A meta-analysis of CXCL12 expression for cancer prognosis

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Background: CXCL12 (SDF1) is reported to promote cancer progression in several preclinical models and this is corroborated by the analysis of human tissue specimens. However, the relationship between CXCL12 expression and cancer survival has not been systematically assessed.

Methods: We conducted a systematic review and meta-analysis of studies that evaluated the association between CXCL12 expression and cancer survival.

Results: Thirty-eight studies inclusive of 5807 patients were included in the analysis of overall, recurrence-free or cancer-specific survival, the majority of which were retrospective. The pooled hazard ratios (HRs) for overall and recurrence-free survival in patients with high CXCL12 expression were 1.39 (95% CI: 1.17–1.65, \(P = 0.0002\)) and 1.12 (95% CI: 0.82–1.53, \(P = 0.48\)) respectively, but with significant heterogeneity between studies. On subgroup analysis by cancer type, high CXCL12 expression was associated with reduced overall survival in patients with oesophagogastric (HR 2.08; 95% CI: 1.31–3.33, \(P = 0.002\)), pancreatic (HR 1.54; 95% CI: 1.21–1.97, \(P = 0.0005\)) and lung cancer (HR 1.37; 95% CI: 1.08–1.75, \(P = 0.01\)), whereas in breast cancer patients high CXCL12 expression conferred an overall survival advantage (HR 0.5; 95% CI: 0.38–0.66, \(P < 0.00001\)).

Conclusions: Determination of CXCL12 expression has the potential to be of use as a cancer biomarker and adds prognostic information in various cancer types. Prospective or prospective–retrospective analyses of CXCL12 expression in clearly defined cancer cohorts are now required to advance our understanding of the relationship between CXCL12 expression and cancer outcome.

A feature of most cancers is heterogeneity with regard to treatment response, recurrence and propensity for metastasis. Biomarkers that decipher this heterogeneity, either independently or in addition to current staging systems can help to guide the suitability of radical surgery and chemoradiotherapy, as well as a tailored approach to follow-up. Despite the promise that prognostic biomarkers hold, relatively few have reached clinical practice. This is because of a failure to translate findings from preclinical models to the clinic, a lack of rigorous prospective biomarker validation studies and poor reproducibility between such studies.

In the past two decades, much scientific endeavour has focused on the role that the immune system has in cancer development (de Visser et al., 2006; Grivennikov et al., 2010). Immune cells contribute to cancer progression, preparation of the premetastatic niche (Psaila and Lyden, 2009) and outgrowth of cancer cells at distant sites. Cytokines are the master regulators of protumorigenic immune cells, orchestrating their recruitment from the bone
CXCL12 expression predicts cancer outcome

MATERIALS AND METHODS

This meta-analysis was performed in accordance with the Meta-analysis of Observational studies in Epidemiology (MOOSE) group (Stroup et al, 2000) and Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidance (Moher et al, 2009).

Identification of relevant literature. MEDLINE (PubMed) and EMBASE (Ovid) were search on 10 November 2016 by a health-care librarian (TP) using the strategy shown in Supplementary Figures 1 and 2. All abstracts generated from the search strategy were read and the full text of selected publications was viewed to determine whether the inclusion criteria were met. References from included studies were also hand searched to identify further studies for inclusion.

Inclusion/exclusion criteria. Studies in humans with any solid cancer reporting the effect of CXCL12 expression on absolute, cancer-specific and/or recurrence-free survival were included. We accepted publications reporting any means of CXCL12 protein quantification including ELISA of serum or tumour lysates, or histological analysis of tumour samples. Included studies must have analysed surgical resection histology rather than tumour biopsy. Studies were excluded if they analysed RNA only, or performed a synthesis of publicly available proteomics or RNA data, as were studies of <20 patients. Studies were also excluded if they were not published in English and duplicated data sets from the same institution were also excluded.

Authors were contacted via email if their publication met the inclusion/exclusion criteria, but reported insufficient information for inclusion in the analysis. If no response was obtained they were contacted a second time within 4 weeks.

