

C-Reactive Protein as a Prognostic Factor in Cancer

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fulfilment of the requirements for the degree of**

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Signed:

A handwritten signature in blue ink, appearing to read 'Cliona M Lorton', with the name 'LORTON' written in small capital letters below the signature.

Cliona M Lorton

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1 Contents

List of Figures	viii
List of Tables.....	xi
List of Abbreviations.....	xiv
Abstract	xix
Lay Abstract.....	xxi
Overall Hypothesis.....	xxiii
Overall Aim.....	xxiii
Output.....	xxiv
Thesis structure	xxviii
1 Introduction	1
1.1 C-reactive protein (CRP).....	2
1.1.1 CRP Function	2
1.1.2 Clinical Use of CRP	3
1.1.3 CRP Synthesis and Structure.....	3
1.1.4 Non-pentameric CRP: structure and function	6
1.1.5 Baseline CRP levels	6
1.1.6 Medications affecting CRP	8
1.1.7 Diet and Supplementation with Vitamins and Minerals.....	11
1.1.8 Other interventions.....	12
1.1.9 Diseases associated with higher CRP.....	12
1.1.10 CRP and disease outcomes.....	15
1.1.11 CRP in pathogenesis.....	16
1.2 Cancer	17
1.2.1 Oesophageal cancer.....	18
1.2.2 Lung cancer.....	18
1.2.3 Cancer-related inflammation.....	19
1.2.4 CRP in Cancer	19

1.3	Prognostication	20
1.3.1	Standard predictors of outcome in cancer	21
1.3.2	Appeal of CRP as a prognostic biomarker in cancer	22
1.4	Novel uses for CRP in Prognostication in Cancer	24
1.4.1	CRP kinetics.....	24
1.4.2	CRP cutpoints and CRP-based scores.....	24
1.4.3	Tumoural CRP	27
1.4.4	CRP and Skeletal Muscle Abnormality	27
2	CRP Kinetics to Predict Survival in Cancer	28
2.1	Introduction.....	29
2.1.1	Specific Hypotheses of this Study	32
2.1.2	Specific Aims of this Study	32
2.2	Methods.....	33
2.2.1	Cleveland Clinic dataset.....	33
2.2.2	St James's Hospital (SJH) dataset.....	35
2.3	Results.....	36
2.3.1	Dataset 1: Cleveland Clinic.....	36
2.3.2	Dataset 2: St James's Hospital	61
2.4	Discussion.....	66
2.5	Conclusions.....	72
3	CRP and CRP -Based Prognostic Scores in Oesophageal Adenocarcinoma: A Systematic Review	73
3.1	Introduction.....	74
	Specific Hypothesis of this Study	77
3.1.1	Specific Aims of this Study: Review Questions	77
3.2	Methods.....	78
3.2.1	Summary	78
3.2.2	Identification of Studies	78
3.2.3	Inclusion criteria	78

3.2.4	Exclusion criteria.....	78
3.2.5	Study Selection.....	79
3.2.6	Data Extraction.....	79
3.2.7	Risk of bias assessment	80
3.2.8	Data Analysis: Narrative synthesis and Meta-analysis	80
3.2.9	Certainty assessment	81
3.2.10	Software	81
3.3	Results	82
3.3.1	Study Identification, Selection and Inclusion.....	82
3.3.2	Included Studies	84
3.3.3	Studies of the prognostic value of CRP in OAC	89
3.3.4	Studies of the prognostic value of CRP-based scores in OAC.....	92
3.3.5	Quality of Study Reports.....	96
3.3.6	Meta-analysis	97
3.4	Discussion	102
3.4.1	Overview	102
3.4.2	CRP	102
3.4.3	GPS/mGPS	102
3.4.4	Other CRP-based scores.....	103
3.4.5	Future possibilities	105
3.4.6	Limitations of evidence	106
3.4.6	Limitations of this review	107
3.4.7	Strengths of this review	108
3.5	Conclusions	110
4	Tumoural CRP in Oesophageal Adenocarcinoma.....	111
4.1	Introduction	112
4.1.1	Specific Hypotheses of this Study.....	114
4.1.2	Specific Aims of this Study.....	114
4.2	Methods.....	115

4.2.1	Western Blot and Immunohistochemistry.....	115
4.2.2	Cell culture.....	119
4.2.3	Enzyme-Linked Immunosorbent Assay (ELISA).....	122
4.2.4	Immunofluorescence.....	124
4.3	Results.....	127
4.3.1	Western Blot	127
4.3.2	Immunohistochemistry.....	128
4.3.3	ELISA: OE33 cell line	136
4.3.4	Immunofluorescence.....	137
4.4	Discussion.....	146
4.5	Conclusions.....	154
5	The use of CRP and Skeletal Muscle measures to predict symptoms and quality of life in cancer: a feasibility study	155
5.1	Introduction.....	156
5.1.1	Specific Hypotheses of this Study	159
5.1.2	Specific Aims of this Study	159
5.2	Methods.....	161
5.2.1	Inclusion and exclusion criteria	162
5.2.2	Study procedures.....	163
5.3	Results.....	168
5.3.1	Iterations 1,2 and 3.....	168
5.3.2	Recruitment in Iteration 3	171
5.3.3	Demographics	173
5.3.4	Primary outcomes: Feasibility	176
5.3.5	Secondary Outcomes.....	178
5.3.6	Associations between baseline variables and outcomes	196
5.3.7	Further Exploratory Analyses	199
5.4	Discussion.....	203
5.4.1	Primary Aim: Feasibility.....	203

5.4.2	Secondary Aims	208
5.4.3	Limitations and strengths of study	215
5.5	Conclusions	221
6	Concluding summary	222
6.1.1	Concluding discussion.....	223
6.1.2	Strengths and limitations	227
6.1.3	Future directions.....	229
6.1.4	Conclusions	231
6.1.5	Summary of thesis findings: Mind map	232
	References.....	233
	Appendices.....	264
	Appendix 1: Search strategy	264
	Appendix 2: QUIPS Tool.....	268
	Appendix 3: Participant Information Leaflet and Consent Form.....	269
	Appendix 4: Data Collection Sheet.....	273
	Appendix 5: EORTC QLQ-C30.....	274
	Appendix 5: Eastern Cooperative Oncology Group (ECOG) Performance Status.....	276

List of Figures

Figure 1-1. Ribbon diagram demonstrating the pentameric structure of human C-reactive protein complexed with phosphocholine (from Black et al., 2004).....	4
Figure 1-2. The ligand-binding and effector molecule-binding faces of the 5 monomers within the CRP pentamer (from Szalai & McCrory 2002)	5
Figure 1-3. Model of the complex between CRP and the C1q globular domain (from Gaboriaud et al., 2003).....	5
Figure 1-4. Effects of glucocorticoids on innate and adaptive immunity (modified from Adorisio et al., 2021)	9
Figure 1-5. Plasma CRP in the Copenhagen General Population Study, illustrating typical CRP levels recorded in adults with various diseases (from Allin & Nordestgaard 2011)...	13
Figure 1-6. CRP values reported in various cancer sites and in healthy adults (from Hart et al., 2020)	14
Figure 2-1. Primary Cancer Sites for Included Patients (n=607) from the Cleveland Clinic cohort	36
Figure 2-2. Kaplan-Meier survival curve illustrating shorter overall survival in people with high baseline CRP (CRP1).....	39
Figure 2-3. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival.....	40
Figure 2-4. Kaplan-Meier survival curve of the prognostic influence of white blood cell count on overall survival.....	41
Figure 2-5. Kaplan-Meier survival curve illustrating shorter overall survival in those age 65 or older	42
Figure 2-6. Kaplan-Meier survival curve illustrating shorter overall survival in males.....	43
Figure 2-7. Kaplan-Meier survival curve illustrating shorter overall survival in people with metastatic disease.....	44
Figure 2-8. Kaplan-Meier survival curve illustrating absence of an association between ethnicity and overall survival.....	45
Figure 2-9. Kaplan-Meier survival curve illustrating absence of an association between CRP change category and overall survival in NMSC	49
Figure 2-10. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival in people with any cancer site except NMSC	51

Figure 2-11. Kaplan-Meier survival curve comparing overall survival in NMSC with overall survival in other cancer primary sites.....	54
Figure 2-12. Kaplan-Meier survival curve comparing overall survival in people with multiple primary cancer sites with those with single primary cancer sites, excluding NMSC	57
Figure 2-13. Kaplan-Meier survival curve illustrating the prognostic influence of CRP change category on overall survival in people with multiple primary sites (n=97)	58
Figure 2-14. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival in people with a single primary cancer site.....	59
Figure 2-15. Kaplan-Meier survival curve illustrating the prognostic influence of baseline CRP (CRP1) on overall survival in people with oesophageal adenocarcinoma.....	63
Figure 2-16. Kaplan-Meier survival curve illustrating absence of an association between CRP change category and overall survival in oesophageal adenocarcinoma.....	64
Figure 2-17. Kaplan-Meier survival curve illustrating no significant association between sex and overall survival in oesophageal adenocarcinoma	65
Figure 3-1. PRISMA 2020 flow diagram(Page et al., 2021) summarising the study identification and selection process	83
Figure 3-2. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, after multimodal treatment or resection alone	97
Figure 3-3. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, after multimodal treatment.....	98
Figure 3-4. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, with no metastatic (M0) disease	99
Figure 3-5. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included only patients with no residual tumour at resection (R0 resection)	99
Figure 3-6. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included chemotherapy and surgery	100

Figure 3-7. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included R0-R2 resection status.....	101
Figure 3-8. Forest plot of studies conducted in the United Kingdom or Republic of Ireland investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ	101
Figure 4-1. Expression of C-reactive protein (CRP) in fresh snap-frozen liver tissue	127
Figure 4-2. Expression and localisation of CRP in Resected OAC Tumours	131
Figure 4-3. Kaplan-Meier survival curve of overall survival in OAC/OGJ in patients with serum CRP > 10mg/L and patients with serum CRP ≤ 10mg/L.....	134
Figure 4-4. Kaplan-Meier survival curve of overall survival in patients where tumoural CRP was positive or absent in resected tumour tissue	135
Figure 4-5. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with serum CRP > 10mg/ and patients with serum CRP ≤ 10mg/L	142
Figure 4-6. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with high digitally-scored CRP-positivity density in tumour cytoplasm in tumour biopsies when compared to low digitally-scored CRP-positivity density	144
Figure 4-7. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with high digitally-scored CRP-positivity density in tumour nuclei in tumour biopsies when compared to low digitally-scored CRP-positivity density	145
Figure 5-1. Summary of Iterations 1,2 and 3 showing location of recruitment and patient population included.....	161
Figure 5-2. Summary of Study Procedures highlighting key data collected at enrolment and at follow-up.....	164
Figure 5-3. Flowchart of recruitment to iteration 3	171
Figure 5-4. Breakdown of included patients.....	172
Figure 5-5. Distribution (median and range) for EORTC QLQ-C30 Summary Score at Enrolment stratified by diagnostic group.....	181
Figure 5-6. Distribution (median and range) for EORTC QLQ-C30 Summary Score at follow-up, stratified by diagnostic group.....	188
Figure 5-7. Prevalence of symptoms above the threshold for clinical importance in the three diagnostic groups at enrolment and at follow-up.....	193
Figure 5-8. Prevalence of functional impairments above the threshold for clinical importance in the three diagnostic groups at enrolment and at follow-up.....	194

List of Tables

Table 1-1. Prognostic scores incorporating CRP reported in lung and oesophageal cancer	25
Table 2-1. Demographics of Included Patients	37
Table 2-2. CRP Change Category (n=607)	38
Table 2-3. Pairwise comparisons of differences in survival between CRP change categories	40
Table 2-4. Relationship between patient characteristics, CRP change category and WBC, and overall survival (whole group n=607): Multivariable analysis.....	46
Table 2-5. Demographics of Included Patients by Diagnostic Subgroup.....	47
Table 2-6. CRP Change Category by Diagnostic Subgroup	48
Table 2-7. Pairwise comparisons of difference in survival between CRP change categories (n=532)	51
Table 2-8. Relationship between patient characteristics, CRP change category and WBC, and overall survival (n=532): Multivariable analysis.....	52
Table 2-9. Demographics of Included Patients: Single and Multiple Primary Cancer Sites	55
Table 2-10. CRP Change Category: Single and Multiple Primary Cancer Sites	56
Table 2-11. Pairwise comparisons of difference in survival between CRP change categories in people with a single primary cancer site, excluding non-melanoma skin cancer (n=435)	59
Table 2-12. Relationship between patient characteristics, CRP change category and WBC, and overall survival in people with a single primary cancer site, excluding non-melanoma skin cancer (n=435): Multivariable analysis	60
Table 2-13. Demographics of Included Patients (n=124)	61
Table 2-14. CRP Change Category and Five Year Overall Survival (n=124)	62
Table 3-1. Characteristics of Included Studies.....	87
Table 3-2. CRP and CRP-Based Scores	90
Table 3-3. HR and 95% Confidence Interval for Survival by CRP / CRP-Based Score in OAC for all included studies	91
Table 3-4. Components and Calculation of Scores Identified in the Review.....	94
Table 3-5. Risk of Bias for Included Studies (QUIPS tool, Hayden et al., 2013).....	95

Table 4-1. Chemotherapeutic agents and concentration used to treat OE33 cells.....	122
Table 4-2. Demographics of Included Patients (n=37).....	129
Table 4-3. Relationship between tumoural CRP and patient and disease characteristics..	133
Table 4-4. Demographics of Included Patients (n=107).....	138
Table 4-5. Number and Percentage of OAC Biopsies with High CRP on digitally-scored immunofluorescence	140
Table 4-6. Relationship between serum CRP and CRP in OAC tumoural biopsy by digitally-scored immunofluorescence.....	141
Table 4-7. Relationship between CRP in OAC tumoural biopsy, assessed by digitally-scored immunofluorescence, and survival	143
Table 5-1. Inclusion and Exclusion Criteria for Iteration 1	162
Table 5-2. Threshold values for CT analysis metrics	166
Table 5-3. Inclusion and Exclusion Criteria for Iteration 2	169
Table 5-4. Inclusion and Exclusion Criteria for Iteration 3	170
Table 5-5. Reason for exclusion for 12 recruited participants excluded from data analysis	172
Table 5-6. Demographic details for n=88 included patients.....	173
Table 5-7. Demographic details for n=88 included patients stratified by diagnostic group	174
Table 5-8. Study completion and reasons for study non-completion by diagnostic group	177
Table 5-9. Distribution of CRP, Albumin, GPS and CAR for n=88 included patients stratified by diagnostic group.....	178
Table 5-10. CT Analysis of L3 Skeletal Muscle: Results Stratified by Diagnostic Group	179
Table 5-11. CT-Measured Body Composition Parameters Categorised as Low or Normal based on published threshold values.....	180
Table 5-12. Comparison of prevalence of symptoms above the threshold for clinical importance at enrolment between diagnostic groups.....	182
Table 5-13. Comparison of prevalence of functional impairments above the threshold for clinical importance at enrolment between diagnostic groups	183
Table 5-14. Associations between CRP, Albumin and CRP-based scores and body composition metrics (operable and inoperable cancer only)	184

Table 5-15. Associations between CRP, Albumin, GPS, CAR and body composition metrics (operable and inoperable cancer only) and EORTC QLQ-C30 Summary Score at enrolment	185
Table 5-16. ECOG performance status at follow-up stratified by diagnostic group.....	187
Table 5-17. Comparison of prevalence of symptoms above the threshold for clinical importance between diagnostic groups at time of follow-up	189
Table 5-18. Comparison of prevalence of functional impairments above the threshold for clinical importance between diagnostic groups at time of follow-up.....	190
Table 5-19. Degree of Change in Summary Score	191
Table 5-20. Associations between CRP, Albumin, CRP-based scores and body composition metrics and Summary Score at follow-up	197
Table 5-21. Associations between CRP, Albumin, CRP-based scores and body composition metrics and post-operative length of stay and complications (operable cancer).....	198
Table 5-22. Associations between high and normal CRP and study completion.....	199
Table 5-23. Associations between CRP, Albumin, CRP-based scores and body composition metrics and study completion	201
Table 5-24. Associations between CRP, Albumin, CRP-based scores and potential to benefit from specialist palliative care	202

List of Abbreviations

ABC: Avidin-biotin complex

ACBS: Aarhus Composite Biomarker score

ACEi: angiotensin-converting enzyme inhibitors

ACS: acute coronary syndrome

AJCC/UICC: American Joint Committee on Cancer / Union for International Cancer Control

AKI: acute kidney injury

AKPS: Australia-modified Karnofsky Performance Scale

ARB: angiotensin receptor blockers

ASC: adipose stromal cells

AU: arbitrary units

BCA: bicinchoninic acid

BMI: Body mass index

BSA: bovine serum albumin

CA-125: cancer antigen 125

CC: Cleveland Clinic

CI: Confidence interval

CNG: Combination of NLR and GPS

CRP: Albumin ratio CAR

CRP: C-Reactive protein

CRT: chemoradiotherapy

CSS: cancer specific survival

CT: chemotherapy

CT: Computerised Tomography

CVD: cardiovascular disease

DAB: Diaminobenzidine peroxidase

DEXA: Dual Energy X-Ray Absorptiometry

ECOG: Eastern Cooperative Oncology Group Scale of Performance Status

EDTA: ethylene-diamine tetra acetic acid

ELISA: enzyme-linked immunosorbent assay

EMR: electronic medical record

EORTC QLQ-C30: The European Organisation for Research and Treatment of Cancer
Quality of Life Questionnaire – Core Questionnaire

ER: endoplasmic reticulum

FBS: foetal bovine serum

FC: score fibrinogen and CRP score

FFPE: formalin-fixed paraffin-embedded

GIST: Gastrointestinal stromal tumour

GPS: Glasgow Prognostic Score: GPS

GRADE: The Grading of Recommendations, Assessment, Development and Evaluation

HbA1c: glycosylated haemoglobin

HGC: Hamburg-Glasgow classification:

HR: Hazard ratio

HRP: horseradish peroxidase

HRT: hormone replacement therapy

hs-CRP: high-sensitivity C-Reactive protein

HU: Hounsfield Units

ICD-9-CM: International Classification of Disease Codes Version 9, Clinical
Modification

ICI: immune-checkpoint inhibitors

IF: immunofluorescence

IHC: immunohistochemistry

IL-1 β : Interleukin-1beta

IL-6: Interleukin-6

IPD: individual patient data

IQR: inter-quartile range

IRB: Institutional Review Board

IT: information technology

LPS: lipopolysaccharide

LoS: length of stay

LSMI: Lumbar Skeletal Muscle Index

mCRP: monomeric CRP

MDD: minimum detectable dose

mg/L: milligrams per litre

mGNRI: modified geriatric nutrition risk index

mGPS: modified GPS

MI: myocardial infarction

MM: multimodal

MMP: matrix metalloproteinase

mRNA: messenger ribonucleic acid

nCRP or pCRP: native pentameric CRP

NFDM: non-fat dry milk

NLR: Neutrophil Lymphocyte Ratio

NMSC: non-melanoma skin cancer

NR: not reported

NSAIDs: non-steroidal inflammatory drugs

NSCLC: non-small cell lung cancer

OAC: oesophageal adenocarcinoma

OC: oesophageal cancer

OCP: oral contraceptive pill

OGJ: oesophago-gastric junction

OPD: outpatient department

OS: overall survival

OSCC: oesophageal squamous cell carcinoma

P-POSSUM: Portsmouth-Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity

Pan-CK: pan-cytokeratin

PBS: phosphate-buffered saline

pCRP*: possible intermediate form of CRP

PET/CT: Positron emission tomography / computerised tomography

PIEC: Prognostic Index for Esophageal Cancer

PMI: psoas muscle index

PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses

PRO: patient-reported outcome

PROM: patient-reported outcome measure

PSA: prostate specific antigen

pt, pN, pM: pathologic T, N and M stage

PVDF: polyvinylidene fluoride

QoL: quality of life

QUIPS: Quality in Prognosis Studies

RCT: randomised controlled trial

REMARK REporting recommendations for tumour MARKer prognostic studies

ROB: risk of bias

ROS: reactive oxygen species

RPM: revolutions per minute

RPMI / cRPMI: complete Roswell Park Memorial Institute 1640 medium

RT: room temperature

SCLC: small cell lung cancer

SD: standard deviation

SE_{lnHR}: standard error of natural logarithm of the Hazard Ratio

SIR: Systemic Inflammatory Response

SJH: St James's Hospital

SJH/AMNCH REC: St James's Hospital and Adelaide and Meath incorporating National Children's Hospital Joint Research Ethics Committee

SLE: Systemic lupus erythematosus

SMD: skeletal muscle radiodensity

SMG: skeletal muscle gauge

SNPs: single-nucleotide polymorphisms

SPC: specialist palliative care

SPPB: short physical performance battery

STEMI ST-elevation MI

TBST: Tris-buffered saline

TIL: tumour-infiltrating lymphocytes
TKI: tyrosine kinase inhibitors
TMA: tissue microarray
TME: tumour microenvironment
TNF- α : Tumour necrosis factor-alpha
TNM: Tumor-Node-Metastasis
TRG: tumour regression grade
TSA: target signal amplification
TUG: timed up and go test
UD: undifferentiated
Upper GI: Upper gastrointestinal
VAT: visceral adipose tissue
VTE: venous thromboembolism
WBC: white blood cell count
ypT, ypN and ypM neoadjuvant pathologic T, N and M stage
lnHR: natural logarithm of the Hazard Ratio
6MWT: six-minute walk test

Abstract

Prognostication, the prediction of future outcomes, is a key aspect of cancer care but remains imprecise. An elevated serum C-reactive protein (CRP) is associated with high symptom burden, poor quality of life and shorter survival in cancer. CRP is inexpensive, widely available and straightforward to measure, making it an appealing potential prognostic marker. This thesis assessed promising, clinically relevant applications of CRP as a predictor of important patient outcomes in cancer, with a focus on oesophageal adenocarcinoma (OAC) and lung cancer.

As a non-specific inflammatory marker, transient CRP elevations could cause inaccurate prognostication. CRP change over time (CRP kinetics) was assessed in mixed cancer and OAC cohorts. It was no better than standard prognostic markers at predicting survival but could have a role in other cancer cohorts.

Numerous prognostic scores incorporate CRP, and a wide range of threshold (cutpoint) values for CRP have been reported in OAC. A systematic review and meta-analysis found inadequate evidence to conclude the best CRP cutpoint in OAC. Evidence was strongest for the Glasgow Prognostic Score (GPS) / modified GPS; future studies should focus on these scores.

Presence of CRP within tumour tissue has been associated with shorter survival in several cancer types. In this thesis, tumoural CRP was demonstrated in pre- and post-treatment OAC tissue, predominantly in the stroma. A possible association with reduced survival was identified. Tumoural CRP was not correlated with serum CRP and an OAC cell line did not secrete CRP. Taken together, the results suggest a stromal origin for tumoural CRP in OAC.

Elevated CRP and CT-assessed abnormalities of skeletal muscle are associated with, and may predict, poor quality of life (QoL) and high symptom burden in cancer. A feasibility study found several barriers to a definitive study of this relationship. Novel findings of associations between skeletal muscle measures (muscle density and psoas muscle index), CRP and patient outcomes (post-operative complications, symptoms and study non-completion) were identified.

This thesis confirmed that CRP can be applied in novel ways and used in combination with other factors, to predict important clinical outcomes in cancer. It also demonstrated the value of robust prognostic factor research and highlighted the areas which should be

examined in future work, to translate this promising prognostic factor into improved patient care.

Lay Abstract

People with cancer and their healthcare teams need accurate information about what is likely to happen with their treatment and with their illness so that they can make the right decisions together. The information we have now is OK but could be better.

A simple blood test, called C-reactive protein (CRP), may help. CRP is a measure of inflammation in the body. CRP levels are high in infection, in some inflammatory illnesses (like Rheumatoid Arthritis) and, sometimes, in cancer. This thesis used four studies to answer the question: can CRP be used to predict symptoms, quality of life and survival in cancer?

Repeat measurements of CRP might give a more accurate picture than single measurements. The first study in this thesis looked at whether repeat measurements of CRP could predict survival in two different groups of people with cancer: one group with a mix of different types of cancer and another group who all had a particular type of oesophageal cancer, called adenocarcinoma (also known as OAC). Repeat measurement of CRP did not provide any extra useful information about survival in either of these groups.

In previous studies, researchers have used lots of different cutpoints to call CRP high or not: some called any CRP above 2.6mg/L “high”, others called CRP “high” only if the level was above 10mg/L. Other researchers looked at CRP together with the results of other blood tests, to see if the combination (a prognostic score) can help predict survival in cancer. It’s not clear which cutpoint or score is best for OAC. In the second study in this thesis, we read all the published studies in this area and put all their results together. It still isn’t clear which CRP cutpoint is best, but the best score seems to be the Glasgow Prognostic Score (GPS) / modified GPS.

CRP is usually found in the blood but scientists have also found CRP inside the cancer itself. Some even found that people who didn’t have CRP inside their cancer lived longer than people who did. The third study in this thesis looked for CRP in samples from OAC cancers. CRP was in some of the cancers, before and after treatment, and may be linked with survival, though this isn’t certain yet. It appears that the CRP inside the cancer may in fact be made by the body’s own cells, rather than the cancer cells, but further studies will be needed to confirm this.

People with cancer often lose muscle – the amount of muscle and the quality of muscle are often less than in healthy people. This loss of muscle can be seen on a CT scan (“CAT scan”). The fourth study in this thesis looked at whether a high CRP, together with loss of muscle, could help predict which people with cancer are most at risk of having a lot of symptoms and a poor quality of life. This study was a feasibility study, meaning the main aim was to see if this type of study can work. This could lead on to bigger studies in the future. The study found a number of problems which would mean that this study would be unlikely to work on a larger scale. However, it did give some useful information. Other researchers can learn from what worked and didn’t work in this study, when they are planning their own study. Also, there were hints of some interesting links between CRP and muscle and how people got on with their treatment. Other studies can take these hints and plan studies to find out more.

Overall, the work in this thesis suggests that CRP could indeed help to predict symptoms, quality of life and survival in cancer. This thesis has also shown what studies should be done next, to work toward a time when CRP can be really used to improve patient care.

Overall Hypothesis

Novel applications of CRP can be used to predict important clinical outcomes, including quality of life, symptoms and survival, in cancer.

Overall Aim

To assess novel potential applications of CRP in prediction of outcomes in cancer, with particular reference to lung and oesophageal cancers.

Specific aims and hypotheses for each study are given in the individual chapter.

Output

Papers

C-Reactive Protein and C-Reactive Protein-Based Scores to Predict Survival in Esophageal and Junctional Adenocarcinoma: Systematic Review and Meta-Analysis.

Lorton CM, Higgins L, O'Donoghue N, Donohoe C, O'Connell J, Mockler D, Reynolds JV, Walsh D, Lysaght J. *Ann Surg Oncol*. 2022 Mar;29(3):1853-1865. doi: 10.1245/s10434-021-10988-x. Epub 2021 Nov 12. PMID: 34773194.

ASO Author Reflections: Can CRP and CRP-Based Scores Predict Survival in Operable Adenocarcinomas of the Esophagus and Esophago-Gastric Junction?

Lorton CM, Reynolds JV, Lysaght J. *Ann Surg Oncol*. 2021 Nov 16. doi: 10.1245/s10434-021-11069-9. Epub ahead of print. PMID: 34783945.

Abstracts

The Prognostic Role of C-Reactive Protein and Albumin in Advanced Cancer Palliative Care Inpatients: a Retrospective Study.

Wrafter S, Lorton CM, Brady B, Ui Dhuibhir P, Joyce D, O'Leary N, Walsh TD. *Palliat Med*. 2016 Jun; 30(6): NP98

CRP and CRP-based Scores in Upper GI Cancer: a Systematic Review

Lorton CM, Higgins L, Donohoe C, O'Connell J, Mockler D, Zgaga L, Walsh TD, Reynolds JV, Lysaght J. *Support Care Cancer*. 2021 Jun 15;1-261 OTHER-26. doi: 10.1007/s00520-021-06285-8. Online ahead of print

C-Reactive Protein, Quality of Life and Symptoms in Inoperable Lung Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD. *Support Care Cancer*. 2021 Jun 15;1-261 PALLIA-38. doi: 10.1007/s00520-021-06285-8. Online ahead of print

Oral Presentations

CRP, Skeletal Muscle Change, Symptoms and Quality of Life: a Feasibility Study

Lorton CM, Cuffe S, Reynolds JV, Lysaght J, Walsh TD

European Association for Palliative Care Research Network (EAPC-RN) / European Palliative Care Research Centre (PRC) pre-conference seminar, Dublin, June 2016

Moving Goalposts: Experience from a Feasibility Study in Advanced Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD

8th International Seminar of PRC and EAPC-RN PhD symposium, Edinburgh, December 2018

Moving Goalposts: Experience from a Feasibility Study in Advanced Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD

Moving Points in Palliative Care conference, Dublin, March 2019

C-Reactive Protein, Quality of Life and Symptoms in Inoperable Lung Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD

Irish Association for Palliative Care (IAPC) Education and Research Seminar, virtual conference, May 2021

Posters

The Prognostic Role of C-Reactive Protein and Albumin in Advanced Cancer Palliative Care Inpatients: a Retrospective Study

Wrafter S, Lorton CM, Joyce D, Ui Dhuibhir P, O'Leary N, Walsh TD

EAPC World Research Congress, Dublin, June 2016

CRP and Skeletal Muscle Change: Results from a Feasibility Study in Advanced Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD

IAPC Education and Research Seminar, Kilkenny, February 2019

Tumoural Expression of the Inflammatory Marker CRP in Oesophageal Adenocarcinoma

Lorton CM, Buckley AM, Davern MB, Conroy MJ, Useckaite Z, Walsh TD, Reynolds JV, Lysaght J

Irish Association for Cancer Research (IACR) 55th Annual Conference, Belfast, February 2019

CRP Kinetics as a Predictor of Survival in Cancer

Lorton CM, Shrotriya S, Lysaght J, Walsh TD

Moving Points in Palliative Care conference, Dublin, March 2019

CRP and CRP-based Scores in Upper GI Cancer: a Systematic Review

Lorton CM, Higgins L, Donohoe C, O'Connell J, Mockler D, Zgaga L, Walsh TD, Reynolds JV, Lysaght J

11th Cancer Conference, Trinity College Dublin, September 2019

CRP and CRP-based Scores in Upper GI Cancer: a Systematic Review

Lorton CM, Higgins L, Donohoe C, O'Connell J, Mockler D, Zgaga L, Walsh TD, Reynolds JV, Lysaght J

Multinational Association of Supportive Care in Cancer (MASCC) / International Society of Oral Oncology (ISOO) Annual Conference, virtual conference, June 2021. *Support Care Cancer* 2021 Jun 15;1-261 OTHER-26. doi: 10.1007/s00520-021-06285-8. Online ahead of print

C-Reactive Protein, Quality of Life and Symptoms in Inoperable Lung Cancer

Lorton CM, O'Connell F, Nadarajan P, Reynolds JV, Lysaght J, Walsh TD

MASCC/ISOO Annual Conference, virtual conference, June 2021. *Support Care Cancer* 2021 Jun 15;1-261 PALLIA-38. doi: 10.1007/s00520-021-06285-8. Online ahead of print

Awards and Prizes

Award: Clinical Research Fellowship (value €6000)

Purpose: To support conduct of the study: the use of CRP and skeletal muscle measures to predict symptoms and quality of life in cancer

Awarding Body: All Ireland Institute of Hospice and Palliative Care (AIIHPC), September 2015

Award: Early Career Researcher Bursary (value €685)

Purpose: To attend European Association for Palliative Care Research Network (EAPC-RN) / European Palliative Care Research Centre (PRC) pre-conference seminar, Dublin, June 2016

Awarding Body: AIIHPC

Prize: Best poster for CRP Kinetics as a Predictor of Survival in Cancer

Moving Points in Palliative Care conference, Dublin, March 2019

Thesis structure

The introductory chapter provides an overview of the three core elements addressed in this thesis: CRP, cancer and prognostication. The gaps in the literature are noted and the potential for further use of CRP highlighted. The introduction also outlines how these will be addressed by the four studies in this thesis. A detailed introduction to each study, including its specific literature and methodology is discussed in the individual chapter, as are the results, discussion and conclusions for each. The final chapter of the thesis is an overall conclusion, including recommendations for future research directions.

1 Introduction

1.1 C-reactive protein (CRP)

CRP is a member of the pentraxin protein superfamily (1). The pentraxins are a superfamily of soluble pattern recognition receptors and are involved in humoral immunity (2). CRP is a short pentraxin and was the first of the pentraxins to be identified (2). CRP has been described as “evolutionarily conserved”, as it has been identified in organisms as diverse as the American horseshoe crab, chickens, dogs and humans (3). However, the structure and function of CRP differ significantly between species (3).

CRP in humans was first described by Tillett and Francis in 1930, when they described “Fraction C”, “a non-protein fraction of somatic origin” which was precipitated by serum from patients with pneumococcal pneumonia (4). In the 1940s, a series of papers by Abernethy, Avery, MacLeod and McCarty confirmed that CRP was present in infections other than pneumococcal disease (5-7). They also corroborated Tillett and Francis’ finding that CRP was an acute phase protein, reporting that CRP was present during acute infection and became undetectable following recovery (4). Improved detection, including the advent of high-sensitivity assays (hs-CRP) in the 1960s and 1970s, has since demonstrated that CRP is, in fact, present in healthy individuals but at very low levels (8, 9). The hs-CRP assays have a much lower limit of detection but measure the same analyte (10), thus the term CRP will be used throughout this thesis.

1.1.1 CRP Function

CRP has been described as a “classical acute phase protein” with serum levels rising dramatically in the presence of inflammation(11). The acute phase response is a key part of immune defence and refers to the complex series of changes which occur in the body in response to inflammation (12, 13). These include changes in circulating plasma protein levels (some increased – positive acute phase reactants and some decreased – negative acute-phase proteins), activation of a cytokine cascade and neuroendocrine changes, which result in fever and behavioural changes such as lethargy (12).

CRP plays an important role in host immune defence but its biological role is still incompletely understood (14). The study of CRP’s function is made more difficult by two major limitations: Firstly, there is no known deficiency state of human CRP which can be used to study its normal function (10). Secondly, as noted above, CRP differs significantly between species, hindering comparisons with animal models (10). CRP has been shown to

be involved in pathogen recognition, activation of the classical complement pathway (by binding C1q) and stimulation of phagocytic cells (via binding of Fc γ receptors)(15)(Fig. 1.2). Pathak and Agarwal speculate that CRP has evolved so that its ligand-binding site is only exposed when it is needed i.e. when inflammation has begun (3).

1.1.2 Clinical Use of CRP

Circulating (serum) CRP is the most widely-used biochemical marker of inflammation in clinical use (16). It is used for three main clinical purposes – to screen for an active infection or inflammation, to monitor the course (including response to treatment of infection or inflammation and to identify intercurrent bacterial infection in certain diseases, most usefully those which don't themselves cause significant elevations of CRP (for example systemic lupus erythematosus (SLE) (17, 18). Persistently elevated levels are seen in chronic inflammatory disease and in cancer (16), which is the focus of this thesis. Individual characteristics, clinical conditions and medications affecting CRP levels are discussed further below.

1.1.3 CRP Synthesis and Structure

Hepatocytes are the main source of CRP (15). CRP is initially synthesised as a monomer, which is then assembled into a pentamer within the endoplasmic reticulum (ER) of the hepatocyte. The pentamer is stored within the ER, bound to two carboxylesterases (19). CRP can increase several hundredfold or even 1000-fold above baseline circulating concentration in the setting of infection or injury (20). In the acute phase response, binding affinity of these proteins drops significantly, allowing secretion of CRP (19). The principal control of CRP levels, however, is at the level of transcription (21). The CRP gene is located on the short arm of chromosome 1(14). As part of the acute phase response, its transcription is rapidly upregulated (14). Interleukin-6 (IL-6) is the main regulator of CRP production but interleukin-1beta (IL-1 β) and tumour necrosis factor-alpha (TNF- α) also increase its transcription (15, 22).

CRP has traditionally been recognised as a pentamer, consisting of five identical subunits, each 206 amino acids long, which are noncovalently associated and arranged symmetrically around a central pore (14), as shown in Figure 1.1. It is this pentameric structure that lends its name to the whole pentraxin family (23). X-ray studies in the 1990s allowed detailed description of the pentameric structure (24, 25). Each protomer is folded into two antiparallel

β sheets and has two surfaces or faces – a ligand-binding or recognition face and an effector-molecule binding, as seen in Figure 1.2. The recognition face binds phosphocholine, in the presence of two calcium ions (14). The effector face is thought to be where complement (C1q) and antibody receptors (Fc γ receptors) bind (14)(Fig. 1.2 and 1.3).

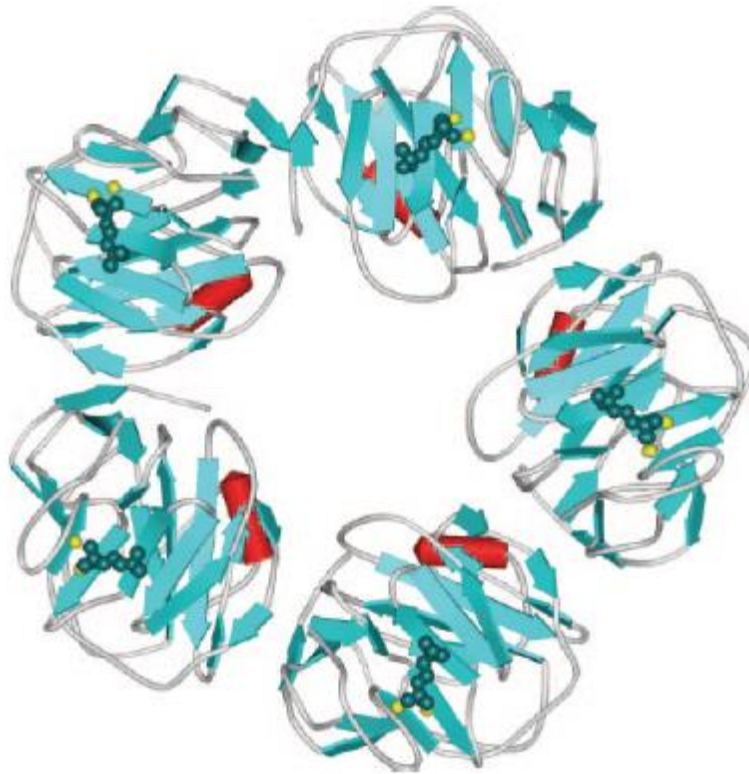


Figure 1-1. Ribbon diagram demonstrating the pentameric structure of human C-reactive protein complexed with phosphocholine (from Black et al., 2004)

Yellow = Calcium ions, green = Phosphocholine, 2 x beta-pleated sheets in each protomer shown as flat blue arrows ending in red

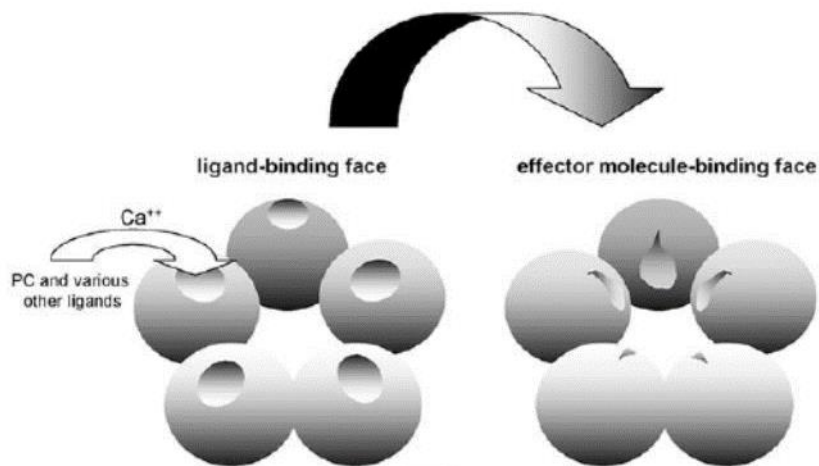


Figure 1-2. The ligand-binding and effector molecule-binding faces of the 5 monomers within the CRP pentamer (from Szalai & McCrory 2002)

Left of diagram: Ligand-binding (recognition) faces which binds phosphocholine and other molecules, right of diagram: effector-molecule binding face which can bind C1q and/or Fcγ receptors.

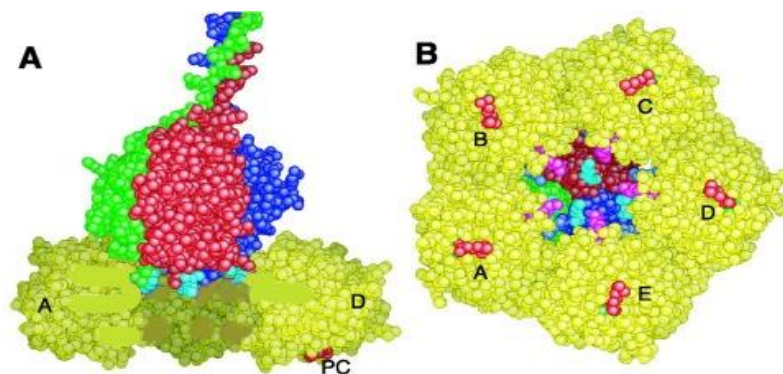


Figure 1-3. Model of the complex between CRP and the C1q globular domain (from Gaboriaud et al., 2003)

A: lateral view, with 3 of the CRP subunits (protomers, labelled A to E) omitted for clarity. C1q is shown at the top of the diagram, with its 3 modules shown in green, red and blue. The lysines at the top of the C1q head are shown in light blue. Phosphocholine (PC) is shown at the bottom right of the image in red. Image B: perpendicular bottom view with all 5 CRP protomers shown and the C1q head in the central pore of the pentamer. PC is shown bound to each protomer with the nearby calcium ion just visible and shown in green.

1.1.4 Non-pentameric CRP: structure and function

The first reports of alternative forms of CRP were from Potempa *et al.*, in the 1980s (26). In 2004, Black *et al.*, noted the possible existence of “modified CRP”, proposing that this may be generated at sites of deposition of CRP (14). It has since become clear that there are two major conformational forms of CRP – native pentameric CRP (pCRP or nCRP) and a monomeric form (mCRP) (15) – with a possible intermediate form, pCRP* (27). The predominant form found in serum is pCRP (15). Although still poorly understood, it is believed that pCRP binds to the cell membrane of activated endothelial cells at sites of inflammation and dissociates into mCRP (28), via modification of the disulfide bond within pCRP(29). Until recently, it was believed that pCRP was the only form found in the blood but more recently it has been shown that mCRP can circulate in the blood, attached to a microparticle(10, 30). Initial reports proposed that mCRP was far more potent in terms of inducing further inflammation than “native CRP” (14). While this idea was initially dismissed by some(31), more recent reports have confirmed that pCRP and mCRP do indeed have very different functions within the inflammatory process, with pCRP having a more anti-inflammatory role than mCRP(22). As discussed above, pCRP activates complement and induces phagocytosis, while mCRP recruits circulating leukocytes to areas of inflammation and may delay apoptosis (22). pCRP inhibits nitric oxide production and mCRP induces it (22). mCRP has been implicated in the pathophysiology of post-stroke dementia (32, 33). The existence of these different forms of CRP may explain the diverging reports in the literature on CRP’s pro-inflammatory versus anti-inflammatory actions (22, 34). Crawford *et al.*, showed that even the most sensitive of assays for serum CRP fail to detect both mCRP and pCRP, if it is attached to a microparticle, rendering some circulating CRP “clinically invisible” (34). Assays to measure mCRP have only been described in very recent years (35-37) and are not yet commercially available(22). Ongoing development of these assays will be essential for understanding the true role of CRP (28).

1.1.5 Baseline CRP levels

The largest large population studies have reported a median CRP of 1.31-1.89mg/L in one study of 22,000 US adults and 0.6 – 1.7mg/L in another of 13,500 European adults (38, 39). Baseline CRP may be affected by a number of factors, including ethnicity, age, sex, hormonal treatment and pregnancy, body mass index (BMI) / body composition, smoking

and socio-economic status. Medications and disease states also affect CRP and are discussed further below.

While the majority of studies of baseline CRP have been conducted in white people, differences in CRP levels between ethnic groups have been identified (40, 41). In a systematic review, highest mean CRP levels were in Black people living in the United States (2.99 mg/L) with lower levels, in descending order, in Hispanic, South Asian, white and East Asian people (mean 1.01mg/L) (40). Much of the ethnic difference appears to be explained by differences in anthropometric and metabolic factors, such as waist circumference, BMI, triglycerides, systolic blood pressure, and glycosylated haemoglobin (HbA1c) (41, 42). Genetic variation is thought to explain the remaining difference, specifically single-nucleotide polymorphisms (SNPs) within the CRP promoter region (43).

Baseline CRP increases with increasing age (38, 39, 44). Although some studies report higher levels in women (45, 46) or men (44), several much larger studies confirm a similar distribution between men and women (38, 39, 47). Hormonal treatment has an impact on baseline CRP levels; oral hormone replacement therapy (HRT), the oral contraceptive pill (OCP) and pregnancy are all associated with higher levels (45). Route of administration of HRT may influence its impact on CRP levels, with transdermal and percutaneous oestrogen reported to have no impact or even reduce CRP (48, 49), for reasons which remain unclear. Increasing BMI is associated with a higher baseline CRP (41, 42, 45, 47), as is smoking (47, 50). Visceral adipose tissue (VAT) in particular is associated with a raised CRP (51). VAT is known to secrete cytokines and adipokines and is associated with the pro-inflammatory condition of metabolic syndrome (52).

Socio-economic and environmental factors also influence baseline CRP. In a study of 34,000 adults across Europe, Layte *et al.*, showed a consistent link between elevated CRP and lower socio-economic position (47). The basis of the relationship between socio-economic factors and inflammation is still being studied. One meta-analysis suggested that BMI was an important mediator in this relationship (53), though Layte *et al.*, found that lifestyle and comorbid disease factors (smoking, BMI, hypertension and diabetes) only partly explained differences in CRP in their cohort (47). Another review suggests that the timing of adverse socio-economic circumstances (childhood versus adulthood) may matter (54). Air pollution has also been associated with elevations in CRP (55).

1.1.6 Medications affecting CRP

The impact of a wide variety of medications on CRP has been studied (56). The most studied are non-steroidal inflammatory drugs (NSAIDs), corticosteroids and statins.

Overall, most NSAIDs do not appear to influence CRP levels (57). However, meta-analysis found some evidence that the selective COX-2 inhibitor, lumiracoxib, may elevate CRP and naproxen, a non-selective NSAID, may lower CRP (57). Aspirin appears to have no significant effect on CRP. Although Ikonomidis *et al.*, reported that aspirin reduced CRP by a median of 0.4mg/L (58), several more recent studies found that it has no impact on circulating CRP (59-61).

Exogenous corticosteroids, specifically glucocorticoids, are widely used to treat inflammatory and autoimmune disease (62). Figure 1.4 illustrates the diverse effects of glucocorticoids on immune cells. Critically for CRP, glucocorticoids block expression and activity of the majority of inflammatory cytokines, including IL-1 β , TNF- α and IL-6, which are key in the upregulation of CRP (15, 63). Glucocorticoids also inhibit IL-1 α secretion by monocytes in vitro(64). Despite being identified as a key regulator of inflammation and implicated in the pathogenesis of cancer, IL-1 α is less studied than IL-1 β (65) and its relationship to CRP remains to be understood fully.

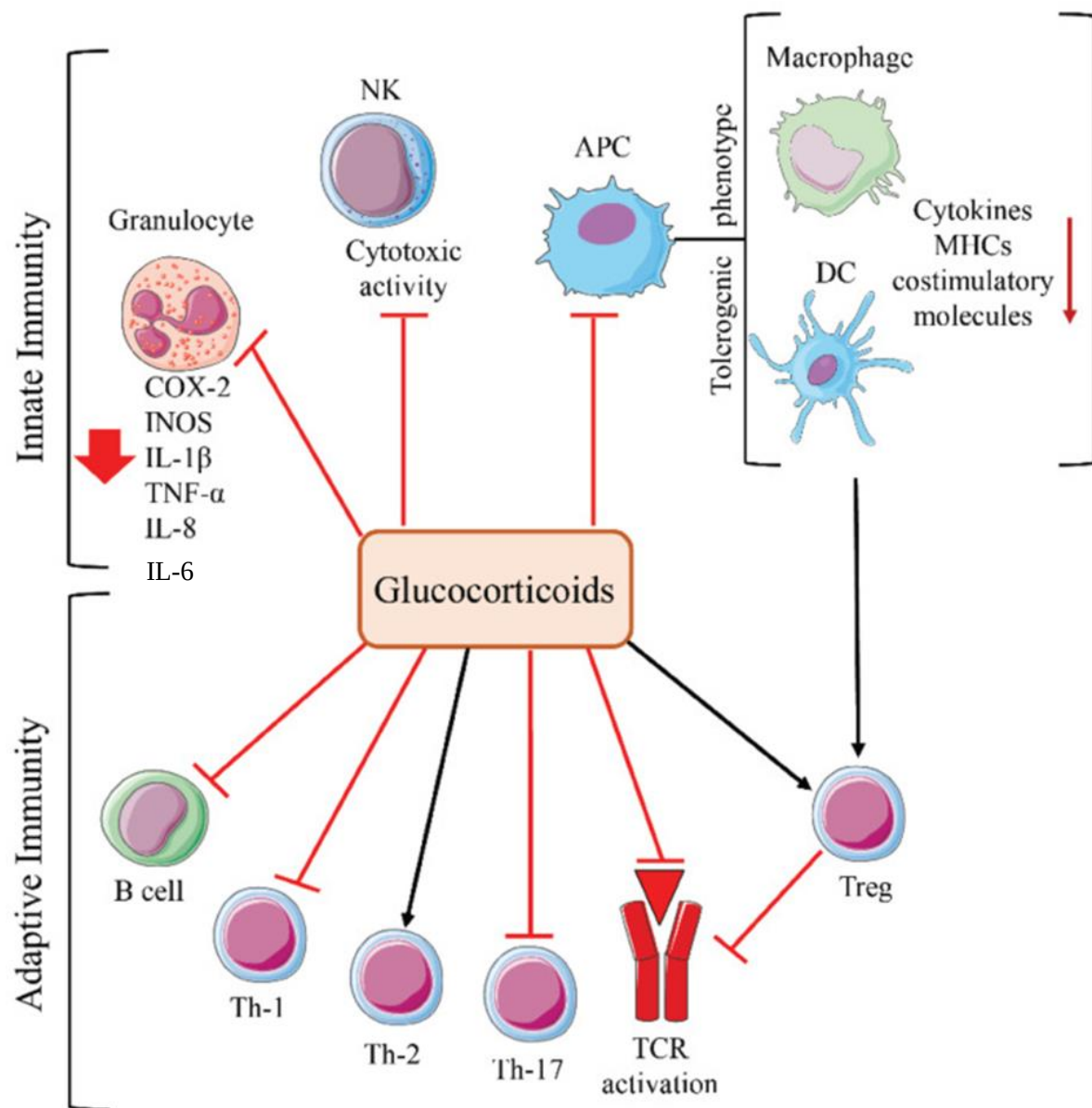


Figure 1-4. Effects of glucocorticoids on innate and adaptive immunity (modified from Adorisio et al., 2021)

Red = inhibitory signalling; black = activating signalling.

Many of the studies which examine CRP levels in patients treated with glucocorticoids are in acute respiratory infection, or ongoing inflammatory disease, such as chronic obstructive pulmonary disease (COPD) or rheumatoid arthritis, and steroids were used in the context of

treating the illness (66-68). As a consequence, reductions in CRP due to steroids may be confounded by the effect of reduced inflammation due to improving illness, and vice-versa (66). However, a study by Bartko *et al.*, showed that dexamethasone reduced systemic production of IL-6 after infusion of endotoxin in healthy volunteers by 90% compared with placebo with a corresponding reduction in CRP (69).

1.1.6.1 Other anti-inflammatory agents

Methotrexate, a folate antagonist drug which is commonly used in inflammatory arthritides and also in cancer, does not reduce CRP (70).

Colchicine has multiple mechanisms of anti-inflammatory action (71). It reduced IL-6 and IL-1 β in the coronary circulation in patients with acute coronary syndrome (ACS) (72) but a recent meta-analysis found that it does not reduce circulating CRP (73). It is noteworthy that the reduced IL-6 in the coronary circulation was not associated with a corresponding reduction in venous IL-6 (72). Local increases in IL-6 may not be sufficient to stimulate systemic production of CRP, which would explain why circulating CRP was not affected.

There has been growing interest in the blockade of various inflammatory cytokines and reduction of CRP in the management of acute cardio- and cerebrovascular events. Antagonism of IL-6 reduces CRP(74, 75). Anakinra, an interleukin-1 (IL-1) antagonist, also lowers CRP(76). Anakinra antagonises both IL-1 α and IL-1 β (65). Canakinumab, which antagonises IL-1 β alone, reduces CRP; it is unclear whether antagonism of IL-1 α alone would have the same effect(77).

1.1.6.2 Statins

The PRINCE (pravastatin inflammation / CRP evaluation) randomised controlled trial showed that pravastatin reduced CRP levels in adults receiving a statin for prevention of cardiovascular disease (78). Subsequent studies confirmed that other statins (atorvastatin, rosuvastatin) also lower CRP, suggesting this is a class effect (79, 80). Other lipid-lowering drug, including ezetimibe and fibrates also reduce CRP (81, 82). It is important to note that, although the percentage reductions are large (up to 60% with ezetimibe and a statin, the absolute values remain small, since trial participants had a mean initial CRP of only

2mg/L(81). The impact of such small absolute reductions in the context of the higher CRP values seen in cancer is unknown.

1.1.6.3 Other drugs

Clopidogrel, an inhibitor of platelet activation and aggregation, may cause an initial reduction in CRP post myocardial infarction but this reduction is not sustained (83). Carvedilol, a beta-adrenoceptor antagonist, reduced CRP by 0.7mg/L in one study but another drug of the same class, propranolol, did not significantly reduce CRP, thus this may not be a class effect (84). Similarly, among drugs which block the renin-angiotension-aldosterone system (angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB), both used to treat hypertension), only some reduce CRP, with others having no impact on CRP levels (56). Calcium-channel blockers, another group of drugs used as anti-hypertensives, make no significant difference to CRP levels (56). It is unclear whether hydrochlorthiazide, a diuretic used as an anti-hypertensive, has any significant effect of CRP (56). A thiazolidinedione anti-diabetic agent, rosiglitazone, reduced CRP in adults with Type 2 Diabetes Mellitus (85, 86), as did another drug of that class, troglitazone (87). In a longer-term study, however, the treatment was associated with weight gain (86), with unknown net impact on risk of cardiovascular disease (CVD). Other anti-diabetic drugs, glyburide and metformin, were associated with lesser reductions in CRP in the same study (86).

1.1.7 Diet and Supplementation with Vitamins and Minerals

Meta-analyses of supplementation with Vitamins C, D and E have found small reductions in CRP (averaging 0.43mg/L, 0.1mg/L and 0.62mg/L, respectively)(88-90). Niacin reduced CRP in adults with ACS by 0.83mg/L (91).

