proteomic biomarkers in colorectal liver metastases

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ABSTRACT

The liver is the most common site of metastasis in colorectal cancer. Multimodal treatment, including liver resection, is potentially curative and prolongs survival for selected patients with colorectal liver metastases (CRLM). However, the treatment of CRLM remains challenging because recurrence is common, and prognosis varies widely between patients despite curative-intent treatment. Clinicopathological features and tissue-based molecular biomarkers, either alone or in combination, are insufficient for accurate prognostication. As most of the functional information in cells resides in the proteome, circulating proteomic biomarkers may be useful for rationalising the molecular complexities of CRLM and identifying potentially prognostic molecular subtypes. High-throughput proteomics has accelerated a range of applications including protein profiling of liquid biopsies for biomarker discovery. Moreover, these proteomic biomarkers may provide non-invasive prognostic information even before CRLM resection. This review evaluates recently discovered circulating proteomic biomarkers in CRLM. We also highlight some of the challenges and opportunities with translating these discoveries into clinical applications.

1. Introduction

The liver is the most common site of metastasis from colorectal cancer (CRC) [1], the second most common cause of cancer-related death worldwide [2]. Despite the overall poor prognosis associated with colorectal liver metastasis (CRLM), liver resection in combination with multimodal therapy is potentially curative [3,4]. A wide variation in long-term survival and cure rates has been reported after curative-intent treatment [4]. Genomic biomarkers such as *KRAS* and *BRAF* have significantly enhanced the prognostication of CRLM beyond reliance on clinicopathological factors [5,6]. However, *KRAS* and *BRAF* mutation status may not be sufficient for precise prognostication [6]. Genes that interact with *KRAS*, such as *SMAD4* and *TP53*, may supplement the prognostication of CRLM [5,6], though further research is needed for their validation. Novel biomarkers such as ctDNA and circulating tumour cells are promising but are not yet routinely recommended for detecting molecular residual disease or relapse [7]. The lack of standardised protocols and high cost in plasma isolation limits the use of ctDNA and circulating tumour cells [8]. These existing limitations highlight the need for a multilevel biological and molecular approach to prognostication, and motivates the exploration of alternative biomarkers.

Proteomic biomarkers provide opportunities for new prognostic methods in clinical practice [9]. These biomarkers are currently utilised in the management of other malignancies, such as breast and prostate cancers [10,11]. The prognostic value of these biomarkers evidences the potential benefits of similar approaches to managing CRLM, which is currently limited to the carcinoembryonic antigen (CEA). CEA is utilised as a tumour marker to aid in the management and prognostication of CRC. Whilst elevated CEA is associated with CRC recurrence and progression [12], CEA lacks

Abbreviations: CD, clusters of differentiation; CEA, carcinoembryonic antigen; COL IV, type IV collagen; CRC, colorectal cancer; CRLM, colorectal liver metastasis; CRP, C-reactive protein; DFS, disease-free survival; ctDNA, circulating tumour DNA; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; EMT, epithelial-mesenchymal transition; HGF, hepatocyte growth factor; HR, hazard ratio; HSP, heat shock protein; IGFBP-2, insulin-like growth factor-binding protein-2; IL-6, interleukin-6; LC, liquid chromatography; MMP, matrix metalloproteinases; MPO, myeloperoxidase; MS, mass spectrometry; OS, overall survival; OR, odds ratio; RAS, rat sarcoma viral gene; RFS, recurrence-free survival; TFF1, trefoil factor 1; TFF3, trefoil factor 3; TIMP1, tissue inhibitor of matrix metalloproteinase-1; YKL40, chitinase-3-like protein-1

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Table 1
Summary of the studies on the proteomic biomarkers of prognostic value in patients with CRLM.

