Trends in the incidence and mortality of vulvar cancer in Australia, and a 29-year overview of management at the Royal Hospital for Women, Sydney.

Author:
Barlow, Ellen

Publication Date:
2021

DOI:
https://doi.org/10.26190/unsworks/22697

UNSW
SYDNEY

Trends in the incidence and mortality of vulvar cancer in Australia, and a 29-year overview of management at the Royal Hospital for Women, Sydney.

Ellen Louise Barlow

A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Women's and Children's Health

Faculty of Medicine

April 2021

Thesis Title

Trends in the incidence and mortality of vulvar cancer in Australia, and a 29-year overview of management at the Royal Hospital for Women, Sydney.

Thesis Abstract

Aims: This vulvar cancer thesis had four major aims. To: (i) analyse incidence and mortality trends in Australian women over the years from 1982 to 2011, (ii) investigate the independent prognostic significance of HPV, p16 and p53 status, (iii) determine the incidence of, and risk factors for morbidity following groin node dissection, and (iv) explore the pattern of local recurrences and determine their relationship with the extent of the histopathological margin.

Methods: Four studies were performed**.** (i) Australian population-based vulvar cancer data were analysed for changes in age-standardized incidence and mortality rates. Subsequently, data collected over a 29-year period from the database of a single-institution were analysed in three studies. (ii) immunohistochemistry was used to determine p53 and p16 status, and HPV status was determined by PCR detection of HPV DNA in 119 patients, (iii) clinical and histopathological data for 333 patients (525 groins) treated with groin node dissection were retrospectively analysed for post-operative morbidity, and (iv) data on 345 patients treated primarily with surgery were retrospectively analysed for risk factors associated with local recurrence.

Results: (i) vulvar cancer incidence was significantly increasing in women under 60 years and mortality decreasing in women over 60 years, (ii) p16, p53 and HPV DNA status were not independent prognostic factors, (iii) the number of lymph nodes resected was the only factor significantly associated with all complications, and (iv) primary site recurrences were increased in patients with histopathological margins < 8mm. Treatment of patients with sub-optimal margins decreased the risk of recurrence.

Conclusions:

(i) Vulvar cancer incidence has increased by more than 80% in women younger than 60 years in Australia, consistent with increased exposure to the human papillomavirus in cohorts of females born after 1950, (ii) vulvar cancer treatment decisions should continue to be based on clinical indicators rather than on p16 or p53 status, (iii) a more extensive lymph node dissection is a significant risk factor for all post-operative groin complications, and (iv) Guidelines should continue to recommend a surgical margin of 1 cm.

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Publication Details #1

Full Title:	Staging for vulvar cancer
Authors:	Neville F Hacker & Ellen L Barlow.
Journal or Book Name:	Best Practice & Research Clinical Obstetrics and Gynaecology
Volume/Page Numbers:	Vol 29: pp 802-811.
Date Accepted/Published:	Published-2015
Status:	published
The Candidate's Contribution to the Work:	Conducted the literature review and contributed equally to the manuscript writing and feedback. Formatting of the manuscript for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is not used in lieu of a chapter. It comprises a section of the literature review in Chapter 2 (2.2.2) under the heading 'Staging and current management of vulvar cancer'.

Publication Details #2

Full Title:	Sentinel node biopsy in vulvar cancer: A critical appraisal
Authors:	Neville F Hacker & Ellen L Barlow.
Journal or Book Name:	Asian Journal of Oncology
Volume/Page Numbers:	Vol 3: pp 5-11
Date Accepted/Published:	Published - 2017
Status:	published
The Candidate's Contribution to the Work:	Conducted the literature review and contributed equally to the manuscript writing and feedback. Formatting of the manuscript for publication.

Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is not used in lieu of a chapter. It comprises a section of the literature review in Chapter 2 (2.3.4) under the sub-heading "Modifications to the lymph node dissection to reduce morbidity".

Publication Details #3

Full Title:	Changing trends in vulvar cancer incidence and mortality rates in Australia since 1982.
Authors:	Ellen L Barlow, Yoon-Jung Kang, Neville F Hacker, & Karen Canfell.
Journal or Book Name:	International Journal of Gynecological Cancer
Volume/Page Numbers:	Vol 25(9): pp 1683-1689
Date Accepted/Published:	Published - November 2015.
Status:	published
The Candidate's Contribution to the Work:	Data retrieval and management. Manuscript draft and writing, formatting of tables and figures, and preparation of manuscript for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is incorporated in my thesis in lieu of Chapter 3.

Publication Details #4

Full Title:	The clinical relevance of p16 and p53 status in patients with squamous cell carcinoma of the vulva
Authors:	Ellen L Barlow, Neil Lambie, Mark W Donoghoe, Zin Naing, & Neville F Hacker.
Journal or Book Name:	Journal of Oncology
Volume/Page Numbers:	Vol 2020: 8 pages
Date Accepted/Published:	Published March 24, 2020
Status:	published

The Candidate's Contribution to the Work:	Concept of project, data retrieval and management. Statistical analysis, manuscript draft and writing, formatting of tables and figures, and preparation of manuscript for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is incorporated in my thesis in lieu of Chapter 4.

Publication Details #5

Full Title:	Morbidity related to the groin lymph node dissection for vulvar cancer.
Authors:	Ellen L Barlow, Mark W Donoghoe & Neville F Hacker.
Journal or Book Name:	International Journal of Gynecology and Clinical Practices.
Volume/Page Numbers:	Vol 6: p 149 (7 pages)
Date Accepted/Published:	November 22, 2019.
Status:	published
The Candidate's Contribution to the Work:	Project concept, data retrieval and management. Statistical analysis, manuscript draft and writing, formatting of tables and preparation of manuscript for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is incorporated in my thesis in lieu of Chapter 5.

Publication Details #6

Full Title:	The prognostic role of the surgical margins in squamous vulvar cancer: A retrospective Australian study.
Authors:	Ellen L Barlow, Michael Jackson & Neville F Hacker.
Journal or Book Name:	Cancers
Volume/Page Numbers:	Vol 12: p 3375 (19 pages)

Date Accepted/Published:	Published November 14, 2020.
Status:	published
The Candidate's Contribution to the Work:	Data retrieval, management and statistical analysis. Manuscript draft and writing, formatting of tables and figures, and preparation of manuscript for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is incorporated in my thesis in lieu of Chapter 6.

Candidate's Declaration

I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

Acknowledgements

Firstly, I would like to thank my Principal Supervisor, Professor Neville Hacker, whose expertise in treating women with vulvar cancer, and the compassion he displayed while doing so, were the inspiration for my undertaking this PhD. I am privileged to have him as a mentor, and extremely grateful for his unlimited guidance and support through the duration of my PhD. His patience and generosity in tolerating my moments of hesitancy and anxiety, and always finding the positive, have helped me immensely.

I would also like to thank my Co-Supervisors, Professor Karen Canfell, and Dr Yoon-Jung Kang, who have both been generous in sharing their expertise in epidemiology, and always approachable and willing to provide whatever support I required. My PhD has been greatly enhanced by my association with them.

I would like to acknowledge my co-authors of the research papers which make up this thesis. I learnt something valuable about vulvar cancer management and/or research from each one of them.

I would like to acknowledge the Mark Wainwright Analytical Centre, University of New South Wales, most particularly Mark Donoghoe, for guidance with statistical analysis.

I would also like to thank my gynaecological oncology nursing colleagues at the Royal Hospital for Women. Their pride in my research, and their encouragement, have been inspirational for me.

My heartfelt thanks and love to all my family. Most especially, my husband Phil, and children, Matthew, Benjamin, Rhiannon, and Nathaniel, and to their partners, Jay, Georgie, Andrew, and Phoebe. They have all endured an endless dialogue around my PhD and coped so empathically with the inevitable highs and lows. I will always be grateful for their unwavering confidence that I would complete and submit my thesis.

Finally, to my grandchildren, Archibald, and Lucia, who were both born in the later stages of this PhD. Time spent with these precious little people has been an antidote to my stress. I hope that in the future my ‘later in life’ PhD will make them proud and provide them with the realisation that education can and should be a life-long pursuit.

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Glossary of Abbreviations

APC	Annual percent change
Av APC	Average annual percent change
AIHW	Australian Institute of Health and Welfare
AJCC	American Joint Commission on Cancer
ASIR	Age-standardised incidence rate
ASM	Age-standardised mortality
AUS	Australia
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CDK's	Cyclin-dependent kinases
CI	Confidence interval
CT	Computed tomographic
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
DSS	Disease-specific survival
dVIN	Differentiated vulvar intraepithelial neoplasia
EAPC	Estimated annual percent change
ECIS	European Cancer Information System
EORTC	European Organisation for Research and Treatment of Cancer.
ESGO	European Society of Gynecological Oncology
FACT-G	Functional Assessment of Cancer Therapy General

FFPE	Formalin-fixed paraffin-embedded
FIGO	The International Federation of Gynecology and Obstetrics
GLOBOCAN	Global Cancer Incidence, Mortality and Prevalence.
GOG	Gynecologic Oncology Group
GROINNS-V	GROningen International Study on Sentinel Nodes in Vulvar Cancer
Gy	Gray (unit of measurement for radiation)
HPV	Human Papillomavirus
HR	Hazard ratio
HSIL	High grade squamous intraepithelial lesion
IARC's	International Agency for Research in Cancer
ICD	International coding of diseases
IMRT	Intensity modulated radiotherapy
ISSVD	International Society for the Study of Vulvovaginal disease
LAST	Lower Anogenital Squamous Terminology
LEG	Lymphoedema and Gynecologic Cancer
LLL	Lower limb lymphoedema
LND	Lymph node dissection
LS	Lichen sclerosus
LSIL	Low grade squamous intraepithelial lesion
LVSI	Lymphovascular space invasion
MAMBO	Morbidity and measurement of the body
NCNN	National Comprehensive Cancer Network

NGOR	National Gynae-Oncology Registry
NPCR	National Program of Cancer Registries
OPSCC	Oro-pharyngeal squamous cell carcinoma
OSCC	Oral squamous cell carcinoma
OS	Overall survival
p16	p16INK4A
PCR	Polymerase chain reaction
PFS	Progression-free survival
p53	TP53 (tumour protein)
PNI	Perineural invasion
pRB	Retinoblastoma protein phosphorylation
QOL	Quality of life
RB	Retinoblastoma protein
RD	Risk difference
RR	Relative risk
RSR	Relative survival ratio (or rate)
SD	Short drainage
SEER	Surveillance, Epidemiology, and End Results
SH	Squamous hyperplasia
SHR	Subdistribution hazard ratio
SLN	Sentinel lymph node
SV	Saphenous vein

SPSS	Statistical Package for the Social Sciences
SRR	Standarised rate ratio
TNM	Tumour (T), nodes (N), and metastases (M)
UK	United Kingdom
UKCIS	United Kingdom Cancer Information System
UICC	Union for International Cancer Control
US	United States
uVIN	Usual-type vulvar intraepithelial neoplasia
VCD	Volume controlled drainage
VIN	Vulvar intraepithelial neoplasia
VMAT	Volumetric modulated arc therapy
VSCC	Vulvar squamous cell carcinoma
WT	Wild type

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List of Publications and Presentations

Publications from this thesis:

- **Barlow EL**, Kang YJ, Hacker NF, Canfell K. Changing trends in vulvar cancer incidence and mortality rates in Australia since 1982. Int J Gynecol Cancer. 2015;25 (9):1683-1689.
- Hacker NF, **Barlow EL**. Staging for vulvar cancer. Best Pract & Res Clin Obstet & Gynaecol. 2015;29: 802-811.
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- Kang YJ, Smith M, **Barlow E**, Coffey K, Hacker N, Canfell K. Vulvar cancer in high-income countries: Increasing burden of disease. Int J Cancer. 2017;141(1):2174-2186.
- **Barlow EL**, Donoghoe MW, Hacker NF. Morbidity related to the groin lymph node dissection for vulvar cancer. Int J Gynecol Clin Prac. 2019;6:149.
- **Barlow EL**, Lambie N, Donoghoe MW, Naing Z, Hacker NF. The clinical relevance of p16 and p53 status in patients with squamous cell carcinoma of the vulva. J Oncol. 2020;2020:3739075.
- **Barlow EL**, Jackson M, Hacker NF. The prognostic role of the surgical margins in squamous vulvar cancer: A retrospective Australian study. Cancers. 2020;12:3375.
- van der Velden J, Pleunis N, **Barlow E**, Zijlmans H, de Hullu J, Hacker NF, Fons G. Radiotherapy is not indicated in patients with vulvar squamous cell carcinoma and only one occult intracapsular groin node metastases. Gynecol Oncol. 2021;160(1):128-133.

Oral presentations from this thesis:

- **Barlow E**, Kang YJ, Hacker NF, Canfell K. 'Trends in incidence and mortality of vulvar cancer in Australia over the period 1982 - 2011', School of Women's and Children's Health, UNSW PhD Seminar, February 10th, 2016.
- **Barlow E**, Kang YJ, Hacker NF, Canfell K. 'Human papilloma virus associated gynaecological cancers and the trends in Australian vulvar cancer incidence and mortality', The Royal Hospital for Women Gynaecological Cancer Centre Research Meeting, 10th October 2017.
- **Barlow EL**, Hacker NF. 'Incidence of short and long-term postoperative

morbidity related to lymph node dissection for vulvar cancer.' International Meeting of the European Society of Gynaecological Cancer (ESGO), Vienna, Austria, November 4th – 7th, 2017.

- **Barlow EL**, Donoghoe, MW, Hacker NF. 'Morbidity related to the groin lymph node dissection for vulvar cancer'. The Royal Hospital for Women Gynaecological Cancer Centre Research Meeting, November 15th, 2018.
- **Barlow, EL**, Lambie, N, Donoghoe, MW, Naing, Z, Hacker, NF. 'The clinical relevance of p16 and p53 status in patients with squamous cell carcinoma of the vulva', The Royal Hospital for Women, Grace Research Meeting, January 10th, 2020.
- **Barlow, EL**. Jackson, M, Hacker, NF. 'The prognostic role of the surgical margins in squamous vulvar cancer: a retrospective Australian study'. School of Women's and Children's Health UNSW PhD Seminar, December 10th, 2020.

Poster Presentations from this thesis:

- **Barlow E**, Kang YJ, Hacker NF, Canfell K. 'Trends in the incidence and mortality of vulvar cancer in Australia'. 16th Biennial Meeting of the International Gynaecological Cancer Society, Melbourne, Australia, October 13th -16th, 2014.
- **Barlow E**, Kang YJ, Hacker NF, Canfell K. 'Trends in the incidence and mortality of vulvar cancer in Australia'. International Meeting of the European Society of Gynaecological Cancer (ESGO), Nice, France, October 24th – 27th, 2015.
- **Barlow EL**, Hacker NF. 'Incidence of short and long-term postoperative morbidity related to lymph node dissection for vulvar cancer.' International Meeting of the European Society of Gynaecological Cancer (ESGO), Vienna, Austria, November 4th – 7th, 2017.

CHAPTER ONE

INTRODUCTION

Vulvar cancer is a rare disease, which has traditionally affected mainly older women. Squamous cell carcinoma is the predominant histological type and is therefore the focus of this thesis. It develops through two separate molecular pathways associated with different pre-neoplastic lesions. One pathway is related to the human papillomavirus (HPV), which is more common in younger women. The other is associated with differentiated vulvar intraepithelial neoplasia (dVIN), lichen sclerosus (LS), and often in the presence of p53 tumour suppressor gene mutations, which is more common in older women. More recently its prevalence in younger women has been increasing, potentially due to increasing levels of exposure to HPV, although this has been inconsistent in different parts of the world.

Traditional treatment of vulvar cancer has been en-bloc radical vulvectomy and bilateral inguino-femoral lymphadenectomy, with or without pelvic lymphadenectomy. For patients with extensive primary disease where primary surgery would necessitate creation of a stoma, preoperative radiation, with or without chemotherapy, is generally used. These treatments are associated with high physical and psychosexual morbidity.

There have been many modifications to the surgical management of squamous vulvar cancer over the last 40 years, with the aim of performing the most conservative surgery consistent with cure of the disease. Because of the rarity of the disease, prospective, randomised studies to test different treatment philosophies have been limited. Hence, most modifications have been introduced without rigorous testing, and the vulvar cancer literature highlights many of these controversial issues.

In relation to the primary vulvar tumour, the move from radical vulvectomy to a more conservative vulvar resection began about 40 years ago, but the appropriate width of the surgical excision margin has remained controversial. For about 30 years, a 1cm margin was generally accepted, which equates to a histological margin of 8mm after tissue shrinkage from formalin fixation. More recently, the relevance of the 1cm margin has been questioned. In addition, some authors have proposed further modifications to treatment of the primary cancer based on the preoperative determination of the HPV status and the immuno-histochemical profile of the tumour. It has been suggested that p53 positive cancers may warrant more aggressive surgery and adjuvant treatment, while HPV/p16 positive cancers may undergo more conservative surgery and less frequent surveillance.

The most important prognostic factor in squamous vulvar cancer is the status of the regional lymph nodes. Groin lymph node dissection has been associated with significant short and long-term post-operative morbidity. The major short-term morbidity has been wound breakdown and infection, and lymphocyst formation, while the major long-term morbidity has been lower limb lymphoedema. The use of a separate groin incision, rather than the en bloc approach, was the first modification introduced to improve wound breakdown, and although never subjected to a randomised prospective study, this approach has generally been accepted into modern practice. The need for preservation of subcutaneous fat, the preservation of the saphenous vein, and the role of groin wound drains remain controversial issues with respect to short-term morbidity. The recent introduction of sentinel node biopsy was an attempt to address lower limb lymphoedema, but this procedure is only applicable if the sentinel node is negative and the primary tumour is < 4 cm in diameter. Hence, more than 50% of patients will still require a complete inguino-femoral lymphadenectomy.

This thesis contains four main studies, each addressing controversial questions mentioned above. The first study sought to identify vulvar cancer incidence and mortality trends in Australian women, and to determine if there was an increased incidence in younger Australian women. This was only the second population-based study of Australian trends in vulvar cancer, and the first Australian study to examine age and mortality trends.

The second study focused on the clinical relevance of the immuno-histochemical biomarkers p16, p53 and the presence of HPV in a cohort of patients with squamous vulvar cancer. Of particular interest was whether these markers could be used to modify the radicality of the surgery for the primary lesion.

The third and fourth studies focused on answering the surgical management controversies related to the acute morbidity associated with groin dissection and the importance of surgical margins in the treatment of the primary lesion in relation to local recurrence. In order to address these controversial issues, data were retrospectively analysed from 345 consecutive patients treated for squamous vulvar cancer at the Gynaecological Cancer Centre at the Royal Hospital for Women between 1987 and 2017. There has been a consistent policy of conservative management at that Centre over the past 30 years.

1.1 | Research Questions

The research questions related to the four studies for this thesis are:

Study 1

(i) What are the temporal trends in the incidence and mortality of vulvar cancer in Australian women?

(ii) Is there evidence of an increasing incidence of vulvar cancer in younger cohorts of Australian women born after 1950?

Study 2.

(i) Is human papillomavirus (HPV) status prognostically meaningful in vulvar squamous cell carcinoma, and is pre-operative determination of p16 or p53 status by immunohistochemistry clinically relevant?

(ii) What are the clinicopathological variables associated with p16 and p53 status?

Study 3.

(i) What is the incidence of short and long-term postoperative morbidity of the groin lymph node dissection in a large cohort of patients treated for invasive vulvar cancer?

(ii) What clinical factors are associated with post-operative morbidity following groin node dissection?

Study 4.

(i) What is the long-term survival of patients with squamous vulvar cancer treated in the era of conservative management?

(ii) Is there a relationship between the extent of the surgical excision margin and local vulvar recurrences in women treated with primary surgery for squamous vulvar cancer?

(iii) Is treatment of close or positive surgical excision margins beneficial in reducing local recurrence?

CHAPTER TWO

LITERATURE REVIEW

Introduction

In this chapter, a review of the relevant background literature to the work presented in this PhD thesis has been performed. This review commenced in 2013, initially to identify potential knowledge gaps in the literature and to determine how my own research could contribute to further understanding of vulvar cancer management, prior to the development of the research proposal.

The original search period was confined to articles published from 1980 to 2013, except for earlier papers considered seminal to vulvar cancer management, or to describe vulvar cancer management from an historical perspective. Over the course of this thesis, regular literature searches have been undertaken to stay abreast of current evidence, and to examine new topics that became relevant as the research questions evolved. The final update of this review was conducted between the 1st of October 2020 and the 31st of January 2021 to include all relevant publications to that date.

2.0.1 | Literature Search Strategy

To identify literature relevant to this review searches of electronic resources including, PubMed, MEDLINE, EMBASE, PsycINFO, CINAHL and the Cochrane Library (providing access to Cochrane reviews) was conducted. Electronic sources, particularly for the epidemiological statistics and vulvar cancer treatment guidelines, were searched through the Internet links to GLOBOCAN, the Australian Institute of Health and Welfare (AIHW), the National Comprehensive Cancer Network (NCNN), and the European Cancer Information System (ECIS). Reference lists of all relevant articles or book chapters were manually searched to identify other relevant literature. The literature search was built around the four main research themes, and due to the broad scope of these topics, multiple search terms and words were used either alone, or in combination with one another (see Appendix 1.) This literature review was limited to English language articles.

2.0.2 | Structure of the Literature Review

The following review has been categorized into three parts. Part 1 briefly introduces vulvar cancer and describes the etiology of vulvar squamous cell carcinoma and its precursor

lesions. The literature on human papillomavirus and its relationship to squamous vulvar cancer is reviewed. This is followed by a detailed review of the literature related to world-wide trends in the incidence of squamous intraepithelial precursor lesions, and the incidence and mortality of invasive vulvar cancer.

Part 2 provides an overview of vulvar squamous cell carcinoma including clinical features, diagnosis, staging, current management, and prognosis. The most recently proposed factors believed to influence prognosis in squamous vulvar cancer, are the biomarkers p16, p53 and the presence of HPV, therefore, an introduction to these biomarkers is provided. In particular, the literature examining the current hypothesis that the presence of these biomarkers may be beneficial in determining prognosis and modifying management is critically appraised.

Part 3, a major component of this thesis was to evaluate outcomes related to the conservative resection of the primary lesion and the management of the inguino-femoral lymph nodes in patients with squamous cell carcinoma of the vulva. Therefore, a critical appraisal was undertaken of published studies that examined surgical excision margins and local vulvar recurrence rates, and studies of acute and chronic morbidity related to the vulvar excision and groin lymph node dissection.

Of note, I have not included my own research publications explicitly in this literature review, although I have noted where relevant how they complement the other literature. I have included two published papers (in their entirety) that were co-authored with my Principal Supervisor, Professor Neville Hacker, which were written during my PhD candidature. One discusses the current vulvar cancer staging system and describes current management (Chapter 2. Part 2), and the other critically reviews the literature on the sentinel node procedure (Chapter 2. Part 3.).

LITERATURE REVIEW: PART ONE

2.1.1 | Introduction to Vulvar Cancer

Vulvar cancer is a relatively rare cancer with an estimated age-adjusted annual incidence rate of between 0.3 – 6.6 per 100,000 women worldwide [1-3]. In the United States (US), Europe and Australia, vulvar cancer is the fourth most common gynaecologic malignancy after cancers of the uterus, ovary and cervix [4,5]. In Australia, around 370 women are diagnosed with vulvar cancer each year, and 434 women died from the disease during the period from 2014 to 2018 [5].

Squamous cell carcinoma is the most common histopathological subtype, accounting for approximately 85% of vulvar cancers [5]. The other less common cell types are melanoma, sarcoma, adenocarcinoma, basal cell carcinoma, Bartholin's gland carcinoma, and invasive Paget's disease. These less common vulvar cancers will not be addressed in any depth in this thesis.

Squamous cell carcinoma of the vulva is generally considered to be a disease of post-menopausal women, with a mean age at diagnosis in Australian women during the period 2012 - 2016, of 68 years [5]. In recent years, there has been an increased incidence of vulvar intraepithelial neoplasia (VIN) and a more moderate increased incidence of squamous vulvar cancer worldwide [6-12]. This increase has predominately been driven by women under 60 years, and has been attributed to a number of risk factors such as smoking [13], changes in sexual behaviour, and the increased level of human papillomavirus (HPV) infection, and immunosuppression [8].

Anatomy of the Vulva

Anatomically, the female external genitalia are referred to collectively as the vulva. The vulva includes the mons pubis, bilateral labia majora, bilateral labia minora, vulvar vestibule, clitoris, and the perineum [14]. The labia majora are the most prominent features of the vulva and contain sebaceous glands hair follicles and subcutaneous fat. The labia minora lie medially and contain sebaceous glands and nerves.

The labia majora and labia minora provide a split covering for the entrances to the vagina and urethra. The clitoris is located beneath the point where the labia minora meet. The vestibule is the inner area of the labia minora and extends from the clitoris to the posterior fourchette. It contains the vaginal orifice and external urethral meatus. The space between the vagina and the anus is the perineum. These vulvar structures are covered by squamous epithelium [14,15].

The vulva's blood supply is via branches of the internal pudendal artery and to a lesser extent the external pudendal artery [17]. Venous drainage is primarily via the pudendal veins, perineal vein, and the deep posterior vein of the clitoris [18]. Lymphatic drainage from the vulva is principally to the superficial inguinal nodes, and to a lesser extent the deep inguino-femoral nodes [17].