Folic acid supplementation reduced CRP, particularly in women with Type 2 diabetes mellitus (92). Zinc supplementation was also recently shown to reduce CRP in healthy adults (mean reduction of 0.75mg/L) and in adults with chronic inflammatory disease (93).

Individual nutrients such as ginseng, nigella seeds and garlic have all been associated with reductions in CRP (94-96). Prebiotics are associated with reductions in CRP in overweight and obese adults (97).

In systematic review and meta-analysis, Pickworth *et al.*, reviewed the impact of dietary patterns on CRP and found that 23 different diets were associated with reductions in CRP, including highly restrictive diets such as the “lemon detox” diet, which allows only organic syrup and lemon juice, and more mainstream whole-grain based diets (98). Overall, the evidence was mixed, of variable quality and the authors note that further interventional studies are required before any clinical implementation would be possible (98).

1.1.8 Other interventions

Smoking cessation is associated with reduction in CRP; this reduction occurs over a period of years rather than acutely (99).

In their review, Black *et al.*, found weak evidence that CRP may be reduced by mindfulness-based stress reduction (100). None of the included studies reported CRP in people with active cancer (100).

Although Djalilova *et al.*, showed in their systematic review that yoga may reduce biomarkers of inflammation, including CRP (101). No significant reductions in CRP were found in the included studies in cancer (101).

Sauna bathing was not found to influence CRP levels in one Finnish study(102). Resistance training may reduce CRP in older adults, possibly through the mediator of muscle mass (103). The relationship between systemic inflammation and skeletal muscle will be explored in Chapter 5 of this thesis.

1.1.9 Diseases associated with higher CRP

Higher baseline CRP levels are found in chronic inflammatory conditions, such as rheumatoid arthritis, chronic infection (such as gingivitis), diabetes mellitus and in cancer (104), compared to healthy adults (82, 105, 106). Hypertension, metabolic syndrome and dyslipidaemia (specifically low high-density lipoprotein cholesterol and high triglycerides) are also associated with a higher CRP(82).

Data from the Copenhagen General Population Study showed the distribution of CRP across that population of some 63,000 adults and the levels typically seen in various diseases (Fig. 1.5).

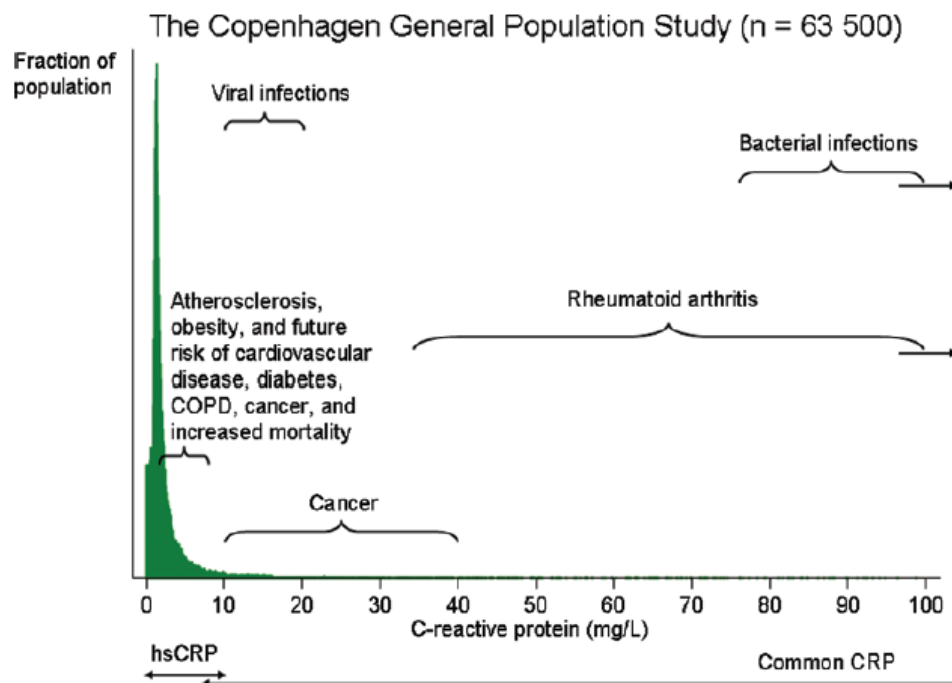


Figure 1-5. Plasma CRP in the Copenhagen General Population Study, illustrating typical CRP levels recorded in adults with various diseases (from Allin & Nordestgaard 2011)

CRP was measured in 63,500 adults using high-sensitivity or standard CRP assays and compared across clinical conditions, including infection, rheumatoid arthritis and cancer and conditions associated with increased cardiovascular risk (10).

It is important to note that, despite the minor variations in baseline CRP seen at a population level with age, sex, ethnicity and the minor elevations of CRP seen in atherosclerosis, diabetes and chronic inflammatory conditions such as obesity, these values still fall well within the normal range, as illustrated in Figure 1.5. A level of up to 10mg/L has traditionally been taken as the upper limit of normal (107). A CRP approaching 10mg/L cannot truly be deemed normal, when this value is estimated to be somewhere between the 97th and 99th percentile in healthy adults (10, 108) but this value remains useful for identifying acute bacterial infection (107). Vanderscheuren *et al.*, and Landry *et al.*, assessed the causes and clinical outcomes of high CRP levels measured at a hospital laboratory, covering inpatients, outpatients and the emergency department (109, 110). Very high (> 100mg/L (110)) and extreme elevations (over 500mg/L(109)) were uncommon and were reported as 3% and less

than 0.1% of all CRP results, respectively (109, 110). Close to 90% of the highest values (>350mg/L in Landry *et al.*, and > 500mg/L in Vanderscheuren *et al.*,) were caused by infection (109, 110). Such extreme elevations are associated with high mortality, particularly in patients with underlying cancer as well as infection (109). In the Landry *et al.*, study, 11% of all CRP measurements above 100mg/L were attributed to malignancy, without evidence of infection (110).

As shown in Figure 1.6, CRP levels in cancer are variable. While it is agreed that CRP tends to be higher in people with cancer (10, 28, 104), reported levels vary substantially from close to normal levels up to extremely elevated (28). Many studies of CRP in cancer omit to stratify CRP levels by stage (28). Overall, higher stage / more advanced cancers are associated with higher CRP levels (28). Figure 1.6 illustrates CRP levels reported in various cancer sites, as collated by Hart *et al.*, in their review paper (28). Highest levels were reported in gastric, pancreatic and lung cancers but with considerable variability between studies (28).

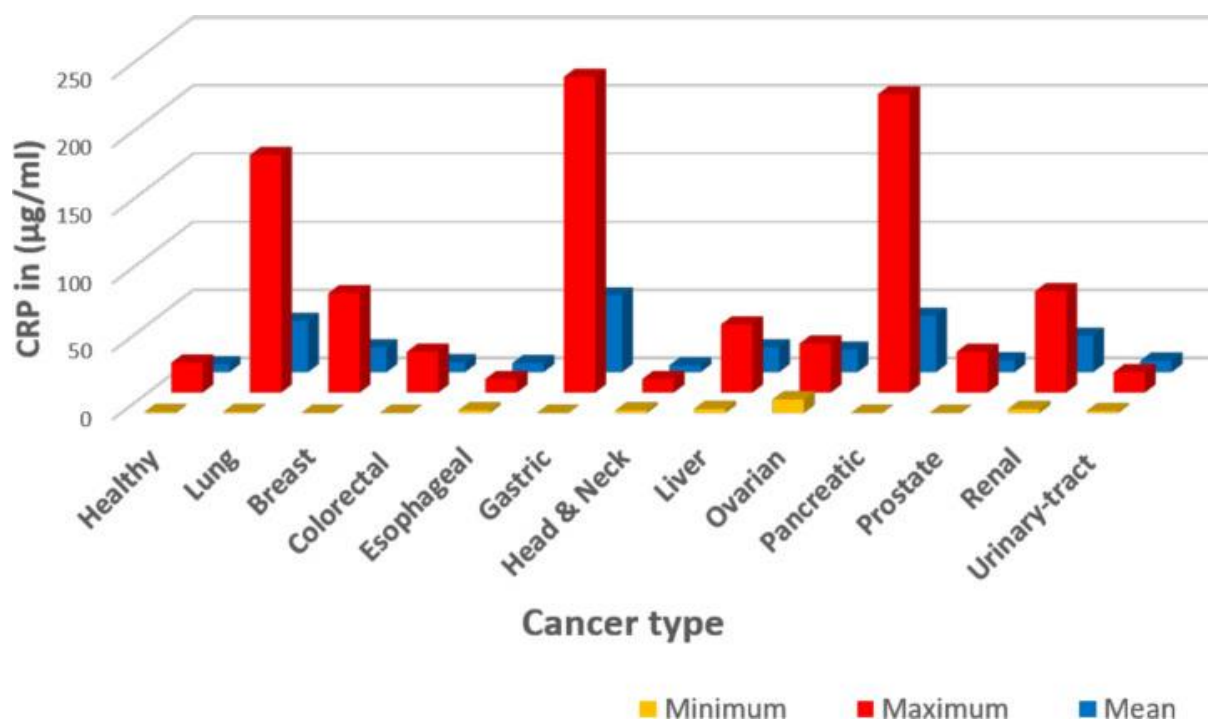


Figure 1-6. CRP values reported in various cancer sites and in healthy adults (from Hart *et al.*, 2020)

Yellow indicates the minimum value reported, red the maximum and blue the mean. All values are in µg/ml, which is equivalent to mg/L.

How relevant factors influencing baseline CRP are to the higher levels seen in cancer remains uncertain. Most studies which demonstrate differences in CRP between males and females, between BMI categories or with medication were in healthy populations or in groups with modest elevations in CRP, such as in CVD prevention studies. Furthermore, the differences between groups were small compared to the overall higher levels reported in cancer. However, it does appear that even potent anti-inflammatory medications, such as steroids, cannot prevent a rising CRP in the context of the acute inflammatory response, and the same may be true of persistent cancer-related inflammation. Patients with rheumatoid arthritis who were taking prednisolone had a similar acute post-operative rise in CRP following joint replacement as those patients who were not taking a corticosteroid (111). Patients with SLE, who typically do not have a high CRP despite their inflammatory condition, still show a rise in CRP in the setting of infection (17). In contrast, Mc Sorley *et al.*, showed that people undergoing resection of colorectal cancer who received dexamethasone at induction of anaesthesia had a significantly lower post-operative CRP than those who did not (112). Interestingly, patients with post-operative complications still developed significantly higher post-operative CRPs, even if they had received dexamethasone (112), again supporting the idea that the massive and rapid increase in CRP in the acute phase response can overcome almost any brake, given sufficient inflammatory stimulus.

1.1.10 CRP and disease outcomes

There is an abundance of literature describing the relationship between CRP and clinical conditions, from Alzheimer's disease(113) to Herpes Zoster infection (113, 114). In the vast majority, an elevated CRP has been associated with adverse outcomes, although there was considerable heterogeneity and evidence of small-study effects in many of the meta-analyses, increasing the risk of false positive results (115). The strongest evidence was for associations between high CRP and cardiovascular deaths and between CRP and venous thromboembolism (VTE) (115). Increasing CRP is independently associated with CVD, even after adjustment for the traditional risk factors of age, sex, diabetes, smoking, weight circumference, BMI, systolic blood pressure and cholesterol (low density and high density) triglycerides, atherosclerosis and ethnicity (42). The American College of Cardiology/American Heart Association Task Force considers CRP $\geq 2\text{mg/L}$ a "risk-enhancer", which would tip the balance toward prescription of a statin for patients with borderline or

intermediate CVD risk (116). In VTE, meta-analysis of population studies found a dose-response relationship has been shown between increasing CRP and risk of VTE (117). Most recently, an elevated CRP has been found to be associated with adverse clinical outcomes in COVID-19, including VTE, acute kidney injury (AKI) and death (118).

1.1.11 CRP in pathogenesis

In 1982, Kushner noted that “much still remains to be learned” about CRP and questioned whether CRP could have a role in disease pathogenesis, as well as its role in acute inflammation (20). A review of Mendelian randomisation studies which assessed CRP levels and health outcomes found little evidence to support causality, that is that an elevated CRP causes adverse outcomes (115). Consistent with this, administration of human CRP to healthy adults failed to elicit any inflammatory response (119). A prior study had shown CRP administration to healthy adults did activate both inflammation and coagulation (120) but these findings may have been due to contamination or bacterial origin of the CRP preparation used (119).

However, there is accumulating evidence, particularly in cardiovascular disease, that does lend some weight to a pathogenic role for CRP (1, 22). Data from the PROVE IT-TIMI 22 study found that patients whose CRP was low after statin therapy had fewer cardiovascular events than those with higher CRP; this was the case regardless of their low-density lipoprotein (LDL) cholesterol (79). The authors speculated that reducing CRP may thus have a role in modifying the process of atherothrombosis (79). In the subsequent JUPITER study, Ridker *et al.*, demonstrated that adults with a high baseline hs-CRP treated with rosuvastatin had a reduced rate of cardiovascular events and death compared to placebo, even where their cholesterol was not high enough to warrant a statin, again suggesting that reduction of CRP may explain the improved survival (80).

The Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) found that canakinumab, a monoclonal antibody to IL-1 β , reduced vascular events and mortality post-myocardial infarction (MI), with the greatest reductions in vascular events seen in those who achieved the lowest tertile of CRP (121). Intriguingly, a reduced incidence of lung cancer was noted in those who received canakinumab, compared with placebo (122). There was a higher rate of fatal infection than with placebo (122) but the concept that reduction of

inflammation could reduce the incidence of lung cancer remained appealing and prompted further studies of canakinumab at different stages in lung cancer treatment (CANOPY-1, CANOPY-2, CANOPY-A and CANOPY-N) (122). At the time of writing, the CANOPY-1 (canakinumab with pembrolizumab and platinum-based doublet chemotherapy) and CANOPY-2 (canakinumab-docetaxel) in locally advanced or metastatic non-small cell lung cancers (NSCLC) did not show significant effects on survival, though Novartis note that further analysis is ongoing in subgroups based on inflammatory biomarkers while CANOPY-A (adjuvant) and CANOPY-N (neoadjuvant) continue to recruit patients (123). Importantly, even if CRP does not play a role in pathogenesis or progression of cancer, it may still have a useful role in prognostication and monitoring treatment in clinical practice and for stratification in clinical trials (10).

1.2 Cancer

Globally, there were an estimated 19.3 million new cases of cancer and 10 million cancer deaths in 2020 (124). In Ireland, there are approximately 24,000 new invasive cancers (excluding non-melanoma skin cancer) diagnosed annually (125). Despite improvements in early diagnosis and advances in treatment, cancer is the cause of almost one in three deaths in Ireland, overtaking circulatory disease (126). Cancer incidence is rising rapidly globally and even conservative estimates predict a rise of almost 50% by 2040, with an accompanying increase in cancer deaths (124). Cancer may become the most common cause of death in many countries by the end of this century (127).

As well as death, cancer is associated with significant morbidity and symptom burden (124). Patients with earlier stage disease have an average of 9 symptoms, while studies which included more patients with metastatic disease found the average number of symptoms to be 11 (128, 129). Cancer survivors can also have a sizeable symptom burden (128). Number of symptoms is strongly associated with quality of life (QoL) (129).

This thesis will focus particularly on the role of CRP in oesophageal adenocarcinoma (OAC) and lung cancer. Both are prevalent, and associated with poor survival, high symptom burden and inflammation.

1.2.1 Oesophageal cancer

Oesophageal cancer is the sixth highest cause of cancer mortality globally; causing over 500,000 deaths in 2020 (124). In Ireland, oesophageal cancer is the fourth highest cause of cancer deaths and seventh highest in females, with over 400 deaths due to the disease each year (125). Oesophageal cancer is associated with significant symptom burden, even in the setting of curative treatment (130). The two main subtypes of oesophageal cancer are oesophageal squamous cell carcinoma (OSCC) and OAC. There is an increasing incidence of adenocarcinoma at the oesophago-gastric junction (OGJ). Emerging research clearly identifies OAC/OGJ as biologically, genomically and clinically distinct from OSCC (131, 132). Globally, the majority of oesophageal cancers are OSCC (133). However, OAC/OGJ incidence is increasing and has surpassed that of OSCC in many developed countries, while the rate of OSCC is predicted to decline (133). This change has major implications for clinical practice and research (133). The 8th AJCC/UICC staging classification acknowledges the survival difference between the two histopathologic cell types (134). Although survival in OAC has improved in the last thirty years, overall the prognosis remains poor with significant disparities between regions (132, 135, 136). Many of the factors which predict prognosis in OAC (pathologic Tumor-Node-Metastasis (TNM) stage, lymph node involvement, resection margins) are only known post-operatively. Accordingly, it would be very useful to have prognostic markers available pre-operatively to predict those most likely to benefit from curative intent therapy such as surgery or multimodal therapy or perioperative chemotherapy (137). Obesity, particularly central obesity, and metabolic syndrome, are associated both with systemic inflammation and with development of OAC (138). CRP above the median (1.9 mg/L) was associated with an 80% increased risk of developing OAC in people with Barrett's oesophagus (139), a strikingly high risk increase for relatively low levels of CRP.

1.2.2 Lung cancer

Lung cancer is the leading cause of cancer deaths in Ireland(125) and worldwide, with dismal 5-year survival of 10-20%(124). There are 1,880 deaths a year from lung cancer in Ireland alone(125). Lung cancer is associated with a high symptom burden, among the highest of all cancers(140). Inflammation has been implicated in the development of the most common group of lung cancers, non-small cell lung cancer (NSCLC)(141). In one large epidemiological study, CRP level in the highest quintile (3.65mg/L or over) was associated with a twofold increase in lung cancer compared to the lowest quintile (< 1.11mg/L)(142).

Prognostic scores which incorporate CRP, the Glasgow Prognostic Score (GPS)/modified GPS (mGPS), and the CRP: Albumin ratio (CAR) have been associated with survival in NSCLC(143-145). The link between inflammation and small cell lung cancer (SCLC) has been less studied(146). There is some evidence that inflammation, as measured by the Neutrophil Lymphocyte Ratio (NLR) and possibly the mGPS, is associated with survival in SCLC(146).

1.2.3 Cancer-related inflammation

The ancient Greek physician, Galenus, is considered the first to have noted the connection between chronic inflammation and cancer(52). In the 1860's, Virchow proposed that the immune infiltrate seen in chronic inflammation provided the origin for cancer(147). Over 100 years later, Dvorak observed that there are similarities between inflammation and tumours, notably development of blood vessels (angiogenesis) and immune cell infiltrate and described cancer as “wounds that do not heal” (148).

Hanahan and Weinberg highlighted that inflammation is a key enabling characteristic of cancer, noting that inflammation enables the development of “multiple hallmark capabilities” by tumours (149). Cancer-related inflammation can play a role in promoting proliferative signalling, avoidance of cell death and angiogenesis, as well as being implicated in invasion and metastasis (149). Indeed, cancer-related inflammation has even been proposed as a seventh hallmark of cancer, being implicated in its development and progression(150), though the most recent paper from Hanahan stops short of labelling it a hallmark(151). Examples of cancers arising in the presence of chronic inflammation include colorectal cancer arising in inflammatory bowel disease (52) and OAC. OAC can develop on a background of Barrett's oesophagus (152, 153) via phenotypic plasticity (transdifferentiation), another proposed hallmark of cancer(151). Obesity, a chronic inflammatory state, is associated with increased risk of cancer (154, 155).

1.2.4 CRP in Cancer

Given its position as the most widely used marker of inflammation in clinical practice (Ryan 2015) and the many advantages CRP has, as outlined below, it is logical that CRP would be of interest in studies examining the link between inflammation and cancer.

CRP has been associated with cancer risk, presence and prognosis. While there is little evidence of causality (i.e. that a high CRP causes cancer) (104, 115), epidemiologic studies have shown that raised serum CRP is associated with increased risk of any incident cancer (142), including some of the most common cancers such as lung and colorectal cancer (156, 157). Exceptions to this appear to be breast and prostate cancer, for reasons which remain unclear (10). In a study of 160,000 adults in primary care, Watson *et al.*, showed that a raised CRP (above 6.8 mg/L, chosen as mean of the upper limits of normal in the laboratories included in the study) was associated with a higher one-year cancer incidence than a normal CRP (158). The risk was highest if CRP had increased on repeat testing within 90 days (158). This theme is picked up in Chapter 2 of this thesis, where the potential for serial CRP measures to indicate prognosis is studied.

As noted above, most studies have found higher CRP levels in people with cancer, compared to healthy controls (104). The corollary - that CRP could thus be used to diagnose cancer – has, however, not been proven (104). Raised CRP in cancer may reflect an aggressive tumour, the degree of inflammation in the tumour microenvironment or differences in the host's response to the cancer (10).

Elevated serum CRP is associated with reduced survival in cancer (142, 159). Confounding by deaths due to cardiovascular disease, rather than cancer itself, has been shown to be unlikely (10). While the association between CRP and survival in cancer has indeed been widely reported, several gaps exist in current understanding of this association. These are the focus of this thesis and are discussed further below.

1.3 Prognostication

The word prognostication derives from Greek, meaning foreknowledge, from the words pro (beforehand) and gnosis (knowledge) (160). It has been stated that the most important aspect of a physician's work in the time of Hippocrates was to prognosticate (160). Modern medical prognostication covers three main areas (160): the prediction of

1. Duration of illness (in Latin: prognosis quoad tempus)
2. Recovery (prognosis quoad restitutionem)
3. Survival (prognosis quoad vitam)

Moons *et al.*, reject as being too broad the definition of prognosis as “the expected course of an illness”, instead defining prognosis as “the probability or risk of an individual developing a particular state of health (an outcome) over a specific time, based on his or her clinical and non-clinical profile” (161). They go on to note that “outcomes are often specific events, such as death or complications, but they may also be quantities, such as disease progression, (changes in) pain, or quality of life” (161). Prognosis, as addressed in this thesis, is studied in line with this definition. The outcomes of interest in this thesis are death, changes in symptoms and changes in quality of life.

Prognosis is important to patients, clinicians and researchers. While the most important reason to estimate prognosis accurately is so that patients’ care is consistent with their values and preferences (162), accurate prognostication is also key for planning clinical services. Identification of which patients are expected to have similar outcomes is likewise essential for clinical trials (163).

1.3.1 Standard predictors of outcome in cancer

Some factors associated with survival vary between cancer sites, with markers specific to cancer type e.g., prostate specific antigen for prostate cancer or B symptoms (weight loss, pyrexia, night sweats) in Hodgkin Lymphoma (164, 165). However, many key predictors of survival apply to most cancers. TNM stage categorises the extent of solid tumours, with increasing stage associated with decreased survival (163). Increasing age is associated with reduced survival in cancer, with patients aged 85 and older having the worst survival of all age groups. Data from over one million people in Europe confirmed observations that, in general, women tend to have longer survival than men (166). Histologic grade is associated with survival in many cancers (163). Consistent with clinical experience, physical function has been confirmed as predictive of survival across a wide range of cancers and treatment modalities (167). Although there was variation in the strength of the association between different metrics of physical function, the association was present for all metrics studied, including grip strength, gait speed, six-minute walk test (6MWT), short physical performance battery (SPPB) and timed up and go test (TUG) (167).

Despite these widely known prognostic factors, clinicians are often inaccurate in estimating prognosis in incurable cancer and may overestimate survival time (168). Even in curable disease, predictors of outcome may not be available pre-operatively.

In their seminal series of papers, the Prognosis Research Strategy (PROGRESS) group highlighted the need for further high-quality research prognosis research (169). In this thesis, the role of CRP as a prognostic factor is investigated. A prognostic factor is a “variable associated with the risk of a subsequent health outcome among people with a particular health condition” (170). Prognostic factors may be measured at the level of the individual (as here for CRP) or a wider “ecological” level (such as socioeconomic group) (171).

More specifically, CRP is a potential prognostic biomarker. While there has been a huge amount of research in recent years on prognostic biomarkers, very few have been incorporated into clinical practice (172). The National Cancer Institute define a biomarker as “a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease” (173). Kerr and Yang recently defined a prognostic biomarker as “a clinical or biological characteristic that provides information on the likely patient health outcome” and note that it should be measured before treatment (172). However, Kerr and Yang also propose that a prognostic biomarker “identifies tumour-specific molecular or histopathological characteristics that are associated with long-term outcome or disease course” (172). This narrowing of the definition to focus only on the tumour and surrounding tissue conflicts with the paradigm proposed by McMillan’s group of “staging the tumour and staging the host”, in a series of papers (174-176). The tumour is staged with TNM staging and host is “staged” through measurement of the systemic inflammatory response (174) This thesis will explore how CRP may be in accord with both concepts, as Chapter 4 explores the role of CRP within the tumour, while Chapters 2, 3 and 5 address CRP as part of the systemic inflammatory response.

1.3.2 Appeal of CRP as a prognostic biomarker in cancer

CRP has considerable appeal as a potential prognostic biomarker due to its accessibility, ease of measurement and its biological properties. Measurement is cheap and straightforward(16). As a protein circulating in the blood, it is easy to sample before, during and after cancer treatment (10). There is no known diurnal variation in serum CRP (10) and measurements do not require fasting, which greatly simplifies its measurement in clinical practice (82). Unlike many novel biomarkers (172), CRP assays are highly standardised and well-validated. There is no inter-observer variability, which hampers use of other

biomarkers(172). Furthermore, serum CRP samples have been found to be very stable when stored, with CRP values unchanged after refrigeration at 4 °C for 5 months and after freezing at -20°C for over a year .

Serum CRP begins to rise within one to two hours of an inflammatory insult, rising exponentially and reaching a peak within 24-48 hours, usually in proportion to the extent of the tissue damage (10, 177, 178). Its half-life is approximately nineteen hours in almost all clinical circumstances studied, including health, cancer, infection and chronic inflammatory disease (11) and serum levels are dependent almost entirely on rate of synthesis (10). The implications of these findings are two-fold: firstly, CRP may be interpreted in the same way in most clinical populations and secondly, a persistently elevated circulating CRP indicates persistent inflammation(10). Aside from intercurrent episodes of infection or inflammation, CRP levels have been shown to remain quite consistent for individuals, with no significant change in healthy adults whose CRP was measured monthly for almost a year (107). An exception to this is the case of fulminant hepatic failure, where CRP production in sepsis has been found to be much lower than would be expected, likely due to synthetic failure (179).

Importantly, it is possible to make more efficient use of existing resources in CRP measurement. Implementation of an education programme and a 48 hour mandatory interval between repeat CRP requests resulted in savings calculated at £5000 per annum in one UK hospital (18). Thus, if more use can be made of CRP data already being gathered, while simultaneously reducing the cost, there would be increased value for the healthcare system. One of the authors who has published most extensively on CRP, Mark B Pepys, has repeatedly commented, in the context of cardiovascular disease risk, that CRP must be measured and interpreted correctly to avoid excess cost, anxiety for patients and missed diagnosis of underlying disease (17, 31). The same may be said for CRP in the context of cancer. Despite its obvious appeal as a biomarker, CRP is not routinely used for prognostication or treatment planning in cancer and further study of its role in cancer has been recommended (28). Concerns remain about the non-specific nature of CRP and about the use of CRP without consideration of other clinical factors. Novel means of using CRP, which have the potential to address these issues, have been proposed but require further study. The work described in this thesis will examine some of these.

1.4 Novel uses for CRP in Prognostication in Cancer

1.4.1 CRP kinetics

As a non-specific marker of inflammation, transient elevations in CRP due to acute episodes of infection or inflammation could lead to inaccurate prognostication (180). Serial measurements of CRP, such as those required for assessment of cardiovascular risk (82), are a possible solution (180). Early studies have suggested that longitudinal change in CRP (CRP kinetics) may be predictive of prognosis, particularly in urological cancers (181). It is currently unknown whether these findings are applicable to the wider cancer population with varied diagnoses, treatment modalities and stages of disease, nor whether it is of value in OAC. The value of CRP kinetics in a mixed cancer population and in OAC is addressed in Chapter 2. In line with recommended procedure in prognostic research, multivariable analysis is used and the prognostic factor is evaluated in two independent cohorts (171).

1.4.2 CRP cutpoints and CRP-based scores

There remains uncertainty as to what level of CRP should be considered high (the cutpoint or threshold), in the context of predicting outcomes in cancer. The cutpoint reported differs dramatically between studies (28, 159), with values from 2mg/L to 50mg/l reported as prognostic in one review (159). Even within a single cancer site there is a huge range of cutpoints reported, with a sixfold difference in the pre-operative CRP reported as prognostic of survival in OSCC, from 2mg/L to 12mg/L (182, 183). A level of 10mg/L is a reasonable indicator of clinically significant inflammation (184). This cutpoint is used in approximately one-third of studies examining CRP as prognostic marker in cancer (159). As noted above, this value is significantly higher than baseline CRP for the great majority of people and it is plausible that the association with adverse outcomes in cancer is present at less elevated levels of CRP. Furthermore, the most appropriate cutpoint may differ between cancer sites. Some studies in cancer have used 5mg/L as the upper limit of normal; this corresponds to the upper limit of the reference range in current clinical use at St. James's Hospital where most of the work for this PhD was conducted (M Neville, Biochemistry lab, St James's Hospital, personal communication). Other studies have derived their own cutpoint, based on medians within the study population or healthy controls (185). Some papers do not even report the cutpoint used (159, 185). As Kushner *et al.*, point out, the acute phase response must be understood as a continuum, rather than an all or nothing phenomenon and, thus, it is problematic to define a single cutpoint between "normal" and "abnormal (184). Moreover, CRP is a continuous variable and dichotomising continuous variables is not

recommended in prognostic research (170). Nonetheless, for real clinical practice, some indication of the upper limit of normal is required (184). The heterogeneity of cutpoint reported in the literature is bewildering and is a major barrier to the use of CRP for prognostication in clinical practice.

It has been proposed that prognostic scores which incorporate more than one biomarker may be more accurate than single biomarkers (186). Such scores could be valuable to support decision-making by clinicians and patients. There is growing consensus that prognostic scores based on markers of the systemic inflammatory response (SIR) have a role in operable and inoperable cancer (187, 188). Numerous prognostic scores, incorporating CRP and other clinical parameters, commonly albumin, have been devised and continue to proliferate in the literature (187, 189). Table 1.1 shows the diverse scores reported in oesophageal and lung cancers alone. However, it is unclear which score is best and which should be used in particular cancer sites.

Systematic review and meta-analysis is crucial for synthesising and summarising published work on prognostic factors(171). This methodology is used in Chapter 3 to assess which CRP cutpoint and CRP-based score are most useful to predict survival in OAC.

Table 1-1. Prognostic scores incorporating CRP reported in lung and oesophageal cancer

Score Name	Components	Year first reported in oesophageal or lung cancer	Scoring
Glasgow Prognostic Score (GPS)	CRP Albumin	2003(190)	Grouped: score 0, 1, 2
Modified Glasgow Prognostic Score (mGPS)	CRP Albumin	2010(191, 192)	Grouped: score 0, 1, 2
GPS mGPS with modified cutpoints <u>CRP modified</u> 5mg/L “new mGPS” (193)) 3mg/L “Highly sensitive mGPS” (194)) ≥6.6mg/L “adapted GPS” (195)	CRP Albumin		Grouped: score 0, 1, 2

<u>Albumin modified</u> <38 g/L(196) “adapted GPS” ≤ 41.4 g/L (195) “Sensitive-mGPS” ≤ 45.6 g/L(197)			
CRP-Albumin Ratio (CAR)	CRP Albumin	2015(198, 199)	Above and below cutpoint Range 0.012 – 0.5(198, 199)
Prognostic Index for Esophageal Cancer (PIEC)	CRP Weight loss cTNM III /IV	2003(200)	Grouped: score 0, 1, ≥ 2
Fibrinogen and CRP score (FC)	CRP Fibrinogen	2016(201)	Grouped: score 0, 1, 2
Predicting score	GPS (CRP and albumin) Number of lymph nodes	2010(202)	Grouped: score 0, 1, 2
NLR and CRP	CRP NLR	2012(203)	Grouped: high/high, low/low, high/low, low/high
Combination of NLR and GPS (CNG)	GPS (CRP and albumin) NLR	2019(204)	Grouped: score 0,1,2,3
Hamburg-Glasgow classification (HGC)	GPS (CRP and albumin) Disseminated tumour cells	2017(205)	Grouped: HGC I-IV
Aarhus composite biomarker score (ACBS)	CRP Albumin Neutrophil count Lymphocyte count Haemoglobin	2019(204)	Grouped: score 0, 1,2,3

cTNM III or IV: clinical Tumour-Node-Metastasis stage III or IV. NLR: Neutrophil:lymphocyte ratio

1.4.3 Tumoural CRP

Although CRP is mainly produced in the liver, in recent years extrahepatic sites of production have been identified including lymphocytes and the kidney (206, 207). CRP has also been identified within tumour cells in cancers including prostate cancer, hepatocellular carcinoma and in OSCC (208-211). Importantly, some of these studies demonstrated that prognosis was worse for individuals with detectable tumoural CRP (209-211). Tumoural CRP may constitute a more specific biomarker of cancer-related inflammation than serum CRP and could be an ideal biomarker of survival and clinical outcome. A recent review of CRP and cancer advocated that CRP be studied using immunohistochemistry (IHC) (28). The study described in Chapter 4 adopts this approach to assess tumoural CRP but also assesses the relationship of tumoural CRP with survival. The source of CRP remains controversial; this study explores two possible sources: circulating CRP and carcinoma cells.

1.4.4 CRP and Skeletal Muscle Abnormality

It has been proposed that many cancer symptoms are linked with inflammation; Laird *et al.*, found several common symptoms of cancer e.g., pain, anorexia, dyspnoea and fatigue and poor QoL were associated with CRP > 10mg/L (212). Systemic inflammation is also associated with abnormalities of skeletal muscle in cancer (loss of volume and loss of density) (213). CRP could be combined with skeletal muscle indices to identify people at risk of high symptom burden and low QoL, potentially enabling early or preventative intervention (212). However, the associations between systemic inflammation, skeletal muscle, symptoms and QoL are currently incompletely understood (214). Chapter 5 describes a feasibility study, intended to inform future studies of these associations.

2 CRP Kinetics to Predict Survival in Cancer

2.1 Introduction

CRP is a non-specific marker of inflammation (10). A potential limitation to its use in prognostication of cancer survival is that acute changes, such as with intercurrent infection or inflammation, could lead to inaccurate prediction of survival time (180). Serial measurements of CRP may provide a solution (180).

CRP has a short half-life and its clearance remains constant in most disease conditions (11). A persistently high CRP reflects ongoing stimulus for its production i.e., ongoing inflammation (215) and changes in CRP over time mirror changes in the inflammatory state. CRP change over time (termed CRP kinetics) could be a better measure of cancer-related inflammation than a single measure. Thus, CRP kinetics may have a role in prognostication in cancer.

There is a precedent for use of serial blood markers in prognostication, with prostate specific antigen (PSA) in prostate cancer being the most widely recognised example. Longitudinal change in PSA, either measured as PSA velocity (change in PSA concentration over time) and PSA doubling time (time taken for PSA concentration to double), is used in follow-up of people with prostate cancer (164). PSA doubling time is the more widely used metric (164) and is an independent predictor of survival in castrate-resistant prostate cancer (216).

Serial measurements of CRP have long been recognised as of value in monitoring disease. Over thirty years ago, Cox *et al.*, reported that a CRP which did not fall in response to treatment for infection either indicated ineffective treatment, a complication such as an abscess or an underlying cancer (217). They noted that an unexplained persistently high CRP was a “grave prognostic sign” (217). More recently, serial measurements of CRP have been examined as predictors of post-operative complications, particularly infection and anastomotic leaks, in cancer surgery. Asmar *et al.*, showed that a persistently high CRP following cytoreductive surgery with hyperthermic intraperitoneal chemotherapy was a better predictor of post-operative complications than a single measure, no matter how high (218). A recent large multi-centre study (219) confirmed a role for CRP kinetics in the prediction of anastomotic leaks requiring intervention following resection for colorectal cancer and for ruling out such leaks, but did not assess survival. In the setting of

cardiovascular risk assessment, two measurements of CRP are recommended in order to get an average value for risk stratification (82).

CRP kinetics has also been studied in non-malignant disease. A high rate of CRP increase was independently associated with acute kidney injury (220), new-onset atrial fibrillation (221) and 30 day mortality in ST-elevation myocardial infarction (STEMI) (222). Increasing CRP over time was associated with all cause and cardiovascular disease-related mortality in a study in peritoneal dialysis patients (223).

The literature relating to CRP kinetics and cancer is relatively sparse but CRP kinetics have been shown to be associated with survival in specific cancer sites and treatments. CRP kinetics have been studied in patients who underwent surgery for colorectal cancer (224), in urothelial cancer treated with chemoradiotherapy (225), in metastatic nasopharyngeal carcinoma treated with chemotherapy (226), advanced pancreatic cancer (227) and advanced gastric cancer (228). In each case, persistently elevated CRP was associated with survival. Fujita, Wang and Yasuda all showed that CRP kinetics were associated with survival in renal cell carcinoma treated with tyrosine kinase inhibitors (TKIs) (229-231). Ito *et al.*, and Tachibana *et al.* also studied renal cell cancer; patients whose CRP failed to normalise post nephrectomy and with combination immunotherapy treatment, respectively, had worse overall survival (232, 233).

There is little published work examining CRP kinetics in lung cancer or in oesophageal cancer. In Stage IV lung cancer, McFarland *et al.*, found that a persistently high CRP was an independent predictor of worse survival, but was not a better predictor than a single CRP measurement (234). In contrast, in their small study in small cell lung cancer, Arpin *et al.*, showed that a rapid increase in CRP in the two days following chemotherapy was associated with treatment response, although they did not examine survival (235). Over twenty years later Ozawa *et al.*, found a similar pattern in non-small cell lung cancer treated with immunotherapy: an initial CRP rise predicted treatment response (236), as did Klümper *et al.*, who also showed that the CRP rise was associated with improved survival (237). However, other studies in immune-checkpoint inhibitors (ICI) found the opposite - a reduction in CRP, rather than a rise, was associated with improved survival following atezolizumab (238). Similarly, Riedl *et al.*, showed that CRP increase (doubling rate) was an independent predictor of shorter survival in NSCLC treated with nivolumab,

pembrolizumab or atezolizumab (239). In oesophageal squamous cell carcinoma, Ibuki *et al.*, found that a persistently elevated CRP at one month post-resection was associated with shorter survival (240). Another study in OSCC found that changes in a CRP-based prognostic score, the modified Glasgow Prognostic Score (mGPS), after neoadjuvant chemotherapy predicted survival (241).

To date, no published study has examined the prognostic role of CRP kinetics in an unselected cancer cohort, as found in clinical practice, nor in oesophageal adenocarcinoma (OAC), a cancer strongly associated with inflammation(153).

2.1.1 Specific Hypotheses of this Study

1. Change in CRP over time can predict survival
2. Change in CRP over time can predict survival more accurately than single CRP measurements
3. Change in CRP over time can predict survival more accurately than standard clinical predictors

2.1.2 Specific Aims of this Study

1. To assess if CRP kinetics can predict survival in
 - a. A large mixed cohort with solid tumours
 - b. A separate, prospectively collected and homogenous cohort with OAC
2. To determine if CRP kinetics are a more powerful predictor of survival in cancer than a single CRP measurement.
3. To determine if change in CRP over time can predict survival more accurately than standard clinical predictors

2.2 Methods

Two separate datasets were examined, the Cleveland Clinic (CC) dataset and St James's Hospital (SJH) dataset.

2.2.1 Cleveland Clinic dataset

This was a retrospective review of a large database which included mixed cancer sites and disease stages. The database had been created for a previous study (242). Data had been extracted from an electronic medical record (EMR) and been imported into an Excel document, an anonymised copy of which was shared for this research. Ethical approval had been granted by the Institutional Review Board (IRB) of the Cleveland Clinic (waiver of consent).

Inclusion criteria were adults aged eighteen or older with solid tumours (as identified by International Classification of Disease Codes Version 9, Clinical Modification (ICD-9-CM, World Health Organisation) who attended the Taussig Cancer Institute from 2006 to 2012, who had two CRP levels at least one week apart and at least one total white blood cell count (WBC)). The seven-day minimum time interval between CRP measures was chosen because an acute event, such as infection, is likely to be treated or improve within a week, and given CRP's short half-life, an acute elevation of CRP should have returned to normal within this time, assuming no ongoing stimulus to its production. Blood results which predated cancer diagnosis were not included. Haematological malignancies had been excluded at the point of database creation. CRP measurements had been undertaken as part of routine clinical care. CRP measurement was by immunoturbidimetry in the hospital laboratory. The dataset included details of sex, age, ethnicity, diagnosis, disease extent (metastatic or not) and date of death or last follow-up. Overall survival (OS) was calculated from date of first CRP to date of death. Those alive at the last visit date, which was defined as the last recorded attendance at the Cancer Institute or the final laboratory measure recorded, whichever was latest, were censored. Only treatment received in the 4 weeks preceding maximum CRP level was recorded (as per the original study's design). No detail was available of treatments received at any other time point. As most conventional predictors of survival in cancer were missing from this dataset, total WBC was examined as a predictive factor, by way of comparison with CRP change. Where WBC was measured within 7 days of the initial CRP

measurement, survival analysis was conducted using WBC. High WBC was defined as any value above the upper limit of the reference range at CC: $11 \times 10^3/\mu\text{L}$.

High CRP was based on the upper limit of the reference range at CC: 10mg/L. First CRP measure is abbreviated as CRP₁ and second CRP measurement is abbreviated as CRP₂. CRP change over time was classified as

1. Persistently high (both CRP₁ and CRP₂ above upper limit of reference range i.e., > 10mg/L)
2. Normal which became high (CRP₁ $\leq$ 10mg/L, CRP₂ > 10mg/L)
3. High which normalised (CRP₁ > 10mg/L, CRP₂ $\leq$ 10mg/L)
4. Persistently normal (both CRP₁ and CRP₂ $\leq$ 10mg/L)

Statistical analysis was conducted using Microsoft Excel 2016 and IBM SPSS software (version 27, IBM). P values of <0.05 were considered significant, two-tailed.

Descriptive analysis was conducted of demographics, CRP and WBC. Mann-Whitney U test was used to compare medians and t-tests were used to compare means. Pearson Chi-Square test compared frequencies of categorical variables between groups. Kaplan-Meier curves were constructed to provide unadjusted estimates of differences in survival between groups defined by prognostic factors (243). The Kaplan-Meier method accounts for censored data (244). Log rank (Mantel-Cox) chi square test were used to assess statistical significance of survival differences between groups (245). Pairwise comparisons were conducted where there was a statistically significant difference and there were more than two groups. Correction for multiple statistical testing was not conducted due to the small number of planned comparisons(246). Multivariable analysis was conducted to yield adjusted estimates of prognostic factor effects (243). Cox proportional hazards regression, which is the most commonly used approach for time-to-event outcomes (243), was used. Results are reported as Hazard Ratios (HR) and 95% confidence intervals (CI).

Subgroup analysis was conducted of clinically relevant subgroups. First, non-melanoma skin cancers alone (NMSC) was examined, then all cancers excluding NMSC and differences between these 2 groups were assessed. To assess the potential impact of an individual having multiple primary cancers, further analysis compared demographics, CRP levels, CRP change category and survival between those with single primary cancer sites

and those with multiple primary cancer sites. Multivariable analysis was repeated excluding first those with NMSC and then those NMSC or multiple primary cancer sites to assess their potential influence on the whole group results.

2.2.2 St James's Hospital (SJH) dataset

This was a secondary analysis of data collected for a previous prospective study (247). Ethical approval was granted by the St James's Hospital and Adelaide and Meath incorporating National Children's Hospital Joint Research Ethics Committee(SJH/AMNCH REC). Inclusion criteria in the original study were adults aged eighteen or older with locally advanced OAC who underwent curative-intent treatment (resection or multimodal treatment) at St James's Hospital between 2011 and 2014. CRP was routinely recorded as part of the study. CRP measurement was by immunoturbidimetry in the hospital laboratory. Data from all participants who had both a pre-operative and a one month post-operative value CRP were included in this study. Other, earlier post-operative measurements (post-operative days 1, 3, 7 and 14) were not used for analysis since these may have reflected post-operative inflammation or infection. SJH maintains an Upper Gastrointestinal (GI) cancer registry, which includes survival and follow-up data. Data from this registry and the SJH electronic record, as well as RIP.ie (a website which announces deaths and funeral arrangements) were used to determine survival time and follow-up. Overall survival was calculated from date of surgery to date of death. Those alive at five years or who were lost to follow-up were censored.

CRP change classification and statistical analysis were as for the CC dataset.

2.3 Results

2.3.1 Dataset 1: Cleveland Clinic

Data from 607 patients were included. Primary cancer site is illustrated in Figure 2.1. Breast cancer, non-melanoma skin cancer (NMSC), colorectal, lung and urological cancers were the five most common cancers. No cancer site was specified in the database for 48 patients. 75 patients had NMSC and no other documented solid tumour. 97 people had 2 or more primary cancer sites.

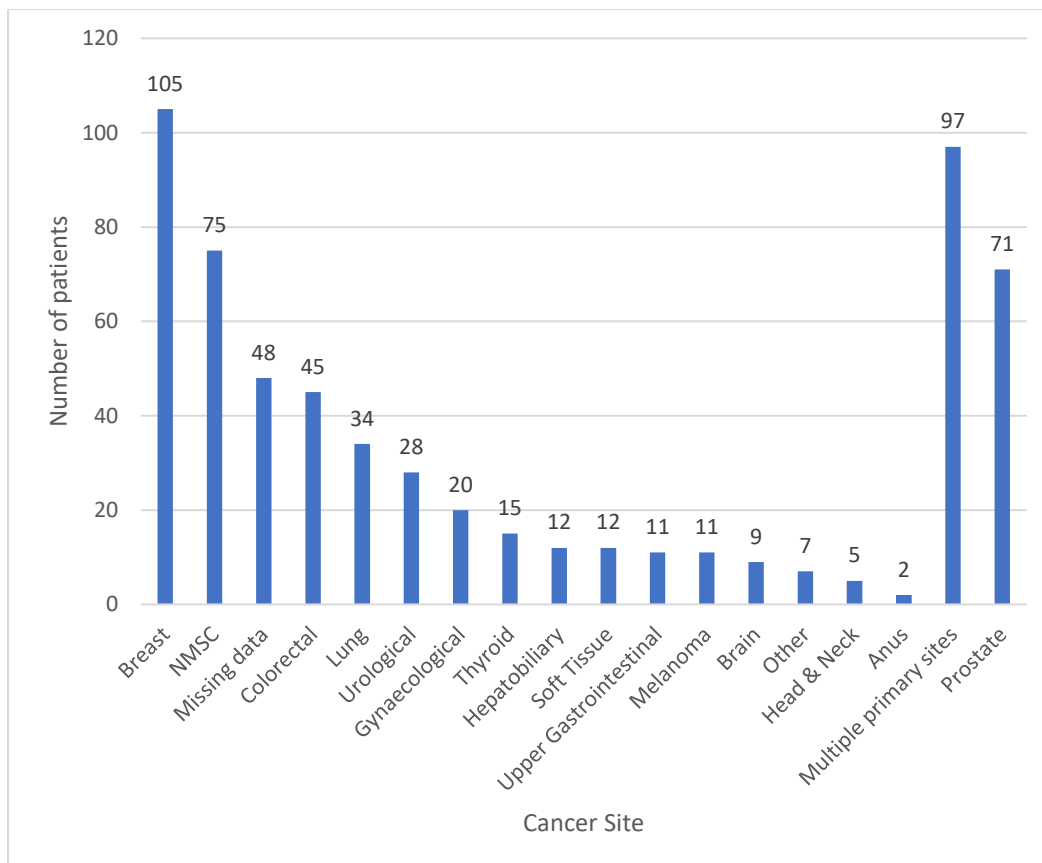


Figure 2-1. Primary Cancer Sites for Included Patients (n=607) from the Cleveland Clinic cohort

Urological: bladder and renal cancers. Gynaecological: cervix, uterus, vulva, ovary and other female genital cancers. Hepatobiliary: pancreatic and liver cancers. Other: cancer of small intestine, other digestive organs, testicular cancer and other endocrine cancer.

Results are presented below, first for the whole group (n=607), then for diagnostic subgroups: people with NMSC alone (n=75), all excluding NMSC (n=532), people with single (n=435) and people with multiple primary cancer sites (n=97).

2.3.1.1 Results for n=607 (whole group)

Table 2.1 shows demographics for all included patients (n=607). Median age was 65 years (range 23-93), with 51% aged 65 or older. 348 (57%) were female. 485 (80%) had local or loco-regional disease and the rest had metastatic disease. 500 (82%) were Caucasian, 85 African-American with 7 people (1%) and 15 people (2.5%) of the cohort recorded as Hispanic or Latino/a and other, respectively.

Table 2-1. Demographics of Included Patients

Characteristic	Whole cohort, including NMSC n=607 No. of patients (%)
Age, years	
median and range	65 (23-93)
< 65	299 (49%)
≥ 65	308 (51%)
Gender	
Male	259 (43%)
Female	348 (57%)
Disease extent	
Local / loco-regional	485 (80%)
Metastatic	122 (20%)
Ethnicity	
Caucasian	500 (82%)
African-American	85 (14%)
Hispanic / Latino/a	7 (1.25%)
Other	15 (2.5%)

2.3.1.1.1 CRP and CRP change category

Median first CRP measurement (CRP₁) was 15mg/L (range 0.9-490). Median second CRP measurement (CRP₂) was 14mg/L (range 0.2-591). Median interval between CRP measures was 93 days (range 7 – 1411).

381 (63%) had high CRP₁ (>10mg/L) and 360 (59%) had high CRP₂.

Table 2.2 shows CRP change category for the whole cohort. 259/607 (43%) had a high CRP at time points 1 and 2 (CRP₁ and CRP₂ high) and were categorised as persistently high. 84 (14%) had a normal CRP₁ and a high CRP₂. 105 (17%) had high CRP₁ and a normal CRP₂. 159 (26%) had normal CRP at both time points.

Table 2-2. CRP Change Category (n=607)

CRP change category	n (%)
Persistently high	259 (43%)
Normal, became high	84 (14%)
High, became normal	105 (17%)
Persistently normal	159 (26%)

White Blood Cell Count

WBC measured within 7 days of CRP₁ was available for 566 people, mean 8.7 ± 4 k/ μ L, median 7.9 (range 2.8- 37.5). 109 people (19%) had a high WBC ($>11 \times 10^3/\mu$ L).

Follow-up and survival

Median follow-up for the whole cohort was 1075 days (range 14-2455). 174 people died (29%). Median survival was not reached.

2.3.1.1.2 Factors associated with survival

Baseline CRP (CRP₁)

People with a high baseline CRP (CRP₁) had significantly worse OS (median survival 2175 days, 95% CI 1601-1806 days) than people with low CRP₁ (median not reached)(Fig. 2.2).

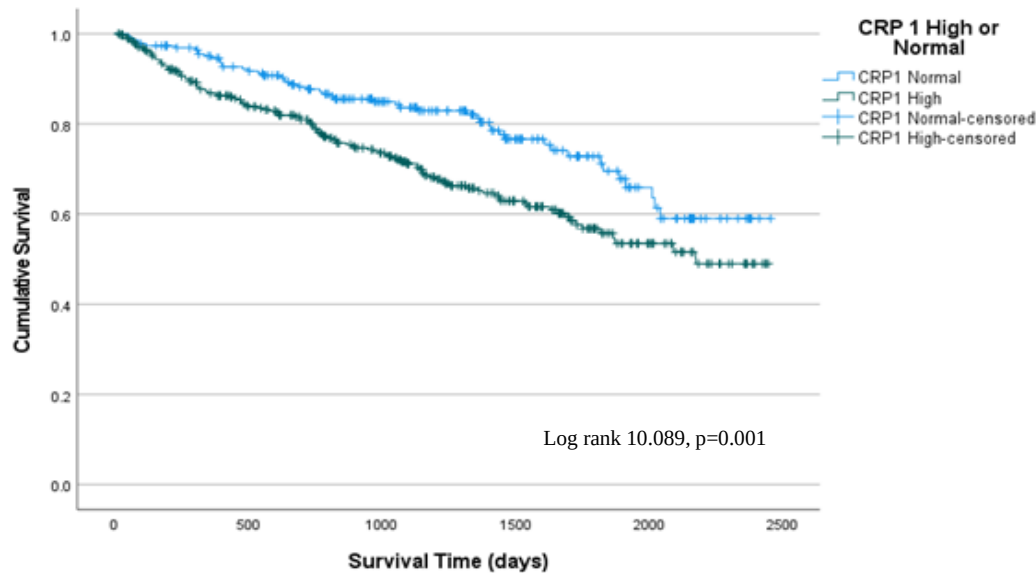


Figure 2-2. Kaplan-Meier survival curve illustrating shorter overall survival in people with high baseline CRP (CRP₁)

The Kaplan-Meier method was used to assess the prognostic impact of baseline CRP(CRP₁) on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between normal CRP (blue line) and high CRP (green line) groups.

CRP Change Category

There was a significant difference in OS between CRP change category groups (Figure 2.3). Median survival was only reached in the persistently high group and was 1816 days (95% CI: 1398-2233 days).

Pairwise comparison (Table 2.3) demonstrated statistically significant survival differences between people with persistently high CRPs and those with a high CRP which normalised (p=0.003), between persistently high and persistently normal CRPs (p<0.001) and between people with a normal CRP₁ and a high CRP₂ and those with persistently normal CRPs (p=0.02). Differences between other CRP change categories were not statistically significant.

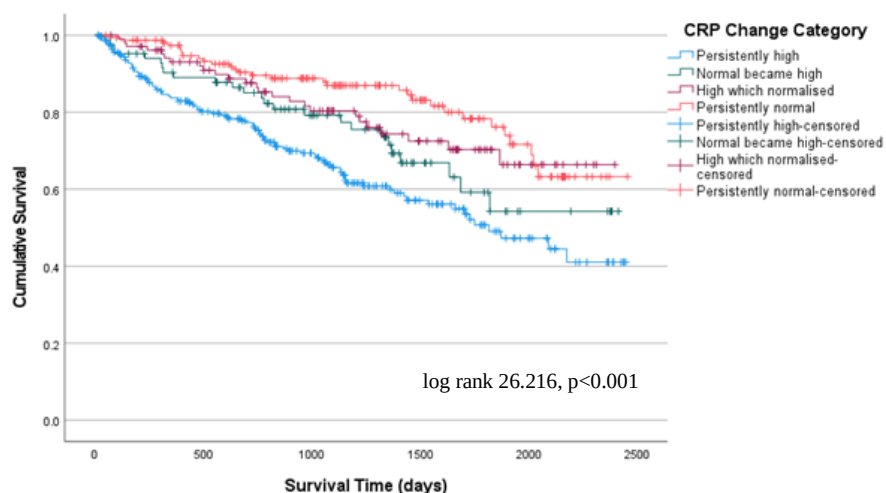


Figure 2-3. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

Table 2-3. Pairwise comparisons of differences in survival between CRP change categories

	Persistently high	Normal became high	High which normalised	Persistently normal
Persistently high	-	p=0.083	p=0.003*	p<0.001*
Normal became high	p=0.083	-	p=0.338	p=0.02*
High which normalised	p=0.003*	p=0.083	-	p=0.256
Persistently normal	p<0.001*	p=0.02*	p=0.256	-

*Statistically significant. Pairwise comparison using Log rank (Mantel-Cox) chi-square test.