Assessment of publication quality and risk of bias. The Quality in Prognostic Studies (QUIPS) tool was used to determine risk of bias (Hayden et al, 2013). Two authors (HS and AGW) independently assessed each publication meeting the inclusion criteria for the quality domains set out in the QUIPS tool and any discrepancies in their assessment were resolved by joint analysis of the quality domain in question. Risk of bias for each domain was reported using a traffic light system, with red, orange or green indicating a high, moderate or low risk of bias, respectively.

Data extraction and statistical analysis. Data was extracted by one author (AGW) into a spreadsheet and cross-checked by a second author (HS). In all included studies, the independent variable under observation was the level of CXCL12 expression and was classified as high vs low CXCL12 level. Our primary aims were to determine firstly whether CXCL12 expression predicts survival in cancer patients, and secondly, whether CXCL12 measurement can be considered a valid prognostic biomarker in cancer.

marrow and blood to the tumour and polarising their phenotype once within the tumour microenvironment. These soluble mediators can also promote intravasation of tumour cells or their migration to metastatic sites, drive angiogenesis and inhibit cytotoxic T-cell activity (Balkwill, 2004; Mantovani et al, 2008; Chow and Luster, 2014). Certain cytokines may therefore be able to provide prognostic information by identifying tumours that are likely to metastasise or display therapeutic resistance (Ludwig and Weinstein, 2005).

The chemokine CXCL12 (SDF1) binds to the chemokine receptor CXCR4 and is constitutively expressed in tissues that serve as sites for metastasis including the lung, bone marrow and liver. Cancer cells migrate to these organs in a CXCL12-dependent manner (Taichman et al, 2002; Ray et al, 2015). Preclinical evidence suggests that migration of cancer cells towards CXCL12 in metastatic sites is dependent on simultaneous gain of CXCR4 expression and loss of CXCL12 in the tumour cell, enabling movement away from the primary tumour and towards the metastatic niche (Wendt et al, 2006, 2008; Murakami et al, 2013). However, this experimentally validated hypothesis is at odds with findings demonstrating that CXCL12 is upregulated in cancer tissues relative to their normal counterparts and that high CXCL12 expression in some human tumours correlates with cancer dedifferentiation and increased tumour grade and stage (Tsuboi et al, 2008; Jaafar et al, 2009; Machelon et al, 2011; Zhong et al, 2012).

Given the complex and multifaceted role that CXCL12 has in the progression of primary cancer to metastasis, the prognostic benefit of determining CXCL12 expression in cancer patients is unclear. In an attempt to address this issue, we have performed a meta-analysis of CXCL12 protein expression in the tumour or plasma of cancer patients with the primary independent variable being high vs low CXCL12 level. Our primary aims were to determine firstly whether CXCL12 expression predicts survival in cancer patients, and secondly, whether CXCL12 measurement can be considered a valid prognostic biomarker in cancer.
### Table 1. Demographics of included studies