First author	Year	Technique	Sample	Population characteristics	Comparison characteristics	Key biomarkers	Key findings
V Rao	2022	ELISA to measure serum extravesicular TIMP1 levels, 3D ECM-remodelling assay.	Serum samples	Discovery cohort: 151 CRLM patients	Validation cohort I: 49 CRLM patients Validation cohort II: 56 CRLM patients	TIMP1 and HSP90AA	Higher expression of extravesicular TIMP1 in patients with shorter overall survival.
K Lin	2022	LC-MS.	Serum samples, taken pre- and postoperatively	Discovery cohort: 56 CRLM patients and 7 patients with benign liver disease	Internal validation cohort: 154 CRLM patients and 78 patients with benign liver disease External validation cohort: 110 CRLM patients	CD14, Serpin A4, CFP, and LBP	The preoperative levels of extravesicular CD14, serpin A4, cyan fluorescent protein, and lipopolysaccharide-binding protein demonstrate significant association with overall survival.
M Lindgren	2022	ELISA to measure plasma type IV collagen level.	Plasma samples and peripheral venous samples were taken preoperatively, within 50 days before surgery	138 CRLM patients that underwent resection	118 healthy volunteers	COL IV	Plasma COL IV in postoperative patients could not predict prognosis.
P Reijonen	2021	Immunofluorometric assay to measure serum MMP-8. ELISA to measure serum MMP-9 and MPO. Immunoturbidimetric method to measure CRP. Immunoenzymatic assay to measure CEA and CA19–9.	Serum samples were drawn before and 3 months after liver resection	419 CRLM patients that underwent resection	Nil	MMP-8, MPO, and CRP	Pre- and postoperatively elevated serum MMP-8 displayed a statistically significant association with a poor 10-year overall survival rate. Postoperatively upregulated MPO correlated to superior 5-year disease-free survival.
R Peltonen	2021	Time-resolved immunofluorometric assay or ELISA.	Serum samples, taken pre- and postoperatively	111 CRLM patients that underwent resection	Nil	MMP-2, MMP-8, and MMP-9	Preoperatively elevated serum MPO demonstrated a statistically significant association with improved disease-free survival and overall survival.
Y Deng	2021	Serum measurement of CRP, albumin, CEA, and CA19–9 levels as well as lymphocyte, neutrophil and platelet count.	Serum samples were collected preoperatively, 7 days before surgery	283 CRLM patients that underwent resection	Nil	CRP-to-albumin ratio	Increased CRP-to-albumin ratio was a statistically significant predictor of reduced overall survival.
Y Sakamoto	2020	Serum measurement of CRP, albumin, CEA and CA19–9 levels.	Serum samples were taken preoperatively, within 3 days before surgery	184 CRLM patients that underwent partial hepatectomy	Nil	CRP-to-albumin ratio	Elevated CRP-to-albumin ratio was associated with poorer overall survival and recurrence-free survival.
R Peltonen	2020	ELISA to measure serum YKL-40 and IL-6 levels. Routine methods of measuring serum CRP, CEA, and CA19–9.	Serum samples, taken pre- and postoperatively	441 CRLM patients undergoing resection	Nil	YKL-40, IL-6, CRP, CEA, and CA19–9	Elevated preoperative YKL-40 and CA19–9 levels were associated with shorter overall survival. Elevated preoperative CA19–9 was associated with shorter relapse-free survival. Elevated postoperative CEA and CA19–9 demonstrated a statistically significant association with shorter relapse-free survival and overall survival.
N Messaoudi	2020	ELISA to measure serum CD73.	Serum samples, taken preoperatively	215 CRLM patients that underwent resection	Nil	CD73	Preoperatively elevated serum CD73 was significantly associated with shorter disease-free survival.
M Vocka	2019	Immunochemistry, to measure serum levels of HSP60, CHI3L1, and IGFBP-2.	Serum samples	64 CRLM patients and 33 CRC patients with metastasis other than liver	79 healthy volunteers	HSP60 and IGFBP-2	Markedly elevated serum HSP60 levels displayed a statistically significant association with shorter overall survival. Elevated serum IGFBP-2 levels displayed a statistically significant association with shorter overall survival.
M Shibutani	2019	Serum measurement of CRP and albumin, to calculate CRP-to-albumin ratio.	Serum samples, taken preoperatively 2 weeks before CRLM resection	119 CRLM patients that underwent resection	Nil	CRP and albumin	Preoperatively elevated systemic inflammatory markers indicate a high risk of CRLM recurrence. Elevated CRP-to-albumin ratio displayed a statistically

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Table 1 (*continued*)