White Blood Cell Count

High WBC ($>11 \times 10^3/\mu\text{L}$) was associated with significantly shorter OS than normal or low WBC (median OS 1425 days, 95% CI 1044-1805 days in the high WBC group, median not reached in the normal or low WBC group) (Fig. 2.4).

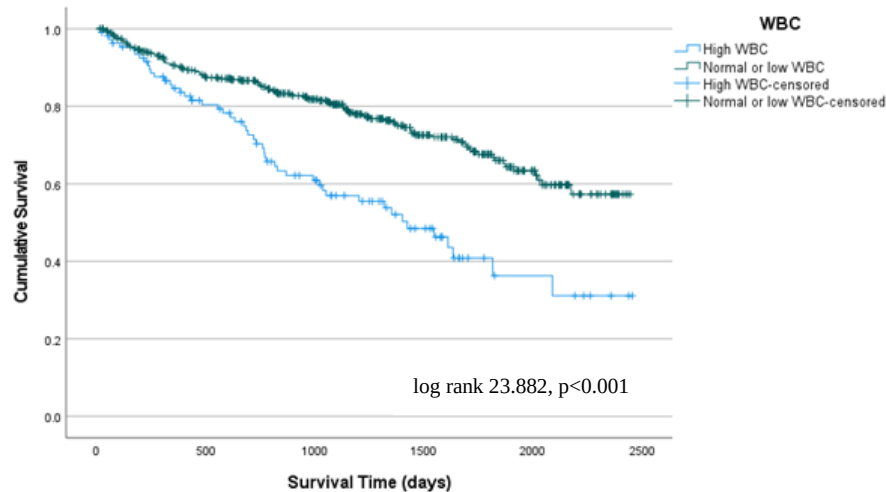


Figure 2-4. Kaplan-Meier survival curve of the prognostic influence of white blood cell count on overall survival

The Kaplan-Meier method was used to assess the prognostic impact of baseline white blood cell count (WBC) on overall survival in $n=566$ adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between normal WBC (green line) and high CRP (blue line) groups.

Age

As shown in Figure 2.5, age greater than 65 was associated with significantly shorter median OS (1866 days, 95% CI 1630 – 2102 days,) than age less than 65 (median not reached).

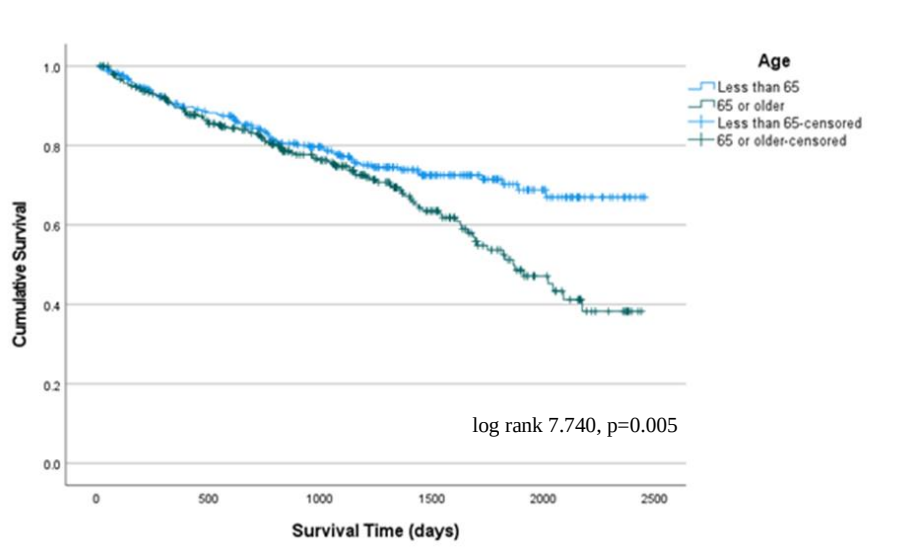


Figure 2-5. Kaplan-Meier survival curve illustrating shorter overall survival in those age 65 or older

The Kaplan-Meier method was used to assess the prognostic impact of age (less than and greater than/equal to 65) on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between age ≥ 65 (green line) and age < 65 (blue line) groups.

Sex

Male sex was associated with shorter OS (median OS 1872 days, 95% CI 1543-1872 days) than female sex (median not reached) (Figure 2.6).

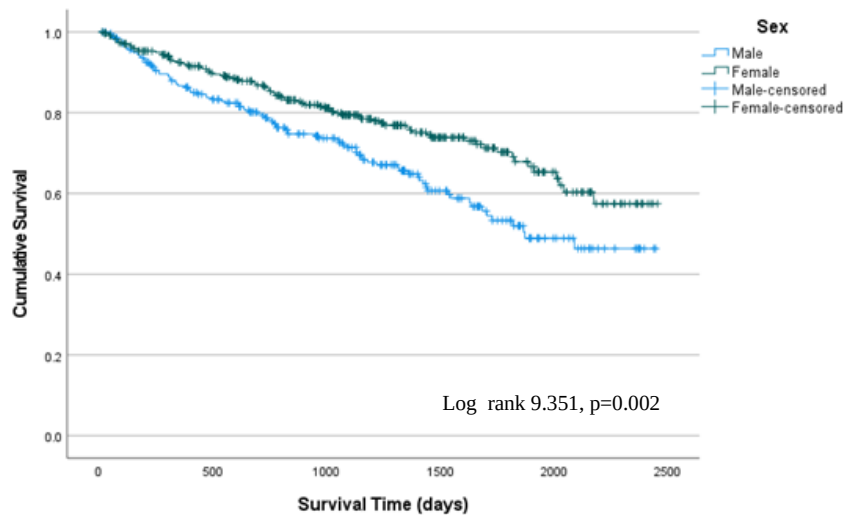


Figure 2-6. Kaplan-Meier survival curve illustrating shorter overall survival in males

The Kaplan-Meier method was used to assess the prognostic impact of sex on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between female (green line) and male (blue line) groups.

Disease extent

Presence of metastases was associated with shorter OS (median 1056 days, 95% CI 833 – 1279 days) than local or loco-regional disease (median not reached). (Figure 2.7).

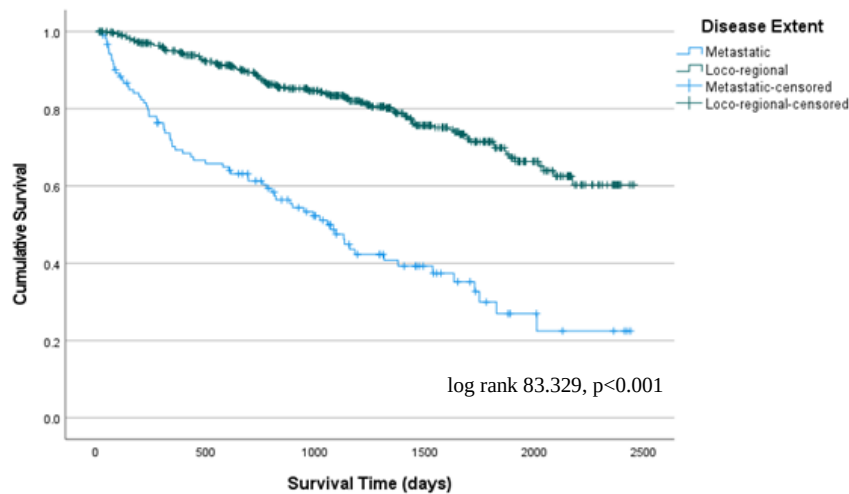


Figure 2-7. Kaplan-Meier survival curve illustrating shorter overall survival in people with metastatic disease

The Kaplan-Meier method was used to assess the prognostic impact of disease extent on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between local / loco-regional (green line) and metastatic (blue line) groups.

Ethnicity

There was no significant difference in survival between different ethnicities (Figure 2.8).

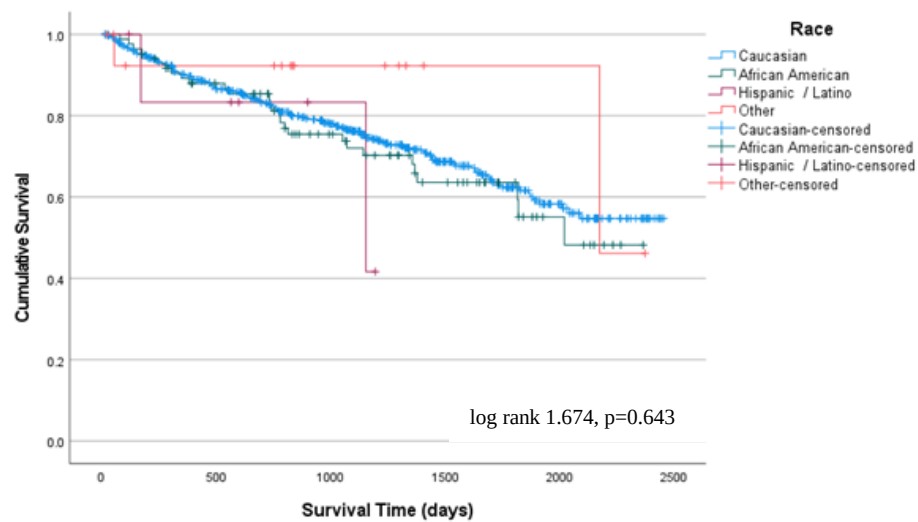


Figure 2-8. Kaplan-Meier survival curve illustrating absence of an association between ethnicity and overall survival

The Kaplan-Meier method was used to assess the prognostic impact of patient ethnicity on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between ethnicity groups.

2.3.1.1.3 Multivariable analysis

The results of multivariable analysis are reported in Table 2.4. Increasing age, presence of metastatic disease, high WBC, high baseline CRP, and a persistently high CRP were all independently associated with overall survival. Presence of metastatic disease was the strongest predictor of overall survival (HR 3.243, 95% CI 2.344 - 4.486, $p < 0.001$). Sex and the other CRP change categories were not significant in multivariable analysis.

Table 2-4. Relationship between patient characteristics, CRP change category and WBC, and overall survival (whole group n=607): Multivariable analysis

Characteristic	HR	95% CI	p-value
Age	1.022	1.007 – 1.036	0.003**
Sex			
Male	1		
Female	1.302	0.949 – 1.787	0.102
Disease extent			
Local/loco-regional	1		
Metastatic	3.243	2.344 – 4.486	<0.001***
WBC	1.037	1.008 – 1.067	0.012*
Baseline CRP	1.002	1.001 – 1.004	0.012*
CRP change category			
Persistently normal	1		
Normal became high	1.19	0.664 – 2.132	0.559
High became normal	0.967	0.549 – 1.704	0.908
Persistently high	1.685	1.045 – 2.717	0.032*

HR: hazard ratio. CI: confidence interval. WBC: white blood cell.

Cox proportional hazards regression * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

2.3.1.2 Subgroup Analyses

2.3.1.2.1 Results for NMSC alone (n=75)

Demographics for the NMSC group are shown in Table 2.5. Median age was 69 (range 28-86) and the group was 53% female. 97% had local or loco-regional disease. 93% were of Caucasian ethnicity.

Table 2-5. Demographics of Included Patients by Diagnostic Subgroup

Characteristic	Excluding NMSC n=532 No. of patients (%)	NMSC only n=75 No. of patients (%)	P value
Age, years			
median and range	64 (23-93)	69 (28-86)	p=0.042
< 65	268 (50%)	31 (41%)	
≥ 65	264 (50%)	44 (59%)	p=0.143
Gender			
Male	224 (42%)	35 (47%)	
Female	308 (58%)	40 (53%)	p=0.455
Disease extent			
Local / loco-regional	412 (77%)	73 (97%)	
Metastatic	120 (23%)	2 (3%)	p<0.001*
Ethnicity			
Caucasian	430 (81%)	70 (93%)	
African-American	81 (15%)	4 (5%)	
Hispanic / Latino/a	7 (1.3%)	0	
Other	14 (2.6%)	1 (1.3%)	p=0.064

Pearson chi-square test. * statistically significant

CRP and CRP change category

Median CRP₁ was 8mg/L (range 2-401). Median CRP₂ was 8mg/L (range 2-185). Median interval between CRP measures was 81 days (range 7 – 961). Of the 75 NMSC patients, 35 (47%) had high CRP₁ (>10mg/L) and 31 (41%) had high CRP₂.

CRP change category distribution is shown in Table 2.6. 43% of patients had a persistently normal CRP while 24% a persistently high CRP and the remaining 33% had a single high CRP.

Table 2-6. CRP Change Category by Diagnostic Subgroup

CRP change category	Excluding NMSC n=532 n (%)	NMSC only n=75 n (%)	P value
Persistently high	241 (45%)	18 (24%)	p=0.001
Normal, became high	72 (14%)	12 (16%)	
High, became normal	92 (17%)	13 (17%)	
Persistently normal	127 (24%)	32 (43%)	

Pearson Chi-Square test

White Blood Cell count

WBC within 7 days of CRP₁ was available for 66/75. Median WBC was $7.5 \times 10^3/\mu\text{L}$ (range 2.9 - 17.9).

Follow-up and survival

Median follow-up was 1030 days (range 14-2455). 12 patients died (16%) and median survival was not reached.

CRP change category and survival

There was no significant difference in survival between CRP change category groups for people with NMSC (Fig. 2.9).

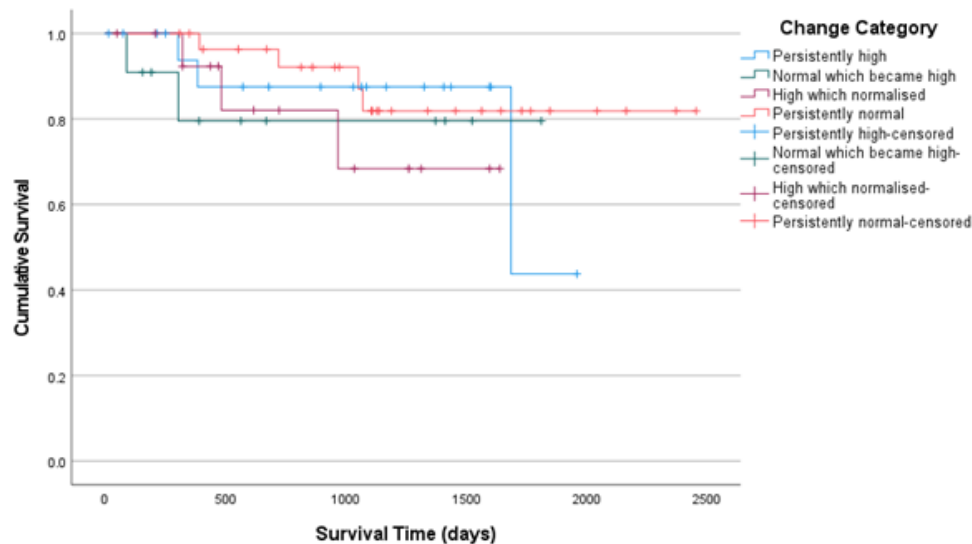


Figure 2-9. Kaplan-Meier survival curve illustrating absence of an association between CRP change category and overall survival in NMSC

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in n=75 adults with NMSC. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

2.3.1.2.2 Results for all cancer sites except NMSC (n=532)

Demographics for the non-NMSC group are shown in Table 2.5. Median age was 64 (range 23-93) and the group was 58% female. 77% had local or loco-regional disease. 81% were of Caucasian ethnicity, 15% African-American, 1.3% Hispanic / Latino/a and 2.6% of other ethnicity.

CRP and CRP change category

Median CRP₁ was 16mg/L (range 0.9-490). Median CRP₂ was 15mg/L (range 0.2-591). The median interval between CRP measures was 95 days (range 7 – 1411). 346 (65%) of patients had high CRP₁ (>10mg/L) and 329 (62%) had high CRP₂.

CRP change category distribution is shown in Table 2.6. 45% of people with cancers other than NMSC had a persistently high CRP, 31% had a single high CRP and 24% had a persistently normal CRP.

White Blood Cell count

WBC within 7 days of CRP₁ was available for 500 of 532 people. Median WBC was $7.9 \times 10^3/\mu\text{L}$ (range 2.8 – 38).

Follow-up and survival

Median follow-up was 1093 days (range 17-2446). 162 died (31%). Median survival was not reached.

CRP change category and survival: All excluding NMSC (n=532)

There was a statistically significant difference in OS when people with cancer other than NMSC were grouped by CRP change category (Fig. 2.10). Median survival was reached only for the persistently high group, median was 1750 days (95% CI 1302-2197 days).

Pairwise comparison (Table 2.7) confirmed that there were statistically significant survival differences between the persistently normal and persistently high CRP groups ($p < 0.001$), persistently normal and normal which became high ($p = 0.048$) and persistently high and high

which normalised groups ($p=0.001$). Differences between other CRP change categories were not statistically significant.

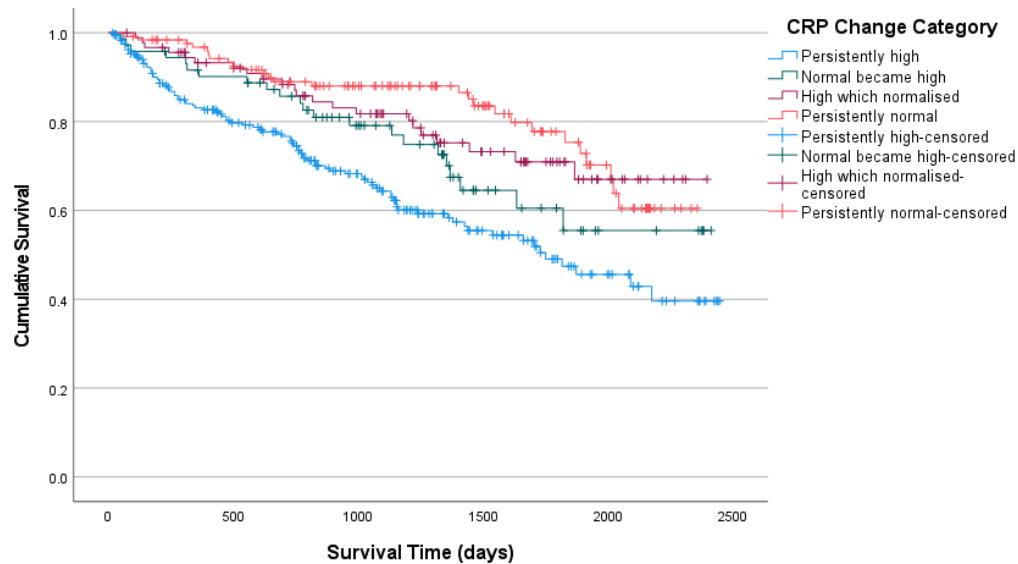


Figure 2-10. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival in people with any cancer site except NMSC

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in $n=532$ adults with cancers other than NMSC. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

Table 2-7. Pairwise comparisons of difference in survival between CRP change categories ($n=532$)

	Persistently high	Normal became high	High which normalised	Persistently normal
Persistently high	-	$p=0.064$	$p=0.001^*$	$p<0.001^*$
Normal became high	$p=0.064$	-	$p=0.307$	$p=0.048^*$
High which normalised	$p=0.001^*$	$p=0.307$	-	$p=0.450$
Persistently normal	$p<0.001^*$	$p=0.048^*$	$p=0.450$	-

*Statistically significant. Pairwise comparison using Log rank (Mantel-Cox) chi-square test.

Multivariable analysis

The results of multivariable analysis are reported in Table 2.8. Increasing age, presence of metastatic disease and high baseline CRP were all independently associated with overall survival. Presence of metastatic disease was the strongest predictor of survival (HR 3.425, 95% CI: 2.461 – 4.768, $p < 0.001$). Sex, WBC and CRP change category were not independent predictors of overall survival.

Table 2-8. Relationship between patient characteristics, CRP change category and WBC, and overall survival (n=532): Multivariable analysis

Characteristic	HR	95% CI	p-value
Age	1.020	1.005 – 1.035	0.008*
Sex			
Male	1		
Female	1.358	0.977 – 1.886	0.068
Disease extent			
Local/loco-regional	1		
Metastatic	3.425	2.461 – 4.768	<0.001*
WBC	1.028	0.996 – 1.061	0.082
Baseline CRP	1.003	1.001 – 1.006	0.001*
CRP change category			
Persistently normal	1		
Normal became high	1.18	0.637 – 2.187	0.599
High became normal	0.882	0.478 – 1.627	0.688
Persistently high	1.612	0.965 – 2.692	0.068

HR: hazard ratio. CI: confidence interval. WBC: white blood cell count

*statistically significant. Mann-Whitney U test to compare medians (age, WBC, CRP). Pearson Chi-Square test to compare frequencies of categorical variables.

Difference between NMSC (n=75) and all excluding NMSC (n=532)

People with NMSC differed significantly from the rest of the group: median age was older and a significantly lower proportion had metastatic disease (Table 2.5). Sex and ethnicity did not differ between the groups (Table 2.5).

There were statistically significant differences between NMSC and the rest of the cohort in terms of CRP₁ and CRP₂ ($p=0.004$ and $p<0.001$, respectively), with NMSC having lower median CRP at both time points (8mg/L versus 16mg/L for CRP₁ and 8mg/L versus 15mg/L for CRP₂).

As shown in Table 2.6, there was also a significant difference in CRP change category between NMSC and all excluding NMSC ($p=0.001$). 45% of people with cancers other than NMSC had a persistently high CRP, compared to 24% in the NMSC subgroup. 24% of the non-NMSC group had a CRP which remained normal, compared to 43% of those with NMSC.

There was no significant difference in WBC between the two groups ($p=0.322$) with median WBC $7.5 \times 10^3/\mu\text{L}$ (range 2.9 - 17.9) in people with NMSC and median $7.9 \times 10^3/\mu\text{L}$ (range 2.8 – 38) in the rest of the group.

Overall survival was not significantly different between people with NMSC and the rest of the group, as seen in Figure 2.11.

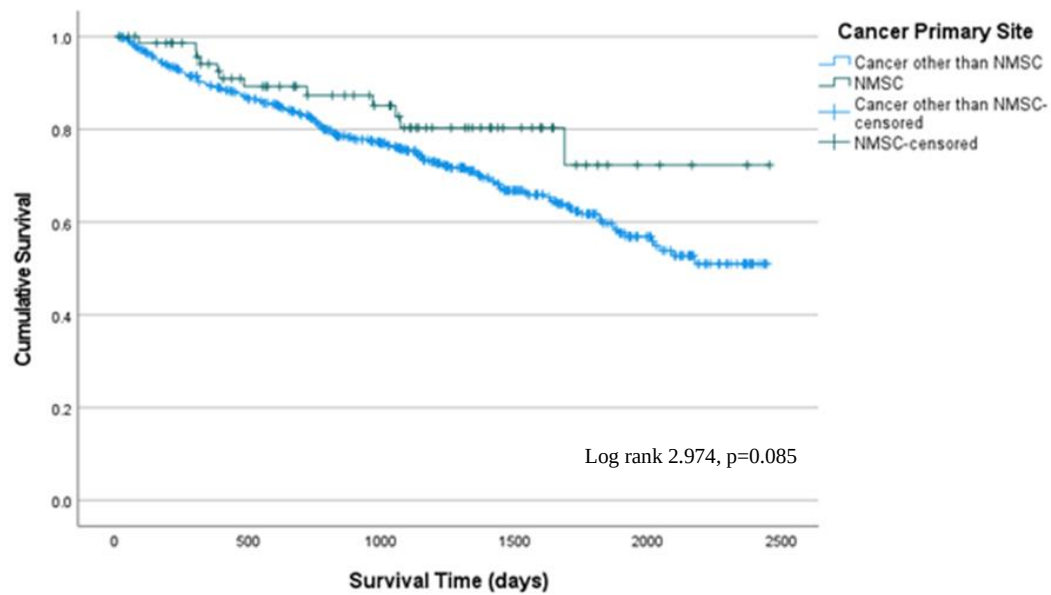


Figure 2-11. Kaplan-Meier survival curve comparing overall survival in NMSC with overall survival in other cancer primary sites

The Kaplan-Meier method was used to assess the prognostic impact of diagnosis (NMSC compared to other cancer primary sites) on overall survival in n=607 adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between NMSC(green line)(n=75) and all other cancer primary site (blue line)(n=532) groups.

2.3.1.2.3 People with multiple primary cancer sites v. single primary site (excluding NMSC)

Demographics are shown in Table 2.9 for people with a single primary cancer site (excluding those with NMSC alone) and for people with multiple primary cancer sites. Age, gender distribution and ethnicity did not differ between the two groups. There was a significantly higher proportion with metastatic disease in the group with multiple primary cancer sites (36%), compared to 20% in the group with a single primary cancer site.

Table 2-9. Demographics of Included Patients: Single and Multiple Primary Cancer Sites

Characteristic	Single primary cancer site n=435 No. of patients (%)	Multiple primary cancer sites n=97 No. of patients (%)	p value
Age, years			
median and range	64 (23-93)	65 (28-87)	p=0.354
< 65	222 (51%)	45 (46%)	
≥ 65	213 (49%)	51 (53%)	p=0.520
Gender			
Male	180 (41%)	44 (45%)	
Female	255 (59%)	53 (55%)	p=0.473
Disease extent			
Local / loco-regional	350 (80%)	62 (64%)	
Metastatic	85 (20%)	35 (36%)	p<0.001*
Ethnicity			
Caucasian	352 (81%)	78 (80%)	
African-American	66 (15%)	15 (16%)	
Hispanic / Latino/a	5 (1%)	2 (2%)	
Other	12 (3%)	2 (2%)	p=0.884

*statistically significant. Mann-Whitney U test to compare median age. Pearson Chi-Square test to compare frequencies of categorical variables.

CRP and CRP change category

There was no significant difference in CRP₁ or CRP₂ between those with multiple primary cancer sites and those with a single cancer site. Mean CRP₁ in the multiple cancer site group was 44 mg/L $\pm$ 68mg/L and 46 mg/L $\pm$ 67mg/L for the single primary site group(p=0.678). Mean CRP₂ for multiple sites was 49 mg/L $\pm$ 68mg/L mg/L $\pm$ 67mg/L for single primary sites (p=0.024).

There was no significant difference in CRP change category between those with single and multiple primary sites, as shown in Table 2.10.

Table 2-10. CRP Change Category: Single and Multiple Primary Cancer Sites

CRP change category	Single primary n=435 n (%)	Multiple primary n=97 n (%)	P value
Persistently high	211 (49%)	44 (45%)	p=0.940
Normal, became high	59 (14%)	15 (15%)	
High, became normal	74 (17%)	17 (18%)	
Persistently normal	91 (21%)	21 (22%)	

Pearson Chi-Square test

Survival

People with multiple primary cancer sites had significantly worse overall survival than people with single primary cancer sites (Figure 2.12). Median survival in those with multiple primary sites was 1639 days (95% CI: 1132-2126 days), median survival was not reached in those with a single primary cancer site.

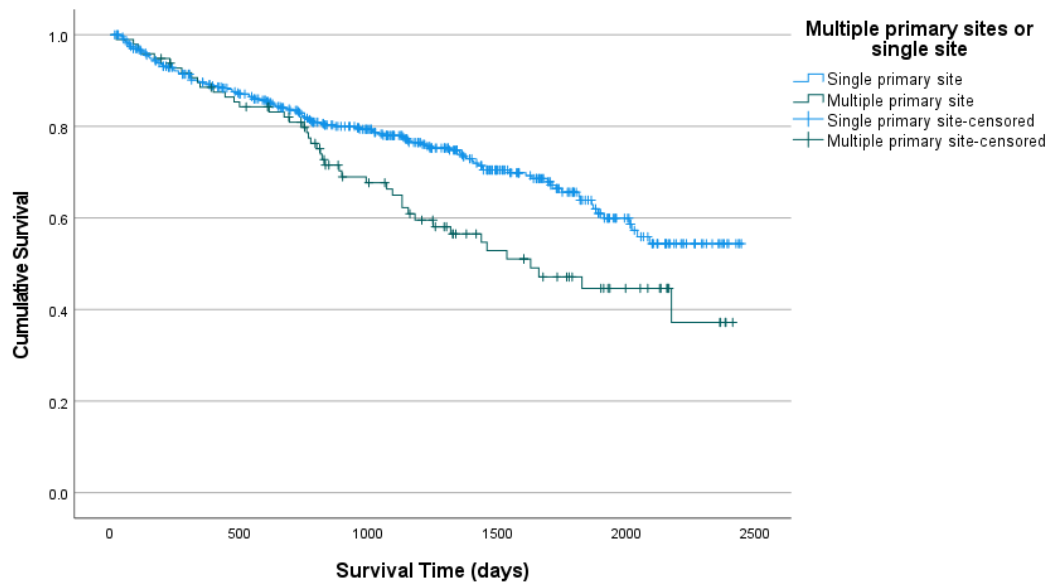


Figure 2-12. Kaplan-Meier survival curve comparing overall survival in people with multiple primary cancer sites with those with single primary cancer sites, excluding NMSC

The Kaplan-Meier method was used to assess the prognostic impact of multiple primary sites (single primary cancer site compared to multiple primary sites) on overall survival in n=532 adults with cancer other than NMSC. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between multiple primary cancer site(green line)(n=97) and single primary site (blue line)(n=435) groups.

Survival when grouped by CRP change category

In people with multiple primary sites, there was a significant difference in survival according to CRP change category (Figure 2.13). Median survival in the persistently high group was 1131 days (95% CI 1017-1244 days), median survival was not reached in the other groups.

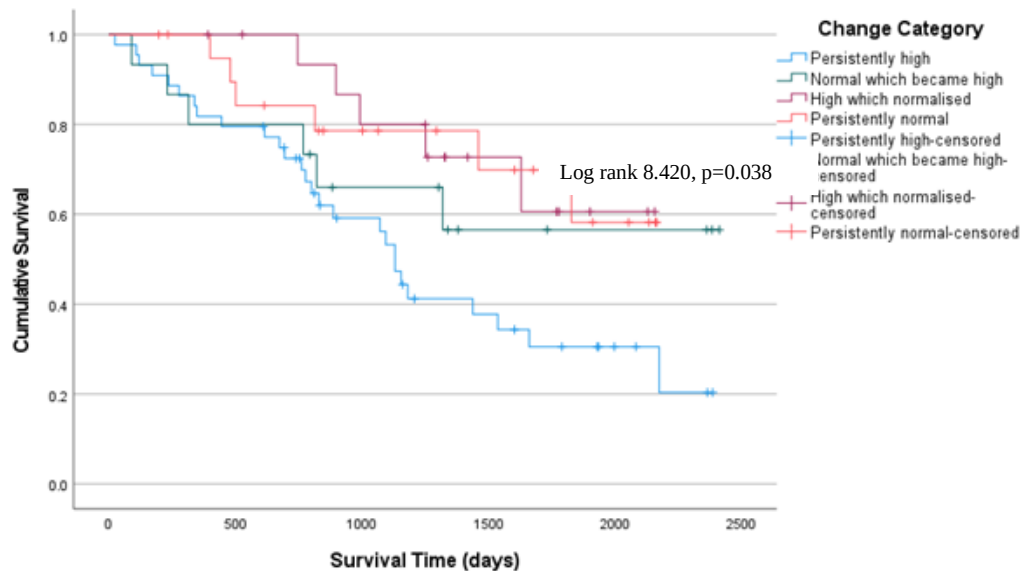


Figure 2-13. Kaplan-Meier survival curve illustrating the prognostic influence of CRP change category on overall survival in people with multiple primary sites (n=97)

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in n=97 adults with multiple primary cancer sites. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

In people with a single primary cancer site, there was a significant difference in survival according to CRP change category (Figure 2.14). Median survival in the persistently high group was 1750 days (95% CI 1302-2197 days), median survival was not reached in the other groups.

Pairwise comparison (Table 2.11) confirmed that there were significant survival differences between the persistently normal and persistently high CRP groups ($p<0.001$) and persistently high and high which normalised groups ($p=0.008$).

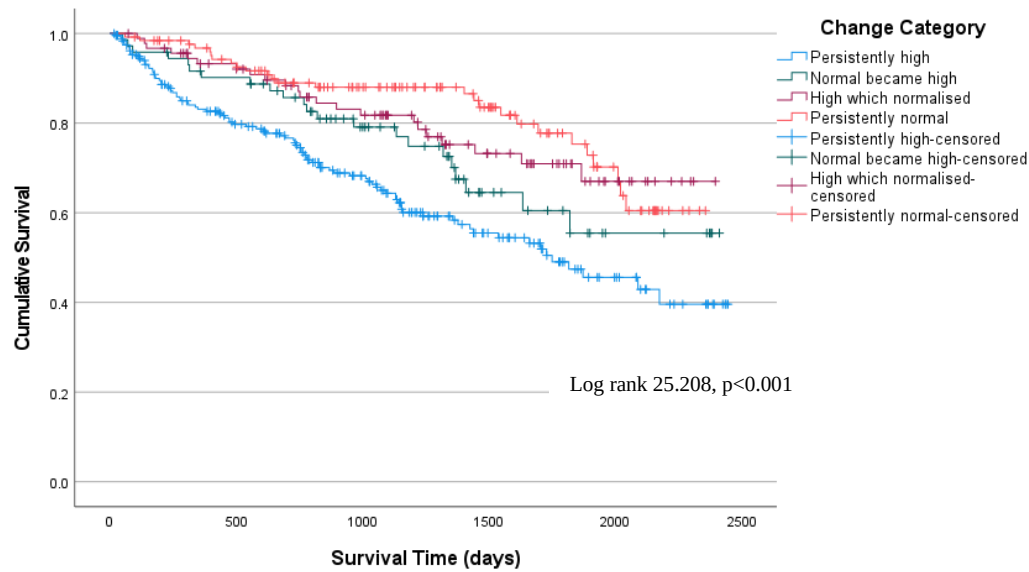


Figure 2-14. Kaplan-Meier survival curve of the prognostic influence of CRP change category on overall survival in people with a single primary cancer site

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in $n=435$ adults with cancer. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

Table 2-11. Pairwise comparisons of difference in survival between CRP change categories in people with a single primary cancer site, excluding non-melanoma skin cancer ($n=435$)

	Persistently high	Normal became high	High which normalised	Persistently normal
Persistently high	-	$p=0.098$	$p=0.008^*$	$p<0.001^*$
Normal became high	$p=0.098$	-	$p=0.481$	$p=0.095$
High which normalised	$p=0.008^*$	$p=0.481$	-	$p=0.522$
Persistently normal	$p<0.001^*$	$p=0.095$	$p=0.522$	-

*statistically significant. Pairwise comparison using Log rank (Mantel-Cox) chi-square test.

The results of multivariable analysis are reported in Table 2.12. Increasing age, presence of metastatic disease and high baseline CRP were all independently associated with overall survival. Presence of metastatic disease was the strongest predictor of survival (HR 2.9, 95% CI: 1.9 – 4.456, $p<0.001$). Sex, WBC and CRP change category were not independent predictors of overall survival.

Table 2-12. Relationship between patient characteristics, CRP change category and WBC, and overall survival in people with a single primary cancer site, excluding non-melanoma skin cancer (n=435): Multivariable analysis

Characteristic	HR	95% CI	p-value
Age	1.020	1.001 – 1.039	0.035*
Sex			
Male	1		
Female	1.423	0.95 – 2.132	0.087
Disease extent			
Local/loco-regional	1		
Metastatic	2.9	1.9 – 4.456	<0.001***
WBC	1.029	0.992 – 1.067	0.125
Baseline CRP	1.003	1.00 – 1.006	0.038*
CRP change category			
Persistently normal	1		
Normal became high	1.13	0.525 – 2.442	0.752
High became normal	0.775	0.365 – 1.678	0.508
Persistently high	1.524	0.815 – 2.852	0.0187

HR: hazard ratio. CI: confidence interval. WBC: white blood cell.

Cox proportional hazards regression * $p<0.05$ *** $p<0.001$

2.3.2 Dataset 2: St James's Hospital

2.3.2.1 Demographics

Data from 124 patients were included. Demographics of included patients are given in Table 2.12. Median age was 63 years (range 40-78). 83% were male. All had oesophageal adenocarcinoma (OAC), none had metastatic disease. All were treated with curative intent: all had surgery, 70% had multimodal treatment (radiotherapy and/or chemotherapy). Ethnicity was not recorded.

Table 2-13. Demographics of Included Patients (n=124)

Characteristic	No of patients (%)
Age, years	
median and range	63 (40-78)
< 65	76 (61%)
≥ 65	48 (39%)
Gender	
Male	103 (83%)
Female	21 (17%)
Cancer site	
OAC	124
Disease extent	
Local / loco-regional	124 (100%)
Metastatic	0
Treatment received	
Multimodal	87 (70%)
Surgery only	37 (30%)

OAC: Oesophageal Adenocarcinoma.

2.3.2.2 CRP and CRP change category

Median CRP₁ was 2.5mg/L (range 0.8-77). Median CRP₂ was 4.6 mg/L (range 1-97).

20 patients (16%) had high (> 10mg/L) CRP₁ and 30 (24%) had high CRP₂.

CRP change category is shown in Table 2.13. Two-thirds of the cohort had a normal CRP at both time points, 28% had a single high CRP and 7% had a persistently high CRP.

2.3.2.3 Follow-up and survival

Median follow up was 1550 days (range 45-2822). 54 people (44%) died. Overall survival at five years in each CRP change category group is shown in Table 2.13.

Table 2-14. CRP Change Category and Five Year Overall Survival (n=124)

CRP change category	n (%)	5 year OS (%)
Persistently high	8 (7%)	37%
Normal, became high	22 (18%)	45%
High, became normal	12 (10%)	42%
Persistently normal	82 (66%)	63%

2.3.2.4 Factors associated with Survival

Baseline CRP (CRP₁)

As shown in Figure 2.15, there was no significant difference in survival between high and low CRP₁ groups (high CRP median survival 1111 days (95% CI 289 – 1932), low group median not reached).

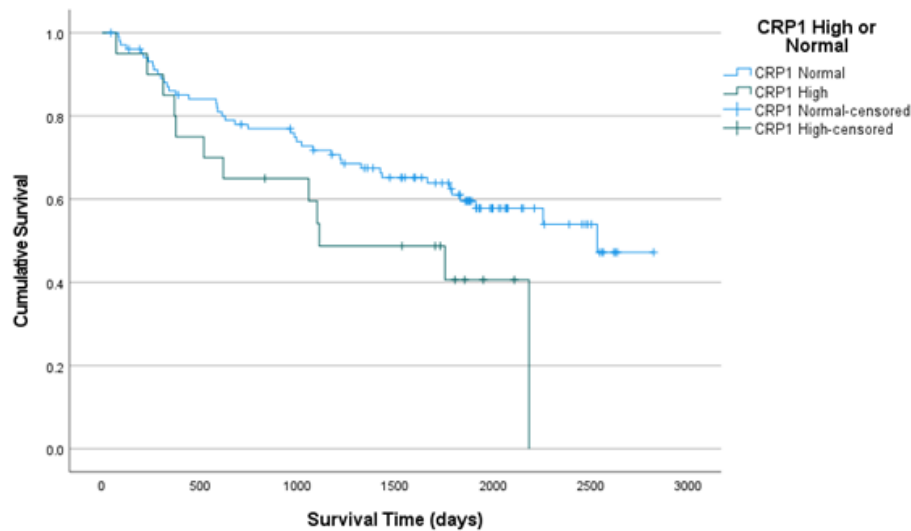


Figure 2-15. Kaplan-Meier survival curve illustrating the prognostic influence of baseline CRP (CRP₁) on overall survival in people with oesophageal adenocarcinoma

The Kaplan-Meier method was used to assess the prognostic impact of baseline CRP(CRP₁) on overall survival in n=124 adults with oesophageal adenocarcinoma. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between normal CRP (blue line) and high CRP (green line) groups.

CRP Change Category

There was no significant difference in survival between CRP change category groups, as shown in Figure 2.16.

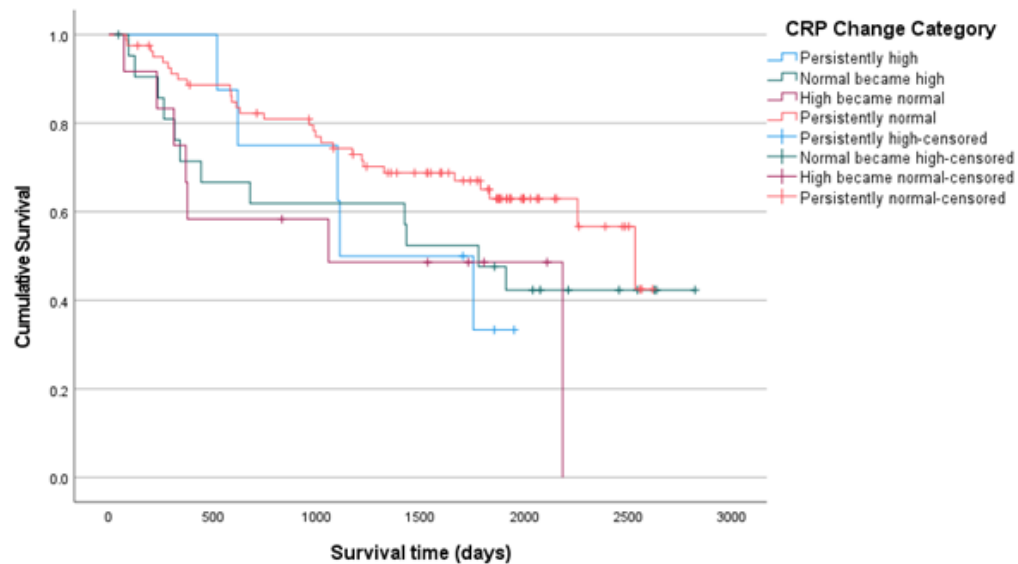


Figure 2-16. Kaplan-Meier survival curve illustrating absence of an association between CRP change category and overall survival in oesophageal adenocarcinoma

The Kaplan-Meier method was used to assess the prognostic impact of CRP change category on overall survival in n=124 adults with oesophageal adenocarcinoma. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between CRP change category groups.

Age

There was no significant difference in survival between people aged greater than and less than 65.

Sex

There was no significant difference in overall survival between men and women,

Treatment Received

There was a statistically significant difference in survival between treatment groups: those treated with surgery only had longer survival (median not reached) than those who received multimodal treatment (median 1789 days, 95% CI 1033-2544 days) (Fig. 2.17).

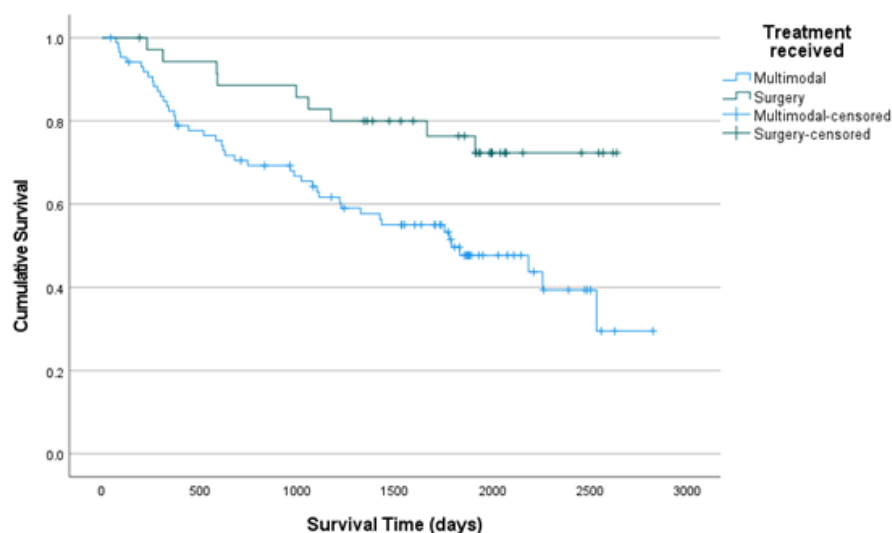


Figure 2-17. Kaplan-Meier survival curve illustrating no significant association between sex and overall survival in oesophageal adenocarcinoma

The Kaplan-Meier method was used to assess the prognostic impact of sex on overall survival in n=124 adults with oesophageal adenocarcinoma. Log rank (Mantel-Cox) chi-square test was used to assess statistical significance of survival differences between surgery only (green line) and multimodal(blue line) groups.

2.4 Discussion

This study assessed the potential of serial CRP measurements, or CRP kinetics, to predict survival in cancer, using two independent datasets.

A large dataset of people with mixed cancer sites was examined first. Older age, male sex and more advanced disease were all associated with worse survival, consistent with the literature (163, 248), although sex lost significance in multivariable analysis. A high initial CRP was associated with worse overall survival. CRP change category also discriminated differences in survival between groups. Both baseline CRP and CRP change category remained significant in a multivariable analysis, although baseline CRP was a very weak predictor. Furthermore, there was no significant difference in survival between people with a single high CRP, compared to those whose CRP remained normal. These observations suggested that serial CRP measures may have a value over that of a single CRP measure. However, on subgroup analyses excluding people with NMSC or multiple primary cancer sites, CRP change category was no longer a significant predictor of survival.

The same approach was used in a separate independent cohort of OAC only. The SJH cohort was a much more homogenous group; all had OAC treated surgically with curative intent. Neither initial CRP nor CRP change category were associated with survival in this cohort. The only independently significant predictor of survival in the SJH cohort was treatment received, with surgery alone associated with better survival. This is an unexpected finding, given that neoadjuvant therapy is superior to surgery alone in OAC (249).

Findings of this work do not provide convincing evidence for CRP kinetics to predict survival in an unselected cancer cohort, nor in OAC. These negative results conflict with reports in the literature, which suggest a promising role for CRP kinetics, as noted in the introduction. Publication bias may account, at least in part, for this conflict. Despite the clear ethical obligation to publish all research results (250), negative studies of prognostic markers in cancer are very rarely reported. Negative reports comprised less than 1.5% of papers retrieved in a systematic review (251).

It is also possible that CRP kinetics may not be a predictor in all cancers but have a predictive role in certain cancers only. Much of the previously published work in CRP kinetics is in

urological cancers (252). Numbers with urological cancers in this present work were too small (n=28) to conduct subgroup analysis of CRP kinetics in that group alone. The literature on CRP kinetics in oesophageal cancer is very limited. Otowa *et al.*, found that change in a CRP-based prognostic score predicted survival in oesophageal squamous cell carcinoma(OSCC) (241). Another study in OSCC found that a persistently high CRP (> 10mg/L) at one month post-operatively was associated with significantly worse overall survival (240). It is notable that both of these studies were in OSCC, whereas the present work is the first to include OAC only. These cancers are increasingly recognised as being clinically distinct (132). It is possible that the findings in OSCC do not apply to OAC.

Another possibility is that CRP kinetics are more strongly predictive in later stage disease, which constituted only one-fifth of the CC cohort and none of the SJH cohort. The majority of studies which reported an association between CRP kinetics and survival were in advanced or metastatic disease (226, 229-231, 236, 253-257).

It is also possible that CRP kinetics do not, in fact, have any meaningful role in the prediction of survival in cancer. The association of a persistently high CRP with survival could, for example, reflect unresolving or recurrent infection. None of the studies which report CRP kinetics to predict survival in cancer (224-226, 229-231, 234, 236, 238, 239, 241, 253-257) address infection as a potential confounder, although Wang *et al.*, and Riedl *et al.*, acknowledge this limitation (230, 239). In this study, as reason for CRP test was not recorded in the CC dataset, it is possible that CRP was measured because of suspected infection, a major possible confounder. The relatively high CRP levels recorded, which may have been due to acute infection, lend weight to this theory, though WBC was normal for most, which argues against the presence of clinically significant infection. CRP was measured routinely in the SJH cohort and levels were lower than in the CC cohort. There was no association found between elevated CRP and survival in the SJH cohort, possibly due to the absence of infection as a confounder.

The influence of another possible confounder in the CC dataset - the inclusion of people with NMSC - was examined through subgroup analysis. Seventy-five people were recorded as having NMSC. NMSC is often excluded from cancer mortality statistics and studies(258). When people with NMSC were excluded from the present study, CRP kinetics were not a significant predictor of survival. Although baseline CRP remained an independent predictor of survival, the HR of 1.003 is of very questionable clinical value. Given that NMSC is often

not fatal(258), it is possible that this group died of diseases other than cancer. Likewise, their elevated CRP may have been due to intercurrent infection or chronic inflammatory disease, which may have been treated in the interval between the two measures. A further subgroup analysis, excluding those with multiple primary cancer sites, showed similar results. The reason for this is not evident. It was not recorded whether the multiple cancers were synchronous or metachronous. It was also unknown which was being treated at the time of CRP measurement and which was referred to when disease extent was recorded. This group had significantly shorter overall survival but their mean CRP was similar to those with a single cancer. There may have been individuals within that group who had multiple synchronous cancers, a high CRP and short survival; this could have influenced the overall group results.

There are several limitations to this study. This was a secondary analysis of two datasets collected for other studies. Different data points were available in each dataset, which hampered comparison between the two, and each dataset lacked some potentially useful information for analysis. WBC was not available for the SJH dataset, thus comparison of its prognostic value to that of CRP and CRP kinetics was not possible. The CC dataset, meanwhile, lacked detail on tumour stage and histology, both key prognostic factors. Information on treatment received was only recorded in line with the protocol for the original study (242) i.e., only treatments and procedures which occurred within four weeks of the person's highest recorded CRP were included. Similarly, albumin was also only recorded if it had been measured at the time of that highest CRP. Since the highest CRP may well have occurred during an infection, there was significant potential for bias in albumin, as another acute phase reactant (259), precluding analysis of GPS/mGPS or CAR in the present study. Information was also lacking on medications which may have influenced CRP levels.

CRP was recorded at varying time-points in the CC cohort, both in terms of time from diagnosis and the intervals between CRP measurement dates, which ranged from one week to almost four years. This reflects a real clinical setting but many confounders may have intervened in that time, which could have influenced survival. It is unclear what the optimum time interval between CRP measurements is, with measurement intervals in other studies ranging from 2 days (235) to six months (234). The answer to this may not be straightforward. Even with a well-established and widely-studied biomarker such as PSA, the optimum frequency of measurement is not clear (164).

The small sample size in the SJH dataset may have led to a Type II error with a true survival difference missed (260).

This study also has a number of strengths. The CC dataset was large and allowed examination of the role of CRP kinetics in a real-world setting. The cohort included a mix of the most common cancer primary sites and was broadly representative of US cancer figures in distribution of cancer primary sites and age profile. Details of treatment modality (ies) received were not available for this group but included the most commonly used modalities of chemotherapy, radiotherapy and surgery. Subgroup analyses were conducted to better understand potential confounders, such as inclusion of people with NMSC in the CC cohort and presence of multiple primary cancer sites for some individuals. The SJH dataset included prospectively collected data with consistent timing of CRP measures. This study applied findings from one cohort to another, independent, cohort. Such external validation is recommended, yet is rarely reported in the literature (261). The proposed classification is straightforward and could potentially be calculated automatically by computer software for clinician ease of use.

Disease extent was the strongest predictor of survival in the CC cohort in this study. Assessment of disease extent is already a cornerstone of planning cancer treatment. This study does not support the routine integration of serial CRP measurement into clinical practice at present, with associated cost and burden to patients, small though these may be. This work highlights that clinicians must beware of studies reporting prognostic markers as the results may not be applicable to their own patients (251). External validation and critical interrogation of the included cohort for possible confounders, as in this study, may demonstrate the limited applicability of a given marker.

Even if future studies were to confirm a role for serial CRP measurement in cancer prognostication, further work would be required before it could be used in clinical practice. In prostate cancer, there are guidelines on methodology to calculate PSA kinetics (PSA doubling time); these address the minimum number of measurements which must be included, advise that the same measurement method should be used and propose minimum intervals between measurements (262). Similar guidance would be required to support eventual clinical use of CRP kinetics.

There are several areas which future research in this area could address. Future studies could examine other ways of analysing longitudinal change in CRP. Most analyse based on grouping by a specific cutpoint value for CRP, as in this study. This system is very straightforward. However, alternative approaches to assessment of CRP kinetics have been described. These include ratios, CRP velocity and absolute change. In an era of electronic patient records (EPR), clinicians have ready access to serial blood results and information technology (IT) could automate calculation of such measures. Arpin *et al.* calculated change based on a ratio of the individual's initial CRP, in a group with small cell lung cancer treated with chemotherapy and found an association between a doubling in CRP and chemotherapy response(235). Rabello *et al.*, used a similar approach in people with cancer with hospital-acquired pneumonia and found people whose CRP reduced quickly had better survival (263). In non-malignant conditions, Zahler and Milwidsky *et al.* showed that a higher CRP velocity (change in CRP divided by change in time, measured in hours) predicted risk of acute kidney injury, new-onset atrial fibrillation and 30 day mortality post STEMI (220-222). Longitudinal change in CRP-based scores (CAR and mGPS) has also been assessed in a small number of studies with a higher mGPS or CAR associated with shorter survival (241, 264). Absolute change in CRP following resection for colorectal cancer was studied in the PREDICT study, with change of ≥ 50 mg/L between two post-operative days associated with anastomotic leak (219). Percentage change in CRP has also been examined; Suzuki *et al.*, found a decrease of $\geq 25\%$ from initial CRP was associated with better response to immunotherapy in metastatic renal cell carcinoma (265).

Use of CRP kinetics in combination with other prognostic markers may be of value. Wang *et al.*, combined CRP kinetics with neutrophil-to-lymphocyte ratio (NLR) kinetics, although they found CRP kinetics alone to have better predictive accuracy than the combined metric (230).

Another avenue for future research is to investigate the role of CRP kinetics in prediction of response to immunotherapy or to track secondary resistance to immunotherapy. Recently, Kijima *et al.*, proposed that early changes in CRP level could be a biomarker for response to pembrolizumab in advanced urothelial cancer (254). They found that a persistently high CRP was independently predictive of shorter overall survival, as did Tachibana *et al.*, in renal cell carcinoma. Similarly, Suzuki *et al.*, found that a reduction in CRP was associated with better response to nivolumab in renal cell carcinoma and Riedl *et al.* showed an increasing CRP with worse survival in NSCLC treated with ICI (239, 265). In contrast,

Fukuda *et al.*, found that an initial flare in CRP, followed by a reduction in CRP, was associated with a better response to ICI and longer survival in metastatic renal cell carcinoma (253). Klümper *et al.* replicated this finding and, very recently, identified the same pattern in NSCLC (237, 266). Ozawa *et al.*, also found that early increase in CRP was associated with response to nivolumab or pembrolizumab in non-small cell lung cancer (NSCLC), though no survival difference was seen(236). This seemingly paradoxical increase in CRP may represent immune activation. Boland *et al.*, proposed that immune cell infiltration into the tumour, which can cause a transient increase in tumour size (267), could also cause an initial increase in tumour markers such as CA-125, though this increase was not associated with a clinical benefit in their ovarian cancer cohort (268). As a host-derived marker of inflammation, it is very plausible that an initial rise in CRP reflects the host response to immunotherapy. Future studies in immunotherapy are needed to determine optimum timing of CRP measurement and understand which kinetic changes are most clinically significant.