| First author | Year of publication | Study period | Country | Cancer type | No. of patients | Age *mean (s.d.)*median (range) | % female | % high CXCL12 expression | Median follow-up |
|--------------|---------------------|--------------|---------|-------------|-----------------|--------------------------------|----------|--------------------------|-----------------|
| Guo et al (2016) | 2016 | ? | China | Pancreatic | 182 | $63 (34–85) | 34.7 | 34.6 | 12.6 |
| Guo cohort 2 (Guo et al, 2016) | 2016 | ? | China | Pancreatic | 153 | $65 (38–90) | 45.1 | 77.1 | 10.9 |
| Uchi et al (2016) | 2016 | 1997–2007 | Japan | Oesophageal | 79 | $59 (44–79) | 89.9 | 78.4 | 68 |
| Stanisavljević et al (2016) | 2016 | 1993–1996 | Norway | Colonic | 263 | *61.9 (9.3) | 52 | 73.0 | ? |
| Stanisavljević cohort 2 (Stanisavljević et al, 2016) | 2016 | 2007–2011 | Norway | Colonic | 239 | *73.5 (11.9) | 51 | 89.5 | ? |
| Ock et al (2017) | 2016 | 2004–2014 | Japan | Gastric | 70 | ? | ? | 48.6 | 37.8 |
| de Cuba et al (2016) | 2016 | 2007–2011 | Holland | Colonic (metastatic) | 52 | *58 (12) | 56.6 | 53.9 | 22.5 |
| Sterlacci et al (2016) | 2016 | 1992–2004 | Germany | Lung (NSCLC) | 295 | ? | 6.9 | 53.2 | ? |
| Izumi et al (2016) | 2016 | 2005–2009 | Japan | Gastric | 110 | ? | 28.2 | 58.2 | ? |
| Tang et al (2016) | 2016 | 2000–2012 | China | Endometrial | 202 | ? | 100 | 52.0 | ? |
| Rave-Frank et al (2016) | 2016 | ? | Germany | Head and neck | 229 | ? | 15.3 | 42.4 | 83 |
| Kadota et al (2015) | 2016 | 1999–2009 | United States of America | Lung adenocarcinoma | 303 | $72 (39–88) | 35.0 | 40.6 | 49 |
| Tang et al (2015) | 2015 | 2009–2014 | China | Glioma | 42 | *44.9 (13) | 31.0 | 21.4 | ? |
| Tabernero et al (2015) | 2015 | ? | Worldwide | Metastatic colorectal | 611 | ? | 37.0 | 72.2 | 4.9 |
| Fu et al (2015) | 2015 | 2005–2007 | China | Cervical | 130 | ? | ? | 60.8 | 68 |
| Amara et al (2015) | 2015 | 1995–2011 | Tunisia | Colorectal | 124 | ? | 43.5 | 71.8 | ? |
| Hong et al (2014) | 2014 | ? | United States of America | Pancreatic | 32 | ? | 15.6 | ? | 38 |
| Wang et al (2014) | 2014 | 2002–2006 | China | Gastric | 84 | ? | 31.3 | 50.0 | 59 |
| Martinetti et al (2014) | 2014 | ? | Italy | Colonic | 50 | $60 (51–58) | 42 | 50.0 | ? |
| D’Alterio et al (2014) | 2014 | ? | Italy | Rectal | 68 | ? | 42.6 | 77.9 | 64 |
| Walentowicz-Sadlecka et al (2014) | 2013 | 2000–2007 | Poland | Endometrial | 92 | *65.1 (9.5) | 100 | 34.8 | ? |
| Wang et al (2013) | 2013 | 1982–2007 | China | Prostatic | 148 | $65.8 (34–85) | ? | 18.9 | 95 |
| Wang et al (2012) | 2012 | 2002–2003 | China | Renal | 38 | $55.4 (21–81) | 38.1 | 48.9 | ? |
| Lee et al (2012) | 2012 | 1998–2009 | Korea | Gallbladder carcinoma | 72 | ? | 54.2 | 80.6 | ? |
| Scheveel et al (2012) | 2012 | 1985–1999 | Holland | Cervical | 103 | $48 (24–87) | 100 | 76.7 | 137 |
| Popple et al (2012) | 2012 | 1984–1997 | United Kingdom | Ovarian | 172 | $61 (24–90) | 100 | 34.3 | 167 |
| Sakai et al (2012) | 2012 | 1999–2007 | Japan | Colorectal (metastatic) | 92 | ? | 37.0 | 55.4 | 38 |
| Machelon et al (2011) | 2011 | 2002–2004 | France | Ovarian | 183 | $59 (25–77) | 100 | 47.0 | 69 |
| Yan et al (2011) | 2011 | ? | Australia | Breast | 236 | $55 (24–87) | 100 | 66.5 | 131 |
| Kobayashi et al (2010) | 2010 | 1995–1999 | Japan | Breast | 223 | $52 (30–82) | 100 | 70.9 | 74 |
| Liang et al (2010) | 2010 | 1990–2005 | United States of America | Pancreatic | 72 | $63 (40–80) | 33.3 | 34.7 | ? |
between studies was assessed using the Cochran Q statistic ($\chi^2$ test) and $I^2$. Heterogeneity was considered high, medium or low if $\geq 75\%$, 50–75\% or $< 50\%$, respectively (Higgins et al., 2003). Funnel plots were constructed for overall, disease-specific and recurrence-free survival analyses and assessed by visual inspection. Subgroup analysis was performed to determine the relationship between CXCL12 expression and outcome in specific cancer types for overall survival. A $P$-value $< 0.05$ throughout was considered statistically significant. We did not correct the $P$-value for multiple comparisons within our subgroup analysis as the Cochrane Handbook (V.5.1.0) (Higgins and Green, 2011) currently recommends against this.

