ESMO Research Fellowship
(August 2021– July 2023)

Dr Madeleine Strach
FINAL REPORT

Host Institute: **The Christie NHS Foundation Trust, Manchester, UK**
Mentor: **Dr Jorge Barriuso**
Project title: **Characterisation of appendiceal adenocarcinoma by multi-omic analysis and correlation with clinical outcomes: a step towards rational therapeutics**
Home Institute: **Chris O'Brien Lifehouse, Sydney, Australia**

Introduction

Appendiceal adenocarcinomas (AA) are rare malignancies with a propensity to spread with isolated peritoneal metastases.[1, 2] Prognosis can be poor with the most aggressive forms having 5-year overall survival as low as 14% and those with more favourable characteristics improved 5-year OS up to 59%.[3-7] Cytoreductive surgery and heated intraperitoneal chemotherapy (CRS/HIPEC) is the main treatment to achieve attempts at cure and the role of systemic chemotherapy has been unclear. Literature surrounding clinical outcomes has been challenging to interpret due to changing histopathological classification,[8] with the most recent classification established with the WHO 2019 system.[9]

The biological understanding of AAs is limited. Genomic analysis of cancers is widely used to guide therapy with improved patient outcomes in many cancer subtypes including colorectal cancer (*RAS/BRAF*). The genomic landscape of AA has been reported in small mixed cohorts of patients (including those with more indolent appendiceal mucinous neoplasms).[10-12] There is growing interest in describing the spectrum of *KRAS* mutations that occur in this tumour type, given the more recent development of $KRAS^{G12C}$ inhibitors and pan-*KRAS* inhibitors under development.[13-16] The literature is sparse on correlating molecular profiles with treatment and survival outcomes. Additionally, there have been little data to describe the molecular basis of peritoneal metastases or to identify new therapeutic targets.

Beyond the tumour genome, we now know that targeting cells in the tumour microenvironment, such as with immunotherapy, has revolutionised the treatment of many cancers. Single-cell/nuclei RNA sequencing (sc/nRNA-Seq) allows the study of individual cells in cancer tissue, providing the potential to vastly improve our understanding of cell identity, diversity, development and function.[17] It has the power to accurately characterise all cells in the tumour microenvironment and to better understand their individual roles in the malignant process.

Rationale and Aim

The aims of this fellowship project were to:

1. evaluate the role of peri-operative chemotherapy and establish predictors of clinical outcomes in patients with appendiceal adenocarcinoma who undergo cytoreductive surgery
2. optimise single-cell/nuclei RNA sequencing techniques for transcriptomic analysis of the tumour microenvironment
3. evaluate the genomics of appendiceal adenocarcinoma and correlate with clinical outcomes

Experimental Design

Aim 1:
We will perform analysis of a prospective database of patients with histologically confirmed appendiceal adenocarcinoma who completed CRS+/-HIPEC. Patients will be selected from the peritonectomy services at The Christie NHS Foundation Trust (Manchester, UK). Demographic, clinicopathological, treatment and follow-up data will be curated. The role of systemic chemotherapy will be investigated. The primary endpoint will be overall survival (OS) and secondary endpoints include progression-free survival and response to systemic chemotherapy. Kaplan-Meier survival analysis will calculate DFS and OS. Univariate and multivariate cox proportional hazard regression models will identify predictive factors of clinical outcomes. Results with a p-value <0.05 will be considered statistically different.

Aim 2:
Experiment 2.1: Optimisation of a single-cell RNA sequencing method for appendiceal adenocarcinoma
Given limited literature for the optimal method in this tumour type, we will evaluate different methods for transcriptomic analysis. Fresh tissue will be obtained from patients at the time of CRS prior to HIPEC administration. Tissue will be dissociated into single cells via methods including micro-dissection and manipulation, flow cytometric cell sorting, microfluidic platforms and droplet-based methods. Cells will be cleaned and lysed to preserve cellular mRNA. Antibody barcoding with microenvironment protein targets could be used to better define cellular subpopulations. Machine learning techniques, such as, T-distributed Stochastic Neighbour Embedding (t-SNE) will be used to define the different cell subpopulations.

Experiment 2.2: Transcriptomic analysis by using single-cell RNA sequencing techniques
We will perform analysis of the tumour microenvironment of appendiceal adenocarcinoma to elucidate potential biomarkers of disease prognosis and treatment response. We will perform characterisation of cell populations in the peritoneal metastatic microenvironment. This will include analysis of intratumoural clonal heterogeneity, infiltrating immune cell populations and identification of peritumoral stromal cells such as cancer associated fibroblasts. Evaluation of genetic signatures and candidate molecules will lead to identification of potential biomarkers. Biological outcomes will be correlated with the clinical outcomes established in Aim 1.

Aim 3:
We will perform whole exome sequencing and bulk RNA sequencing of primary AA with matched peritoneal metastasis. DNA and RNA will be extracted from archival tissue samples obtained from an institutional biobank program. Following quality control and library preparation, next generation sequencing will be performed and analysis using existing pipelines for genomic processing and variant calling. Differential gene expression analysis and gene set enrichment analysis will be performed for RNA analysis using existing analysis packages. Results will be correlated with clinical outcomes.

Results, Conclusions and Future Perspectives

Aim 1:
We analysed 216 patients with AA who had CRS and 97 patients who had systemic chemotherapy. Our key findings were a survival benefit of systemic chemotherapy in patients with positive lymph nodes compared to no chemotherapy and exceptional survival in patients with low grade peritoneal metastases or peritoneal acellular mucin. Full description of methods and results can be found in the published manuscript, attached to this report as **Appendix 1** (https://doi.org/10.1016/j.esmoop.2023.101619).

Additionally, we analysed a cohort of 177 patients with goblet cell adenocarcinoma (GCA) which on univariate analysis found improved survival in patients with positive lymph nodes who had perioperative

chemotherapy compared to no chemotherapy and changes in stratification of patients using the current 3-tier WHO grading system compared to the previous Tang grading. More details regarding these findings are described in our abstract from ASCO GI 2023 (https://doi.org/10.1200/JCO.2023.41.4_suppl.50).

Finally, we performed a combined analysis of the tumour molecular characteristics and outcomes for both the AA and GCA cohorts. Our main findings were that *KRAS*^{G12D} mutations were most common in our cohort and that anti-EGFR therapy was associated with worse outcomes in *KRAS*^{WT} mostly GCAs. More details regarding these findings are described in our abstract from ESMO Sarcoma and Rare Cancer Congress 2023 (https://doi.org/10.1016/j.esmoop.2023.101032).

Future avenues of research stemming from these projects includes concept development regarding prospective trials for systemic chemotherapy, improving systemic chemotherapy response criteria and broader molecular classification and correlation with clinical outcomes.

Aim 2:
We evaluated two different methods for extracting single nuclei from AA snap-frozen tissue samples. The first was a mechanical method which results in a significant amount of cellular debris and background RNA which we decided was not the optimal method to take forward. The second method was based on enzymatic tumour lysis and gentle manual dissociation of cells which resulted in successful nuclei extraction. However, we found that overall nuclei yield was highly dependent on the starting material for this tumour type such that for more mucinous tumours more starting material was necessary to achieve adequate nuclei counts for downstream applications. The tissue optimisation process and development of this protocol took longer than planned such that the snRNAseq analysis was not completed during the fellowship.

Aim 3:
Analysis of this part of the project remains underway and we are looking forward to presenting and disseminating the results at future conferences and peer-reviewed manuscripts.

List of Publications and Presentations Resulting from the Translational Research Project "Characterisation of appendiceal adenocarcinoma by multi-omic analysis and correlation with clinical outcomes: a step towards rational therapeutics"

Publications

1. **Strach, M. C.,** Chakrabarty, B., Nagaraju, R. T., Mullamitha, S., Braun, M., O'Dwyer, S. T., Aziz, O. and Barriuso, J. (2023). Defining a role for systemic chemotherapy in local and advanced appendix adenocarcinoma. *ESMO Open*, *8*(5), p.1-13. DOI: 10.1016/j.esmoop.2023.101619
2. **Strach, M. C.,** Mahon, K. and Barriuso, J. (2023). Genomic Subtypes of Appendiceal Adenocarcinoma: Enough to Guide Clinical Decision Making? *Journal of Clinical Oncology*, *41*(19), 3559–3559. pp. DOI: 10.1200/jco.22.02895