First author	Year	Technique	Sample	Population characteristics	Comparison characteristics	Key biomarkers	Key findings
							significant association with shorter relapse-free survival.
S Loosen	2018	ELISA to measure serum osteopontin.	Serum samples, taken pre- and postoperatively	125 CRLM patients that underwent resection	65 healthy volunteers	Osteopontin	Upregulation of serum osteopontin, either pre- or postoperatively, was associated with poor prognosis.
S Lee	2016	Serum measurement of laboratory biomarkers, including CEA, CRP, ferritin, and albumin.	Serum samples	75 CRLM patients and 45 CRC patients with metastases other than liver	Nil	CRP, CEA, and ferritin	Elevated serum CRP, CEA, or ferritin levels displayed associations with poor survival outcomes. Serum ferritin can independently predict prognosis in metastatic CRC patients.
A Køstner	2016	Serum measurement of CRP and albumin levels.	Serum samples were taken preoperatively, within 20 days before surgery	492 patients with CRLM who underwent resection, with post-operative follow and chemotherapy	Nil	CRP and albumin	Elevated CRP significantly correlated to reduced overall survival time, acting as a significant prognosticator post-operatively.
H Nystrom	2015	ELISA to measure plasma type IV collagen levels, and laboratory measurement of CEA.	Plasma samples, taken preoperatively, within 2 weeks before resection	85 CRLM patients undergoing resection, of whom 27 received long-term follow-ups	118 healthy volunteers	COL IV	Elevated preoperative plasma COL IV levels indicated a high risk of recurrence after resection of CRLM. Downregulated plasma COL IV and CEA displayed an association with longer survival.
M Vocka	2015	ELISA to measure serum TFF1–3, CEA, and CA19–9 levels.	Serum samples, collected at the time of distant metastasis diagnosis or at the time of progression validated by CT	64 CRLM patients and 33 CRC patients with metastasis other than liver	79 healthy volunteers	TFF3, CEA, and CA19–9	Elevated TFF3 levels displayed associations with significantly worse outcomes. Serum TFF3 levels strongly correlated with the extent of metastatic spread in the liver.
K Bunatova	2012	ELISA to measure plasma TIMP1 levels.	Plasma samples, taken preoperatively and postoperatively	87 CRLM patients that underwent resection	Nil	TIMP1	Elevated preoperative plasma TIMP1 displayed a statistically significant association with shortened overall survival after resection. Postoperative plasma TIMP1 level did not demonstrate statistically significant results in predicting prognosis.
F Hoogwater	2011	ELISA to measure serum CD95L.	Serum samples, taken pre- and postoperatively	62 CRLM patients that underwent resection	Nil	CD95L	Preoperative elevation of serum CD95L levels demonstrated statistically significant associations with both poor overall survival and poor recurrence-free survival.
H Nystrom	2011	ELISA to measure plasma type IV collagen levels. Immunohistochemistry/CEA analysis.	Plasma samples were taken preoperatively. For CRLM patients, plasma was taken before and during chemotherapy and at the time of CRLM diagnosis	15 CRLM patients and 32 CRC patients	8 patients undergoing surgery for benign pathologies	COL IV	Plasma COL IV level correlated to the extent of metastatic spread in the liver.
V Wong	2007	Serum measurement of CRP, CEA and CA19–9 levels.	Serum samples were taken preoperatively, a day before surgery	170 patients with CRLM, who underwent resection	Nil	CRP	Preoperative elevation of serum CRP levels demonstrated statistically significant associations with both shorter overall and disease-free survival.
K Jong	2004	ELISA to measure serum EGF, HGF, CRP, SAA, and IL-6 levels. Radioimmunoassay to measure serum IGF-1.	Serum samples were taken preoperatively 2 days before surgery. Additionally, portal and systemic circulation serum on postoperative days 0, 1, 4, 7, and 10	33 CRLM patients, of whom 24 were treated with partial hepatectomy, and 9 had laparotomy	Nil	HGF, CRP, and IGF-I	Elevated serum CRP was associated with shortened disease-free survival and overall survival. Elevated serum HGF was associated with shortened disease-free survival and overall survival.

sensitivity [13]. Therefore, there is the need for alternate biomarkers which can independently prognosticate CRLM.

The prospect of identifying protein biomarkers for CRLM has been realised by the advances in proteomic techniques, such as the development of high-throughput mass spectrometry [14]. Techniques such as liquid chromatography-mass spectrometry (LC-MS) enable protein identification, characterisation, and quantification in complex biological samples [15]. If prognostic markers can be found in serum or plasma samples, the management of CRLM would benefit from informed prognostication before liver resection. This review aims to highlight the recent developments in circulating proteomic biomarkers of CRLM and discuss their prognostic significance. The identified primary studies on prognostic proteomic CRLM biomarkers have been summarised in Table 1.