RESULTS

Search results. The study flow is shown in Figure 1. A total of 38 studies were included in the meta-analysis of one or more outcome measures totalling 5807 patients (Table 1). Twenty-eight studies reported absolute survival, 16 recurrence-free survival and 4 reported cancer-specific survival. A further 18 studies met the inclusion criteria, but failed to report sufficient data to be included. Of these studies, five authors responded to requests for further data. Of the 11 who failed to respond, 5 reported no association between CXCL12 expression and outcome in the original manuscript.

Study demographics. The demographics of included studies can be seen in Table 1. The total study period ranged from 1982 to 2014, although in 27\% of publications, the study period was not identifiable. Forty-seven per cent of studies analysed patients from Australasia, 39\% from Europe, 11\% from North America and 3\% from Africa and 30\% of studies analysed data from more than 200 patients. Eighteen studies analysed patients with gastrointestinal cancer (49\%), 7 with gynaecological cancer (19\%), 4 with breast cancer (11\%), 3 with urological cancer (8\%) and 2 with lung cancer (5\%). The proportion of patients considered to express high levels of CXCL12 varied widely from 18.9\% (Wang et al., 2013) to 89.5\% (Stanisavljevic et al., 2016). The median follow-up period ranged from 4.9 months in a study of metastatic colorectal cancer (Tabernero et al., 2015) to 167 months in a study of ovarian cancer (Popple et al., 2012) and 13 studies (35\%) did not provide the median follow-up period.

Study methodology and assessment of study quality. The technical detail for included studies, study methodology and technique for CXCL12 protein quantification is shown in Table 2, while an analysis of risk of bias as determined using the QUIPS tool is shown in Supplementary Table 1. There were 3 studies that analysed serum CXCL12 concentration and 34 studies that quantified tumour protein expression using IHC. We did not identify any study that quantified CXCL12 expression in protein from tumour lysate. Most studies simultaneously analysed the expression of other factors with CXCR4 analysed by 23 studies. The antigen retrieval technique and details of the antibody used, sufficient that the methodology could be repeated by readers, were documented by 16 studies (43\%). Interestingly, one study reported the use of an antibody with specificity for CXCR4 for the analysis of CXCL12 (Ishigami et al., 2007).

The method for defining low and high CXCL12 expression level was reported in 89\% of studies, with 65\% using an arbitrary method not related to data distribution and only 22\% of studies determining CXCL12 value cutoffs based on ROC curve analysis. In 10 studies (26\%), the CXCL12 expression data was linked to follow-up data collected in a prospective manner.