Presentations

1. **Strach, M. C.,** Chakrabarty, B., Nagaraju, R., Mullamitha, S., Braun, Clouston, H., Fish, R., Renehan, A., Selvasekar, C., Sutton, P., Wild, J., Wilson, M., O'Dwyer, ST., Aziz, O.,* and Barriuso, J. (2023). Outcomes for patients with appendix adenocarcinoma and the role of systemic chemotherapy. [Encore] Poster presentation. The Association of Coloproctology of Great Britain & Ireland Annual Meeting 2023. *Manchester.* 3-5 Jul 2023. *presenting author
2. **Strach, M. C.,** Barriuso, J., Chakrabarty, B., Nagaraju, R., Clouston, H., Fish, R., Renehan, A., Selvasekar, C., Sutton, P., Wild, J., Wilson, M., Aziz, O.,* and O'Dwyer, S. (2023). Clinical outcomes of appendix goblet cell adenocarcinoma and role of systemic chemotherapy. [Encore] Poster presentation. The Association of Coloproctology of Great Britain & Ireland Annual Meeting

2023. *Manchester.* 3-5 Jul 2023. *presenting author

3. **Strach, M. C.,** Chakrabarty, B., Nagaraju, R., Mullamitha, S., Braun, M., O'Dwyer, S., Aziz, O. and Barriuso, J. (2023). 8O Outcomes for patients with appendix adenocarcinoma and the role of systemic chemotherapy. Oral presentation at ESMO Sarcoma and Rare Cancer Congress 2023, Lugano, March 2023. *ESMO Open*, 8(1), 101030. DOI: 10.1016/j.esmoop.2023.101030
4. **Strach, M. C.** Chakrabarty, B., Nagaraju, R., Burghel, G. J., Schlecht, H., Mullamitha, S., Braun, M., O'Dwyer, S., Aziz, O.and Barriuso, J. (2023). 10P Molecular mutations in appendix cancers. Poster at ESMO Sarcoma and Rare Cancer Congress 2023, Lugano, March 2023. *ESMO Open*, 8(1), 101032. DOI: 10.1016/j.esmoop.2023.101032
5. **Strach, M. C.,** Chakrabarty, B., Nagaraju, R., Aziz, O., O'Dwyer, S., & Barriuso, J. (2023). Abstract 50: Clinical outcomes of appendix goblet cell adenocarcinoma and role of systemic chemotherapy. Poster at ASCO GI, San Francisco, January 2023. *Journal of Clinical Oncology*, 41(4_suppl), 50–50. DOI: 10.1200/jco.2023.41.4_suppl.50
6. **Strach, M.C.,** Chakrabarty, B., Nagaraju, R. T., Mullamitha, S., Braun, M., O'Dwyer, S. T., Barriuso, J. and Aziz, O. (2022). Appendiceal adenocarcinoma treated at a national peritoneal tumour centre. Prize for best poster. The Association of Coloproctology of Great Britain & Ireland Annual Meeting 2022. *Edinburgh.* 4-6 Jul 2022.
7. **Strach, M.C.,** Chakrabarty, B., Nagaraju, R. T., Mullamitha, S., Braun, M., O'Dwyer, S. T., Barriuso, J. and Aziz, O. (2022). Appendix Adenocarcinoma: Long-term outcomes for this rare and aggressive tumour. Oral Presentation for Manchester Medical Society. *Manchester 15th Mar 2022.*

List of Publications and Presentations Resulting from other projects during the fellowship period (if applicable)

Publications

1. Coulson, K., Day, N., **Strach, M. C.,** & Sutton, P. A. (2023). Emergency surgery for patients with cancer receiving systemic anticancer therapy. *British Journal of Surgery*. *110*(6), 631–634. pp. DOI: 10.1093/bjs/znad007
2. **Strach M.C.,** Sutherland, S., Horvath, L. G. and Mahon K. (2022) The role of chemotherapy in the treatment of advanced appendiceal cancers: summary of the literature and future directions. *Therapeutic Advances in Medical Oncology*. 14;1-36. DOI: 10.1177/17588359221112478
3. Irawati, N., Moghadam, A., Abdul-Razak, M., **Strach, M.,** Elliott, M., Ch'ng, S., Shannon, K., Palme, C. E., Clark, J., Wykes, J. and Low, T. (Hubert). (2022). Outcomes after definitive treatment for head and neck angiosarcoma. *ANZ Journal of Surgery*, *92*(6), 1407–1414. pp. DOI: 10.1111/ans.17695
4. **Strach, M. C.,** Grimison, P. S., Hong, A., Boyle, R., Stalley, P., Karim, R., Connolly, E. A., Bae, S., Desai, J., Crowe, P., Singhal, N. and Bhadri, V. A. (2022). Mesenchymal chondrosarcoma: An Australian multi-centre cohort study. *Cancer Medicine*, *12*(1). 368–378. pp. DOI: 10.1002/cam4.4849
5. Connolly, E. A., Bhadri, V. A., Wake, J., Ingley, K. M., Lewin, J., Bae, S., Wong, D. D., Long, A. P., Pryor, D., Thompson, S. R., **Strach, M. C.,** Grimison, P. S., Mahar, A., Bonar, F., Maclean, F. and Hong, A. (2022). Systemic treatments and outcomes in CIC-rearranged Sarcoma: A national multi-centre clinicopathological series and literature review. *Cancer Medicine*, *11*(8), 1805–1816. pp. DOI: 10.1002/cam4.4580

Presentations

1. Zhang, B., Wilson-Smith, A., Ussher, N., Connolly, E. A., **Strach, M. C.** and Bhadri, V. A. (2023). 51P Outcomes of adult patients undergoing extrapleural pneumonectomy for sarcoma at a specialised centre. Poster at ESMO Sarcoma and Rare Cancer Congress 2023, Lugano, March 2023. *ESMO Open*, *8*(1), 101088. p. DOI: 10.1016/j.esmoop.2023.101088

2. Connolly, E. A., Thomson, K., King, D., Schilling, K., Ryan, J., Grimison, P., Zhou, D., Zhang, B., **Strach, M. C.,** Baker, A., Sibbald, T. and Bhadri, V. A. (2023). 115TiP MYTH Study: Methotrexate for AYA in the home: A study of safety, feasibility, patient acceptability and cost effectiveness of an ambulatory model for AYA osteosarcoma patients. Poster at ESMO Sarcoma and Rare Cancer Congress 2023, Lugano, March 2023. *ESMO Open*, *8*(1), 101152. p. DOI: 10.1016/j.esmoop.2023.101152
3. **Strach, M. C.,** Yeung, N., Lin, H.-M., Ansari, N., Koh, C., Shin, J.-S., Kench, J., Horvath, L. and Mahon, K. L. (2023). Characteristics of immune-infiltrating cells in the tumor microenvironment of appendiceal cancer with peritoneal disease. Poster at ASCO GI, San Francisco, January 2023. *Journal of Clinical Oncology*, *41*(4_suppl), 217–217. pp. DOI: 10.1200/jco.2023.41.4_suppl.217
4. **Strach, M.C.,** Chan, W.Y., Zhang, B.Z., Connolly, E.A., Bhadri, V. and Grimison, P. (2022). Characteristics of patients with bone and soft tissue sarcomas referred to a dedicated early phase clinical trial clinic. *CTOS Annual Meeting,* Vancouver, November 2022.
5. **Strach, M.C.,** Zhang, B.Z. (joint), Connolly, E.A., Boyle, R. Stalley, P. Karim, R., Hong, A.M., Grimison, P.S. and Bhadri, V. (2022) Abstract ID 2206836: To treat or not to treat: an audit of initial management of patients with desmoid tumours at a specialised centre. *CTOS Annual Meeting*, Vancouver, November 2022.
6. **Strach, M. C.,** Yeung, N., Apostolov, E., Lin, H.-M., Nagaraju, R. T., Ansari, N., Koh, C., Shin, J.-S., Kench, J., Aziz, O., Swarbrick, A., Horvath, L. G., Barriuso, J. and Mahon, K. (2022). 1713P Single-cell transcriptomic analysis of appendiceal cancer peritoneal disease. Poster at ESMO Congress 2022, Paris, France September 2022. *Annals of Oncology*, *33*, S1322. p. DOI: 10.1016/j.annonc.2022.07.1791
7. **Strach, M. C.,** Ansari, N., Koh, C., Solomon, M., Horvath, L. and Mahon, K. (2022). Outcomes of appendiceal cancer treated at a state peritonectomy service. Poster at ASCO 2022, Chicago, USA, June 2022. *Journal of Clinical Oncology*, *40*(16_suppl), 3629–3629. pp. DOI: 10.1200/jco.2022.40.16_suppl.3629
8. **Strach, M. C.,** Yeung, N., Lin, H.-M., Ansari, N., Koh, C., Shin, J.-S., Kench, J., Centenera, M., Butler, L., Horvath, L. and Mahon, K. (2022). Patient-derived explant model of appendiceal cancer. Poster at ASCO 2022, Chicago, USA, June 2022. *Journal Of Clinical Oncology, Suppl 16 Abstr 4160*(40). DOI: 10.1200/jco.2022.40.16_suppl.4160
9. Subbiah, V., Bhadri, V., Bui, N., Batty, K., **Strach, M.,** Zakharian, M., Smith, S., Yee, N. A., Srinivasan, S., Saville, M. W., Oneto, J. M. M. and Guminski, A. (2021). 547P Early pharmacokinetic data from a phase I study of SQ3370 in patients with advanced solid tumors provides proof-of-concept for the click chemistry-based CAPAC platform. Poster at ESMO Congress 2021, Virtual Meeting, September 2021. *Annals of Oncology*, *32*, S609. p. DOI: 10.1016/j.annonc.2021.08.1069

Selection of Courses and Workshops Attended During the Fellowship

1. Ongoing enrolment in PhD, The University of Sydney, Australia
2. ESMO Adolescent and Young Adult Malignancies Preceptorship, Athens, Greece, October 2022
3. Informatics Training Scheme 2021-2022, Translation Manchester, The University of Manchester, UK

Acknowledgements

I would like to thank my mentor, Dr Jorge Barriuso, who not only provided intellectual input into my fellowship projects with essential assistance in all aspects of design, analysis, interpretation and presentation of results but embedded me within the lower GI medical oncology unit at The Christie, taught me important clinical aspects of managing patients with appendiceal malignancies and demonstrated to me the art of balancing

busy clinical loads with a thriving academic career. Additionally, Dr Barriuso confirmed for me the importance of maintaining collegiate networks within rare cancer and clinician scientist communities.