2. Methodology

A literature search was conducted on the PubMed database using keywords such as “prognostic”, “prognosis”, “serum”, “circulating”, “non-invasive”, “proteomic”, “protein”, “collagen”, “peptide”, “CRLM”, “colorectal liver metastasis”, “CRCLM”, “colorectal cancer liver metastasis”, and “hepatic metastasis”. Studies were deemed eligible if they reported on circulating proteins with prognostic value in patients with surgically resected CRLM. Exclusion criteria comprised: studies about patients with CRLM undergoing non-surgical treatment; studies about organoids, 3D cell cultures, cell lines, and animal models; and studies reporting about biomarkers of therapeutic efficacy or diagnostic value without data on prognostic value. The name of the first author, year published, protein quantification technique, sample, population characteristics, comparison characteristics, key biomarkers studied, and key findings were extracted from the included studies.

3. Circulating extravesicular proteins

Extracellular vesicles initiate and sustain the tumour microenvironment that influences cellular function and the remodelling of the extracellular matrix (ECM) [16]. Extracellular vesicles are upregulated in patients with cancer compared to healthy controls [16]. Additionally, extracellular vesicles can circulate to distant sites and alter the ECM complex to facilitate metastatic spread [16]. Tissue inhibitor of matrix metalloproteinase-1 (TIMP1) is a secretory protein that is upregulated in CRLM microenvironments [16]. Notably, the upregulation of extravesicular TIMP1 leads to ECM remodelling [16]. Rao et al. [16] investigated extravesicular TIMP1 as a potential prognostic marker and therapeutic target in patients with CRLM. Serum extravesicular TIMP1 levels were measured using ELISA within a discovery cohort of CRLM patients (n=151) [16]. Upregulation of TIMP1 (extravesicular TIMP1 > 1537 pg/mL) was associated with shorter overall survival ($p < 0.01$) [16]. This was validated through two independent cohorts, which demonstrated similarly reduced overall survival (OS) with elevated TIMP1 ($p < 0.01$). This finding was corroborated by multivariate analysis of the discovery (OR: 1.90, 95% CI: 1.22–2.97, $p < 0.01$) and validation I (OR: 5.70, 95% CI: 1.95–16.61, $p < 0.01$) and II (OR: 18.25, 95% CI: 5.59–59.61, $p < 0.01$) cohorts, respectively.

The negative prognostic characteristic of elevated extravesicular TIMP1 was consistent with an earlier study by Bunatova et al. [17], who compared pre- and postoperative plasma TIMP1 levels in CRLM patients (n = 72) with resected or ablated liver metastases. Elevated preoperative plasma TIMP1 (> 165 ng/mL) was significantly associated with shortened OS after treatment, irrespective of treatment type ($p = 0.02$) [17]. Postoperative plasma TIMP1 levels did not demonstrate statistically significant results in predicting prognosis or early recurrence after treatment, albeit this result was limited by a small sample size (n = 29) [18].

Similarly, Lin et al. [18] analysed serum extravesicular proteins as a potential tool for risk stratification and prediction of treatment response after CRLM resection. LC-MS assessed extravesicular proteins in pre- and post-operative samples from the discovery cohort, which comprised CRLM patients (n = 56) and patients with benign liver disease (n = 7) [18]. Increased levels of extravesicular proteins demonstrated statistically significant associations with shorter OS both pre-operatively ($p < 0.01$) and post-operatively ($p < 0.01$) for patients with CRLM [18]. Extravesicular protein signature was an independent, high-risk predictor of reduced OS ($p < 0.01$) [18]. Moreover, in the independent validation cohort, extravesicular protein concentrations were raised in patients with CRLM cohort in comparison to the patients with benign liver disease ($p = 0.04$) [18].

4. Extracellular matrix proteins

The tumour stroma contains non-cancerous components such as non-malignant cells, blood vessels, and ECM [19]. The enzymatic remodelling of ECM upregulates collagen, and increased collagen deposition promotes tumour proliferation [19]. COL IV is a stromal protein with potential prognostic ability in patients with CRLM [19]. Lindgren et al. [19] used ELISA to compare preoperative plasma COL IV levels in CRLM patients that underwent resection (n = 138) and in a healthy control group (n = 118). Preoperative plasma COL IV was elevated in CRLM patients in comparison to controls, especially for those with advanced stage and large metastatic burden ($p < 0.01$) [19]. COL IV levels above 135.2 ng/mL had a sensitivity and specificity of 74.6% and 83.9%, respectively, for differentiating between patients with CRLM and healthy controls [19]. However, circulating COL IV did not correlate with OS for CRLM patients [19].