Survival analysis. The pooled HR for overall survival in patients with high CXCL12 expression compared with low expression was
| First author          | Sample            | Quantification method | Other factors analysed | Antigen retrieval method       | Sample storage | Antibody/ELISA source (CAT number)                  | Definition of expression level cutoffs | Follow-up collection | Outcome measures |
|----------------------|-------------------|-----------------------|------------------------|-------------------------------|----------------|---------------------------------------------------|----------------------------------------|---------------------|------------------|
| Guo et al, 2016      | Tumour tissue     | IHC                   | CXCR7                  | Autoclave + citrate           | FFPE           | R&D Systems, Minneapolis, MN, USA                  | Arbitrary                             | Retrospective       | OS               |
| Guo cohort 26 (Guo et al, 2016) | Tumour tissue     | IHC                   | CXCR7                  | Autoclave + citrate           | FFPE           | R&D Systems                                       | Arbitrary                             | Retrospective       | OS               |
| Uchi et al (2016)    | Tumour tissue     | IHC                   | CXCR4                  | Target retrieval solution     | FFPE           | R&D Systems (MAB350)                               | Arbitrary                             | Retrospective       | RFS              |
| Stanisavljević et al (2016) | Tumour tissue     | IHC                   | CXCR4                  | Target retrieval solution     | FFPE           | R&D Systems (MAB350)                               | Arbitrary                             | Prospective         | RFS              |
| Stanisavljević cohort 2 (Stanisavljević et al, 2016) | Tumour tissue     | IHC                   | CXCR4                  | Target retrieval solution     | FFPE           | R&D Systems (MAB350)                               | Arbitrary                             | Prospective         | RFS              |
| Ock et al (2017)     | Serum             | Protein array         | Multiple (>10)         | N/A                           | Frozen         | Bio-Rad Laboratories (Hercules, CA, USA) (Bio-Plex 220 assay) | Data distribution                     | Retrospective       | OS               |
| de Cuba et al (2016) | Tumour tissue     | IHC                   | HIF1a, CXCR4, VEGF     | ?                             | FFPE           | R&D Systems                                       | Arbitrary                             | Prospective         | OS               |
| Sterlacci et al (2016) | Tumour tissue     | IHC                   | CXCR4, pCXCR4          | ?                             | FFPE           | Abcam (Cambridge, UK)                              | ROC curve analysis                    | Retrospective       | OS               |
| Izumi et al (2016)   | Tumour tissue     | IHC                   | CXCR4                  | ?                             | FFPE           | R&D Systems AF-310-NA                              | Arbitrary                             | Retrospective       | OS               |
| Teng et al (2016)    | Tumour tissue     | IHC                   | CXCR4                  | ?                             | FFPE           | Abcam                                             | Arbitrary                             | Retrospective       | CSS              |
| Rave-Frank et al (2016) | Tumour tissue     | IHC                   | CXCR4                  | Heat (100 °C, 60 min)         | FFPE           | R&D Systems                                       | Arbitrary                             | Retrospective       | OS               |
| Kadota et al (2015)  | Tumour tissue     | IHC                   | Multiple (>10)         | ?                             | FFPE           | R&D Systems                                       | Arbitrary                             | Prospective         | OS               |
| Tang et al (2015)    | Tumour tissue     | IHC                   | CXCR4                  | Citrate (pH 6.0, 100 °C, 15 min) | FFPE           | R&D Systems (MAB350)                               | Arbitrary                             | ?                  | OS               |
| Tabemero et al (2015) | Serum             | ELISA                 | Multiple (>10)         | N/A                           | Frozen         | Assay Gate (Ijamsville, MD, USA)                  | ROC curve analysis                    | Prospective         | OS, RFS          |
| Fu et al (2015)      | Tumour tissue     | IHC                   | N/A                    | ?                             | FFPE           | ?                                                 | Arbitrary                             | Retrospective       | OS               |
| Amara et al (2015)   | Tumour tissue     | IHC                   | CXCR4                  | Citrate (pH 9.0, microwave 2–5 min) | FFPE           | R&D Systems (?)                                   | Arbitrary                             | Retrospective       | OS               |
| Hong et al (2014)    | Tumour tissue     | IHC                   | CEA, CA19-9, HGF       | ?                             | FFPE           | Biovision (Milpitas, CA, USA) (?)                 | ?                                     | Prospective         | OS, RFS          |