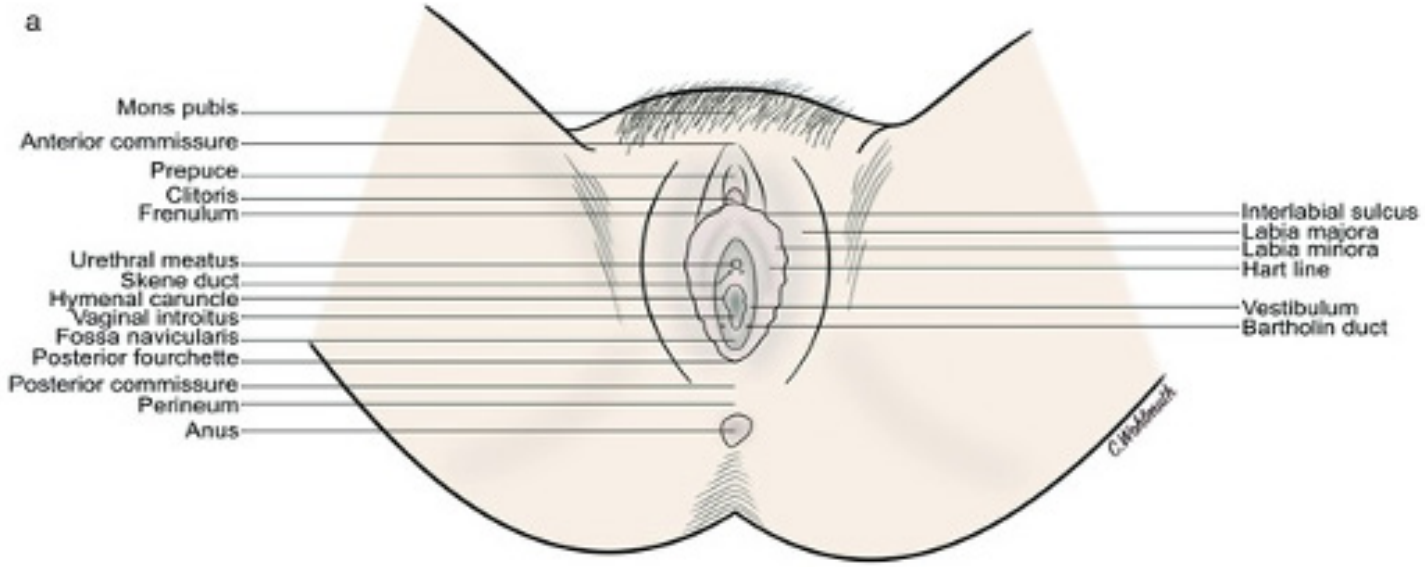

Figure 2.1.1 | Diagram depicting the structures of the vulva [16].

(Figure reprinted from Journal of the German Society of Dermatology, Vol 17(12), Wohlmuth C, Wohlmuth-Weisner I, Vulvar malignancies: an interdisciplinary perspective, Pages 1257-1274, Copyright (2019), with permission under the Creative Commons License Deed).

2.1.2 | Etiology of Vulvar Squamous Cell Carcinoma (VSCC) and Precursor Lesions

VSCC develops through two separate etiologic pathways. The first, keratinizing type of vulvar cancer generally occurs in elderly women, is often associated with lichen sclerosus, and/or differentiated vulvar intraepithelial neoplasia (dVIN) and is unrelated to smoking or the human papillomavirus (HPV) infection. The second type of vulvar cancer, which is characterized by a warty or basaloid histopathology, is generally seen in younger women, has been associated with smoking and HPV infection, and is characterized by the presence of 'usual type' vulvar intraepithelial neoplasia (uVIN), in association with the invasive component [19-21]. These two types of vulvar squamous cell carcinomas have different epidemiological, pathological, and clinical features, and should therefore be considered as two distinct entities [22,23].

HPV DNA has been detected in approximately 20 - 40% of vulvar cancers [21,24-28]. HPV 16 is the most common type, with the next most frequent types being 18 and 33 [8,21,24,25,28].

Squamous Precursor Lesions of the Vulva

Squamous precursor lesions of the vulva have been variously classified for more than 100 years [23]. In 1912, Bowen was the first to report on these intraepithelial lesions, and they were commonly referred to as Bowen's disease [29]. Since its inception in 1970,

the International Society for the Study of Vulvovaginal disease (ISSVD) has been one of the leaders in developing and defining the histologic classification of vulvar disease and precursor lesions of cancer of the vulva [30].

Vulvar Intraepithelial Neoplasia (VIN) is a cellular abnormality of the vulvar epithelium. Previously, the classification for squamous VIN was sub-divided in a similar way to cervical intraepithelial neoplasia (CIN), i.e., VIN 1 (showing mild atypia), VIN 2 (moderate atypia), or VIN 3 (severe atypia, carcinoma in-situ) according to the degree of cellular histologic abnormality. In 2004, the ISSVD revised this classification, as there was no evidence to suggest a disease biological continuum from VIN 1 to VIN 3 as was implied by this classification [31,32].

The terms VIN 1, VIN 2 and VIN 3 are no longer used. The histological equivalent of low-grade VIN lesions is now described as wart or HPV infection [31,32]. The term VIN now applies to histologic high grade squamous intraepithelial lesions, of which there are two categories: (i) VIN, usual type, uVIN (subcategorized histologically as warty, basaloid, or mixed), and (ii) VIN differentiated type, dVIN.

(i). *VIN, usual type*, encompasses VIN 2, VIN 3, and the previous older histologic terms: Bowen's disease, bowenoid papulosis, dysplasia, and carcinoma in situ. These lesions are usually associated with high-risk HPV types, most commonly HPV 16. Clinically they may be a unifocal or multifocal lesion, and typically present as patches, erosions, plaques, or papules, which may appear hyperkeratotic, verrucous, pigmented, or have red or white changes [31]. Invasive squamous cancer of the warty or basaloid type is associated with this type of VIN [27,33].

(ii). *VIN, differentiated type* (dVIN), is much less common. It is seen particularly in older women and is not associated with HPV. dVIN is generally apparent in a background of lichen sclerosus, and the lesion is usually seen as an ulcer, papule, or hyperkeratotic plaque [31].

In 2012, the Lower Anogenital Squamous Terminology (LAST) was introduced to provide a combined terminology for all squamous HPV lesions of the lower anogenital tract [34]. The LAST classification included only two pathological categories, low grade squamous intraepithelial lesions (LSIL), and high grade squamous intraepithelial lesions (HSIL) [34]. In relation to vulvar cancer, this revised terminology raised concerns about the absence of reference to dVIN, and the potential for overtreatment of low-grade squamous intraepithelial lesions [30]. These LAST classifications were subsequently accepted by the ISSVD, but with differentiated VIN now included as a separate category [30].

Table 2.1.1 Current ISSVD classification for squamous vulvar intraepithelial neoplasia (VIN), and the previous 2004 ISSVD VIN classification.

Current ISSVD Classification [29] Lower Anogenital Squamous Terminology, 2012	2004 ISSVD Classification [30]
Low-grade squamous intraepithelial lesion of the vulva or vulvar LSIL (including flat condyloma, or HPV infection)	Flat condyloma, or HPV infection
High-grade squamous intraepithelial lesion of the vulva or HSIL of the vulva	Vulvar intraepithelial neoplasia (VIN), usual type, (uVIN) (subcategorized histologically as warty, basaloid, or mixed)
Vulvar intraepithelial neoplasia, differentiated type (dVIN)	VIN differentiated type, (dVIN).

ISSVD, International Society for the study of vulvovaginal disease.
(Modified from Bornstein et al.[29] and Sideri et al.[30]).

As the terminology for vulvar squamous intraepithelial lesions changed over the course of this thesis, the pathological identification of VIN was based on earlier ISSVD classification systems. In this literature review, the terminology used for pre-invasive diseases of the vulva refers to the classification system as described in the individual research publications. For the subsequent data analysis of vulvar squamous intraepithelial lesions, patients treated prior to the use of the ISSVD 2004 classification system were reclassified using this terminology (see Table 2.2.1).

VIN and its Relationship to Squamous Vulvar Cancer

VIN had generally been considered to have a low malignant potential. However, in 2005, Jones et al. [19] reported a series of 405 cases of usual VIN (described as warty/basaloid or mixed VIN) seen over a 40-year period in Auckland, New Zealand. They found that 10 of 63 (15.9%) women with persistent untreated usual VIN progressed to malignant disease, and 3.8% of women progressed after treatment. In 2012, Wallbillich et al. [35] conducted a retrospective chart review of 303 American patients treated for VIN 2/3 (subtype not described) between 1993 and 2011. They reported that only 7 of 303 (2.3%) patients who received treatment for VIN recurred with an invasive vulvar cancer.

There have been numerous other studies investigating the malignant potential of VIN. They have consistently shown differentiated VIN to have a higher rate of progression to VSCC than usual VIN, but to be much less common [36-38].

Differentiated VIN (dVIN) has also been shown to have a shorter time interval between the preinvasive VIN phase and invasive cancer than usual VIN (uVIN/HSIL) [39,40]. A 2021 study by Thuijs et al. [40] analysed Dutch Pathology Registry data to determine VSCC incidence rates in women diagnosed with both types of high-grade VIN. Between 1991 and 2011, there were 1148 patients diagnosed with high-grade VIN, 254 were excluded from the analysis due to concurrent VSCC. Of the 894 patients remaining, the authors reported that 100/882 (11.3%) patients with usual VIN (HSIL) and 7/12 (58.3%) patients with dVIN progressed to VSCC during follow-up. The median progression time to VSCC for patients with uVIN/HSIL was 4.1 years, and for patients with dVIN it was only 1.4 years.

In a population based Dutch study over the years from 1992 to 2005, van de Nieuwenhof et al. [36] reported that 104 of 1,826 patients (5.7%) with uVIN, progressed to squamous cell carcinoma (SCC), while 20 of 67 patients (32.8%) with differentiated VIN progressed. Despite this, incidence rates of VSCC in the Netherlands remained stable.

2.1.3 | Human Papillomavirus and its Relationship to VIN and VSCC

A 2009 systematic review [21] of HPV type distribution in vulvar (56 studies) and vaginal carcinomas (11 studies), reported that HPV DNA was detected in approximately 40% of the 1,379 invasive vulvar cancers, and in 80% of the 1,340 VIN 2/3 lesions. HPV DNA prevalence was significantly higher among vulvar cancers associated with the warty-basaloid subtypes compared to keratinizing squamous cell carcinomas (85.9% versus (vs.) 6.4%, respectively). HPV type 16 was by far the most frequently detected (29.3% and 71.2% respectively) followed by HPV type 33 (5.6% and 7.7% respectively). Interestingly, the highest prevalence of HPV associated vulvar cancers was found in North America (59.2%), compared to Asia (42.2%), Europe (33.3%), and South America (24.2%). HPV prevalence data for Australia were not available for inclusion in this review.

Similar findings were reported from a meta-analysis of studies investigating HPV prevalence in vulvar, vaginal, and anal cancers between 1986 and 2008. HPV DNA was detected in 40.4% of 1,873 vulvar carcinomas, and in 87.7% of 856 cases of VIN3 (the histological subtypes of VIN were not defined). Again, HPV DNA was more commonly detected in North American women (63.2%) than in women from Asia (38.2%), Europe (34.7%), Australia and New Zealand (28.6%), and Latin America (24.2%), and HPV type 16 was the most prevalent (71.9% of cases of VIN2/3 and 32.2 % of vulvar carcinomas). The strongest variation in HPV prevalence was found by histopathological type of vulvar cancer, with HPV DNA detected in only 13.2% of keratinizing carcinomas, which are generally found in older women, compared to 64.9% of warty-basaloid carcinomas, which

are more common in younger women. In accordance with these findings, HPV DNA was more frequently detected in women younger than 60 years, compared to women older than 71 years [24].

More recently, a systematic review and meta-analysis of 92 papers published between 1990 and 2015 was reported [28]. It included 5,015 cases of vulvar cancer (64 papers) and 2,764 cases of VIN (48 papers) and reported a pooled HPV-positive prevalence rate of 40% for vulvar cancers, and 76% for VIN. For the two VIN sub-categories, uVIN and dVIN, the HPV prevalence was 86.2% and 2.0%, respectively. The most prevalent high-risk HPV type was HPV 16, followed by HPV 33 and HPV 18. In this meta-analysis, the authors postulated that the 'negligible' number of patients who had HPV detected in association with differentiated VIN suggested that these lesions were not driven by HPV [28].

There may be other factors affecting the incidence of vulvar cancer in addition to the HPV infection. In Australia in 2004, the Department of Pathology at the Royal Darwin Hospital reviewed vulvar biopsies taken between 1989 and 2002. They reported that the majority of women diagnosed with high-grade VIN and vulvar cancer were young Aboriginal women from remote communities in East Arnhem Land, in the Northern Territory [41]. A subsequent investigation of this geographical cluster by Condon et al. [42] found the age-adjusted incidence rate for squamous vulvar cancer in indigenous women, under 49 years of age was 31.1 per 100,000 women (95% CI: 13.1- 49.1). This was more than 50 times higher than the average Australian rate.

The cause of this vulvar cancer cluster is unknown, but the high prevalence of other anogenital lesions (58% of women in the study group), supported the concept that an oncogenic HPV infection was a key causal factor [42]. This concept was confirmed three years later when Rumbold et al. [43] undertook a cross sectional community-based study of 551 Indigenous women residing in this region to assess the prevalence of oncogenic ano-genital HPV. They found that genital HPV infection (most commonly HPV 16) was significantly more prevalent in the vulva/vaginal/perianal area than in the cervix. The cervical HPV infection rate was comparable to rates reported in other Australian studies. The authors postulated that the large discrepancy between genital sites may be due to more persistent vulvar infections, or potential environmental factors which had impaired host immunity in these women [43]. Subsequently, a genetic case-control study of this vulvar cancer cluster was undertaken from 2011 to 2013 [44]. Thirty Indigenous women from Arnhem Land, previously diagnosed with invasive vulvar cancer or VIN2/3 between 1996 and 2011 were recruited to the study, as well as 62 unaffected controls, matched for age and community. From genomic DNA extracted from saliva samples, no evidence of an effect of genome-wide homozygosity on invasive vulvar cancer or VIN was found in

this community of Aboriginal women. The authors suggested that their results did not definitively exclude the involvement of genetic risk factors and suggested that the use of a different analytical strategy and genetic model may be more successful [44].

Given that HPV DNA 16 and 18 are the most represented types in HPV related vulvar cancers, the current prophylactic vaccines against HPV type 16 and 18 have the potential to reduce the incidence of HPV related vulvar cancers in the future [21,28,45,46]. The newer nono-valent HPV vaccine should also protect against VIN and VSCC caused by HPV types 31, 33, 45, 52, and 58 [47]. As the majority of squamous vulvar cancers are HPV independent, it is therefore estimated that only around 30 - 40% of vulvar cancers would be prevented by these prophylactic vaccines [48].

Non-Squamous Precursor Lesions of the Vulva Associated with VSCC.

Lichen sclerosus (LS) is a chronic inflammatory dermatosis, characterized by a remitting and relapsing lymphocytic response, which usually occurs on the anogenital skin. It occurs in women between 6 and 10 times more frequently than in men [49,50]. Although lichen sclerosus is seen in women of all ages, the incidence peaks during periods of lower oestrogen production (puberty, peri, and post-menopause) [50-52].

Lichen sclerosus is associated with severe pruritis, which leads to vulvar and perianal soreness and may lead to architectural changes and sexual dysfunction [52-54]. One early hypothesis proposed that irritants such as urine, vaginal secretions, or smegma, modified by a psychological component, may cause itch and subsequent scratching in sensitive women. In many women these irritants simply cause itching and scratching leading to lichen simplex chronicus, but women who have the relevant immune genetic profile may be prone to develop lichen sclerosus [55]. The vulvar skin develops a thin, tissue paper like appearance, often accompanied by papules and/or white plaques. Over time, fissures and tears may develop, and scarring can cause fusion of the labia, introital stenosis and agglutination of the clitoral hood [51].

Lichen sclerosus can sometimes be asymptomatic and not diagnosed. For this reason, the true prevalence is unknown [56]. A recent study of histological data retrieved from the Dutch Pathology Registry found that the incidence rate of biopsy-proven lichen sclerosus had almost doubled from 7.4 per 100,000 women diagnosed between 1991 and 1995 to 14.1 per 100,000 women between 2006 and 2011. This rise was attributed to increasing awareness. As women became less hesitant to report vulvar symptoms to their General Practitioners, there was a corresponding increase in the rate of vulvar biopsies [57].

The etiology of lichen sclerosus is uncertain but there is evidence to suggest that

autoimmune mechanisms [58-60], and in some patients, genetic factors are involved in its pathogenesis. Around 10 - 12% of patients diagnosed with LS report having a positive family history [61,62]. Vulvar LS is often seen in association with differentiated VIN [63], but it is unclear whether there is any causal association between the two or simply, coexistence [64].

Squamous cell carcinoma arising within LS only occurs with anogenital disease. The long-term risk of a woman with LS developing vulvar cancer is generally considered to be low, with a lifetime risk of less than 5% [50,65]. Patients with lichen sclerosus in association with squamous hyperplasia have been shown to be at an increased risk for progression to vulvar cancer [66,67].

The goals for treatment of lichen sclerosus are to alleviate the symptoms and associated discomfort, and to prevent anatomical changes to lessen the possibility of malignant transformation [50-52,65]. The mainstay of treatment for lichen sclerosus is the long-term use of potent topical corticosteroids (TCS). There is evidence that non-compliance with this treatment may increase the risk of malignant transformation [65]. In 2015, Lee et al. [65] conducted a prospective longitudinal study of 507 Australian women with biopsy-proven vulvar lichen sclerosus. During the study period, the women were treated with various regimens of potent TCS, and during follow-up were observed for symptoms of vulvar lichen sclerosus, scarring, and/or the development of vulvar cancer. Patients who adhered strictly to the treatment protocol (n = 357) were considered compliant with treatment and the others were considered partially compliant (n = 150). The results showed that biopsy-proven vulvar squamous cell carcinoma or VIN occurred in 0 of the 357 compliant patients (0%) vs. 7 of the 150 partially compliant patients (4.7%) ($P < .001$). Symptoms were suppressed in 333 compliant patients (93.3%) vs. 87 partially compliant patients (58.0%) ($P < .001$), and scarring occurred in 12 compliant patients (3.4%) vs. 60 partially compliant patients (40.0%) ($P < .001$).

2.1.4 | Worldwide Trends in the Incidence and Mortality of Squamous Vulvar Cancer

There have been limited population-based data available on the trends in incidence and mortality of vulvar cancer, presumably attributable to the rarity of the disease [9], and potentially to the previous disease classification system ICD-9, where vulvar cancer was classified as ‘malignant neoplasm of other and unspecified female genital organs’ [68].

Almost 30 years ago, Sturgeon et al. [69] identified an increasing incidence of vulvar squamous cell carcinoma in-situ (now classified VIN) and suggested that this would lead to an increase in the incidence of vulvar cancer. Since that time, many countries have

reported increases in squamous vulvar cancer but with variable trends.

Early studies from the United Kingdom [70] and Australia [71] reported overall vulvar cancer incidence rates to be unchanged, whereas, more recent data from the United Kingdom [12], South Africa [72], and pooled data from several high income countries [11,73], have shown an increase in incidence in vulvar cancer. This mirrors other reported trends in the United States [6,74] and Canada [75], although increased incidence rates in the US are not consistently reported [11,73]. Compared to Germany [76,77], the increased incidence of vulvar cancer is reported to be somewhat lower in other European countries [9,36,78]. Several studies that have examined age-specific incidence trends have reported a significantly increased incidence of vulvar cancer in younger women [9-12,73,78], while some others have not [6,70,74,75].

Early studies reported stable vulvar cancer incidence rates over time, despite a substantial increase in the incidence of VIN. Data from the Norwegian Cancer Registry, for the period 1973-1992, showed that despite a three-fold increase in VIN, only 16 of 468 (3.4%) women with VIN progressed to invasive vulvar cancer. It was also reported from Norway that although squamous cell carcinoma of the vulva increased in incidence during the period 1956 to 1990, the age-adjusted incidence rate remained constant [79].

A later Swedish study, conducted to determine time trends in the incidence of cervical and other genital squamous cell carcinomas for the period 1958 to 1996, also found that despite a 22-fold increase in the incidence of in-situ SCC in the vulvar/vaginal cohort, there was only a small increase in the incidence of VSCC over the 38-year study period.

In these early studies, the authors postulated that the marked discrepancy between the increased incidence of in-situ vulvar disease compared to the relatively stable invasive vulvar cancer incidence rates may be attributed to: (i) women who had encountered the change in social mores in the 1960's, with increased sexual freedom, having not yet reached an age to have developed invasive vulvar carcinoma; (ii) the early diagnosis and treatment of in-situ vulvar disease may have alleviated any increase in invasive vulvar carcinoma incidence [69]; and (iii) the pronounced increased incidence of in-situ disease may have been related to the increased prevalence of HPV infection, but different etiological factors may have been associated with the majority of invasive squamous vulvar cancers [80].

Judson et al. [6] performed a review of 13,176 in-situ and invasive vulvar carcinomas from the Surveillance Epidemiology and End Results (SEER) database for the period 1973 – 2000. They also identified a 411% increase in the incidence of in-situ VSCC, from 0.56 cases per 100,000 women in 1973, to 2.86 cases per 100,000 women in 2000. Despite this 411% increase in VIN, there was only a modest 20% increase in the incidence of invasive

vulvar carcinoma (from 1.8 cases per 100,000 in 1973, to 2.2 cases per 100,000 in 2000). However, when age-specific trends were examined, the peak incidence for in-situ disease was in the 40-49 years age group, which then steadily declined. In comparison, the authors observed almost no change in the incidence of invasive vulvar cancer, especially in women under 50 years of age, but for women older than 50 years, their invasive vulvar cancer risk increased rapidly with age.

Several subsequent population-based studies have reported variable vulvar cancer trends in women of different age groups. The first of these studies was a 2009 analysis of data collected by the Thames Cancer Registry of women diagnosed with vulvar cancer in Southeast England between 1960 and 1999. Somoye et al. [70] found the overall rate of vulvar cancer to be unchanged but reported there was an increased incidence of 1.10/100,000 women in those aged ≥ 80 years, in each 5-year calendar period (95% CI: 0.36 - 1.84/100,000). In contrast, women aged 60-69 years experienced a decrease in incidence of 0.34 per 100,000 women in each of the 5-year periods (95% CI: 0.50 - 0.18/100,000).

In 2013, Schuurman et al. [78] performed a Dutch population-based study of 4,614 women diagnosed with invasive vulvar cancer between 1989 and 2010. They reported an overall increase in incidence from 2.0/100,000 in 1989 to 2.7/100,000 in 2010. There was no observed increase from 1989 to 2002, but a statistically significant annual increase of 5% (95% CI: 2.7-7.7) in all women between 2002 and 2010. For women under 60 years, there was a significant increase (Estimated annual percentage change (EAPC) 3.5%; 95% CI: 2.0 - 4.9) in incidence over the whole study period, and in women older than 60, an increase was observed between 2004 and 2010 (EAPC 5.0%; 95% CI: 1.5 - 8.6).

Similar results were observed in an earlier cross-sectional analysis of US cancer incidence data. In 2008, Saraiya et al. [74] reviewed data on in-situ and invasive squamous vulvar cancer from 39 population-based cancer registries, covering the years 1998 to 2003. They reported that the incidence of in-situ disease increased with age until ages 40 to 49 years, and then declined gradually, whereas the incidence rates of invasive squamous vulvar cancer gradually increased until 60 to 69 years, following which there was a strong increase with advancing age. Of interest in this analysis was the differentiation between race and ethnicity. The authors determined that there were similar age-specific incidence rates of invasive cancer for white and black women under 50 years, but after 50, the rates increased more rapidly among white women compared to black women. It was also found that squamous vulvar cancer mortality rates increased with age, particularly in white women after 79 years of age.

The following year, another population-based study also reported a rising trend in the

age-adjusted incidence of invasive vulvar cancer in the United States. Bodelon et al. [81] analysed 6,632 new cases of invasive vulvar cancer diagnosed between 1973 to 2004, registered in nine US cancer registries. They reported a 1% yearly increase in invasive vulvar cancer over the period (Annual percent change (APC) 1%; 95% CI: 0.6% - 1.4%). Furthermore, these authors also reported the increase in incidence was most evident in white women compared to black women, or women from other ethnic groups. In contrast to the earlier US study by Saraiya et al. [74], the rise in invasive cancer incidence rates was apparent in all age categories.

Similar incidence trends have been noted in Danish women. Following a review of data from the Danish Cancer Registry for the period 1978 to 2007, Baandrup et al. [9] reported a significant increase in the age-standardised incidence rate of squamous cell vulvar carcinoma in-situ of 1.97% per year (95% CI: 0.99% - 2.96%). There was a 1.6% per year (95% CI: 0.50% - 2.71%) increased incidence of invasive squamous vulvar cancer in women below 60 years of age. However, unlike two of the earlier reviews [74,78], the incidence of invasive cancer among women older than 60 years remained relatively stable.

Although not based on population data, two retrospective reviews of women treated for vulvar cancer in single institutions also identified an increasing presentation of younger women with squamous vulvar cancer. In 2000, Joura et al. [7] reported a 157% increase in VSCC in Austrian women under 50 years of age when they compared data from two four -year periods, 10 years apart. Likewise, in 2008, Hampl and colleagues [82] reported on their experience in a German University Hospital over the preceding 27 years. They compared data from three-time frames; 1980-89, 1989-98, 1998-2007. They compared the latest cohort with the earliest and found that the number of women treated for invasive squamous vulvar cancer had nearly doubled, and the percentage of women aged 50 years or younger had increased 4-fold (11.3% vs. 41.2%).

A later analysis of population-based data from the United States and Canada also showed an increased incidence of invasive squamous vulvar cancer in the US (between the years 1973-2010), and in Canada (1992-2008). The greatest increase for the Canadian cohort occurred in the 80 years and older age group, where the incidence increased from approximately 9.0 per 100,000 to more than 11.5 per 100,000 ($p = 0.05$). The authors also reported that two and five-year relative survival rates had decreased over time for all age groups, but this was most apparent in the 80 years and older cohort [75].