These findings were partially corroborated by Nyström et al. [20,21]. In their 2011 study of 15 patients with resected CRLM, Nyström et al. [20] reported a correlation between elevated preoperative plasma COL IV levels and the extent of metastatic spread in the liver. Moreover, in their 2015 study of 27 patients getting long-term follow-up after CRLM resection, Nyström et al. [21] demonstrated a correlation between elevated COL IV levels (200.1 ± 136.9 ng/mL) and risk of recurrence after resection ($p < 0.01$). Elevated COL IV levels more accurately predicted the presence of CRLM when compared to elevated CEA levels [21]. Contrary to Lindgren et al. [19], Nyström et al. [21] found that upregulated preoperative plasma COL IV was associated with shorter recurrence-free survival (RFS) following liver resection ($p < 0.01$).

5. Changes in circulating epithelial-mesenchymal transition regulators in CRLM

Epithelial-mesenchymal transition (EMT) is a process of reverting polarised epithelial cells to mesenchymal cells that release ECM components, enabling tumour cell metastasis and resistance to apoptosis [22]. Osteopontin is a glycoprotein that regulates EMT in the metastatic process of CRC [23]. A 2018 study by Loosen et al. [24] measured serum osteopontin levels in CRLM patients that underwent resection (n = 125) and compared these with healthy controls (n = 65). CRLM patients have higher serum osteopontin levels, and serum osteopontin above 130.25 ng/mL was a moderately sensitive (63.2%) and specific (87.7%) marker for differentiating CRLM patients from healthy controls [24]. Upregulation of serum osteopontin, either pre- or postoperatively, was associated with poor prognosis [24]. Patients with preoperative serum osteopontin levels above 264.4 ng/mL had a median OS of 304 days, compared to a median OS of 1394 days in patients with osteopontin levels below 264.4 ng/mL (HR: 3.20, 95% CI: 1.41–7.29, $p < 0.01$) [24]. In multivariate analysis, serum osteopontin levels could independently predict OS (HR: 3.08, 95% CI: 1.05–9.02, $p = 0.04$) [24].

Trefoil factor 1 (TFF1) and trefoil factor 3 (TFF3) are trefoil proteins produced in the gastrointestinal mucosa and are implicated in the EMT of CRC [25]. A 2015 study by Vocka et al. [26] surveyed 97 patients with metastatic CRC, including 64 patients with CRLM, and compared them with age-matched healthy controls (n = 79). In 58 patients, second serum samples were obtained 3 months after the first sample. Vocka et al. [26] demonstrated a significant decline in TFF3 levels in patients with partial remission of disease, with a mean reduction of -2.33 ng/mL ($p < 0.01$) within 3 months after treatment. TFF3 levels increased (mean change of 1.31 ng/mL) in patients with disease progression, albeit without statistical significance ($p = 0.10$). TFF3 levels strongly correlated to extent of metastatic spread in the liver. Patients with elevated TFF3 levels (> 3.4 ng/mL) had a median OS of 7.8 months, significantly shorter ($p < 0.01$) than median OS (17.9 months) for patients with TFF3 levels below 1.7 ng/mL [26]. TFF3 serum levels above 1.7 ng/mL were highly specific (97.47%) and moderately sensitive for detecting CRC, however, sub-analysis of the CRLM population was not available. Further, significant elevation in TFF1 levels was observed in patients with poorly differentiated tumours when compared to those with well or moderately differentiated tumours ($p < 0.05$). Further studies are needed to validate the prognostic values of TFF1 and TFF3.

IGFBP-2 is a downstream component of the oncogene *MACC1*, and it is implicated in CRC metastasis, especially CRLM [27]. IGFBP-2 encourages tumour proliferation and resistance to apoptosis [28]. In their 2019 study, Vocka et al. [28] compared IGFBP-2 levels in patients with metastatic CRC (n = 97), of whom 64 had CRLM, against healthy controls (n = 79). In metastatic CRC patients, serum IGFBP-2 levels (mean: 613.4 ng/mL, range: 427.9–968.6 ng/mL) were elevated ($p < 0.01$) in comparison to healthy controls (mean: 308.1 ng/mL, range: 219.6–417.8 ng/mL) [28]. Elevated IGFBP-2 levels, both slightly elevated (630–1260 ng/mL) or markedly elevated (> 1260 ng/mL), were associated with shorter OS ($p < 0.01$) [28]. IGFBP-2 serum cut-off of 630.0 ng/mL demonstrated low sensitivity (48.96%) but high specificity (94.52%) for discriminating between the CRC cohort and healthy controls ($p < 0.01$) [28]. However, sub-cohort analysis for patients with CRLM was not conducted. Another novel protein considered by Vocka et al. [28] was HSP60 – a protein involved in protein folding and translocation and implicated in diseases such as Crohn's disease. Marked upregulation of HSP60 (≥0.84 ng/mL) displayed a statistically significant association with shorter OS ($p < 0.01$) [28]. HSP60 had high specificity (94.74%) for differentiating CRC and healthy controls ($p < 0.01$) [28], however, sub-cohort analysis was not available for the CRLM cohort. Moreover, these results were limited by a small number of patients with CRLM (n = 64).