| Wang et al (2014)    | Tumour tissue     | IHC                   | N/A                    | ?                             | FFPE           | R&D Systems (MAB350)                               | ROC curve analysis                    | Retrospective       | OS               |
| Martinetti et al (2014) | Serum             | ELISA                 | VEGF, PDGF, osteopontin, CEA | N/A                           | Frozen         | Bio-Rad Laboratories (Bio-Plex 220 assay)          | ROC curve analysis                    | Prospective         | OS, RFS          |
| D’Alterio et al (2014) | Tumour tissue     | IHC                   | CXCR4, CXCR7           | ?                             | FFPE           | R&D Systems (MAB350)                               | Arbitrary                             | Retrospective       | OS               |
| Walentowicz-Sadlecka et al (2014) | Tumour tissue     | IHC                   | CXCR4, CXCR7           | Epitope Retrieval Solution (Dako) | FFPE           | Abcam (AB9797)                                    | Arbitrary                             | Retrospective       | OS               |
| First author               | Sample      | Quantification method | Other factors analysed | Antigen retrieval method | Sample storage | Antibody/ELISA source (CAT number) | Definition of expression level cutoffs | Follow-up collection | Outcome measures |
|---------------------------|-------------|-----------------------|------------------------|--------------------------|----------------|-----------------------------------|----------------------------------------|---------------------|-----------------|
| Wang et al (2013)         | Tumour tissue | IHC                   | VEGF, MMP9             | 0.1% zymine (37 °C, 30 min) | FFPE | R&D Systems (?) | Arbitrary | ?                     | RFS             |
| Wang et al (2012)         | Tumour tissue | IHC                   | CXCR4, CXCR7           | Citrate (pH 6.0, 100 °C, 10 min) | FFPE | R&D Systems (MAB350) | ROC curve analysis | Retrospective | OS, RFS         |
| Lee et al (2012)          | Tumour tissue | IHC                   | N/A                    | Target Retrieval Solution (DAKO) | FFPE | R&D Systems (MAB350) | ROC curve analysis | Retrospective | CSS             |
| Schaevel et al (2012)     | Tumour tissue | IHC                   | N/A                    | Citrate (pH 6.0, microwave, 12 min) | FFPE | R&D Systems (MAB350) | ?                     | Retrospective | RFS             |
| Popple et al (2012)       | Tumour tissue | IHC                   | CXCR4                  | EDTA (pH 9.0, microwave, 10 min) | FFPE | R&D Systems (MAB350) | Arbitrary | Retrospective | OS              |
| Sakai et al (2012)        | Tumour tissue | IHC                   | CXCR4, CD133           | ?                        | FFPE | R&D Systems (MAB350) | Arbitrary | Retrospective | OS, RFS         |
| Machelon et al (2011)     | Tumour tissue | IHC                   | N/A                    | Citrate (pH 6.0, microwave) | FFPE | Abcam (AB10395) | Arbitrary | Prospective | OS, RFS         |
| Yan et al (2011)          | Tumour tissue | IHC                   | FoxP3                  | Tris/EDTA (pH 9.0, microwave) | FFPE | R&D Systems (MAB350) | Arbitrary | retrospective | CSS             |
| Kobayashi et al (2010)    | Tumour tissue | IHC                   | CXCR4                  | 0.5% Tween-20 in PBS     | FFPE | R&D Systems (MAB350) | Arbitrary | ?                     | OS, RFS         |
| Liang et al (2010)        | Tumour tissue | IHC                   | N/A                    | EDTA (pH 9.0, 100 °C, 20 min) | FFPE | R&D Systems (?) | ROC curve analysis | prospective | OS, RFS         |
| Mirsola et al (2009)      | Tumour tissue | IHC                   | CXCR4                  | ?                        | FFPE | Dianova (Hamburg, Germany) (?) | ROC curve analysis | ?                     | OS, RFS         |
| Akishima-Fukasawa et al  (2009) | Tumour tissue | IHC                   | N/A                    | Citrate (pH 6.0, 121 °C, 10 min) | FFPE | R&D Systems (?) | Arbitrary | Prospective | OS, RFS         |
| Hassan et al (2009)       | Tumour tissue | IHC                   | CXCR4                  | ?                        | FFPE | R&D Systems (MAB350) | Arbitrary | ?                     | OS              |
| Gilbert et al (2009)      | Tumour tissue | IHC                   | CXCR4                  | ?                        | FFPE | R&D Systems (MAB350) | Arbitrary | Retrospective | RFS             |
| Sasaki et al (2009)       | Tumour tissue | IHC                   | CXCR4                  | Citrate buffer (120 °C, 10 min) | FFPE | R&D Systems (MAB350) | Arbitrary | Retrospective | OS              |
| Ishigami et al (2007)     | Tumour tissue | IHC                   | N/A                    | ?                        | FFPE | R&D Systems (MAB172) | ?                     | ?                     | OS              |
| Pils et al (2007)         | Tumour tissue | IHC                   | CXCR4                  | ?                        | FFPE | R&D Systems (MAB350) | ?                     | ?                     | OS              |