Contrary to these results, a review of English Cancer Registry data for the years 1990 to 2008 identified an overall increase in the incidence of invasive vulvar cancer in women aged up to 70 years ($p = 0.018$) but a decrease in incidence for women 80 years and older

($p < 0.001$). There was also a statistically significant decrease in mortality in women 60 years and older ($p < 0.001$) [12].

The first population-based study to analyse Australian vulvar cancer trends was published in 2010. It was conducted ostensibly to review the epidemiological evidence for the role of HPV in cancer. From this study, Grulich et al. [71] reported little change in the incidence of HPV associated squamous vulvar cancer over the previous two decades. The estimated annual percentage change in age-standardised incidence rates was only 0.15% (0.39% to 0.70%). Note that our study (Barlow et al.) was published in 2015 [10], and provided an updated analysis of vulvar cancer rates in Australia between 1982 and 2009. The main conclusion of our analysis was that although age-standardised incidence rates of vulvar cancer in women across all ages did not significantly change between the periods 1982 - 1984 compared to 2007 - 2009, there was a significant 84% increase in vulvar cancer incidence in women younger than 60 years (SRR 1.84; 95% CI: 1.49-2.26), with no change for women 60 + years (SRR 0.90; 95% CI: 0.79-1.04). We also identified a 22% decrease in age-standardised vulvar cancer mortality rates between the years 1982-86 and 2007-11 (from 0.7 to 0.5 per 100,000 women; SRR 0.78; 95% CI: 0.66-0.93). This decrease was driven by declines in women 60 + years, (SRR 0.76; 95% CI: 0.63 - 0.91) where there was a 24% decrease in mortality. Mortality rates were stable in women younger than 60 years (SRR 1.05; 95% CI: 0.62-1.79), (see Chapter 3 for the full details).

Two recent studies from Germany have reported an increasing incidence of vulvar cancer but at somewhat variable rates. In 2015, an analysis of population-based data retrieved from eight German cancer registries was published. The results from 12,711 cases of invasive vulvar cancer (12,205 squamous cancers), indicated that age-standardised incidence rates had increased by 6.7% per year (95% CI: 5.6 – 7.9), from 1.7 per 100,000 in 1999 to 3.7 per 100,000 in 2011. This correlated to a standardised rate ratio (SRR) of 2.1 (95% CI: 1.9 – 2.4), with the trend across all age groups, but especially in the under 70 years age groups [76]. A 2018 analysis of Cancer Registry data from Southwest Germany confirmed this increasing trend in German women, but at a much greater rate. Holleczek et al. [77] reported an increase in the age-standardised incidence rate of all vulvar cancers from 1.6 cases per 100,000 women, per year in 1974 – 1978 to 7.9 in 2009 – 2013, representing an increase across all age groups. The authors attributed the observed increase in incidence almost entirely to the squamous cell carcinomas which had an increased age-standardised rate from 1.7 to 7.1 (320%), and largely occurred between 1999 and 2013. Of interest in this study was the finding of an almost exclusive increase in the incidence of small tumours ($\leq$ 2cm) from 1.2 to 6.6 which was observed between the years 1989 – 2013, whereas the number of larger tumours and other invasive cancers remained relatively constant. Over the study period, the age-standardised rate of mortality increased by about 120% from 0.6 to 1.3 deaths per 100,000 women per year.

Their mortality rate increase seems incongruous with the authors' reporting that the rate of clinical stage 1 cancers increased from 31% to 52% over the study period, whereas metastatic stage IV cancers decreased from 21% in 1989 - 93 to 10% in 2009 - 13.

Further evidence of an increasing incidence of vulvar cancer in women younger than 60 years has been emerging over the last four years. In 2017, Kang et al. [11] reported their comprehensive analysis of vulvar cancer data obtained from the International Agency for Research on Cancer (IARC)'s, Cancer Incidence in Five Continents, for the years 1988 – 1992 to 2003 – 2007 (including the Australian vulvar cancer data shown in Chapter 3). This study assessed trends in the age-specific incidence of vulvar cancer in 13 high-income countries which included Canada, the United States, nine European countries, Australia, and Japan. During the study period, the 5-yearly estimated annual percent change (APC) across all countries increased by 4.6% in women of all ages ($p = 0.005$), and 11.6% in those < 60 years ($p = 0.02$). No change was observed in women aged 60 years and older (5-yearly annual percentage change (APC) 0.1%, $p = 0.94$). The SRR for 2003 – 2007 vs. 1988 – 1992 was significantly elevated in women under 60 years of age (SRR 1.38; 95% CI: 1.30–1.46), but not in women aged 60 years and older (SRR 1.01; 95% CI: 0.97–1.05). Notably, when these authors analysed the data for each individual geographical region, they observed a significant 21% and 18% increase in vulvar cancer incidence in women of all ages in Europe and Oceania/Asia, respectively. This was driven by a 51% increase in incidence in women < 60 years of age in Europe, and a 69% increase in Oceania/Asia over the study period [11].

In 2019, an analysis of South African Cancer Registry data for the years 1994 to 2013 was published. Chikandiwa et al. [72] analysed age-standardised incidence and mortality trends for several HPV-related cancers, including vulvar cancer. The authors reported an overall increase in vulvar cancer incidence rates between 1994 and 2012, with the greatest increase in women aged 30 – 39 years (0.1/100,000 women in 1994-98 vs. 1.7/100,000 in 2009-12). Accordingly, they found median age at diagnosis had declined from 64 years in 1994 to 46 years in 2012. Mortality rates for vulvar cancer had increased by 2.6% over the same period.

In 2020, Bray et al. [73] examined 68 Cancer Registries for international variations in the incidence rates of vulvar cancers, and further assessed time trends in Australia, China, Colombia, India, Norway, Slovakia, the U.S., and the U.K. over the period from 1983 to 2012. They reported a moderate overall increase in incidence of vulvar cancer in Australia, Norway, the United Kingdom, and Slovakia, with a more rapid rise in incidence rates in women aged < 60 years. Vulvar cancer incidence rates in the United States were reported to be stable.

Conclusion

Overall, this review of world-wide population-based data confirms an increased incidence of vulvar intraepithelial neoplasia and stable or increasing rates of invasive vulvar cancer for women of all ages over the last 40 years. These rates vary somewhat across geographical locations, in women of different age groups, and in some instances in women of different ethnicity. More recent studies have reported an increased incidence of vulvar cancer in younger women [9-12,73,78]. Of the few studies to review trends in vulvar cancer mortality, a decrease in mortality, particularly in older women, was evident in the United Kingdom [12] and Australia [10] whereas, the reverse was evident in the United States and Canada [75], Germany [77], and South Africa [72].

The literature identifies several factors which may contribute to the variations described in this review of population-based trends in vulvar cancer, including (i) variations in the time periods examined, (ii) the pooled analysis of data across age groups which masked age trends [11], (iii) geographical variations in the prevalence of the human papillomavirus infection [24,25,83], (iv) the differential case mix of histological types of vulvar cancer rather than the predominant squamous cell carcinoma type which makes comparisons between studies difficult [73], (v) variations in vulvar cancer coding practices in different population-based cancer registries [11,77], as well as the fact that various subtypes of vulvar cancer have been shown to be inconsistently recorded in cancer registry data due to the lack of clarification of the histological subtypes in pathology reports [21,84]. In addition, the implementation of the new disease classification coding system for vulvar cancer from the International Coding of Diseases (ICD)-9 (malignant neoplasms of other and unspecified female genital tract organs) [68] to ICD-10 (vulva, vagina and other unspecified female genital organs) [85] which took place over different time periods in different countries, may also have led to misclassification of vulvar cancer registrations across various regions [11].

Despite potential inconsistencies, population-based cancer registries are acknowledged as being essential for defining and monitoring trends in cancer incidence, and in guiding public health interventions to decrease cancer rates and improve survival [86,87]. The availability of reliable, comprehensive clinicopathologic data is particularly important for research on comparatively rare cancers, such as vulvar cancer [74,88].

In my thesis, I sought to determine both the incidence and mortality trends for all vulvar cancers in Australia, neither of which had previously been addressed. This research will be addressed in Chapter 3.

Table 2.1.2. Summary of trends in the incidence and mortality of invasive vulvar cancer from population-based data.

Author (Publication year) Time period	Geographic region Source of data (% Population covered)	Number of invasive vulvar cancers	Age-adjusted incidence rates of vulvar cancer per 100,000 women	Age specific survival, and/ or mortality rates per 100,000 women	Comments
Sturgeon et al.[66] (1992) *1973 - 1987*	United States *SEER data (10%)	2,346 *VSCC	1.3 (1973-76) 1.2 (1985-87)	Not determined	Stable incidence rates
Iversen & Tretli [76] (1998) *1956 - 1990*	Cancer Registry of Norway (100%)	1,268 VSCC	1.1 (1956-60) 0.9 (1986-90)	5 -year *RSR stable	Stable incidence rates Stable survival rates. Increasing age-excess death rate
Hemminki et al. [77] (2002) *1958 - 1996*	Swedish-Family Cancer Database (100%)	2,289 VSCC	1.77 (1958-69) 2.35 (1990-96)	Not determined	Small increase in incidence
Judson et al. [6] (2006) *1973 - 2000*	United States SEER data (14%)	5,716 All vulvar cancers	1.31 (1973) 1.57 (2000)	Not determined	20% increase in incidence Increasing with age rapidly after age 50
Saraiya et al. [71] (2008) *1983 - 2003*	United States *CDC, *NPCR and SEER data (83%)	13,549 VSCC	(1983-2003) All women- 1.72 White women-1.77 Black women–1.3 Asian/Pacific Islander-0.63	5-year RSR-86% Mortality rates increased with age after 70-79 years	Gradual increasing incidence to age 60-69 years, then sharp increase. After 50 years, higher incidence in white than black women
Bodelon et al. [78] (2009) *1973 - 2004*	United States SEER data (9%)	6,352 All vulvar cancers	Since 1973 *EAPC -1%	Not determined	Increased incidence across all age groups Most evident in white women
Somoye et al. [67] (2009) *1960 - 1999*	South East England Thames Cancer Registry	Not stated All vulvar cancers	3.7 (1960) 3.5 (1999)	Decreased mortality in women aged ≥ 60 in years 1984-99	Incidence unchanged overall Increased in women ≥ 80 yrs Decreased in women 60-69 yrs
Van de Nieuwenhof et al. [35] (2009) *1992 - 2005*	Netherlands *PALGA Foundation (100%)	2701 VSCC	2.6 (1992) 2.5 (2005)	Not determined	Stable incidence rates overall Gradual and strong increase ≥60 years
Grulich et al. [68] (2010) *1982 - 2005*	Australia *AIHW (81%)	Not stated	EAPC -0.15%	Not determined	Overall, incidence stable Aboriginal women ≤ 49 years from Arnhem land incidence rate of 31.1 (50 times the national rate)

Author (Publication year) Time period	Geographic region Source of data (% Population covered)	Number of invasive vulvar cancers	Age-adjusted incidence rates of vulvar cancer per 100,000 women	Age specific survival, and/ or mortality rates per 100,000 women	Comments
Baandrup et al. [9] (2011) *1978 - 2007*	Denmark Danish Cancer Registry (100%)	1865 VSCC	Overall - 1.15 (1978-79) 1.40 (2006-07) < 60 years -0.29 (1978-79) 0.62 (2006-07)	Not determined	Slightly increasing incidence overall. Significantly increased trend in women < 60 years
Schuurman et al. [75] (2013) *1989 - 2010*	Netherlands Netherlands Cancer Registry (95%)	4614 VSCC	2.0 (1989) 2.7 (2010) Overall, EAPC increase of 1.4% EAPC of 3.5% < 60 years EAPC of 5% ≥ 60 years (only from 2004)	Stable survival rates	Incidence rates increased in all women from 2002 Over the whole study period increased incidence strongest in women aged < 60 years.
Aktar-Danesh et al. [72] (2014) *US-1973 – 2010* *Canada-1992 - 2008*	United States SEER data (26%) Canada Canadian Cancer Registry	15,041 VSCC	USA- rates not provided Canada 3.1 (1992) 3.8 (2008) 0.04 increase per year	2- and 5-year RSR decreased for all ages in USA and Canada	Yearly increase in incidence Greatest increase in women aged 80+ years.
Lai et al. [12] (2014) *Incidence 1990 – 2010* *Mortality 1990 - 2009*	United Kingdom (UK) UKCIS (10%)	All vulvar cancers	Overall 2.13 (1990-92) 2.51 (2007-09)	Overall 0.85 (1990-92) 0.64 (2008-10)	Increased incidence in women 20–70 years. Decreased incidence in women ≥80 years Significant decrease in mortality in women aged ≥60 years
Barlow et al. [10] (2015) *Incidence 1982 – 2009* *Mortality 1982 - 2011*	Australia AIHW (84%)	5,715 All vulvar cancers	Overall 2.1 (1982-84) 2.5 (2007-09) SRR, 1.13, later to earlier period. APC (0.5%)	*ASM, 0.7 (1982-86) 0.5 (2007-2011)	Overall, relatively stable incidence. 84% increase in women < 60 years 22% decrease in mortality, driven by women 60+ years

Author (Publication year) Time period	Geographic region Source of data (% Population covered)	Number of invasive vulvar cancers	Age-adjusted incidence rates of vulvar cancer per 100,000 women	Age specific survival, and/ or mortality rates per 100,000 women	Comments
Buttmann-Schweiger et al. [73] (2015) *1999 - 2011*	Germany Eight Cancer Registries (60%)	12,711 All vulvar cancers	1.7 (1999) 3.6 (2011) *ASIR annual increase - 6.7%	Not determined	Increased trend in all age groups, greatest in women aged 30–49 years and 50–69 years.
Kang et al.[11] (2017) *1988 - 2007*	Canada, United States, nine European countries, Australia, and Japan *IARC's, Cancer Incidence in Five Continents (1 – 100%)	All vulvar cancers	Overall, 1988-2007 pooled data - 14% increase driven by Europe -21% increase Oceania/Asia – 18% increase	Not determined	Overall < 60 years -38% increase ≥ 60 years – stable rates < 60 years Oceania/Asia – 69% increase Europe -51% increase
Holleczek et al.[74] (2018) *1974 - 2013*	South West Germany Saarland Cancer Registry (1%)	1,136 All vulvar cancers	1.6 (1974-78) 7.9 (2009-13)	Not determined	390% increase in incidence from 1974 to 2013 which essentially occurred from 1999
Chikandiwa et al.[69] (2019) *1994 - 2013*	South Africa South African National Cancer Registry (% coverage unclear)	Not defined	EAPC 1994 -2012 = 16.1% increase	1997-2013 APC- 2.6% increase	ASIR increased in women younger than 50 years. 0.1 (1994-98) 1.7 (2009-12)
Bray et al.[70] (2020) *1983-2012*	Incidence in 68 countries Time trends in 8 countries IARC's GLOBOCAN database IARC's, Cancer Incidence in Five Continents (1 – 100%)	Not defined	Overall, moderately increasing incidence rates in Aus, Norway, UK., and Slovakia. Stable rates in the US	Not determined	In women < 60 years of age more rapid rise in incidence rates in Aus, Norway, UK., and Slovakia.

*Abbreviations: **SEER**, National Cancer Institute's Surveillance, Epidemiology, and End Results; **VSCC**, Vulvar squamous cell carcinoma; **RSR**, Relative survival ratio or rate; **CDC**, Centers for Disease Control and Prevention; **NPCR**, National Program of Cancer Registries; **uVIN**, Usual vulvar intraepithelial neoplasia; **dVIN**, Differentiated vulvar intraepithelial neoplasia; **EAPC**, Estimated Annual Percent Change, **PALGA Foundation**; The nationwide network and registry of histo- and cytopathology in the Netherlands; **ASIR**, Age-standardised incidence rate; **AIHW**, Australian Institute of Health and Welfare; **UKCIS**, The United Kingdom Cancer Information System; **ASM**, Age-standardised mortality; **IARC's**, International Agency for Research in Cancer; ***AUS***, Australia; **US**, United States.

LITERATURE REVIEW: PART TWO

2.2.1 | Overview of Vulvar Squamous Cell Carcinoma

Clinical Features

Most women present with a vulvar lump or lesion, frequently describing a long history of vulvar pruritis, which is generally related to vulvar dystrophy. Other less common presenting symptoms are pain, bleeding, ulceration, dysuria, and vaginal discharge. Even less commonly, a patient may present with a lump in the groin due to metastases to the lymph nodes [89].

VSCC's can develop anywhere on the vulva but occur most often on the labia majora. The labia minora, clitoris, and perineum are less common primary sites. In 2008, a German single institution study of patients with vulvar cancer treated over a 28-year period reported that tumour localisation had changed from the labia to the area between the clitoris and the urethra. Tumour location in the latter site had increased from 19% in the earlier period to 37% in the most recent period ($p = 0.05$) [82]. This change in localisation has not been reported elsewhere. Multifocal growth pattern is uncommon and is only present in about 5% of cases. Occasionally, the primary cancer is advanced and occupies most or all of the vulva [89].

Diagnosis of Vulvar Cancer

Vulvar cancer can only be diagnosed after histological examination of biopsied tissue. The biopsy can usually be easily performed under local anaesthesia in the outpatient setting. Even if the lesion is small, the primary lesion should preferably be left in-situ to allow the surgeon to determine appropriate surgical margins [89].

Routes of Spread of Vulvar Cancer

Vulvar cancer spreads locally by direct extension to nearby organs, including the vagina, urethra, and anus, and by lymphatic permeation to regional lymph nodes [90,91]. Lymphatic metastases are usually to the superficial inguinal nodes initially and then to the femoral nodes. Most studies report an overall incidence of metastases to the inguino-femoral nodes of around 30% [90,92-96]. Metastases to the pelvic and paraaortic nodes are uncommon, with an overall reported incidence of about 9%, and this rarely occurs without involvement of the ipsilateral inguinal nodes [92]. Haematogenous spread of vulvar cancer is rare without lymph node metastases, and more commonly occurs in patients with three or more positive groin nodes [90].

2.2.2 | Staging and Current Management of Vulvar Cancer

Surgery is the cornerstone of both the staging and treatment of vulvar cancer due to the prognostic significance and therapeutic implications of the status of the lymph nodes [97]. The following paper titled ‘Staging for vulvar cancer’[98] was written to comprise a review of the literature on vulvar cancer staging and to provide an overview of the current management of squamous cell carcinoma of the vulva.

Best Practice & Research Clinical Obstetrics and Gynaecology 29 (2015) 802–811

Contents lists available at ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

4

Staging for vulvar cancer

Neville F. Hacker, AM, MB BS (Hons I Qld), MD (UNSW), FRANZCOG, FRCOG, FACOG, FACS, Professor of Gynaecological Oncology, Conjoint, Director [a, b, *],
Ellen L. Barlow, BN, MN (Hons), Clinical Nurse Consultant [b]

[a] *University of New South Wales, Sydney, Australia*
[b] *Gynaecological Cancer Centre, Royal Hospital for Women, Sydney, Australia*

Keywords:
FIGO
staging
vulvar cancer

Vulvar cancer has been staged by the International Federation of Gynaecology and Obstetrics (FIGO) since 1969, and the original staging system was based on clinical findings only. This system provided a very good spread of prognostic groupings. Because vulvar cancer is virtually always treated surgically, the status of the lymph nodes is the most important prognostic factor and this can only be determined with certainty by histological examination of resected lymph nodes, FIGO introduced a surgical staging system in 1988. This was modified in 1994 to include a category of microinvasive vulvar cancer (stage IA), because such patients have virtually no risk of lymph node metastases. This system did not give a reasonably even spread of prognostic groupings. In addition, patients with stage III disease were shown to be a heterogeneous group prognostically, and the number of positive nodes and the morphology of those nodes were not taken into account. A new surgical staging system for vulvar cancer was introduced by FIGO in 2009. Initial retrospective analyses have suggested that this new staging system has overcome the major deficiencies in the 1994 system.

* Corresponding author. Gynaecological Cancer Centre, Royal Hospital for Women, Barker St, Randwick, NSW 2031, Australia. Tel.: +61 2 9382 6290; Fax: +61 2 9382 6200.
E-mail address: n.hacker@unsw.edu.au (N.F. Hacker).

http://dx.doi.org/10.1016/j.bpobgyn.2015.01.004
1521-6934/

Staging is used to describe the extent of an individual's cancer. Four basic stages are described, and these are assigned by dividing the extent of the disease into four categories, based on increasingly poor prognostic features. Ideally, the 5-year survival for the four stages should be reasonably evenly distributed between 0% and 100%.

For an individual patient with vulvar cancer, an accurate knowledge of the extent of her disease is critical for optimal management, and for determining the prognosis. Staging is also important beyond the individual patient, because it allows patients to be placed in reasonably homogeneous groups, so that results can be compared between treatment centres internationally. It also facilitates entry of reasonably homogeneous groups of patients on to clinical trials.

The most widely used staging system for vulvar cancer is the one defined by the International Federation of Gynaecology and Obstetrics (FIGO) [1], but vulvar cancer may also be staged according to the TNM classification, which is used by both the American Joint Commission on Cancer (AJCC) and the Union for International Cancer Control (UICC) [2]. There was close collaboration between FIGO, AJCC and UICC in developing the 2009 staging system for vulvar cancer.

Earlier FIGO staging systems for vulvar cancer

The first FIGO staging system for vulvar cancer was introduced in 1969. The system was based on a clinical evaluation of the primary tumour and regional lymph nodes, and a limited search for distant metastases [3]. Basically, patients with stage I disease had a primary tumour confined to the vulva $\leq$2 cm in diameter, with no suspicious groin nodes; patients with stage II disease had a tumour confined to the vulva >2 cm in diameter with no suspicious groin nodes; patients with stage III disease had a tumour that had spread to the urethra, distal vagina or anus, or clinically suspicious groin nodes; and patients with stage IV disease had infiltration of the bladder, rectum or proximal urethral mucosa, fixation to bone or distant metastases.

This clinical staging was easy to apply, and it gave a reasonable distribution of prognostic groups, the 5-year survivals being 90.4%, 77.1%, 51.3% and 18% for patients with stages I, II, III and IV, respectively [3]. This prognostic distribution reflected the fact that the status of the lymph nodes is the single most important prognostic factor in vulvar cancer [4,5], and the incidence of lymph node metastases increased with each stage, with 10.7% for patients with stage I disease, 26.2% for stage II, 64.2% for stage III and 88.9% for stage IV [3].

Both microscopic and macroscopic metastases may be present in lymph nodes that are not palpable, and suspicious nodes may be enlarged because of inflammatory changes only. Clinical evaluation of lymph nodes is therefore inaccurate in approximately 20–30% of cases [6,7]. Because vulvar cancer is virtually always treated surgically and the true status of the lymph nodes can only be determined histologically, FIGO introduced a surgical staging system for the disease in 1988.

The 1988 FIGO surgical staging system was modified in 1994, with the subdivision of stage I into IA and IB. Stage IA was a lesion up to 2 cm in diameter, with stromal invasion not greater than 1 mm. Such patients have virtually no risk of lymph node metastases [3], so they can be treated by radical local excision alone. The 1994 FIGO staging is shown in Table 1.

In 1991, the Gynecological Oncology Group (GOG) reported a retrospective analysis of 588 patients with vulvar cancer available from their database [8]. This analysis highlighted a number of problems with the new surgical staging system.

The first problem was that the new system did not give a reasonably even spread of prognostic groupings. The GOG study demonstrated that when the tumour had negative lymph nodes, even primary lesions with up to 8-cm diameter had an excellent prognosis [8]. An analysis of 121 cases of stages I and II squamous cell carcinoma of the vulva managed at the Royal Hospital for Women in Sydney from 1987 to 2005 showed no difference in recurrence rates, time to recurrence or survival between patients with 1988 FIGO stages I or II disease. The 5-year actuarial survival for patients with stage I disease was 97%, compared to 95% for patients with stage II ($p = 0.83$) [9].

A second problem was that patients with stage III disease were a heterogeneous group prognostically, with survivals ranging from 100% to 34% [8]. For example, the GOG study reported six patients with tumours $\leq$2 cm in diameter with negative nodes, but with involvement of the distal vagina and/or

Table 1
1994 FIGO surgical staging for vulvar cancer.

FIGO stage	Clinical/pathologic findings
	C
Stage I	Tumour ≤2 cm in greatest diameter, confined to the vulva or perineum; nodes are negative
IA	As above with stromal invasion ≤1.0 mm[a]
IB	As above with stromal invasion >1 mm
Stage II	Tumour confined to the vulva and/or perineum, >2 cm in greatest dimension, nodes are negative
Stage III	Tumour of any size with: 1. Adjacent spread to the lower urethra and/or the vagina and/or the anus 2. Unilateral regional lymph node metastasis
Stage IVA	Tumour invades any of the following: Upper urethra, bladder mucosa, rectal mucosa, pelvic bone or bilateral regional node metastasis
Stage IVB	Any distant metastasis including pelvic lymph nodes

[a] The depth of invasion is defined as the measurement of the tumour from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion.

urethra. Their survival was 100%. There were 47 patients who had a tumour <2 cm in diameter with one positive node, and their survival was 95%. On the other hand, 28 patients had a tumour >8 cm in diameter with two positive nodes, and their survival was only 34%, yet all of these patients were officially classified as having FIGO stage III disease [8]. In addition, Rouzier et al. reported a cohort of 895 patients with FIGO stage III vulvar cancer who had been registered with the Surveillance, Epidemiology, and End Results (SEER) database from 1988 through 2004. The 5-year overall survival (OS) for patients with regional metastatic nodal disease (39%) was significantly worse than that of patients with locally advanced tumours but negative nodes (62%; $p < 0.0001$) [10].

A third problem with the 1988 FIGO staging was that the number of positive nodes, and the morphology of those nodes, was not taken into account. The GOG study reported a 5-year survival of 90.9% for 385 patients with negative nodes, 75.2% for 125 patients with one to two positive nodes, 36.1% for 40 patients with three to four positive nodes, 24.0% for 19 patients with five to six positive nodes and 0% for 16 patients with seven or more positive nodes [8].