2.5 Conclusions

This study assessed the relationship between longitudinal change in CRP and CRP kinetics in two datasets. Each dataset had its own value and own limitations. The CC dataset was large and included a diverse range of cancer sites, mimicking clinical practice, but was marred by the unknown indication for CRP measurement and absence of data on traditional prognostic indicators. The SJH dataset was small but comprised a homogenous cohort of surgically-treated OAC with data collected as part of a prospective study and at pre-specified time points. CRP kinetics, as assessed by a straightforward classification based on values above and below 10mg/L in two serial CRP measurements, was studied in both datasets. CRP kinetics was associated with survival in the CC dataset. In multivariable analysis, a persistently high CRP was independently predictive of overall survival but statistical significance was lost when people with NMSC were excluded. This may be due to confounding by infection or concurrent chronic inflammatory disease. Statistical significance was also lost when people with more than one primary cancer site were excluded, reason for this is not evident but may include confounding by individuals with synchronous primary cancers, a high tumour burden and CRP and short survival.

In the SJH dataset, there was no association between CRP kinetics and survival. Possible explanations include a Type 2 error due to small sample size or that CRP kinetics is not prognostic in this group.

The present study does not support the clinical application of a CRP kinetics classification system for prediction of survival in unselected cancer cohorts nor in OAC. It highlights that clinicians must beware of novel prognostic markers, particularly those which have not been thoroughly assessed for potential confounders and validated in separate cohorts, as there is a publication bias toward positive results and any published results may not be generalisable to the clinician's own patient population.

Future areas for research in this area include investigation of alternative measures of CRP change over time, such as CRP percentage change or change ratios, combination with other prognostic markers and studies of the role of CRP kinetics in novel treatment modalities, particularly immunotherapy.

3 CRP and CRP -Based Prognostic Scores in Oesophageal Adenocarcinoma: A Systematic Review

3.1 Introduction

In recent years, a high CRP has been proposed as a prognostic marker for survival in most common cancers (242), including oesophageal cancer (269). There has also been growing interest in prognostic scoring systems based on the systemic inflammatory response (187). As the most commonly used marker of inflammation (16), it is unsurprising that many of these scores incorporate CRP. Several combine CRP with other laboratory parameters, frequently albumin (189). Examples include the Glasgow Prognostic Score (GPS) (190), modified GPS (mGPS)(270) and CRP-Albumin Ratio (CAR) (271). The GPS was first described in 2003 by Forrest *et al.*, (190) and includes albumin and CRP, scoring is shown in Table 3.4 in Results. The same group later developed the modified GPS (mGPS), as reported by McMillan *et al.*, in 2007 (270). The mGPS, as its name suggests, modifies the scoring system but includes the same parameters, as shown in Table 3.4. CAR was first reported in acute medical admissions (271) and sepsis (272). In 2015, several papers were published which reported associations between a high CAR and shorter survival in cancer, including hepatocellular carcinoma (273), renal cell carcinoma (274) and oesophageal squamous cell carcinoma (275). As shown in Table 3.4, the CAR is a ratio of CRP to albumin. Aside from these well-known scores, as noted in the introduction to this thesis, there are also multiple new scores in the recent literature (187).

Prognostic scores are widely used in clinical practice (161). From the Apgar score in newborns (where a low score at 5 minutes post birth is associated with a dramatically increased risk of cerebral palsy) (276) to the P-POSSUM score which predicts in-hospital mortality after surgery (277), clinicians are familiar with using scores to prognosticate in various settings. Evidence suggests that clinicians are in favour of using prognostic scores to prognosticate in cancer: Watt *et al.*, (278) found 80% of surveyed clinicians believed a measure of systemic inflammation should be included in future clinical guidelines for cancer care. A recent systematic review of systemic inflammation-based prognostic scores in operable cancer concluded that such scores should become part of routine pre-operative workup and follow-up (187). Another review by the same group concluded that these scores should be used in future randomised controlled trials(279).

Despite this, markers of systemic inflammation as prognostic tools are still not widely used in routine clinical practice (278). A barrier to the clinical implementation of CRP and CRP-based scores in clinical practice is the range of different CRP cutpoints and scores reported in the literature, with no guidance for clinicians regarding which is best. CRP cutpoints ranging from 2.6mg/L (280) to 10mg/L (281) have been reported as prognostic in oesophageal cancer. Furthermore, the literature on CRP and CRP-based scores is mixed. In oesophageal cancer, Feng *et al.*, (282) found that GPS predicted survival, Kimura *et al.*, (283) that the mGPS predicted survival and Kunizaki *et al.*, (284) that the CAR did so. However, Jomrich *et al.*, found none of the 3 scores prognostic of survival in their oesophageal cancer cohort (137). Many studies of CRP and CRP-based scores include small numbers and may lack power to detect significant differences between groups (159). A systematic review uses a scientific strategy to collect, appraise and synthesise all the relevant studies which address a particular clinical question (285), while meta-analysis uses statistical methods to summarise the results of multiple primary studies (285). As the number of studies into prognostic factors grows, often with contradictory findings, systematic review and meta-analysis is essential to guide practice and research (170). Riley *et al.*, recently described an “urgent need” for systematic reviews of prognostic factors (170).

Previous systematic reviews have examined the value of CRP (269) and CRP-based scores (286-289) in oesophageal cancer. Unfortunately, they included either exclusively or a large majority of studies in OSCC (195, 290). Wang *et al.*, (288) found the GPS prognostic in OSCC but could not examine its association with OAC as data were not reported for OAC alone in included studies. Dolan *et al.*, found an association between GPS/modified GPS(mGPS) and survival in operable OC but did not distinguish between OSCC and OAC (187). Emerging research from the Cancer Genome Atlas clearly identifies OAC/OGJ as biologically (131), genomically (131) and clinically (132) distinct from OSCC thus findings from studies in OSCC may not be generalisable to patients with OAC. Furthermore, almost all included studies were conducted in East Asia (269, 288) and, as Wu *et al.*, (286), note, the results may not be applicable to patients in other regions. Similarly, Dolan *et al.*, speculate that there may be differences in the tumour and the host inflammatory response between ethnicities (187).

The role of CRP in OAC has been identified as requiring further study (291). To our knowledge, no review to date has systematically examined the prognostic value of CRP and

CRP-based scores in this group. The aim of the current study was to establish which pre-treatment CRP cutpoint and CRP-based score best predict survival in OAC/OGJ.

Specific Hypothesis of this Study

CRP and CRP-based scores can be used to predict survival in OAC and OGJ

3.1.1 Specific Aims of this Study: Review Questions

Primary:

1. What CRP cutpoint is best supported by current evidence to predict survival in OAC / OGJ?
2. Which CRP-based score is best to predict survival in OAC / OGJ?

Secondary:

1. What CRP threshold levels are reported as being used in OAC / OGJ?
2. What CRP-based scores are reported as being used in OAC / OGJ?

3.2 Methods

3.2.1 Summary

A systematic literature review and meta-analysis was performed and is reported in line with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guidelines(292). The review was registered in the PROSPERO international prospective register of systematic reviews (CRD42020212520).

3.2.2 Identification of Studies

Six electronic databases were searched (EMBASE, Medline, Web of Science, Cochrane database, Scopus, CINAHL) from inception to 1st October 2020. The search strategy was developed with a specialist subject librarian, Mr David Mockler, and is shown in Appendix 1. A hand search of four key journals in the field (Annals of Surgical Oncology, Journal of Gastrointestinal Surgery, Journal of Surgical Oncology, Journal of Clinical Oncology) was undertaken for 2011-2020. Reference lists of included studies and of relevant systematic reviews were reviewed. Forward citation searching of seminal articles was also conducted.

3.2.3 Inclusion criteria

Articles were included if they met all of the following criteria:

- (1) included adults (aged ≥ 18) with oesophageal adenocarcinoma. Studies of OAC which included junctional tumours were included; other cancer sites were excluded.
- (2) reported blood CRP and / or a blood CRP-based score and
- (3) provided Hazard Ratios (HR) for overall (OS) or cancer-specific survival (CSS) based on CRP and / or a CRP-based score, or where HR could be calculated from data provided in the paper

3.2.4 Exclusion criteria

Articles were excluded if they were

- (1) Conference abstracts
- (2) editorials, expert opinion, reviews
- (3) case series of fewer than five OAC cases

- (4) were based on nonhuman research;
- (5) included histology other than carcinoma
- (6) were written in languages other than English or French

Where studies reported duplicate or overlapping reports of the same study, only the most complete report (largest and / or most recent) was included (293).

3.2.5 Study Selection

Two investigators (CL and either Dr Larissa Higgins (LH), Dr Jim O’Connell (JOC) or Dr Niamh O’Donoghue (NOD)) independently screened all papers retrieved for eligibility for full-text review, based on title and/or abstract. All potentially eligible full text articles were independently screened by two investigators (CL and LH, JOC or NOD). Any discrepancies were resolved by discussion.

3.2.6 Data Extraction

A standardised data collection form was piloted, amended and agreed by two investigators (CL, LH). One author (CL) used a standardised data extraction form to extract data from included full text studies. A second author (LH, JOC or NOD) independently checked accuracy of data extracted. Any discrepancies between the two reviewers were discussed.

Extracted data included: country(ies) where the study was conducted, study design (retrospective or prospective), sample size, demographics of included patients (age, gender, cancer site, stage, histology, histopathologic and clinical operative findings including tumour regression grade and resection margin status), treatment(s) received, timing of CRP measurement, whether infection / inflammatory disease status was considered, distribution of CRP levels, CRP cutpoint used and rationale for that cutpoint, CRP-score(s) used and how calculated, follow-up period, outcomes reported (OS / CSS / both). HR and 95% confidence intervals were recorded. Study authors were contacted 1. where it was unclear if OAC was included 2. to request HR for OAC alone, where OAC was included and 3. where there was missing data or it was unclear if a paper was eligible for inclusion.

3.2.7 Risk of bias assessment

The Quality in Prognosis Studies (QUIPS) (294) tool (Appendix 2), which is appropriate for prognostic factor reviews (170), was used to assess risk of bias (RoB) for all included studies. As is recommended (295), two authors (CL and LH, JOC or NOD) independently assessed RoB.

3.2.8 Data Analysis: Narrative synthesis and Meta-analysis

All studies meeting eligibility criteria were included in narrative synthesis.

Where two or more studies reported the same population, CRP cutpoint or CRP-based score, treatment received and outcome measure (OS or CSS), meta-analysis was conducted.

A random effects model was used as recommended for prognostic factor reviews due to the high anticipated heterogeneity in such reviews (170). The random effects model for meta-analysis assumes that the associations between the prognostic factor and the outcome of interest are different but related (296). This approach has been recommended since it is considered unlikely that the true prognostic effect of a given factor will be identical in different studies (170).

Where both univariable and multivariable analysis were reported, HR from multivariable analysis was used, as recommended (170). The method described by Parmar (297) and Tierney (298) and the associated calculation spreadsheet (298) was used to calculate the natural logarithm of the HR ($\ln HR$) and its standard error ($SE_{\ln(HR)}$) from HR and 95% CI. mGPS and GPS were combined for meta-analysis as in other reviews by the group which devised these scores (187, 188). Where studies differed in their analysis of mGPS/GPS e.g., score 0 / 1 versus 2 or score 0 versus 1 / 2, their results were combined as “any high score”. This is addressed further in the discussion below.

Sources of potential heterogeneity were noted during data collection. Statistical heterogeneity was quantified using the I^2 statistic (299). I^2 is a measure of how much of the variability in effect estimates is due to heterogeneity, as opposed to being due to chance (300). As per the Cochrane handbook (300), I^2 values of 0-40% were deemed “might not be

important, 30-60% “may represent moderate heterogeneity”, 50-70% “may represent substantial heterogeneity” and 75-100% “considerable heterogeneity”.

Subgroup analyses were conducted based on important clinical and study characteristics: treatment type, TNM stage, resection margin status and geographical region where the study was undertaken (United Kingdom (UK) or Ireland, the remaining studies from mainland Europe were too dissimilar to combine). Subgroup analysis by Tumour Regression Grade (TRG) was not conducted because of variable reporting of this characteristic. Subgroup analyses of OAC and OGJ alone were not possible as the two relevant studies in OAC alone were not sufficiently similar and there was only one study which reported OGJ alone. A funnel plot was not appropriate as there were fewer than ten studies included in meta-analysis(301).

P values were two-tailed and $p < 0.05$ was considered statistically significant.

3.2.9 Certainty assessment

The PRISMA 2020 statement recommends that authors of systematic reviews report an assessment of certainty in the body of evidence (292). The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach to quantify confidence in estimates of a body of evidence is commonly used and was devised for interventional studies (302). Although it has been proposed to apply GRADE to prognostic factor research (302), more recent expert guidance has cast doubt on the applicability of the GRADE domains to this type of review (170). Thus, the GRADE domains have not been used in assessing the strength of evidence for this review but the limitations of the available evidence are addressed in detail in the discussion.

3.2.10 Software

Microsoft Excel (version 2102, Microsoft Corporation, Washington) was used for data extraction and analysis and EndNote 20 (Clarivate, Philadelphia) for reference management. Covidence systematic review software (Veritas Health Innovation, Melbourne) was used for reference screening and meta-analysis was conducted with RevMan (*Review Manager (RevMan). Version 5.4, The Cochrane Collaboration, 2020*).

3.3 Results

3.3.1 Study Identification, Selection and Inclusion

Figure 3.1 summarises study selection: the total number of studies identified through electronic and manual searches, those excluded and reasons for exclusion. Total number of records identified through database searching was 1567. After de-duplication 819 records remained for title and abstract screening. 720 were excluded, leaving 99 records to be retrieved for full-text review. All were retrieved and assessed for eligibility for inclusion by two independent reviewers, as outlined in the Methods section. 91 did not meet inclusion and exclusion criteria, as itemised in Figure 3.1, and were excluded. 5 were conference abstracts only and therefore no full text was available.

No additional records were identified through hand-searching. 28 corresponding authors were contacted for further information, 12 responded.

Eight reports (of eight studies) were included for narrative review (Table 3.1).

Six studies were included for meta-analysis. Two studies (280, 303) were excluded from meta-analysis because they were insufficiently similar to other studies to combine.

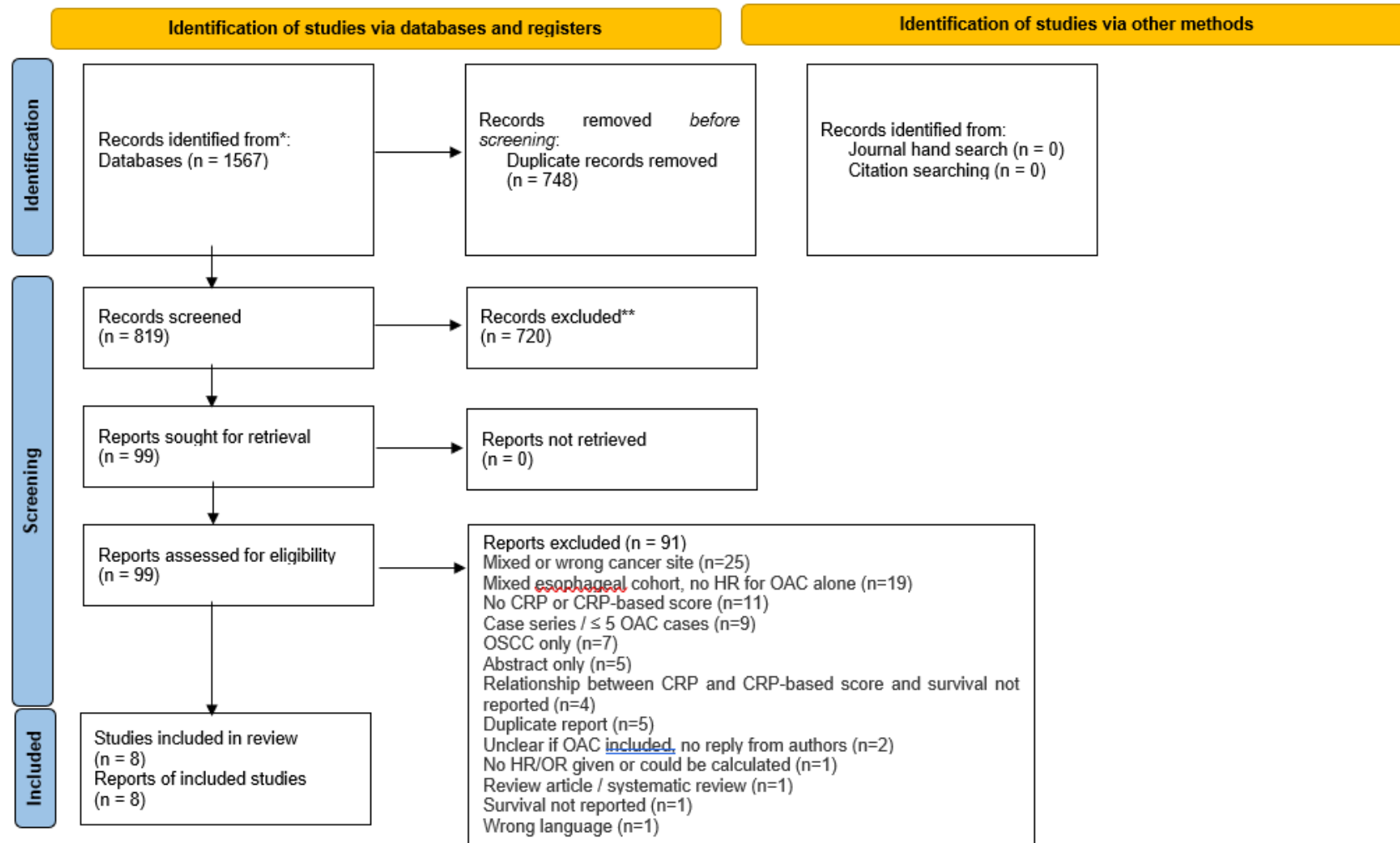


Figure 3-1. PRISMA 2020 flow diagram(Page et al., 2021) summarising the study identification and selection process

3.3.2 Included Studies

3.3.2.1 Study Characteristics

Table 3.1 lists author name, year of publication, country, included cancer sites, histologies, number of included patients, sex and age distribution of included patients, treatment modalities, disease stage (TNM), tumour regression grade, resection margin status. Study design, median follow-up and survival outcome (OS/CSS) for all included studies.

All included studies were published between 2011 and 2020. Four of the eight papers (50%) had been published in the two years preceding the review.

All of the included studies were conducted in Europe, five in Ireland or the United Kingdom and three in mainland Europe. One study included patients from two hospital sites, one study was unclear about how many sites were included and six were single-centre studies. All were retrospective. Three of the included studies provided data on how many patients were excluded and / or missing data (195, 304, 305).

3.3.2.2 Patient characteristics

Data from 1475 patients with OAC were included in this review. The age range of included patients was from 23 to 86. Exact numbers of male and female patients with OAC included were not provided for all studies, as some studies which included other histologies other than OAC reported overall breakdown of sex but not sex breakdown for each histological group. The total number of female patients in included studies was 462 and total number of male patients was 1870.

3.3.2.3 Cancer site and histology

As shown in Table 3.1, three studies included oesophageal cancer only, one included OGJ cancers only, three included both oesophageal and OGJ cancers and one study included oesophageal. Four studies included only patients with adenocarcinoma, the other four studies included both squamous cell carcinoma (OSCC) and adenocarcinoma.

3.3.2.4 Disease extent

Studies differed in how stage was reported, as seen in Table 3.1, variously reporting the range of included ypT, ypN and ypM (neoadjuvant pathologic) stages, pT, pN and pM (pathologic) TNM stages or simply reporting T, N and M stages. One study reported the range of overall Union for International Cancer Control (UICC) stages included.

The range of TNM stages reported by studies included in this review was T0-T4, N0-N3 and M0-M1 disease. However, only two studies included patients with M1 disease (304, 305). The proportion of patients with M1 disease was relatively low in the Vashist study (18%)(305) and very low in the Wen study (3%)(304), thus very few patients with M1 disease were included in the meta-analysis.

3.3.2.5 Treatment(s) Received

As detailed in Table 3.1 and, as discussed in further detail below, the studies in this review differed in terms of treatment modalities received by included patients. All included patients had undergone surgery but some studies also included patients who had chemotherapy and/or radiotherapy. Neoadjuvant chemotherapy was most common with seven of the eight studies including patients who had this treatment, three of these included some patients who had chemoradiotherapy. Two studies included patients who also had adjuvant chemotherapy. Only one study included patients who had surgery alone.

3.3.2.6 Histopathological findings at resection

Mandard Tumour Regression Grade (TRG) (306) was reported in three studies (Table 3.1). Walsh (307) and Jagadeham (195) reported TRG in the same way (as TRG 1-2) but found different proportions with TRG 1-2: 37% and 10%, respectively. Jomrich (308) categorized TRG differently (as TRG 1, 2-4 and TRG 5), hampering comparison with the other two studies.

Resection margin status was reported by seven of the eight included studies (Table 3.1). Three studies (195, 305, 308) included only patients with R0 classification (no residual tumour) (309). Dutta (303) and Powell (310) also included patients with R1 classification (microscopic residual tumour) (309), 23% and 39%, respectively. Walsh (307) and Wen (304) included patients with R2 disease (macroscopic residual tumour) (309) but neither study provided detail of how many patients had R2 disease.

3.3.2.7 Follow-up and Survival

Median follow-up time ranged from 21 to 90 months but was not reported in four studies (Table 3.1). All but one study(303) reported OS (Table 3.1).

3.3.2.8 Measurement of CRP

All studies reported timing of CRP measurement, as detailed in Table 3.2. Anciaux (280) reported CRP at diagnosis while all seven other studies reported pre-operative CRP measurements. Timing of pre-operative CRP measurement varied from within one month before surgery to within 3 days before surgery. Jomrich (308) reported CRP measured at two different timepoints – prior to neoadjuvant treatment and pre-operatively.

Jomrich (308) reported immunoturbidometry was used to analyse CRP; the other studies did not comment on laboratory method used.

Table 3-1. Characteristics of Included Studies

Author, Year	Country	Primary cancer site	Included histologies	Included n (OAC) ^a Total male/female	Age (years) (median, range or mean±SD ^a	Treatment modality/ies	Stage	Tumour Regression Grade	Resection margin status	Study design	Median follow-up (months)	OS or CSS
Anciaux 2020(280)	Belgium	Oesophageal	OAC OSCC	78 109/36 ^a	60 (IQR 54-67) ^a	Surgery (all) Neoadjuvant CT(n=35) Neoadjuvant CRT(n=22)	ypT1- T4	NR	NR	Retrospective	NR	OS
Dutta 2012(303)	UK	Oesophageal OGJ	OAC OSCC	98 83/15	60% were < 65 years	Surgery (all) Neoadjuvant CT (n=45) Adjuvant therapy (n=17)	UICC 1-3	NR	R0-R1 (R1 n=23 (23%))	Retrospective	90	CSS
Jagadesham 2017(195)	UK	Oesophageal OGJ	AC	94 173/26 ^a	63 (23-79) ^a	Surgery (all) Neoadjuvant CT (all)	ypT0-T4, ypN0-ypN3, M0	TRG 1-5 (1-2 in n=20, 10%)	R0	Retrospective	NR	OS
Jomrich 2018(308)	Austria	OGJ	AC	155 131/24	62± 11	Surgery (all) Neoadjuvant CT(all) Neoadjuvant CRT(n=11) Adjuvant CT (n=44)	ypT0-T4, ypN0-N3, M0	TRG 1-5 (TRG 1 n=13(8%), TRG 2-4 n=93 (60%), TRG 5 n=49(32%))	R0	Retrospective	64	OS
Powell 2021(310)	UK	Oesophageal	OAC	294 250/44	69 (IQR 62-74)	Surgery (all), Neoadjuvant CT (n=207)	T1-T4, N0-N3, M0	NR	R0-R1 (R1 n=115 (39%))	Retrospective	60	OS

Vashist 2011(305)	Germany	Oesophageal	OAC OSCC	244 391/104 ^a	63 (34-85) ^a	Surgery (all)	pT1-pT4, N0-N3, M0-M1 (M1 n=91 (18%))	Not applicable	R0	Retrospective	NR	OS
Walsh 2016(307)	Ireland	Oesophageal OGJ	OAC	223 187/36	63 (30-86)	Surgery (all), neoadjuvant CRT (n=109), neoadjuvant CT (n=66)	T1-T3, N0-N3, M0	TRG 1-5 (TRG 1-2 n=38 (37%), TRG 3-5 n=63 (63%))	R0-R2	Retrospective	21	OS
Wen 2018(304)	UK	Oesophageal OGJ Gastric	AC OSCC	184 546/177 ^a	66 ± 11 ^a	Surgery (all) Neoadjuvant CT(NR for OAC alone but 76% of all OC)	T0-T4, N0-N3, M0-M1 (M1 n=18 (3%))	NR	R0-R2 (R1/R2 n=192 (27%))	Retrospective	NR	OS

OAC: oesophageal adenocarcinoma. OGJ: Adenocarcinoma of oesophago-gastric junction. AC: adenocarcinoma. OSCC: oesophageal squamous cell carcinoma. SD: standard deviation. IQR: inter-quartile range. CT: chemotherapy. CRT: chemoradiotherapy. OC: Oesophageal cancer. UICC: Union for International Cancer Control. NR: Not reported. OS: overall survival. CSS: Cancer-specific survival.

a. Gender breakdown and age given for whole cohort where not available for OAC alone

3.3.3 Studies of the prognostic value of CRP in OAC

Three studies reported the prognostic impact of high CRP (280, 308, 310). Two included patients with oesophageal cancer only (280, 310) and one (308) included only OGJ cancers. All patients received surgery. All patients in one study (308) and the majority in the two other studies (280, 310) received neoadjuvant treatment (as detailed in Table 3.1).

All studies analysed CRP as a categorical variable, with a different cutpoint used in each study, ranging from 2.8 – 10mg/L (Table 3.2). The cutpoint was based on median CRP in one study (280), based on GPS in another (310) whilst the rationale was unclear in the third study (308).

Distribution of CRP in each study cohort is shown in Table 3.2. Included studies reported CRP distribution in different ways. In the Anciaux study (280), CRP distribution was reported as median and interquartile range (IQR), with relatively low CRP levels recorded (median 2.8mg/L, IQR 1.2-7.7). Jomrich (308) reported CRP as a mean value of 1.88 mg/L. Powell (310) reported that 84% of patients in their study had a normal CRP (≤ 10 mg/L).

Jomrich (308) found a high pre-op CRP (>5 mg/L) was independently predictive of reduced survival in OGJ cancers but that a high CRP before neoadjuvant treatment was not predictive. Anciaux (280) and Powell (310) found a high CRP at diagnosis and pre-operatively, respectively, were not predictive of OS in OAC.

HR and 95% CI reported for OS or CSS for CRP in each included study are shown in Table 3.3.

Meta-analysis was not appropriate given the widely differing cutpoints for CRP and small number of studies.

Table 3-2. CRP and CRP-Based Scores

Author, Year	Timing of CRP	CRP Cutpoint (mg/L)	CRP Score(s)	Distribution of CRP in cohort (median, IQR, or mean $\pm$ SD, mg/L) and/or % with normal CRP or score
Anciaux 2020(280)	At diagnosis	2.8	-	2.8 median, IQR 1.2-7.7 ^a
Dutta 2012(303)	Pre-op (within few days)	-	mGPS	89% mGPS 0
Jagadesham 2017(195)	Within one week prior to surgery	-	GPS adapted GPS	Median 5, IQR 5-5 92% mGPS 0 69% adapted mGPS 0
Jomrich 2018(308)	Pre-neoadjuvant treatment Pre-op (both within 3 days)	5	mGPS	Pre-neoadjuvant treatment: mean CRP 1.6 $\pm$ 0.3, 68% mGPS 0 Pre-op: mean CRP 1.88 $\pm$ 0.1, 73% mGPS 0
Powell 2021(310)	Pre-op	10	mGPS	84% normal CRP 84% mGPS 0
Vashist 2011(305)	max 7 days pre-op	-	GPS	54% GPS 0
Walsh 2016(307)	Pre-op (within 1 month)	-	mGPS	78% normal CRP 78% mGPS 0
Wen 2018(304)	Pre-op (1-4 weeks)	-	mGPS PI	Median 4 (IQR: < 3-14) ^a 69% normal CRP ^a 69% mGPS 0 ^a 64% PI 0 ^a

CRP: C-reactive protein. NR: not reported. mGPS: modified Glasgow Prognostic Score. GPS: Glasgow Prognostic Score. PI: Prognostic Index. IQR: inter-quartile range. SD: standard deviation. ^aCRP / CRP-based score distribution given for whole cohort where not available for adenocarcinoma alone

Table 3-3. HR and 95% Confidence Interval for Survival by CRP / CRP-Based Score in OAC for all included studies

Author, Year	HR for CRP	HR for GPS	HR for mGPS	HR :other score
Anciaux 2020(280)	2.3 (1.2 – 4.6) p=0.012 ^a as per authors, CRP not independent predictor in multivariable model (No HR available)	-		-
Dutta 2012(303)	-		2.91 (1.51– 5.62) p=0.001 ^b	
Jagadesham 2017(195)	-	1.58 (0.62 – 4.06) p=0.337	-	NR
Jomrich 2018(308)	Pre-neoadjuvant treatment: 1.06 (0.7-1.59) p=0.781 ^a Pre-op: 1.59 (1.06 – 2.4) p=0.027 ^a		Pre-neoadjuvant treatment: 1.25 (0.82 – 1.90) p=0.308 ^a Pre-op: mGPS 0 v 1 or 2: HR 1.72 (1.10 – 2.67) p=0.017	
Powell 2021(310)	1.448 (0.811 – 2.584) p=0.211		0.477 (0.188-1.210) p=0.119 mGPS 1 v 0: HR 2.014 (1.040-3.899) p=0.038 mGPS 2 v 0: HR 0.961(0.428 - 2.157) p=0.923	
Vashist 2011(305)		GPS 1 V 0: 1.7 (1.3-2.2) p <0.001 GPS 2 V 0: 2.5 (1.7-3.6) p<0 .001		
Walsh 2016(307)			Score 1 or 2 v 0: HR 1.24 (0.69-2.22) p=0.47	
Wen 2018(304)			mGPS 1: HR 1.03 (0.67-1.59) p=0.877 mGPS 2: HR 3.85(1.76 – 8.46) p=0.001 ^a	

All HR (hazard ratios) given refer to results of multivariable analysis for overall survival, unless otherwise indicated NR: Not reported.

a. Univariate analysis. b. Cancer-specific survival

3.3.4 Studies of the prognostic value of CRP-based scores in OAC

Seven studies reported the prognostic value of CRP-based scores (195, 303-305, 307, 308, 310). Four scores were reported: GPS (195, 305), mGPS(303, 307, 308, 310), an adapted GPS(195), and Prognostic Index (PI)(304) (Table 3.2). Detail of how each score was calculated by investigators is in Table 3.4.

As shown in Table 3.1, four studies included patients with both OC and OGJ (195, 303, 304, 307), two OC only (305, 310) and one (308) included only OGJ. All patients received surgery. The proportion of patients receiving neoadjuvant treatment differed significantly between studies. All patients in two studies (195, 308) and the majority in another two (304, 310) received neoadjuvant treatment. Approximately half of those in the Dutta (303) and Walsh(307) studies received neoadjuvant treatment, while patients who had received any neoadjuvant treatment were excluded from the study reported by Vashist (305). Two studies included patients who had received adjuvant treatment: Dutta (303) reported that 17 patients (17%) received any adjuvant therapy and Jomrich (308) included 44 patients (28%) who were treated with adjuvant chemotherapy.

CRP was measured pre-operatively in all studies which examined CRP-based scores; exact timing ranged from within 1 month (307) to within 3 days pre-operatively (Jomrich 2018) (Table 3.2). Jomrich(308) also assessed GPS and adapted GPS prior to neoadjuvant treatment.

Distribution of scores in each study cohort is shown in Table 3.2. In Vashist's study, over half of all participants had a normal GPS (305). mGPS score was 0 in the majority of patients in all the studies which reported this score, with exact percentage ranging from 69% (304) to 92% (195). Approximately two-thirds of patients had a normal adapted GPS(195) or Prognostic Index(304) in the studies which reported these scores.

Analysis of the mGPS and GPS differed across studies. Three studies presented separate HR for score 0 v score 1 and score 0 v score 2 (304, 305, 310) one reported HR for score 0 or 1 v 2 (308) and one reported HR for score 0 v 1 or 2 (307). Three studies reported a single HR for any change in GPS (195, 303, 310).

Findings from included studies for each CRP-based score are discussed below and HR and 95% CI reported for OS or CSS for CRP-based scores in each included study are shown in Table 3.3. Results of meta-analysis are below.

3.3.4.1 GPS

Jagadesham (195) showed high pre-operative GPS was not independently predictive of OS after multi-modal treatment in OAC and OGJ. Vashist (305), in contrast, found a high pre-operative GPS predicted survival in an OAC cohort treated by resection only.

3.3.4.2 mGPS

Five studies reported the prognostic role of mGPS. Wen (304) reported data from a cohort with OAC and OGJ, most of whom had neoadjuvant treatment, and found mGPS score of 2 predicted shorter survival. Dutta (303) also found an elevated mGPS was a significant predictor of (cancer-specific) survival in a cohort with OAC and OGJ, although fewer than half had neoadjuvant treatment. Walsh(307), however, found a high mGPS did not predict overall survival in a similar cohort.

Pre-operative mGPS was not predictive of survival in a study of OAC where most were treated neoadjuvantly (310) but was in OGJ where all had neoadjuvant treatment (308). The same study found mGPS measured pre-neoadjuvant treatment, however, was not predictive of overall survival (308).

3.3.4.3 adapted GPS

Jagadesham (195) devised their own adapted GPS, based on the upper and lower quartiles of CRP and albumin in the study cohort (as outlined in Table 3.4). It was not predictive of survival in their cohort with OAC and AEG after multimodal treatment.

3.3.4.4 Prognostic Index

Wen (304) reported that PI, which is based on CRP and white blood cell count (detail in Table 3.4), was not an independent predictor of survival in their OC subgroup.

Table 3-4. Components and Calculation of Scores Identified in the Review

Score	Components	Calculation
GPS (195, 305)	CRP, albumin	GPS 0: CRP $\leq$ 10mg/L and albumin $\geq$ 35g/L GPS 1: CRP > 10mg/L OR albumin < 35g/L GPS 2: CRP > 10mg/L and albumin < 35g/L
mGPS(303, 304, 307, 308, 310)	CRP, albumin	mGPS 0: CRP $\leq$ 10mg/L and any albumin level mGPS 1: CRP > 10mg/L and albumin $\geq$ 35g/L mGPS 2: CRP > 10mg/L and albumin < 35g/L
Adapted GPS(195)	Upper (CRP) and lower quartiles (albumin) in data	Adapted GPS 0: CRP < 6mg/L and albumin > 41.4g/L Adapted GPS 1: CRP $\geq$ 6mg/L OR albumin $\leq$ 41.4g/L Adapted GPS 2: CRP $\geq$ 6mg/L and albumin $\leq$ 41.4g/L
PI(304)	CRP, white blood cell count (WBC)	PI 0: CRP $\leq$ 10mg/L and WBC $\leq$ 11 x 10 ⁹ /L PI 1: CRP > 10mg/L OR WBC > 11 x 10 ⁹ /L PI 2: CRP > 10mg/L and WBC > 11 x 10 ⁹ /L

Studies reporting each score are in brackets. GPS: Glasgow Prognostic Score. mGPS: modified Glasgow Prognostic Score. PI: Prognostic Index.

Table 3-5. Risk of Bias for Included Studies (QUIPS tool, Hayden et al., 2013)

	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting
Anciaux 2020(280)	Low	Moderate	Low	Moderate	Low	Low
Dutta 2012(303)	Low	Moderate	High	Low	Moderate	Moderate
Jagadeshram 2017(195)	Low	Moderate	Moderate	Low	Low	Low
Jomrich 2018(308)	Low	Moderate	Moderate	Low	Low	Moderate
Powell 2021(310)	Moderate	Moderate	Moderate	Low	Low	Low
Vashist 2011(305)	Low	Moderate	Moderate	Low	Low	Low
Walsh 2016(307)	Moderate	High	Moderate	Moderate	Moderate	Moderate
Wen 2018(304)	Low	Low	High	Moderate	Low	Low

3.3.5 Quality of Study Reports

Only one study (308) excluded patients with active infection, a potential confounder.

Risk of bias for included studies is shown in Table 3.5. Five studies scored low or moderate risk of bias in all domains and no study had a high risk of bias on more than one domain. Subgroup assessment based on risk of bias was not conducted because of the small number of studies.

3.3.6 Meta-analysis

3.3.6.1 Overall

Six studies which reported OS and pre-operative GPS/mGPS in OAC and/or OGJ were included in meta-analysis (Fig. 3.2). There was a significant association between elevated GPS/mGPS and OS: HR 1.81 (95% CI 1.25 – 2.62, $p=0.002$) with moderate heterogeneity ($I^2=48\%$).

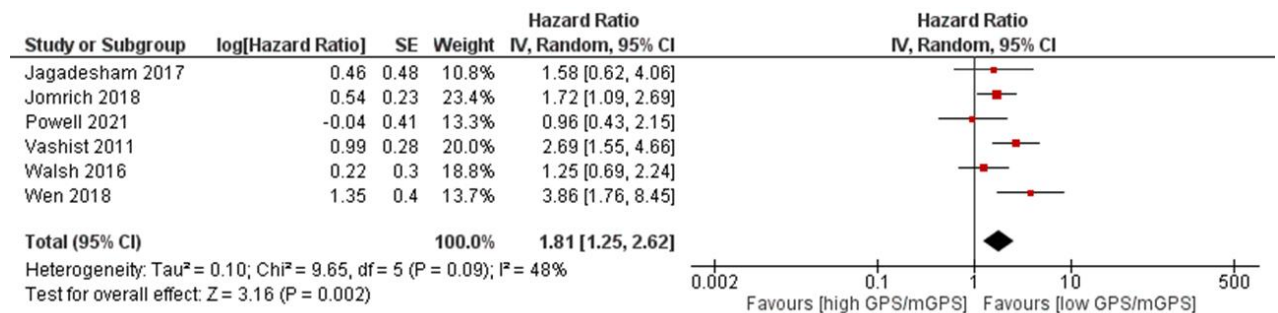


Figure 3-2. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, after multimodal treatment or resection alone

Meta-analysis was conducted, using a random-effects model, of six studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment or resection alone.

3.3.6.2 Subgroup Analyses

Multimodal Treatment

Results were similar when one study of resection only (305) was excluded for a subgroup analysis of five studies which included multimodal treatment: HR 1.64 (95% CI 1.09 – 2.47, $p=0.02$, $I^2=44\%$), (Fig. 3.3).

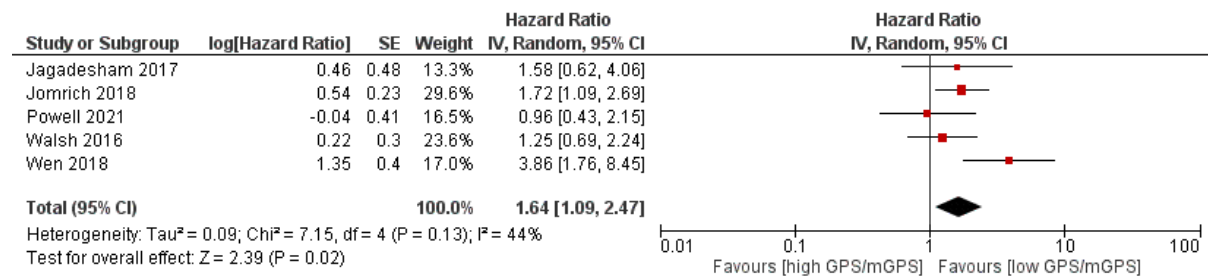


Figure 3-3. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, after multimodal treatment

Subgroup analysis was conducted, using a random-effects model, of five studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment.

Studies including only M0 disease, studies including only R0 resection

Further subgroup analyses of four studies including only M0 stage disease and three which only included patients with R0 resection status also showed significant associations between elevated GPS/mGPS and OS, HR 1.43 (95% CI 1.05 – 1.95, $p=0.02$, Fig. 3.4) and HR 1.99 (95% CI 1.44 – 2.76, $p<0.0001$, Fig. 3.5), respectively and with minimal heterogeneity (both $I^2=0\%$).

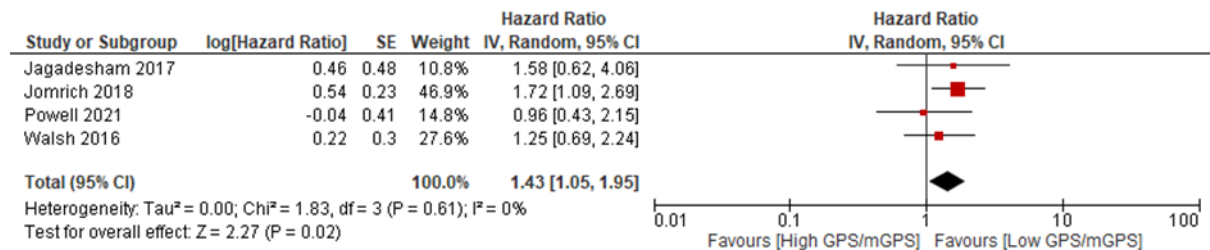


Figure 3-4. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, with no metastatic (M0) disease

Subgroup analysis was conducted, using a random-effects model, of four studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment and where only patients with no metastatic (TNM stage M0) disease were included

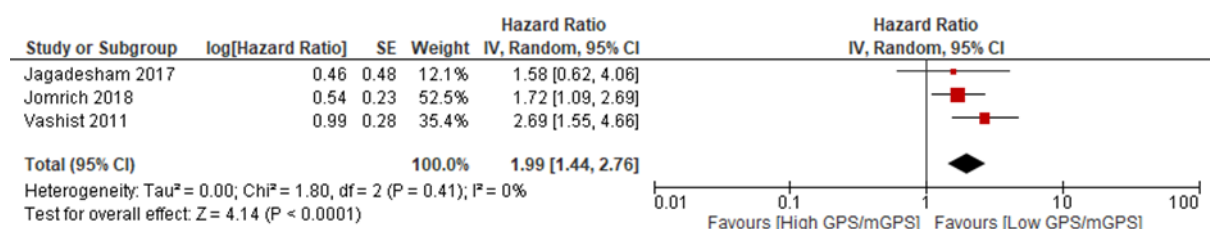


Figure 3-5. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included only patients with no residual tumour at resection (R0 resection)

Subgroup analysis was conducted, using a random-effects model, of three studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment or resection alone and where only patients with no residual tumour at resection (R0 resection) were included.

Other subgroup analyses

When the three studies of surgery plus chemotherapy, the three studies including R0-R2 resection status and the four studies conducted in the United Kingdom or Ireland were analysed separately, associations were not statistically significant. HR for OS for studies including surgery and chemotherapy was 1.82 (95% CI 0.78-4.23, $p=0.05$, $I^2=67\%$)(Fig. 3.6).

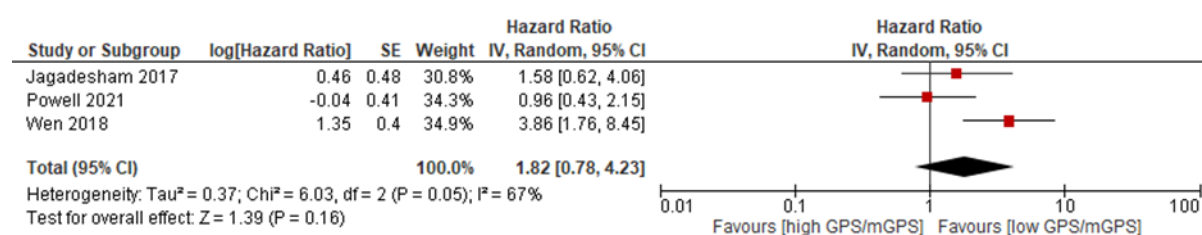


Figure 3-6. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included chemotherapy and surgery

Subgroup analysis was conducted, using a random-effects model, of three studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, and where studies included patients who had received chemotherapy and surgery as well as patients who had surgery.

The HR for OS for studies which included any resection status from R0-R2 was 1.65 (95% CI 0.75 – 3.6, $p=0.03$, $I^2= 72\%$)(Fig. 3.7) and HR for OS for studies carried out in the United Kingdom or Ireland was 1.63 (95% CI 0.9 – 2.93, $p=0.07$, $I^2= 58\%$)(Fig. 3.8).

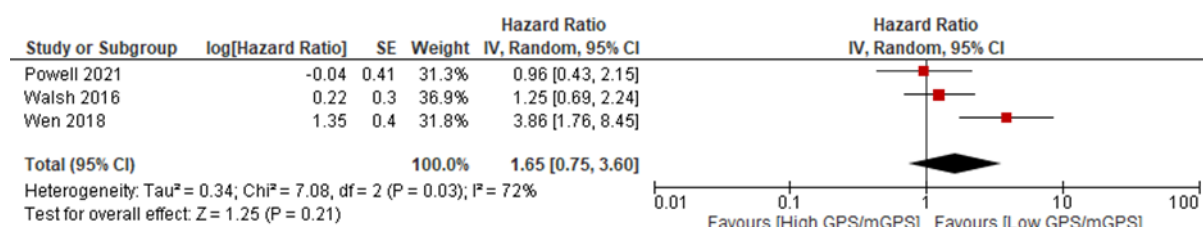


Figure 3-7. Forest plot of studies investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ, where studies included R0-R2 resection status

Subgroup analysis was conducted, using a random-effects model, of three studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment and where patients with any resection status (R0 no residual tumour; R1 microscopic residual tumour and R2 macroscopic residual tumour) were included.

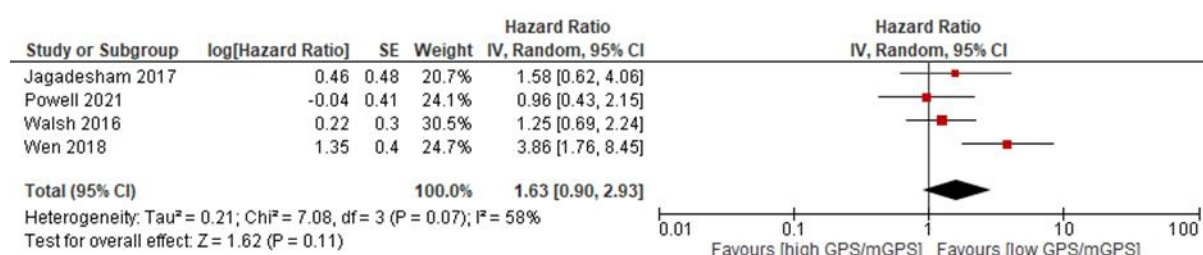


Figure 3-8. Forest plot of studies conducted in the United Kingdom or Republic of Ireland investigating the prognostic value of pre-operative GPS/mGPS to predict overall survival in patients with OAC, including OGJ

Subgroup analysis was conducted, using a random-effects model, of four studies which reported Hazard Ratios for overall survival based on pre-operative GPS/mGPS in patients with OAC, including OGJ, who were treated with multimodal treatment, and where studies were conducted in the United Kingdom or Republic of Ireland.

3.4 Discussion

3.4.1 Overview

To our knowledge, this is the first review to evaluate comprehensively the evidence for CRP and CRP-based scores in OAC, including OGJ. Meta-analysis demonstrated that an elevated pre-operative GPS or mGPS was significantly associated with reduced OS. Subgroup analyses in five studies which included multimodal treatment only, four which included only M0 disease and three which included only R0 resection showed similar findings. As the incidence of OAC increases(136), the need for reliable prognostic markers in this cancer becomes more pressing; our results suggest these scores have a role to play.

3.4.2 CRP

There was inadequate evidence to draw conclusions on the role of CRP alone as a predictor of survival in OAC. This review identified only three studies which assessed the prognostic role of CRP alone. Comparison between studies was hampered by the use of different cutpoints for CRP in each of the three studies (2.8mg/L, 5 mg/L and 10mg/L). This is a known problem in prognostic factor research(170). CRP was also measured at a different timepoint (at time of diagnosis) in one of the studies; this presented a further barrier to meta-analysis(170). It is noteworthy that all three studies which assessed CRP analysed it as a categorical (binary) variable rather than continuous. Categorisation of a continuous variable is a very common practice but is associated with reduced statistical power(261).

CRP has been shown in a previous systematic review to predict survival in OC(269). However, that review included very few patients with OAC, with no analysis of OAC alone (with or without OGJ). Thus, the review's conclusions were based almost entirely on patients with OSCC. Given the previously noted significant differences between OSCC and OAC, great caution should be applied in extrapolating findings from predominantly OSCC data.

3.4.3 GPS/mGPS

The results of the current review are consistent with the literature on the relationship between GPS/mGPS and survival in surgically-treated cancers. An elevated GPS/mGPS has been shown to predict shorter survival in most operable cancers (187). Similar results were found in a systematic review of the GPS/mGPS in oesophageal cancer, published in 2019 (288).

However, the included studies included predominantly patients with OSCC (288). Lindenmann (311) reported that elevated GPS was an independent prognostic marker in a cohort which included both OAC and OSCC treated with curative oesophagectomy. Unfortunately, data for OAC from that study were not available for inclusion in this review. Of note (OAC v. OSCC) was also a significant independent predictor of survival in that study, with the OSCC group having worse survival (311). This underlines the need to analyse each histological group separately.

Lindenmann also showed that high GPS and high CRP were associated with shorter survival after palliative treatment (various treatment modalities which were individualised for each patient)(312). GPS also predicted survival post stenting for inoperable OC in two UK studies (313, 314). Each of these studies included a mix of OAC and OSCC.

3.4.4 Other CRP-based scores

As detailed in the introduction to this thesis, numerous CRP-based scores have been devised. However, only a few have been reported in OAC. Apart from the GPS/mGPS and a modification of the GPS, the studies included in this review reported only one other score - the PI, which was reported by Wen(304) as not predictive of survival. Kudou (315) also assessed the PI in surgically-treated adenocarcinoma of OGJ and upper gastric cancer. Data from that study were not available for this review but, in contrast to the Wen study (304), they found that PI was an independent predictor of overall survival (315).

None of the studies included in this review reported use of CAR, which has been proposed as a prognostic marker in cancer, with a high CAR associated with shorter survival in several cancer sites (286). In a recent meta-analysis, CAR was shown to predict survival in OC (287). However, only 0.5% of patients included in the meta-analysis had OAC (287). One study in a mixed OC cohort but where the majority (over 70%) had OAC, found that CAR was not an independent predictor of survival (137). Data from that study were not included in this review as the authors did not respond to requests for information. Our review has not identified any other studies which examined CAR in OAC. As a ratio, CAR is a continuous variable and, as such, has potential to be more informative than categorical scores such as the GPS and mGPS(286). Some have argued, however, that scores which are based on reference ranges, rather than ratios, may be simpler and more useful in the clinical setting

(316). Moreover, a recent review found that most published studies analysed CAR as a categorical variable (above and below a certain cutpoint) with various different cutpoints for CAR reported, hampering comparisons between studies (286). Many studies use the value which created the largest difference between groups, which is a very significant source of potential bias (317). It would be useful for future studies to assess the role of CAR, if any, in OAC.

Several studies in OC have assessed the prognostic role of GPS with various modified cutpoints for CRP and albumin. As detailed in Table 3.4 and in the results section, Jagadesham(195) found their “adapted GPS” was not predictive of survival in OAC. In OSCC, Nakamura (193) examined three different cutpoints for CRP in calculation of their “new mGPS (NmGPS)” and found that only a cutpoint of 5mg/L was an independent predictor of survival. Chen found that an even lower cutpoint for CRP, of 3mg/L, was independently predictive of survival in OSCC with their “high-sensitivity mGPS (HS-mGPS)”(318). Also in OSCC, Tian devised the “sensitive-modified GPS (S-mGPS)” with an albumin cutpoint of 45.6g/L but a CRP cutpoint of 10mg/L, and found this to be an independent predictor of survival (197). All three studies reported that their novel score was more strongly predictive of survival than the traditional mGPS(193, 197, 318). Use of study data to devise adapted scores may be useful in that group of patients but this approach limits applicability to other cohorts and makes meta-analysis more complex(319).

Other novel CRP-based scores whose potential to predict survival in OSCC have been reported include the CRP-prealbumin ratio (320), the modified geriatric nutrition risk index (321) and the plasma fibrinogen and CRP score (FC score) (201).The CRP-prealbumin ratio (CRP/PALB) was found in a recent study to predict survival in an OC cohort where the vast majority of patients had OSCC (320). Kouzu modified another prognostic score, which originally did not include CRP, the geriatric nutrition risk index (322), by substituting CRP for one of the scores components, creating the modified GNRI (mGNRI) and reported that the mGNRI predicted survival in elderly adults with OC, the majority of whom had OSCC (321). The FC score was an independent predictor of survival in OSCC in one study (201).

3.4.5 Future possibilities

Although beyond the scope of this review, the potential of CRP and CRP-based scores to predict response to chemotherapy or chemoradiotherapy is of interest, as over 50% of patients do not respond to neoadjuvant therapy, with delays in surgery and possible impact on outcomes. Zingg proposed that CRP could be used as part of the re-staging process following neoadjuvant treatment, based on their finding that CRP, measured post neoadjuvant treatment, predicted survival in their study (290). However, this information would be far more useful if available prior to commencement of the neoadjuvant therapy. Jomrich assessed the role of CRP and mGPS measured prior to neoadjuvant therapy in OGJ and found neither was predictive of overall survival (137). Further studies are needed.

A potentially useful future approach is to combine CRP-based scores with other prognostic markers, as in Reeh's study (205) which combined GPS with disseminated tumour cell count or with haematological markers of the systemic inflammatory response (SIR), such as the neutrophil-lymphocyte count (NLR). McSorley recently demonstrated that a combination of mGPS and NLR predicted survival in a large mixed cohort of OC, OGJ and gastric cancers, independent of traditional prognostic markers including performance status and TNM stage (176). As those authors noted, this introduces the exciting possibility of "staging" both the cancer and the patient (176). The subgroup analyses of M0 disease and R0 resection status in the present study support this idea and suggest that GPS/mGPS may have a role in identifying those patients with more localised disease who go on to have worse clinical outcomes. One possible explanation is that a high CRP (and thus GPS/mGPS) reflects a more inflammatory tumour micro-environment, which promotes tumour progression and metastasis (28). An alternative explanation is that an elevated GPS/mGPS is related to sarcopenia and evolving cancer cachexia (213), which are both associated with shortened survival (323, 324).

As has been discussed, the literature includes a diverse range of CRP cutpoints and CRP-based scores, many of which are reported in a very small number of studies and are not validated in external datasets, as is recommended (261). The study described in the previous chapter of this thesis illustrates that a promising finding in one dataset may not be borne out in data from another group of patients.