I would also like to thank Mr Omer Aziz for facilitating my appointment with The Christie Peritoneal Oncology Centre (CPOC), integration with the surgical service, support in database and biobank project setup, facilitating presentation opportunities, providing a very structured supervisory environment and of course assistance with the revision process for abstracts, posters, presentations and manuscript preparation.

I also extend special thanks to our labs postdoctoral fellow Dr Rags Nagaraju who was always willing to act as a sounding board for project ideas and help with experimental design and assistance with lab experiments.

I would like to thank staff at The Christie and The University of Manchester/Manchester Cancer Research Centre for welcoming me so warmly, particularly our project manager Ms Mamoona Ahmed who helped with all the important paperwork, and to the patients and their families for supporting our research.

I would like to thank the Royal Australasian College of Physicians (RACP) who supported me with a travel grant.

Finally, I would like to thank ESMO for supporting me in this endeavour and providing an empowering forum for clinical, academic and professional development with the focus on improving care and opportunities for our patients.

Personal Statement

The ESMO fellowship has provided me with a life- and career-defining opportunity. It has been an unparalleled experience being able to learn from the expertise of a place like The Christie and bring back this knowledge to my home institute. I've had immersion in translational research and excellent mentorship for life as a clinician scientist. Understanding the research environment in different countries provides me with additional insights which is only gained on fellowship such as these. This fellowship has been formative in encouraging me to continue to pursue an ongoing career as a clinician scientist.

While I complete the final stages of my PhD candidature, I am delighted to have received ongoing support for continuing my research in rare malignancies as I take up the role of the Australia and New Zealand Sarcoma Association (ANZSA) Sarcoma Clinical Research Fellow and continue my clinical career back home in Sydney. I am grateful for the lifelong personal, professional and collaborative relationships that I have fostered during this ESMO fellowship and would definitely recommend interested young oncologists apply for this. If anyone would like more information on my experience, I am happy to be contacted (Twitter/X: @MaddyStrach).

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SIGNATURES	
Award Recipient full name	***Signature and Date***
Madeleine Strach	18th September 2023

Research Mentor full name	***Signature and Date***
Jorge Barriuso	18th September 2023

Appendix 1:

Strach, M. C., Chakrabarty, B., Nagaraju, R. T., Mullamitha, S., Braun, M., O'Dwyer, S. T., Aziz, O. and Barriuso, J. (2023). Defining a role for systemic chemotherapy in local and advanced appendix adenocarcinoma. *ESMO Open*, *8*(5), 101619. p. https://doi.org/10.1016/j.esmoop.2023.101619

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

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ORIGINAL RESEARCH

Defining a role for systemic chemotherapy in local and advanced appendix adenocarcinoma☆

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Available online xxx

Background: Appendix adenocarcinomas (AAs) are rare tumours that often present late, with a propensity for peritoneal metastases (PMs). This study aimed to evaluate outcomes of AA patients undergoing cytoreductive surgery (CRS) with curative intent and determine the role of systemic chemotherapy.

Materials and methods: Data were collected from a prospective database and classified according to World Health Organization (WHO) 2019 classification. Tumour clearance from CRS was described using a completeness of cytoreduction (CC) score ranging from 0 [no residual disease (RD)] to 3 (>2.5 cm RD). Patients with CC0-2 CRS received hyperthermic intraperitoneal chemotherapy (HIPEC). Systemic chemotherapy was categorised as 'prior' (>6 months before), 'neoadjuvant' (<6 months before), 'adjuvant' (<6 months after CC0-1 CRS) or 'palliative' (after CC2-3 CRS). Analyses used Kaplan–Meier and Cox regression methods.

Results: Between January 2005 and August 2021, 216 AA patients were identified for inclusion. Median age was 59 years (21-81 years). CRS/HIPEC was carried out in 182 (84%) patients, of whom 164/182 (76%) had mitomycin C HIPEC. CC0-1 was achieved in 172 (80%) patients. Systemic chemotherapy was given to 97 (45%) patients from the whole cohort and to 37/46 (80%) patients with positive nodes. Median overall survival (OS) was 122 months (95% confidence interval 61-182 months). After multivariate analysis, patients with acellular and lower-grade PM had similar OS to those with localised (M0) disease ($P = 0.59$ and $P = 0.19$). For patients with positive nodes, systemic chemotherapy was associated with reduced risk of death compared to no chemotherapy ($P < 0.0019$).

Conclusion: This study identifies AA patients with positive lymph nodes derive the most benefit from systemic chemotherapy. We confirm the prognostic importance of stage and peritoneal grade, with excellent outcomes in patients with acellular mucin and lower-grade PM.

Key words: appendix adenocarcinoma, appendix cancer, peritoneal metastases, chemotherapy, treatment outcomes

INTRODUCTION

Appendix adenocarcinomas (AAs) are rare, heterogeneous and aggressive tumours often presenting with peritoneal metastases (PMs).[1-4] The incidence is estimated to be 1-2 per million per year.[1,5] Prognosis from small retrospective and heterogeneous cohorts is poor, with a 5-year survival ranging from 14% to 59%.[6-11] Due to changing nomenclature over the years, these cohorts have been mixed including indolent appendiceal mucinous neoplasms (AMNs).[3,12] The current World Health Organization (WHO) 2019 classification provides stricter guidance for classification of primary AAs as mucinous adenocarcinomas (MACs), adenocarcinomas not otherwise specified (ANOS) and signet ring adenocarcinomas (SRCs); goblet cell adenocarcinomas (GCAs) are classified separately.[13] This also

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†Shared senior authorship.

☆Note: This study was previously originally presented as an oral presentation at the ESMO Sarcoma and Rare Cancers Congress 2023 (ID: 8O). Abstract reference: Strach MC, Chakrabarty B, Nagaraju R, Mullamitha S, Braun M, O'Dwyer ST, et al. Outcomes for patients with appendix adenocarcinoma and the role of systemic chemotherapy. *ESMO Open*. 2023;8(1 suppl 3):101030. https://doi.org/10.1016/j.esmoop.2023.101030.

incorporates the American Joint Committee on Cancer (AJCC) tiered grading.[14] PMs are recommended to be graded separately to that of primary tumours due to potential for discordant grading.[3,15] The WHO 2019 terminology for PM has equivalence to the Peritoneal Surface Oncology Group International (PSOGI) 2016 consensus.[15] We describe both these systems in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Cytoreductive surgery and heated intraperitoneal chemotherapy (CRS/HIPEC) is a potentially curative treatment for AA, either for patients with existing PMs or for those at high risk of developing PMs. During CRS, the surgeon aims to remove all macroscopic disease, after which, HIPEC at 42°C is instilled intraperitoneally to eradicate occult tumour cells.[16,17] The peritoneal cancer index (PCI) is an operative scoring system to quantify the volume and distribution of PM (range 0-39) and the completeness of cytoreduction (CC) score records the volume of disease at the end of surgery (range 0-3).[18,19] A lower PCI and CC score has been shown to be prognostic with longer survival outcomes in AA.[6,20-22] The addition of HIPEC cannot be conclusively said to improve outcomes over CRS alone in AA, particularly in the setting of complete cytoreduction, and supportive data are based on heterogeneous pathology cohorts.[6,23,24] Systemic chemotherapy can be considered, and in the palliative setting regimens are extrapolated from colorectal cancer treatments, but the role is yet to be defined in the perioperative setting.[25-28]

This study aimed to analyse patients with AAs who underwent curative intent CRS/HIPEC, and to evaluate the role of systemic chemotherapy. The primary endpoint of this study was overall survival (OS) and secondary endpoints progression-free survival (PFS), radiological and pathological response rates to previous systemic chemotherapy. This study was approved by the institutional clinical audit committee (reference 3091).