A recent study from Brazil retrospectively analysed 234 patients who underwent inguinal lymphadenectomy for vulvar squamous cell carcinoma between January 1980 and February 2010. Lymph node metastases were present in 107 patients (45.7%). Patients with negative nodes had a disease-specific 5-year survival of 78.2%, compared to 48.7% for patients with one to two positive nodes ($p = 0.004$) and 30% if there were three or more positive nodes ($p = 0.025$) [11].

The significance of the morphology of the positive nodes was not appreciated until 1992, when Origoni et al. demonstrated survivals of 90.0%, 41.6% and 20.6% for nodal metastases <5, 5–15 and >15 mm in diameter, respectively [12]. They also demonstrated that patients whose lymph node metastases had extracapsular spread had a much worse prognosis (25%) than patients in whom the metastatic disease was confined to the node (85.7%; $p = 0.001$). Other studies have subsequently confirmed these findings [13,14]. The percentage of the lymph node replaced by tumour has also been shown to be significant in multivariate analysis [14,15].

The fourth problem was that the number of positive nodes is the critical prognostic factor, not the bilaterality of the positive nodes, yet the 1988 FIGO staging classified patients with unilateral regional lymph node metastases as having stage III disease, and patients with bilateral regional lymph node metastases as having stage IVA disease. Although the majority of reported studies have indicated that bilaterality was not an independent prognostic factor [4,8,12,16], others have suggested that it was [17–19]. Papers suggesting that bilaterality was an independent risk factor included patients with only one positive node in the analysis. This is clearly invalid, because such patients are not at risk of having bilaterally positive nodes.

Just prior to the publication of the 2009 FIGO staging, a Dutch group reported 134 patients with stage III/IVA vulvar cancer. They demonstrated that the presence of bilateral lymph node metastases was not a significant prognostic factor if the correction was made for the number of positive nodes [20].

New 2009 FIGO staging for vulvar cancer

In view of the above considerations, the 1994 FIGO staging was modified in 2009 (Table 2). Stage IA was not changed, but the former stages IB and II were combined to create a new stage IB. This is now a tumour of any size greater than stage IA, confined to the vulva and with negative lymph nodes. All of these patients should have a good prognosis. A tumour of 1-cm diameter with 2 mm of stromal invasion would be stage IB, as would a tumour 3 cm in diameter with a maximum depth of invasion of 1 mm.

The former stage III has been divided into new stages II and III, to overcome the heterogeneity of the former stage III. Patients with spread to the lower third of the urethra and/or the lower third of the vagina or anus with negative nodes form a fairly homogeneous group prognostically, and they are classified as stage II.

All stage III patients now have positive nodes, with subdivision into stages IIIA, B and C to take into account the prognostic implications of the morphology of the nodes. Bilateral involvement is no longer included in the staging, but only the number of the positive nodes, the diameter of the metastatic foci and the presence or absence of extracapsular spread.

Stages IVA and B remain essentially unchanged, except that patients with bilaterally positive groin nodes are no longer classified as having stage IVA disease. They would be classified as having at least stage IIIA (ii).

The 2009 FIGO vulvar cancer staging has been shown to be an improvement on the 1988 staging in several retrospective institutional reviews.

The first report was from Nijimegen in the Netherlands, where 269 patients with vulvar squamous cell carcinoma treated from 1988 to 2009 were retrospectively staged according to the old and revised FIGO staging systems [21]. As a result of the restaging, 113 patients (42.4%) were reclassified into a lower stage. No patients were upstaged. The greatest change was in patients with tumours confined to the vulva with negative lymph nodes – all 81 patients (30.1%) with old stage II disease were downstaged to IB. The 76 patients (28.3%) with old heterogeneous stage III disease were reclassified into stage II (7 patients, 9.2%), stage IIIA (40 patients, 52.6%), stage IIIB (eight patients, 10.5%) and stage IIIC (21 patients, 27.6%). The 31 patients (11.5%) with old stage IVA disease were reclassified into stage IIIA (four patients, 12.9%), stage IIIB (10 patients, 32.3%), stage IIIC (11 patients, 35.5%) and stage IVA (six patients, 19.3%).

Table 2
FIGO staging of carcinoma of the vulva (2009).

Stage I	Tumour confined to the vulva
IA	Lesions ≤2 cm in size, confined to the vulva or perineum and with stromal invasion ≤1.0 mm,[a] no nodal metastasis
IB	Lesions >2 cm in size or with stromal invasion >1.0 mm,[a] confined to the vulva or perineum, with negative nodes
Stage II	Tumour of any size with extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with negative nodes
Stage III	Tumour of any size with or without extension to adjacent perineal structures (1/3 lower urethra, 1/3 lower vagina, anus) with positive inguino-femoral lymph nodes
IIIA	(i) With one lymph node macrometastasis (≥5 mm), or (ii) One to two lymph node micrometastasis(es) (<5 mm)
IIIB	(i) With two or more lymph node macrometastases (≥5 mm) or (ii) Three or more lymph node micrometastases (<5 mm)
IIIC	With positive nodes with extracapsular spread
Stage IV	Tumour invades other regional (2/3 upper urethra, 2/3 upper vagina), or distant structures
IVA	Tumour invades any of the following: (i) upper urethral and/or vaginal mucosa, bladder mucosa, rectal mucosa or fixed to pelvic bone, or (ii) fixed or ulcerated inguino-femoral lymph nodes
IVB	Any distant metastasis including pelvic lymph nodes

FIGO Committee on Gynecologic Oncology. Revised FIGO staging for carcinoma of the vulva, cervix and endometrium. *Int J Gynecol Obst* 2009;105:103–104.

[a] The depth of invasion is defined as the measurement of the tumour from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion.

This study confirmed that, in patients with negative nodes, the tumour diameter was not predictive of OS ($p = 0.475$) or disease-specific survival ($p = 0.915$). There was also a decreasing OS for patients with stages IIIA, IIIB and IIIC disease ($p = 0.005$). The 5-year disease-specific survival was 77% for patients with one positive node, 62% for two or three positive nodes and 28% for four or more positive nodes. Patients with intranodal lymph node metastases ($n = 54$) had a 5-year disease-specific survival of 72% compared to 45% for 32 patients with extranodal spread ($p = 0.004$). There was no significance to the presence or absence of bilaterally positive nodes when the individual stages of disease were taken into account [21].

A retrospective review of 468 patients undergoing surgery for vulvar cancer from the Mayo Clinic and the Medical University, Gdansk, Poland, reported that the new staging system downstaged 31% of patients ($n = 155$), with only one patient upstaged from III to IVA because of grossly ulcerated lymph nodes [22]. The new staging failed to separate stages IB and II in terms of 10-year cause-specific survival ($p = 0.52$), but the authors felt that the complexity and morbidity of treating tumours involving the urethra, vagina and anus were much higher, and would justify the assignment of such tumours to a higher stage. Only 31 patients had stage II disease, limiting the power to find a statistically significant survival difference. Similarly, they were unable to show a statistically significant difference between the substages of stage III, probably because of small numbers of cases in each substage, but they did find a strong trend toward worse survival in patients with extracapsular spread. Their study supported the omission of bilateral lymph node involvement from the new staging system.

The group at the Queensland Centre for Gynaecological Cancer retrospectively reviewed 394 patients treated in Brisbane from 1988 until 2009 [23]. Seventy-two patients were downstaged (18.3%) and five upstaged (1.3%), because of ulcerated groin nodes. Their data confirmed the wisdom of combining the old stages IB and II. OS and relapse-free survival (RFS) for substages IIIA and IIIB were similar and ranged between 50% and 60% at 5 years, but the number of patients in each substage was relatively small. Patients with stage IIIC disease had an RFS of only 18% at 5 years. They concluded that the FIGO 2009 staging system successfully addressed some of the concerns of the 1988 system, and they noted that it especially identified high-risk patients within the heterogeneous group of lymph-node-positive patients.

From the three major retrospective reviews above, it is apparent that the new staging system has seen a major downstaging of about 30% of patients (Table 3). This has mainly involved old patients with stage II disease being downstaged to stage IB. This has generally been considered appropriate, although Tabbaa et al. suggested that tumours >4 cm in diameter had a less favourable prognosis. Less than 1% of patients were upstaged by the new system (Table 3).

A potential problem with the new staging is that the number of patients with stage II disease will be very low. As shown in Table 4, about 20% of patients were classified as stage II in the 1988 FIGO staging system, whereas it is likely to be <5% in the new system.

Implications for pathologists

Accurate histological assessment of diagnostic biopsies and the final resected specimen are important both for staging and for appropriate management. Distinguishing early stromal invasion from differentiated vulvar intraepithelial neoplasia (VIN) can be challenging, particularly as differentiated VIN often occurs in a background of lichen sclerosus. The latter may be misinterpreted as a fibrotic stromal response [24].

By convention, the depth of invasion has been measured from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion [25]. This can be a challenge,

Table 3
Staging changes based on 2009 *FIGO classification*.

Author	Number	Downstaged	Upstaged
van der Steen et al. 2010[21]	269	113 (42%)	0
Tabbaa et al. 2012[22]	468	155 (31%)	1 (0.2%)
Tan et al. 2012[22]	394	72 (18.3%)	5 (1.3%)
	1131	**340 (30.1%)**	**6 (0.5%)**

Table 4
Number of patients with FIGO stage II vulvar cancer.

Author	1988	2009	Total cases
van der Steen et al. 2010[21]	81	7	269
Tabbaa et al. 2012[22]	108	31	468
Tan et al. 1012[23]	51	6	394
	240 (21.2%)	**44 (3.9%)**	**1131**

particularly if the tumour is polypoid or ulcerated, or if it occurs in a location lacking dermal papillae [24]. In these circumstances, tumour thickness (measured from the overlying surface to the deepest point of invasion) may be measured. Fu estimated that the average difference between tumour thickness and the conventional measurement of depth of invasion was 0.3 mm [26].

As part of the new vulvar cancer staging, the pathologist must report not only the number of nodes with metastatic disease but also the size of the metastases (<5 or >5 mm) and the presence or absence of extranodal spread. Tumour within a lymphatic space outside a lymph node is not equivalent to extranodal extension, as it does not represent infiltrative spread of tumour into the surrounding soft tissues. Unequivalent extension of tumour beyond the node must be present for this parameter to be included in the histological report [24].

Management

Management of patients with carcinoma of the vulva must be individualized, and the most appropriate operation must be determined independently for the primary tumour and the groin lymph nodes. In considering the appropriate management of the primary tumour, the presence or absence of associated VIN and the presence or absence of multifocal invasive disease must be taken into account. Any associated VIN will usually be best treated by wide superficial excision, while patients with multifocal invasive disease will often justify radical vulvectomy rather than radical local excision.

For patients with stage IA disease, radical local excision, without lymphadenectomy, is all that is required, because tumours up to 20 mm in diameter with stromal invasion not greater than 1 mm have virtually no risk of positive lymph nodes. It is important to be certain that the patient's tumour has both a diameter of $\leq$20 mm *and* <1 mm of stromal invasion if no lymph node dissection is to be performed. A 30-mm tumour with <1-mm stromal invasion will have a small but definite incidence of positive nodes, and the wider the tumour diameter, the more likely that a sampling error may have occurred.

Patients with stage 1B disease can usually be treated by vulvar-conserving surgery, with at least ipsilateral inguinal–femoral lymph node dissection [9,27]. The surgical margins of excision should be at least 10 mm [28]. If the tumour is close to the urethra, this will mean resection of the distal urethra, which can be performed safely without loss of urinary continence (Figs. 1A and B). Patients with small tumours may be offered sentinel lymph node biopsy [29], after proper discussions of risks and benefits [30].

In a study of patients with disease confined to the vulva and negative lymph nodes treated at the Royal Hospital for Women from 1987 to 2005, current FIGO stages 1A and 1B, 16 of 121 patients (95.9%) were treated with radical local excision, 19 (15.7%) had no lymphadenectomy, and 54 (44.6%) had unilateral inguino-femoral lymphadenectomy. Only five patients (4.1%) underwent radical vulvectomy, in all cases for tumour multifocality. The 5-year OS for the 121 patients was 96.4% [9].

Treatment of patients with stage II disease will create a greater challenge. Although by definition patients with stage II disease will have negative groin nodes, this will only be known after the nodes have been removed. A computed tomographic scan of the pelvis and abdomen will help determine the presence of any enlarged nodes in the pelvis or groin.

If the anus is involved, preoperative radiation, with or without concurrent chemotherapy, is usually used to treat the primary tumour, in order to spare the patient a colostomy [31,32]. Neoadjuvant chemotherapy has also been advocated in this situation, although reported experience is more limited [33,34]. The groins should normally be dissected prior to the radiation therapy, although some may elect to incorporate the groins and pelvis into the primary radiation fields [35,36]. Following radiation

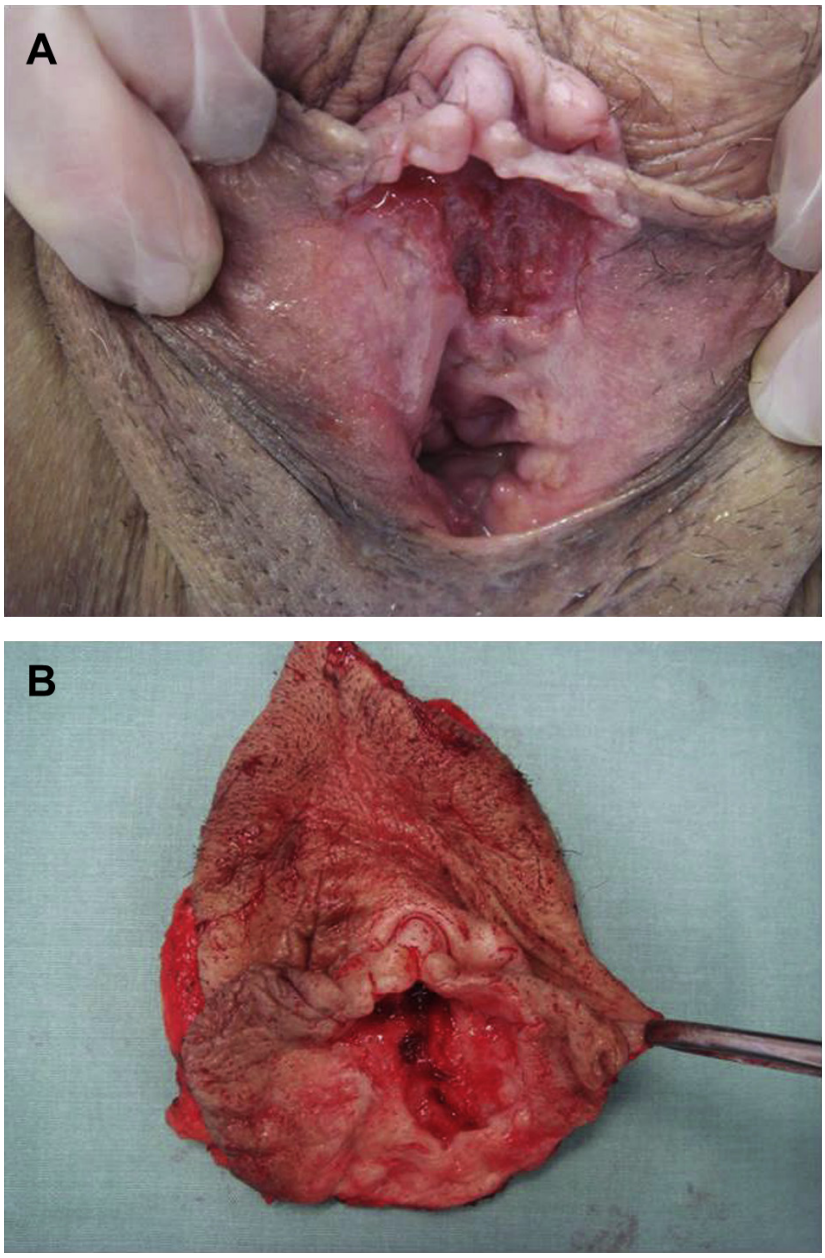

Fig. 1. (A) Ulcerated primary tumour in the anterior vulvar vestibule, close to the urethra. (B) Specimen following radical local excision. In order to achieve margins of at least 10 mm, it was necessary to resect the distal urethra.

to the primary tumour, the tumour bed should be either resected or biopsied extensively to exclude the presence of persistent disease.

If the disease involves the distal urethra or vagina, it may be possible to resect it primarily with clear surgical margins. It is possible to resect about 2 cm of the distal urethra without compromising urinary continence. Primary surgery is usually preferable to primary radiation therapy, and if the surgical margins are <5 mm, consideration should be given to a small field of adjuvant postoperative radiation therapy to try to prevent a local recurrence [37].

All patients with stage III disease have positive lymph nodes. From the reported experience in the literature, there seems to be no justification for adjuvant radiation for patients with one micrometastasis (metastatic deposit ≤5 mm) [38]. Patients with three or more micrometastases, one macrometastasis (>5-mm diameter) or any evidence of extracapsular spread should receive bilateral groin and pelvic radiation [39]. There are insufficient data on patients with two micrometastases to draw definitive conclusions.

Patients with stage IV disease will have either extensive primary disease not amenable to surgical resection without a stoma or fixed, possibly ulcerated groin nodes. Patients with positive pelvic nodes

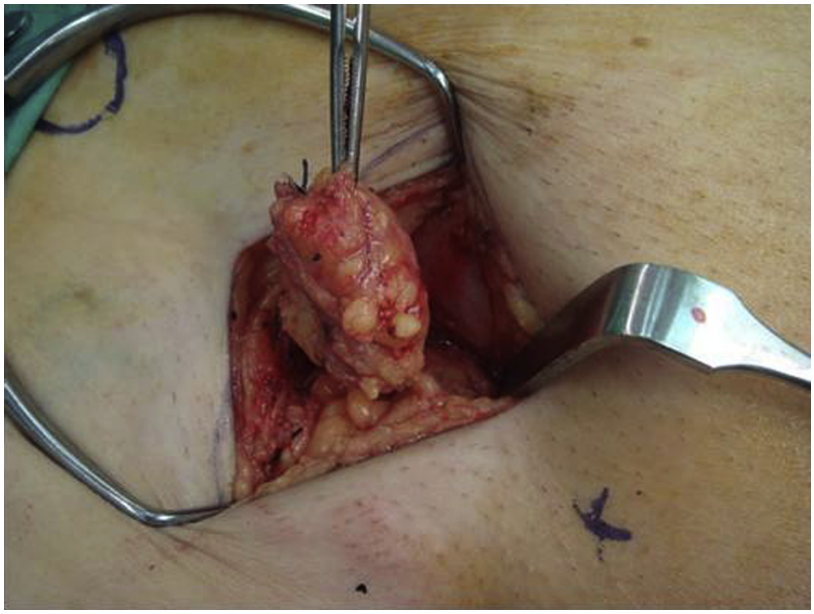

Fig. 2. A bulky groin node being resected. Full groin dissection is not necessary if a frozen section confirms that the node is positive. The patient will require postoperative bilateral groin and pelvic radiation therapy.

are classified as having stage IVB disease, and such patients almost invariably have bulky positive nodes in the groin.

Treatment again needs to be individualized. If primary surgery would necessitate a stoma, preoperative radiation, with or without chemotherapy, is usually used [31,32]. Bulky groin or pelvic nodes should be resected prior to radiation therapy. If a frozen section confirms the presence of metastatic disease, it is not necessary to do a complete groin or pelvic node dissection, but all nodes larger than about 1.5 cm diameter should be resected [40] (Fig. 2).

Summary

The 2009 FIGO staging for vulvar cancer has addressed the poor prognostic spread of the 1994 FIGO staging by combining the old stages IB and II into the one stage, IB. This has meant that about 30% of patients, all of whom have negative lymph nodes and a generally good prognosis, will be downstaged. It has addressed the heterogeneity of the old FIGO stage III disease by classifying all patients with positive groin nodes that are not fixed or ulcerated as having stage III disease. The number of positive nodes and the morphology of those nodes have been used to subclassify stage III into three substages, IIIA, IIIB and IIIC.

Practice points

1. Patients with a primary vulvar cancer up to 20 mm in diameter with up to 1 mm of stromal invasion do not require groin node dissection.
2. All other patients require at least an ipsilateral inguinal–femoral lymph node dissection.
3. Patients with small unifocal primary tumours, certainly not larger than 4 cm in diameter, may be offered sentinel-node biopsy after appropriate counselling regarding risks and benefits.
4. A surgical margin of at least 10 mm around the primary tumour is necessary to prevent a local recurrence.
5. Patients with advanced vulvar cancer who would require a stoma if treated with primary surgery should be offered primary (chemo)radiation, and subsequent resection, or at least extensive sampling, of the tumour bed.

Research agenda

1. Should stage 1B disease be further subdivided based on a primary tumour diameter of ≤4 cm versus >4 cm?
2. Is survival for patients with FIGO stage II significantly different from that of patients with stage IB?
3. Is neoadjuvant chemotherapy as effective as preoperative (chemo)radiation in avoiding primary exenterative surgery in patients with advanced vulvar cancer involving the anus or proximal urethra?
4. What is the optimal size of a groin or pelvic lymph node that can be reliably sterilized with a standard dose of external beam radiation therapy?

Conflict of interest

Neither author has any conflict of interest.

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CORRIGENDUM

On page 29, second paragraph, for *unequivalent* read *unequivocal*.

Prognosis for Squamous Vulvar Cancer

Managed appropriately, the prognosis for vulvar cancer is generally good [89]. Lymph node involvement, most particularly the number of positive nodes, is the most important prognostic factor [90,99-103] and this is reflected in the current FIGO staging system for squamous vulvar described in section 2.2.2 of this chapter.

Following the introduction of radical vulvectomy and inguino-femoral lymphadenectomy, early studies reported overall 5-year survival rates for patients of between 60 and 70% [104-106]. More recent studies, which included patients undergoing conservative surgical resections have reported 5-year overall survival rates of 66 [101] and 76% [107]. Five-year disease specific survival has been reported to be 80% for patients with negative nodes, compared to 68% for those with positive nodes [101]. These later studies would have included more patients with earlier stage disease.

There is well documented evidence that extracapsular nodal spread, and the extent of nodal replacement by tumour, are poor prognostic factors in vulvar cancer [108-110]. In 2006, Raspagliesi et al. [111] performed a retrospective study of 389 patients treated for squamous vulvar cancer at one institution in Milan to identify the clinical and pathological factors related to prognosis. They reported that the 10-year survival for patients with positive lymph nodes without extracapsular nodal spread was 71% compared to 29.8% for patients with extracapsular spread ($p < 0.01$), and 34.3% for patients with > 50% of nodal replacement compared to 55% for patients with < 50% of nodal replacement ($p < 0.01$). On multivariable analysis, percentage of nodal replacement with metastases (HR 6.99) and extracapsular nodal spread (HR 4.88) were the most significant predictors of survival.

A recent study has also shown a poor prognosis for patients with extracapsular spread. In 2016, Luchini et al. [112] conducted a systematic review and meta-analysis to determine the prognostic impact of extra-nodal extension (extracapsular spread) in vulvar cancer. Their review included 13 studies (2,419 patients). They examined the number of deaths and/or recurrences, and calculated hazard ratios for the time-dependent risk associated with the presence of extra-nodal extension. Pooled results showed patients with extra-nodal extension, compared to patients without extra-nodal extension, had significantly higher rates of all-cause mortality in 6 studies (RR = 3.18, 95% CI: 2.02-5.00, $p < 0.0001$), cancer-specific mortality in 3 studies (RR = 2.03, 95% CI: 1.12-3.69, $p < 0.02$), and recurrence in 4 studies (RR = 2.69, 95% CI: 1.61-3.76, $p < 0.0001$).

Other factors reported to be associated with a worse prognosis are tumour size greater than 4cm [95,113,114], lymphovascular space invasion [111,114], depth of invasion [114] and perineural invasion [114-116].

The width of the surgical resection margin is also reported to be prognostically important, although this has recently become a controversial issue. In addition, the presence of epithelial abnormalities in the excised tumour specimen and/or at the resection margin have been suggested to be associated with local disease recurrence. Both these factors will be discussed in depth in Part 3 of this chapter.

Another recent focus of research has been on the influence of HPV or its surrogate biomarker p16INK4A (p16), and the tumour suppressor gene TP53 (p53), on prognosis for VSCC.

2.2.3| Biomarkers p16, p53 and their Relationship to Vulvar Cancer

p16 and its Relationship to HPV

Human cell division and growth occurs through an ordered and tightly regulated process. The cell cycle contains checkpoints that guarantee normal cell cycle progression. These checkpoints consist of four phases, GAP 1 (G1), synthesis (S), GAP 2 (G2), and mitosis (M) [117]. Cyclins and cyclin-dependent kinases (CDKs) are key cellular proteins that regulate the transition from one phase of the cell cycle to the next [117-119]. Once activated, CDKs stimulate downstream progression by phosphorylating selected proteins [119].

The cell cycle is also influenced by negative regulators called CDK inhibitors which impede CDK activity [117,119]. There are two families of CDK inhibitors, one of these is the INK4 family which inhibit cyclin-dependent-kinase 4 [117]. p16 INK4a (p16), a member of the INK4 family, is an important tumour suppressor protein that inactivates cyclin-dependent kinases that phosphorylate the retinoblastoma protein (pRb). This leads to disruption of progression through the G1-S transition checkpoint of the cell cycle [120]. Rb phosphorylation (pRb) status subsequently influences expression of p16. In the presence of a human papillomavirus (HPV) infection, p16 is overexpressed due to the inactivation of pRb by the HPV oncogenes E6 and E7 [121]. p16 overexpression is generally now considered to be a surrogate biomarker of HPV infection (particularly high-risk HPV types) making it effective for evaluating HPV associated squamous abnormalities of the lower genital tract [122-124] and vulvar squamous cell carcinomas [125].

p53 and its Relationship to p16 and Vulvar Cancer

p53 is a tumour suppressor gene involved in cell cycle control and DNA repair, and is inversely related to p16 [126]. After DNA damage occurs in a cell, the p53 pathway produces a set of proteins that can directly aid in DNA repair processes [127].