The evidence base is too weak at present to recommend use of CRP or CRP-based prognostic scores for clinical use in OAC. This review has found that the strongest evidence is for GPS/mGPS. Future studies should focus on further validating these well-established scores, rather than developing new models and scores, a practice which is rampant in the literature (325). This recommendation is supported by the findings of a survey of clinicians (mainly surgeons and oncologists) where a majority reported a preference for the GPS / mGPS over other measures of the systemic inflammatory response (278). The same survey found that clinicians were open to use of these scores, particularly for stratification in clinical trials and to support decisions about adjuvant and palliative treatments (278). Future studies should investigate these possibilities specifically.

3.4.6 Limitations of evidence

This review has limitations known to be associated with prognostic factor reviews. The quality of prognostic factor research and reporting is known to be generally poor (169). All included studies were retrospective, which can be associated with poorer quality of data in prognostic factor studies(261). Future studies should examine these scores prospectively. Statistical assessment of possible publication bias was not possible due to the small number of included studies, but publication bias and selective reporting are well-documented problems in prognostic research (170). Overall, the risk of bias was lower than has been reported in other prognostic factor reviews (251).

The studies included in this review report predominantly male patients, with data from fewer than 400 women included. All included studies were conducted in Europe. It is not possible to conclude whether the findings of this review are applicable to women or to other regions.

Timing of CRP measurement varied, which may affect the reliability of these results. Furthermore, there is significant potential selection bias if CRP sampling was not routine. If patients only had CRP measured where infection or inflammation was suspected clinically, that infection or inflammation could also have influenced their survival. The study by Wen was the only one in this review which addressed this potential confounder (304). They found no difference in OS between people with and without CRP measurements(304) but the impact on other studies is unknown.

The REporting recommendations for tumour MARKer prognostic studies (REMARK) guidelines (326) recommend that studies report the flow of patients through a study, including those who were ineligible and those who dropped out. Only three studies in this review reported this information.

Results of multivariable analyses were not available for all studies. While adjusted (multivariable) estimates are preferable, there can still be issues as studies rarely adjust for all the same factors(170). Separate meta-analyses for adjusted and unadjusted estimates are recommended but were not possible in the present study due to the small number of studies(170).

The small number of studies is a limitation, particularly for the subgroup analyses and these should be interpreted with caution. It is notable that the available evidence for GPS/mGPS as predictors of survival is much less for OAC/OGJ than for OSCC (1194 patients in this meta-analysis compared to over 6000 patients in a meta-analysis of OSCC (288), reflecting the fact that the majority of studies conducted in this field are conducted in East Asia, where OSCC is more prevalent than OAC, as noted in the introduction to this thesis.

3.4.6 Limitations of this review

This review was limited to studies in English or French, which is a possible limitation. However, only one study was excluded on this basis. Furthermore, language restrictions have not been shown to introduce systemic bias in meta-analysis (327).

Conference abstracts were not included in this review. The need to include conference abstracts in systematic reviews is far from clear (328). Quality assessment would not have been possible with the limited information in each abstract. This is a known barrier to inclusion of conference abstracts in systematic reviews (328). Furthermore, conference abstracts have previously been noted to be “unclear or misleading” in many cases (329).

The QUIPS tool used for RoB assessment has limitations. Interrater agreement has been found to be relatively low and there is no clarity on how to derive an overall score for each study based on the different domains (295). Nonetheless, it remains the most recommended tool for RoB assessment in prognostic reviews (170, 295).

Studies differed in their categorisation of mGPS / GPS, as noted in the results. This has been identified in other reviews of these scores (288, 330). There were too few studies to stratify by choice of categorisation. HR were combined notwithstanding this difference. Since some studies considered a mGPS/GPS of 1 to be normal, when these studies were combined with studies which considered the same score to be high, our conclusion that “any high mGPS/GPS” is associated with reduced OS is valid. If anything, combining such studies risked missing a real difference.

The forest plots in this systematic review were presented in alphabetical order. This presentation does not maximise the information presented, whereas ordering by effect size or year of publication can give some indication of publication bias and change over time, respectively(331). Alphabetical order is, nonetheless, the most commonly used in most published reviews, including Cochrane reviews(331).

Summary data were used in this review, rather than individual patient data (IPD). While the use of IPD has been recommended for meta-analysis (332), it is very time-consuming (332) and still doesn't overcome the problem of publication bias (317). Moreover, the low response rate from authors contacted to provide information for this review calls into question the feasibility of this approach.

3.4.7 Strengths of this review

The search strategy was comprehensive, including six databases and supplementary searches, and was devised with an expert librarian.

Previous reviews have been unable to report on OAC as these data were not published (288). To address this, we contacted all authors of mixed OC studies for HR for OAC alone. Multiple attempts were made to contact authors in order to maximise available data for the review. Despite this, HR were not available for several studies, which may have affected our results. Nonetheless, this is the first review of which we are aware which has combined all available existing data on OAC.

The most appropriate measure for a time-to-event outcome (Hazard Ratios), was used in this review (as opposed to Odds Ratio or Relative Risk, which do not take into account the timing of the event (death))(298).

3.5 Conclusions

This systematic review and meta-analysis assembled, appraised and synthesised the available evidence on CRP and CRP-based scores as predictors of survival in OAC, including OGJ.

A range of CRP cutpoints were reported as prognostic, with differences in the population studied and meta-analysis was not possible. At present there is insufficient evidence to support the use of CRP alone in this context.

The most widely reported CRP-based score was the GPS/mGPS. Meta-analysis found that a high pre-operative GPS/mGPS is independently predictive of worse OS in adults with OAC, including OGJ, treated with multimodal treatment or surgery alone. Findings were similar in subgroup analyses of only studies which included multimodal treatment, studies in patients with no metastatic disease (M0) and studies in patients with complete surgical resection (R0 resection). It is notable that the available evidence for GPS/mGPS to predict survival is less than that for OSCC. Future studies should examine the role of the GPS/mGPS in OAC prospectively, both alone and in combination with other prognostic markers, and assess scores at defined timepoints.

One possible explanation for the link between these markers and survival is that a high CRP (and thus GPS/mGPS) reflects a more inflammatory tumour micro-environment, which promotes tumour progression and metastasis. This idea will be explored further in Chapter 4 of this thesis. An alternative explanation is that an elevated CRP (and GPS/mGPS), as markers of systemic inflammation, are related to skeletal muscle abnormalities and evolving cancer cachexia. The study reported in Chapter 5 of this thesis examines the link between systemic inflammation, skeletal muscle abnormalities, symptoms and quality of life, in greater detail.

4 Tumoural CRP in Oesophageal Adenocarcinoma

4.1 Introduction

As noted previously, CRP is widely used clinically and clinicians are familiar with CRP as a blood-borne marker. It is also well-established that serum CRP is made in the liver(15). However, in recent years CRP has been found within many different body tissues(206, 333) and, furthermore, it has been shown that CRP can be made in tissues outside the liver(207, 334). CRP has been identified within several different tumours – termed “tumoural” or “intratumoural” CRP(159). Importantly, its presence in tumours has been associated with shorter survival(208). This chapter will examine tumoural CRP in OAC.

The first documented site of extra-hepatic synthesis of CRP was in lymphocytes, in 1986(206). Since then, CRP has been identified in tissue from systems as diverse as the respiratory system (in pleural fluid(335), nasal aspirate fluid(336) and nasal polyps(336), the nervous system (in neurones in Alzheimer’s disease(334), the cardiovascular system (in aortic valves(333) and atherosclerotic plaques(337)), as well as in the oral cavity (gingival crevicular fluid(338) and the kidney(207).

In 2003, Nozoe *et al.*,(208) first reported that CRP was identified by immunohistochemistry in resected OSCC tumours. They also found that tumoural CRP expression was an independent predictor of shorter survival in that group(339). Nearly a decade later, Nakatsu *et al.*, replicated those findings(209). CRP within tumour tissue has also been reported in prostate cancer(210, 340), renal cell carcinoma(341-343), hepatocellular carcinoma(344) and colorectal cancer(345, 346). However, not all cancers express CRP. Janik *et al.*, showed that resected thymomas did not express CRP(347). It is not known which other tumour types do not express CRP, as it may either not have studied or not published, due to the known publication bias against negative results(348). There is only one published report of tumoural CRP in OAC, a conference abstract from 2006 by Räsänen *et al.*, (349). They showed that CRP was present in 11 of 18 resected OAC tumours and in 4 of 8 samples from adjacent normal oesophageal tissue(349). This study was never further disseminated (Räsänen J, personal communication).

For tumoural CRP to be of clinical use, it would need to have some prognostic or therapeutic value. In the non-cancer setting, presence of CRP within a tissue is associated with adverse prognostic features or worse outcomes: expression of CRP within atherosclerotic plaques

was associated with plaque ulceration(350) and local CRP expression was associated with rejection episodes in renal transplant(351). The initial report by Nozoe *et al.*, raised the exciting possibility of tumoural CRP as a novel marker for survival or even an eventual therapeutic target(209, 339). However, the literature is mixed with regard to the relationship between tumoural CRP and survival. While studies in renal cell cancer(343) and prostate cancer(340) found no relationship, another study in prostate cancer(210) and studies in hepatocellular carcinoma(344), and colorectal cancer(346) did show a link with survival. The relationship between tumoural CRP and survival has not been reported in OAC.

There is still uncertainty about the source of CRP identified in non-hepatic tissue. In certain tissues, such as pleural fluid and gingival crevicular fluid, which are transudates or exudates of serum(352), the source may well be systemic CRP. However, some authors have identified CRP mRNA, within aortic valves(333) and aneurysmal tissue(353), in neuronal tissue in Alzheimer's disease(334) and in adipose tissue(354), suggesting a local source. In cancer, Jabs *et al.*, showed CRP mRNA in renal cell tumours and, further, speculated that this local CRP may contribute to serum CRP levels, which were correlated with local CRP(341). Conflicting with this, Nakatsu *et al.*, found no correlation between tumoural and serum CRP in OSCC(209). There is no published information on the relationship between tumoural CRP and serum CRP in OAC.

Only a few studies of tumoural CRP specify where in the tumour CRP was located. Given that the tumour microenvironment (TME) consists not just of the carcinoma cells themselves but also a variety of stromal cells which interact with one another and with the carcinoma cells(149), this is important to clarify. The precise location of tumoural CRP may be a vital clue to its source.

No published study to date has examined in detail tumoural CRP in OAC. One study abstract reported that CRP could be found in resected OAC tissue(349), but these findings have not been confirmed. No detail is available about the localisation of CRP within the OAC tumour. It is unknown whether tumoural CRP is present at time of diagnosis or if it represents a consequence of cancer treatment. The relationships between tumoural CRP and serum CRP and between tumoural CRP and survival have yet to be elucidated.

4.1.1 Specific Hypotheses of this Study

1. CRP is present within OAC tumours, both before and after treatment
2. CRP is associated with serum CRP in OAC
3. CRP is associated with survival in OAC

4.1.2 Specific Aims of this Study

1. To confirm the presence of CRP within resected OAC tumours
2. To establish if CRP is present within OAC tumours at time of diagnosis
3. To describe the localisation of CRP within OAC tumours

Supplementary exploratory aims

4. To explore the relationship between tumoural CRP and serum CRP in OAC
5. To examine the relationship between tumoural CRP and overall survival in OAC
6. To assess if an OAC cell line can secrete CRP, when cultured in normal conditions, in conditions mimicking the TME and after treatment with chemotherapy or radiotherapy

4.2 Methods

Particular thanks to Dr Joanne Lysaght, as my supervisor, and to Dr Stephen Maher, Dr Amy Buckley, Dr Zivile Useckaite, Dr Maria Davern, Dr Melissa Conroy, Dr Margaret Dunne and Dr Mark Bates for kindly providing me with the relevant experiment protocols and for their invaluable assistance with writing the following Methods section.

Immunohistochemistry was used to assess presence and localisation of CRP in resected OAC tissue. Matched clinical data, including demographics, serum CRP and survival were examined. Next, an oesophageal cancer cell line was cultured in normal conditions, in conditions mimicking the TME and was treated with chemotherapy or radiotherapy. Enzyme-Linked Immunosorbent Assay (ELISA) was used to assess secretion of CRP from the cells. Finally, presence and localisation of CRP in a separate cohort of pre-treatment OAC tumour biopsies were assessed using immunofluorescence (IF). Matched clinical data, including demographics, serum CRP and survival were examined for that cohort also.

4.2.1 Western Blot and Immunohistochemistry

The SJH/AMNCH REC granted ethical approval for this study.

4.2.1.1 Western Blot

Western blot was undertaken, in collaboration with Dr Stephen Maher, to confirm the activity of the antibody which was to be used for immunohistochemistry.

Liver tissue was snap frozen and homogenised in RIPA buffer containing 6M urea. The tissue was then incubated on ice for 5 minutes. The cell lysate was then collected using a cell scraper. A 29-gauge needle was used to shear insoluble protein. In order to remove any remaining insoluble protein, the sample was centrifuged for 2 minutes at 4,000 x g at 4°C. The supernatant was aspirated and placed in a fresh tube on ice.

Bicinchoninic acid (BCA) assay was used to quantify protein. The Thermo Scientific Pierce BCA Protein Assay Kit (ThermoFisher Scientific, Waltham, Mass., USA) was used. The supplied stock solution of bovine serum albumin (BSA) was used to create standards of 1000 µg/ml, 800 µg/ml, 600 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml and 0 µg/ml were

prepared. The liver protein solution and 10 µg of each standard concentration were added to a 96-well plate, with triplicates of each. BCA Reagents A and B were prepared as 50:1 solutions. 200 µg of each was added to each well of the 96-well plate. Next, the plate was incubated for 30 minutes at 37°C. The plate was cooled to room temperature. A VersaMax microplate reader, using SoftMax Pro software, was used to read absorbance at 562nm. A standard curve was plotted by plotting the average measurement for each BSA standard versus its concentration in µg/ml. The standard curve was then used to determine the protein concentration of the liver protein sample. 10% SDS-PAGE gels were cast between upright glass plates (Bio-Rad Laboratories, CA, USA). To exclude air and to aid polymerisation, 70% ethanol was layered on top. Once polymerisation had occurred, the ethanol was removed. A stacking gel was placed on top of the gel and a 12-well comb inserted. Using the protein concentration determined by BCA assay, an equal volume of 2X Laemmli buffer was added to dilute the sample. The protein was then denatured by heating for 10 minutes at 95°C. Electrophoresis by 90 minutes at 50V in electrode buffer then separated the samples. 60 µg total tissue lysate was electrophoresed. A recombinant human (rh) CRP standard was used as a positive control. B-actin was employed as a loading control.

The gel was placed in 1x transfer buffer for 10 minutes. A polyvinylidene fluoride (PVDF) membrane was activated with 100% methanol for 1 minute. Whatman filter paper (Cytiva, Marlborough, MA, USA) and the PVDF membrane were soaked in 1x transfer buffer. A “transfer sandwich” was created of filter paper-gel-membrane-filter paper), using a wet transfer apparatus (BioRad Laboratories, Hercules, CA, USA). Having ensured no bubbles were trapped in the sandwich, it was placed in the transfer tank with an ice block. Proteins were then transferred for 2 hours at 225 mA.

The membrane was placed on a shaker for one hour at room temperature with 5% non-fat dry milk (NFD) in TBST (Tris-buffered saline, 0.1% Tween 20). Next, the membrane was incubated for 1.5 hours at room temperature in the primary antibody, a polyclonal rabbit anti-human CRP antibody (1:1000 dilution in 0.5% BSA solution). Membranes were washed in three washes of TBST, 5 minutes each. The membrane was then incubated with the conjugated secondary antibody, a horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (1:5000 dilution in 0.5% BSA solution) for one hour at room temperature. The membranes were again washed in three washes of TBST, 5 minutes each.

The Supersignal West Pico PLUS Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL, USA), an enhanced chemiluminescent HRP substrate, was used to detect the antibody complexes. As it only remains stable for 8 hours at room temperature, the substrate working solution was prepared shortly before use. The blot was incubated in the working solution for 5 minutes, then removed and excess reagent drained. The blot was placed in clear plastic wrap and bubbles were removed. The membranes were exposed to X-ray film (Fujifilm, Minato, Japan) for 2 minutes. The X-ray film was developed using an Agfa film processor (Agfa-Gevaert N.V., Mortsel, Belgium).

4.2.1.2 Immunohistochemistry

Immunohistochemistry (IHC) was used to examine resected OAC specimens for presence of CRP. This study utilized tissue microarrays (TMAs) previously constructed through collaboration between staff in the Central Pathology Laboratory and the Department of Surgery at St James's Hospital, Dublin. Briefly, resected tumour from patients with OAC was fixed overnight in 10% formalin and then embedded in paraffin wax using an embedder (Leica EG 1140H, Leica Microsystems, Wetzlar, Germany). A pathologist had marked areas of tumour on each block; cores of tissue (0.6mm each) were taken from the blocks and used to construct the TMAs. In collaboration with Dr Zivile Useckaite, a Leica RM 2253 microtome (Leica Microsystems, Wetzlar, Germany) was used to cut sections of 5 µm thickness from each TMA. These were floated onto Superfrost Plus poly-L-lysine-coated glass slides (ThermoFisher Scientific, Waltham, Mass., USA). The slides were baked overnight in a tissue-drying oven at 37°C and were processed immediately. A slide of resected liver tissue was included as a positive control.

Slides were incubated in TrilogTM (Cell MarqueTM, Merck, Darmstadt, Germany), which had been diluted 1:20 with distilled water, in a pressure cooker (Princess DYB350) on low pressure for 10 minutes. Slides were then placed in a solution of hydrogen peroxide (20ml of 30% hydrogen peroxide in 180ml methanol), covered and left for 30 minutes to block endogenous peroxidase activity. Slides were then washed 3 times for 5 minutes by being placed in phosphate-buffered saline (PBS) solution and left on an agitator. Normal blocking serum, provided as part of the Vectastain Kit, was added to slides, ensuring all tissue was covered and incubated at RT for 30 minutes. The primary antibody, a recombinant rabbit monoclonal antibody to human CRP (ab32412, Abcam, UK), diluted to 1 in 500 with PBS, was applied to the slides, ensuring full coverage of the slides. The slides were incubated for 1 hour at RT. Slides were then washed 3 times for 5 minutes by being placed in PBS solution and left on an agitator. A negative control was created by adding PBS only to one slide. Biotinylated antibody from the Vectastain kit was added to blocking serum and applied to the slides before incubation at RT for 30 minutes. The 3 x 5-minute PBS wash step was repeated. Next, avidin-biotin complex (ABC) reagent solution, from the Vectastain kit, was added to the slides and they were incubated at RT for 30 minutes. The 3 x 5 minute PBS wash step was repeated. The slides were covered in diaminobenzidine peroxidase solution (DAB) and incubated for 5 minutes in the dark. When colour development was seen, DAB

was rinsed off using distilled H₂O. Haematoxylin was added for 30 seconds to counterstain. Slides were placed in PBS for 5 minutes.

Slides were then transferred into 100% methanol and moved up and down 10 times. This step was repeated with fresh methanol. The slides were then moved into Xylene for 5 minutes and into fresh Xylene for another 5 minutes. Slides were left in another bath of fresh Xylene overnight. The following day, DPX mountant (Sigma-Aldrich, Saint Louis, MO, USA) was used to mount coverslips onto the slides, which were left to dry in a fume hood.

Slides were scanned and immunoreactivity on the digital images was assessed at 20X magnification. Immunoreactivity was assessed independently by 2 researchers (CL and Dr Amy Buckley). Any discrepancies were reviewed independently by a third researcher (Dr Jason McGrath). Each researcher noted whether or not CRP staining was present and, if so, whether it was present in stroma, epithelium or both.

4.2.1.3 Matched clinical data

The St James's Upper GI biobank and the hospital electronic patient record (EPR) was used to obtain matched clinical data including age, sex, tumour location and precise histology, disease extent, tumour differentiation, treatment received, Mandard TRG ((306), resection margin status, vital status and date of last follow-up or date of death. Matched serum CRP was recorded where available.

4.2.1.4 Statistical analysis

As the primary aim of immunohistochemistry was to determine whether or not tumoural CRP could be found in OAC tissue, tumoural CRP was analysed as present or absent. Relationship between serum CRP and presence of tumoural CRP was assessed using Pearson's product-moment correlation coefficient. The Kaplan-Meier method was used to assess differences in survival between high and low serum CRP and between tumoural CRP present and absent groups.

4.2.2 Cell culture

In collaboration with Dr Maria Davern, OE33 an oesophageal adenocarcinoma cell line was cultured under various conditions. The cell line originated from a patient with a poorly

differentiated tumour of the lower oesophagus and was obtained from the European Collection of Authenticated Cell Cultures. OE33P (radiosensitive) and OE33R (radioresistant) cells were generated by Lynam-Lennon *et al.*, as described in 2010(355).

4.2.2.1 Cell culture maintenance

The OE33 cells were maintained in Roswell Park Memorial Institute 1640 medium (RPMI 1640), which was supplemented with 1% penicillin-streptomycin(PS) (Lonza, Basel, Switzerland) and 10% foetal bovine serum (FBS)(Lonza, Basel, Switzerland). They were maintained as monolayers in 75cm² filtered culture flasks (Nunc™ EasYFlask™, Thermo Scientific, Waltham, Mass., USA), in a humidified chamber and 37°C and 5% CO₂. Cells were tested regularly for mycoplasma, as per laboratory policy, using PCR.

4.2.2.2 Cell sub-culture

Upon reaching 70-80% confluence, sub-culture was carried out. Growth media was decanted. Cells were then washed with 5ml PBS. Trypsin ethylene-diamine tetra acetic acid (EDTA) solution (0.5% trypsin, 0.02% EDTA) was used to detach the cells: 2 ml was added to the flask. Cells were then incubated for 3-5 minutes at 37°C until the cells had detached. An equal volume of complete RPMI containing foetal bovine serum was added to inactivate the trypsin. A 10ml sterile pipette (Starstedt, Newton, NC, USA) was used to remove any cells adhering to the flask base. The OE33 cells were seeded at a density of 0.1 x 10⁶ cells/ml.

4.2.2.3 Cell counting

A 9 square, 3 x3 glass haemocytometer (Neubauer glass haemocytometer 0.01mm depth, Marienfeld-Superior, Germany) and trypan blue dye were used to assess cell viability and number. Cells were trypsinised as previously described. Cells were centrifuged at 1300 PRM for 3 minutes. The supernatant was discarded and 1ml of RPMI 1640 was used to resuspend the cell pellet. 10 µL of cell suspension was diluted (1 in 20) in 190 µL of trypan blue solution (comprising a 1:1 ratio of trypan blue to PBS). 10 µL of the resulting solution was added to the haemocytometer and viewed immediately with a light microscope at 20X magnification (Nikon Eclipse E200, Nikon Corp., Chiyoda, Japan). Viable cells have an intact cell membrane and thus do not stain blue, while dead cells are stained. Viable (unstained) cells were counted in the four corner squares of the haemocytometer. Any cell in contact with the inner side of the square was excluded but cells touching the outsides were

included in the count. The total number of viable cells per ml was calculated using the following formula: average number of cells per square x dilution factor(20) x 10^4 .

4.2.2.4 Nutrient deprivation and hypoxia treatment

OE33 cells were cultured for 48 hours in the following nutrient and atmospheric oxygenation conditions. The Whitley H35 hypoxystation (Don Whitley Scientific, Bingley, UK) was used to generate hypoxic conditions.

- Complete RPMI, normoxia (21% atmospheric O₂, 5% CO₂, 37°C)
- Complete RPMI, hypoxia (0.5% atmospheric O₂, 5% CO₂, 37°C)
- Serum-free RPMI (0% FBS, 1% PS), normoxia
- Serum-free RPMI, hypoxia
- Glucose-free RPMI ((Gibco™, product code 11560406), (10% FBS, 1% PS)), normoxia
- Glucose-free RPMI, hypoxia
- Glucose and serum-free RPMI ((Gibco™, product code 11560406), (0% FBS, 1% PS)), normoxia
- Glucose and serum-free RPMI ((Gibco™, product code 11560406), (0% FBS, 1% PS)), normoxia

4.2.2.5 Irradiation

An XStrahl RS225 cabinet X-ray irradiator (XSTRAHL, Surrey, UK) was used to irradiate cells at a dose rate of 1.74Gy/minute to a total dose of 1.8 Gy (195kV, 15mA) or to mock-irradiate (0Gy).

4.2.2.6 Chemotherapy

OE33 cells were treated with chemotherapeutic agents as follows. The cells were seeded in a 12-well plate in complete RPMI at a density of 0.2×10^6 cells/ml and incubated overnight (at 37°C and 5% CO₂). The media was replaced with fresh RPMI and the cells treated for 48 hours with an IC₅₀ dose of either cisplatin, docetaxel, epirubicin or 5-FU, as detailed in Table 4.1. The supernatant was then harvested and stored at -80°C.

Table 4-1. Chemotherapeutic agents and concentration used to treat OE33 cells

Agent	IC₅₀
Cisplatin	2.39 μ M
Docetaxel	0.005 μ M
Epirubicin	4.479 μ M
5-FU	2.475 μ M

4.2.2.7 Generation of cell-conditioned media

OE33 cells were seeded in T25 flasks, at a density of 1×10^6 cells per flask. The media was changed the next day. Once 40-50% confluency was reached, the cells were cultured in the nutrient / oxygenation conditions described above or were irradiated, mock-irradiated or treated with chemotherapy drugs as outlined above. Conditioned media was harvested and stored at -80°C for CRP ELISA.

4.2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

The human C-reactive protein / CRP Quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc., MN, USA) was used to quantify the secretion of CRP in supernatant from OE33 cells cultured in the various nutrient and oxygenation conditions outlined above, from OE33 cells treated with chemotherapy or radiation as outlined above and in the vehicle controls (complete RPMI in normoxic conditions, in 0.5% hypoxic conditions or irradiated with 1.8Gy; 0.9% saline; and dimethyl sulfoxide (DMSO)).

All reagents were brought to room temperature before use. The wash buffer, substrate solution, calibrator diluent RD5P were prepared as per manufacturer's instructions. Using the Human CRP standard provided in the kit, 8 solutions of known concentration of CRP (50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.13ng/ml, 1.56ng/ml, 0.78ng/ml and 0ng/ml) were prepared to allow calculation of a standard curve.

100 μ L of assay diluent was added to each well of the polystyrene 96-well plate, provided with the kit, which was pre-coated with a monoclonal antibody to human CRP. 50 μ L of sample, control or standard was added per well and the well was covered with the adhesive strip provided. The plate was then incubated at room temperature for 2 hours. The plates

were washed by dipping the plate in wash buffer, shaking twice and patting dry on clean paper towel. Human CRP conjugate from the kit was added, 200 μ L to each well, and the plate was covered with a new adhesive strip. The plate was incubated at room temperature for 2 hours. Next, the plates were washed by dipping the plate in wash buffer, shaking twice and patting dry on clean paper towel. 50 μ L of Substrate Solution was added to each well before incubation at RT for 30 mins, under cover for protection from light. After the incubation, 50 μ L of Stop Solution was added to each well. A microplate reader, set to 450nm, was used to determine the optical density (O.D).of the contents of each well. The average O.D. from duplicate samples for each standard, sample and control was calculated. The average zero standard OD was subtracted from each result to give the corrected O.D. for each well.

A standard curve was generated in Microsoft Excel 2016 (Microsoft Corporation, Redmond Washington, USA) from the OD for the CRP standards and was used to determine the concentration of each sample tested.

4.2.4 Immunofluorescence

In collaboration with Dr Margaret Dunne and Dr Mark Bates, immunofluorescence (IF) was used to assess presence of CRP in pre-treatment OAC biopsies.

The SJH/AMNCH REC granted ethical approval for this study.

4.2.4.1 Immunofluorescence

Formalin-fixed paraffin-embedded (FFPE) biopsies from n=107 patients of St James's Hospital Dublin with OAC were reviewed. Biopsies had been taken pre-treatment. A pathologist reviewed the specimens and identified tumour and stromal areas. Cases which contained $< 2\text{mm}^2$ of tumour were excluded.

3 μm sections were taken from each FFPE block. A Thermo Scientific™ Shandon™ Sequenza™ Immunostaining centre (ThermoFisher Scientific, Waltham, Mass., USA) was used to manually stain the sections. Sections were de-waxed and rehydrated. Hydrogen peroxide (H_2O_2) 3% w/w solution (Sigma Aldrich), was used for peroxidase blocking for 10 minutes. Antigen retrieval was performed for five minutes using citrate buffer (pH6) in a pressure cooker. Tissue slides were incubated with 10% goat serum solution (from Tyramide SuperBoost kit (Thermo Fisher Scientific Inc Waltham, Mass., USA)) for ten mins at RT.

The primary antibody (recombinant rabbit monoclonal antibody to human CRP [ab32412, Abcam, UK] diluted to 1 in 500 with PBS) was added to the slides for 30 minutes at RT. Slides were washed three times with PBS containing 0.1% Tween 20 (PBST). A goat anti-rabbit HRP-tagged secondary antibody was then applied for 1 hour at RT. Following this, slides were incubated for 30 minutes with an Alexa Fluor tyramide-based target signal amplification (TSA) probe, which was supplied as part of a Tyramide SuperBoost kit (Thermo Fisher Scientific Inc Waltham, Mass., USA). A microwave (Daewook KOK-457 on setting: defrost 850g meat) and citrate buffer (pH6) were used for heat-stripping. An Alexa Fluor™ TSA probe, FITC (488 nM), was used to visualise CRP. The epithelial cell marker pan-cytokeratin (Pan-CK), (Agilent DAKO, polyclonal wide spectrum screening), was visualised using a streptavidin conjugated Alexafluor 750nM fluorescent probe (Thermo Fisher Scientific Inc) and a biotinylated anti-rabbit secondary antibody (Thermo Fisher Scientific Inc, Waltham, Mass., USA). Slides were counterstained using Hoescht dye

for 30 minutes (Thermo Fisher Scientific Inc. Waltham, Mass., USA). Slides were washed twice in PBST. Alcohol (80%) was used to dehydrate slides for 1 minute. Finally, slides were mounted using ProLong™ Gold Anti-fade mounting media (Thermo Fisher Scientific Inc, Waltham, Mass., USA) and coverslips were added. A Zeiss Axioscan Z1 whole slide scanner (Zeiss, Germany) was used to capture whole slide images for each biopsy at 10x magnification.

Definiens image analysis software packages (Tissue Studio® and Developer XD™ (Definiens AG, Munich)) were used to import and analyse whole-slide images. Tissue Studio's "Tissue-Background Separation" algorithm was used to identify the whole tissue biopsies. Regions of interest were automatically segmented. They were then manually verified and, where necessary, corrected. False nuclei, cells and other artifacts were detected and removed using Developer XD™ software. Pan-CK staining was used to distinguish areas of tumour and areas of stroma, with areas positive for Pan-CK staining considered tumour and areas negative for Pan-CK considered stroma. CRP positivity was assessed in stromal nuclei, tumoural cytoplasm and tumour nuclei. CRP positivity was recorded as density (number of positively stained cells per area of stroma, tumour or total area (μm^2)) and percentage (%) (number of positively stained cells/total number of cells in the region of interest).

4.2.4.2 Matched clinical data

The St James's Upper GI biobank was used to obtain matched clinical data including age, sex, disease extent, tumour differentiation, treatment completed, Mandard TRG(306), vital status and date of last follow-up or date of death. Matched serum CRP was recorded where available, this had been measured using ELISA as part of a larger study(356).

4.2.4.3 Statistical analysis

Chi-square Test for Independence was used to analyse the relationship between two categorical variables. Absolute values for CRP positivity measures were analysed as continuous variables. CRP measures were also categorised using X-tile software (Camp 2004) was used to determine optimal cutpoints to separate the cohort into high and low subsets for each measure. Relationship between serum CRP and CRP positivity was assessed using Pearson's product-moment correlation coefficient. The Kaplan-Meier method was

used to assess differences in survival between high and low serum CRP and between high and low CRP positivity groups in stromal nuclei, tumoural cytoplasm, tumour nuclei and in the tissue as a whole; and assessed as density and as a percentage of cells. All significance testing was two-sided and a p value of <0.05 was considered statistically significant.

4.3 Results

4.3.1 Western Blot

Western blot confirmed specificity of the antibody which was to be used for immunohistochemistry. A clear band was demonstrated in the liver tissue and in the positive control (Figure 4.1).

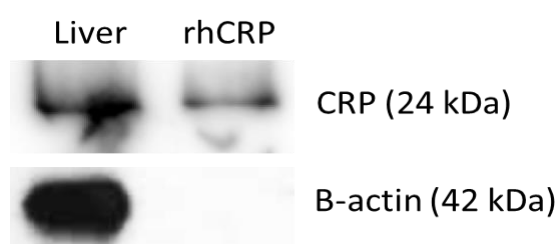


Figure 4-1. Expression of C-reactive protein (CRP) in fresh snap-frozen liver tissue

Liver tissue was probed with a polyclonal rabbit anti-human CRP antibody (1:1000 dilution in 0.5% BSA solution) and a secondary antibody goat anti-rabbit-HRP (1:5000 dilution in 0.5% BSA solution). A recombinant human (rh) CRP standard was used as a positive control. B-actin was used as a loading control. Bands were visualised via chemiluminescence using an Agfa film processor (Agfa-Gevaert N.V., Mortsel, Belgium).

4.3.2 Immunohistochemistry

4.3.2.1 Demographics

Data from 37 patients were included. All included patients had adenocarcinoma but 8 also had other histological types noted within the tumour – six signet-ring cell carcinoma, one neuroendocrine and one GIST (GI stromal tumour). Tumour location was lower oesophagus for 4 and OGJ for 33. Demographics of included patients are given in Table 4.2. Median age was 63 years (range 41-76), 84% (n=31) were male; ethnicity was not recorded. A small majority (57% (n=21) had pT3 or pT4 disease, with 41% (n=15) having pT0-p2 disease. 49% (n=18) had pN0 disease and another 49% (n=18) had pN1-pN4 disease. None had documented metastatic (M1) disease and only one patient had unknown (Mx) disease. Almost half (49%, n=18) had moderately or well- differentiated tumours, 43% (n=16) had poorly or undifferentiated tumours. Five patients (14%) had Mandard TRG of 1-2, 35% (n=13) TRG 3-5 and data were missing for one patient (3%).

All were treated surgically with 49% (n=18) having neoadjuvant chemoradiotherapy and 8% (n=3) neoadjuvant chemotherapy.

Table 4-2. Demographics of Included Patients (n=37)

Characteristic	No of patients (%)
Age, years Median (range)	63 (41-76)
Gender Male Female	31 (84%) 6 (16%)
Tumour Location Lower oesophagus OGJ	4 (11%) 33 (89%)
Histology OAC OAC + Other OAC + signet cell OAC + neuroendocrine OAC + GIST	29 (76%) 8 (22%) 6 1 1
Disease extent pT0 - pT2 pT3 - pT4 Missing data on pT category pN0 pN1 pN2 pN3 Missing data on N category cM0 cMx	15 (41%) 21 (57%) 1 (3%) 18 (49%) 4 (12%) 5 (15%) 9 (24%) 1(3%) 36 (97%) 1 (3%)
Tumour Differentiation Well or moderately differentiated Poorly differentiated Missing data	18 (49%) 16 (43%) 3 (8%)
Treatment received Surgery Multimodal: surgery + chemotherapy Multimodal: surgery and chemoradiotherapy Missing data	15 (41%) 3 (8%) 18 (49%) 1 (3%)
Tumour Regression Grade 1-2 3-5 Not applicable (No neoadjuvant treatment) Missing data	5 (14%) 13 (35%) 15 (41%) 4 (11%)
Resection margin status R0 R1 Missing data	28 (76%) 8 (22%) 1 (3%)

OGJ: adenocarcinoma of gastro-oesophageal junction. pT / pN / pM: pathological TNM category.
cM: clinical M category.

4.3.2.2 Serum CRP

Serum CRP was available for 34 patients. Median CRP was 3.4 mg/L (range 1 – 246). Eleven people (30%) had a CRP > 10mg/L.

4.3.2.3 Tumoural CRP IHC

Tumoural CRP was detected in 25 (68%) of samples. Tumoural CRP was identified in stroma alone in 16 (43%) and stroma and epithelium in 9 (25%), as seen in Figure 4.2.

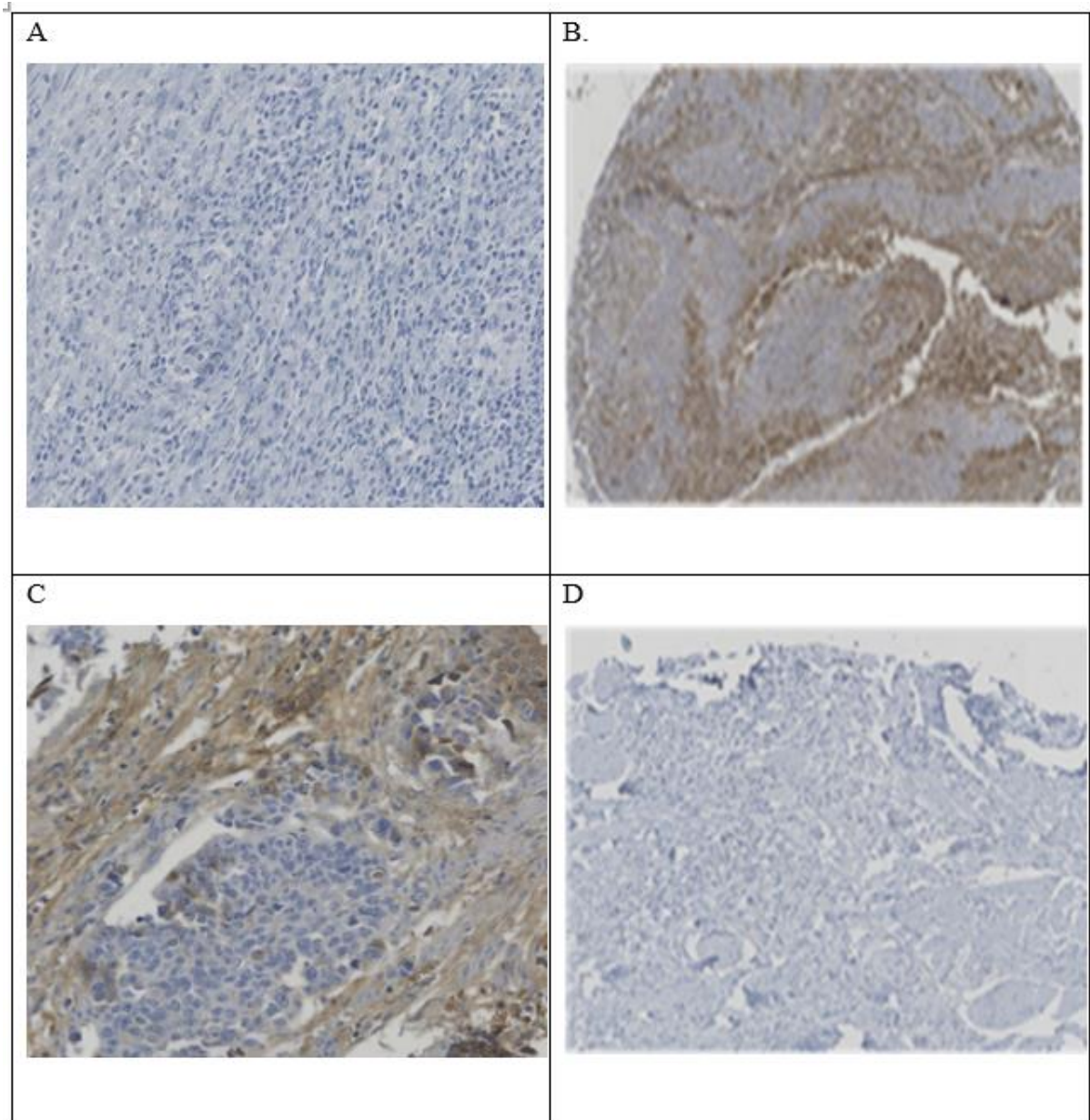


Figure 4-2. Expression and localisation of CRP in Resected OAC Tumours

A. Negative control with absence of any staining for CRP. B. Stroma and epithelium stained positively for CRP. C. Stroma only stained positively for CRP. D. Absence of CRP staining (neither stroma nor epithelium).

Expression and localisation of CRP was examined in tumour tissue microarrays (TMAs) from n=37 resected OAC tumours, using a monoclonal antibody to human CRP (ab32412, Abcam, UK). For the negative control, PBS was added to one slide in lieu of the antibody.

4.3.2.4 Relationship between tumoural CRP and patient and disease characteristics

There was no statistically significant correlation between increasing age and presence of tumoural CRP, $r=0.032$, $p=0.853$. Table 4.3 details the relationship between tumoural CRP and other patient and disease characteristics. There were no statistically significant associations between presence of tumoural CRP and sex, tumour location (lower oesophagus or OGJ), histology (OAC or OAC and other), pathological T stage, pathological N stage, tumour differentiation, treatment received (multimodal or surgery alone), TRG or resection status.

Table 4-3. Relationship between tumoural CRP and patient and disease characteristics

	Sex		Tumour location		Histology		Disease extent (T stage)		Disease extent (N stage)		Differentiation		Treatment received		TRG		R status	
	M	F	Oes	OGJ	OAC	OAC+ Other	pT0-pT2	pT3-pT4	pN0	pN1-pN4	UD / poor	Mod/well	MM	Surgery	1-2	3-5	R0	R1
CRP present in tumour	19	6	1	24	20	5	9	15	10	14	11	12	13	11	3	8	18	6
CRP not present in tumour	12	0	3	9	8	3	6	6	8	4	5	6	8	4	2	5	10	2
Statistic	NR		NR		NR		$\chi^2=0.514$, p=0.473		$\chi^2=2.00$, p=0.157		$\chi^2=0.17$, p=0.897		$\chi^2=0.514$, p=0.473		NR		NR	

M: male, F: female, Oes: Oesophagus, OGJ: Oesophago-gastric junction, UD: Undifferentiated, MM: Multimodal, TRG: Tumour Regression Grade, R: Resection margin status, NR: not reported as assumptions for the relevant statistical test (Chi-Square Test for Independence) are violated and no other statistical test is appropriate.

4.3.2.5 Relationship between serum CRP and tumoural CRP

There was no statistically significant correlation between serum CRP level and presence of tumoural CRP: $r=0.096$, $p=0.591$.

4.3.2.6 Follow-up and survival

Median follow-up was 37 months (range 4 – 114). Thirty people (81%) died during the follow-up period.

4.3.2.7 Relationship between serum CRP and survival and CRP in tumour biopsy and survival

4.3.2.7.1 Serum CRP and Overall Survival

There was no statistically significant difference in overall survival between people with a high CRP ($> 10\text{mg/L}$) (median survival 20 months (range 0-53)) and with a normal CRP ($\leq 10\text{mg/L}$) (median survival 37 months (range 30-44)), as seen in Figure 4.3.

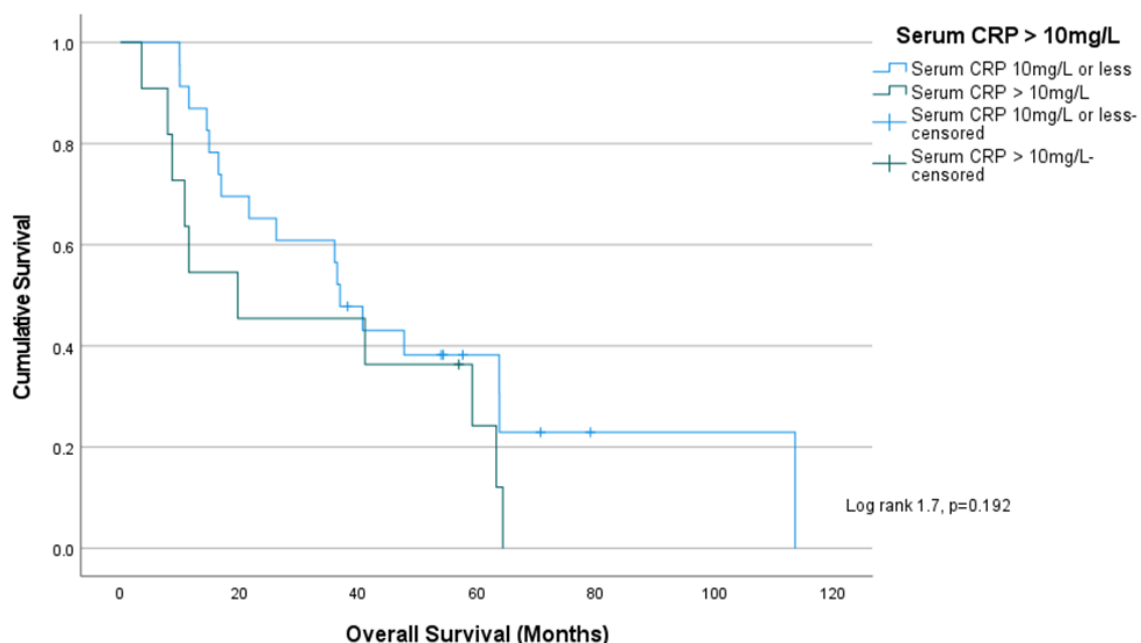


Figure 4-3. Kaplan-Meier survival curve of overall survival in OAC/OGJ in patients with serum CRP $> 10\text{mg/L}$ and patients with serum CRP $\leq 10\text{mg/L}$

The Kaplan-Meier method was used to assess the prognostic impact of serum CRP on overall survival in 37 adults with OAC or OGJ adenocarcinoma. Log rank (Mantel-Cox) chi square test was used to assess statistical significance of survival differences between normal CRP, here defined as less than or equal to 10mg/L (blue line) and high CRP (green line) groups.

4.3.2.7.2 Tumoural CRP present in resected tumour tissue and survival

There was no statistically significant difference in overall survival between patients where tumoural CRP was present (median survival 22 months, 95% CI 11-33 months) and not present (median survival 41 months, 95% CI 25-58 months), as seen in Figure 4.4.

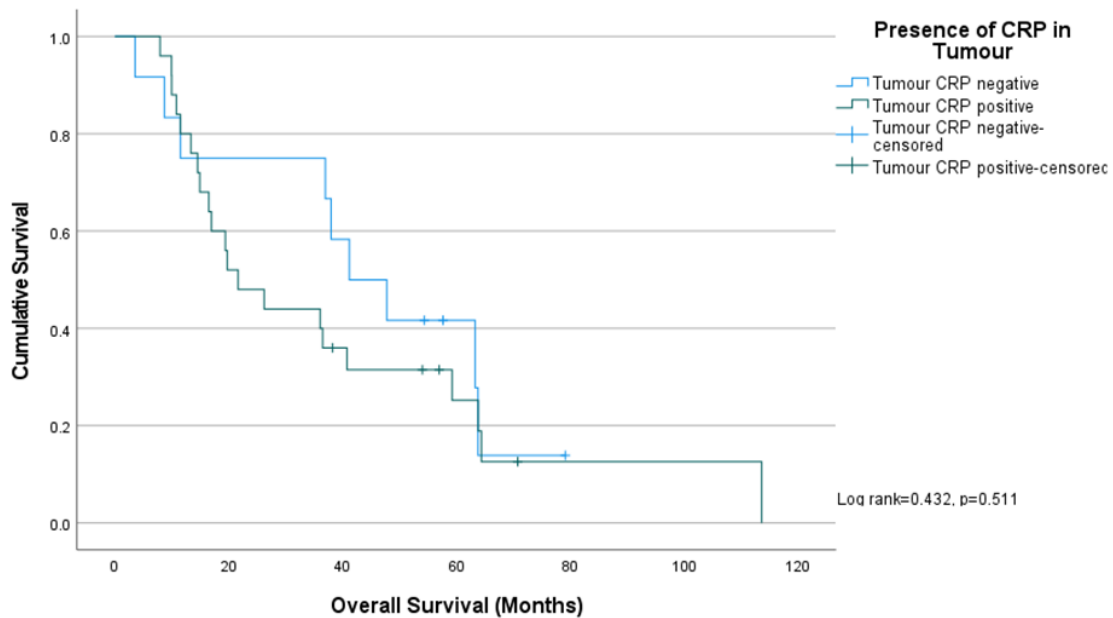


Figure 4-4. Kaplan-Meier survival curve of overall survival in patients where tumoural CRP was positive or absent in resected tumour tissue

The Kaplan-Meier method was used to assess the prognostic impact tumoural CRP-resected tumour samples on overall survival in 37 adults with OAC or OGJ adenocarcinoma. Log rank (Mantel-Cox) chi square test was used to assess statistical significance of survival differences between tumour CRP negative (blue line) and tumour CRP positive (green line) groups.

4.3.3 ELISA: OE33 cell line

The mean minimum detectable dose (MDD) for the CRP assay was 0.010 ng/mL. All samples tested had CRP concentration less than the mean minimum detectable level of the assay. i.e., all samples had undetectable CRP levels.

4.3.4 Immunofluorescence

4.3.4.1 Demographics

Data from 107 patients were included. All included patients had OAC. Demographics of included patients are given in Table 4.4. Median age was 64 years (range 30-80), 92% were male and ethnicity was not recorded. Data were missing regarding TNM stage in almost half. Where TNM stage was available, most (n=47) had T3 or T4 disease, while 29 had N0 disease and 28 had N1 disease. Only 2 people were recorded as having metastatic (M1) disease. Almost half (49%) had poorly differentiated tumours, well or moderately differentiated in 36% with missing data for 15%. 72% had Mandard TRG of 3-5, 26% TRG 1-2 and data were missing for 2%. All received multimodal treatment with 57% having chemoradiotherapy and surgery and 43% receiving chemotherapy and surgery.

Table 4-4. Demographics of Included Patients (n=107)

Characteristic	No of patients (%)
Age, years	
Median (range)	64 (30-80)
Gender	
Male	98 (92%)
Female	9 (8%)
Disease extent	
T1 or T2	6 (6%)
T3 or T4	47 (44%)
Missing data on T stage	54 (50%)
N0	29 (27%)
N1	28 (26%)
Missing data on N stage	50 (47%)
M0	45 (42%)
M1	2 (2%)
Missing data on M stage	60 (56%)
Tumour Differentiation	
Well or moderately differentiated	39 (36%)
Poorly differentiated	52 (49%)
Missing data	16 (15%)
Treatment received	
Surgery and chemotherapy	46 (43%)
Surgery and chemoradiotherapy	61 (57%)
Tumour Regression Grade	
1-2	28 (26%)
3-5	77 (72%)
Missing data	2 (2%)

4.3.4.2 Serum CRP

Serum CRP was available for 35 patients. Median CRP was 1.87mg/L (range 0.09 – 73). Four people had a CRP > 10mg/L. All four patients were male, aged 63-79 and all were deceased at time of follow-up.

4.3.4.3 Follow-up and survival

Median follow-up was 27 months (range 6 – 115 months). Sixty-two people (58%) died during the follow-up period.

4.3.4.4 Tumoural CRP Immunofluorescence: digital score

As detailed in Table 4.5, the majority of OAC biopsy samples studied stained highly-positive for CRP in stromal nuclei and tumour nuclei, whether scored as density or as a percentage of cells. Tumour cytoplasm was highly-positive for CRP in 56% of samples, when scored by percentage but only 43% when scored by density. Similarly, tumour cytoplasm was highly-positive for CRP in 65% of samples, when scored by density but only 48% when scored by percentage.

Table 4-5. Number and Percentage of OAC Biopsies with High CRP on digitally-scored immunofluorescence

	High CRP-positivity density	High % CRP-positivity
Stromal Nuclei	74 (69%)	82 (77%)
Tumour Cytoplasm	46 (43%)	60 (56%)
Tumour Nuclei	51 (48%)	70 (65%)
Total	58 (54%)	73 (68%)

4.3.4.5 Relationships between serum CRP and CRP in tumour biopsy

As seen in Table 4.6, there was no statistically significant correlation between serum CRP and CRP positive cells in stromal nuclei, tumour cytoplasm or tumour nuclei, as assessed by digital scoring, whether CRP positivity was measured in terms of density or as a percentage.

Table 4-6. Relationship between serum CRP and CRP in OAC tumoural biopsy by digitally-scored immunofluorescence

	CRP- Positive Stromal Nuclei: Density	% CRP- Positive Stromal Nuclei	CRP- Positive Tumour Cytoplasm Density	% CRP- Positive Tumour Cytoplasm	CRP- Positive Tumour Nuclei Density	% CRP- Positive Tumour Nuclei	Total CRP- Positive Nuclei: Density	% CRP- Positive Nuclei
Serum CRP	$r=-0.014$ $p=0.937$	$r=0.011$ $p=0.949$	$r=0.099$ $p=0.583$	$r=0.112$ $p=0.522$	$r=-0.098$ $p=0.576$	$r=-0.095$ $p=0.586$	$r=-0.041$ $p=0.816$	$r=0.118$ $p=0.500$

r : Pearson's product-moment correlation coefficient

4.3.4.6 Serum CRP and Overall Survival

All people with a high CRP were censored i.e., were alive at time of last follow-up (median follow-up in this subgroup was 50 months, range 18-61 months). There was no statistically significant difference in overall survival between people with a high CRP ($> 10\text{mg/L}$) and with a normal CRP ($\leq 10\text{mg/L}$)(Figure 4.5).

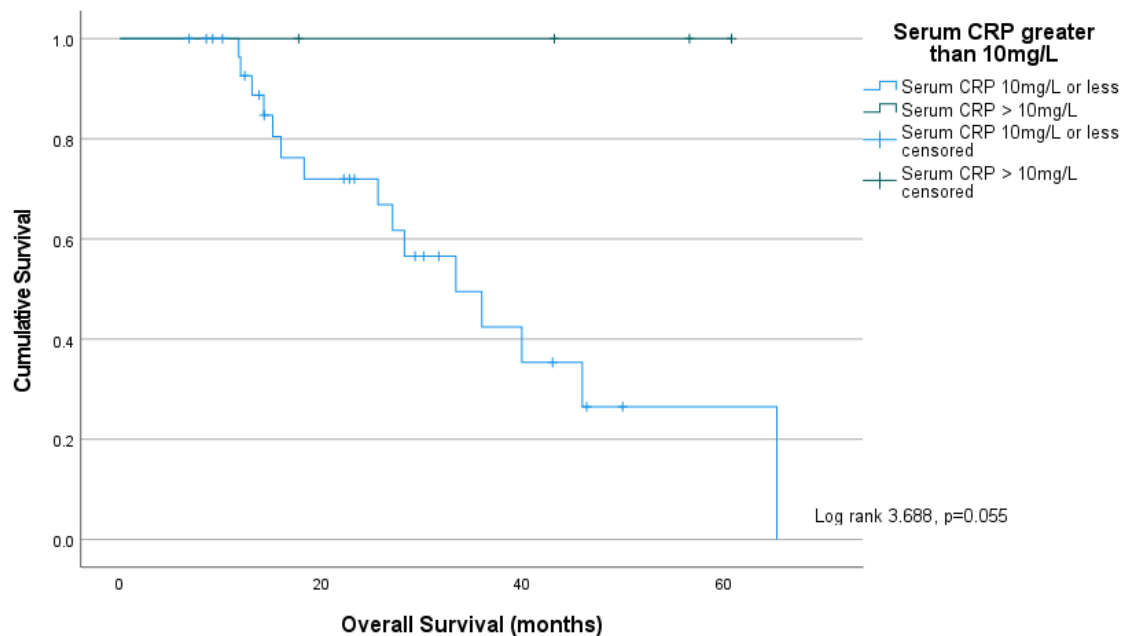


Figure 4-5. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with serum CRP $> 10\text{mg/L}$ and patients with serum CRP $\leq 10\text{mg/L}$

The Kaplan-Meier method was used to assess the prognostic impact of serum CRP on overall survival in $n=35$ adults with OAC. Log rank (Mantel-Cox) chi square test was used to assess statistical significance of survival differences between normal CRP, here defined as less than or equal to 10mg/L (blue line) and high CRP (green line) groups.