MATERIALS AND METHODS

Population

Between January 2005 and August 2021, patients with histologically confirmed AAs were identified from a prospectively collected database at a peritoneal tumour centre in the UK. All who were intended for curative CRS/HIPEC were included. Patients were discussed in a specialised peritoneal tumour multidisciplinary team (MDT) and all pathology slides reported at external institutions were imported and re-reported by specialist pathologists.

Operative technique

A standardised operative technique was used and has been described in detail previously.[22] The PCI was calculated by the surgeons at the time of CRS and scored 0-39.[18] The CC score was calculated after maximal resection and defined as: CC0, no residual disease (RD); CC1, <0.25 cm RD; CC2, 0.25-2.5 cm RD; and CC3, >2.5 cm RD.[19]

Chemotherapy

Treatments with intraperitoneal and intravenous chemotherapies were given following standard institutional protocols (Supplementary Appendix S1, available at https://doi.org/10.1016/j.esmoop.2023.101619). Chemotherapy was grouped as: 'prior' chemotherapy >6 months before CRS, 'neoadjuvant' chemotherapy as <6 months before CRS, 'adjuvant' chemotherapy defined as <6 months after CC0-1 CRS and 'palliative' chemotherapy as that given after CC2-3 CRS or for those with recurrent unresectable disease.

Data collection and pathological classification

Data including demographics, clinicopathological variables, treatment characteristics and survival status were extracted from the database. All patients had pathology reports reviewed (MCS, BC); missing data were referenced from the hospital record. Diagnoses were strictly documented based on the WHO 2019 classification defined in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.101619, with the exception of retaining the classification of peritoneal acellular mucin (AM) from the PSOGI classification.[13,15] Classification according to the prior PSOGI 2016 consensus was recorded which formed the basis for grade reclassification of the ANOS subgroup based on tumour differentiation: 'well' as grade 1, 'moderate' as grade 2 and 'poor' as grade 3.[15]

Follow-up

Patients were routinely followed up from the date of CRS/HIPEC clinically, biochemically with tumour markers [including carcinoembryonic antigen (CEA)] and with computed tomography (CT) of chest/abdomen/pelvis every 6 months for 2 years until 5 years and then at years 8 and 10. Patients who received systemic chemotherapy had more frequent follow-up while receiving treatments.

Outcome measures

The primary outcome of OS was defined as the time from diagnosis to death of any cause, censored for the time of last known follow-up. The secondary outcome of PFS was defined as the time from diagnosis to first of disease recurrence, progression or death, censored for the time of last known follow-up. Response rates and all suspected recurrence and progression were confirmed radiologically using RECIST1.1 criteria,[29] discussed and documented at MDT and the date of first event used even if observed in retrospect. Pathological response was defined using the tumour regression grade (TRG): grade 0, complete response with no remaining viable tumour cells; grade 1, moderate response with only small cluster or single cells remaining; grade 2, minimal response with residual cancer remaining, but with predominant fibrosis; grade 3, poor response with minimal or no tumour death with extensive residual cancer.[30]

Statistical analysis

Descriptive epidemiological methods were used to describe the cohort. Tumour marker analysis used the clinical threshold of CEA ≤5 as normal. Survival analysis was carried out using the Kaplan–Meier method and the log-rank test was used to assess statistical significance. Univariate and multivariate analysis of prognostic variables was carried out using Cox regression. Statistical analysis was carried out using IBM SPSS (IBM SPSS Statistics, version 28.0, Armonk, NY) and R 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) (23 June 2022).

RESULTS

Baseline characteristics

We identified 1077 patients with appendix tumours referred to our UK centre between 2005 and 2021. Of these, 704 AMNs, 146 GCAs and 11 non-appendix tumours were excluded, resulting in 216 AA patients: 141 MAC, 71 ANOS and 4 SRC (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.101619). Baseline demographics and clinical characteristics are summarised in Table 1.

At initial diagnosis, 82 (38%) patients had localised disease (M0) on CT imaging and preoperative pathology, 134 (62%) with metastatic disease (M1) and 132 (61%) with PM (M1b). Subsequently, 12 (15%) patients were found to have PM (M1b) confirmed pathologically at CRS and 1 patient had confirmed extraperitoneal metastasis (M1c). In this confirmed M0 group, 48/69 (70%) patients had CRS within 6 months of their initial diagnostic procedure. This was 92/147 (63%) for the M1 group.

Ninety-seven patients had systemic chemotherapy; 28 had neoadjuvant or adjuvant chemotherapy (N/ACT). Reasons for systemic chemotherapy recommendations are presented in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Survival outcomes related to clinicopathological factors

At a median follow-up of 56 months (range 1-286 months), the median OS was 122 months [95% confidence interval (CI) 62-182 months] with 5-year and 10-year OS of 63% and 51%, respectively (Figure 1). The median PFS was 41 months (95% CI 28-54 months) with 5-year and 10-year PFS of 43% and 40%, respectively (Figure 1). Beyond 5 years, we found two progression events. Results of univariate and multivariate analysis of prognostic factors for OS are presented in Table 2 and for PFS in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Univariate analysis. Variables associated with prognosis on univariate analysis included CEA, PCI, CC score, nodal stage, metastasis stage, peritoneal grade and the presence of signet ring cells (srcs) (Supplementary Figures S2 and S3, available at https://doi.org/10.1016/j.esmoop.2023.101619). Patients with lower-grade PM or AM demonstrated similar OS and PFS compared to patients with no PM [median OS not reached (NR), $P = 0.4$ and median PFS NR versus 194 months, $P = 0.68$]; Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2023.101619. The prognostic impact of positive compared to negative lymph nodes on OS and PFS was maintained even in the presence of confirmed metastatic (M1) disease (median OS 29 versus 70 months, $P = 0.0011$ and median PFS 18 versus 30 months, $P < 0.0001$); Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Similar OS and PFS outcomes were seen following analysis of AA subtype (Figure 1), presence of mucin (including ANOS with 1%-50% mucin; Supplementary Figure S6, available at https://doi.org/10.1016/j.esmoop.2023.101619) and primary tumour grade (Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2023.101619). The latter remained so when the localised disease (M0) cohort was analysed separately for OS and PFS ($P = 0.68$ and $P = 0.88$; Supplementary Figure S7, available at https://doi.org/10.1016/j.esmoop.2023.101619).

Multivariate analysis. After multivariate analysis for impact on OS (Table 2), variables that retained significance for poorer prognosis included SRC subtype ($P < 0.001$), CC2/3 cytoreduction ($P < 0.001$), positive lymph nodes ($P < 0.001$) and stage M1b/c ($P = 0.004$). Multivariate analysis revealed that female sex predicted increased risk of death compared to male sex ($P < 0.001$), and confirmed that age, CC1 cytoreduction and stage M1a (AM) were not associated with worse OS outcomes. After multivariate analysis for impact on PFS (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.101619), variables that retained significance for increased risk of recurrence or progression included CC2/3 cytoreduction ($P = 0.002$), elevated CEA ($P = 0.032$), positive lymph nodes ($P = 0.004$) and stage M1b/c ($P < 0.001$). While PCI score was prognostic in univariate analysis, even the highest PCI score group did not remain prognostic for OS or PFS after multivariate analysis including cytoreductive status in the model.

Outcomes for patients treated with systemic chemotherapy

Whole cohort. Unselected patients who received chemotherapy had worse OS and PFS compared to those not who did not ($P < 0.0001$); Table 2, Figure 2 and Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Lymph node-positive patients. On multivariate analysis, N/ACT, prior and palliative chemotherapy settings were associated with reduced risk of death compared to no chemotherapy ($P = 0.005$, $P = 0.011$ and $P < 0.001$, respectively); Table 2 and Figure 2. On multivariate analysis for PFS, palliative chemotherapy was associated with a lower risk of recurrence ($P = 0.01$). There was no statistical difference for prior ($P = 0.055$) and N/ACT ($P = 0.08$); Figure 2 and Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Patients with peritoneal signet ring cells. The presence of peritoneal srcs did not impact outcomes of prior ($P = 0.22$)

Table 1. Baseline demographics and clinical characteristics of patients with appendix adenocarcinoma