6. Circulating matrix metalloproteinases and myeloperoxidase as prognostic markers in CRLM

Matrix metalloproteinases (MMPs) mediate metastasis by stimulating angiogenesis and cellular migration in metastatic processes [29]. Abnormal levels of MMPs, such as MMP-8 and MMP-9, have been suggested to predict prognosis in malignancies such as gastric and breast cancer. MMP-8 and MMP-9 activity is promoted by myeloperoxidase (MPO), which may help predict prognosis after CRLM resection [29]. Using ELISA and immunofluorometric assay, Reijonen et al. [29] measured serum levels of MMP-8, MMP-9, MPO, CRP, CEA, and CA19–9 in CRLM patients that underwent resection (n = 419). Pre- and postoperatively elevated serum MMP-8 (HR: 1.53, 95% CI: 1.07–2.19; HR: 1.45, 95% CI: 1.01–2.09, respectively) displayed a statistically significant association with poor 10-year OS rate [29]. Postoperatively high MMP-8 indicated worse 10-year OS only in the cases of synchronous disease (HR: 1.81, 95% CI: 1.13–2.89) [29]. This pattern was not replicated in patients with metachronous disease [29]. Preoperatively upregulated MPO demonstrated a significant association with better 10-year OS (HR: 0.51, 95% CI: 0.31–0.85) [29]. Postoperatively upregulated MPO correlated to superior 5-year disease-free survival (DFS) and 10-year OS (HR: 0.70, 95% CI: 0.54–0.90; HR: 0.79, 95% CI: 0.61–1.03, respectively) [29].

Comparable results were reported by Peltonen et al. [30] in their 2021 study of 111 CRLM patients who underwent liver resection. Peltonen et al. [30] found that preoperatively elevated serum MPO demonstrated a statistically significant association with improved DFS ($p < 0.01$) and OS ($p < 0.05$). Preoperatively, both downregulated (≤29.6 ng/mL) and upregulated serum MMP-8 (> 76.2 ng/mL) were associated with shorter OS when compared to intermediate levels ($p = 0.02$) [30]. Down- and upregulated MMP-8 had similar associations with shorter DFS, albeit without statistical significance [30]. Postoperatively upregulated serum MMP-9 (> 77.7 ng/mL) displayed statistically significant associations with shorter DFS in patients aged over 65 ($p < 0.01$) [30]. Whilst these results are encouraging, further research is needed to validate these findings in a larger cohort of patients with CRLM.

7. Circulating immune cell surface molecules as prognostic markers in CRLM

The clusters of differentiations (CDs) are proteins on the cell surface that mediate communication and signalling between cells [31]. The expression of CDs in malignant cells regulates metastatic progression [31] and thus may possess prognostic value in patients with CRLM. In their study of 193 patients with CRLM who underwent surgery with curative intent, Messaoudi et al. [32] found that preoperatively elevated serum CD73 (> 7.2 ng/mL) was significantly associated with shorter DFS. The DFS in 7.2% of patients with serum CD73 levels over 7.2 ng/mL was 36.0 months, with a statistically significant difference ($p = 0.01$) when compared to the rest of the patients (58.0 months) [32]. The association between preoperatively elevated serum CD73 and shorter DFS was stronger in patients that received neoadjuvant chemotherapy before liver resection [32]. Elevated serum CD73 was not associated with decreased time to recurrence (HR: 1.00, 95% CI: 0.89–1.13, $p = 0.98$) and disease-specific survival (HR: 1.08, 95% CI: 0.94–1.23, $p = 0.27$), irrespective of other variables.

Hoogwater et al. [33] reported that CD95 ligand (CD95L) levels could independently predict the prognosis of patients with resected CRLM, after analysis of serum samples from 62 patients with resected disease. Preoperative elevation of serum CD95L levels (> 0.1762 ng/mL) demonstrated statistically significant associations with poor OS ($p = 0.02$) and RFS ($p < 0.05$). Patients with preoperatively upregulated serum CD95L had a median of 8.08 months (95% CI: 4.37–11.79) and a median OS of 31.57 months (95% CI: 20.30–42.84). In comparison, patients with downregulated serum CD95L (< 0.1762 ng/mL) had a much greater median RFS of 15.13 months (95% CI: 10.63–21.63) and a median OS of 58.38 months (95% CI: 44.45–72.31) [34]. These results on the prognostic value of CD95L were independent of overall tumour load or chemotherapy [33].