4.3.4.7 Relationship between CRP in tumour biopsy and survival

Density of CRP-positivity in tumour cytoplasm and density of CRP-positivity in tumour nuclei were associated with overall survival, with higher density being associated with statistically significantly shorter survival, as detailed in Table 4.7 and illustrated in Figures 4.6 and 4.7, respectively.

Other measures of CRP-positivity in tumour samples were not associated with statistically significant differences in survival (Table 4.7).

Table 4-7. Relationship between CRP in OAC tumoural biopsy, assessed by digitally-scored immunofluorescence, and survival

	CRP-Positive Stromal Nuclei: Density	%CRP-Positive Stromal Nuclei	CRP-Positive Tumour Cytoplasm Density	%CRP-Positive Tumour Cytoplasm	CRP-Positive Tumour Nuclei Density	%CRP-Positive Tumour Nuclei	Total CRP-Positive Nuclei: Density	%CRP-Positive Nuclei
Median survival and 95% CI high group (months)	36 (25-46)	36 (25-47)	29 (17-41)	33 (22-45)	27 (6-47)	29 (19-40)	33 (22-44)	33 (22-44)
Median survival and 95% CI low group (months)	73 (28-118)	73 (31-115)	67 (36-97)	73 (22-124)	53 (25-82)	72 (31-113)	44 (18-80)	72 (22-115)
p value	0.144	0.105	0.035*	0.065	0.028*	0.073	0.087	0.127

*p<0.05. 95% CI: 95% confidence interval

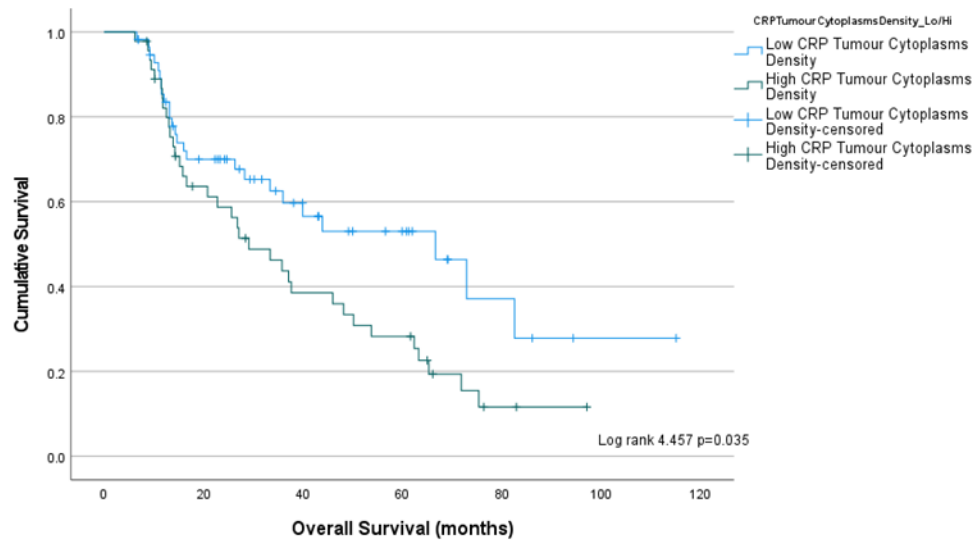


Figure 4-6. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with high digitally-scored CRP-positivity density in tumour cytoplasm in tumour biopsies when compared to low digitally-scored CRP-positivity density

The Kaplan-Meier method was used to assess the prognostic impact of digitally-scored CRP-positivity in tumour cytoplasm in tumour biopsy samples on overall survival in 103 adults with OAC. Log rank (Mantel-Cox) chi square test was used to assess statistical significance of survival differences between high CRP-positivity density in tumour cytoplasm (green line) and low CRP-positivity density (blue line) groups.

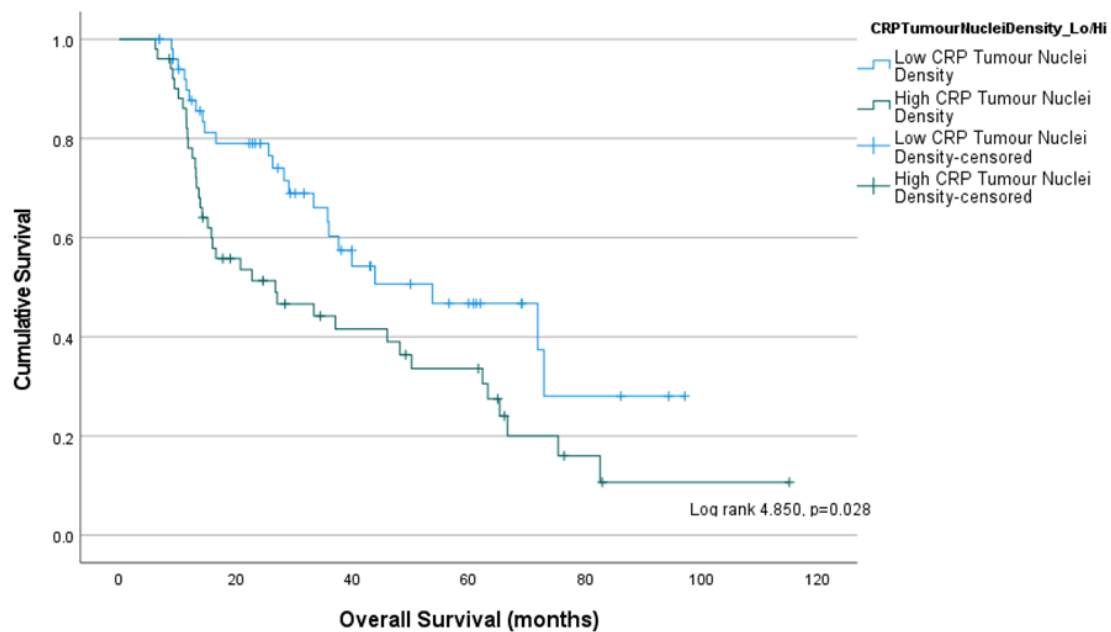


Figure 4-7. Kaplan-Meier survival curve illustrating overall survival in OAC in patients with high digitally-scored CRP-positivity density in tumour nuclei in tumour biopsies when compared to low digitally-scored CRP-positivity density

The Kaplan-Meier method was used to assess the prognostic impact of digitally-scored CRP-positivity in tumour cytoplasm in tumour biopsy samples on overall survival in 103 adults with OAC. Log rank (Mantel-Cox) chi square test was used to assess statistical significance of survival differences between high CRP-positivity density in tumour cytoplasm (green line) and low CRP-positivity density (blue line) groups.

4.4 Discussion

The study described in this chapter has provided new information about CRP in OAC tissues. This work has confirmed that CRP is present within some but not all OAC tumours. It has also shown that CRP can be present within OAC tumours at time of diagnosis and also following treatment, whether multimodal or surgery alone. This work outlined in this chapter reveals that CRP may be found in OAC cancer cells (both within the nucleus and the cytoplasm) and in stromal tissue (both nucleus and cytoplasm). CRP within tumours was not correlated with serum CRP in the cohorts studied here, which supports a local origin. Interestingly, CRP was not secreted from an OAC cell line, regardless of culture conditions or treatments applied to the cell line, suggesting that the cancer cells in isolation cannot produce CRP. CRP in tumour cytoplasm and tumour nuclei on pre-treatment biopsy was associated with shorter overall survival. Each aspect of these findings and the implications are discussed in detail below.

This work has demonstrated conclusively that CRP can be found within OAC tumour tissue. Moreover, and for the first time, CRP was visualised in pre-treatment biopsy as well as in resected tumour specimens. This novel finding is important as it shows that tumoural CRP is not simply a response to treatment-induced inflammation but is present at time of diagnosis. This may reflect the inflammatory carcinogenic process in OAC(153). Both manual immunohistochemistry and automated immunofluorescence were able to identify CRP within OAC tumour tissue.

CRP was present in most but not all OAC tumours studied. Its presence was demonstrated in both pre- and post-treatment cohorts. CRP was identified in two-thirds of resected tumour samples studied and was present in tumours treated with multimodal treatment, as well as those treated with surgery alone. The only previously published work in this area, reported in a conference abstract by Räsänen *et al.*, found that CRP was expressed in 11 of 18 OAC tumours(349) – a strikingly similar proportion. The present work has confirmed those findings in a resected cohort twice the size of the Räsänen cohort and gone on to show that CRP can also be present pre-treatment. Digital scoring identified some level of CRP in all pre-treatment biopsies with over half having CRP levels graded “high” based on software-calculated cutpoints. Presence of CRP within OSCC tumours was reported in 49% (339) – 59%(209, 357) in studies of that histological type. In other cancer sites where tumoural CRP

has been identified, the percentage of samples where was present ranged widely, from 28% in hormone-naïve prostate cancer(340) to 79% in renal cell carcinoma(341).

Using chromogenic staining, CRP was visualised in resected OAC tissue. When present, it was always in the stroma and in some cases (25%) was also in the epithelium. It was not identified in epithelium alone. Immunofluorescence staining of pre-treatment OAC biopsies explored this localisation further and demonstrated that, when present, CRP was localised to stromal nuclei, tumour cytoplasm and tumour nuclei. The proportion of samples with high CRP was highest in stromal nuclei, then tumour nuclei and lastly tumour cytoplasm (digitally-scored). In their OAC sample, Räsänen *et al.*, identified CRP in glandular epithelial cells in OAC tumours, as well as in fibroblasts, mononuclear phagocytes and endothelial cells(349). They did not describe the intracellular location i.e. cytoplasm or nuclear distribution. In prostate cancer, both McCall *et al.*, (210) and Elsberger(340) identified CRP within the cytoplasm and the nuclei of tumour cells. Studies in renal cell carcinoma(341, 342) and colorectal carcinoma(345) also reported this localisation, while Powell reported CRP within tumour cytoplasm(346). None of the studies in OSCC(209, 339, 357) reported precise localisation of CRP in their cohorts, nor did studies in other cancer sites (211, 344, 358).

The findings of this work are mixed with regard to the relationship between tumoural CRP and survival. No relationship was found between presence of tumoural CRP in resected OAC tumour and survival, although the sample size was small, leaving the possibility of a Type II statistical error(359). In pre-treatment biopsies, a statistically significant relationship was identified between shorter overall survival and high CRP positivity (density) in tumour cytoplasm and tumour nuclei. However, when the same factors (i.e., high CRP positivity in tumour cytoplasm and tumour nuclei) were analysed differently (as percentage, rather than as density), the statistical relationship was not seen. Furthermore, the relationship between survival and presence of tumoural CRP was examined using 8 different metrics of CRP-positivity, as well as serum CRP, with an increasing number of comparisons being associated with an increased risk of a Type I statistical error(246). The hypotheses for this study had been pre-specified and this is exploratory initial work on the topic, hence it was appropriate to refrain from correction for multiple testing e.g. Bonferroni testing(246) but, by the same logic, the findings are the basis for future work, rather than definitive.

Previous studies differ in their findings with regard to the relationship between tumoural CRP and survival, while several studies, including the previous work in OAC(349), did not report the relationship ((342, 345, 358) (341). Both of the published studies in OSCC(208, 209) reported an association between tumoural CRP and shorter survival. Shin *et al.*, in hepatocellular carcinoma(211) and Powell *et al.*, in colorectal carcinoma(346), also showed a statistically significant association, though the latter was only in left-sided cancers. In prostate cancers, the data are mixed with McCall *et al.*, finding an association between high levels of tumoural CRP (either nuclear or cytoplasmic) and shorter survival(210) but Elsberger *et al.*, found no association(340). Meanwhile, in renal cell carcinoma, Can *et al.*, noted a trend toward shorter survival when CRP was present in the tumour but this was not significant in multivariate analysis(343).

This study also provides new insights into the possible source of CRP within OAC tumours. In contrast to tumour tissue, where CRP was identified in most samples, CRP was not present in supernatant from an OAC cell line. To replicate conditions in the tumour microenvironment, the OE33 cells were cultured under stress conditions (low pH, reduced nutrient supply, hypoxia), as well as non-stress conditions, but this still did not stimulate CRP production. Application of anti-neoplastic treatments (chemotherapy or radiotherapy) also failed to stimulate the OE33 cells to secrete detectable levels of CRP. These findings suggest that OAC cancer cells in isolation may not be able to produce CRP. Very few studies have addressed the ability of cultured cancer cells to produce CRP. Grimm *et al.*, showed that scc-4 (an oral squamous cell carcinoma cell line) expressed CRP(360). Smith *et al.*, and Yoshida *et al.*, demonstrated that the hepatoma cell lines HepG2 and Hep3B, respectively, could secrete CRP(361, 362). Given that the liver is the predominant source of CRP, this is perhaps not surprising, but, in fact, Shin *et al.*, found that only certain hepatocellular cancer cell lines secrete CRP(211), suggesting that there is variation even within cancer cell types and future work could assess additional OAC cancer cell lines.

The source of tumoural CRP remains uncertain(340). In a non-cancer setting, it has been suggested that CRP localised within coronary artery atherosclerotic lesions originates in the blood and is deposited on arterial walls when serum CRP levels are high(363). In cancer, it is plausible that serum-derived CRP could enter the TME through tumour microvasculature, which is known to be “leaky”(149). On the other hand, locally-produced CRP could contribute to circulating CRP, as proposed by Nozoe *et al.*,(208) and Jabs *et al.*,(341).

Nozoe *et al.*, found an association between presence of CRP visualised with IHC within resected OSCC and serum CRP(208). Jabs *et al.*, showed a positive correlation between intratumoural CRP mRNA expression and serum CRP levels in renal cell carcinoma(341). There may also be no connection between the serum and tumoural CRP. Nakatsu *et al.*, in their study in OSCC, found no relationship between tumoural and serum CRP(209). Consistent with this, in the current work, there was no statistically significant correlation between serum CRP and presence of CRP in either OAC resected tumour or in pre-treatment biopsies. Furthermore, tumoural CRP was associated with shorter survival in the pre-treatment cohort, but a high serum CRP was not, suggesting that serum CRP and tumoural CRP are not directly related in OAC.

Elsberger speculated that tumour cells absorb CRP from “their surrounding environment”(340). The results set out in this chapter suggest that the stroma is the most likely source in OAC. The only previous work in OAC supports this theory, having demonstrated CRP expression in endothelial cells, fibroblasts and mononuclear phagocytes(349). Motoyama *et al.*, found a statistically significant association between a CRP genetic polymorphism and IHC-detected tumoural CRP in OSCC but no association between the polymorphism and serum CRP levels in that cohort(357), suggesting distinct mechanisms of production for serum and tumoural CRP. The exact process through which local CRP could be made remains unclear, though McArdle *et al.*, hypothesised that host inflammatory cells could produce IL-6, which in turn stimulates CRP production(364).

The tumour microenvironment (TME) is now understood to be a dynamic environment, where diverse cell types are present and interact with the neoplastic carcinoma cells(149). Several of these cells are potential sources of CRP. Endothelial cells are present in the TME(149). Endothelial cells at other sites have been shown to be capable of CRP production when exposed to stress or toxins. In the aorta, endothelial cells exposed to atherogenic stress secreted CRP(365) while endothelial cells in brain microvasculature secreted CRP on exposure to aluminium(366). Since almost every cancer is infiltrated with immune cells, these are another potential source of CRP. Lymphocytes were the first proven extra-hepatic site of CRP synthesis(206). In 2006, Haider *et al.*, found that peripheral blood mononuclear cells, harvested from volunteers who had been given lipopolysaccharide (LPS), made and secreted CRP(367). *In vitro*, monocytes stimulated with LPS are able to produce CRP(368), as are macrophages activated by angiotension II, IL-6 or nicotine(369-

371). Adipocytes are another possible source of CRP. Adipocytes are capable of producing CRP(354, 372) after exposure to any of the inflammatory cytokines IL-1 β , IL-6 or resistin. Epigenetic reprogramming of cells in the TME by local factors, including cytokines, is proposed to play a role in their recruitment to support the tumour's growth(151). In a mouse model, adipose stromal cells (ASCs), derived from white adipose tissue, migrated via the blood to tumours and were able to engraft within the tumour stroma as adipocytes(373). ASCs were also shown to promote cancer progression, highlighting a possible mechanistic link between obesity (where ASCs are abundant) and cancer progression(373). ASCs are known to interact with immune cells in the TME and potentially to influence critical elements of cancer development and progression including chronic inflammation, epithelial-to-mesenchymal transition and angiogenesis(154).

The pathway of production of CRP within these extra-hepatic sites is not yet clear. Reactive oxygen species (ROS) may play a part. ROS plays a key role in inflammation in cancer; particularly through the induction of proinflammatory transcriptional factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)(52), which in turn mediate cellular stress response. Ming Li *et al.*, showed that CRP generation in macrophages may occur via angiotensin receptor (AT₁) and a ROS-MAPK signalling pathway, but also suggest that the precise pathway may differ between cells(369).

If CRP is indeed produced locally, it may be produced at lower levels than secreted by the liver, as has been noted in human coronary artery smooth muscle cells(374). Nakatsu *et al.*, hypothesised that, in OSCC, tumoural CRP levels may be too low to affect serum CRP levels(209). They propose that tumoural CRP may exert its effect locally, rather than by influencing circulating CRP(209). The precise nature of how it may exert that effect, however, is unclear. A possible parallel is in atherosclerosis. It has been shown that CRP, produced locally by endothelial cells and infiltrating monocytes and lymphocytes, may contribute to neovascularisation within the atherosclerotic plaque(350). Given that angiogenesis is a hallmark of cancer (149), the potential for CRP to have a similar role in the TME is clear. Other work in atherosclerosis showed that CRP stimulates expression of matrix metalloproteinase-1 (MMP-1) by histiocytes, another mononuclear immune cell(375). The MMP family of endopeptidases has been implicated(376) in another of the hallmarks of cancer, invasion and metastasis(149). MMP-1 has been found to promote tumour growth and metastasis in OSCC(377). MMP-1 is also strongly associated with proliferation in Barrett's oesophagus and OAC, as well as lymph node metastasis in

OAC(378). Consistent with this potential link between tumoural CRP and metastasis, Elsberger *et al.*, found an association between CRP in tumour nuclei and presence of metastases in prostate cancer(340). The role of the TME is far from fully understood but it is interesting to note that, in colorectal cancer, a high percentage of stroma relative to carcinoma cells is an independent prognostic marker(379). This finding has been applied in the Glasgow Microenvironment Score, which incorporates a measure of inflammatory infiltrate as well as tumour stroma percentage(380), and recently was confirmed as a prognostic tool in colorectal cancer(381), highlighting the key role the TME, and its inflammatory and stromal cells, plays in cancer(382). Local secretion and local action of CRP is consistent with this growing understanding.

There are several aspects of tumoural CRP which are not fully understood and which future studies should address. As outlined above, the nature of the relationship, if any, between serum CRP and tumoural CRP remains unclear. The source of tumoural CRP is uncertain. The work presented in this chapter suggests that the stroma is a likely source in OAC. Future studies could assess for the presence of mRNA in the stroma, which would provide strong supporting evidence of that hypothesis. Furthermore, it remains unclear which cell or cells in the stroma may be responsible, co-culture of OAC cells with each individual stromal cell type and subsequent ELISA to assess for CRP secretion could help to answer this question. The synthetic pathways and stimuli to local CRP secretion are being studied extensively in atherosclerotic disease; ongoing research in this area could be used to guide studies in cancer. The microbiome, both within the gut and within the tumour itself, is a topic of significant interest to cancer researchers(151). Given that CRP is a key part of the body's response to bacterial infection(14) and that an elevated serum CRP is associated with lower gut microbial diversity(383), the relationship between CRP (systemic and tumoural) and the microbiome in the development and progression of cancer is a potentially fascinating avenue of further study.

The connection with obesity is another area of great interest. There is increasing evidence of links between obesity, chronic inflammation and cancer(154). Intriguingly, Anty *et al.*, found that CRP gene expression was increased in adipose tissues of obese patients compared with controls(384). The interplay between CRP and body composition will be explored further in Chapter 5 of this thesis. Finally, and most importantly, the impact of tumoural

CRP on patient outcomes, particularly survival, warrants further study. Larger studies, designed to address this question, are required.

Other future work might include combining tumoural CRP with other prognostic markers. Ali *et al.*, suggested that serum CRP and intratumoural CRP could be combined as a potential prognostic indicator(342). The findings of chapter 3 of this thesis found that there is insufficient evidence to conclude on the value of serum CRP as a prognostic indicator and this would need to be confirmed before exploring its addition to another marker. An alternative would be the GPS/mGPS, which, as shown in the previous chapter of this thesis, predicts overall survival in OAC. Tumoural CRP could also be combined with other markers in tissue. CD8⁺ tumour-infiltrating lymphocytes (TIL) have been suggested for combination with CRP-based scores to predict response to neoadjuvant treatment in rectal cancer(385), addition of tumoural CRP could prove valuable.

Similar to the hypothesis explored in chapter 2 of this thesis, Elsberger *et al.*, examined whether change in expressed tumoural CRP over time might be associated with survival(340). Although they noted a trend toward shorter survival, this was not statistically significant. This could be studied further in a larger dataset but the practicalities of this approach, which requires repeat samples of tissue, might limit its usefulness. A potentially interesting avenue to circumvent the difficulty of repeatedly sampling the cancer tissue itself would be to find a reliable circulating marker of tumoural CRP. Kaplan *et al.*, found that there was a positive correlation between CRP in circulating macrophages (human monocytes derived macrophages, HMDM) and CRP levels identified with IHC in carotid artery atherosclerotic lesions, while systemic CRP did not correlate with CRP levels within the lesion(386). They suggest that CRP levels in HMDM could be tested to evaluate the inflammatory status of the atherosclerotic lesion, which is much more difficult to access(386), much like cancer tissue.

This study has several strengths. The presence of tumoural CRP in OAC was confirmed but the location, possible source and clinical impact were also addressed. This work examined the presence of and impact of tumoural CRP in pre-treatment and post-treatment cohorts, providing the most comprehensive analysis to date in this cancer site. Both manual and automated techniques were used. The laboratory findings were strengthened by analysis of

matched clinical data with long and accurate follow-up for both datasets, as part of the professionally-maintained Upper GI registry.

Limitations of this work included the small numbers of patient samples available for the post-treatment cohort, which may have prevented identification of a significant relationship between tumoural CRP and survival. Similarly, although the pre-treatment cohort was larger, a matched serum CRP data was not available for many in that group, limiting assessment of the relationship between tumoural and serum CRP.

Owing to resource restrictions, it was not possible to assess for the presence of CRP mRNA in tumour samples. As noted above, this would be of great interest for future studies to examine. The post-treatment cohort was, unavoidably, a biased sample as people with excellent response to neoadjuvant treatment and therefore no residual tumour were necessarily excluded. However, once presence of CRP had been demonstrated in this cohort, the subsequent immunofluorescence study addressed this limitation by examining pre-treatment samples.

4.5 Conclusions

This chapter has provided several new insights into the under-researched area of tumoural CRP. CRP was confirmed to be present in resected OAC tumour tissue and, in a novel finding, in pre-treatment biopsies. Density of cells with high CRP in tumoural nuclei or cytoplasm on pre-treatment biopsy was associated with shorter survival, while no association was found between other metrics of tumoural CRP, nor between presence of tumoural CRP in resected tissue and survival. No correlation was found between serum CRP and tumoural CRP, suggesting a local origin for tumoural CRP. When present, CRP was found in stromal cells +/- carcinoma cells, but never in carcinoma cells alone. Furthermore, CRP was not secreted by an OAC cell line, even when they were exposed to environmental conditions mimicking the TME or when exposed to chemotherapy or radiotherapy. Taken together, these findings suggest a stroma origin for tumoural CRP in OAC. Possible cells responsible for secretion of CRP in the TME were discussed, with immune cells and adipocytes appearing likely candidates. Both immune cell infiltrates and adipocyte abundance are dramatically altered in obesity. The link between body composition, inflammation and clinical outcome is explored in depth in the next chapter.

5 The use of CRP and Skeletal Muscle measures to predict symptoms and quality of life in cancer: a feasibility study

5.1 Introduction

This chapter reports findings from a feasibility study which examined relationships between CRP, skeletal muscle and patient-reported outcomes in cancer (quality of life and symptoms) and the potential to use CRP and skeletal muscle measures to predict these outcomes. Since CRP is both widely available and has been linked with patient outcomes, it is an attractive candidate for this role.

It is known that cancer can cause a significant symptom burden and poor quality of life and that these can be present at diagnosis or develop during treatment(387). It would be very useful if prognostic tools could predict which individuals will have poor QoL and high symptom burden. This could enable early intervention, more intensive symptom screening or preventative action; a proactive and patient-centred, rather than a reactive one(212). This objective information could potentially even have a role in identifying people who might benefit from early specialist palliative care (SPC) input or could be used as screening to prompt a more comprehensive assessment of palliative care needs. Despite recommendations to refer all patients with certain advanced cancers, including lung cancer, for SPC(388), there are concerns about availability of adequate SPC resources to support this practice; a needs-based model may be better than systematic referral simply based on diagnosis(389).

There is evidence from advanced cancer that inflammation is associated with cancer symptoms, particularly anorexia, fatigue and reduced performance status, as well as poor quality of life (QoL)(212, 390, 391). Inflammation is also associated with abnormalities in skeletal muscle in cancer, with reductions in muscle mass (myopenia) and muscle density (sometimes termed myosteatorsis) (213). Although the majority of work in this area has focused on operable cancers(213), a recent retrospective study in mixed advanced cancers identified a significant independent association between a high mGPS and low skeletal muscle density, though not muscle mass(392). Indeed, it has been proposed that inflammation may even mediate skeletal muscle change in cancer(213, 393). Myopenia and reduced muscle density are associated with poor survival in cancer(394). A high symptom burden is also associated with shorter survival(395).

CT imaging and blood tests are a standard part of the cancer diagnostic process. It would be straightforward to measure serum CRP as a component of the staging workup. Skeletal muscle can be assessed on Computerised Tomography scan (CT), prior to and post treatment. Large international studies have analysed images from routine staging CT to assess skeletal muscle mass(394). Skeletal muscle at the third lumbar vertebra (L3) is evaluated to calculate Lumbar Skeletal Muscle Index (LSMI), which is correlated with whole body skeletal muscle mass(396). The same technique is used to assess skeletal muscle radiodensity (SMD), as a marker of muscle quality or possible fatty infiltration(393, 394). Thus, information on skeletal muscle change and inflammation can be easily accessed, without any additional burden to the patient. In future, information on skeletal muscle change could become part of routine radiology CT reports. The authors of a recent systematic review concluded that body composition should be assessed together with other relevant factors, particularly systemic inflammation, when assessing people with cancer(397), again highlighting the concept of “staging the host” as well as the tumour, in order to then treat the host and the tumour(398).

It has been stated that there is an urgent need to characterise the relationships between inflammation, body composition, symptoms and quality of life in advanced cancer(214). A large multicentre study published in 2020 evaluated factors influencing QoL in advanced cancer, including performance status, body composition and the systemic inflammatory response(391). That study recruited patients at different timepoints and many had undergone chemotherapy in the weeks before recruitment(391). Given that oncologic therapies may influence the systemic inflammatory response, this may have influenced their findings(399). Bye *et al.*, also examined the relationship between body composition and QoL and suggested a link between LSMI and SMD and selected QoL subscales(400). In both studies, the investigators examined QoL and symptoms at a single timepoint and thus were unable to examine longitudinal change in these parameters. Understanding longitudinal change in these parameters of this is key in planning services to best meet patient need.

Clinical research is needed to further evidence-based care for patients with incurable cancer(401). Patients with advanced disease are willing to be involved in research(402, 403) but the challenges of research in advanced cancer and palliative care are well-documented. These include funding, institutional and researcher capacity, perceptions of palliative care as associated with death and the challenges of studies in a vulnerable population(403, 404).

Clinical research in seriously-ill individuals presents particular challenges including recruitment, “gatekeeping” by professionals and in longitudinal studies, retention of participants(402-407). As a result, pilot or feasibility studies have been described as “practically a prerequisite” before embarking on a larger study (408). In 2021 alone, feasibility studies have been reported in advanced cancer or palliative care in areas as diverse as opioid-induced constipation, peer mentorship and virtual reality (409-411), highlighting the widespread acceptance of their necessity. The terms “pilot” and “feasibility” are used somewhat interchangeably and inconsistently in healthcare literature(412, 413). Feasibility has been conceptualised as an umbrella term, which includes pilot studies and studies looking at feasibility outcomes(414). The study described in this chapter assessed feasibility in the formal sense of the word(415), with primary outcomes of recruitment and completed datasets and pre-specified feasibility criteria(413) and seeking to assess the viability of larger, pivotal study of CRP and skeletal muscle to predict symptoms and QoL in inoperable cancer.

5.1.1 Specific Hypotheses of this Study

1. A longitudinal study of CRP and skeletal muscle to predict symptoms and quality of life in inoperable cancer is feasible.
2. High CRP is associated with abnormal skeletal muscle in cancer.
3. High CRP and abnormal skeletal muscle at time of diagnosis are associated with poor quality of life in cancer.
4. High CRP and abnormal skeletal muscle at time of diagnosis are associated with high symptom burden in cancer.
5. There is a significant difference in prevalence rates of high CRP and abnormal skeletal muscle between non-cancer, operable lung cancer and inoperable lung cancer cohorts.*
6. CRP and abnormal skeletal muscle could predict study completion*
7. CRP and abnormal skeletal muscle could predict potential to benefit from SPC*

5.1.2 Specific Aims of this Study

Primary Aims

1. Assess the feasibility of this study design as indicated by
 - Enrolment rate
 - Proportion of completed datasets

Secondary Aims

1. Describe the prevalence of high CRP in adults with newly diagnosed inoperable cancer.
2. Describe the prevalence of abnormal skeletal muscle (mass and quality) in adults with newly diagnosed inoperable cancer.
3. Describe symptom burden and quality of life in adults with newly diagnosed inoperable cancer.
4. Describe change over time in symptom burden and quality of life in adults with newly diagnosed inoperable cancer.
5. Investigate the potential role of high CRP and presence of abnormal skeletal muscle at diagnosis in the prediction of future symptom burden and QoL in people with inoperable cancer.

6. Compare the prevalence of high CRP and abnormal skeletal muscle at time of diagnostic investigations in non-cancer, operable lung cancer and inoperable lung cancer cohorts. *
7. Compare symptom burden and quality of life in non-cancer, operable lung cancer and inoperable lung cancer cohorts, at time of diagnostic investigations and 6-8 weeks later (6-8 weeks post-operatively for the operable lung cancer cohort).*
8. Examine the relationships between high CRP, abnormal skeletal muscle and symptoms, performance status and QoL in non-cancer, operable lung cancer and inoperable lung cancer cohorts. *
9. Investigate the potential role of high CRP and abnormal skeletal muscle in the prediction of post-operative complications and length of stay in surgically-treated lung cancer. *
10. Explore if CRP and abnormal skeletal muscle could predict study completion*
11. Explore if CRP and abnormal skeletal muscle could predict potential to benefit from SPC*

*denotes additional hypotheses and objectives for Iteration 3 of this study

5.2 Methods

This was a prospective observational feasibility study, conducted in St James's Hospital (SJH), Dublin, which recruited adults (aged 18 or over). Results are reported in line with the STROBE and CONSORT 2010: extension to randomised pilot and feasibility trials statements(416, 417), as has been recommended for non-randomised feasibility studies(418).

As a feasibility study, there was periodic review of study progress after eight weeks of recruitment. At each review, recruitment rate to the study was analysed and potential barriers to recruitment were assessed. There were three iterations of the study, henceforth termed Iterations 1, 2 and 3, as seen in Figure 5.1.

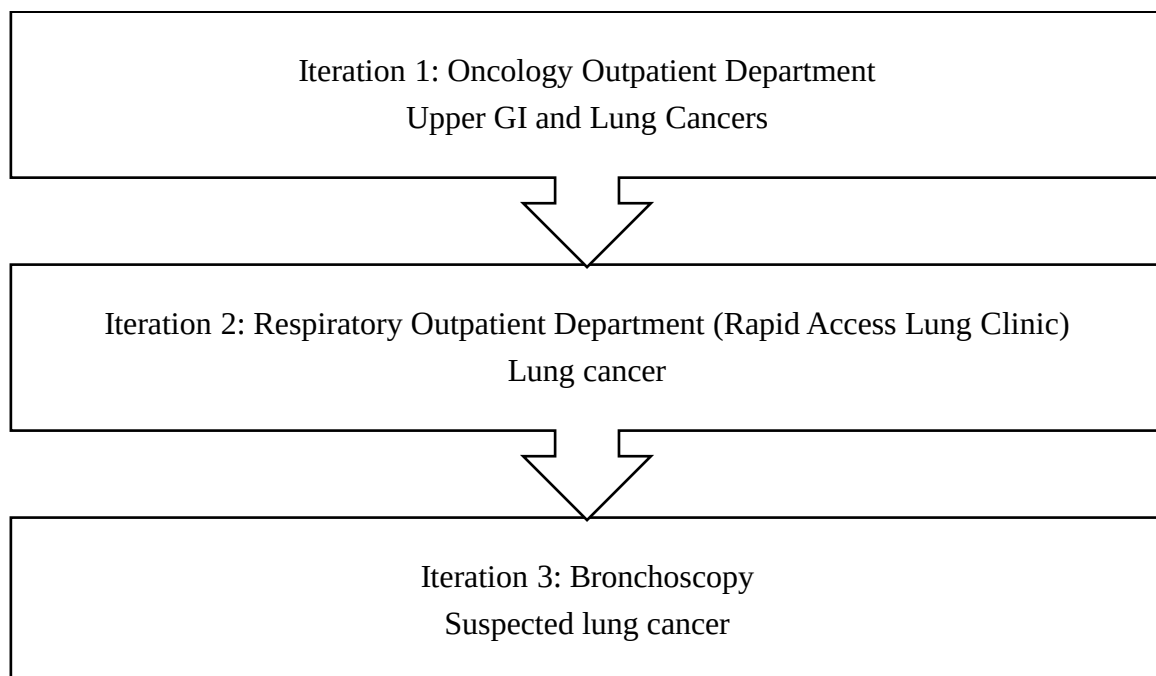


Figure 5-1. Summary of Iterations 1,2 and 3 showing location of recruitment and patient population included

Upper GI: Upper gastrointestinal cancers (oesophageal, gastro-oesophageal and gastric)

Iteration 1 recruited patients with inoperable Upper GI cancers or lung cancers at the SJH Oncology Outpatient Department (OPD) prior to receipt of anti-cancer treatment. Iteration 2 recruited patients with inoperable lung cancer at the SJH Rapid Access Lung Clinic prior to receipt of anti-cancer treatment. Iteration 3 recruited patients with suspected lung cancer at the time of diagnostic bronchoscopy, in the SJH Day ward.

Numbers recruited, challenges identified at each point, adaptations made in response to these and rationale for these changes are addressed in detail in the results section. Study procedures, shown in Figure 5.2, remained unchanged between the three iterations, while recruitment procedures and inclusion criteria were amended.

5.2.1 Inclusion and exclusion criteria

Inclusion and exclusion criteria for Iteration 1 are shown in Table 5.1 Inclusion criteria for Iterations 2 and 3 are shown in Table 5.3 and Table 5.4, respectively.

Table 5-1. Inclusion and Exclusion Criteria for Iteration 1

Inclusion	Exclusion
Inoperable lung or upper GI cancer	Based on the judgement of the Oncology clinician, study participation is not appropriate
Has not started new systemic cancer treatment since diagnosis of inoperable disease	Documented diagnosis of dementia
CT scan of thorax and abdomen in last one month	Unable to complete study assessments
Able to understand and speak English	

Ethical approval was granted by the SJH/AMNCH REC (Reference 2015-10(16)). After Iterations 1 and 2, the modifications to the study protocol necessitated updated ethical approval; this was sought and obtained from the same committee before the study recommenced (Reference 2016-04 List 13(10) and Reference 2018-01 List 1(7), respectively).

An intended sample size of 30 participants with inoperable cancer was chosen by consensus between the researcher and her supervisors; it was agreed that this sample size was likely to provide a good snapshot of feasibility in this cohort, as well as being realistic in the timelines of a PhD. Feasibility criteria were recruitment rate of 1 participant per week and 60% complete datasets. There is no agreed criterion on how much missing data is too much (and thus warrants specific statistical techniques to address it) but it has been proposed that if more than 40% of key variables are missing, results are likely compromised(419).

5.2.2 Study procedures

Study procedures were as follows and as summarised in Figure 5.2: Potentially eligible participants were invited to speak with the researcher by the clinical team. The researcher (Dr. Lorton) outlined the study, including provision of a patient information leaflet (PIL), Appendix 3. If potential participants were willing to take part, they provided written consent.

Demographic details, co-morbidities, medications, smoking status and most recent blood results (CRP, full blood count (FBC), albumin) were noted in a data recording sheet (Appendix 4) from the medical record. Evidence of infection, in the form of documented clinical suspicion or current antibiotic treatment, was noted. CRP was included only if it had been measured within the preceding six weeks, as it was felt that this timeframe would be feasible in practice, while still being close enough to the date of recruitment to be informative. Where albumin had been measured within one week of CRP measurement, GPS and CAR were calculated as follows: GPS 0: $\text{CRP} \leq 10\text{mg/L}$ and $\text{albumin} \geq 35\text{g/L}$, GPS 1: $\text{CRP} > 10\text{mg/L}$ OR $\text{albumin} < 35\text{g/L}$, GPS 2: $\text{CRP} > 10\text{mg/L}$ and $\text{albumin} < 35\text{g/L}$ (190). CAR was calculated as $\text{CRP (mg/L)} / \text{albumin g/L}(271)$. Height and weight were obtained from the medical record and used to calculate BMI, as previously described. Where none was documented patient's self-reported height and weight were noted.

The medical record was reviewed to record the dates of any cross-sectional imaging undertaken and which type (PET-CT, CT thorax abdomen and pelvis or CT thorax).

5.2.2.1 Assessment of symptoms and quality of life

The researcher completed a validated symptom and quality of life questionnaire with the patient: the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire – Core Questionnaire (EORTC QLQ-C30) (420)(Appendix 5). The EORTC QLQ-C30 is a widely used research tool, which includes functional, symptom and quality of life scales(421). If the questionnaire was not completed, reason why not was recorded. Performance status was assessed by the researcher using the Eastern Cooperative Oncology Group Scale of Performance Status (ECOG) scale, which is extensively used to assess and describe the functional status of people with cancer (422)(Appendix 6).

5.2.2.2 Follow-up

For the non-cancer and inoperable cancer groups, follow-up was conducted six to eight weeks later. The researcher contacted participants by telephone to arrange completion of follow-up questionnaires, the EORTC QLQ-C30 and the ECOG. Questionnaires were completed in person or over the telephone, depending on patient preference. EORTC QLQ-C30 is validated for completion in person or over the telephone (421). As most of the operable cohort were in the early post-operative phase at this point and assessment of symptoms and QoL would have been heavily influenced by this, the operable cohort were followed up six to eight weeks post-operatively, at the Cardiothoracic OPD. Where follow-up questionnaires were not completed, reason for this was recorded. Anti-cancer treatments received by the patient since the initial assessment were recorded from the clinical record, as was confirmed histology. For the surgically-treated group, post-operative complications and length of stay were noted.

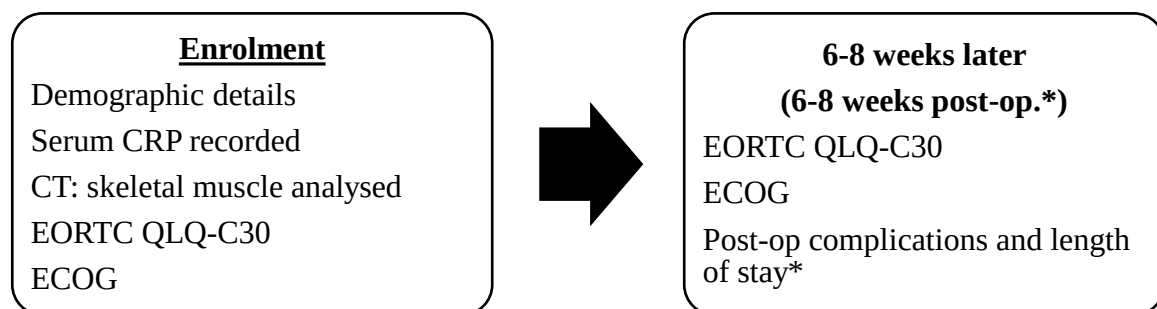


Figure 5-2. Summary of Study Procedures highlighting key data collected at enrolment and at follow-up

*operable cohort only

5.2.2.3 Analysis of EORTC QLQ-C30

EORTC QLQ-C30 scores are reported as a summary score, which summarises the symptom and functional scales components of the questionnaire; this approach is recommended by the EORTC Quality of Life Group(423). The summary score is reported as 0-100, with 0 worst and 100 best. Change in score over time was analysed as little change if 5-10% change

in score, moderate if 10-20% change and very much change if >20% change as per EORTC guidance(424). Moderate and very much change groups were analysed together as significant change. The five functional and nine symptom scales of the questionnaire were also analysed, using the thresholds for clinical importance (TCIs) established by Giesinger *et al.*,(425). These thresholds dichotomise the continuous score for each scale and have been proposed for use in symptom screening in clinical practice, to identify the most significant impairments for an individual patient, as well as to provide prevalence rates in research(425).

5.2.2.4 Skeletal muscle analysis

Where recent CT imaging included L3, the imaging was reviewed to assess skeletal muscle, using specialised software (Slice-O-Matic (Tomovision, Canada), as follows: The CT slice (single image in the axial plane) best demonstrating the disc space between the third and fourth lumbar vertebra (L3-L4) was identified by Dr Bryan Dalton (BD), Research Registrar in Radiology, who used using the software's inbuilt functions to demarcate each tissue type in that slice, with manual correction where necessary. Radiation attenuation was used to identify each tissue type Hounsfield Unit (HU) thresholds were (396).

-29 to 150 for skeletal muscle

-190 to -30 HU for subcutaneous and intramuscular adipose tissue

-50 to -150 HU for visceral adipose tissue

Cross-sectional skeletal muscle area (SMA) was calculated and corrected for height (SMA /height squared) to give skeletal muscle index (SMI), measured in cm^2/m^2 (396). Mean Muscle Attenuation (MA) for whole muscle area, measured as the average HU radiodensity for the whole skeletal muscle area in that section(394) and henceforth referred to as skeletal muscle density (SMD), was recorded. Skeletal muscle gauge (SMG) was calculated as per Weinberg *et al.*, as $\text{SMI} \times \text{SMD}$ and reported in arbitrary units (AU)(426). Total psoas muscle surface area (cm^2) was also measured and divided by height squared (m^2), to give psoas muscle index (PMI) in cm^2/m^2 (427). Threshold (cutpoint) values for SMI and SMD were as per Martin *et al.*, and are shown in Table 5.2 below (394). In the absence of validated cutpoints for SMG (428), SMG was analysed as a continuous variable only. PMI cutpoints

are also shown in table 5.2 and were chosen as having been validated in both operable(429, 430) and inoperable lung cancer(431).

Table 5-2. Threshold values for CT analysis metrics

Metric	Cutpoint for men	Cutpoint for women
L3 SMI (cm ² /m ²) ^a	BMI ≤ 24.9 : 43 BMI ≥ 25: 53	Any BMI: < 41
L3 SMD (HU) ^a	BMI ≤ 24.9 : 41 BMI ≥ 25: 33	BMI ≤ 24.9 : 41 BMI ≥ 25: 33
PMI (cm ² /m ²) ^b	< 6.36	< 3.92

^a SMI and SMD threshold (cutpoint) values as per Martin *et al.*,(394). ^b PMI threshold (cutpoint) values as per Nakamura *et al.*.(429).

5.2.2.5 Statistical analysis

Distribution of demographics, CRP, albumin, GPS, CAR, baseline body composition measures (BMI, LSMI, SMD, SMG, PMI) and EORTC QLQ-C30 scores at enrolment and at follow-up (summary score and percentage of patients reporting impairment above the TCI in each of the symptom and functional scales) were compared between non-cancer, inoperable and operable cancer groups. Chi-square test was used to assess for differences in proportion of cases between groups for categorical variables. Independent *t*-test and Mann-Whitney *U* test used to test for differences between 2 groups in normally and non-normally distributed continuous variables, respectively. Kruskal-Wallis Test was used to compare non-normally distributed continuous variables between the three groups and one-way between-groups ANOVA was used to compare non-normally distributed continuous variables between them.

Correlations between CRP, albumin, GPS, CAR, baseline body composition measures and initial and follow-up EORTC QLQ-C30 scores, post-operative complications and LoS were assessed using Pearson product-moment correlation coefficient (*r*) for normally-distributed variables and Spearman rank-order correlation coefficient (*rho*) for those which were not

normally-distributed. Correlation was considered small where r/ρ was 0.1-0.29, medium if 0.3-0.49 and large/strong where r/ρ was 0.5-1.00(432). Analysis of relationships between enrolment variables and outcome (follow-up EORTC QLQ-C30 scores) was repeated after exclusion of participants where there was evidence of infection, as a potential confounding cause of CRP elevation. Two further exploratory analyses were conducted: whether CRP or body composition measures were associated with study completion and whether they might be associated with need for specialist palliative care. Individuals who had a significantly worse summary score at follow-up, were too unwell to complete the study or who had died were considered to have had potential to benefit from SPC and were compared to those who completed the study and whose summary score was stable or improved. P values below 0.05 were considered significant and all statistical tests were two-tailed.

Data analysis was conducted using Microsoft Excel (version 2102, Microsoft Corporation, Washington) and IBM SPSS (version 27, IBM Corporation, Armonk, NY).

5.3 Results

As noted in the Methods, interim review of study recruitment took place after eight weeks in each study iteration. Recruitment rate, challenges identified, adaptations made in response to these and rationale for these changes are discussed for each iteration. Next, feasibility data with respect to study completion and missing data is outlined. Finally, data collected from participants (CRP, body composition and symptom / QoL data) will be reported.

5.3.1 Iterations 1,2 and 3

Iteration 1

After eight weeks of recruitment at Oncology OPD (March-May 2016), no patients had been recruited, even though clinic attendance records showed that 18 potentially eligible patients had attended in that time. One person had been deemed too unwell by the clinical team. Two were already involved in another study, hence the clinical team did not ask them about participation in the present study. In nine, their most recent CT had been done over a month before the date of OPD. Six were eligible but had not been asked about the study. The researcher met with the Consultant Oncologist and discussed study progress. Both agreed that it was essential for the researcher to be present for the full duration of each clinic to prompt clinicians regarding potentially eligible patients (it had initially been thought that researcher attendance at the beginning of the clinic would suffice). Both also noted that the clinic was a busy environment and that patients had a tiring day, as they would often see four healthcare professionals in a row, as well as having blood tests. This was identified as a barrier to referral of patients to the researcher and, potentially, to patient's willingness to meet. It was agreed that an ethical amendment should be sought to change the protocol to state that analysis of skeletal muscle would be on the "most recent CT" instead of "CT from the last one month" to "recent CT".

Iteration 2

Upon study recommencement in September 2017, a significant obstacle to the planned study became apparent. Another study had commenced recruitment at the same Oncology OPD, recruiting an identical cohort. Following meetings with the Consultant Oncologist and with her PhD supervisors, the researcher identified an alternative recruitment site: the SJH Rapid Access Lung clinic. This outpatient clinic provides rapid assessment and diagnosis of people

with suspected lung cancer. Following their investigation, patients with confirmed lung cancer return to the clinic to receive their diagnosis and to discuss the next steps in their care. It was agreed that the clinicians at the clinic would invite the newly diagnosed patients to meet the researcher to discuss the study. Inclusion and exclusion criteria for Iteration 2 are shown below. The exclusion criterion “in the judgement of the Oncology clinician, study participation is not appropriate” was amended to “in the judgement of the Respiratory Medicine clinician”. Furthermore, not all patients had a CT abdomen but all had either had a Positron-Emitted CT (PET/CT) or one was awaited, thus this criterion was also modified. Recruitment began in November 2017.

Table 5-3. Inclusion and Exclusion Criteria for Iteration 2

Inclusion	Exclusion
Inoperable lung cancer	In the judgement of the Respiratory Medicine clinician, study participation is not appropriate
Has not started new systemic cancer treatment since diagnosis of inoperable disease	Documented diagnosis of dementia
CT scan of thorax and abdomen OR PET-CT in last one month or planned	Unable to complete study assessments
Able to understand and speak English	

As before, a scheduled review of study progress was undertaken after eight weeks of recruitment. In that time, four patients had agreed to speak with the researcher. Of these, two agreed to take part in the study and asked the researcher to telephone them to complete the study questionnaires a few days later. When contacted by the researcher, however, one declined to participate. The researcher discussed study progress with the Respiratory Medicine team and together they identified some possible challenges to recruitment. Patients were, unsurprisingly, often very distressed at their diagnosis and the clinical team reported that it was difficult to introduce the topic of research, or to suggest meeting another healthcare professional on that day. The clinical team kindly offered to telephone patients a few days later and obtain consent for the researcher to contact them. This was not felt to be a sustainable model and was deemed unduly burdensome on the clinical team. On further

discussion, another possibility emerged. The study could recruit people with suspected lung cancer at bronchoscopy, capturing symptom burden and QoL at a very early point in the patient's disease journey. A further advantage of this structure would that it would also allow result in recruitment of a control group of clinically-similar patients who turn out not to have cancer, and a third group with operable cancer, potentially providing interesting data to inform future studies in those groups. It was agreed that the researcher should attend the weekly lung multidisciplinary team (MDT) meeting, to ascertain the outcome of investigations, including diagnosis, histology, staging and treatment plan and in order to group the patients appropriately as non-cancer, inoperable and operable cancer.

Iteration 3

Table 5-4. Inclusion and Exclusion Criteria for Iteration 3

Inclusion	Exclusion
Suspected lung cancer (as per treating Consultant in Respiratory Medicine)	In the judgement of the Respiratory Medicine clinician, study participation is not appropriate
	Documented diagnosis of dementia
	Unable to complete study assessments

An amendment to ethical approval was obtained to allow recruitment of adults with suspected lung cancer at time of bronchoscopy. The study opened in February 2018 in the Endoscopy Unit at SJH. As before, the clinical team (who were seeing the patient to obtain informed consent for the bronchoscopy), invited the patient to speak to the researcher. If they agreed, the researcher spoke to the patient, outlined the study and gained informed consent. The researcher then proceeded to complete the EORTC-QLQ C-30 and ECOG with the patient, before they had their bronchoscopy. The paper and electronic medical records were reviewed and data recorded as detailed in the Methods.

As previously, a scheduled review of study progress was undertaken after eight weeks of recruitment. In that time, 28 people had been recruited to the study. Of these, 9 had inoperable lung cancer, 9 operable lung cancer and 10 had been confirmed not to have cancer. It was agreed to continue recruitment until 100 people had been recruited, on the

basis of the breakdown of those already recruited and on the original intended sample size of 30 people with inoperable cancer.

5.3.2 Recruitment in Iteration 3

The study completed recruitment in October 2018. Recruitment is summarised in Figures 5.3 and 5.4. As seen in Figure 5.3, of 219 eligible patients, the researcher approached 120. Numbers who declined to speak to the researcher were not formally recorded, however in the majority of cases the researcher had verbal approval from the patient to speak to them but was unable to do so before they were called for their bronchoscopy. Of the 120 patients who were approached and informed about the study, 100 agreed to take part. Of those who declined to take part, nine did not specify a reason, six reported feeling too anxious or upset, four felt too unwell and one declined on the basis of being involved in another study.

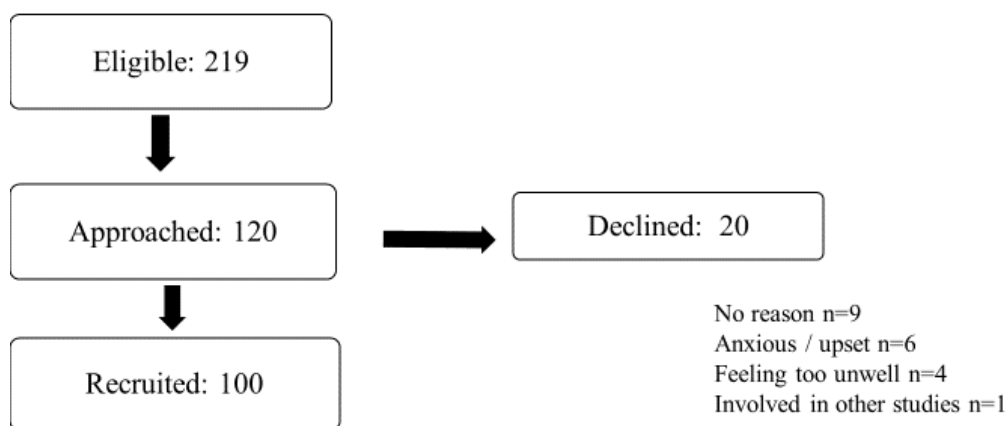


Figure 5-3. Flowchart of recruitment to iteration 3

Figure 5.4 shows the breakdown of diagnoses of study participants. For final analysis, 12 people (8 female, 4 males; median age 68 (range 40-90)) were excluded: 11 had recurrent cancer or a primary cancer site other than lung and one person declined subsequent investigations thus a diagnosis was never reached (details in Table 5.5). This left an overall included number of 88 of whom 37 did not have cancer, 35 had inoperable lung cancer and 16 had inoperable lung cancer. This group of 88 will be the focus of the remainder of the results.

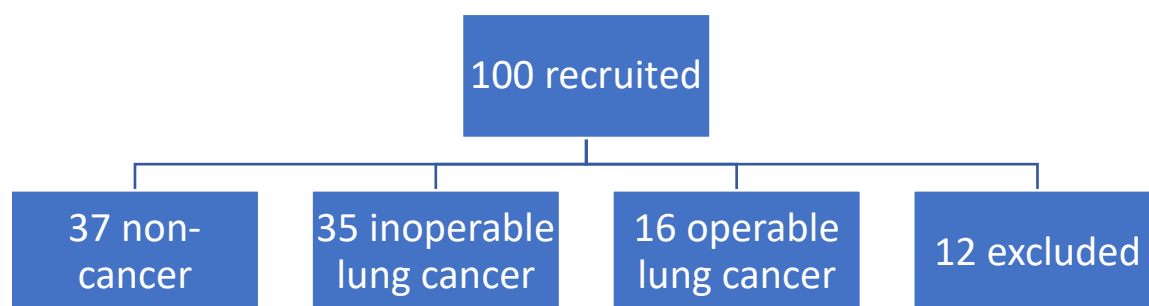


Figure 5-4. Breakdown of included patients

Table 5-5. Reason for exclusion for 12 recruited participants excluded from data analysis

Reason for Exclusion	Number of recruited participants
Recurrence of previous lung cancer	2
Confirmed primary site not lung	9
Primary site	
Lymphoma	2
Bladder	1
Colorectal	1
Endometrial	1
Laryngeal	1
Oesophageal	1
Renal cell carcinoma	1
Thyroid	1
Declined further investigations, no diagnosis confirmed	1

5.3.3 Demographics

Table 5.6 details demographic data for the 88 included patients. Table 5.7 compares demographics between the three diagnostic groups: non-cancer (n=37), inoperable lung cancer (n=35) and operable lung cancer (n=16).