Variable	Whole cohort *n* (%)	Localised (M0) *n* (%)	Metastatic (M1) *n* (%)
	216 (100)	**69 (100)**	**147 (100)**
Age (median, range)	59 (21-80)	60 (29-79)	58 (21-80)
Sex			
Male	90 (42)	24 (48)	57 (39)
Female	126 (58)	36 (52)	90 (61)
Subtype			
MAC	141 (65)	36 (52)	105 (71)
ANOS	71 (33)	32 (46)	39 (27)
SRC	4 (2)	1 (1)	3 (2)
CEA (mean, range)	22 (<3-990)	7 (<3-446)	25 (<3-990)
<6	139 (65)	58 (84)	81 (55)
≥6	55 (26)	5 (7)	50 (34)
Missing	20 (9)	6 (9)	16 (11)
PCI (median, range)	8 (0-39)	2 (0-27)	14 (0-39)
Missing	7 (3)	0	7 (5)
CRS[a]	214 (99)	66 (96)	146 (99)
HIPEC	182 (84) [100]	66 (96) [100]	116 (79) [100]
Mitomycin C	164 (76) [90]	61 (88) [92]	103 (70) [89]
Oxaliplatin	16 (7) [9]	5 (7) [8]	11 (5) [9]
Missing	2 (1) [1]	0	2 (1) [2]
CC score			
0	133 (62)	68 (99)	65 (44)
1	39 (18)	1 (1)	38 (26)
2-3	44 (20)	0	44 (30)
Stage at CRS			
N0/X	168 (79)	57 (83)	113 (77)
N1/2	46 (21)	12 (17)	34 (23)
M0[b]	69 (32)	69 (100)	0
M1a[c]	25 (12)	0	25 (17)
M1b/c[d]	122 (56)	0	122 (83)
Primary tumour grade[e]			
MAC	141 (65) [100]	36 (52) [100]	105 (71) [100]
Grade 2	108 (50) [77]	27 (39) [75]	81 (55) [77]
Grade 3[f]	30 (14) [21]	9 (13) [25]	21 (14) [20]
Missing	3 (1) [2]	0	3 (2) [3]
ANOS	71 (38) [100]	32 (46) [100]	39 (27) [100]
Low grade	53 (25) [75]	26 (38) [81]	27 (18) [69]
High grade	18 (8) [25]	6 (9) [19]	12 (8) [31]
Primary tumour differentiation[g]			
Well	68 (32)	21 (30)	49 (33)
Moderate	131 (61)	43 (62)	87 (59)
Poor	11 (5)	5 (7)	7 (5)
Missing	4 (2)	0	4 (3)
Peritoneal tumour grade[h]	116 (54)	0	116 (79)
Mucinous carcinoma peritonei	83 (38)		83 (56)
Grade 1	7 (3)		7 (5)
Grade 2	13 (6)		13 (9)
Grade 3	23 (11)		23 (16)
Non-mucinous metastases	33 (15)		33 (22)
Low grade	3 (1)		3 (2)
High grade	30 (14)		30 (20)
Mucinous[i]			
Yes	182 (84)	47 (68)	135 (92)
No	34 (16)	22 (32)	12 (8)
Signet ring cells[j]			
Yes	46 (22)	10 (15)	37 (25)
No	167 (78)	59 (86)	109 (74)
Missing	1 (0.5)	0	1 (1)
Radiotherapy	10 (4)	1 (1)	9 (6)
Systemic chemotherapy	97 (45)	20 (29)	77 (52)
None	119 (55)	49 (71)	70 (48)
Neoadjuvant or adjuvant[k]	28 (13)	11 (16)	17 (12)
Neoadjuvant	12 (6)	2 (3)	10 (7)
Adjuvant	16 (7)	9 (13)	7 (5)
Both	0	0	0
Prior (>6 months before CRS)	18 (8)	4 (6)	14 (10)
Palliative	51 (24)	5 (7)	46 (31)

Continued

Table 1. Continued

Variable	Whole cohort *n* (%)	Localised (M0) *n* (%)	Metastatic (M1) *n* (%)
	216 (100)	69 (100)	147 (100)
First-line chemotherapy agents			
Oxaliplatin + fluoropyrimidine[l]	63 (29)	13 (19)	50 (34)
Fluoropyrimidine	13 (7)	6 (9)	7 (5)
Irinotecan + fluoropyrimidine	10 (5)	0	10 (7)
Mitomycin + capecitabine	5 (2)	1	4 (3)
+Bevacizumab	2 (1)	0	2 (1)
+Cetuximab/panitumumab[m]	2 (1)	0	2 (1)
Other[n]	1 (0.5)	0	1
Missing	5 (2)		

ANOS, adenocarcinoma not otherwise specified; CRS/HIPEC, cytoreductive surgery and heated intraperitoneal chemotherapy; MAC, mucinous adenocarcinoma; SRC, signet ring cell carcinoma; WT, wild type.
[a]Of 34 cases that had initial debulking, 3 proceeded to have subsequent CRS/HIPEC, CC1 ($n = 2$), CC2 ($n = 1$). Two cases did not proceed with CRS and had caecectomy ($n = 1$) and diagnostic biopsy ($n = 1$).
[b]M1 at subsequent CRS ($n = 13$).
[c]Staged as M1c due to acellular mucin and visceral metastasis ($n = 1$).
[d]Five patients had M1c disease only (i.e. no peritoneal metastasis).
[e]Primary tumour grading based on World Health Organization (WHO) 2019 classification, grade 1 MAC is a low-grade AMN which is considered a different histological subtype of appendiceal tumour.
[f]This group is defined as presence of ≤50% signet ring cells.
[g]Includes all subtypes of mucinous and non-mucinous adenocarcinoma.
[h]This group categorises patients with cellular peritoneal metastases and excludes patients with M1c only extraperitoneal metastases ($n = 5$) and a case with peritoneal acellular mucin and visceral metastasis ($n = 1$).
[i]Any proportion of mucin, includes presence of mucin in ANOS/non-mucinous metastasis ≤50%.
[j]In either primary or peritoneal disease.
[k]±6 months of CRS.
[l]Changed from 5-fluorouracil to raltitrexed chemotherapy for cardiotoxicity ($n = 2$).
[m]RAS WT ($n = 1$), RAS status unknown ($n = 1$).
[n]First-line carboplatin/paclitaxel pre-CRS for initial diagnosis of ovarian cancer ($n = 1$).

and N/ACT ($P = 0.89$) on OS, Table 2, noting the small numbers in this group.

Response rate to systemic chemotherapy

Radiological response. There were 77/97 (79%) patients in whom disease was potentially evaluable during the first chemotherapy regimen. This excludes those who received adjuvant chemotherapy and includes patients post-CC2-3 CRS with RD evident on imaging. The objective response rate was 13% and disease control rate 30% (Table 3).

Pathological response. There were 15/97 (15%) patients who had preoperative chemotherapy which allowed assessment for pathological response on the resected specimen. The pathological response rate (TRG 0-1) was 20% (Table 3).

Exploratory pathological analyses

Discordant primary and peritoneal grade. We identified 75 patients with discordant grade (40 higher-grade primary tumour to lower-grade PM, 35 lower-grade primary tumour to higher-grade PM); Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2023.101619. Thirty-one (41%) patients in this group received chemotherapy, 13 before CRS (6 prior and 7 neoadjuvant).

When this subgroup was classified by primary tumour grade, there was no significant difference on OS ($P = 0.42$) although a difference was noted for PFS ($P = 0.014$); Supplementary Figure S8, available at https://doi.org/10.1016/j.esmoop.2023.101619. When classified by PM grade, there was a significant difference in both OS and PFS outcomes ($P = 0.0075$ and $P < 0.0001$); Supplementary Figure S8, available at https://doi.org/10.1016/j.esmoop.2023.101619. Patients with higher-grade primary tumour and lower-grade PM had longer OS and PFS compared to patients with lower-grade primary tumour and higher-grade PM (median OS 122 versus 48 months, $P = 0.00094$ and median PFS NR versus 15 months, $P < 0.0001$); Supplementary Figure S9, available at https://doi.org/10.1016/j.esmoop.2023.101619.

Discordant primary and peritoneal signet ring cell status. We identified 18 patients from our cohort who had discordant srcs (10 had srcs in primary tumour and not in PM; 8 had srcs in PM and not in primary tumour); Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2023.101619. Analysis of the impact of srcs in different disease settings revealed that despite the presence of srcs in the primary tumour, a small cohort of patients ($n = 12$) with peritoneal AM or no disease relapse had excellent OS and PFS outcomes; Supplementary Figure S10, available at https://doi.org/10.1016/j.esmoop.2023.101619.