Abbreviations: CSS = cancer specific survival; ELISA = enzyme-linked immunosorbent assay; FFPE = formalin-fixed paraffin-embedded; IHC = immunohistochemistry; N/A = not applicable; OS = overall survival; RFS = recurrence-free survival.
Figure 2. Forrest plot of overall survival for all studies meeting the inclusion criteria listed in order of effect size.

Figure 3. Forrest plot of recurrence-free survival for all studies meeting the inclusion criteria listed in order of effect size.

Figure 4. Funnel plots for included studies reporting overall (left) and recurrence-free survival (right).
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1.39 (95% CI: 1.17–1.65, P = 0.0002), but with a significant degree of heterogeneity ($I^2 = 86\%$) (Figure 2), while the pooled HR for recurrence-free survival was 1.12 (95% CI: 0.82–1.53, P = 0.48), again with a high degree of study heterogeneity ($I^2 = 88\%$) (Figure 3). The pooled HR for cancer-specific survival, which was only analysed in four studies, was 1.67 (95% CI: 0.43–6.50, P = 0.46) again with high study heterogeneity ($I^2 = 87\%$) (results not shown). Funnel plots for overall, recurrence-free and cancer-specific survival demonstrated no evidence of publication bias or small study effects (Figure 4).

Subgroup analysis. Following subgroup analysis, high CXCL12 expression served as a marker of reduced overall survival in oesophagogastric (HR 2.08; 95% CI: 1.31–3.33, P = 0.002), pancreatic (HR 1.54; 95% CI: 1.21–1.97, P = 0.0005) and lung (HR 1.37; 95% CI: 1.08–1.75, P = 0.01) cancers (Figure 5). For colorectal and ovarian cancer, however, there was no relationship between CXCL12 expression and overall survival (HR 1.21; 95% CI: 0.64–2.51, P = 0.49) and (HR 1.23; 95% CI: 0.75–2.03, P = 0.42), respectively. For breast cancer patients, high CXCL12 predicted better overall survival (HR 0.5; 95% CI: 0.38–0.66).

Figure 5. Subgroup analysis by cancer type demonstrating meta-analysis of high vs low CXCL12 expression for overall survival.

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CXCL12 expression predicts cancer outcome

The primary objective of this meta-analysis was to determine whether CXCL12 expression was associated with survival in cancer patients. We found that high CXCL12 expression was associated with reduced absolute survival in patients with oesophageal, pancreatic or lung cancer, while the converse was the case for breast cancer patients. Indeed, the major cause of heterogeneity in our meta-analysis resulted from heterogeneity in the relationship between CXCL12 expression and outcome between breast and other cancer types. Our data indicate that determination of CXCL12 expression could be useful for predicting outcome in these cancer types. Although studies of RNA expression were excluded from this meta-analysis, published studies that have assessed CXCL12 mRNA expression in oesophageal (Goto et al., 2017) or breast cancer (Razis et al., 2012) support our findings, with an association between increased CXCL12 expression and adverse outcome in oesophageal cancer, but the converse in breast cancer.

The cause of the different effect of high CXCL12 expression and outcome in breast compared with other cancers is unclear. The publications of breast, pancreatic, lung and oesophageal cancer included in our study all analysed primary rather than metastatic tumours; thus, differences are unlikely to result from sampling differences between cancer types. However, they may result from clinicobiological differences between these cancers.