Table 5-6. Demographic details for n=88 included patients

	Whole group n=88
Age (median, range)	67 (46-89)
Sex (n,%)	
Male	45 (51%)
Female	43 (49%)
Marital status (n,%)	
Married	45 (51%)
Widowed	18 (21%)
Single	16 (18%)
Separated or divorced	4 (5%)
Missing	4 (5%)
Ethnicity	
White	88 (100%)
Number of co-morbidities (mean, SD, range)	3 ± 2, 0-8
Outpatient / inpatient (n,%)	70 (80%) / 18 (20%)
Smoking status (n,%)	
Smoker	45 (51%)
Ex-smoker	24 (27%)
Non-smoker	13 (15%)
Missing	6 (7%)
Evidence of infection	
Yes	14 (16%)
No	74 (84%)

Table 5-7. Demographic details for n=88 included patients stratified by diagnostic group

	Non-cancer n=37	Inoperable lung cancer n=35	Operable lung cancer n=16	p value
Age (median, range)	66 (46-89)	68 (52-88)	65 (57-75)	0.122
Sex (n,%)				
Male	21 (57%)	16 (46%)	8 (50%)	0.642
Female	16 (43%)	19 (54%)	8 (50%)	
Marital status (n,%)				
Married	18 (49%)	20 (57%)	7(44%)	0.602
Widowed	5 (14%)	10 (29%)	3 (19%)	
Single	8 (22%)	3 (9%)	5 (31%)	
Separated or divorced	3 (8%)	1 (3%)	0	
Missing	3 (8%)	1 (3%)	1 (6%)	
Number of co-morbidities (mean,SD)	3 ± 2	3 ± 2	3 ± 2	0.909
Out/ inpatient (n,%)	28(76%) / 9(24%)	27(77%) / 8(23%)	15(94%) / 1 (6%)	0.294
Smoking status(n,%)				
Smoker	20 (54%)	18 (55%)	7 (58%)	0.094
Ex-smoker	7 (19%)	13 (39%)	4 (33%)	
Non-smoker	10 (27%)	2 (6%)	1 (8%)	
ECOG				
0	20 (54%)	12 (34%)	7 (44%)	0.286
1	7 (19%)	9 (26%)	6 (38%)	
2	6 (16%)	7 (20%)	0	
3	4 (11%)	7 (20%)	2 (13%)	
4	0	0	0	
missing	0	0	1 (6%)	
Histology				
NSCLC	Not applicable	20	11	0.006**
SCLC		12	0	
Neuroendocrine		0	2	
Missing/no histology		3	3	
Evidence of infection				
Yes / No	10(27%)/27(73%)	4(11%)/31 (99%)	0 / 16 (100%)	0.031*

** $p < 0.01$. * $p < 0.05$. Chi-square test (for categorical variables). Kruskal-Wallis Test (median), one-way between-groups ANOVA (mean)

5.3.3.1 Treatment received by inoperable lung cancer cohort

Of 35 people in the inoperable lung cancer cohort, 16 (46%) received both chemotherapy and radiation, 8 (23%) received radiotherapy alone (one of whom had radical radiotherapy, 5 (14%) had chemotherapy alone and 4 received best supportive care (11%). The remaining 2 people died before a final decision on treatment had been made.

5.3.4 Primary outcomes: Feasibility

5.3.4.1 Recruitment rate

In the third iteration of the study, recruitment rate was 12 patients per month.

5.3.4.2 Completed datasets

5.3.4.2.1 CRP and CRP-based scores

A CRP within the preceding six weeks was available for 61 patients (69%), 27 in the non-cancer group, 27 in the inoperable group and 7 in the operable group. Albumin was available for 51 patients (58%) and GPS and CAR could be calculated for 50 patients (57%). Distributions of CRP, albumin, GPS and CAR are in Table 5.9 below.

5.3.4.2.2 Body composition

PET-CT was available for 53 (60%) overall but the images were unsuitable for analysis for four patients (file corrupted for one, poor quality image / artefact at L3 level for three). Availability of PET-CT images differed between the diagnostic groups with useable images available for 31 (89%) of the inoperable lung cancer group and 15 (94%) of the operable group, compared to n=3 (8%) of the non-cancer group. The remainder of the study participants had CT thorax, which does not include L3, or had no cross-sectional imaging. This is discussed further in the Discussion. Height was missing for 7 (8%), meaning body composition indices which incorporate height (SMI, SMG, PMI, BMI) could not be calculated for these individuals. Weight was missing for 16 (18%). BMI could be calculated for 68 (77%) of participants. Reference values for SMI and SMD are based on gender and/or BMI(394) , thus comparison to these was not possible for all participants, even where their body composition metrics were available.

5.3.4.2.3 Symptom / QoL scores

Day 1 EORTC QLQ-C30 questionnaires were incomplete for 31 (35%) of participants overall – one participant had requested not to complete the questionnaires that day but was willing to take part in the study, five were missing some part of the required data to calculate a summary score and the remainder were called for their bronchoscopy before completing the questionnaire.

5.3.4.2.4 Study completion

Sixty-nine patients of 88 (78%) completed the study. Of the 19 (22%) who did not complete the second assessment, six (7%) were lost to follow-up (unable to contact), six (7%) had died, 4 were too unwell (5%) and 3 withdrew (3%).

Table 5.8 shows study completion according to diagnostic group. There was no significant difference between groups in proportion of patients who completed the study. Absolute numbers were too small for statistical analysis of differences in reasons for study non-completion. Possible differences are discussed further in the Discussion.

Nineteen people had entirely complete datasets. Of the inoperable cancer group, 14 had entirely complete datasets (40% of that cohort). Of the operable cancer group, 5 (31%) had entirely complete datasets. However, study design meant that data analysis was possible for a much greater number of patients as all patients had some usable data.

Table 5-8. Study completion and reasons for study non-completion by diagnostic group

	Non-cancer n=37	Inoperable lung cancer n=35	Operable lung cancer n=16	p value
Study completion (n, %)				
Yes	30 (81%)	27 (77%)	12 (75%)	
No	7 (19%)	8 (23%)	4 (25%)	0.861
Reason for non-completion (n, %)				
Unable to contact	4 (11%)	1 (3%)	1 (6%)	
Too unwell	1 (3%)	2 (6%)	1 (6%)	
Withdrew	0	2 (6%)	1 (6%)	
RIP	2 (5%)	3 (9%)	1 (6%)	

Chi-square test.

5.3.5 Secondary Outcomes

5.3.5.1 CRP and CRP-based scores

There was no significant difference in distribution of CRP, albumin, GPS or CAR between the diagnostic groups ($p=0.525$, $p=0.153$, $p=0.416$, $p=0.982$ respectively), as shown in Table 5.9.

Table 5-9. Distribution of CRP, Albumin, GPS and CAR for n=88 included patients stratified by diagnostic group

	Non-cancer	Inoperable lung cancer	Operable lung cancer	p value
CRP (mg/L) (median, range)	n=27 4.95 (0.99-104)	n=27 7.44 (0.9-157)	n=7 9.9 (1-84)	0.525
≤ 10mg/L (n,%)	18 (67%)	16 (59%)	4 (57%)	0.817
> 10mg/L (n,%)	9 (33%)	11 (41%)	3 (43%)	
Albumin (g/L) (median, range)	43 (30-49)	40 (35-46)	43 (34-46)	0.153
GPS				0.416
0	12 (32%)	16 (45%)	3 (19%)	
1	7 (19%)	1 (23%)	1 (6%)	
2	2 (5%)	0	1 (6%)	
missing	16 (43%)	11 (31%)	5 (31%)	
CAR (median, range)	0.124 (0.021 – 3.47)	0.154 (0.022 – 4.24)	0.109 (0.023 – 2.47)	0.982

Chi-square test (categorical variables), Kruskal-Wallis Test (medians)

5.3.5.2 Body Composition

Median BMI for the non-cancer group (n=28) was 26 kg/m² (range 15-42), for the inoperable cancer group (n=28) was 25 (16-38) and 22 (18-35) for the operable cancer group (n=12). BMI did not differ significantly between diagnostic groups ($p=0.667$).

Table 5.10 details the distribution of key body composition metrics from CT analysis. Values for the non-cancer cohort are provided. However, statistical analysis of differences

between the non-cancer and cancer cohorts is not shown, in view of the very small number of non-cancer patients with available data for comparison (n=3). Mean LSMI, SMG and PMG did not differ significantly between inoperable and operable lung cancer groups (p=0.732, p=0.088 and p=0.485, respectively). Mean SMD was significantly lower in the inoperable group (mean 27 HU) than the operable group (mean 35), p=0.015. Table 5.11 shows LSMI, SMD and PMI, stratified by diagnostic group and categorised as low or normal, based on published threshold values, as detailed in the Methods. There was no significant difference between diagnostic groups in any of the three metrics (p=0.732, p=0.057, p=0.537). When the inoperable and operable cancer groups were analysed together, 55% had low LSMI, 78% had low SMD and 27% had low PMI.

Table 5-10. CT Analysis of L3 Skeletal Muscle: Results Stratified by Diagnostic Group

Mean, SD	Inoperable lung cancer n=31 ^a	Operable lung cancer n=15	p value ^b	Non-cancer n=3
LSMI (cm ² /m ²)	44.6, 9	43.6, 9	0.732	50, 10
SMD (HU)	27, 10	34.6, 6.7	0.015*	35, 9.9
SMG (AU)	1208, 626	1539, 516	0.088	1830, 793
PMI (cm ² /m ²)	5.7, 1.5	6, 1.7	0.485	10, 2.3

^a n=31 except for LSMI and SMG, where n=28. ^b refers to analysis of difference in means between inoperable and operable lung cancer groups, Independent *t* test *p<0.05. LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index

Table 5-11. CT-Measured Body Composition Parameters Categorised as Low or Normal based on published threshold values

	Inoperable lung cancer	Operable lung cancer	p value ^c	Non-cancer
LSMI ^a	n=27	n=12	0.732	n=3
Low	15	7		1
Normal	12	5		2
SMD ^a	n=25	n=12	0.057	n=3
Low	22	8		1
Normal	3	4		2
PMI ^b	n=30	n=14	0.537	n=3
Low	9	4		0
Normal	21	11		3

^aLSMI and SMD threshold values as per Martin *et al.*(394). ^bPMI threshold values as per Nakamura *et al.*(429). ^crefers to analysis of difference between inoperable and operable lung cancer groups (Chi-square test). LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index

5.3.5.3 EORTC QLQ-C30 Score at Enrolment

Summary score

There was no significant difference in summary score at enrolment between groups ($p=0.518$), as shown in Figure 5.5. Median and range scores were 84 (28-96) for non-cancer, 76 (51-97) for inoperable cancer and 84 (64-98) for operable cancer.

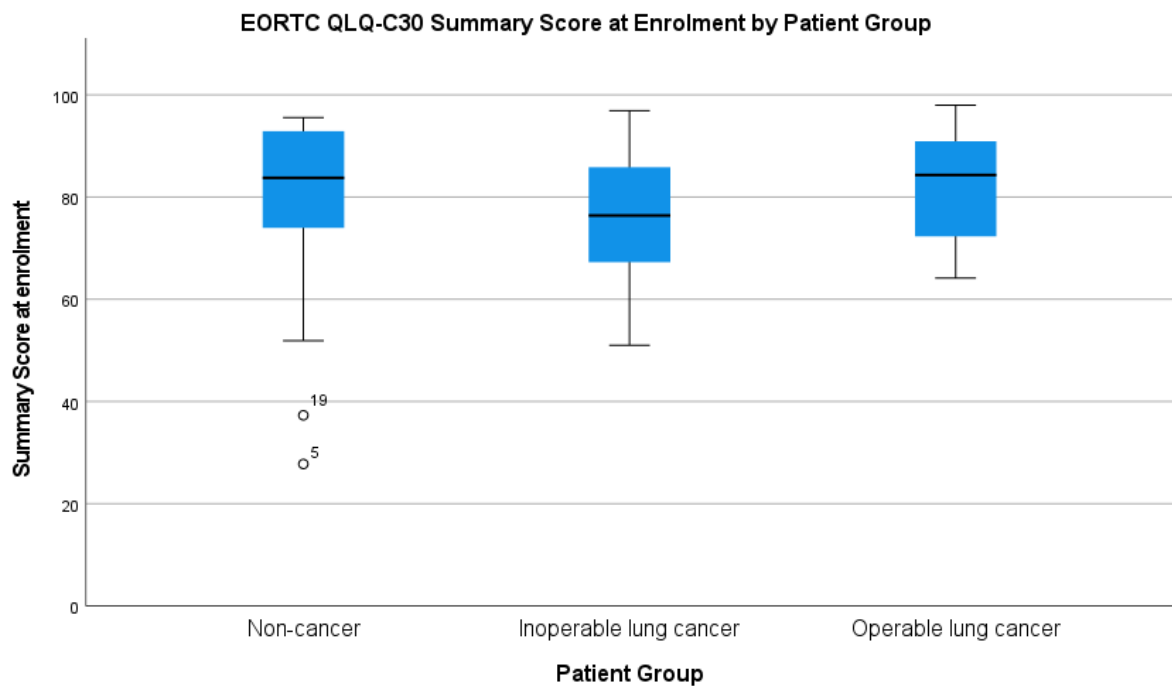


Figure 5-5. Distribution (median and range) for EORTC QLQ-C30 Summary Score at Enrolment stratified by diagnostic group.

Kruskal-Wallis Test.

Impairment in symptom and functional domains

Table 5.12 compares the prevalence of symptoms above the threshold for clinical importance between the diagnostic groups at enrolment. There were no statistically significant differences in prevalence at enrolment. Dyspnoea was the most common symptom in all three groups at enrolment, with a prevalence of 65% in the non-cancer group, 62% in the inoperable cancer group and 46% in the operable cancer group. Appetite loss was more frequent in the inoperable cancer group (20%) than in the non-cancer (11%) and operable groups (0%), but this difference was not statistically significant.

Table 5-12. Comparison of prevalence of symptoms above the threshold for clinical importance at enrolment between diagnostic groups

Patients with symptom above TCI (n, %^a)	Non-cancer	Inoperable lung cancer	Operable lung cancer	p value
Fatigue	10 (31%)	10 (37%)	3 (21%)	0.594
Nausea/vomiting	7 (21%)	4 (13%)	1 (7%)	0.418
Pain	10 (30%)	11 (37%)	4 (26%)	0.706
Dyspnoea	23 (65%)	18 (62%)	7 (46%)	0.442
Sleep disturbance	11(31%)	10 (34%)	6 (40%)	0.842
Appetite loss	4 (11%)	6 (20%)	0	0.129
Constipation	4 (12%)	5 (17%)	1 (7%)	0.648
Diarrhoea	4 (12%)	2 (6%)	2 (14%)	0.691

TCI: threshold for clinical importance from Giesinger et al., 2020. ^a Number of patients reporting symptom above TCI expressed as a percentage of all patients in that group with a valid response. Note that there was slight variation in the number of patients who had a valid response for each symptom, thus percentages vary. Chi-Square test.

Table 5.13 compares the prevalence of functional impairments above the threshold for clinical importance between the diagnostic groups at enrolment. There were no statistically significant differences.

Table 5-13. Comparison of prevalence of functional impairments above the threshold for clinical importance at enrolment between diagnostic groups

Patients with impairment above TCI (n, %^a)	Non-cancer	Inoperable lung cancer	Operable lung cancer	p value
Physical	10 (29%)	16 (51%)	6 (40%)	0.189
Role	9 (25%)	13 (43%)	3 (20%)	0.181
Emotional	12 (40%)	11 (40%)	8 (53%)	0.644
Cognitive	9 (30%)	9 (33%)	4 (26%)	0.901
Social	9 (29%)	7 (25%)	2 (14%)	0.566

TCI: threshold for clinical importance from Giesinger et al., 2020. ^aNumber of patients reporting an impairment above the TCI expressed as a percentage of all patients in that group with a valid response. Note that there was slight variation in the number of patients who had a valid response for each impairment, thus percentages vary. Chi-Square test.

Financial impact

Significant financial impact was reported by seven people (23% of those with available data) in the non-cancer group at enrolment, compared to seven (26%) in the inoperable group and two (14%) in the operable group.

Relationships between CRP, body composition metrics and EORTC QLQ-C30 Score at Enrolment

As shown in Table 5.14, there were no statistically significant associations found between CRP, albumin, GPS, CAR and BMI in the cancer cohort. Of the CT-measured body composition metrics, only PMI was significantly associated with CRP, GPS and CAR (Table 5.14).

Table 5-14. Associations between CRP, Albumin and CRP-based scores and body composition metrics (operable and inoperable cancer only)

	BMI	LSMI	SMD	SMG	PMI
CRP	p=0.523	p=0.114	p=0.811	p=0.627	<i>rho</i> =0.436 p=0.014*
Albumin	p=0.651	p=0.626	p=0.169	p=0.111	p=0.621
GPS	p=0.294	p=0.067	p=0.742	p=0.539	<i>rho</i> =0.497 p=0.01*
CAR	p=0.438	p=0.244	p=0.604	p=0.673	<i>rho</i> =0.446 p=0.023*

BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index. * p<0.05. Spearman rank-order correlation coefficient

Table 5-15. Associations between CRP, Albumin, GPS, CAR and body composition metrics (operable and inoperable cancer only) and EORTC QLQ-C30 Summary Score at enrolment

Summary Score at enrolment	CRP	Albumin	GPS	CAR	BMI	LSMI	SMD	SMG	PMI
Non-cancer	p=0.102	p=0.545	p=0.559	p=0.209	p=0.633	-	-	-	-
Inoperable cancer	p=0.657	p=0.417	p=0.560	p=0.66	p=0.327	p=0.963	p=0.627	p=0.323	p=0.390
Operable cancer	n=5 $\rho=0.9$ p=0.037*	p=0.6	p=0.225	p=0.2	p=0.420	n=9 $\rho=0.867$ p=0.002**	p=0.067	n=9 $\rho=0.9$ p<0.001***	p=0.99

* p<0.05. **p<0.01. *** p<0.001. Spearman rank-order correlation coefficient

BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index.

For clarity, correlation coefficient only given where result was statistically significant.

When the analyses of associations between CRP, Albumin, GPS, CAR and EORTC QLQ-C30 Summary Score at enrolment were repeated excluding any patients where there was evidence of infection, results were overall unchanged.

5.3.5.4 ECOG at follow-up

Unlike at enrolment, there was a statistically significant difference in performance status between diagnostic groups at time of follow-up, with the non-cancer group having better performance status (Table 5.16).

Table 5-16. ECOG performance status at follow-up stratified by diagnostic group

	Non-cancer n=37	Inoperable lung cancer n=35	Operable lung cancer n=16	p value
ECOG				
0	18 (49%)	4 (11%)	1 (6%)	
1	5 (14%)	8 (23%)	5 (31%)	
2	3 (8%)	6 (17%)	3 (19%)	
3	2 (5%)	7 (20%)	2 (13%)	
4	0	1 (3%)	0	
Missing (incl. RIP)	9 (24%)	9 (26%)	5 (31%)	p=0.024*

* p<0.05. Chi-Square test.

5.3.5.5 EORTC QLQ-C30 score at follow-up

There was a significant difference in summary score at follow-up between the non-cancer and the inoperable groups with median score 89 (range 49-99) versus median score 76 (range 40-97), respectively, $p=0.022$. There was no significant difference between the non-cancer and operable groups (median 85, range 61-94), $p=0.343$, as illustrated in Figure 5.6.

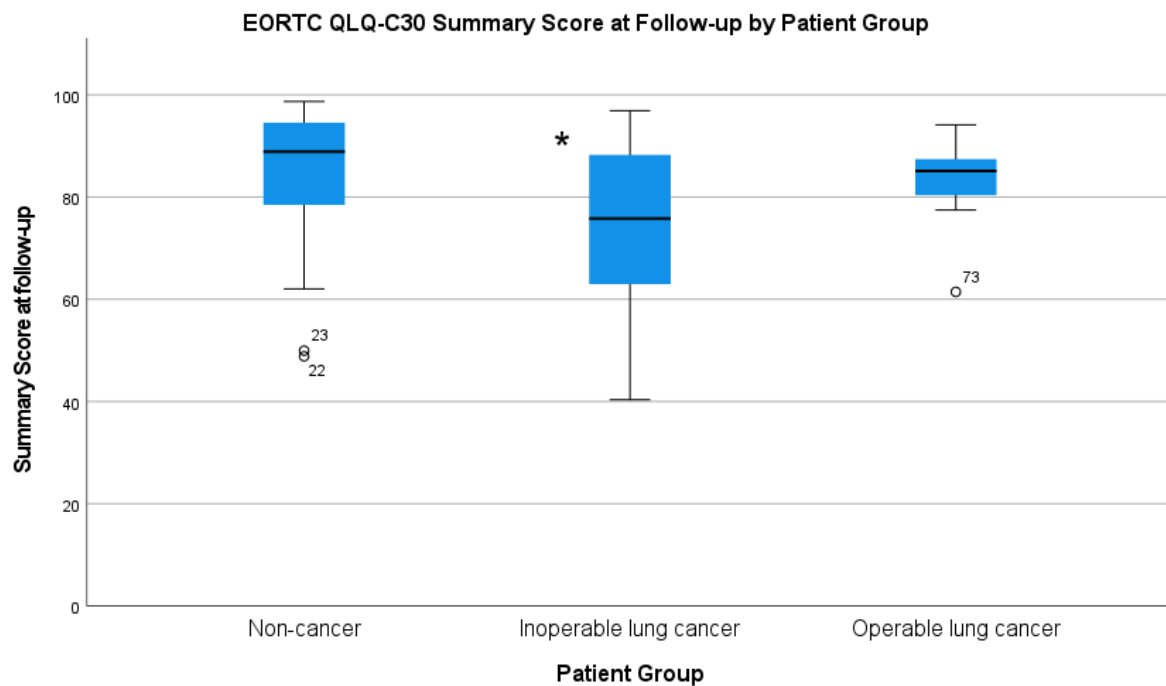


Figure 5-6. Distribution (median and range) for EORTC QLQ-C30 Summary Score at follow-up, stratified by diagnostic group.

Kruskal-Wallis Test.* $p<0.05$

Table 5.17 compares the prevalence of symptoms above the threshold for clinical importance between the diagnostic groups at follow-up. Dyspnoea was the most common symptom overall, with a prevalence of 56% in the non-cancer group and 75% in the operable cancer group, though only 30% in the inoperable cancer group; this difference was statistically significant ($p=0.025$). Appetite loss was reported by 29% of the inoperable cancer group and by none of the non-cancer and operable groups, this difference was highly statistically significant ($p<0.001$). The inoperable group were also significantly more fatigued than the other groups ($p=0.041$), with over half reporting clinically important levels of fatigue.

Table 5-17. Comparison of prevalence of symptoms above the threshold for clinical importance between diagnostic groups at time of follow-up

Patients with symptom above TCI (n, %^a)	Non-cancer	Inoperable lung cancer	Operable lung cancer	p value
Fatigue	6 (20%)	14 (51%)	4 (33%)	0.041*
Nausea / vomiting	2 (6%)	8 (29%)	4 (33%)	0.046*
Pain	13 (44%)	13 (48%)	3 (25%)	0.383
Dyspnoea	17 (56%)	8 (30%)	9 (75%)	0.025*
Sleep disturbance	8 (26%)	6 (22%)	5 (41%)	0.451
Appetite loss	0	8 (29%)	0	<0.001***
Constipation	1 (3%)	6 (23%)	1 (8%)	0.067
Diarrhoea	2 (6%)	6 (22%)	1 (8%)	0.191

TCI: threshold for clinical importance from Giesinger et al., 2020. ^a Number of patients reporting symptom above TCI expressed as a percentage of all patients in that group with a valid response. Note that there was slight variation in the number of patients who had a valid response for each symptom, thus percentages vary. * $p<0.05$. *** $p<0.001$. Chi-Square test.

Table 5.18 compares the prevalence of functional impairments above the threshold for clinical importance between the diagnostic groups at follow-up. There were statistically significant differences between the groups in physical and role functional impairments, with a higher prevalence of impairment among both cancer groups than the non-cancer group. Emotional role functional impairment was highest in the non-cancer group (43%), this difference was statistically significant ($p=0.039$). While the inoperable group reported a higher rate of social functional impairment, this difference was not statistically significant ($p=0.106$). There were no significant differences between groups in cognitive function impairment.

Table 5-18. Comparison of prevalence of functional impairments above the threshold for clinical importance between diagnostic groups at time of follow-up

Patients with impairment above TCI (n, %^a)	Non-cancer	Inoperable lung cancer	Operable lung cancer	p value
Physical	8 (26%)	18 (56%)	7 (58%)	0.008**
Role	5 (16%)	14 (51%)	5 (41%)	0.018*
Emotional	13 (43%)	8 (30%)	0	0.039*
Cognitive	6 (20%)	7 (26%)	2 (18%)	0.772
Social	5 (16%)	10 (37%)	1 (10%)	0.106

TCI: threshold for clinical importance from Giesinger et al., 2020 ^a Number of patients reporting an impairment above the TCI expressed as a percentage of all patients in that group with a valid response. Note that there was slight variation in the number of patients who had a valid response for each impairment, thus percentages vary. ** $p<0.01$. * $p<0.05$. Chi-Square test.

Financial impact

Significant financial impact was reported by two people (7% of those with available data) in the non-cancer group at follow-up, compared to six (23%) in the inoperable group and by none of the patients in the operable group.

5.3.5.6 Change in EORTC QLQ-C30 From Enrolment to Follow-up

Change in Summary Score

Change in summary score was available for n=54, as shown in Table 5.19.

Table 5-19. Degree of Change in Summary Score

	Significantly better	Little change	Significantly worse
Non-cancer n=25	7 (28%)	15 (60%)	3 (12%)
Inoperable cancer n=20	2 (10%)	13 (65%)	5 (25%)
Operable cancer n=9	3 (33%)	4 (44%)	2 (22%)

Categorised as per Fayers *et al.*, 2001

Change in prevalence of impairment in symptom and functional domains of EORTC QLQ-C30

Figure 5.7 illustrates the percentage of patients in each group with clinically important levels of symptoms, as measured on the EORTC QLQ-C30, at enrolment and at follow-up. Figure 5.8 illustrates the percentage of patients in each group with clinically important functional impairments at the same time points. The key findings illustrated by these figures are now summarised.

Non-cancer group

The prevalence of clinically significant symptoms fell from enrolment to follow-up, with the exception of pain (Figure 5.7A). Figure 5.8A shows some increase in role and cognitive function impairments, with little change in the other functional domains.

Inoperable cancer group

The prevalence of clinically significant symptoms increased for all symptoms except dyspnoea and sleep (Figure 5.7B). There was increased prevalence of significant impairment in physical, role and social function at follow-up, while there was a slight reduction in cognitive and emotional function impairments (Figure 5.8B).

Operable cancer group

Prevalence of clinically significant fatigue, nausea and vomiting and dyspnoea all increased during follow-up, with little change in prevalence of pain, sleep disturbance and bowel symptoms (constipation and diarrhoea) (Figure 5.7C). Significant impairments in social, cognitive and emotional function reduced from enrolment to follow-up, although impairments in physical and role function increased (Figure 5.8C).

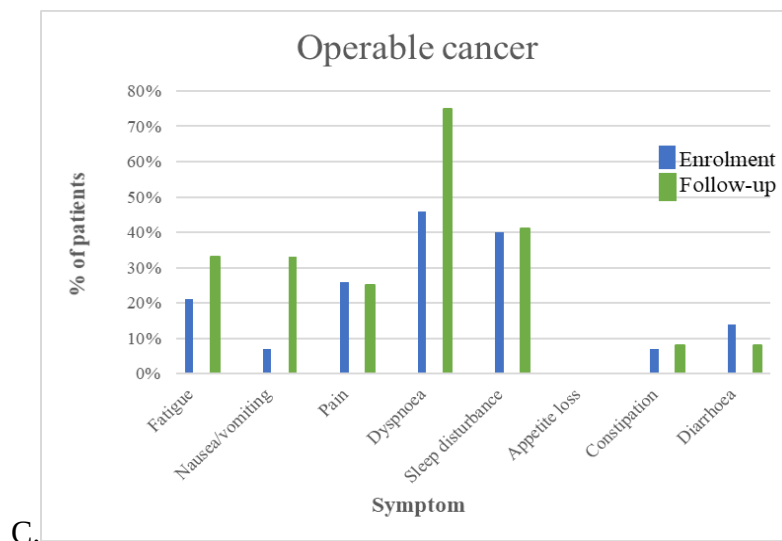
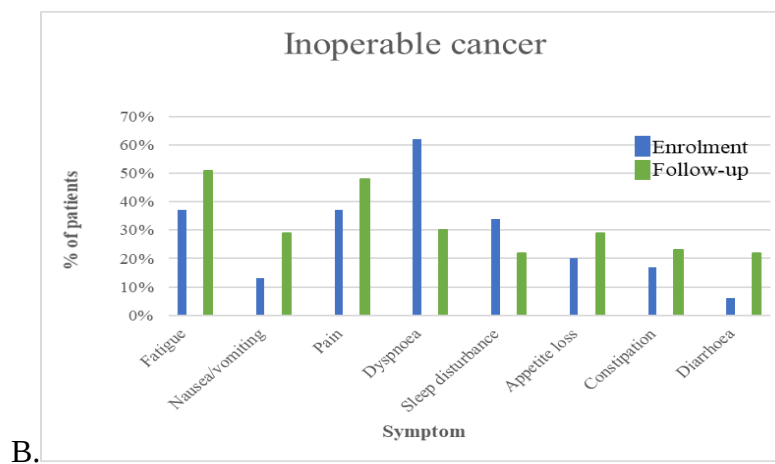
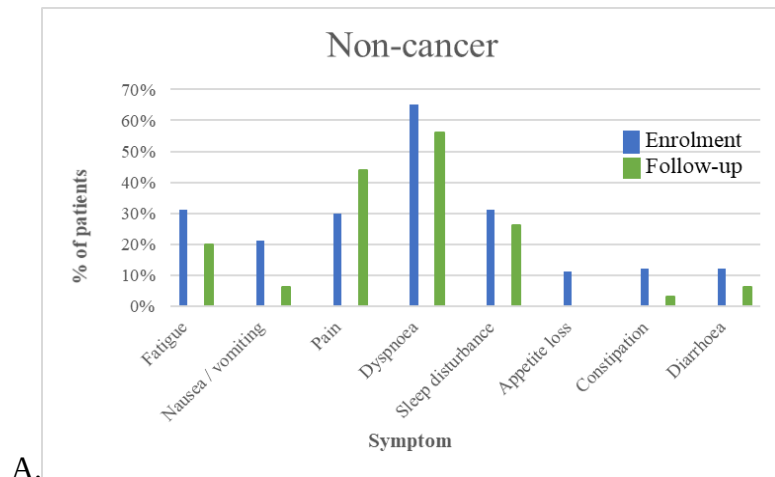
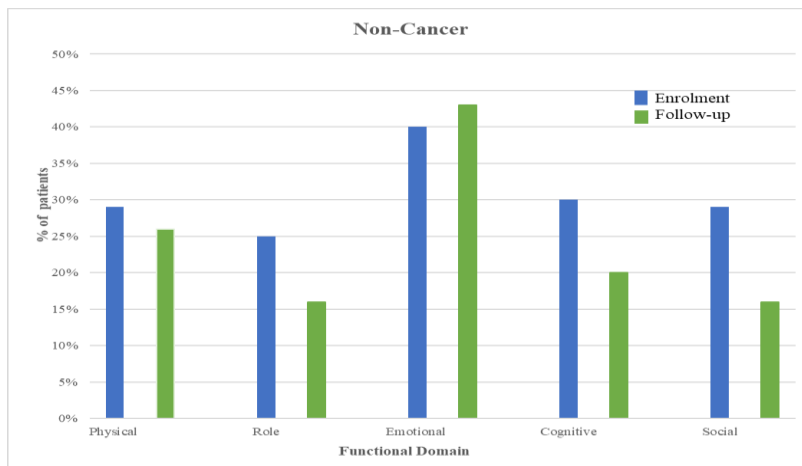


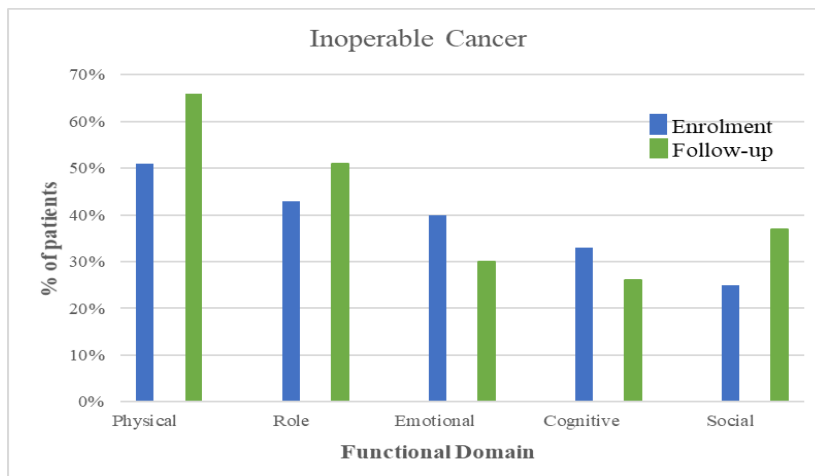
Figure 5-7. Prevalence of symptoms above the threshold for clinical importance in the three diagnostic groups at enrolment and at follow-up

Threshold for clinical importance from Giesinger et al., 2020. A. Non-cancer B. Inoperable Cancer and C. Operable Cancer. Enrolment shown in blue, follow-up shown in green.

A.



B.



C.

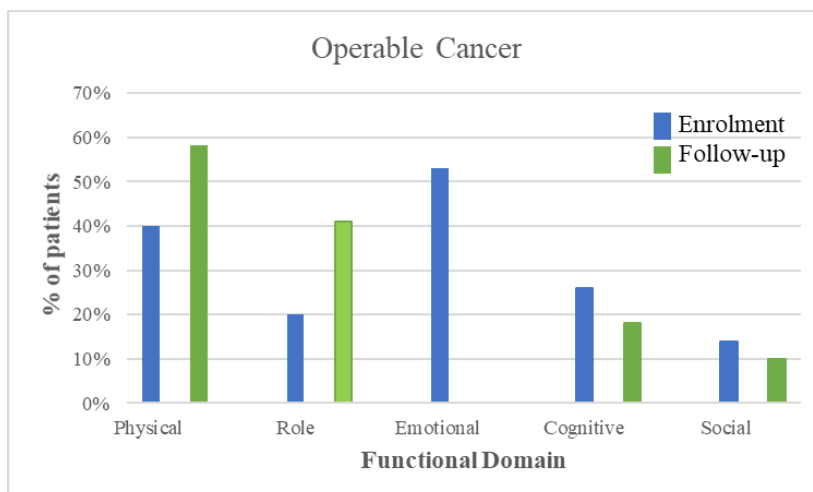


Figure 5-8. Prevalence of functional impairments above the threshold for clinical importance in the three diagnostic groups at enrolment and at follow-up

Threshold for clinical importance from Giesinger et al., 2020. A. Non-cancer B. Inoperable Cancer and C. Operable Cancer. Enrolment shown in blue, follow-up shown in green

5.3.5.7 Post-operative complications and length of stay (operable cohort only)

Of 16 patients, 9 had documented evidence of post-operative complications. There was no statistically significant relationship between number of co-morbidities ($p=0.197$), sex ($p=0.614$), ECOG ($p=0.142$), histology ($p=0.633$) and development of post-operative complications. Median length of stay was 9 days (range 5 – 150).

5.3.6 Associations between baseline variables and outcomes

Associations between baseline variables and the following outcomes are reported below.

1. Symptoms / QoL at follow-up
2. Post-operative complications and length of stay (operable cancer group only)
3. Study completion

5.3.6.1 Symptoms / QoL at follow-up

Table 5. 20 reports associations between CRP, Albumin, GPS, CAR and EORTC QLQ-C30 summary score at follow-up. In the non-cancer group, a high CRP was negatively correlated with summary score at follow-up ($\rho = -0.478$, $p=0.033$). In the inoperable cancer group, a high GPS ($\rho = 0.651$, $p=0.005$) and high CAR ($\rho = 0.617$, $p=0.008$) were positively correlated with follow-up summary score, as was a high PMI ($\rho=0.448$, $p=0.042$). There were no significant associations in the operable group.

When the same analyses were repeated excluding any patients where there was evidence of infection, there were a number of changes in which results were statistically significant. In the non-cancer group, the association between CRP and follow-up summary score was no longer statistically significant ($p=0.121$). In the inoperable cancer group, the association between CRP and follow-up summary score became statistically significant ($\rho = 0.556$, $p=0.031$), that between GPS and follow-up summary score was no longer significant ($p=0.17$) and the association between CAR and follow-up summary score remained significant ($\rho = 0.793$, $p<0.001$).

Table 5-20. Associations between CRP, Albumin, CRP-based scores and body composition metrics and Summary Score at follow-up

Summary Score at follow-up	CRP	Albumin	GPS	CAR	BMI	LSMI	SMD	SMG	PMI
Non-cancer	$\rho = -0.478$ $p=0.033^*$	$p=0.545$	$p=0.272$	$p=0.405$	$p=0.81$	-	-	-	-
Inoperable cancer	$p=0.094$	$p=0.281$	$\rho = 0.651$ $p=0.005^{**}$	$\rho = 0.617$ $p=0.008^{**}$	$p=0.506$	$p=0.762$	$p=0.636$	$p=0.791$	$\rho=0.448$ $p=0.042^*$
Operable cancer	$p=0.873$	$p=0.6$	$p=0.225$	$p=0.2$	$p=0.456$	$p=1.0$	$p=0.765$	$p=0.932$	$p=0.332$

* $p<0.05$. ** $p<0.01$. BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index. Spearman rank-order correlation coefficient. For clarity, correlation coefficient only given where result was statistically significant.

5.3.6.2 Degree of Change in Summary Score

When the cancer cohorts were analysed together, to maximise power to identify difference, there was no significant difference between CRP or CAR and degree of change in summary score ($p=0.457$ and $p=0.552$, respectively).

5.3.6.3 Post-operative complications and length of stay (operable cancer group only)

There were no significant associations between post-operative length of stay or post-operative complications and CRP, albumin, GPS or CAR. There were also no significant associations between length of stay and BMI or CT-analysed body composition but there was a weak but statistically significant negative correlation between SMD and post-operative complications ($\rho = -0.288$, $p=0.045$) (Table 5.21)

Table 5-21. Associations between CRP, Albumin, CRP-based scores and body composition metrics and post-operative length of stay and complications (operable cancer)

	CRP	Albumin	GPS	CAR	BMI	LSMI	SMD	SMG	PMI
Length of stay	$p=0.355$	$p=0.873$	$p=0.858$	$p=0.624$	$p=0.282$	$p=0.18$	$p=0.09$	$p=0.068$	$p=0.216$
Post-operative complications	$p=0.471$	$p=0.753$	$p=0.329$	$p=0.982$	$p=0.511$	$p=0.632$	$\rho = -0.288$ $p=0.045^*$	$p=0.214$	$p=0.808$

* $p<0.05$. BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index. Spearman rank-order correlation coefficient. For clarity, correlation coefficient only given where result was statistically significant.

5.3.7 Further Exploratory Analyses

5.3.7.1 Study Completion

When CRP was categorised as high ($> 10\text{mg/L}$) or normal ($\leq 10\text{mg/L}$), there was a significant association between a high CRP and non-completion of the study for all those with cancers (Table 5.22). Although on visual inspection the same trend appeared to be present for the whole study cohort and for the inoperable group, these were not statistically significant.

Table 5-22. Associations between high and normal CRP and study completion

	Completed study	Did not complete study	p value
Whole group			
High CRP	15	8	0.087
Normal CRP	32	6	
All cancer			
High CRP	8	6	0.026*
Normal CRP	18	2	
Inoperable cancer			
High CRP	6	5	0.055
Normal CRP	14	2	

* $p < 0.05$. Chi-Square test. High CRP $> 10\text{mg/L}$.

Table 5.23 shows the associations between CRP, albumin, GPS, CAR and body composition metrics and study completion. A higher CRP, GPS and CAR were all associated with study non-completion in the cohort ($n=88$) as a whole. When these relationships were analysed for the combined cancer cohort and for the inoperable cohort, only CRP and CAR had a significant association with study completion. None of the body composition metrics were associated with study completion.

Number of co-morbidities ($p=0.307$), age ($p=0.913$) and ECOG ($p=0.816$) were not significantly associated with study completion. In the lung cancer groups, histology was also not associated with study completion ($p=0.467$).

When analysis of the associations between CRP, CAR and study completion was repeated excluding all patients where there was evidence of infection, the association remained

significant only for CRP and only for the inoperable cohort (correlation coefficient $\rho=0.433$, $p=0.039$).

Table 5-23. Associations between CRP, Albumin, CRP-based scores and body composition metrics and study completion

Study completion	CRP	Albumin	GPS	CAR	BMI	LSMI	SMD	SMG	PMI
Whole group n=88	p=0.040*	p=0.338	p=0.029*	p=0.029*	p=0.229	p=0.37	p=0.415	p=0.598	p=0.861
All cancer n=51	ρ =0.46 p=0.006**	p=0.792	p=0.052	ρ =0.458 p=0.012*	p=0.815	p=0.447	p=0.34	p=0.458	p=0.4
Inoperable cancer n=35	ρ =0.467 p=0.014*	p=0.444	p=0.169	ρ =0.423 p=0.04*	p=0.312	p=0.774	p=0.846	p=0.608	p=0.575

* p<0.05. **p<0.01. BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index. Spearman rank-order correlation coefficient. For clarity, correlation coefficient only given where result was statistically significant

5.3.7.2 Potential to benefit from SPC

When those in the inoperable group who had a significantly worse summary score at follow-up, were too unwell to complete the study or who had died were compared to those who completed the study and whose summary score was stable or improved, there were no significant differences in median CRP, CAR or body composition measures as shown in Table 5.24.

Table 5-24. Associations between CRP, Albumin, CRP-based scores and potential to benefit from specialist palliative care

	CRP	Albumin	CAR	BMI	LSMI	SMD	SMG	PMI
Worse summary score, unwell or RIP	p=1.0	p=0.357	p=1.0	p=1.0	p=0.165	p=0.908	p=0.648	p=0.39

BMI: body mass index, LSMI: lumbar skeletal muscle index, SMD: skeletal muscle radiodensity, SMG: skeletal muscle gauge, PMI: psoas muscle index. Spearman rank-order correlation coefficient.

5.4 Discussion

5.4.1 Primary Aim: Feasibility

Despite extensive consultation and consideration in the study planning phase, several issues arose during the course of the study. This underlines the value of a feasibility study.

The first 2 iterations of the study identified a number of barriers to recruitment. These were a mixture of study design (inclusion criteria), practical realities (busy Oncology OPD, another study starting recruitment in the same clinic) and gatekeeping by the clinical team. Excessively strict inclusion criteria are a known barrier to successful recruitment in cancer trials (433). Gatekeeping too has been described as an obstacle to recruitment (403). In this study, however, it is arguable that the gatekeeping was appropriate, given the low rate of acceptance of the study by those patients who were approached in iteration 2 (1 in 4), which contrasts with the over 80% acceptance in iteration 3.

The third iteration of the study recruited successfully. The majority of patients (83%) who were invited to participate agreed to do so. This compares favourably with other observational studies in advanced disease, where rates of 50-70% are reported (403, 434). However, several feasibility issues were identified, particularly missing data, study attrition and a heterogenous study cohort.

Missing data has been identified as a significant threat to the validity and wider application of trials in palliative care populations (435). Trial duration and volume of data collected are associated with missing data in trials in palliative care (435). This study was designed with a view to minimise missing data, with a short follow-up period and only two patient-reported outcome measures. However, missing data remained a major challenge. Some data were missing due to study design: the time constraints of the recruitment setting led to an increased chance of incomplete questionnaires. Recent weight was not always documented. CRP was not measured as part of the study, rather CRP values taken as part of routine clinical care were used for analysis. This was at the request of the clinical team, who felt that routine testing of CRP was not appropriate in the context of a feasibility study and had concerns about medical governance of any unexpectedly elevated CRP results. As a consequence, 31% of patients did not have a CRP available for analysis. While using existing CRP values was in keeping with the overall premise of this study, in making

additional use of existing data, it may have introduced bias if participants had infection at the time of CRP measurement. Evidence of infection was sought, in order to minimise the impact of this. Had the requirement for an additional CRP been present, it is possible that this may have reduced patient's acceptance of the study. However, a recently published study in advanced cancer found that very few patients declined to participate because of the need for a blood test(403). Furthermore, in the present study, many patients had blood samples taken at the time of cannulation for bronchoscopy so the extra burden would have been minimal. Thus, it seems unlikely that this addition would have significantly impacted on feasibility.

Missing data was also a difficulty with respect to PET-CT. The majority of the non-cancer cohort had CT Thorax only and thus did not have L3 level imaging available. They were excluded from analysis of skeletal muscle as a result, meaning that body composition could not be compared between the cancer and non-cancer cohorts. An option would have been to analyse skeletal muscle at thoracic vertebral level instead, as the use of thoracic level images for this purpose has been described (436). However, imaging at L3 is considered the “*de facto* gold standard”(428), with internationally-validated reference values available (394). Cutpoints derived from studies at L3 should not be applied to patients with only chest imaging (437). Comparison of thoracic levels measurements for the non-cancer group to L3 levels from the cancer cohort was also not possible, as it is known that L3 measurements differ significantly from those at other vertebral levels (437). Among the cancer cohort, availability of PET-CT images was much higher but 4 patients images could not be analysed due to image corruption or quality. This is a known problem when using images gathered as part of routine care for research purposes (428).

Attrition was a significant problem, with almost a quarter of all participants not completing the study. Interestingly, there was no significant difference in rates of study completion between the diagnostic groups. There was a difference in the reasons for attrition between the groups, although numbers were too small for meaningful statistical comparison of these. In the non-cancer group, four of the seven people did not complete the second assessment because they were uncontactable and three were too unwell or died. In the similarly-sized inoperable group, only one was contactable but seven were unwell, asked to withdraw or died. The latter three reasons are recognised as the main contributors to attrition in studies in this group (438). While unsurprising, this observation highlights the major challenge of

attrition in longitudinal studies in advanced cancer, even in studies with short follow-up (402). This study was devised considering the recommended ways to overcome attrition (short duration of study, simple methodology) and having assessments at times and locations convenient to participants (402, 439). Most participants completed follow-up from their homes, in line with anticipated preference in this group (402). These factors may explain why attrition was less than in other longitudinal studies of QoL or symptoms, where reported attrition ranged from 33% at 5 weeks in one study to over 50% at 3 weeks in another (407, 440). However, study structure alone cannot fully address this research challenge. Simply restricting studies to those most likely to complete the study is appealing but will not address the evidence gap for frailer patients (438). One possible means to improve follow-up is to identify those who are most likely to drop out and implement processes to address this.

A recent review of randomised controlled trials (RCTs) of interventions to improve QoL in advanced life-limiting illness found that participants with worse performance status on the Australia-modified Karnofsky Performance Scale (AKPS) were more likely to have missing data (441). This was not the case in the present study, where no significant association was found between performance status and study completion. Possible explanations are that the cohort in this study had relatively good performance status, with 80% of the inoperable cohort having ECOG of 2 or less, and that ECOG may be a less sensitive measure of performance status than the AKPS (441). Missing data at enrolment was also associated with missing data at subsequent timepoints (441). The authors suggest that participants with low performance status and/or missing data should be identified early in order to provide additional support to ensure complete data is collected and note that other factors which may affect missing data and study completion should be identified (441). The present study suggests that CRP may be one of these factors. CRP was associated with study non-completion in the inoperable cancer cohort, even when those with suspected infection were excluded, suggesting that it may be a useful additional marker of non-completion risk. CRP may have been acting as proxy marker of the disease burden, symptoms or functional status or perhaps even a combination of all three. This hypothesis should be investigated in future studies.

The current study design results in a heterogeneous study cohort. Heterogeneity among recruited participants is very common in studies in advanced cancer, with 79% including more than one tumour type and half included any cancer type (401). Although the present

study recruited only people with suspected lung cancer, the inclusion of people with operable cancer and non-cancer controls meant the overall study population was very heterogeneous. It was valuable for the researcher to include people with operable cancer and non-cancer controls, as this provided interesting insights into these groups and an excellent opportunity to understand the impact of cancer alone and cancer stage on the parameters measured. However, this model would be inefficient in a larger scale study looking at potential triggers for SPC referral. Even among the inoperable cohort, there was heterogeneity in treatments planned – some were to have chemotherapy, some radiotherapy, and some best supportive care. The study location, a tertiary referral centre with availability of radical radiotherapy for NSCLC and the inclusion of patients with SCLC compounded this heterogeneity since some patients with inoperable disease may still have been treated with curative intent. Such heterogeneity could confound results of a full-scale study to the point of making the study invalid, unless sufficiently large numbers were recruited to account for this. In turn, a very large sample size would increase the time taken to complete the study, based on recruitment and retention findings from this work. Given the high rate of attrition in this study, a sample size calculation would also have to account for that anticipated loss of participants and, potentially, a significant amount of missing data (441). At present, most published palliative care studies (60%) are conducted in one centre, with resulting challenges in reaching the required sample size (401). Multisite collaborations are strongly recommended in order to ensure adequate power and timely completion of studies (408).

Overall, the feasibility issues outlined above mean that the study design would not be feasible for a definitive study. However, the specific local findings and experience have informed another study recruiting the same cohort in the same setting. Furthermore, the study has been disseminated widely in poster and oral presentations nationally and internationally, with more output in preparation. The researcher also received verbal feedback after conference presentations that sharing her experience and learning would help others to plan and to persevere with their own studies. While much research goes unpublished (442), this phenomenon appears especially common in palliative care research, with less than one-fifth of closed studies having results published in PubMed (401). Publication bias against negative results has been discussed throughout this thesis and may certainly contribute. However, the lack of dissemination conflicts with the very clear ethical obligation to report all research results to avoid duplication, help to plan future studies and ultimately to promote better patient care(250). Arguably, this ethical imperative is even

stronger for studies in advanced disease, with all the noted research challenges, and for feasibility studies, as problems and solutions identified in a feasibility study may help the design of other studies (415).

As a secondary aim, this work has provided further insights into the link between inflammation, body composition, symptoms and quality of life. These are discussed in the next section.

5.4.2 Secondary Aims

5.4.2.1 CRP

Although CRP levels appeared slightly higher in the cancer groups than in the non-cancer group, there was no significant difference in distribution of CRP and CRP-based scores between the groups. It is notable that CRP levels were relatively high, compared to the general population, in all three groups. Elevated CRP levels in the non-cancer group, where one-third of the group had a CRP greater than 10mg/L, may have been due to co-morbidities, such as COPD, and possibly infection, which may also have caused the symptoms which led to their investigations for lung cancer. These findings would suggest that CRP is not a useful adjunctive investigation in the diagnosis of suspected lung cancer, since it did not distinguish between those who did and did not have the condition. The literature is inconsistent, with some work suggesting higher levels in lung cancer than in benign lung disease, while others suggest the contrary (104). A very recent epidemiological UK Biobank study showed a consistent association between higher CRP and risk of incident lung cancer (443). It may be that an elevated CRP predicts risk of cancer but is not sufficiently specific to be used at an individual diagnostic level.

Albumin level was normal for the majority of all participants and most had GPS of 0. Although CRP levels in the present study were elevated compared to baseline population levels, they were not sufficiently elevated to score on the GPS, Other studies have found a higher prevalence of high GPS/mGPS – up to 57% in one large study of mixed advanced cancers, though that study included people at a variety of disease points, unlike the present study where GPS was assessed at time of diagnosis, which may provide an explanation (391).

5.4.2.2 BMI

Median BMI was in the normal or overweight range for all three diagnostic groups, including those with inoperable lung cancer, a cancer often associated with weight loss (444). It is known that many people with cancer remain overweight, due to pre-existing obesity, and this may mask weight loss and muscle loss (394, 445) . In the present study, BMI was not associated with any of the outcomes assessed. This is consistent with the work

by Martin *et al.*, which highlighted the superiority of CT analysis over BMI as a measure of body composition (394).

5.4.2.3 LSMI and SMD

LSMI measurements in the cancer cohorts in the present study (mean LSMI 45 cm²/m² in the inoperable group and 44 cm²/m² in the operable group) were almost identical to those reported in other studies in lung cancer (400, 446, 447). When the inoperable and operable cancer groups were analysed together, 55% had low LSMI. This is very similar to other reports, with a median frequency of low LSMI in lung cancer found to be 50% (with a range from 42-61%) in a recent systematic review (397).

By contrast, SMD values in the cancer cohorts in this study are lower than reported in other studies in lung cancer, with a mean SMD of 27 HU in the inoperable group, compared to 35 HU in Cortellini *et al.*, and 37 HU in Sjøblom *et al.*, / Bye *et al.*,’s cohort (400, 446, 447). When the inoperable and operable cancer groups were analysed together, using the cutpoints validated by Martin *et al.*, to define normal and low SMD, 78% had low SMD (394). Despite the Cortellini and Sjøblom studies using a lower threshold, they found only 23% and 10% to have low SMD (446, 447). The latter two studies included patients with a similar age profile to the present study and also looked at people with inoperable disease, but only included patients having chemotherapy (446, 447). The inclusion of people in the present study who were not fit for any oncology treatment may be reflected in the lower SMD. The prevalence of low SMD in the present study is closer to Daly *et al.*, who, using the same thresholds in their large study of advanced cancers, found 54% had SMD (391). One-third of their study cohort had cancers other than GI and lung, including breast cancer which is likely to have influenced their results(391).

In the present study, interesting associations were identified for both of these measures of body composition but neither one was clearly superior to the other. LSMI did not differ between people with operable and inoperable cancers, where SMD did. Neither measure was associated with CRP, albumin or CRP-based scores, contrary to other reports in lung cancer (448, 449). Kim *et al.*, showed a statistically significant though weak correlation between higher CRP and lower LSMI in small cell lung cancer (448). In NSCLC, Tenuta *et al.*, found an association between high CRP and reduced muscle mass on Dual Energy X-

Ray Absorptiometry (DEXA) (449). It is possible that patient numbers in the present study were too small to detect a real association.