8. Circulating inflammatory proteins as prognostic markers in CRLM

Inflammation is a hallmark of oncogenesis [34]. Saliently, inflammatory cytokines promote acute phase reaction, which upregulates C-reactive protein (CRP) and downregulates albumin levels [35]. The prognostic value of CRP is largely consistent across current research. An analysis of 170 patients with resected CRLM by Wong et al. [36] found that preoperatively elevated CRP levels (> 10 mg/L) were significantly associated with shorter DFS (HR: 4.07, 95% CI: 1.36–12.19, $p = 0.01$), compared to those with lower CRP levels. Similarly, in their study of 120 patients with metastatic CRC, Lee et al. [37] demonstrated a statistically significant association between

elevated serum CRP levels (≥10 mg/L) and poor survival outcomes (HR: 2.87, 95% CI: 1.92–4.28). However, these results were skewed due to the inclusion of patients with metastatic CRC without liver metastases (n = 45). These results were corroborated by Køstner et al. [38], whose analysis of 492 patients with resected CRLM demonstrated the negative prognostic value of CRP. Patients with low CRP levels (< 10 mg/L) had significantly increased overall median survival of 4.27 years, in comparison to patients with moderately elevated CRP levels (11–30 mg/L) with OS of 2.59 years ($p < 0.01$) [38]. Contrary to the above, a study of 441 patients with resected CRLM by Peltonen et al. [39] found that preoperatively elevated CRP levels had no significant association with shorter OS (HR: 0.91, 95% CI: 0.77–1.06). Postoperatively elevated CRP levels were associated with a shorter OS upon univariate analysis (HR: 1.11, 95% CI: 1.02–1.20), however, this significance was not appreciated on multivariate analysis (HR: 1.09, 95% CI: 0.99–1.20, p = 0.09) [39].

The CRP-to-albumin ratio may offer a more accurate prognostic value. Shibutani et al. [40] investigated preoperative serum CRP and albumin levels in patients that underwent CRLM resection (n = 119). Elevated CRP-to-albumin ratio (> 0.0471) displayed a statistically significant association with shorter RFS (p = 0.02), with moderately low sensitivity (62.0%) and specificity (58.5%) [41]. These findings were supported by Sakamoto et al. [41] in their retrospective analysis of 184 CRLM patients who underwent resection. Sakamoto et al. [41] reported that elevated CRP-to-albumin ratio was not only significantly associated with shorter OS (HR: 2.82, 95% CI: 1.63–4.72) and RFS (HR: 1.62, 95% CI: 1.02–2.49) after CRLM resection, but also with greater risks of post-operative sequelae. Similarly, in their study of 283 patients with CRLM who underwent radical resection, Deng et al. [42] found that increased CRP-to-albumin ratio in patients with CRLM could independently predict OS (HR: 2.22, 95% CI: 1.39–3.55) and RFS (HR: 1.49, 95% CI: 1.09–2.06) after liver resection. CRP and CRP-to-albumin ratio possess potential value in prognosticating CRLM after resection, though the existing evidence is limited by possible population and institutional bias, the lack of clinical application, and the lack of a standardised cut-off value.

9. Prognostic value of IL-6-associated peptides

Inflammation influences the spread of malignant cells through a variety of molecular mechanisms, which involve many different inflammatory cytokines [34]. IL-6 is an inflammatory cytokine that stimulates the production of proteins such as hepcidin and ferritin, to promote immunosuppression and cancer proliferation [35]. Lee et al.'s [37] report on patients with metastatic CRC (n = 120) found that serum ferritin could independently predict prognosis in this cohort. Elevated serum ferritin levels (≥150 ng/mL) displayed an association with significantly shortened survival (HR: 1.88, 95% CI: 1.27–2.78) [37].