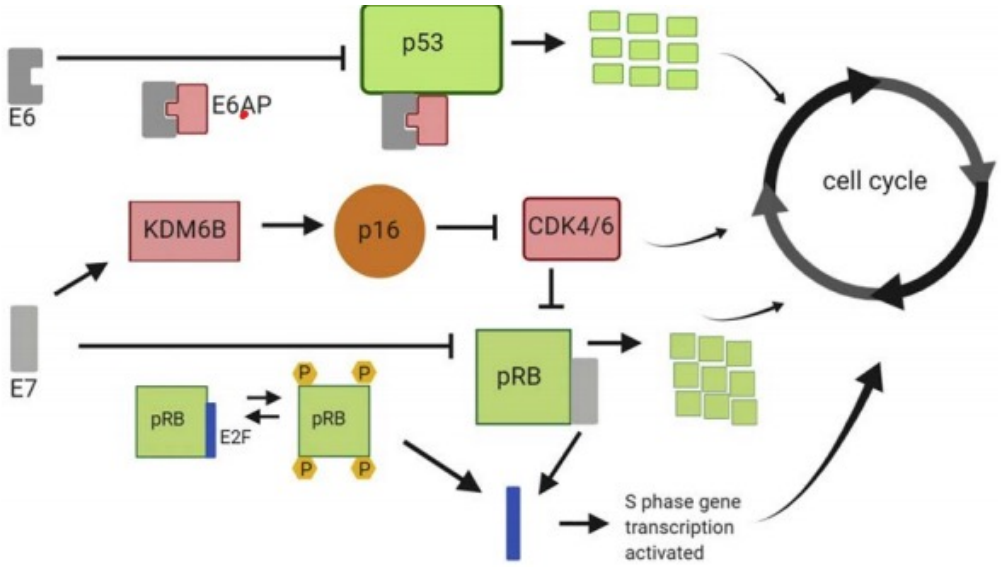

Figure 2.2.1. | The mechanisms of action of HPV E6 and E7 proteins causing p53 and pRB degradation respectively, and finally cell cycle upregulation [128].
(Figure and legend reprinted from Gynecologic Oncology, Vol 158, Zieba S, Chechlinksa M, Kowalik A, Kowalewska M, Genes, pathways, and vulvar carcinoma - new insights from next-generation sequencing studies, Pages 498 - 506, Copyright (2020), with permission from Elsevier).

Abnormal expression of oncogenes and the eradication or mutation of tumour suppressor genes, such as p53, play a critical role in carcinogenesis [126]. Consistent with this role, p53 activity is compromised in a high proportion of all cancer types, either through mutation of the TP53 gene (encoding p53) or changes in the status of p53 modulators. p53 detects oncogenic events in cancer cells and eliminates them through senescence or apoptosis [129]. The molecular mechanism of senescence, which is the irreversible arrest of cell growth, involves both p16 and p53 [130], with different molecular mechanisms resulting in inactivation of p53 [131].

The p53 tumour suppressor gene is recognised as central to the development of many solid tumours [132]. The presence of alterations in p53 activity seem to be a causal factor in the development of vulvar cancer [133], with p53 mutations and p53 overexpression reported in 28 – 68% of vulvar carcinomas [126,132,134-137].

The Presence of HPV-DNA as a Prognostic Factor

Several studies have looked exclusively at the relationship between the presence of HPV-DNA and disease outcomes in patients with vulvar cancer and reported contradictory results. In 1992, Brandenberger et al. [138], retrospectively analysed 44 Swiss patients to determine 5-year overall survival in HPV-positive compared to HPV-negative patients, and were not able to show any significant relationship between HPV status and prognosis. Hording et al. [139], also found no relationship in their retrospective analysis of 62 Danish patients using a similar methodology. Both these studies were disadvantaged by small patient numbers. However, in 2004, Pinto et al. [140] retrospectively investigated the influence of HPV DNA status (and lymph node pathological features) on prognosis in a larger cohort of patients with invasive vulvar cancer. They reported that 23.6% of the 161 tumours were HPV-positive, and like the previous authors found that HPV status was of no prognostic significance.

Two other small studies reported contrary findings. The first was a 1994 retrospective Dutch study of patients with squamous vulvar cancer treated at the one institution. Ansink et al. [141] reported 19 of the 60 (32%) vulvar cancers were HPV-positive, and women with an HPV-positive tumour had a better prognosis than those with a HPV-negative tumour ($p = 0.03$). The following year, Monk et al. [142] from the United States reported similar findings from their prospective study. They found HPV DNA present in 33 of 55 (60%) newly diagnosed VSCC's. On multivariate analysis, when they controlled for lesion size, age, tumour grade, and nodal metastases, HPV positive status remained an independent prognostic factor for improved survival ($p = 0.009$). In line with this, in 2020, Eva et al. [143] performed a retrospective study of 390 patients with VSCC treated between 1990 and 2016 in a single-centre in New Zealand to investigate the incidence of HPV infection and survival by etiology. They reported superior 5-year survival for patients with HPV-dependent compared to those with HPV-independent VSCC (93% vs. 68% respectively, $p < 0.001$).

P16 as a Surrogate Biomarker

Several recent studies from Europe and Canada, using either p16 immunohistochemistry as a surrogate for HPV status, or combined HPV DNA and p16 immunohistochemistry, have also reported HPV/p16 positive status to be prognostically beneficial [8,26,27,144]. In one of these studies, Hinten et al. [27] concluded that HPV related VSCCs should be regarded as a separate entity. Of interest in this study, HPV related cancers were more commonly located on the perineum compared to the non-HPV related cancers, 30% and 14% respectively ($p = 0.001$). The authors postulated that this could be due to the perineum being more susceptible to micro-trauma during sexual intercourse facilitating entry of HPV into the basal cell layer.

In contrast to these results in 2007 Tringler et al. [145] retrospectively evaluated the prognostic significance of p16 expression in 80 patients treated for VSCC. They reported improved disease-free survival for patients with p16-positive tumours in univariate analysis, but failed to show p16 expression to be idependently prognostic in multivariable analysis.

A 2011 single institution study from Spain reported no differences in vulvar cancer survival related to HPV status using combined HPV and p16 status. Alonso et al. [146] retrospectively evaluated the prognostic significance of HPV status and its association with response to adjuvant treatment in 98 women with VSCC. HPV DNA was detected in 19.4% of patients, with p16 staining in 100% of the HPV-positive, and in only 1.3% of the HPV-negative tumours ($p < 0.001$). No differences were detected between HPV-positive and HPV-negative tumours, in terms of either DFS (39.8% vs. 49.8% at 5 years; $p = 0.831$), or OS (67.2% vs. 71.4% at 5 years; $p = 0.791$), although median follow-up in this study was only 45 months.

A 2020 Austrian study performed by Gensthaler et al. [147] to evaluate HPV and p16 status as prognostic factors in 135 patients with VSCC reported p16 status to be an independent factor for DFS (HR 2.12) but found no association with DSS (HR 1.03).

In 2018, two systematic reviews have evaluated the prognostic influence of HPV status in squamous vulvar cancer. Zhang et al. [148] examined 33 studies published between 2000 and 2017, and subsequently included nine studies (the number of patients were not indicated) in their meta-analysis. Their pooled results demonstrated that HPV-positive vulvar cancers were associated with better OS (HR 0.64; 95% CI: 0.47 – 0.87, $p = 0.004$), and DFS (HR 0.66; 95% CI: 0.45 – 0.97, $p = 0.03$) than HPV-negative cancers.

The other meta-analysis by Rasmussen et al. [149] included 18 studies (1,638 patients) published between 1992 and 2017. Again, pooled results showed women with HPV-positive cancers had a significantly improved survival compared to women with HPV-negative cancers with hazard ratios of 0.61 and 0.75 for 5-year OS and DFS, respectively. There was some overlap of data between the two systematic reviews.

p53 as a Prognostic Factor

The correlation between p53 expression and prognosis in patients with squamous vulvar carcinomas is controversial. As early as 1994, a US study hypothesised that HPV-negative tumours of the vulva may have a high incidence of inactivating mutations of p53 while HPV-positive vulvar tumours would seldom have p53 mutations. Lee et al. [133] identified HPV DNA sequences in 12 of 21 (57%) cancers of the vulva, but only one of these 12

(8%) HPV-positive samples had a missense mutation of p53. In contrast, four of the nine (44%) HPV-negative vulvar tumours had point mutations of p53. The p53 mutations were only detected in metastatic lesions and recurrent tumour samples, implying that the acquisition of p53 mutations may be related to neoplastic progression.

Similarly, in 1999, Scheistroen et al. [150] evaluated the pathogenic and prognostic significance of p53 expression in 167 Norwegian women with vulvar cancer. They reported the prognostic impact of p53 overexpression was only evident in stage III disease, where there was a significantly reduced 5-year survival for patients with p53 overexpression compared to those without ($p = 0.004$).

In contrast, a 1997 study from the Netherlands investigated the prevalence of HPV infection and its relationship with p53 overexpression in 66 patients with VSCC. Kagie et al. [126] reported p53 overexpression in 35 of 66 carcinomas (53%). Overexpression of p53 was reported in 54 (57%) of the HPV-negative carcinomas, and 12 (33%) of the HPV-positive carcinomas ($p = 0.23$). The authors concluded that p53 overexpression was common in both HPV-positive and HPV-negative carcinomas, and they found there was no relationship between either HPV infection or p53 overexpression and disease-free survival.

The same year, McConnell et al. [137] also reported that p53 expression failed to correlate with either overall or disease-free survival in a population-based series of 115 squamous cell vulvar cancers from the United Kingdom, but they did find a strong association between p53 overexpression and poorly differentiated tumours.

More recent studies have reported contradictory results. A 2015 study retrospectively reviewed the morphology, immune phenotype, and select molecular features of a consecutive series of 97 American patients with VSCC. Dong et al. [151] reported p16 expression correlated with younger age at presentation ($p < 0.001$), basaloid and warty histologic subtypes ($p < 0.001$), and usual VIN ($p < 0.0001$), and was negatively associated with p53 immuno-positivity ($p = 0.008$). In this study, five of the keratinizing SCCs showed p16 and p53 co-expression, but only one was positive for high-risk HPV. Early clinical stage ($p = 0.006$), p16 expression ($p = 0.002$), and absent p53 expression ($p = 0.02$) were independent predictors of improved overall survival. The authors proposed these results supported an HPV-associated and HPV-independent pathogenesis of VSCC's and validated p16 and p53 immunohistochemistry as markers of disease biology and clinical outcome.

The following year, another US study evaluated p53, p16 and HPV status on local recurrence and DSS in women with early-stage vulvar cancer (FIGO stage 1A-1B).

Hay et al. [134] analysed 92 women treated between 1998 and 2007. HPV testing was done on 39, p16 immunohistochemistry (IHC) on 50, and p53 IHC on 47 tissue samples. Univariate analysis indicated that patients with p16-positive tumours were less likely to have a recurrence (HR 0.31), as were those with HPV-positive tumours compared to those with HPV-negative tumours (HR 0.21). There were also no vulvar cancer-related deaths in either of the p16-positive or the HPV-positive cohorts. The patients with p53-positive tumours were 3 times more likely to have a recurrence (HR 3.23; 95% CI: 1.15-9.06), and almost 7 times more likely to die from their vulvar cancer (HR 6.85; 95% CI: 1.70-27.68) than those with p53-negative tumours. Based on their findings, they proposed that more aggressive surgical and adjuvant treatment may be indicated in patients with p53-positive VSCCs.

In 2017, Nooij et al. [152] performed targeted next-generation sequencing (17 genes), p53 IHC and HPV testing on 36 vulvar cancers and 82 precursor lesions. The three subtypes identified were HPV positive, HPV negative with a TP53 mutation, and HPV negative without a TP53 mutation (wild type). They then analysed the prognostic significance of these three subtypes in a cohort of 236 vulvar cancer patients. They demonstrated that patients with HPV-positive tumours were younger, had earlier FIGO stage disease, and had a lower incidence of positive lymph nodes. Overall, DSS was better for patients with HPV related tumours compared to non-HPV related squamous vulvar cancers ($p = 0.05$). In multivariate analysis HPV positivity remained a favourable independent prognostic factor ($p = 0.02$). Of note, the third HPV negative/p53 wild type subtype of vulvar cancer was found to be associated with frequent Notch 1 and HRAS (transforming protein 21) mutations and to have a five-year local recurrence-free survival intermediate between the other two subtypes (75% for patients with HPV-positive tumours, 67.2% for patients with HPV-negative/p53-wild type tumours, and 56.3% for patients with HPV-negative/p53-mutated tumours). Nooij et al. [152] suggested that a potential clinical implication of these findings might be to perform more radical surgery when HPV was not detected, followed by a more stringent follow-up schedule due to the increased risk of recurrence.

In 2020, another Dutch study was conducted to define the prognostic significance of stratifying VSCCs based on the molecular classification described in the above-mentioned study. Kortekaas et al. [153] retrospectively reviewed 413 patients treated surgically for squamous vulvar cancer in two university hospitals. Of these patients, 18% were HPV-positive, 66% were HPV-negative with a TP53 mutation, and 15% were HPV-negative without a TP53 mutation. They found in their univariate analysis that patients with HPV-negative/p53-mutated tumours had significantly worse overall survival, relative survival, and recurrence free survival compared to patients with HPV-negative/p53-wild type tumours, or those with HPV-positive/p53-wild type tumours. This association was

maintained in the multivariable analysis, but only for recurrence free survival. In both the univariate and multivariable survival outcomes, the HPV- negative/p53-wild type group also had survival intermediate between the other two molecular subtypes.

A 2019 systematic review and meta-analysis by Sand et al. [154] was conducted to determine the prognostic significance of p16 and p53 expression on survival from vulvar cancer. It included 12 studies (475 patients) examining survival according to p16 expression status, and 10 studies (310 patients) according to p53 expression status. Pooled results showed patients with p16-positive tumours had a significantly better 5-year OS compared to those with p16-negative tumours (HR 0.40; 95% CI: 0.29-0.55), while patients with p53-mutated tumours had a significantly lower 5-year OS when compared to those with p53-wild type tumours (HR 1.81; 95% CI: 1.22-2.68). The authors concluded that p53 mutations, and particularly p16 expression, were of prognostic importance for women diagnosed with squamous vulvar cancer.

HPV/p16-positive squamous head and neck [155-158] and anal cancers [159,160] have also been shown to have a better prognosis than HPV/p16-negative cancers. They have also been shown to be more responsive to radiation therapy [156,157,161].

Fakhry et al. [156] conducted a prospective analysis of 96 American patients with stage III or IV SCC of the oropharynx or larynx who received two initial cycles of chemotherapy, followed by concomitant weekly chemotherapy and standard radiation therapy. Patients with HPV-positive tumours had higher response rates after induction chemotherapy when compared to those with HPV-negative tumours (82% vs. 55%, $p = 0.01$), and after chemo-radiation treatment (84% vs. 57%, $p = 0.007$). Patients with HPV-positive tumours also had improved overall survival, lower risk of progression, and death from any cause.

Similar results have subsequently been reported from two retrospective studies from the United States [157] and China [158]. In 2015 Iyer et al. [157] reported on 201 American patients with oropharyngeal cancer (OPC) treated with surgical resection with/without adjuvant radiotherapy between 1985 and 2005. HPV positivity was inferred based on p16-immunohistochemistry. Patients with p16-positive cancers had superior overall survival (74% vs. 44 %, $p < 0.001$).

In 2017, Wang et al. [158] from China investigated the relationship between HPV status and various clinicopathological parameters and survival in 93 patients with oropharyngeal squamous cancers (OPSCC), and 95 with oral squamous cancers (OSCC). They reported that overall survival (OS) of the patients with HPV-positive (p16 positive) OPSCCs was significantly longer than that of those with HPV-negative OPSCCs ($p = 0.004$). Tumour stage and p16 status were independent prognostic factors. Among patients with OPSCCs

who received radio-chemotherapy, those with HPV-positive tumours had improved survival compared to those with HPV-negative tumours (p = 0.015). No differences were observed in patients with OSCCs based on HPV status, but only 9.5 % of patients in the OSCC group had tumours which were HPV/p16-positive, limiting the power of the statistical analysis.

p16/HPV Status and Radiotherapy Response in Squamous Vulvar Cancer

Consistent with findings from other cancer types, in 2016, Lee et al. [162] retrospectively reviewed 57 patients treated with radiotherapy for vulvar cancer between 1985 and 2011 in a US institution. Women with p16-positive tumours had a significantly lower rate of in-field relapse compared to women with p16-negative tumours (HR 0.2; 95% CI: 0.06- 0.6). PFS (65% vs. 16%, p = 0.01), and OS (65% vs. 22%, p = 0.01) were also significantly better for patients with p16-positive tumours, which they attributed to the lower rates of in-field relapse in the p16-positive group.

Likewise, Horne et al. [163] from the US performed a retrospective study to investigate p16 (as a surrogate for HPV positivity) as a predictor for better response rates to neoadjuvant, or definitive chemo-radiation for 76 vulvar cancer patients treated at the Hillman Cancer Center between 2000 and 2016. They reported that p16-positive cancers were more likely to achieve a complete clinical response when compared to p16-negative cancers (63.6% vs. 35% respectively, p = 0.014) and to have a significantly better complete pathological response (63.6% vs. 30% respectively, p = 0.004).

Recently published data further support the concept that p16 positivity may be useful as a prognostic indicator for radiotherapeutic response in patients with vulvar cancer. In 2019, Dohopolski et al. [164] conducted a small retrospective study of 39 women with VSCC treated with surgery and adjuvant RT in one US institution between 2004 and 2016. Ten patients (25.6%) were p16-positive, and 29 (74.4%) were p16-negative. On multivariable analysis, p16 positivity was associated with fewer in-field relapses (HR 0.05; 95% CI: 0.00-0.70, p = 0.026) and improved PFS (HR 0.05; 95% CI: 0.00-0.65, p = 0.022), but not overall survival. The results from this study are compromised by the limited number of patients.

In 2020, Proctor et al. [165] conducted a multi-centre retrospective study to determine if HPV status was a predictor of response to radiotherapy in patients with vulvar squamous cell carcinoma treated with primary radiotherapy. This multi-centre US and Canadian study compared 26 patients with p16-positive/HPV-associated tumours and 22 patients with p16-negative/HPV-independent tumours. The authors reported that patients with p16 -positive VSCCs demonstrated a significantly superior overall survival (HR 0.39, p =

0.03) and progression-free survival (HR 0.35, p = 0.02). In women treated with definitive radiotherapy, p16 positivity was associated with superior overall survival (HR 0.29, p < 0.01) and progression-free survival (HR 0.21, p < 0.01). The results from this study are again compromised by small numbers, and only a univariate analysis was undertaken.

Conclusion

The use of biomarkers to distinguish HPV from non-HPV related cancers in the histopathological evaluation of oropharyngeal cancers is now routine and is increasingly utilised in other HPV related cancers. There is also consensus on the prognostic importance of p16 and HPV in both oropharyngeal and anal cancers.

The review of the early vulvar cancer literature highlights the ambiguous evidence regarding the prognostic relevance of HPV DNA status in squamous vulvar cancer. The evolution of the use of p16 immunohistochemistry as a surrogate marker for HPV infection, along with the use of combined testing (HPV/p16), has provided more conclusive evidence that there are at least two distinct pathways to tumorigenesis in VSCC, and that HPV/p16-positive vulvar cancers have a better prognosis than HPV/p16 -negative cancers.

The early evidence for the prognostic significance of p53 expression was also controversial but more recent studies have confirmed its prognostic importance. It is clear from the literature that the HPV-negative and the HPV-positive pathways do not comprise two homogeneous groups, as clinical and pathological features commonly overlap, and there is evidence of a third pathway. Despite this, several authors have proposed treating patients either more or less aggressively based on the immunohistochemical profile of the tumour.

My thesis sought to further investigate these issues and add data from Australian patients with vulvar cancer to the available evidence. It is apparent from the literature that studies on the combined testing of HPV, p16 and p53 are uncommon in vulvar cancer so we elected to test for all three parameters. This research will be addressed in Chapter 4.

LITERATURE REVIEW: PART THREE

2.3.1 | Implications of the Surgical Excision of Vulvar Cancer

Short-Term Complications Associated with Vulvar Excision.

The most common short-term wound complications after surgery to the primary cancer are vulvar wound infection, and dehiscence (breakdown) [166-170]. Reported incidence rates in studies published since 2000, range from 6 [167] to 75% [166] for postoperative vulvar wound infections, and from 9 [169] to 47% [166] for vulvar wound breakdowns.

The now redundant en bloc procedure was associated with the highest incidence rates for vulvar wound infection (75%) and breakdown (47%) [166]. Other factors found to be associated with an increased risk of vulvar wound complications include, a body mass index (BMI) greater than 25, central or bilateral location of the tumour [166], and the extent of the surgical excision (radical local excision versus (vs.) radical vulvectomy) [167]. One small study reported fibrin sealant (glue) used for inguinal wound closure increased the risk of a vulvar wound infection compared to routine inguinal wound closure (33.3% vs. 14.3%, respectively; $p = 0.01$) [170].

Long-Term Complications Associated with Vulvar Excision.

Long-term complications associated with radical vulvectomy have been reported to include stenosis of the vaginal introitus, alterations to the urinary stream, urinary incontinence [171-173], long term genital numbness resulting in decreased perception of sexual arousal [174], and significantly detrimental effects on sexual function and body image [175,176]. The introduction of radical local excision has been important in reducing long term surgical morbidity [177], but physical and psychological changes can still include urinary incontinence [107], long term genital numbness, and/or decreased genital sensitivity, fear of sexual intercourse, and negative perceptions of body image [178-180].

2.3.2 | Implications of the Surgical Excision of the Groin Lymph Nodes

Despite the evolving surgical options for the management of the groin lymph nodes, any lymph node removal is associated with a risk of both short-term and chronic (long-term) morbidity. Following groin node dissection, groin wound breakdown, wound infection, and lymphocyst formation are the most frequently reported acute postoperative complications [166,169,170,181-188], while lower limb lymphoedema (LLL) and lower limb cellulitis are the most commonly reported long-term (chronic) complications [166,169,170,181-186,188-196]. However, the reported incidence and/or prevalence

rates of these complications, and their association with surgical and clinical risk factors, vary widely (see Tables 2.3.1 and 2.3.2).

Short-Term Complications

Groin wound infection, a wound infection can be difficult to diagnose and is generally inadequately defined in terms of objective clinical factors [197]. The initial clinical indicators for wound infection are erythema, wound heat and increased local pain, which can also be caused by an inflammatory response to tissue injury [198].

A **groin wound breakdown** or dehiscence is a partial or total separation of previously closed wound edges, and typically occurs when healing is still in the early stages following surgery [199].

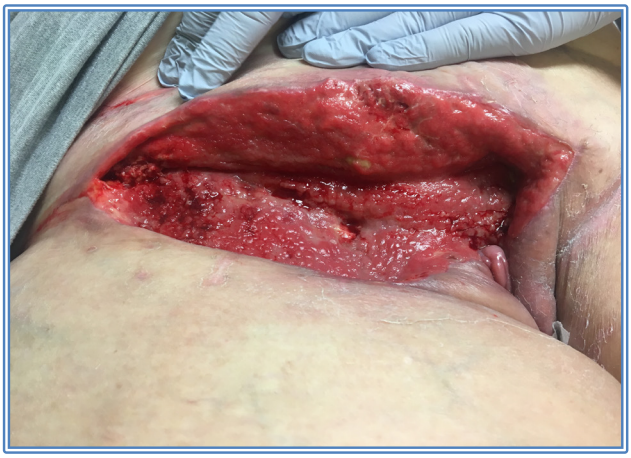

Figure 2.3.1. | Photograph depicting groin wound dehiscence with healing by secondary intention. Note that there is no longer any evidence of infection.
(Photograph taken and reproduced with subject's consent)

A **Lymphocyst** (also termed lymphocele or seroma) is a cyst-like collection of lymphatic fluid which accumulates in the dead space occurring as a result of the surgical dissection of the lymph nodes [200,201]. Lymphocysts may be asymptomatic but can cause considerable morbidity due to infection or pressure [200].

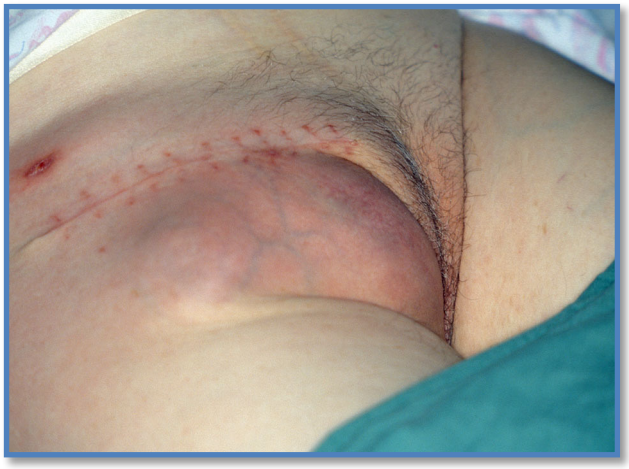

Figure 2.3.2. | Photograph depicting a lymphocyst following inguino-femoral lymph node dissection. Note the primary wound healing.
(Photograph taken and reproduced with subject's consent)

Incidence of Short-Term Complications

Overall, the reported incidence of groin wound infection (or cellulitis), groin wound breakdown (dehiscence), and lymphocyst formation ranges from 4.5 [185] to 59.2% [188], 8 [187] to 47% [166], and 4 [181] to 60% [187] respectively. There is no standard definition for wound infection, surgical wound breakdown, and lymphocyst formation in the vulvar cancer literature, so this may be a contributory factor to the variable rates reported.