While LSMI was associated with a higher (better) score on the EORTC QLQ-C30 at enrolment, this was only true for the operable group and there was no relationship with outcomes in that group (follow-up scores, post-operative complications or length of stay). Survival was not assessed in this work but a low LSMI has previously been associated with shorter survival in surgically-treated NSCLC (450). SMD, meanwhile, had no association with symptom scores but was associated with post-operative complications. A possible explanation for this is the association between a lower SMD and more severe co-morbidity identified by Grønberg *et al.*, in advanced NSCLC (451). No published study has examined SMD as a predictor of outcomes, either surgical or PROMs in resectable lung cancer. Bye *et al.*, found a non-linear relationship between SMI and SMD and QoL in advanced NSCLC (400). Their study, although retrospective, was much larger than the present study and, again, the smaller number may have led to a Type II statistical error.

5.4.2.4 SMG

Skeletal muscle gauge (SMG) incorporates both LSMI and SMD. This novel metric, first described in 2016 (452), has recently been shown to be a better predictor of outcomes in advanced cancer than skeletal muscle area or radiodensity alone, potentially because it assesses both quantity and quality of the muscle (428). Based on their finding that SMG was more closely correlated to physical function than was skeletal muscle mass, Williams *et al.*, proposed that it may be more valuable than muscle mass in capturing muscle performance (453). Low SMG has previously been associated with shorter survival in colorectal cancer, diffuse large B-cell lymphoma, melanoma and ovarian cancer (454-457). It has also been associated with post-operative complications in gastric cancer, hospitalisation and treatment toxicity in breast cancer, treatment delay in endometrial cancer (458-460). In sarcoma, SMG was not associated with survival but was a predictor of surgical wound complications and chemotherapy toxicity (461, 462). However, in the present study, SMG did not appear to provide any additional information to that provided by SMD. A limitation to the use of SMG is that reference ranges have yet to be established (428). Further, larger studies are needed to assess the value of SMG in lung cancer.

5.4.2.5 PMI

PMI did not distinguish between operable and inoperable cancer groups but was the only measure of body composition which was associated with CRP and the CRP-based scores. PMI was also positively correlated with symptom and quality of life summary score at follow-up in the operable group. Despite an editorial in 2017 by Professor V. Baracos, one of the leading authorities in the area, condemning the use of psoas muscle measures (463), studies using this measure have proliferated in the years since, with studies in oesophageal cancer (464), pancreatic cancer (465) and several in lung cancer (430, 466, 467) reporting associations between low psoas muscle area and adverse clinical outcomes. The confusion is exacerbated by some authors using the term “SMI” when they in fact measured PMI (431). Other authors report psoas muscle area without any correction for height (468, 469), or psoas volume index, based on psoas muscle volume adjusted by height cubed (470). This presents a major challenge to meaningful comparison between studies, including this one (463). When compared to other studies using the same metric, mean PMI in the present study was higher ($5.7 \text{ cm}^2/\text{m}^2$) than that reported by Nakamura *et al.*, ($4.8 \text{ cm}^2/\text{m}^2$) (429). When PMI was categorised as normal or low, 27% of those with cancer in the present study had low PMI. This figure is considerably lower than that reported in other lung cancer studies (429-431). Miura *et al.*, had an older population, with slightly more women (430), both factors associated with lower PMI (429). Tsukagoshi *et al.*, included only people with advanced disease, which may explain the prevalence of low PMI in their groups (69% and 43%, respectively) (430, 431). However, Nakamura *et al.*, in their study of resectable NSCLC, had a slightly older and more male-dominated population than the present study, yet found that 55% had a low PMI (429). The origin of the cutpoints for PMI adds further interest to this observation. Hamaguchi *et al.*, defined the threshold values used in all three of these studies based on healthy young adult living organ donors (427). It might reasonably be expected that a cohort such as that described here, with cancer and/or co-morbidities, would have a dramatically lower PMI than that group. The key may be in the geography. Skipworth highlighted the importance of considering body composition “in the context of local geographic and ethnic norms” (398). The Hamaguchi study and all three cancer studies were conducted in Japan. The Asian Working Group for Sarcopenia noted that reference values for muscle mass measures are likely to differ from Western populations because of a number of factors, including ethnicity, body size, lifestyle and cultural background and made recommendations for Asia-specific threshold values (471). Results of the present study

suggest that the converse is true also and that caution must be applied when applying Asian-derived reference values to Western populations.

5.4.2.6 Symptoms, quality of life and performance status

There was no difference in EORTC QLQ-C30 scores at enrolment between the diagnostic groups, whether considered in terms of summary score, symptoms above the TCI or functional impairments above the TCI, nor in ECOG performance status. This baseline similarity makes all the more striking the subsequent divergence between those with cancer and those without. At follow-up, the cancer cohorts had a significantly worse performance status than the non-cancer group and a higher prevalence of symptoms and functional impairments above the TCI. The non-cancer group had a significantly better summary score than the inoperable group, though not than the operable group. Yet it is interesting to note the relatively high ongoing levels of symptom and function impairments in the non-cancer group. While 28% were significantly better in terms of summary score at follow-up, the majority were unchanged. Over half continued to reported dyspnoea and over 40% reported pain. More than 40% reported impairment in emotional functioning. These findings likely reflect the burden of chronic disease. Three-quarters of the group were current or ex-smokers and the majority had multiple medical conditions. Chronic non-malignant respiratory disease is associated with significant symptom distress and reduction in physical function (472).

The operable group had a very high prevalence of dyspnoea at follow-up. This is unsurprising, given that they had undergone lung resection (pneumonectomy or lobectomy) and were assessed relatively early in their post-operative recovery. In their study in resected NSCLC, Kenny *et al.*, found that some study participants had ongoing symptoms and functional impairment 2 years after surgery (473). CRP, albumin and the CRP-based scores were not associated with overall summary score at follow-up in that group. Of the body composition measures, only PMI was significantly associated with follow-up score. As for post-operative complications, only SMD was associated, making it hard to conclude which metric is of most value in this cohort. It would be of interest to re-assess this group at a later timepoint and determine whether any of these measures had a role in predicting longer term symptom, functional and QoL outcomes.

In the inoperable group, performance status deteriorated even in the short period of the study. Impairments in function worsened overall as did prevalence of most symptoms. Interestingly, prevalence of dyspnoea and sleep disturbance reduced, perhaps due to disease or symptom-focused treatment received once their diagnosis was confirmed. The significantly higher prevalence of appetite loss and fatigue at follow-up in this group, compared to the other groups, is consistent with other reports (474, 475). Within the inoperable group, ten deteriorated between enrolment and follow-up: three died, two were too unwell to complete the study and five had a significantly worse symptom and QoL summary score. Contrary to the initial hypothesis underlying this study, none of the CRP or body composition measures were significantly associated with this deterioration, nor did analysis suggest that these measures have a role in identifying who might benefit from early specialist palliative care. Average summary score of 76 in the inoperable cohort in this study was remarkably similar to the average score of 74 in Daly *et al.*'s work (391). That study found a significant relationship between this score and low SMD (391). It is worth noting, however, that the present study was a feasibility study and not powered to detect an association. Furthermore, the inclusion of SCLC in the present study (which constituted almost one-third of the inoperable cohort) may have confounded the results as SCLC is typically very chemosensitive (476) and rapid disease response on starting treatment may have led to improvements in symptom scores. Patient number in this study was too small for subgroup analysis based on histology but future studies should consider this. Overall, the high attrition rate and rapid deterioration in EORTC QLQ-C30 parameters in the inoperable group justify the short follow-up interval chosen for this study and underlines the importance of further study in this area.

Some of the findings of this study are mixed and unexpected. A higher (better) summary score at follow-up was strongly correlated with a higher (worse) GPS and CAR in the inoperable group, contrary to what would be expected. Exclusion of those with documented evidence of infection changed the results somewhat but did not explain this unexpected finding. Possible explanations include that infection was present but not documented, that it was a Type I statistical error due to multiple comparisons (477) or the inclusion of SCLC.

The high burden of symptom and functional impairments in both cancer cohorts is consistent with the findings of Bubis *et al.*, who identified a high level of symptoms among people with newly diagnosed cancer, with respiratory cancers associated with among the highest

symptom burdens of all cancer sites (387). Their findings, together with those of this study, support the concept of supportive care for those with earlier stage disease (478). Interestingly, Bubis *et al.*, also found that urban residence, lower income and higher comorbidity burden were associated with increased risk of worse symptom scores (387), which is of relevance to this cohort recruited at an inner-city hospital which serves several areas of socio-economic deprivation. As noted in the introduction to this thesis, lower socio-economic position is also associated with a higher CRP. The interrelationships between these factors are not yet well understood but they have potentially significant public health and social implications (47).

5.4.3 Limitations and strengths of study

For this study, a sample size of 30 people with inoperable cancer was felt to be sufficient to examine feasibility issues and to allow some exploratory comparison between groups. There are no specific recommendations about what sample size is adequate for a feasibility study (413). The figure of 30 is often considered a “rule of thumb” for comparing groups (479). Although that “rule of thumb” may be an oversimplification (479), it is interesting that the median sample size of published palliative care studies registered on clinicaltrials.gov, which might be expected to include higher quality research from research groups with the wherewithal to register their study, was just 60 (401), not much more than the 51 people with cancer eventually recruited to the present study.

Patient acceptability and burden of study processes were not formally assessed in this study. While these are recommended components of a feasibility study (413), the study procedures were very brief in this study, taking approximately 10-15 mins on each occasion. Anecdotally, several patients commented to the researcher that the study had been “very easy” and “easier than I thought”. Given that some patients were called for their bronchoscopy before completion of the EORTC QLQ-C30, addition of a feasibility questionnaire may have resulted in further missing data.

Number of co-morbidities per patient may have been underestimated, as only co-morbidities documented in the medical notes were recorded for the study. Time constraints meant a full history and questioning of each participant about co-morbidities was not possible.

A further limitation was the lack of consensus around which CRP-based score should be used in lung cancer. The GPS, mGPS, HS-mGPS and CAR have all been reported as prognostic in lung cancer (145, 194, 480, 481), but it remains to be established which inflammation-based score is most accurate and whether the optimal score differs between disease stages and histology (194). Fan *et al.*, showed that the GPS was a better predictor of survival than the mGPS in NSCLC (482) but results of other studies are mixed. The mGPS was shown to be an independent predictor of survival in inoperable NSCLC by Leung *et al.*, (481) but Pinato *et al.*, found the mGPS did not predict survival in operable NSCLC (483). In contrast, Miyazaki *et al.*, in their study of older adults with operable NSCLC, found the mGPS was an independent predictor of survival (484). One study showed that the

HS-mGPS (where the cutpoint for CRP is lower than the standard mGPS, at 3mg/L rather than 10mg/L) was a better predictor of survival in resectable NSCLC than the GPS or the mGPS (194). It is worth noting that the latter study was conducted in Japan (194). Other East Asian studies of inflammatory markers in cancer have also used lower CRP thresholds, consistent with the lower baseline CRP typically seen in that region (187). A systematic review concluded that elevated GPS was associated with shorter overall survival in lung cancer (143) and it was this which informed the choice to use GPS for the present study. However, the majority of included studies were conducted in East Asia and it is unclear how generalisable findings based on lower CRP cutpoints and from populations with lower CRP levels are to the Irish population assessed in the present study. The CAR has also been found to predict survival in lung cancer but the included studies used widely diverging cutpoints, ranging from 0.028 – 0.424 (145), thus it remains unknown which should be used. The evidence for which CRP score to use is even less for small cell lung cancer. The systematic reviews by Jin *et al.*, and Deng *et al.*, did not distinguish between NSCLC and SCLC in their meta-analysis (143, 145). Another review, which did look at SCLC, found that almost all of the published work focused on scores based haematological markers, such as the NLR (146).

Body composition analysis was only undertaken at one timepoint. The underlying hypothesis at the outset of the study was that information available at point of diagnosis, and not serial measures, would predict patient outcomes. Moreover, it was anticipated that not all patients would have serial CT or PET imaging. It has been proposed, however, that assessment of muscle changes over time may be more valuable than single measures (398). Higher levels of skeletal muscle loss over time are associated with reduced survival in people receiving palliative chemotherapy (485-487), even after adjustment for radiological disease response (487). CT analysis was also undertaken at one level only, as in most published studies (428). A recent study found that assessment at multiple vertebral levels more accurately predicted outcomes in cancer than single-level measurements could, but this was beyond the available resources for the current study (428). Automation of muscle segmentation may make such approaches more feasible (428, 463). Intra-observer variability was not assessed, due to resource constraints. Other published studies have also relied on CT analysis by a single researcher (487, 488). Furthermore, a study which assessed intra-observer variability in CT analysis using the same software as the present study found such variability was very low, supporting single investigator assessments (489).

The study recruited people referred for bronchoscopy. As a result, people with a clinical diagnosis of lung cancer who were too unwell for bronchoscopy were excluded; this is reflected in the performance status of study participants, with no participants having ECOG 4. Furthermore, individuals who had a lung lesion that was amenable to biopsy by another route (particularly CT-guided biopsy of a peripheral lesion) may have been missed. Either of these factors could have introduced bias.

Weight change was not collected, as this was felt to be beyond the scope of the study. Information on weight loss in the preceding 3-6 months could have been combined to diagnose cancer cachexia, a syndrome of skeletal muscle loss and / or weight loss which is associated with symptoms and, often, systemic inflammation (490). Although assessment of cachexia was not the goal of the present study, the high prevalence of appetite loss and skeletal muscle loss in the inoperable group suggests that, even at point of diagnosis, cachexia may be present in many of this cohort. Estimates of the prevalence of cancer cachexia vary; rates from 36% to 64% in older adults with lung cancer have been reported (491, 492).

Since the study was completed, a voice script for telephone administration of the EORTC QLQ-C30 has been validated (493). As this did not exist at the time of the study, it is possible that variability in the script used by the researcher may have influenced the results, although the researcher endeavoured to maintain a consistent approach between patients. Furthermore, all assessments were conducted by one researcher, reducing potential variability. Hussain *et al.*, recently found that missing data in RCTs was associated with increasing number of research staff (441). The basis for this relationship was unclear (441) but, in the current study, the consistent presence of and processes employed by the single researcher were noted to contribute to successful recruitment and study completion. An example of this was a participant who advised the researcher when she telephoned to arrange follow-up questionnaire that he was about to hang up until he recognised her voice.

A challenge to any study in the field of skeletal muscle analysis is the lack of consensus in this area. Multiple different definitions of myopenia / sarcopenia have been published (494). Reported prevalence of low muscle mass in cancer varies, with comparisons difficult because of the diverse cutpoints used to define low muscle mass (397, 495). Many studies use their own cutpoints (495). As noted elsewhere in this thesis, this approach severely limits

comparisons between studies. The same is true of muscle quality, there is currently a lack of agreed thresholds for SMD (451). The cutpoints used in the current study are among the most widely used in recent years and take into account gender and BMI (394, 397). Even the terminology in this field is contentious (495). While the term sarcopenia is often used in oncology research, most studies do not consider physical function (494, 495), even though the most accepted definition of sarcopenia covers three domains: muscle strength, quantity and quality of muscle and physical performance (496). Some authors have proposed that the term myopenia be used when referring only to reduced skeletal muscle mass or volume (453) and it is this term which is used here since no assessment of muscle function was conducted.

Total psoas cross-sectional area and total abdominal muscle area at the L3 level are the most widely-reported measures for the assessment of muscle mass in cancer (495). As discussed above, there is some controversy about which of these to use. Heterogeneity in measures used and cutpoints chosen is a significant barrier to progress in the field (397). Given the concerns about the value of PMI (463), it is interesting that, in the present study, it was the only body composition metric which was correlated with symptoms and with a high CRP.

This study did not include a specific tool to measure psychological symptoms, including anxiety and depression. These are highly prevalent in cancer (474). Depression is associated with poor quality of life and with systemic inflammation. Remarkably, a recent study found that depression was associated with a higher tumour mutational burden and, independently, with a high CRP (497), highlighting the interaction between the tumour and the host response in the patient's symptom experience. Further studies in this area are needed to understand these relationships and should include tools to assess psychological symptoms.

Finally, there is no clear level of symptom or functional impairment that defines a need for SPC, nor any one agreed assessment tool (498). In this study, a significant worsening in EORTC QLQ-C30 summary score, being too unwell or death by the time of follow-up were chosen as reasonable indicators of a person's potential to benefit from SPC, however this could be debated. There is an urgent need for research into predictors of SPC need, as it is recognised that survival time is not an adequate indicator of this need (499, 500). While this work did not show a role for CRP or body composition measures, the results of this exploratory analysis, within a feasibility study, should not be seen as conclusive and this is an area that future studies could examine in more detail.

There is a major drive to increase patient-centred research (401). This study used the patients' own report of QoL, which is the most meaningful way to assess QoL (501). Patient-reported outcomes (PROs) and patient-reported outcome measures (PROMs) are increasingly used in clinical practice and in clinical trials(502). A PRO is defined as “any report of the status of a patient's health condition that comes directly from the patient without interpretation of the patient's response by a clinician or anyone else” (503). PROs are frequently the outcomes which are most important to patients and families (504). This study assessed both of the main types of outcome measure: functional status and symptom-based (504). Although numerous scales to assess QoL exist, the EORTC QLQ-30 chosen for this study is the scale most frequently reported in the literature (502). Since the absolute scores generated by the EORTC QLQ-C30 can be difficult to interpret (501), this study used the summary score and the recently-derived thresholds for clinical importance, as recommended by the EORTC group (423, 425). The summary score is at least as good as individual scales in assessment of change over time and avoids the risk of Type I statistical error when making comparisons using multiple individual scales (423). Keaver *et al.*, recently highlighted the value of this approach, as compared to the Global Health Status Score, which has been used in the past (501).

The final recruitment structure provided a comparator group of people who did not have cancer. As discussed above, body composition parameters should be interpreted with reference to appropriately-derived reference values, including regional considerations, as well as BMI and gender. Unfortunately, lack of L3 level imaging in the non-cancer group precluded comparison of skeletal muscle parameters. The study design still provided normative data for CRP and longitudinal patterns in symptom and QoL. The non-cancer group were very closely matched to the cancer cohorts, with no significant differences in age, gender, smoking status, number of co-morbidities, performance status or BMI as well as attending the same hospital as the cancer group and referred through the same clinical pathways. Thus, the differences identified are likely attributable to the presence of cancer and the impact of a cancer diagnosis and treatment.

This was a prospective study, unlike the vast majority of work examining the prognostic value of skeletal muscle measures in cancer(494). Prospective cohort studies are recommended to improve quality in prognostic factor research and inception cohort studies like this one (where participants are enrolled at the time of onset of a condition and then

followed over time to assess the outcome, as in the present study) are particularly recommended(505). Given the lack of consensus about assessing skeletal muscle in cancer, this study examined a number of different metrics, including the novel metric SMG, to better understand the relationship with outcomes, as has been advised (453).

The study used the imaging modality and vertebral level currently most widely-recommended for assessment of skeletal muscle (428) and utilised well-validated tools to assess symptoms, QoL and performance status. The most significant potential confounder of CRP, current infection or inflammation, was noted and accounted for in the analysis, as much as was possible with the study design. As noted in Chapters 2 and 3 of this thesis, studies of prognostic factors frequently do not consider confounding variables, despite being strongly recommended (171).

The iterative process was key to the conduct of this study. Interim review of study progress, allowed for changes to be made in time to inform the next iteration, rather than at the study's end. The notes made by the researcher in the course of each interim review, effectively field notes, together with discussions with the clinical team, informed the changes in study design, which finally led to successful recruitment. It was these field notes and the researcher's observations on the recruitment process that were of value to another researcher planning a study in the same location, consistent with the assertion by Antunes *et al.*, that the identification of challenges specific to that location is key to devise successful solutions(498). The development and maintenance of a good working relationship with the clinical teams was pivotal to the study's success, as has been noted elsewhere(439). Hagen *et al.*, advocate for the use of a kinaesthetic learning model in feasibility studies(415). They explain this as an iterative process where learning from one cycle of the study feeds into the next cycle and the study changes based on the "emerging experience"(415). As in the present study, they note that amendments to the study protocol, including changing inclusion criteria and location of recruitment, may be required(415). The idea of an iterative process during research is already accepted, for example in the development of complex interventions in healthcare(506).

5.5 Conclusions

This study assessed feasibility of a prospective observational study of CRP and skeletal muscle change as potential predictors of symptom burden and quality of life in advanced cancer, using well-validated assessment tools. The study was conducted as an iterative process, with three iterations of the study before eventual successful recruitment. While recruitment to the study was steady in the third iteration, several feasibility issues were identified, specifically missing data, study attrition and a heterogeneous study cohort. A definitive study, with this design, would not be feasible as a result.

The final iteration of the study recruited three groups – people with inoperable lung cancer, operable lung cancer and a non-cancer control group. The three groups were closely matched on all parameters measured at enrolment. Skeletal muscle mass was similar to that reported in other studies but skeletal muscle density was remarkably low. At follow-up, the non-cancer group continued to report symptom and functional impairments but these were significantly worse in the inoperable cancer group, even at a short interval from enrolment. The operable group also reported significant impairments. Given the similarity of the three groups at enrolment, the differences identified are likely attributable to the presence of cancer and the impact of a cancer diagnosis and treatment. Of the CT-assessed body composition measures in this study, none showed clear superiority over the others, although some interesting associations were noted. Particularly interesting were the findings that low skeletal muscle density was associated with post-operative complications and that a high CRP was associated with study non-completion, even after accounting for infection as a potential confounder. Also interesting was the finding that PMI, a somewhat controversial metric, was the only body composition metric which was correlated with symptoms and with a high CRP. Future studies should investigate these intriguing possibilities further.

Ultimately, this study demonstrates the value of a feasibility study – to highlight which issues can be addressed and which remain, as well as giving an indication of likely recruitment rates in an eventual full-scale trial. This helps to ensure scarce resources and patient time are used efficiently(415). The experience of this study supports the assertion that feasibility should be formally assessed for most studies conducted in palliative care.

6 Concluding summary

6.1.1 Concluding discussion

Prognostication, the prediction of future outcomes, is an important part of cancer care. Accurate prediction of future symptom burden, quality of life and likely survival is key for patients, families and clinicians in planning treatment but also for broader life decisions (162). It plays a crucial role in planning clinical services and in the conduct of clinical trials (163). However, prognostication in cancer remains imprecise (168). Further research into prognostic factors in cancer is needed but must be of high-quality in order to advance knowledge and ultimately translate into improved patient-care (169).

Inflammation has been associated with cancer development and progression (150). An elevated CRP, an acute phase protein and circulating marker of inflammation, has been associated with adverse outcomes in cancer, including shorter survival, higher symptom burden and worse quality of life (10, 212). It is cheap and widely available but is not routinely used for prognostication or treatment planning in cancer (16). Concerns remain about the non-specific nature of CRP and use of CRP without consideration of other clinical factors (180). Novel means of using CRP as a prognostic factor, which have the potential to address these issues, have been proposed but remain under-investigated.

In this thesis, a diverse suite of research methodologies, from bench to bedside, was used to assess four promising and clinically relevant potential applications of CRP as a predictor of important outcomes in cancer, including quality of life, symptoms and survival, with particular reference to oesophageal adenocarcinoma and lung cancer. Both are prevalent cancers and are associated with poor survival, high symptom burden and with inflammation (125, 130, 140, 141, 153).

The summary below outlines the key findings from this thesis, highlights the novel findings and the overall contribution to current understanding of CRP as a prognostic factor. Strengths and limitations of the work are noted and future research directions are identified. A mind map is shown at the end of this concluding summary, which illustrates key findings of the thesis.

Monitoring change in CRP over time (CRP kinetics) could address concerns about transient elevations in CRP, as occur with infection, leading to inaccurate prognostication (180). A

persistently elevated CRP has been associated with shorter prognosis in selected cancer sites (227, 228, 255). Chapter 2 investigated the potential role for CRP kinetics in predicting survival in an unselected mixed cancer cohort, as seen in clinical practice, and in OAC, through retrospective review of two independent datasets. No prior studies had assessed the role of CRP kinetics in either of these groups. Both baseline CRP and longitudinal change in CRP were independent predictors of survival in the mixed cancer cohort, although significant potential for bias was noted and significance was lost on subgroup analyses. Neither CRP nor longitudinal change in CRP was associated with survival in the OAC cohort. The findings do not support the clinical application of a CRP kinetics classification system for prediction of survival in unselected cancer cohorts nor in OAC. It is possible that CRP kinetics may still have a role in other specific cancer sites and treatments. The findings also highlight the need for clinicians to be cautious of novel prognostic markers until they are studied in a population representative of their own patients, assessed for potential confounders and validated in multiple independent studies (171).

There is uncertainty about which level of CRP should be considered high (the cutpoint or threshold) in the context of predicting outcomes in cancer (28). Cutpoints reported as prognostic vary dramatically between published studies in OAC. Similarly, while there is growing consensus that prognostic scores incorporating CRP have a role in cancer (187, 188), numerous different scores are reported, with little clarity for the clinician about which to use (189). The systematic review and meta-analysis described in Chapter 3, the first to be conducted in OAC, synthesised current evidence for pre-treatment CRP cutpoints and CRP-based scores as predictors of survival in OAC. There was inadequate evidence to draw conclusions on the role of CRP alone as a predictor of survival in OAC, with three different cutpoints, ranging from 2.8mg/L to 10mg/L, used in the three studies which reported this outcome. The strongest evidence was for GPS/mGPS. Meta-analysis found that a high pre-operative GPS/mGPS was independently predictive of worse OS in adults with OAC, including OGJ, treated with multimodal treatment or surgery alone. Findings were similar in subgroup analyses of only studies which included multimodal treatment, studies in patients with no metastatic disease (M0) and studies in patients with complete surgical resection (R0 resection). The evidence base is too weak at present, due to the small number and potential bias in the existing literature, to recommend immediate clinical use of CRP or CRP-based prognostic scores for clinical use in OAC but the GPS/mGPS do show significant promise in this regard.

Although best known as a circulating protein, CRP has also been identified in tumour tissue – termed “tumoural CRP”. Tumoural CRP could be a more specific biomarker of cancer-related inflammation than serum CRP and provide an ideal biomarker of survival and clinical outcome. Its presence in tumours has been associated with shorter survival (208, 210, 211). There is only one published report of tumoural CRP in OAC, a conference abstract from 2006 by Räsänen *et al.*, (349). The relationships between tumoural CRP, serum CRP and survival have never been reported. In Chapter 4 of this thesis, tumoural CRP in OAC was examined in detail: its presence, localisation, two possible sources of tumoural CRP and association with survival were examined using appropriate laboratory techniques (Western Blot, IHC and ELISA). Its presence was examined in two separate groups, pre-treatment biopsies and resected OAC tissue, each with matched clinical data. The study confirmed that CRP is present within some but not all OAC tumours. CRP was shown to be present in resected OAC tumour tissue and, for the first time, in pre-treatment biopsies. When present, CRP was usually located in stromal cells, sometimes together with carcinoma cells, but was not seen in carcinoma cells alone. In order to assess the potential source of tumoural CRP, an oesophageal cancer cell line was cultured under normal conditions and conditions replicating the TME. Cells were also treated with chemotherapy or radiotherapy. Regardless of culture conditions or treatments applied, CRP was also not secreted from the cells. Furthermore, tumoural CRP was not correlated with serum CRP. These findings, taken together, support a local origin, likely stromal, for tumoural CRP, a new and important finding for understanding the TME in OAC. This work also showed, for the first time, that a high CRP in tumoural nuclei or cytoplasm on pre-treatment biopsy, but not in resected OAC tissue, was associated with shorter survival.

Systemic inflammation, as indicated by a raised CRP, is associated with high symptom burden and poor quality of life in cancer (212). Systemic inflammation is also associated with loss of skeletal muscle mass and density (213). Chapter 5 reports a feasibility study which examined relationships between CRP, skeletal muscle and patient-reported outcomes in cancer (quality of life and symptoms) and the potential to use CRP and skeletal muscle measures to predict these outcomes. Identification of those patients likely to experience a high symptom burden and poor quality of life could allow early intervention and possibly even prevention or mitigation of these outcomes (212). No prior study had investigated this possibility. The study was conducted as an iterative process, with successful recruitment on

the third iteration of the study, which was expanded to include operable cancer and a non-cancer control group. Missing data, study attrition and a heterogeneous study cohort were identified as significant feasibility issues, which would impede an eventual definitive study with this study design. However, the study has informed a different study recruiting at the same site and has been widely disseminated, as a feasibility study should be (415). As secondary aims, the study investigated relationships between CRP and CRP-based scores, body composition (both BMI and CT-assessed measures of skeletal muscle), symptoms and QoL at enrolment and at follow-up in the three diagnostic groups. The three groups were closely matched on all parameters measured at enrolment. At follow-up, all three groups reported ongoing symptom and functional impairments but these were significantly worse in the cancer cohorts. The high attrition and rapid deterioration in symptom / QoL parameters in the inoperable group underlines the importance of further study in this area. The study also highlighted potential unmet supportive care needs in the operable group.

No single CT-assessed skeletal muscle metric proved to be of more prognostic value than the others, though skeletal muscle radiodensity (SMD) was shown for the first time to be associated post-operative complications. In another novel finding, a somewhat controversial metric, psoas muscle index (PMI), was the only one to correlate both with CRP and a symptom outcome. Two further exploratory analyses were conducted: whether CRP or body composition measures were associated with study completion and whether they might be associated with need for specialist palliative care. The study showed for the first time that a high CRP was associated with study non-completion, even after accounting for infection as a potential confounder, which has implications for the design of future studies.

6.1.2 Strengths and limitations

A strength of the work described in this thesis is its strong clinical focus. The applications of CRP studied were all selected as having potential for clinical implementation. CRP is inexpensive and already widely used in clinical practice, meaning clinicians are familiar with it and much of the infrastructure for its use is already in place. The data used in Chapter 2 were gathered as part of routine clinical practice (CC dataset) and an existing biobank (SJH dataset). The aim of the systematic review and meta-analysis in Chapter 3 was to provide clarity for clinicians, as well as other researchers, in selecting a CRP cutpoint or CRP-based score to use in OAC. It would be relatively straightforward to include CRP-staining, as outlined in Chapter 4, as part of the routine histological analysis of tumour specimens in hospital laboratories. The final study in this thesis was designed with clinical realities in mind, to maximise the value of data already being collected. The potential for automated CT-segmentation and analysis could make routine assessment of body composition a practical option(463). Furthermore, the decision to conduct a feasibility study was grounded in an understanding of the challenges of clinical research in cancer; the need for this was borne out by the experience of the study. Finally, the outcomes chosen for study (symptoms, QoL and survival) are those of most importance to patients (504).

An associated limitation to using existing clinical data for aspects of the studies described in Chapters 2, 4 and 5 was missing data and possible selection bias. In addition, sample size was limited by the number of patients with available data, leading to potential Type II statistical error, where a true effect or significant association is missed(359).

Statistical correction for multiple comparisons was not conducted as part of data analysis. Multiple testing increases the risk of Type I statistical error, where results are found to have statistical significance due to chance; this is also known as multiplicity (477). The Bonferroni correction has been used widely to correct for this (477). However, both correction for multiple testing in general and the Bonferroni correction has been criticised for increasing the risk of Type II statistical error, with the risk of missing potentially interesting findings. As the studies forming this thesis had a limited number of outcomes and analysis was based on pre-specified hypotheses rather than “data dredging” (246, 477), statistical correction for multiple tests was not used.

An important strength of this thesis was the use of research methodologies and approaches recommended for prognostic factor research. The study of CRP kinetics was not confined to a single dataset; findings from the first part of the study were assessed in a separate dataset which addressed many of the limitations of the first. External validation of prognostic factor findings is highly advised yet is rarely reported (171, 261). The impact of important confounders was considered with multivariable analysis or subgroup analysis (171). There is an “urgent need” for more systematic reviews of prognostic factors, like that conducted for this thesis (170). Unlike much prognostic factor research, the clinical study used a prospective design, as is recommended (171).

6.1.3 Future directions

This thesis has identified a number of areas for future work.

Prognostic factors have been described as the “building blocks” for prognostic models (171). In this thesis, CRP has been shown to be a useful prognostic marker, alone and in combination with albumin as part of the GPS/mGPS. An important future direction will be to combine CRP with other markers, which could be tumoural (well-established factors such as TNM stage or newer factors like mutational status or tumoural CRP) and / or host-related (haematological markers like NLR or CT-assessed body composition).

A key theme throughout this thesis has been the diversity of cutpoint / threshold values in use for CRP and for body composition metrics. For research in this field to translate to clinical practice, future studies should focus on a small number of scores and thresholds. Evidence in this thesis indicates that GPS/mGPS and serum CRP thresholds of 5 mg/L and 10mg/L show the most promise in OAC.

Immunotherapy continues to change the face of cancer care. The studies reported in this thesis were conducted before immune-checkpoint inhibitors (ICI) were mainstream. It is as yet unclear which biomarkers are most useful in prediction of response to ICI, though some including PD-L1, tumour mutational burden and microsatellite instability have been identified (249). CRP has shown potential as a predictor of outcomes with ICI (185). There is early work suggesting CRP kinetics has a role in predicting response to immune-checkpoint inhibitors(233, 239). Further studies should explore this further, particularly to clarify the optimum timing of CRP measurements and understand which kinetic changes are most clinically significant. Loss of skeletal muscle mass has also been associated with worse outcomes with ICI (507). Understanding how these different tumoural and patient factors impact on ICI treatment and interact with each other is essential to ensure the best care for people with cancer in the future.

The work in this thesis has shown the importance of considering confounders, especially infection and chronic inflammation, when assessing CRP as a prognostic marker. Future studies must report these factors and analyse appropriately, either through exclusion or subgroup analysis.

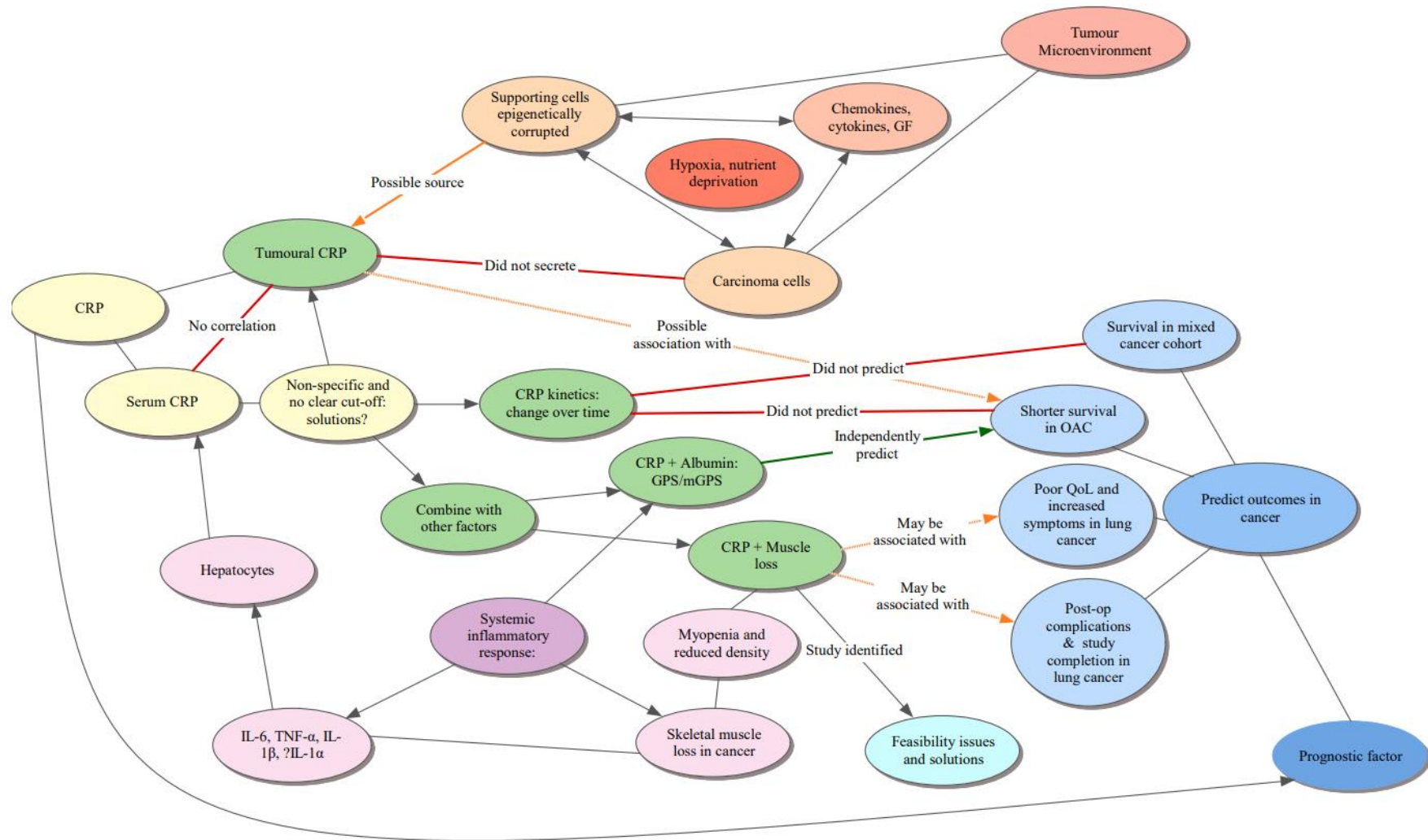
The full spectrum of CRP's biological function is not yet understood. There is growing understanding of the functional differences between the monomeric (mCRP) and dissociated pentameric forms (pCRP*) and circulating CRP, which is the form measured in all standard assays(22). Both mCRP and pCRP* appear to have a strongly pro-inflammatory role(1). Once a commercial antibody for these forms of CRP becomes available, it would be extremely interesting to repeat the study of tumoural CRP, and particularly to assess the relationship with survival identified in the present study, using that antibody. Such a study would provide key comparative data into the relative roles of pentameric pCRP and mCRP and further insights into the structure and function of the protein. In turn, this could inform new therapeutic approaches to modify inflammation within the TME.

The final chapter of this thesis highlighted the significant symptom burden and functional impairments in people with operable and inoperable cancer. Particularly interesting were the findings that low skeletal muscle density was associated with post-operative complications and that a high CRP was associated with study non-completion, even after accounting for infection as a potential confounder. Future multi-centre clinical studies should investigate these intriguing possibilities further. The study also highlighted the value of a feasibility study and underscores the need for future studies in advanced disease to include a feasibility component.

6.1.4 Conclusions

The work outlined in this thesis has advanced current understanding of CRP as a prognostic factor in cancer. It has confirmed that CRP can be applied in novel ways and in combination with other factors to predict important clinical outcomes in cancer. The thesis demonstrates the importance of robust prognostic factor research, with close attention to potential confounders and validation of findings in independent cohorts. It has highlighted the research areas which should be examined in future work, to translate this promising prognostic factor into improved patient care.

6.1.5 Summary of thesis findings: Mind map



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Appendices

Appendix 1: Search strategy

EMBASE

1. 'esophagus tumor'/exp
2. 'Esophageal adenocarcinoma'/exp
3. 'Esophagus carcinoma'/exp
4. 'Esophagus cancer'/exp
5. (esophag* NEAR/3 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma*)):ti,ab
6. (oesophag* NEAR/3 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma*)):ti,ab
7. #1 OR #2 OR #3 OR #4 OR #5 OR #6
8. 'gastroesophageal cancer'/exp
9. (('gastro oesophageal' OR gastroesophageal OR 'gastro esophageal' OR gastroesophageal) NEAR/3 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)):ti,ab
10. ((Esophagogastric OR oesophagogastric OR 'oesophago gastric' OR 'esophago gastric') NEAR/3 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)):ti,ab
11. #8 OR #9 OR #10
12. #7 OR #11
13. 'c reactive protein'/exp
14. ('c-reactive protein' OR CRP OR 'creactive protein'):ti,ab
15. 'glasgow prognostic score'/exp OR 'modified glasgow prognostic score'/exp
16. ('glasgow prognostic score' OR GPS OR 'modified glasgow prognostic score' OR mGPS):ti,ab
17. (('C- reactive protein' OR CRP) NEAR/2 (albumin OR ALB)):ti,ab
18. ('CRP/albumin ratio' OR 'CRP/ALB'):ti,ab
19. #13 OR #14 OR #15 OR #16 OR #17 OR #18
20. #12 AND #19
21. 'conference abstract':it OR 'conference review':it OR 'editorial':it OR 'letter':it
22. #20 NOT #21

Medline

1. Esophageal Neoplasms/
2. (esophag* adj3 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma)):ti,ab.
3. (oesophag* adj3 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma)):ti,ab.
4. or/1-3

5. ((gastro oesophageal OR gastrooesophageal OR gastro esophageal OR gastroesophageal) adj3 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)).ti,ab.
6. ((Esophagogastric OR oesophagogastric OR oesophago gastric OR esophago gastric) adj3 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)).ti,ab.
7. or/5-6
8. or/4,7
9. C-Reactive Protein/
10. (c-reactive protein OR CRP OR creactive protein).ti,ab.
11. (glasgow prognostic score OR GPS OR modified glasgow prognostic score OR mGPS).ti,ab.
12. ((C- reactive protein OR CRP OR creactive protein) adj2 (albumin OR ALB)).ti,ab.
13. (CRP albumin ratio OR CRP ALB).ti,ab.
14. or/9-13
15. 8 AND 14

CINAHL

1. (MH "Esophageal Neoplasms")
2. TI (esophag* N3 (cancer OR neoplasm*OR adenocarcinoma* OR tumo?r* OR carcinoma*)) OR AB (esophag* N3 (cancer OR neoplasm*OR adenocarcinoma* OR tumo?r* OR carcinoma*))
3. TI (oesophag* N3 (cancer OR neoplasm*OR adenocarcinoma* OR tumo?r* OR carcinoma*)) OR AB (oesophag* N3 (cancer OR neoplasm*OR adenocarcinoma* OR tumo?r* OR carcinoma*))
4. S1 OR S2 OR S3
5. TI (((“gastro oesophageal” OR gastrooesophageal OR “gastro esophageal” OR gastroesophageal) N2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)) OR AB (((“gastro oesophageal” OR gastrooesophageal OR “gastro esophageal” OR gastroesophageal) N2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*))
6. TI ((Esophagogastric OR oesophagogastric OR “oesophago gastric” OR “esophago gastric”) N2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)) OR AB ((Esophagogastric OR oesophagogastric OR “oesophago gastric” OR “esophago gastric”) N2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*))
7. S5 OR S6
8. S4 OR S7
9. MH "C-Reactive Protein"
10. TI (“C-Reactive Protein” OR “C Reactive Protein” OR “creactive protein”) OR AB (“C-Reactive Protein” OR “C Reactive Protein” OR “creactive protein”)

11. TI ("glasgow prognostic score" OR GPS OR "modified glasgow prognostic score" OR mGPS) OR AB ("glasgow prognostic score" OR GPS OR "modified glasgow prognostic score" OR mGPS)
12. TI (('C- reactive protein' OR CRP OR "creactive protein") N2 (albumin OR ALB)) OR AB (('C- reactive protein' OR CRP OR "creactive protein") N2 (albumin OR ALB))
13. TI ("CRP albumin ratio" OR "CRP ALB") OR AB (('C- reactive protein' OR CRP OR "creactive protein") N2 (albumin OR ALB))
14. S9 OR S10 OR S11 OR S12 OR S13
15. S8 AND S14

Web of Science

TS =(((esophag* NEAR/2 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma*)) OR (oesophag* NEAR/2 (cancer* OR neoplasm* OR tumor? OR tumour? OR adenocarcinoma* OR carcinoma*)) OR (((“gastro oesophageal” OR gastrooesophageal OR “gastro esophageal” OR gastroesophageal) NEAR/2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)) OR ((Esophagogastric OR oesophagogastric OR “oesophago gastric” OR “esophago gastric”) NEAR/2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma*)))) AND (('c-reactive protein" OR CRP OR "creactive protein") OR ("glasgow prognostic score" OR GPS OR "modified glasgow prognostic score" OR mGPS) OR (('C- reactive protein" OR CRP) NEAR/2 (albumin OR ALB)) OR ("CRP albumin ratio" OR "CRP ALB")))

Cochrane Library

1. [mh "Esophageal Neoplasms"]
2. (esophag* NEAR/3 (cancer OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma* OR carcinoma*)):ti,ab,kw
3. (oesophag* NEAR/3 (cancer OR neoplasm* OR tumor* OR tumour OR adenocarcinoma* OR carcinoma*)):ti,ab,kw
4. #1 or #2 or #3
5. (((“gastro oesophageal” OR gastrooesophageal OR “gastro esophageal” OR gastroesophageal) NEAR/2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma* OR carcinoma*)):ab,ti,kw
6. ((Esophagogastric OR oesophagogastric OR “oesophago gastric” OR “esophago gastric”) NEAR/2 (cancer* OR neoplasm* OR tumor* OR tumour* OR adenocarcinoma* OR carcinoma*)):ab,ti,kw
7. #5 OR #6
8. #4 OR #7
9. [mh "C-Reactive Protein"]

10. (“c-reactive protein” OR “c reactive protein” OR CRP OR “creactive protein”):ti,ab,kw
11. (“glasgow prognostic score” OR GPS OR “modified glasgow prognostic score” OR mGPS):ti,ab,kw
12. (“C-reactive protein” OR CRP OR “creactive protein”) NEAR/2 (albumin OR ALB):ti,ab,kw
13. (“CRP albumin ratio” OR “CRP ALB”):ti,ab,kw
14. #9 OR #10 OR #11 OR #12 OR #13
15. #8 AND #14

Appendix 2: QUIPS Tool (Hayden *et al.*, 2013)

Author and year of publication				
Study identifier				
Reviewer				
Biases	Issues to consider for judging overall rating of "Risk of bias"	Study Methods & Comments	Rating of reporting	Rating of "Risk of bias"
Instructions to assess the risk of each potential bias	These issues will guide your thinking and judgment about the overall risk of bias within each of the 6 domains. Some issues may not be relevant to the specific study or the review research question. These issues are taken together to inform the overall judgment of potential bias for each of the 6 domains.	Provide comments or text excerpts in the white boxes below, as necessary, to facilitate the consensus process that will follow.	Click on each of the blue cells and choose from the drop-down menu to rate the adequacy of reporting as yes, partial, no or unsure.	Click on the green cells; choose from the drop-down menu to rate potential risk of bias for each of the 6 domains as High, Moderate, or Low considering all relevant issues.
1. Study Participation	Goal: To judge the risk of selection bias (likelihood that relationship between PF and outcome is different for participants and eligible non-participants).			
Source of target population	The source population or population of interest is adequately described for key characteristics (LIST) .			
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care).			
Recruitment period	Period of recruitment is adequately described.			
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described.			
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or 'zero time' description).			
Adequate study participation	There is adequate participation in the study by eligible individuals.			
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics (LIST) .			
Summary study participation	The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.			
2. Study Attrition	Goal: To judge the risk of attrition bias (likelihood that relationship between PF and outcome are different for completing and non-completing participants).			
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.			
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.			
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.			
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics (LIST) . There are no important differences between key characteristics (LIST) and outcomes in participants who completed the study and those who did not.			
Study Attrition Summary	Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.			
3. Prognostic Factor Measurement	Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).			
Definition of the PF	A clear definition or description of PF is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).			
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall). Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.			
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.			
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.			
Method used for missing data	Appropriate methods of imputation are used for missing PF data.			
PF Measurement Summary	PF is adequately measured in study participants to sufficiently limit potential bias.			
4. Outcome Measurement	Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).			
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.			
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).			
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.			
Outcome Measurement Summary	Outcome of interest is adequately measured in study participants to sufficiently limit potential bias.			
5. Study Confounding	Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).			
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model (LIST)), are measured.			
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposure).			
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).			
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.			
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.			
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups). Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).			
Study Confounding Summary	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between PF and outcome.			
6. Statistical Analysis and Reporting	Goal: To judge the risk of bias related to the statistical analysis and presentation of results.			
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.			
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model. The selected statistical model is adequate for the design of the study.			
Reporting of results	There is no selective reporting of results.			
Statistical Analysis and Presentation Summary	The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.			

Appendix 3: Participant Information Leaflet and Consent Form

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: CRP and Skeletal Muscle Change – Association with Symptoms and Quality of Life

NAME OF LEAD INVESTIGATOR: Dr Cliona Lorton

You are invited to participate in a pilot research study. Please read this document and ask any questions you may have before agreeing to participate.

WHAT IS THE PURPOSE OF THIS STUDY?

The aims of this study are to

- Help understand how inflammation in the body is linked with symptoms and quality of life
- Provide information about any connection between inflammation in the body and changes in muscle

This is a pilot study to see if this type of study can work. It could then lead on to other bigger studies in the future to decide exactly how and when it should be used.

WHY HAVE I BEEN CHOSEN?

You have been invited to take part in this study because you are having a bronchoscopy at St James's Hospital. It is hoped that your participation in the study will help researchers to understand if there is any link between inflammation, muscle change, symptoms and quality of life and whether we can make more use of information we already collect.

WHAT WILL HAPPEN IF I VOLUNTEER?

Taking part is entirely voluntary. If you initially decide to take part, you can change your mind later. This won't affect any future treatment in any way. Your doctor may also decide to withdraw you from this study if he/she feels it is not in your best interest.

If you agree to take part, you will first be asked to complete some very short questions to make sure the study is suitable for you.

The study itself happens in 2 parts.

DAY 1 (Maximum 15 minutes)

The researcher will complete a questionnaire with you about your symptoms and quality of life.

They will review your medical notes to confirm details like what medications you are taking.

They will look at your recent CT scan to calculate how much muscle you have, and whether there is fat inside this muscle. This is called myosteatosis.

CRP is a very commonly used blood test which can show inflammation in the body. The researcher will look up your results to see what your levels of CRP were on your recent blood tests.

DAY 2 (in 6-12 weeks, maximum 15 mins)

The researcher will complete the same questionnaire with you about your symptoms and quality of life. This can be done in person or over the phone, at a time that suits you – whichever you prefer.

They will look again at your medical records to see what, if any, treatment you have had and how those treatments went.

WILL I NEED TO HAVE ANY EXTRA TESTS, SCANS OR HOSPITAL APPOINTMENTS FOR THIS STUDY?

No, you will not need to have any extra blood tests, scans or hospital appointments for this study.

ARE THERE ANY BENEFITS FROM MY PARTICIPATION?

You will not benefit directly from taking part in this study. However, your participation in this research will help to improve understanding of how muscle, inflammation, symptoms and quality of life may be related. This may help to improve how we care for people in hospital in the future.

ARE THERE ANY RISKS INVOLVED IN PARTICIPATING?

There is a small risk that study participation may reveal a muscle abnormality that you were not previously aware of. In this event, we will inform both you and your doctor within 24 hours. A copy of the study records will be provided to your doctor who will advise you if further investigation or treatment is required.

WHAT HAPPENS IF I DO NOT AGREE TO PARTICIPATE?

Participation is entirely voluntary. You have the right to decline or withdraw participation at any time. This would not affect your current or future treatment.

CONFIDENTIALITY

A study number will identify you and ensure confidentiality is maintained at all times. Your identity will not be disclosed to anyone outside of the research team. In the final report of the study no identifiable details will be published or disclosed.

All the information collected in this study will be kept securely in a password protected file until 5 years after the study ends and then will be destroyed.

COMPENSATION

You will not be paid for taking part in this study.

WHO IS ORGANISING AND FUNDING THIS RESEARCH?

This study is organised by Trinity College Dublin, in collaboration with researchers from St James's Hospital.

HAS THIS STUDY BEEN REVIEWED BY AN ETHICS COMMITTEE?

St. James's Hospital / Tallaght Hospital Joint Research Ethics Committee has reviewed and approved this study.

CONTACT DETAILS

Dr Cliona Lorton

Tel:

Email:

Thank you for taking the time to consider participating in the study.

PARTICIPANT CONSENT FORM

PLEASE CIRCLE YES OR NO

I have read and understood the Participant Information Leaflet YES NO

I had the opportunity to ask questions and discuss the study YES NO

I have received satisfactory answers to all my questions YES NO

I have received enough information about this study YES NO

I understand that the researcher will access my medical records and will treat all my information confidentially YES NO

I understand that I am free to withdraw from the study at any time without giving a reason and without this affecting my future medical care YES NO

I agree to take part in the study YES NO

Participant's Signature: _____ Date: _____

Participant's Name in print: _____

Investigator's Signature: _____ Date: _____

Investigator's Name in print: _____

Appendix 4: Data Collection Sheet

DATE OF RECRUITMENT ____/____/____

DEMOGRAPHICS

Study No: _____

Sex: M F D.O.B: _____

Marital Status (Circle): M S W D Other

Race (Circle): W African Asian Other

If Inpatient, evidence infection? _____

CO-MORBIDITIES (Circle)

IHD CCF Valvular PVD CVA HTN PE/DVT

COPD Asthma IPF DM

Crohn's U.C. Other colitis PUD

RA OA Psoriatic Arthritis

Parkinson's Renal failure DM

Other: _____

SMOKER Y N ____ pack yrs

MEDICATION

NSAID Y N Name + Dose

Corticosteroid Y N

Statin Y N

Vitamin D Y N

Hormones Y N

Immune-mod Y N

Opioid Y N

Height ____ m / feet

Weight ____ kg / stone

BMI ____ kg/m²

ECOG (circle)

0 = Normal activity, no limitation

1 = Able to do light / sedentary work

2 = Self-caring but not able for any work

3 = Spends >50% of day in bed or chair

4 = Totally confined to bed or chair

EORTC QLQ-C30

Completed Y N

If no, why not? _____

QLQ-LC13

Completed Y N

If no, why not? _____

BLOODS	Date		Date
CRP		Bili	
Urea		ALT	
Creat		AST	
Na		ALP	
K		GGT	
TWBC		Albumin	
Neutrophils		Corr Ca	
Lymphocytes			
Hb			
Platelets			

MDT

Date of MDT ____/____/____

Lung cancer Y/N Operable Y/N

Mets (Circle) Brain Bone Liver Lung

LN Other: _____ None

Planned Cancer Therapy (Circle):

Chemo Rad Hormone Immuno Supportive

Surgery (what, when) _____

Any comment re MDT / diagnosis

CT SCAN

Date of Scan ____/____/____

Body part scanned _____

Indication for scan (circle)

Restaging Diagnostic Other

Total skeletal muscle surface area ____ (cm²)

Adipose tissue surface area ____ (cm²)

Visceral fat area ____ (cm²)

Subcutaneous fat area ____ (cm²)

LSMI ____ (cm²/m²)

Mean Muscle Attenuation ____ (HU)

CT Analysis Comment

DATE SECOND ASSESSMENT ____/____/____

Face to face Telephone (circle)

Appendix 5: EORTC QLQ-C30



EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

--	--	--	--	--

Your birthdate (Day, Month, Year):

--	--	--	--	--	--	--	--	--	--

Today's date (Day, Month, Year):

31

--	--	--	--	--	--	--	--	--	--

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a <u>long</u> walk?	1	2	3	4
3. Do you have any trouble taking a <u>short</u> walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your <u>social</u> activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor

Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor

Excellent

Appendix 5: Eastern Cooperative Oncology Group (ECOG) Performance Status (Oken *et al.*, ECOG group, 1982)

0. Fully active, able to carry on all pre-disease performance without restriction.
1. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light sedentary nature e.g. light house work, office work.
2. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4. Completely disabled, cannot carry out any self-care. Totally confined to bed or chair.
5. Dead