IL-6 promotes the transcription of hepatocyte growth factor (HGF) and Chitinase-3-like protein 1 (YKL40) [39,43]. Whilst increased IL-6 levels have been demonstrated in CRC, they do not reliably predict OS and RFS in patients with resected CRLM [39,43]. de Jong et al. [43] found a statistically significant association between upregulated serum HGF and shorter OS (p = 0.08) and RFS (p = 0.04) in CRLM patients (n = 33) who underwent laparotomy or partial hepatectomy. Moreover, a report on 441 patients with resected CRLM by Peltonen et al. [39] demonstrated that preoperatively elevated YKL40 levels were significantly associated with reduced OS (HR: 1.29, 95% CI: 1.16–1.44) and RFS (HR: 1.19, 95% CI: 1.07–1.32). However, no existing report has corroborated such prognosticative value of YKL40, except Vocka et al. [28] who reported no significant correlation between slightly increased (p = 0.28) or largely elevated (p = 0.68) YKL40 levels and OS.

10. Challenges and opportunities in clinical applications

Despite years of dedicated global research, proteomic biomarkers for CRLM are limited to the discovery phase. This limitation is twofold and can be encapsulated by considering the technical and clinical issues. These issues hinder biomarker development progression through key stages such as biomarker discovery, verification, validation, and assay development [44].

Clinically, CRLM disease and patient population are largely heterogenous, leading to sample diversity. This diversity increases the risk of serendipitous protein discovery, which may not reflect the true nature of the CRLM proteome. In the identified studies, this is exacerbated by small sample sizes, with some studies including less than 100 patients, as well as the inclusion of patients with extrahepatic disease. This may cause reporting bias, where identified proteins are reportedly more significant in certain studies and patient populations, and thus not replicable across all CRLM cohorts. Furthermore, study designs also limit clinical applicability. Across identified studies, various milestones of disease progression and recurrence are indiscriminately used. Thus, the results only provide prognostic snapshots, rather than a longitudinal view of disease progression.

The discovery of proteomic biomarkers is an arduous and technically challenging task. Experimental design and techniques cause various limitations in biomarker discovery. Saliently, ELISA-based biomarker discovery can be limited as it may fail to detect proteins present in very low concentrations due to the washout process. Whilst this issue is resolved through LC-MS, this technique is limited by inter-institutional design and LC-MS machine calibration. Altogether, this variability between institutions has led to a general lack of independent verification and validation of the reported results. This is especially problematic for proteins such as COL IV and YKL40, which have conflicting reports of their prognostic values. This issue is exacerbated by limited inter-institutional collaboration. As opposed to other fields which are undertaking proteomic biomarker discovery, the field of CRLM has limited sample set collaboration. Alongside sample heterogeneity, this prevents independent replication and validation of findings.

This review identified exploratory studies focused on biomarker discovery. Future research may follow these biomarkers through further phases of biomarker development, such as clinical assay development and longitudinal repository studies [44]. Further preclinical investigation of the proteomic biomarkers may also prove beneficial, ideally with larger sample sizes to strengthen the validity of the results. The utilisation of MS-based techniques may enable the discovery of many more proteins that can prognosticate CRLM, with reports that MS-derived proteomic profiles can predict the risk of CRLM recurrence after resection [45]. Comparison of serum proteomic results with tissue specimen or urine proteomics results may provide insights into the molecular signature of CRLM, as would comparison or integration with results from genomic or transcriptomic studies of CRLM. More efforts for collaboration in proteomics research may prove valuable, such as promoting the use of a centralized data repository of proteomic findings [46].

11. Promising biomarkers

Most of the protein biomarkers identified in the existing literature have weak evidence of their prognostic values, with reports often lacking external validation. However, several proteins had promising and consistent prognostic value in multiple studies on resected CRLM. TIMP1 predicted OS across two studies, with statistically significant results, despite a small cohort size in one study (n = 29). These results may be explained by the crucial role of TIMP1 in regulating apoptosis, cellular proliferation, and angiogenesis [17]. Similarly, MMP-8 and MPO both displayed significant results in

predicting OS, consistent with the prominent roles of these enzymes in modulating tumour microenvironments and metastatic processes [29]. These three proteins are known to interact with each other; MPO inactivates TIMP1, and TIMP1 inhibits the activity of MMPs [47]. Research has also revealed the prognostic significance of these proteins in other solid tumours, such as hepatocellular carcinoma [48].

12. Conclusion

Proteomic analysis of plasma and serum from patients with CRLM can detect and quantify proteins that indicate the likely course or outcome of the disease. Circulating protein biomarkers identified to date reflect the complex pathways and processes that bring about tumour progression or recurrence. Ongoing advancements in high throughput proteomics are expected to accelerate the translation of proteomic biomarker discovery to a non-invasive serial sampling of protein biomarkers. Further validation and biomarker development are needed to enable the clinical application of these proteomic biomarkers that may allow a simple and precise method of prognosticating CRLM after curative-intent treatment.

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