Risk Factors for Short-Term Complications Associated with Inguino-Femoral Lymphadenectomy

Several studies have retrospectively investigated the risk factors associated with short-term morbidity following inguino-femoral lymphadenectomy. Diabetes mellitus has been significantly associated with an increased risk for any short-term complication [186]. Obesity has been associated with increased rates of groin wound cellulitis and groin wound breakdown [166,183]. Older age and extent of lymphadenectomy have been associated with increased rates of groin wound breakdown [183,186]. A 2008 study designed to investigate the use of a fibrin sealant for the groin wound, unexpectedly found that the use of a particular type of groin drain (Blake vs. Redivac) was associated with an increased risk of vulvar and groin wound breakdowns [170]. In one small study, higher rates of lymphocyst formation were found in patients in whom staples were used for wound closure compared to a subcuticular suture [192].

Strategies to Reduce Short-Term Complications.

Groin wound drains and their relevance to lymphocyst formation.

The use of groin wound suction drains after lymphadenectomy is generally accepted as a method of reducing the incidence of lymphocyst formation. However, disadvantages of drain insertion can be discomfort, retrograde migration of bacteria [202], and increased length of hospital stay [203,204]. In the vulvar cancer literature, there is limited evidence on the benefit of groin drains in preventing or decreasing the incidence of lymphocysts, and no consensus on a standardised protocol for postoperative drain management to guide practice.

Only four studies have looked specifically at inguinal drains and their association with postoperative morbidity, and three of these were retrospective. In 2011, Walker et al. [192] conducted a retrospective study of 56 patients to determine complication rates associated with different surgical techniques used during groin node dissection. Groin suction drains were used in 44 patients with a median duration of drain usage of 5 days. They reported that overall, groin drains were not associated with an increased risk of complications, but short duration of drain usage (1 – 3 days) was associated with higher rates of groin wound breakdown than usage of 4 – 6 days, or ≥ 7 days ($p < 0.001$). There was also an increased risk of lymphoedema associated with drain usage of ≥ 7 days ($p = 0.01$) [192].

The same year, Hinten et al. [186] conducted a retrospective review of 148 patients following an inguino-femoral lymphadenectomy (IGFLND) to determine risk factors associated with short and long-term complications. All patients had suction drains placed in the groin. They described no standardised drain protocol, but drains were generally left in-situ for a minimum of 5 days and removed when the fluid output was less than 50 – 100 ml per day. Results indicated higher drain production on the day the drain was removed was associated with an increased risk for any short-term complication (Hazard ratio (HR, 1.11), and conferred a small but increased risk for lymphocyst formation (HR, 1.05), and wound infection (HR, 1.05).

The first, and to date only, nationwide prospective study to examine inguinal drain management in patients with vulvar cancer following IGFLND was the ‘Morbidity and Measurement of the Body Study (MAMBO) [187]. This study (2017) involved two observational studies conducted in all eight oncology centres in the Netherlands. One study examined volume-controlled drainage where the inguinal drain was removed when fluid drainage was < 30 ml, or no later than 28 days postoperatively (139 groins). In the other study, the inguinal drain was removed five days postoperatively, regardless of fluid output (112 groins). Volume-controlled drainage (median duration of 13 days) was

associated with significantly fewer complications than short duration drainage (median 5 days), and the incidence of lymphocysts after volume-controlled drainage was 10% compared to 54% after short-term drainage (Risk difference (RD) 44%; 95% CI: 31-56, $p < 0.001$).

In contrast to this result, in 2018, Pontre et al. [188] retrospectively examined postoperative drain management after IGFLND in 71 Australian patients treated in a single centre. They compared 48 patients who had groin drains to 23 patients without drains. No significant differences were observed between the two groups for lymphocyst formation or LLL. One complication found to be causally related to the use of a drain was postoperative groin cellulitis, which was significantly higher in patients who had groin drains (25.4% vs. 8.7%, $p = 0.04$), respectively.

There are several limitations in these studies of drain usage. In two studies, the small sample size limited the power to detect differences between the groups. There were only 10 patients in the 'no drain group' in one study [192], and 23 patients in the other [188]. The remaining two studies only examined postoperative inguinal drain management where all patients had suction drains [186,187]. The retrospective design of three of the studies [186,188,192] may have led to selection bias. In addition, the 'no drain' group in the Pontre et al. study [188] had a much lower median lymph node count compared to the 'drain group' (5 vs. 13) which could suggest a less complete surgical resection that may have influenced the results. It was also the policy of only one of the five surgeons to not use groin drains, and the results may have been subject to surgical performance bias. In all four studies, there would have been several surgeons performing inguino-femoral lymphadenectomy which would have resulted in variations in surgical techniques which could have also introduced bias. It therefore remains unclear from current vulvar cancer literature whether inguinal drains significantly reduce lymphocyst formation following IGFLND.

Breast Cancer Surgery and Suction Drainage

Seroma or lymphocyst formation is the most common short-term post-operative complication after axillary dissection for breast cancer [204,205], and the importance of suction drainage has been more widely examined after axillary lymphadenectomy than after inguino-femoral lymphadenectomy for vulvar cancer. In the breast cancer literature, several studies have reported the duration of drain usage to have no effect on seroma formation [206-208], while others have shown suction drains to be associated with increased patient discomfort and longer duration of hospital stay [203,204].

The standard use of axillary drains has been widely questioned and several studies

have examined the morbidity associated with a policy of not using drains after axillary node dissection [203,204,209]. More than 20 years ago, Jeffery et al. [209] examined women undergoing wide local excision of the breast with axillary lymph node dissection, to evaluate the outcomes of the node dissection without utilising postoperative closed suction drainage. They reported that 34 of 81 women (42%) developed seromas, which accumulated over the first 2 weeks postoperatively and resolved within 4 weeks of surgery. The authors concluded that lymphadenectomy without axillary drainage did not increase seroma formation but reduced the discomfort women experienced associated with drains.

In 2013, Taylor et al. [204], conducted a prospective multi-centre study designed to evaluate the impact of a 'no drains policy' on seroma formation and other complications following surgery for breast cancer. Drains were used in 261 patients, and not in 335 patients. The presence or absence of a drain did not significantly affect the incidence of seromas or wound infection rates, whereas the presence of a drain was associated with a longer hospital stay ($p < 0.001$). In 2015, Troost et al. [203] performed a retrospective cohort study of 44 patients with breast cancer who had an axillary drain compared to 52 patients who did not. Like the previous authors they also found no difference between the two groups for seroma formation (84.6% vs. 90% respectively, $p = 0.30$).

Despite no clear evidence of benefit, groin drains are routinely used in most gynaecological oncology centres. It could be argued based on the evidence that patients who have extended groin drainage experience more discomfort associated with the drain insertion site than is warranted. In my thesis I sought to address this gap in the vulvar cancer literature by examining a large cohort of patients treated over 29 years in the one institution with two different policies for drain usage at different time frames. To date, no vulvar cancer study has examined stopping drains completely, as was done at two time points in our institution. These supplementary research questions will be addressed in Chapter 5.

Supplementary research questions:

- What is the incidence of lymphocyst formation in patients with and without a groin drain following inguino-femoral lymphadenectomy?
- Is short term post-operative morbidity increased in patients with a groin drain?

Table 2.3.1. Table of studies related to short-term morbidity associated with groin lymph node dissection.

Author (Country)	Year	Number of patients (Number of groins)	Groin wound infection % per patient (per groin)	Groin wound breakdown % per patient (per groin)	Postoperative cellulitis % per patient (per groin)	Lymphocyst % per patient (per groin)
Zhang et al. [177] United States	2000	83 (139 groins) *(SV spared - 62 groins) (SV ligated - 77 groins)	--- --- 	(26.6%) (13%) (38%)	--- (29.5%) (18%) (39%)	--- (10.8%) (10%) (4%)
Leminen et al. [162] Finland	2000	149 Radical vulvectomy - 60 Modified vulvectomy - 89	--- --- ---	--- --- ---	--- --- ---	7.4% 8% 7%
Gould et al. [178] United States	2001	67	---	19.4%	35,4%	13.1%
Gaarenstroom et al. [165] Belgium	2003	101 (187 groins)	39% (27%)	17% (11%)	--- ---	40% (27%)
Rouzier et al. [179] France	2003	194 (355 groins) (SV spared - 130 groins) (SV ligated - 225 groins)	--- --- --- ---	--- (29%) (16.2%) (36.4%)	--- (25.3%) (17.7%) (29%)	--- --- --- ---
Judson et al. [180] United States	2004	61 Sartorius transposition – 28 Not transposed - 33	--- --- ---	22.9% 25% 21.2%	57.3% 67.8% 44.5%	26.2% 39.2% 15%
Dardarian et al. [186] United States	2006	29 (49 groins) (SV spared - 18 groins) (SV ligated - 31 groins	--- --- --- ---	--- --- (0%) (25%)	--- --- (0%) (45%)	--- --- --- ---
Zhang et al. [206] China	2007	64 (128 groins) (SV spared – 62 groins) (SV ligated – 66 groins)	--- --- --- ---	--- --- --- ---	--- --- 67.7%) (72.7%)	--- --- (25.8%) (31.8%)
Carlson et al. [166] United States	2008	137 Sutured closure - 67 Fibrin sealant closure - 70	--- 35% 36%	--- 13% 13%	--- --- ---	 16.4% 12.9%

Author (Country)	Year	Number of patients (Number of groins)	Groin wound infection % per patient (per groin)	Groin wound breakdown % per patient (per groin)	Postoperative cellulitis % per patient (per groin)	Lymphocyst % per patient (per groin)
Van der Zee et al. [181] European	2008	Sentinel node (SLN)- 264 SLN + *IGFLND - 47	--- ---	11.7% 34%	4.5% 21.3%	--- ---
Walker et al. [188] United Kingdom	2011	56 (98 groins)	28%	19.6%	---	30%
Hinten et al. [182] Netherlands	2011	164	28.6%	18.8%	---	29.2%
Soliman et al. [189] Germany	2012	34 (64 groins)	--- ---	--- (10%)	--- (24%)	--- (13%)
Pouwer et al. [183] Netherlands	2017	141 (251 groins) *VCD – 77 (139 groins) *SD – 64 (112 groins)	--- 52% (40%) 52% (43%)	--- 8% (5%) 11% (7%)	--- --- ---	--- 16% (10%) 60% (52%)
Pontre et al. [184] Australia	2018	71	59.2%	---	25.4%	32.4%

* Abbreviations; **SV**, Saphenous vein; **SLN**, Sentinel lymph node; **IGFLND**, Inguino-femoral lymph node dissection; **VCD**, Volume controlled drainage; **SD**, Short drainage

Long-Term Morbidity

Lymphoedema is the accumulation of protein rich extracellular fluid within the interstitial spaces of tissues. It arises from a disparity between lymph production and lymph transportation to the systemic circulation [211]. Secondary lymphoedema is a chronic condition that occurs following some extrinsic interruption to lymphatic transportation, where the lymphatic system is unable to maintain tissue fluid homeostasis. As a consequence, the lymphatic fluid accumulates in the interstitial spaces of the subcutaneous tissue and causes an increase in the circumference of the affected limb and progressive fibrosis [212]. In the Western world, the most common cause of secondary lymphoedema is related to malignancy, most particularly surgery, +/- radiotherapy/chemotherapy, or inflammation from metastases [211]. For vulvar cancer patients following a groin node dissection (+/- radiotherapy), lymphoedema manifests as a chronic progressive swelling of the lower limbs (LLL) and/or the genitals, resulting in permanent disfigurement and skin changes [213].

Wide variations in the incidence of LLL have been reported from individual vulvar cancer studies, ranging from 3 to 67% [181,196]. This variation has been attributed to differences in study design, the methods used to detect lymphoedema, i.e. patient self-reporting, clinician observation, or the more precise interventions involving circumferential limb measurements, as well as to the timing of lymphoedema assessment [213].

Risk Factors Associated with Lymphoedema

Multiple factors have been associated with an increased (or decreased) risk of developing LLL in vulvar cancer patients following IGFLND, but these are somewhat inconsistent.

One retrospective study of 164 vulvar cancer patients reported younger age to be associated with an increased risk [186], whereas another smaller retrospective study (n = 28), reported older age and higher body mass index (BMI) to be associated with increased severity of LLL [214].

In 2003, a French study of 194 patients by Rouzier et al. [183] reported that the combined use of radiotherapy, and the extent of the lymphadenectomy were associated with increased rates of LLL. In this study, patients who had a complete inguino-femoral lymphadenectomy had an incidence of lymphoedema of 47%, while those having various modifications of the groin nodes dissection had incidence rates ranging from 11.5 to 32% ($p < 0.001$).

Subsequently, other studies have basically confirmed these findings. Berger et al. [168]

performed a retrospective study of 146 patients with vulvar cancer from the United States. They reported multi-modality treatment, and the greater number of lymph nodes removed, were associated with an increased incidence of LLL. These findings were confirmed in a later prospective study of Australian gynaecological cancer patients (22 patients in the vulvar cancer cohort) which reported that there was a significantly increased risk of LLL in those patients who: (i) had a higher BMI, (ii) were older, (iii) received combined modality treatment and (iv) had a greater number of nodes removed [215].

Another retrospective review (2011) of 56 patients with vulvar cancer from the United Kingdom confirmed that the incidence of lymphoedema was increased if more than seven nodes were removed per groin. Walker et al. [192] also reported that the longer duration of drain use increased the risk of developing LLL. The association between groin drain use and subsequent development of LLL was not confirmed in two other retrospective studies [186,188].

Two studies have used sentinel node biopsy (SNB) as a surrogate for fewer lymph nodes removed and compared the incidence of lower limb lymphoedema in patients having this procedure to that in patients having complete inguino-femoral lymphadenectomy (IGFLND). The first, a large multi-centre observational study conducted in the Netherlands [185], reported LLL incidence rates in 1.9% of 264 patients having a SNB compared to 25.2% of 119 patients having a SNB + IGFLND ($p < 0.001$)). The second study was a 2018 retrospective review of 93 Spanish patients. Rodriguez-Trujillo et al. [216] reported that no patients (0%) having a SNB developed LLL, compared to 33% of patients having SNB + IGFLND ($p < 0.001$).

The influence of ligation of the saphenous vein at the time of groin dissection on the incidence of lower limb lymphoedema is much more controversial. Three studies have reported increased incidence rates of lymphoedema in association with saphenous vein ligation [181,190,210], whereas two other studies were unable to find any association [169,193]. Given that lymphoedema is related to lymphatic, not venous obstruction, and that venous anastomoses in the legs are common, it seems counterintuitive that there would be an association with saphenous vein ligation.

Table 2.3.2 Studies reporting incidence or prevalence of chronic morbidity following groin node dissection.

Author (Location) Year	Study design	Lymphoedema assessment method	Number of patients (Number of groins)	Lymphoedema incidence	Chronic cellulitis	Risk factors associated with Lymphoedema
Zhang et al. [177] (USA) 2000	Retrospective	Clinician diagnosed	(74 groins) *(SV spared - 44 groins) (SV ligated – 30groins)	(32%) (3%) (32%)	--- --- ---	Ligation of the SV
Leminen et al. [162] (Finland) 2000	Retrospective	Clinician diagnosed	149	48.9%	---	Extent of surgery (en-bloc)
Gould et al. [178] (USA) 2001	Retrospective	Clinician diagnosed	67 (112 groins)	29.5%	22.2%	No correlation found
Gaarenstrom et al. [165] (Belgium) 2003	Retrospective	Clinician diagnosed	101 (187 groins)	28% (21%)	---	Occurrence of any early complications
Ryan et al. [185] (Australia) 2003	Retrospective	Self-reported leg swelling	68	47%	---	Not determined
Rouzier et al. [179] (France) 2003	Retrospective	Clinician diagnosed	194 (355 groins) (SV spared - 130 groins) (SV ligated - 225 groins)	--- (37.2%) (23%) (45.3%)	--- ---	Extent of LND Sartorius transposition Adjuvant Rx
Judson et al. [180] (USA) 2004	Randomized clinical trial	Clinician diagnosed	61 (99 groins) Sartorius transposition – 28 Not transposed - 33	26.2% 17.8% 33.3%	--- --- ---	No correlation found
Dardarian et al. [186] (USA) 2006	Retrospective	Clinician diagnosed	29 (49 groins) (SV spared - 18 groins) (SV ligated - 31 groins)	(11%) (38%)	(6.4%) (0%)	Ligation of the SV
Beesley et al. [187] (Australia) 2007	Cross-sectional Gynaecological Cancer	Clinician diagnosed	Vulvar cohort - 53	35.8%	---	Not determined
Zhang et al. [206] (China) 2007	Prospective	Clinician diagnosed	(114 groins) (SV spared – 56 groins) (SV ligated – 58 groins)	25% 48.3%	21.4% 41.4%	

Author (Location) Year	Study design	Lymphoedema assessment method	Number of patients (Number of groins)	Lymphoedema incidence	Chronic cellulitis	Risk factors associated with Lymphoedema
Carlson et al. [166] (USA) 2008	Randomized clinical trial	Circumference measurement	137 Sutured closure - 67 Fibrin sealant closure - 70	63.5% 67% 60%	--- --- ---	No correlation found
van der Zee et al. [181] (European) 2008	Observational European Multi- centre	Clinician diagnosed	403 Sentinel node (SLN) - 264 SLN + *IGFND - 119	1.9% 25.2%	0.4% 16.2%	SLN decreased risk
Hinten et al. [182] (Netherlands) 2011	Retrospective	Clinician diagnosed	160	48.8%	33.8%	Younger age
Walker et al. [188] (UK) 2011	Retrospective	Clinician diagnosed	53	13.2%	----	>7 nodes removed Longer duration of drain use
Soliman et al. [189] (Germany) 2012	Retrospective	Clinician diagnosed	34 (64 groins)	(21.4%)	----	No correlation found
de Melo Ferreira et al. [210] (Brazil) 2012	Prospective	Clinician diagnosed	28	67.9%	----	Older age Higher BMI
Novackova et al. [191] (Czech-Republic) 2012	Prospective	Circumference measurement	29 SLN-12 IGFND -17	31% 25% 37.5%	--- --- ---	No correlation found
Farrell et al. [192] (Australia) 2014	Cross-sectional	Self-reported leg swelling	60	73%	---	Not determined
Berger et al. [164] (USA) 2015	Retrospective	Clinician diagnosed	146 (266) IGFLND – (110) IGFLND + RT – (37) NCRT – (90) NCRT = IGFLND – (29)	--- --- (10.9%) (13.5%) (6.7%) (17.2%)	--- --- 	Extent of lymphadenectomy Not determined otherwise

Author (Location) Year	Study design	Lymphoedema assessment method	Number of patients (Number of groins)	Lymphoedema incidence	Chronic cellulitis	Risk factors associated with Lymphoedema
Hayes et al. [211] (Australia) 2017	Prospective	Self-reported swelling + Bioimpedance Spectroscopy All gynaecological cancers	Vulvar cohort - 22	66.7%	----	**Extent of lymphadenectomy Chemotherapy/ Radiotherapy Increasing BMI, older age Vulvar/vaginal cancer
Pontre et al. [184] (Australia) 2018	Retrospective	Clinician diagnosed	71	12.7%	----	No difference drain/no drain No correlation found
Rodriguez-Trujillo et al. [212] (Spain) 2018	Retrospective	Clinician diagnosed	93 Sentinel node (SLN) - 42 IGFLND +/ - SLN - 51	 0% 33.3%	---	IGFLND
Carlson et al. [190] (USA) 2020	Prospective LEG Study (GOG-244) Multi-institutional	Circumference measurement *Included all gynaecological cancer*s	Vulvar cancer cohort- 42	43%	---	No risk analysis for vulvar cancer cohort

* Abbreviations; **SV**, Saphenous vein; **SLN**, Sentinel lymph node; **IGFLND**, Inguinofemoral lymph node dissection; **LLL**, Lower limb lymphoedema; **LEG**, The Lymphedema and Gynecologic Cancer; **GOG**, Gynecologic Oncology Group; **RT**, Radiotherapy; **NCRT**, Neo-adjuvant chemo-radiotherapy.

** Risks associated with all gynaecological cancers-not specific to vulvar cancer.

2.3.4 | Strategies to Limit Morbidity of Groin Lymph Node Dissection

Saphenous Vein Preservation

The saphenous vein (SV) is a superficial vein of the lower limb which drains medially into the femoral vein [217]. The classical technique of the inguino-femoral lymph node dissection for vulvar cancer was to resect a segment of the saphenous vein in order to facilitate the lymphadenectomy [104,105]. Preservation of the saphenous vein was originally proposed by Catalona et al. [218] for surgery for carcinoma of the penis as an attempt to decrease the risk of lymphoedema, and was adapted for vulvar cancer by Plaxe and colleagues in 1993 [219].

In 2000, Zhang et al. from the US, conducted a retrospective study to investigate the hypothesis that preserving the saphenous vein during lymphadenectomy would decrease postoperative morbidity. They compared two groups of patients, 77 who had the saphenous vein ligated, and 62 who had the vein preserved. They reported higher rates of complications in the group who had the vein ligated: cellulitis (39% vs. 18%, $p = 0.006$), groin wound breakdown (38% vs. 13%, $p = 0.001$), and chronic lymphoedema (32% vs. 3%, $p = 0.003$). The authors recommended that the saphenous vein should be preserved during lymphadenectomy [181].

Two later retrospective reviews also advocated that the saphenous vein should be preserved at the time of groin dissection [183,190]. A French study of 194 patients with vulvar cancer treated in a single institution was reported by Rouzier et al. in 2003. In univariate analysis preservation of the fascia lata and saphenous vein were associated with a decreased risk for groin wound breakdown (16.2% vs. 36.4%, $p < 0.001$), acute cellulitis (18% vs. 29.8%, $p = 0.01$), and chronic LLL (23.1% vs. 45.3%, $p < 0.001$). However, the beneficial effect of venous preservation did not maintain significance for any of these complications in logistic regression analysis. Despite this, the authors still recommended that the saphenous vein should be preserved [183].

A small US study of 29 patients (49 groins) reported short and long-term morbidity following groin dissection. The saphenous vein was ligated in 31 groins and preserved in 18. The clinical characteristics of both groups were similar, and closed suction drains were used in all groins. Cellulitis (45% vs. 0%, $p < 0.001$), groin wound breakdown (25% vs. 0%, $p = 0.02$), and chronic lymphoedema (38% vs. 11%, $p = 0.05$) were all more common in the saphenous vein ligation group, and the authors concluded that preservation of the saphenous may reduce the incidence of acute and chronic morbidity [190]. These results need to be considered with caution due to the small sample size, and the use of only univariate analysis.

The first of two studies to refute the benefit of saphenous vein preservation was conducted ostensibly to determine postoperative complications following vulvectomy and lymphadenectomy. It included 101 patients with vulvar cancer from two centres in the Netherlands. The saphenous vein was ligated in 150 groins and preserved in 19 (status unknown in 18 groins). Saphenous vein ligation was not found to be a risk factor for acute or chronic morbidity following groin lymphadenectomy [169]. The other study from Germany [193] of 34 patients with vulvar cancer undergoing 64 lymphadenectomies, also reported saphenous vein ligation was not a significant risk factor for either acute or chronic morbidity, although the number of patients undergoing the intervention was not specified. Neither study was designed to specifically examine the approach to the saphenous vein. In addition, the ability to detect differences between ligation versus preservation would have been further limited by the small sample size for venous preservation (19 groins) in the first study [169], and the total sample size (34) in the later study [193].

To date, there has only been one randomised study comparing SV ligation with SV preservation in patients with vulvar cancer. Zhang et al. [210] from China evaluated outcomes for 64 patients randomised to either ligation or preservation. Univariate analysis found short-term complication rates were similar between the groups, whereas the incidence of LLL (25% vs. 45.3%, $p = 0.01$), and chronic cellulitis (21.4% vs. 41.4%, $p = 0.05$) were lower in the venous preservation group. The authors concluded that the saphenous vein should be routinely preserved. In this study, randomisation will have eliminated any potential selection bias, but no information on other possible confounding risk factors, such as body mass index, smoking status, and comorbidities has been provided by the authors. It is therefore unclear if the two groups were evenly balanced, and if the results may have been influenced by other factors.

Sartorius Muscle Transposition

Sartorius transposition was initially proposed by Stanley Way in 1960 to protect the femoral blood vessels if a groin wound breakdown and infection occurred [105]. There is limited evidence in the vulvar cancer literature on the effectiveness of this technique and to my knowledge, only two vulvar cancer studies have investigated sartorius transposition.

Rouzier et al. [183] investigated the possible benefits of sartorius muscle transposition in combination with other surgical modifications in their large retrospective French study of 194 patients and found no benefit. Judson et al. [184] from the US conducted a prospective randomised trial. Patients were randomised between groin lymphadenectomy with ($n = 28$) or without ($n = 33$) sartorius transposition. Similar rates of cellulitis, wound breakdown and lymphoedema were reported in both groups. Although lymphocyst incidence was higher in the sartorius transposition group, after adjustment for age, the

groups were similar leading the authors to conclude that sartorius transposition did not reduce wound morbidity.

Modifications to the Lymph Node Dissection to Reduce Morbidity.

Over the last 40 years there have been many other attempts to modify the lymph node dissection to try to decrease the risk of LLL. The sentinel node biopsy is by far the most effective method to date. The procedure selectively limits the number of lymph nodes removed, thereby decreasing acute and chronic morbidity. The following article titled 'Sentinel node biopsy in vulvar cancer: A critical appraisal' [220] has been included in this literature review. It describes the early surgical modifications which attempted to reduce the incidence of lymphoedema, provides a description of lymphatic mapping, and critically reviews the outcome of the sentinel node procedure in vulvar cancer.