ANOS three-tier grade reclassification. Seventy ANOS cases were available for three-tier grade reclassification, which was predictive for OS ($P = 0.028$); Supplementary Table S5 and Figure S11, available at https://doi.org/10.1016/j.esmoop.2023.101619. This is mostly driven by the difference in OS between grade 1 and 3 reclassification (median 94 versus 49 months, $P = 0.016$). Reclassification of the ANOS subgroup into three-tiered grading also suggests

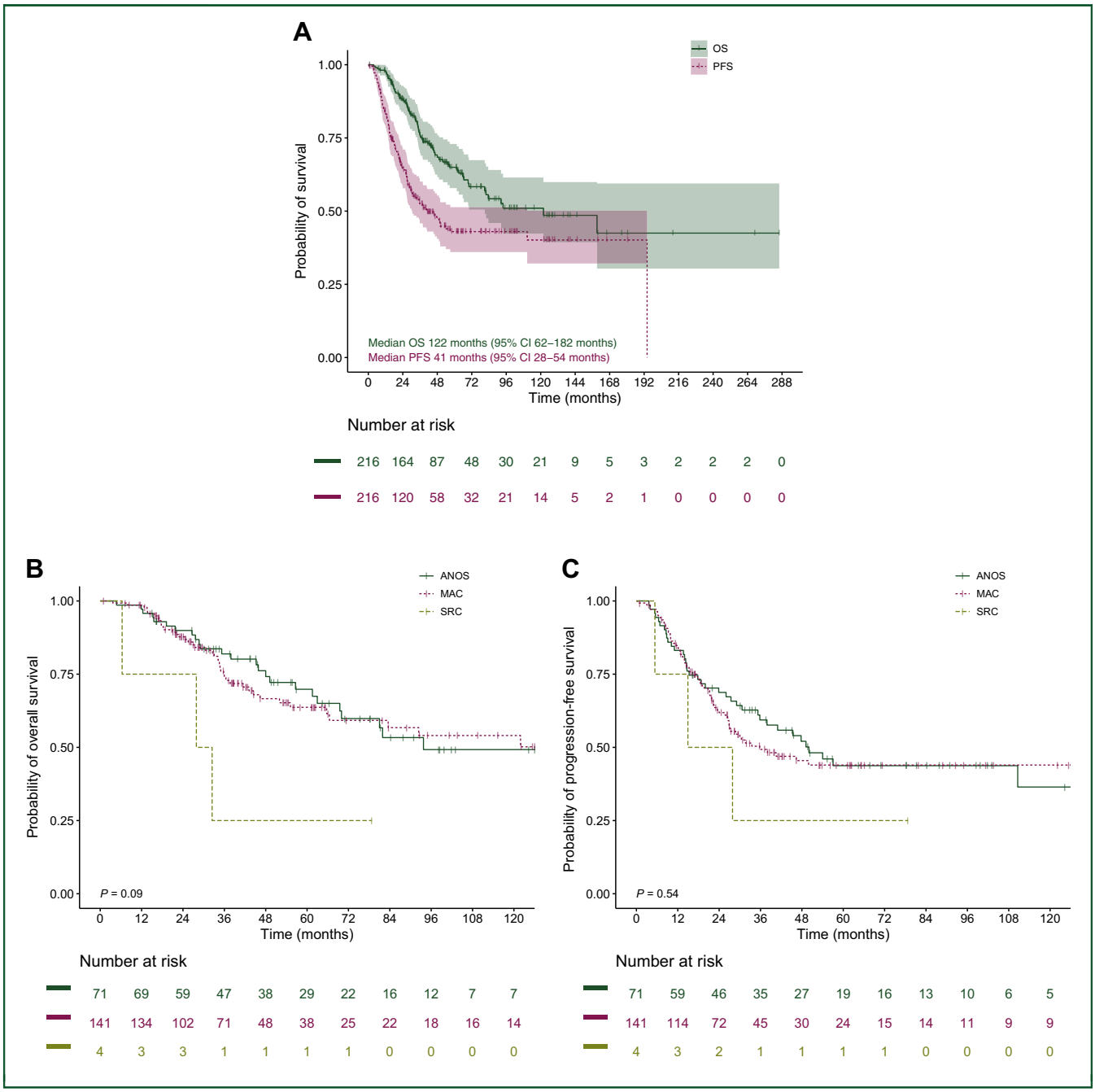

Figure 1. Survival outcomes of patients with appendix adenocarcinoma. (A) Whole cohort. (B) Overall survival by histological subtype. (C) Progression-free survival by histological subtype.
ANOS, adenocarcinoma not otherwise specified; CI, confidence interval; MAC, mucinous adenocarcinoma; OS, overall survival; PFS, progression-free survival; SRC, signet ring cell adenocarcinoma.

improved stratification for predicting PFS for the whole cohort ($P = 0.0077$) and for those with PM ($P = 0.041$); Supplementary Table S5 and Figure S11, available at https://doi.org/10.1016/j.esmoop.2023.101619.

DISCUSSION

Summary

Our study is the largest homogeneous cohort of patients with AA classified according to the WHO 2019 system to evaluate the impact of systemic chemotherapy on survival. We have demonstrated that in patients with positive lymph nodes, chemotherapy was associated with longer survival compared to no chemotherapy. Other key findings from this study include prolonged survival for patients with peritoneal AM and lower-grade PM alongside confirmation of the prognostic impact of PM grading in those with discordant disease, and preliminary validation of a three-tier grade classification for the ANOS subtype.

Survival outcomes

This cohort demonstrated better survival outcomes than expected from previous studies in this aggressive

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival

Variable	Univariate, *n* = 216						Multivariate, *n* = 208			
	n	Median OS, months	95% CI	HR	95% CI	*P* value	*n*	HR	95% CI	*P* value
Subtype										
MAC	141	NR	—	Reference			135	Reference		
ANOS	71	94	55-132	1.0	0.6-1.6	0.88	70	1.6	0.8-2.9	0.17
SRC	4	28	2-53	3.3	1.0-10.8	0.044*	4	20.7	4.5-94.6	<0.001*
Age at Dx, years										
<60	116	122	54-190	Reference			112	Reference		
≥60	100	84	—	1.1	0.7-1.7	0.71	97	1.2	0.7-2.2	0.43
Sex										
Male	90	NR		Reference			85	Reference		
Female	126	82	55-108	1.4	0.9-2.3	0.16	124	3.9	1.9-7.9	<0.001*
PCI										
<10	110	NR	—	Reference			110	Reference		
10-19	49	70	48-92	2.5	1.3-4.8	0.005*	49	0.8	0.3-1.7	0.49
≥20	50	33	24-41	7.3	4.1-13.1	<0.001*	50	0.5	0.1-1.6	0.23
Cytoreduction										
CC0	134	NR		Reference			133	Reference		
CC1	38	82	—	2.0	1.0-4.0	0.058	38	1.9	0.8-4.5	0.17
CC2/3	44	25	14-35	11.6	6.8-20.0	<0.001*	38	29.2	8.3-102.6	<0.001*
CEA										
≤5	139	NR		Reference			137	Reference		
>5	55	38	25-51	3.6	2.2-6.0	<0.001*	52	1.5	0.8-2.8	0.24
Missing	22	49	40-58				20	1.4	0.6-3.4	0.44
N stage										
N0/X	170	159	56-262	Reference			164	Reference		
N1/2	46	48	28-68	2.1	1.4-3.2	0.002*	45	19.1	5.8-62.4	<0.001*
M stage										
M0	69	NR		Reference			69	Reference		
M1a	25	NR		2.2	0.5-10.1	0.29	24	1.4	0.2-8.7	0.70
M1b/c	122	49	32-66	13.7	5.0-37.8	<0.001*	116	5.4	1.7-16.9	0.004*
Primary tumour grade										
ANOS LG	53	NR		Reference			—	—	—	—
MAC G2	108	NR		1.1	0.6-2.0	0.75	—	—	—	—
ANOS HG	18	62	15-108	1.9	0.9-4.3	0.10	—	—	—	—
MAC G3	34	66	21-111	1.9	0.9-3.8	0.076	—	—	—	—
Peritoneal tumour grade										
No disease	74	NR	—	Reference			—	—	—	—
AM[a]	26	NR	—	1.5	0.4-5.8	0.59	—	—	—	—
MCP G1[b]	6	92	—	2.9	0.6-14.2	0.19	—	—	—	—
ANOS LG[b]	4	NR								
MCP G2	53	66	42-89	7.3	2.9-18.2	<0.001*	—	—	—	—
ANOS HG	30	57	35-78	9.8	4.0-14.3	<0.001*	—	—	—	—
MCP G3	23	30	20-41	15.5	6.2-38.9	<0.001*	—	—	—	—
Mucinous										
No	34	122	55-189	Reference			—	—	—	—
Yes	182	82	—	1.1	0.6-2.1	0.76	—	—	—	—
Signet ring cells (peritoneal)										
No	190	159	—	Reference			—	—	—	—
Yes	25	32	22-43	3.9	2.3-6.5	<0.001*	—	—	—	—
Systemic chemotherapy										
None	119	NR	—	Reference			118	Reference		
Neoadjuvant or adjuvant	28	NR	—	1.0	0.4-2.5	1.0	28	1.5	0.4-5.8	0.58
Prior	18	82	78-86	2.3	1.0-5.0	0.038*	18	1.9	0.5-6.7	0.33
Palliative	51	36	30-42	3.9	6.2-3.6	<0.001*	45	3.8	1.8-8.2	<0.001*
Systemic chemotherapy for N+[c]										
None	9	27	4-49	Reference			9	Reference		
Neoadjuvant or adjuvant	15	NR	—	0.6	0.2-1.7	0.37	15	0.04	0.01-0.40	0.005*
Prior	9	81	23-139	1.4	0.6-3.4	0.41	9	0.09	0.01-0.57	0.011*
Palliative	13	28	12-43	5.5	3.1-9.6	<0.001*	13	0.06	0.02-0.25	<0.001*
Systemic chemotherapy for presence of peritoneal signet ring cells										
None	9	41	15-68	Reference			—	—	—	—
Neoadjuvant or adjuvant	2	28	—	1.1	0.4-2.7	0.89	—	—	—	—