Thus, breast cancer is rarely fatal unless metastatic, whereas oesophageal, lung and pancreatic cancers often cause mortality through local invasion. CXCL12 is able to promote local invasion of cancer cells, while loss of CXCL12 promotes tumour cell migration to organs expressing high levels of CXCL12 such as the liver, bone, marrow and lung. Breast cancers may therefore rely on downregulation of CXCL12 to metastasise, whereas in pancreatic, oesophageal and lung tumours, high CXCL12 expression may be associated with poor outcome because it promotes local invasion, in turn contributing to mortality.

Alternatively, differences between breast and other cancer types may reflect systemic differences between the demographics of the studies, or the methodologies used. It should also be considered that the source of CXCL12 within the tumour may be important. The majority of included studies did not investigate the cellular source of CXCL12, and while the immunohistochemical images presented in most publications indicate the primary source of CXCL12 is the tumour cell, it is possible that stromal and tumour cell CXCL12 production have different roles in cancer progression. Finally, there is significant redundancy in the chemokine network such that analysis of a single chemokine alone may be insufficient. Thus, the relative ratio of CXCL12 to its receptors CXCR4 and/or CXCR7 may be a better indicator of CXCL12 activity (Luker et al., 2012; Wani et al., 2014) and there may be differences in these ratios between cancer types.

Our second objective was to determine whether CXCL12 measurement can be used as a prognostic biomarker in cancer patients. The gold standard evidence level for a prognostic biomarker study is a randomised controlled trial (RCT) designed in such a way that participants are randomised to the prognostic

**DISCUSSION**

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Our second objective was to determine whether CXCL12 measurement can be used as a prognostic biomarker in cancer patients. The gold standard evidence level for a prognostic biomarker study is a randomised controlled trial (RCT) designed in such a way that participants are randomised to the prognostic...
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The potential clinical validity of CXCL12 is tested in the subgroup analysis presented here. These data indicate that CXCL12 has clinical validity as a biomarker for breast, pancreatic, lung and oesophagogastric cancer. Studies of colon cancer, whether primary or metastatic, demonstrated heterogenous results over a large number of patients, indicating that measuring CXCL12 alone in colon cancer patients is less likely to be useful for prognostication. Despite this, two colorectal cancer studies assessed the effect of the CXCL12:CXCR4 ratio on survival, with both finding that this approach provided prognostic information. Indeed, the publications by Stanisavljević et al (2016) and D’Alterio et al (2014) found that patients with a combination of low CXCL12 and high CXCR4 expression in the primary tumour experienced reduced recurrence-free or overall survival, respectively. Unfortunately, we were unable to identify other studies that combined the measure of CXCL12 and CXCR4 expression in this way, but the data from these studies indicate that this approach may provide more useful information than measuring either factor alone.

The data identified in our meta-analysis provide only limited information about the precise clinical utility of CXCL12 in specific groups of cancer patients. This is in part because of our broad inclusion criteria that identified a heterogeneous set of studies, and also because of a failure of many included studies to define adequately their cancer population. We found that even simple demographic data such as age, sex and tumour stage was not always reported. Future studies in this area should therefore clearly report the analysis of CXCL12 expression in a subset of cancer patients that are defined on the basis of clinical, histopathological and preferably genomic data such that the clinical utility of CXCL12 in clearly defined cancer patients can be better determined.

In summary, the strengths of this meta-analysis are a wide search strategy identifying multiple studies from differing populations and a pragmatic subgroup analysis highlighting potential differences in the relationship between CXCL12 expression and prognosis between cancer types. Through critical and systematic appraisal, this review has led to guidance points that, if followed, will ensure the generation of higher quality data in the investigation of CXCL12 as a prognostic biomarker. These strengths need to be balanced against the fact that our conclusions are drawn from predominantly retrospectively analyses of survival data, which are by definition prone to bias. The majority of included studies also failed to blind the outcome assessor to participants CXCL12 status, leading to a risk of reporter bias in such studies. Overall, the quality of research in this field needs to improve if progress is to be made in better defining the role of CXCL12 as a prognostic biomarker.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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