Published online: 2019-04-16

Review Article

Sentinel node biopsy in vulvar cancer: A critical appraisal

ABSTRACT

Since the incorporation of inguinal-femoral lymphadenectomy into the management of patients with vulvar cancer in the mid-20th century, there have been attempts to modify or eliminate the groin dissection to decrease the risk of lower limb lymphedema. Early attempts were significantly flawed and resulted in much unnecessary loss of life because recurrence in an undissected groin is usually fatal. The best compromise yet to decrease the risk of lymphedema is sentinel node biopsy, but accumulated evidence now suggests that the false-negative rate for this procedure, if used for lesions up to 4 cm in diameter, is between 5% and 10%. Most women, properly informed of risks and benefits, are not prepared to take a 1% risk of dying from recurrent vulvar cancer to avoid lymphedema. This is the risk involved, assuming a false-negative rate of 5% and an incidence of positive nodes of 20%. For this reason, sentinel node biopsy should not be considered to be standard practice for patients with early vulvar cancer.

Keywords: Lymphadenectomy, lymphedema, sentinal node biopsy, vulvar cancer

INTRODUCTION

The prognosis for patients with vulvar cancer was very poor until the pioneering work of Taussig[1] in the United States and Way[2] in the United Kingdom in the mid-20th century. By paying careful attention to the dissection of the groin lymph nodes, they were able to improve the survival from 20%–25% to 60%–70% although at the cost of considerable morbidity. This was particularly true after the *en bloc* approach to radical vulvectomy and bilateral inguinal-femoral lymphadenectomy popularized by Way, after which patients often spent several weeks in hospital healing their groin wounds.

The use of a separate incision approach for the groin dissection slowly became accepted as the standard of care after the 1981 report of 100 patients treated with this approach by Hacker *et al*.[3] In 1990, Micheletti *et al.* reported that the femoral nodes were located in the fossa ovalis medial to the femoral vein, so there was no need to remove the fascia lata.[4] In 1995, Nicklin *et al.* reported that there could be a 25% reduction in the lateral extent of the groin incision, which helped preserve some lateral lymphatics from the leg which went directly to the axillary nodes.[5] These three modifications significantly improved primary groin healing and did not compromise the removal of all groin nodes [Figure 1].

The status of the groin lymph nodes is the most important prognostic factor for patients with vulvar cancer, but an inevitable consequence of their removal is the later development of lymphedema.[6,7] Ryan *et al.* from our hospital reported lower limb lymphedema in 62% of patients after inguinal-femoral lymphadenectomy.[8] Lymphedema is a lifelong affliction, which requires daily attention to massage and support stockings, so it is not surprising that several attempts have been made over many years to reduce or eliminate this risk.

Neville F. Hacker[1,2], Ellen L. Barlow[1]

[1]Gynaecological Cancer Centre, Royal Hospital for Women, [2]Department of Gynaecological Oncology, School of Women's and Children's Health, University of New South Wales, Randwick, NSW, Australia

Address for correspondence: Dr. Neville F. Hacker, Gynaecological Cancer Centre, Royal Hospital for Women, Locked Bag 1000, Barker Street, Randwick 2031, NSW, Australia.
E-mail: n.hacker@unsw.edu.au

Access this article online	
Website: www.asjo.in	Quick Response Code
DOI: 10.4103/2454-6798.209328	

How to cite this article: Hacker NF, Barlow EL. Sentinel node biopsy in vulvar cancer: A critical appraisal. Asian J Oncol 2017;3:5-11.

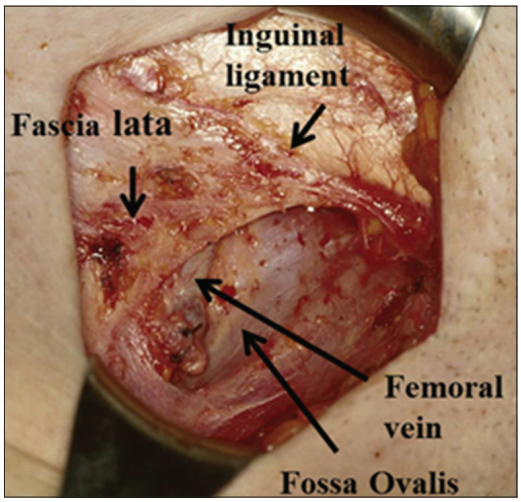

Figure 1: Inguinal-femoral lymphadenectomy. Note the preservation of the fascia lata and the femoral vein in the fossa ovalis

EARLY ATTEMPTS TO DECREASE LOWER LIMB LYMPHEDEMA

The first attempt to decrease the incidence of lymphedema from groin dissection was from Wharton *et al.* at the M.D. Anderson Cancer Hospital in Houston in 1974. They defined "microinvasive carcinoma of the vulva" as a lesion ≤2 cm in diameter with ≤5 mm of stromal invasion.[9] They reported 25 such patients, none of whom had lymph node metastases, and suggested that lymph node dissection could be omitted from this group of patients.

It soon became apparent that this concept of microinvasion was seriously flawed. Further experience revealed that the only patients at virtually no risk of lymph node metastases were those with a tumor ≤2 cm diameter and with ≤1 mm of stromal invasion. Even patients with 1.1–2 mm invasion had a 7.6% incidence of positive nodes in combined series.[10]

The second attempt was from DiSaia *et al.* in 1979. They suggested "superficial inguinal lymphadenectomy" for patients with a lesion ≤1 cm with ≤5 mm stromal invasion. They hypothesized that the superficial inguinal nodes would act as sentinel nodes and that by preserving the femoral nodes, the incidence of lymphedema would be reduced. They reported 18 patients, all of whom had negative nodes, and the survival was 100%.[11]

It soon became apparent that this approach was also flawed. In 1983, Hacker *et al.* reported seven patients from four different Cancer Centers in California who recurred in the groin after a superficial inguinal lymphadenectomy.[12] Subsequently, the Gynecologic Oncology Group (GOG) in the United States conducted a prospective study of superficial inguinal lymphadenectomy for patients with clinical Stage 1 vulvar cancer, (i.e., ≤2 cm diameter) with ≤5 mm stromal invasion and no clinically suspicious inguinal lymph nodes.[13] Once again, the recurrence rate in the groin and subsequent mortality was found to be unacceptably high.

The third attempt to decrease lymphedema involved the use of radiation therapy instead of groin dissection to treat the groin nodes. The GOG conducted a prospective, randomized trial in patients with no clinically suspicious groin nodes. They compared groin irradiation with inguinal-femoral lymphadenectomy (and postoperative radiation for patients with positive nodes) and reported their results in 1992.[14] The study was stopped prematurely after only 49 patients had been entered because there was a 19.2% recurrence rate (5 of 26) in the radiation arm versus 0% recurrence rate in the surgical arm ($P = 0.02$).

With all of these failed attempts to prevent lymphedema, it also became apparent that recurrence in an undissected groin carried a very high mortality. About 90% of these patients were dying of their disease, and this remains true to the present time.[10]

LYMPHATIC MAPPING

The hypothesis behind lymphatic mapping is that the lymphatic drainage from a tumor occurs in an orderly fashion and will initially go to one or more "sentinel" nodes. If the sentinel node is negative, the remainder of the regional nodes will be negative, so complete lymphadenectomy can be avoided in such patients, thereby decreasing the incidence of lymphedema without compromising survival.

This concept was initially introduced by Cabanas in 1977 for the management of men with penile cancer[15] and was later pioneered by Morton *et al.* in 1992 for the management of melanomas.[16]

Two complementary techniques have been used to identify the sentinel node(s): The intradermal injection of technitium^{99} labeled sulfur colloid around the tumor the day before the surgery, and the intradermal injection of a vital blue dye (e.g., isosulfan blue) around the tumor immediately preoperatively.[17-19] The sentinel node(s) is identified by dissecting the groin and identifying the blue node(s) and by the use of a gamma counter intraoperatively [Figure 2]. Ultrastaging is undertaken on all negative sentinel nodes using serial sectioning and immunoperoxidase staining for cytokeratin.

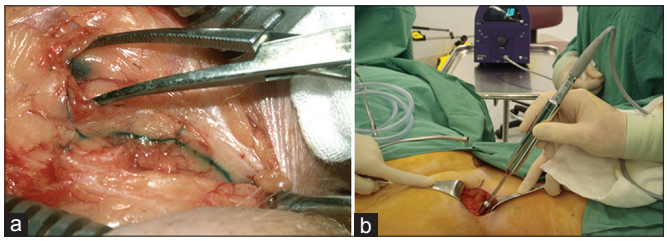

Figure 2: Sentinel node biopsy. (a) Note the blue lymphatics and blue sentinel lymph node, and (b) gamma counter used to identify the radioactive node or nodes

STUDIES OF SENTINEL NODE IDENTIFICATION IN VULVAR CANCER

In 2008, results were published from the multicenter, GROningen International Study on Sentinel nodes in Vulvar cancer study (GROINSS-V).[17] It was an observational study, and to be eligible, patients had to have a squamous cell carcinoma of the vulva <4 cm diameter. There were 403 patients recruited to the study, and they underwent 623 sentinel node dissections. Metastatic sentinel nodes were found in 163 groins (26.2%).

Long-term follow-up from this study was reported on 377 patients with unifocal disease in 2016.[20] The median follow-up was 105 months. As expected, local recurrence was still a problem, being 24.6% at 5 years and 36.4% at 10 years in sentinel node–negative patients. The isolated groin recurrence rate was 2.5% in sentinel node-negative patients, and all 6 patients died of disease. As expected, short- and long-term morbidity were significantly decreased in patients undergoing sentinel node biopsy.

The median diameter of the vulvar cancers in the long-term follow-up of the GROINSS-V study was only 20 mm (range 3–65 mm), yet the 5-year disease-specific survival was only 93.5%. This is low for such small, node-negative tumors. In 2009, we reported the experience with 121 patients with 2009 International Federation of Gynecology and Obstetrics Stage 1B vulvar cancers of all dimensions, treated at our institution. Five patients (4.1%) underwent radical vulvectomy for multifocality, and the remainder underwent radical local excision. All underwent unilateral or bilateral inguinal-femoral lymphadenectomy and were node negative. With a median follow-up of 84 months, the median overall survival at 5 years was 96.4%.[21]

Results from other large studies show higher false-negative rates for sentinel nodes.

The results of a multicenter German study were also published in 2008.[18] This study enrolled 127 patients with primary T1–T3 vulvar cancer. All patients underwent complete inguinal-femoral lymphadenectomy, and positive nodes were identified in 39 cases (30.7%). Three patients had a false-negative sentinel node, and the authors reported a false-negative rate of 7.7%. However, an additional patient with a midline lesion had a positive sentinel node on one side, but a false-negative node on the other, giving an overall false-negative rate of 10.3%. They concluded that sentinel node biopsy was feasible, but not highly accurate and that the false-negative rate was too high except for T1 (2 cm diameter) tumors. Even with these small tumors, the authors reported a false-negative rate of 6.7%, but the patient with the true positive node on one side and false-negative node on the other side had a primary tumor only 18 mm diameter, so the false-negative rate for T1 tumors was 13.3%.

A Polish study of 56 patients and 109 groin dissections was published in 2010.[22] The maximum diameter of the primary tumor was 4 cm, and 99% of patients had both blue dye and lymphoscintigraphy with intraoperative radio localization for sentinel node identification. There were 19 (17%) positive sentinel nodes, but the false-negative rate was 27% (7 cases). The authors concluded: "It is highly probable that the main factor responsible for the high false-negative rate was the surgeon's experience. Although all the operations were performed by surgeons with at least 15 years' experience, the procedure was performed only a few times by each surgeon." This is clearly a problem when dealing with an uncommon disease.

In 2010, the GOG published their results on sentinel node biopsy for squamous cell vulvar cancer.[19] The study included patients with primary tumors up to 6 cm diameter and no clinically suspicious nodes. In all, 452 patients underwent the planned procedures and 418 (92.5%) had at least one sentinel node identified. At least, a unilateral groin dissection was performed on all patients. There were 132 patients (31.6%) with positive sentinel nodes, including 11 (8.3%) with false-negative nodes. For tumors <4 cm, the false-negative rate was 5.6% (4 of 71).

Long-term follow-up of sentinel node biopsy in patients with vulvar cancer was published from Brown University in 2014.[23] They reported results on 69 patients undergoing 111 sentinel node dissections. With a median follow-up of 58.3 months, the groin recurrence rate for patients with negative sentinel nodes was as follows: 0% (0/11) for patients with primary tumors <10 mm, 3.3% (1/30) for tumors 10–20 mm, and 14.3% (2/14) for tumors >20 mm. They concluded that sentinel node dissection was a viable option for patients

with squamous cell carcinomas less than 2 cm diameter. However, as we will show below, the majority of patients would not be prepared to accept a 3.3% risk of recurrence and probable death.

In a recent systematic review and meta-analysis of sentinel node biopsy in patients with vulvar cancer, Meads *et al*. reviewed 29 studies involving 1779 women.[24] They reported a false-negative rate of 9% for clinical follow-up of patients with negative sentinel nodes and concluded that this high false-negative rate highlighted the importance of the learning curve effect. However, the learning curve relates more to the detection of sentinel nodes, and only Levenback *et al.* have looked specifically at this problem. They reported that the failure rate for sentinel node detection was 16% in the first 2 years of their study versus 7% in later years.[25]

For sentinel node identification in patients with breast cancer, Bass *et al.* estimated that 23 patients were required by an individual surgeon to achieve a 90% ±4.5% success rate and 53 patients to achieve a 95% ±2.3% success rate.[26]

Unlike the situation with breast cancer, experience of the individual surgeon with vulvar cancer will always be a problem because the disease is so uncommon.

RECURRENCE RATE FOLLOWING GROIN DISSECTION

One of the assertions made by Van der Zee *et al.* in the GROINSS-V paper was: "The groin recurrence rate in sentinel node-negative patients in the current study (2.3%) seems to be at least comparable to that reported for patients… treated by formal lymphadenectomy of any type."[17]

The 2.3% recurrence rate is favorable compared to patients having a superficial inguinal lymphadenectomy, but this technique was discredited by the GOG study of superficial lymphadenectomy previously discussed.[13] Robison *et al.* claimed that the risk of groin recurrence was 5%–7% for patients with disease confined to the vulva having negative nodes after a superficial inguinal lymphadenectomy,[23] and Hacker and Eifel, in a literature review, reported a groin recurrence rate of 5.3% (31 of 585 patients) for such patients.[10]

By contrast, the risk of recurrence in patients having negative nodes after an inguinal-femoral lymphadenectomy is virtually zero. In a literature review, Hacker and Eifel found only 3 groin recurrences out of 780 reported cases (0.4%).[10]

QUALITY OF LIFE

Oonk *et al.* studied the quality of life for patients from the Groningen study after a sentinel node procedure only and compared it to that of patients having an inguinal-femoral lymphadenectomy because of a positive sentinel node.[27] The study was performed using the EORTC Quality of Life Questionnaire-Core 36 vulvar-specific questionnaire, and they found no difference in overall quality of life between the two groups, in spite of increased complaints of lymphedema in patients having complete groin dissection. They also compared their results to those of two studies in a healthy population of women over 60 years of age and found that the quality of life for their study population was comparable to that of a general age-matched population. They stated: "Our present study does not support our original idea that a decrease in especially long-term morbidity also translates into an improved quality of life for vulvar cancer patients."

The critical issue is not about morbidity, predominantly lymphedema – it is about risk. The question that has to be asked is; "What risk of death is a properly informed patient prepared to take, to avoid the risk of lymphedema?"

Farrell *et al*. undertook a preference study on sixty patients with early vulvar cancer whose treatment at our Institution included at least an ipsilateral inguinal-femoral lymphadenectomy; almost 40% of the patients had lymphedema.[28] The patients were asked for their preference between two stated treatment options: Complete groin dissection, which would result in a 60% risk of lymphedema, but a negligible risk of groin recurrence if the lymph nodes were negative, or sentinel node dissection, which would result in a negligible risk of lymphedema, but a 1:100 risk of a groin recurrence, which would usually be fatal. The 1:100 risk was based on a hypothetical false-negative rate of 5% and an incidence of positive groin nodes of 20%.

Given these two choices, 32 patients (53%) said they would take no risk at all with their life, and a further 6 patients (10%) said they would take a 1:1,000,000 risk. Only nine patients (15%) were prepared to take a 1:2 to 1:100 risk, which would be the risk involved if a sentinel node procedure were to be performed [Figure 3].

An earlier study by de Hullu *et al*. reported similar results on 107 patients previously treated for vulvar cancer with at least an ipsilateral inguinal-femoral lymphadenectomy.[29] Sixty percent of patients said they would choose complete lymphadenectomy rather than risk death from the 5% false-negative rate associated with the sentinel node

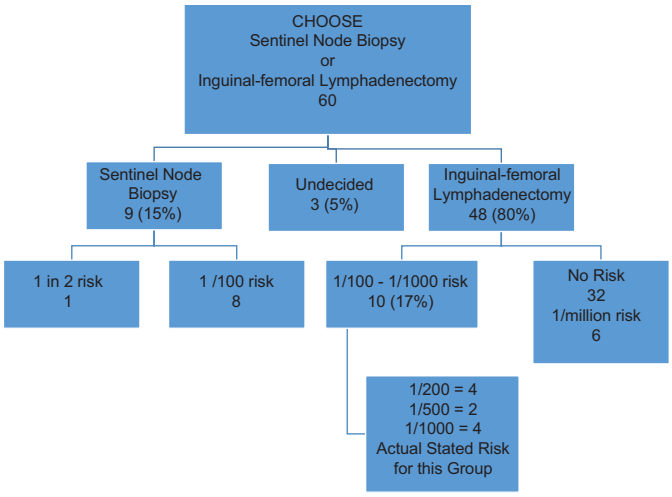

Figure 3: Women's preference for sentinel node biopsy versus inguinal-femoral lymphadenectomy and the degree of risk each woman would take of missing positive lymph nodes with the sentinel node procedure , modified from Farrell *et al*[28]

procedure. Interestingly, 60% of 80 gynecologists filling in structured questionnaires were willing to accept a 5%–20% false-negative rate for the sentinel node procedure.

A study from the United States of patient preferences and physician perceptions in the management of breast cancer revealed that women have a strong desire to be involved in the decision-making regarding their treatment, and physicians are unable to consistently predict the treatment decisions that their patients would make.[30]

INFORMED CONSENT

The decision to undertake sentinel node biopsy clearly needs careful discussion of risks and benefits between surgeon and patient. The benefits, particularly the avoidance of lymphedema, would certainly be attractive to the patient, but full disclosure of the risks, namely, a significantly increased likelihood of dying with a groin recurrence, is critical. Many surgeons, unfortunately, take a paternalistic approach, discussing only the benefits, without concern for the risks. This is presumably based on the false assumption that the recurrence rate in the groin will be the same as that following inguinal-femoral lymphadenectomy, and the mistaken belief that the prevention of lymphedema is of paramount importance to the patient.

There is no question that, once acquired, lymphedema is a lifelong affliction requiring daily management by the patient. However, there is also little doubt that as a patient accommodates to her diagnosis of cancer, her mindset changes regarding the type of morbidity, she is prepared to accept to stay alive. In a study of patients with breast cancer, Ganz *et al.* reported that the cancer experience enriched them, deepened the compassion they felt for others, and changed many of their priorities forever.[31]

Although the majority of patients are not prepared to take even the slightest risk with their life in return for avoiding lymphedema, some patients are prepared to take the small risk involved. In the senior author's experience, such patients include the frail or the elderly, who fear that they will not be able to manage the support stockings successfully, and younger women whose professional career depends on them having slim legs, such as dancers or models.

SENTINEL NODE BIOPSY IN BREAST CANCER

In contrast to the situation with vulvar cancer, where sentinel node biopsy is controversial, it is regarded as the standard of care for patients with early breast cancer. In fact, there is now discussion about whether or not it is necessary to undertake complete axillary dissection even in patients with positive sentinel nodes.

Two recent systematic reviews and meta-analyses have evaluated the safety and efficacy of sentinel node dissection alone versus complete axillary lymph node dissection in patients with early breast cancer and sentinel lymph node metastases. A 2013 paper reported three studies with 50,120 patients who had positive sentinel nodes and indicated similar 5-year survival and regional recurrence rates between the two groups of patients.[32]

A 2015 paper evaluated 12 studies which included 130,575 patients from five randomized controlled trials and seven observational studies – 26,870 patients had undergone sentinel node biopsy alone, while 103,705 had undergone complete axillary node dissection. Although paresthesia and lymphedema were more common in patients having complete axillary node dissection, there were no differences in overall survival ($P = 0.35$), disease-free survival ($P = 0.96$), or locoregional recurrence ($P = 0.73$).[33]

This excellent outcome is not because the false-negative rate for sentinel node biopsy is any lower in patients with breast cancer than it is for patients with vulvar cancer. In a 2016, systematic review of 24 prospective studies involving 15,462 patients with breast cancer, He *et al.* reported a pooled false-negative rate of 7.5%.[34] The difference is clearly related to the fact that most patients with breast cancer receive adjuvant chemotherapy or hormonal therapy, and respond very well to this treatment, such that axillary nodal recurrence rates are in the order of 0.1%–0.3%.[17] There is no effective

adjuvant chemotherapy or hormonal therapy available for patients with node-negative vulvar cancer.

In the future, multimodal therapy for breast cancer will be dependent on features in the primary tumor, including molecular markers, potentially rendering the staging information obtained through axillary lymph node dissection inconsequential.[35]

CONCLUSIONS

Sentinel node biopsy is the best strategy yet developed for the virtual elimination of lymphedema in patients with node-negative vulvar cancer, but it should not be regarded as the standard of care. Vulvar cancer is an uncommon tumor, so individual experience is limited, unlike the situation with breast cancer. Nevertheless, from accumulated data, the false-negative rate for both breast and vulvar cancer seems to be between 5% and 10%.

The reason that the axillary node recurrence rate is so low in patients with breast cancer is that most patients will receive adjuvant hormonal or chemotherapy, which is presumably effective against microscopic nodal metastases. By contrast, there is no effective adjuvant therapy for patients with vulvar cancer, so patients with false-negative sentinel nodes will recur in the groin and usually die of their disease.

Although the risk of groin recurrence and death is very low, the majority of patients properly informed about risks and benefits are not prepared to take this risk. In a study at our own institution, about 80% of patients reported that they would rather take a 60% risk of developing lymphedema than a 1% risk of dying of a groin recurrence.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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CORRIGENDUM

On page 11, last paragraph, for *technitium* read *technetium*

Current Status of the Sentinel Lymph Nodes

Since this article was written almost 5 years ago, there has been a shift in acceptance of sentinel node biopsy for early-stage vulvar cancer patients who fit the protocol described by the GROINSS-V-1 study group [185]. Many more centres have acquired the skills necessary to perform the sentinel node biopsy (SNB) technique and the procedure has been increasingly offered to selected women [221].

In 2018, a Chinese study utilised the Surveillance, Epidemiology, and End Results (SEER) database to obtain information on US patients with vulvar cancer who were registered between 2003 and 2013. Zhou et al. [222] reported that of the 1,475 patients identified, 1,346 (91.3%) underwent lymphadenectomy and 129 (8.7%) underwent SNB. They reported a significant increase in the proportion of patients receiving SNB between 2008 and 2013 compared to the years 2003-2007 (13.9% vs. 3.7% respectively, $p < 0.001$). Another US population-based study reviewed data on women with vulvar cancer who had undergone vulvectomy and lymphadenectomy in 500 US hospitals between 2006 and 2015. Cham et al. [223] reported that 618 women (27.2%) underwent SNB and 1655 (72.8%) underwent lymphadenectomy. The sentinel node biopsy rate had increased from 17.0% in 2006 to 39.1% in 2015. Sentinel node biopsy is now included in vulvar cancer treatment guidelines from the National Comprehensive Cancer Network (NCNN) [224], and the European Society of Gynaecological Oncology [225].

The largest study of oncological outcomes for patients having undergone sentinel node biopsy remains the long-term results from the GROINNS-V study [226]. This study reported an isolated groin recurrence rate of 2.3% in patients who had negative sentinel lymph nodes at biopsy. In the last two years, three further studies on sentinel node biopsy for patients with vulvar cancer have been published. There have been two smaller retrospective studies from the US [227], and Spain [216], and one multi-centre prospective study from Australia and New Zealand [221]. These studies reported isolated groin recurrence rates of 1.2% (2 groin recurrences in 169 groins) [227], 2.7% (2 of 74 patients) [221], and 4.8% (2 of 42 patients) [216] respectively.

Although these false-negative rates have always been acceptable to clinicians [228], patients are much less likely to find them acceptable given that the likelihood of death from recurrence in the groin is about 90% [196,228]. Of note, the authors of the afore-mentioned Spanish study reported that the disease-specific survival rate at 5 years was 83.3% in the negative SLN group and 92.2% in the negative IFL plus SLN group ($p = 0.214$) [216]. It is difficult to believe that future patients would choose sentinel node biopsy at this institution if informed of these results.

Two studies (described earlier in this chapter) that investigated patient choices regarding informed consent reported that almost two-thirds of patients would prefer a full groin dissection rather than a sentinel node biopsy when given a 50% risk of developing lymphoedema following a full groin dissection, and a 1% risk of death following a sentinel node biopsy [196,228]. In fact, in the Australian study, 50% of patients said they would not take a 1:1,000,000 risk of death, and only 15% were prepared to take the 1:100 risk required [196].

The risk of death is based on clinical follow-up of all patients, waiting for a palpable node to become apparent before intervening. It is highly likely that regular ultrasonic surveillance of the groin would allow detection of subclinical nodal metastases, which could probably be cured by earlier active intervention. There has now been tacit recognition that simply following these patients with groin palpation is not enough [229].