Continued

Table 2. Continued

Variable	Univariate, $n = 216$						Multivariate, $n = 208$			
	n	Median OS, months	95% CI	HR	95% CI	*P* value	*n*	HR	95% CI	*P* value
Prior	3	81	0-169	1.7	0.7-3.9	0.22	—	—	—	—
Palliative	10	21	13-29	5.5	3.2-9.7	<0.001*	—	—	—	—

Only age, sex and variables that had a *P* value of significance <0.10 in the univariate model were introduced in the multivariate Cox model. Peritoneal grade was excluded from the multivariate Cox model due to overlap with M stage variables which introduced instability to the model.
95% CI, 95% confidence interval; AM, acellular mucin; ANOS, adenocarcinoma not otherwise specified; CC, cytoreductive score; Dx, diagnosis; G1, grade 1; G2, grade 2; G3, grade 3; HG, high grade; HR, hazard ratio; LG, low grade; MAC, mucinous adenocarcinoma; MCP, mucinous carcinoma peritonei; N+, node positive; OS, overall survival; PCI, peritoneal cancer index; SRC, signet ring cell carcinoma.
[a]This group of AM includes a case of M1c disease (peritoneal AM and visceral metastasis).
[b]These groups were combined for analysis due to small patient numbers.
[c]This variable is included in the multivariate model as an interaction factor between systemic chemotherapy group × nodal status.
**P* value <0.05 is significant.

cancer.[6-11] In the localised M0 cohort, the high 5-year OS of 93% can be explained by early CRS/HIPEC with 70% receiving this procedure within 6 months of their initial diagnosis. Importantly, 15% of initially M0 patients had PM confirming a low threshold for MDT recommendation for CRS/HIPEC in this population. A similar approach was demonstrated in a small cohort of 39 patients who had CRS/HIPEC at a median of 4 months from diagnosis, resulting in median OS of 110 months and 5-year OS of 83%.[31] Despite higher proportions of lower-grade primary tumours in this cohort (85%), there was no difference in survival on univariate analysis.

In the metastatic cohort with a 5-year OS of 52%, the improved outcomes compared to the literature is influenced by selection bias at our institution, where patients with higher-risk disease that impacts resectability (such as small bowel involvement) are usually not deemed suitable for CRS/HIPEC. Thus, patients who proceed to CRS are thought to have a better prognosis.

While these improved survival outcomes are also reflected in the PFS data, there remain a small number of patients who have progression after 5 years. This supports our current surveillance schedule incorporating follow-up to 10 years and we support other peritonectomy centres taking this approach.

We emphasise the importance of complete cytoreduction (CC0) regardless of tumour burden. PCI is prognostic in the univariate analysis but after adjusting for covariates this is not retained. This means that even for patients with high PCI scores (>20), complete CC0 CRS is associated with more favourable outcomes compared to CC2-3 CRS.

A major strength of this cohort is the adherence to the WHO 2019 classification. This was to reconcile the challenges of interpreting survival outcomes from the existing literature where AA patients are included within the broad 'pseudomyxoma peritonei' group of appendix tumours that also includes AMNs.[3,12] We have analysed the ANOS subgroup by reclassification of tumour grade from two-tier into three tier. Analysis suggests that three-tiered grading was more discriminatory, with grade 1 (well differentiated) tumours associated with the best prognosis. We also show discrimination between grade 2 and grade 3 tumours in predicting both OS and PFS. Further validation in a larger cohort is needed.

Discordant cohort

Discordant peritoneal grade compared to primary tumour grade has been previously documented in appendiceal tumours but the prognostic implication for AA specifically has been suggested but not confirmed.[12,26,32] A recent study evaluated 37 cases of discordant grade.[32] These patients had no deaths and three recurrences, but this smaller cohort had a lower PCI and a higher rate of CC0-1 CRS favouring good outcomes. We confirm these findings for the first time in a larger cohort of 75 patients with pure AA with discordant grade and demonstrate the improved outcomes of those with lower peritoneal grade and higher primary grade compared to patients with higher peritoneal grade and lower primary grade. This confirms that peritoneal and primary grade should continue to be reported separately and for those with discordance, the peritoneal grade is most prognostic. The excellent survival outcomes of AM and lower-grade PM seen in our study have previously been demonstrated in mixed cohorts of primary AMNs, and this study confirms these outcomes in a larger cohort of pure AA.[32-34] The implication of this finding is that patients with AA primary diagnosis who experience AM or lower-grade PM can be reassured of expected good longer-term outcomes.

The presence of srcs is associated with poor prognosis and some authors have questioned the role of CRS/HIPEC for patients with AA who have evidence of these.[6,8,27,35] Our study confirms that the presence of srcs was most relevant in the PM setting and that patients who have srcs in the primary tumour can still have a good outcome.

Lymph node status

Our cohort demonstrates that positive nodes are predictive of survival even in the metastatic setting. On multivariate analysis, patients with positive lymph nodes received benefit from chemotherapy. Other authors have suggested focusing chemotherapy on this positive node group, which has been shown in some studies to predict outcomes.[36,37] There are also studies that have shown that lymph node status is not a

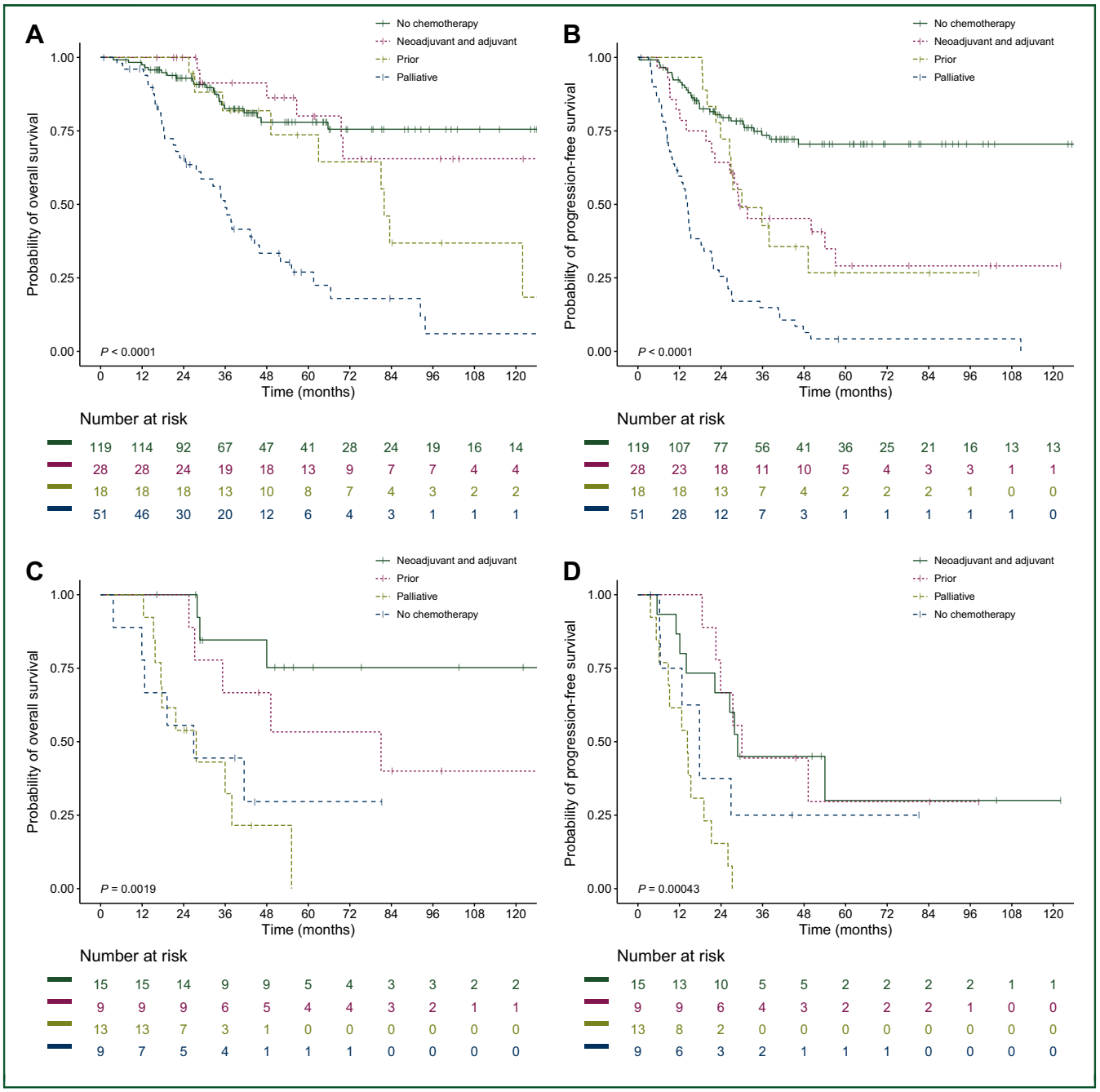

Figure 2. Outcomes of patients with appendix adenocarcinoma receiving chemotherapy. (A) Overall survival. (B) Progression-free survival. (C) Overall survival in patients with positive lymph nodes. (D) Progression-free survival in patients with positive lymph nodes.

significant predictor of survival after CRS/HIPEC.[38,39] For lymph nodes to be fully assessed, patients require a nodal resection, such as during right hemicolectomy. However, data suggest that for MAC with peritoneal seeding and low-risk ANOS (T1, low grade, no lymphovascular invasion) there is no survival advantage for including right hemicolectomy over appendicectomy or partial colectomy.[38,40,41] Our institution has a selective strategy for right hemicolectomy based on tumour grade, radiological findings and patient factors. This is appropriate, given the 21% rate of lymph node involvement in our current cohort is consistent with the literature.

Systemic chemotherapy

There have been few prospective studies and several retrospective reviews attempting to evaluate the role of systemic chemotherapy in AA.[42] Much of the literature has focused on the poor outcomes of those receiving perioperative chemotherapy, but this is in mixed disease cohorts, with those selected for chemotherapy having the worst risk factors, which we have seen in our results on univariate analysis.[28,43] When these groups are further interrogated, patients with non-mucinous, poorly differentiated, positive lymph nodes and srcs may derive benefit from

Table 3. Radiological and pathological responses to systemic chemotherapy[a] in patients with appendix adenocarcinoma

	All (n = 97)	%	95% CI
Evaluable for radiological response (RECIST1.1)	77	100	
PD	22	29	19-39
SD	13	17	9-25
ORR	10	13	5-21
PR	9	12	—
CR	1	1	—
Missing	32	42	—
Pathological response[b]	15	100	
TRG 0-1	3	20	4-48
TRG 0	1[c]	7	—
TRG 1	2	13	—
TRG 2-3	12	80	52-95
TRG 2	1	7	—
TRG 3	11	73	—

CI, confidence interval; CR, complete response; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; TRG, tumour regression grade.
[a]This assessment is for first systemic chemotherapy regimen received.
[b]For patients who had preoperative chemotherapy.
[c]One case had acellular mucin only with no neoplastic component.

chemotherapy.[26,27,36,44] We are unable to draw conclusions from our analysis of the role of systemic chemotherapy in patients with peritoneal srcs due to small numbers, but cannot exclude a potential benefit in the N/ACT or prior chemotherapy groups.

Oxaliplatin and fluoropyrimidine chemotherapy has been the preferred first-line systemic regimen used worldwide and this is reflected in our cohort.[25,45-47] We demonstrated lower chemotherapy response rates compared to the literature where one study demonstrated response rates to neoadjuvant chemotherapy up to 58%, another with up to 45% stable disease rate and 24% partial response rate.[25,46,48] However, given the retrospective nature of these studies and differing referral patterns, it is difficult to compare these results. Furthermore, radiological response is challenging to assess in PM and these studies do not always use RECIST, with some authors suggesting that a modified peritoneal RECIST should be developed.[49] A limitation to our study is that we have not carried out centralised review of response imaging; however, most of our patients have their imaging either at our institution or the images are re-reported by our expert radiologists.

There are limited data on pathological response to neoadjuvant chemotherapy and our cohort of 15 patients who had this approach provides further insights. One prospective cohort of 34 patients with high-grade PM were treated with preoperative oxaliplatin and 5-fluorouracil (FOLFOX4).[50,51] Although there was no improvement in OS in patients who received preoperative chemotherapy compared to those who did not (median 51 versus 37 months, $P = 0.56$), 10 (29%) patients had a pathological response, slightly higher than our pathological response rate of 20%. It is unclear what the impact of the TRG score for the pathological response is on predicting outcomes as this has been validated for response to chemoradiotherapy in rectal cancer and the application to non-rectal cancer adenocarcinoma is unclear.[30]

There is some literature that suggests in AA that regression in grade (from high to low) could be driven by systemic chemotherapy.[51] In our cohort, 13 patients in the discordant grade cohort received systemic chemotherapy before CRS which is challenging to draw conclusions from. The biology of this potential phenomenon is poorly understood, and theories include cancer cell heterogeneity, grade regression and stimulation of mucin production as a chemotherapy resistance mechanism.[51-53]

There have been studies that demonstrate a benefit of adjuvant chemotherapy for patients with AA PM following CRS/HIPEC.[28,54,55] The rationale for chemotherapy referral has not been systematically evaluated in these retrospective studies. One of the key reasons for referral for perioperative chemotherapy that emerged from our study was the presence of positive lymph nodes. Our findings confirm that selection of these patients for chemotherapy is appropriate. There were also a number of patients who historically had an external decision for chemotherapy to be given after their initial local procedure before referral to our specialist centre. Some of these patients have been selected by potential referrers as unresectable due to a high burden of disease and this reinforces the need for early referral to a specialist MDT.

Limitations

A limitation of our study was that despite the large total cohort, we still had small numbers of patients in all examined subgroups. As such, the evaluation of neoadjuvant versus adjuvant chemotherapy could not be carried out. Advantages of neoadjuvant chemotherapy include assessment of biological response to help select for future CRS and which could inform choice of future treatments; it deals with occult metastases earlier and there is improved deliverability of systemic therapy as there is no waiting period for operative recovery. Benefits of adjuvant chemotherapy include improved knowledge of disease histology, staging and amount of RD; it minimises the risk of progression through a treatment window and maintains patients' fitness for CRS. Furthermore, it needs to be emphasised that there is heavy selection bias to improved outcomes for patients selected for multimodality treatments and an aggressive chemotherapeutic approach.

Selection bias also influences patients who are referred to specialist centres and subsequent MDTs. Patients with rapidly progressing, higher-grade disease are more likely to be managed in the community without subsequent referral for specialist MDT opinion as they present with advanced and likely inoperable disease. Therefore, the biology of disease that is referred to specialist centres likely biases to improved outcomes.

Another limitation is our study is not representative of all patients with advanced disease. Patients who undergo debulking surgery with higher burden RD (CC2-3) may still derive benefit from a reduced burden of disease. However, literature evaluating AA regarding these questions is sparse and further study is needed on all patients with advanced disease.

Patients who undergo, or planning for, chemotherapy may have more frequent intervals for radiological imaging. These patients are more likely to have worse PFS outcomes because of detection bias from more frequent imaging, meaning events are picked up earlier.

Finally, loss to follow-up is an inherent limitation of retrospective cohorts. The median follow-up of our cohort was almost 5 years; therefore, the 5-year data are robust. However, the longer-term outcomes are less accurate, which is depicted by the widening CI. Regardless, these results remain highly informative to ongoing clinical practice and raise the possibility of generating AA-specific society guidelines.

Conclusions

This study represents the single largest series of pure AA with long-term follow-up data that evaluates the role of systemic chemotherapy in different disease settings and confirms that positive lymph node status identifies a subgroup of patients with AA who derive the most benefit from systemic chemotherapy. Patients with high-grade disease, peritoneal srcs and positive lymph nodes had the poorest outcomes and should be the focus for clinical trials and development of novel therapies. Finally, we demonstrate the importance of prognostication based on disease stage and PM grade, and, the relevance of continuing to report primary tumour and PM grade separately. In particular, we can reassure patients with AM and lower-grade PM of excellent outcomes, akin to those with localised (M0) disease.

GLOSSARY

Cytoreduction score—a score of residual peritoneal disease calculated after maximal resection during cytoreductive surgery defined as CC0 (no residual disease), CC1 (<0.25 cm residual disease), CC2 (0.25-2.5 cm residual disease) and CC3 (>2.5 cm residual disease).

Cytoreductive surgery—a radical surgical procedure where the surgeon resects the peritoneal lining and if needed associated internal organs of the abdomen.

Heated intraperitoneal chemotherapy—cytotoxic chemotherapy agents that are heated to 42°C and instilled into the abdominal cavity after surgical cytoreduction to treat occult cancer cells.

Peritoneal cancer index—the total score of individually scored regions of peritoneal cancer by the surgeons at the start of cytoreductive surgery (scored 0-39).

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DISCLOSURE

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DATA SHARING

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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