The use of serial groin ultrasonography to facilitate earlier detection of a groin recurrence has recently been proposed by Pouwer et al. [229] from the Netherlands. They conducted a prospective study of 76 patients who had negative sentinel nodes. Groin palpation was combined with an ultrasonic examination of the groins by a radiologist every 3 months. In the first two years of follow-up routine groin ultrasonography detected two asymptomatic groin recurrences (one non-palpable). Both patients underwent IGFLND and received adjuvant radiotherapy. Both remain disease-free 10 years and 39 months respectively after their groin recurrence. The sensitivity of ultrasound to detect a groin node recurrence was 100% (95% CI:16 - 100) and specificity 92% (95% CI: 89 - 95) for the 348 groin ultrasounds performed during follow-up [229].

If ultrasonographic surveillance can be shown to allow early detection and cure of patients with false-negative groin nodes after sentinel node biopsy, this should become the treatment of choice for all patients, because of the significantly decreased morbidity.

Conclusion

This review of the literature has identified a wide variation in the reported incidence of short and long-term complications associated with the removal of the inguino-femoral lymph nodes, and the risk factors associated with their occurrence. The immediate post-operative management of these patients may well decrease the risk of some complications, but this is unclear. Therefore, my thesis sought to determine the incidence of short-and long-term morbidity following groin lymphadenectomy in a large cohort of patients, to investigate causal factors, and to examine strategies to reduce morbidity. This research will be addressed in Chapter 5.

2.3.5 | The Relevance of the Surgical Margin in VSCC

Surgical resection with tumour free surgical margins has been the foundation of effective primary treatment for squamous vulvar cancer [230,231]. Radical vulvectomy was the standard of care in the mid-20th century [104,105]. Since the early 1980s radical local excision, which involves a wide and deep excision of the primary tumour, has progressively been practised [232-234].

Irrespective of the type of surgery performed, VSCC has high local recurrence rates, with one recent retrospective study reporting a local recurrence rate as high as 76% (in patients with dVIN and LS at the surgical margin), up to ten years after treatment [235]. Other recent retrospective studies have reported local recurrence rates ranging between 14 [236] and 41% [237].

The width of the surgical margin has been considered to be an important prognostic factor for local vulvar recurrence. Since the study by Heaps et al. [238] in 1990 a surgical margin of at least 1cm has generally been accepted. With tissue shrinkage of approximately 20% during formalin fixation, this equates to a histopathological margin of 8mm measured from the invasive carcinoma to the inked peripheral surgical margin [239]. Several retrospective cohort studies have shown an increased risk for local recurrence in patients with pathologic margins less than 8mm [238,240-242], and there has been a long-standing recommendation to achieve clear surgical excision margins of at least 1cm [224,243]. Surgical re-excision is recommended when the pathologic peripheral resection margin is less than 8 mm. If there is close proximity to the clitoris or anus, vulvar radiation is usually recommended.

The width of the surgical margin has become a controversial issue recently, with several retrospective studies challenging the need for an 8mm pathologic margin [235,237,244-250]. The first, a Dutch study of 79 vulvar cancer patients, reported no difference in local vulvar recurrence rates in patients with histological margins less than 8mm compared to margins of 8mm or more [245]. In 2015, Baiocchi et al. [244] analysed a series of 205 patients treated for VSCC in Brazil between 1980 and 2007. They also found no difference in local recurrence rates when they categorised patients into 3 tumour-free margin groups (< 3mm, 3mm to < 8mm, and ≥ 8mm).

The following year, Woelber et al. [246] analysed a subgroup of patients from a large multi-centre German study, using the same margin distance cut-offs as the previous study. In 286 surgically treated node negative patients, they reported no difference in local recurrence rates between margin groups, or when margins were analysed as a continuous variable.

Since 2016, two systematic reviews (only one with a meta-analysis) have addressed tumour-free surgical margins and local recurrence rates following vulvar cancer surgery. Nooij et al. [247] performed a meta-analysis of 10 studies (1,278 patients). The pooled analysis found tumour free margins < 8mm were associated with a higher risk of recurrence than margins ≥ 8 mm (pooled risk ratio, 1.99; 95% CI: 1.13 – 3.51). However, in an analysis of 148 patients from their own institution, they found no difference in the risk of local recurrence in patients with margins < 8 mm compared to ≥ 8 mm.

In 2018, te Grootenhuis et al. [249] performed a systematic review of studies that had evaluated prognostic factors associated with local recurrence rates. Data from 22 studies were included (3,657 patients), but only eleven studies analysed local recurrence rates in relation to pathologic tumour-free margins. Although six of these studies reported a decreased risk of local recurrence in patients with a pathological margin distance ≥ 8 mm versus < 8 mm, te Grootenhuis et al. concluded that the quality of the included data did not allow for any evidence based clinical decisions to be made.

The Effect of Precursor Lesions on Local Recurrence Risk.

Some retrospective studies have reported the presence of differentiated VIN or lichen sclerosus, either at the excision margin or in the excised tumour specimens, to be associated with an increased risk for local recurrence. As early as 2000, Preti et al. [251] reported the presence of associated VIN 2 or 3 was an independent prognostic factor for local recurrence when they analysed data on 101 patients with VSCC. More recently (2016), Yap et al. [248] investigated data on 201 patients with VSCCs. Their multivariable analysis revealed that although margin distance was not a significant risk factor for local recurrence, lichen sclerosus adjacent to the tumour was associated with a significantly increased risk (sub-hazard ratio (SHR) 3.39, p = 0.010). Subsequently, Pleunis et al. [250] also reported that although resection margins (< 8mm versus ≥ 8 mm) were not significant, the presence of lichen sclerosus, was significantly related to local recurrence risk in 167 patients with VSCCs treated in the Netherlands.

Another study from the Netherlands incorporated precursor lesions, as well as three margin cut-offs (8mm, 5mm, and 3mm) into their analysis of potential risk factors for local recurrence in 287 patients with VSCC. te Grootenhuis et al. [235] reported that although there was no margin cut-off that influenced the risk of recurrence (HR 1.03), patients with dVIN and LS in the margin (HR 2.76; 95% CI: I.62 - 4.71), or dVIN alone in the margin (HR 2.14; 95% CI:1.11 - 4.12) had higher local recurrence rates. These authors recommended reducing the histological surgical margin to ≥ 3mm.

In contrast to these results, the largest and most recent study to have investigated the

relationship between pathologic tumour free margins and local vulvar recurrence is from the Mayo Clinic. In 2020, Yang et al. [252] reported on 335 patients treated between 2000 and 2018 at the three Mayo Clinic sites. Like several earlier studies, they categorised patients into 3 tumour-free margin groups (< 3mm, 3mm to < 8mm, and ≥ 8mm). Their results showed that patients with margins less than 8mm had a higher rate of local recurrence (HR 1.98; 95% CI:1.13 - 3.41) compared to patients with margins ≥ 8mm. Five--year local disease-free survivals were 48.2%, 81.5%, and 84.6%, ($p < 0.001$), for the margin groups < 3mm, 3mm to < 8mm, and ≥ 8mm, respectively.

The Role of Adjuvant Treatment in Reducing Local Recurrence Rates.

There is little evidence in the literature on the influence of additional treatment for close surgical margins (either surgical re-excision or adjuvant vulvar radiotherapy) on local recurrence rates. A limitation of many of the studies described in this review was that they were either missing data on patients having additional treatment [245,246], had excluded such patients from multivariable analysis [235,247], or the number of patients having additional treatment were insufficient to have a significant effect [244,250]. In addition, most studies did not perform a separate analysis of patients with close or positive margins to compare those treated with or without re-excision or radiotherapy [244-247,250]. An adequately powered subgroup analysis would be beneficial to determine if patients with close or positive margins benefited from additional treatment [253].

To my knowledge, only two studies have focused specifically on the effect of adjuvant radiotherapy for close or positive margins and local recurrence rates. Both are retrospective studies from separate institutions in the United States. The first of these, performed by Faul et al. [254], included 62 patients with close or positive excision margins (< 8mm). They compared two groups: 31 patients who were observed after their surgery, and 31 patients who were treated with adjuvant radiotherapy. Local recurrences occurred in 58% of patients in the observed group, compared to 16% in the radiotherapy group. Adjuvant radiotherapy was associated with significantly decreased local recurrence rates in patients with both positive ($p = 0.005$), and close histopathological margins ($p = 0\ 04$). Adjuvant radiotherapy and surgical margins were found to be significant predictors of local control in both univariate and multivariable analyses. In this report the dose of radiotherapy given to the patients was not described.

In 2013, Viswanathan et al. [255] reported similar results when they assessed the relationship between margin status, radiation dose and recurrence in 205 patients. Of these, 116 patients had close histological margins (defined as < 10mm) and 20 had positive margins. Both close (HR 3.03) and positive margins (HR 7.02) were associated with a significantly increased risk of vulvar recurrence, and patients with ≤ 5mm margins were

at greatest risk. Radiotherapy was associated with a significantly lower risk of vulvar recurrence, but patients who received ≥ 56 Gray (Gy) had a lower risk for recurrence than those who received ≤ 50.4 Gy (p = 0.05).

In 2019, Bedell et al. evaluated 150 patients with FIGO stage 1 VSCC to determine whether additional treatment (re-excision or vulvar radiotherapy) for close or positive surgical margins improved recurrence-free survival [97]. Unlike the previous studies, they were unable to demonstrate that either treatment significantly reduced local recurrence rates, although there was a trend towards improvement in local recurrence-free survival. Given that there were only four patients in the radiotherapy subgroup, the effect of radiotherapy remains unclear from this study.

Patterns of Local Recurrence

Many of the previously described studies dispute the notion that the width of the surgical margin is a consistent prognosticator of local recurrence rates. This may be explained by the fact that they do not distinguish between primary and remote site recurrences.

Almost 20 years ago, Rouzier et al. [94] was the first to separately analyse local recurrence patterns. Their study included 215 patients with vulvar cancer and their primary endpoints were local relapse and cancer-related death. They classified local vulvar recurrences as follows: (i) those at the primary site (within 2cm of the primary excision scar), (ii) those at a remote site (>2cm from the primary excision), and (iii) those in the skin bridge between the vulvar and groin incisions. They determined that a recurrence at the primary site (relative risk (RR) 6.35; 95% CI: 2.07-15.76) or in the skin bridge (RR 6.48; 95% CI: 2.54-16.49) had a significantly detrimental effect on disease-specific survival, by contrast, local recurrences distant to the primary tumour had a good prognosis and were considered to be a new primary cancer.

The first study to adopt this concept came from my own institution in 2009 [242]. The authors retrospectively evaluated patterns of recurrence in 121 patients with early stage VSCC. They reported 26 local recurrences, which included 13 at a primary vulvar site, 12 at a remote site, and one in the skin bridge. Primary site recurrences occurred at a median of 21 months and were significantly more commonly associated with margins < 8mm. Remote site recurrences occurred at a median of 69 months and were more commonly associated with lichen sclerosus or differentiated VIN. Unlike the Rouzier et al. study [94], survival for both primary and remote site recurrences was particularly good, the actuarial overall 5-year survival being 96.1% for patients with any vulvar recurrence. The one patient with a skin bridge recurrence died of disease.

Following on with this concept, seven years later Yap et al. [248] performed a retrospective analysis of 201 patients with vulvar cancer treated in a single-centre in the United Kingdom. These authors utilised the same localisation classification for vulvar recurrences as described by the two previous authors, but they called primary vulvar recurrences 'local relapses' and remote recurrences 'second field tumours'. They reported that lichen sclerosus present in the epithelium adjacent to the tumour was associated with a significantly increased risk for all local vulvar recurrences, local relapses and second field tumours (SHRs: 3.4, 2.7 and 4.4 respectively), but that 'sub-optimum' pathologic excision margins were not found to increase the risk of any vulvar recurrence.

Many vulvar cancers develop in a background of abnormal surrounding skin such as usual VIN (now called vulvar high grade squamous intraepithelial lesions (HSIL)), dVIN and lichen sclerosus. Such patients may relapse on the vulva but at sites well removed from the original tumour. It has been suggested that many such 'recurrences' may be second primary tumours which arise in a 'field of cancerization' [256].

Field Cancerization

The concept of field cancerization has been proposed based on the hypothesis that an area, or field, of epithelium may be transformed by regional carcinogenic activity, which causes irreversible cumulative epigenetic and genetic changes in numerous cells, which may eventually manifest as cancer [257-260]. This concept was first proposed by Slaughter et al. [259] almost 70 years ago. In their seminal report, they examined 783 squamous oropharyngeal cancers histologically. They identified 'abnormal and hyperplastic, often atypical epithelium', in adjacent mucosa for varying distances in all cases, even though there was no gross clinical evidence of disease. They termed this 'field cancerization' and attributed the high local recurrence rates of oral cancers to these histological abnormalities in the surrounding mucosa [259].

Over the ensuing years several studies have discussed the clinical importance of this concept in various cancer types [258,260-263]. Brakhuis et al. [262] discussed this concept in relation to head and neck squamous carcinomas. They described the surrounding squamous abnormalities which were left behind after surgical excision as a 'field effect' and felt that they posed a continuous risk for a recurrence. As the recurrent tumour developed from cells genetically related to the primary tumour, they proposed this type of cancer be designated a 'second field tumour'. They felt it should be differentiated from a recurrence due to residual cancer after surgical excision, or a second primary cancer that developed autonomously.

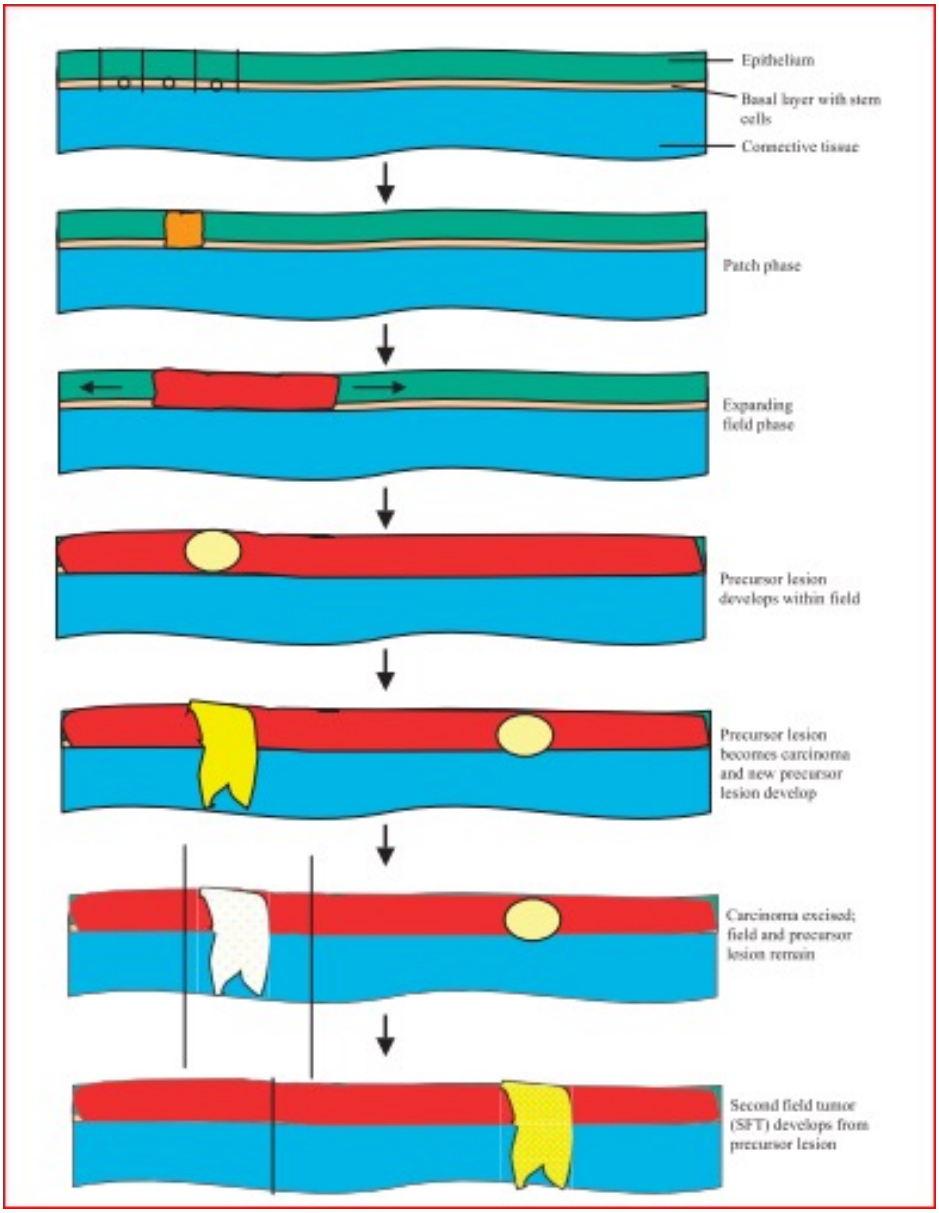

Figure 2.3.3 | Schematic overview of the proposed concept of carcinogenesis of head and neck squamous cell carcinoma [264]. At the top, the epithelium is shown (green) with the basal cell layer (light orange) including the stem cells (three are shown) and connective tissue (blue). The second stage ('patch phase') shows the formation of a patch (dark orange) as a clonal unit of genetically altered cells. Next, the formation of an expanding field (red) from a patch is visualized. Within such field of genetically altered cells, a more progressed preneoplastic lesion develops (light yellow). In the next stage, that lesion develops into cancer (yellow) and another preneoplastic lesion emerges. The carcinoma is removed by the surgeon, but the field and the preneoplastic lesion remain. At the bottom the development of a second field tumor is shown.
(Figure and legend reprinted from Annals of Oncology, Vol 16(2), Braakhuis BJ, Brakenhoff RH, Leemans CR., Head and neck cancer: molecular carcinogenesis, Pages 249-250., Copyright (2005), with permission from Elsevier).

Dakubo et al. [258] examined the clinical relevance of this concept in vulva cancer, in addition to head and neck, lung and other cancers. They proposed two types of local recurrence – those that occurred at the primary site and those that occurred at a distant site. They described recurrences that were genetically related to the primary tumour as 'second field tumours' and those that were genetically dissimilar as true 'second primary tumours' [258]. They felt that future molecular assessment of skin margins around the excised tumour to detect genetic 'field effect' abnormalities may help to identify lesions that were pre-neoplastic and could ultimately lead to a reduction of tumour recurrences if such margins were treated [258].

Drawing from these published studies, my thesis sought to investigate the clinical and pathological variables associated with local vulvar recurrences by investigating the largest vulvar cancer series to date from a single institution. My hypothesis is that patients who have a histological excision margin of less than 8mm are at a higher risk for local recurrence at the primary tumour site, and that sub-optimal margin distance is not associated with remote site vulvar recurrences. I will also strive to determine if treatment by either radiotherapy or re-excision is beneficial in reducing local recurrence rates as this is poorly addressed in the literature. This research will be addressed in Chapter 6.

CHAPTER THREE

CHANGING TRENDS IN VULVAR CANCER INCIDENCE AND MORTALITY RATES IN AUSTRALIA SINCE 1982.

3.1 | Precis

This chapter is derived from the first area of investigation in my thesis which was to identify vulvar cancer incidence and mortality trends in Australian women, and to determine if there was an increased incidence in younger Australian women. An analysis of incidence was performed on all confirmed cases of invasive carcinoma of the vulva diagnosed in Australia between 1982 and 2009; and an analysis for mortality on all vulvar cancer deaths for the period 1982 to 2011.

This chapter addresses the following research questions:

- What are the temporal trends in the incidence and mortality of vulvar cancer in Australian women?
- Is there evidence of increasing incidence of vulvar cancer in younger cohorts of Australian women born after 1950?

ORIGINAL STUDY

Changing Trends in Vulvar Cancer Incidence and Mortality Rates in Australia Since 1982

Ellen L. Barlow, RN, BN, MN (Hons), Yoon-Jung Kang, BA, MPH (Hons), PhD,† Neville F. Hacker, AM, MD FRANZCOG,‡§ and Karen Canfell, DPhil†*

Background: The objective of this study was to assess trends in vulvar cancer incidence and mortality in Australia.

Methods: Case numbers for invasive carcinoma of the vulva (1982–2009) and vulvar cancer deaths (1982–2011) were obtained from the National Cancer Statistics database. Standardized rate ratios (SRRs) were used to assess changes in age-standardized incidence and mortality rates, for all ages and for younger than 60 years and 60+ years.

Results: Age-standardized incidence rates in women across all ages did not significantly change from 1982–1984 to 2007–2009 (from 2.1 to 2.5 per 100,000 women; SRR from the later to the earlier period, 1.13 [95% CI, 1.00–1.27]). However, there was a significant 84% increase in incidence in women younger than 60 years (SRR, 1.84 [95% CI, 1.49–2.26]), with no change for women 60+ years (SRR, 0.90 [95% CI, 0.79–1.04]). Age-standardized mortality in women across all ages significantly decreased by 22% from 1982–1986 to 2007–2011 (from 0.7 to 0.5 per 100,000 women; SRR, 0.78 [95% CI, 0.66–0.93]). However, this was driven by declines in older women, with stable rates in women younger than 60 years (SRR, 1.05 [95% CI, 0.62–1.79]); rates in 60+ years decreased by 24% (SRR, 0.76 [95% CI, 0.63–0.91]).

Conclusion: Since the early 1980s, vulvar cancer incidence has increased by more than 80% in women younger than 60 years in Australia, but there has been no increased incidence in older women. These findings are consistent with the possibility of increased exposure to the human papillomavirus in cohorts born after 1950. By contrast, age-standardized vulvar cancer mortality rates have been stable in younger women, but have declined in older women.

Key Words: Vulvar carcinoma, Human papillomavirus, Incidence, Mortality, Population trends

Received April 25, 2015, and in revised form July 5, 2015.
Accepted for publication July 5, 2015.

(*Int J Gynecol Cancer* 2015;25: 1683–1689)

*Gynaecological Cancer Centre, Royal Hospital for Women, Randwick, NSW, Australia; †Cancer Screening Group, Lowy Cancer Research Centre, Prince of Wales Clinical School, UNSW, Randwick, NSW, Australia; ‡Gynaecological Cancer Centre, Royal Hospital for Women, Randwick, NSW, Australia; and §School of Women's & Children's Health, University of New South Wales, Randwick, NSW, Australia.
Address correspondence and reprint requests to Ellen L. Barlow, RN, BN, MN (Hons), Gynaecological Cancer Centre, The Royal Hospital for Women, Locked Bag 1000, Barker St, Randwick, 2031 NSW, Australia. E-mail: ellen.barlow@sesiahs.health.nsw.gov.au.

ISSN: 1048-891X
DOI: 10.1097/IGC.0000000000000547

Competing interests: K.C. is co–principal investigator of a trial of primary human papillomavirus screening for cervical cancer in Victoria ("Compass"), which is conducted and funded by the Victorian Cytology Service. A funding and equipment contribution for the trial has been received by Victorian Cytology Service from Roche Molecular Systems and Ventana Inc, USA.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Current affiliation for Dr Yoon-Jung Kang and Associate Professor Karen Canfell is Cancer Research Division, Cancer Council of NSW, Woolloomooloo, NSW, Australia.

In Australia, approximately 300 women are diagnosed as having invasive vulvar cancer each year, and 79 women died of the disease in 2009.[1] Squamous carcinoma of the vulva, which comprises approximately 83% of these vulvar cancers,[2] is known to be of mixed etiology. The keratinizing histopathologic type generally occurs in elderly women and is often associated with lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia. The warty or basaloid type is generally seen in younger women. It has been associated with sexually transmitted human papillomavirus (HPV) infection and is often characterized by the presence of "usual type" vulvar intraepithelial neoplasia in association with the invasive component.[3–5] Human papillomavirus DNA is found in approximately 40% of vulvar cancers overall.[6,7] Human papillomavirus type 16 is associated with most of these, with the next most frequent HPV types being 18 and 33.[5,6]

Human papillomavirus is associated with a number of cancers, including cancers of the oropharynx and anus in both sexes, cancer of the penis in men, and cancers of the cervix, vagina, and vulva in women. A number of prior analyses in developed countries have documented increases in the overall rates and/or the HPV-attributable fraction of HPV-related cancers of the anogenital tract and oropharynx over the past 20 years. It has been proposed that both the overall increase in rates and the increase in the HPV-attributable fraction of these cancers are due to increasing exposure to HPV in younger cohorts.[8,9] For example, a 2010 Australian analysis found that the incidence of cancers of the anus and oropharynx had increased significantly in both men and women over the 2 decades prior.[10] Although no significant change in the overall age-standardized incidence of vulvar cancer was identified in this prior analysis, we hypothesized that if such a change occurred, it would be evident only in younger women. Therefore, the aim of the current study was to perform a more detailed analysis of trends in vulvar cancer in Australia in women of different age groups, to determine if there was evidence of an increasing incidence in younger cohorts born after 1950 (ie, <60 years in 2010), consistent with increased exposure to HPV over time. We also aimed to characterize any changes in mortality rates from vulvar cancer in women of different age groups.

MATERIALS AND METHODS

Data Sources

This analysis of vulvar cancer incidence was based on all histologically confirmed cases of invasive carcinoma of the vulva diagnosed in Australia between 1982 and 2009; data were obtained from the Australian Institute of Health and Welfare National Cancer Statistics database.[1] The analysis for mortality included all verified vulvar cancer deaths for the period 1982 to 2011; mortality data were obtained on request from the Australian Institute of Health and Welfare National Mortality Database. Because of very small numbers of deaths from vulvar cancer in younger women (and thus concerns about small cell size and the implications for privacy), the mortality data were supplied as aggregate data over calendar years 1982 to 1986 and then in 5-year intervals up to 2007 to 2011, and in age strata from 0 to 54 years and then in 5-year age groups up to 85+ years.

Human Research Committee Ethics approval was not required for this study because we collated and analyzed publicly available aggregate data.

Statistical Analysis

Age-standardized incidence and mortality rates were determined using the Australian 2001 Standard Population for standardization. We then calculated standardized rate ratios (SRRs) as the ratio of the average standardized rate at the end of the period relative to the average rate at the beginning of the period and 95% confidence intervals for the SRRs using Poisson approximation.[11] Because of differences in data availability, we used 3-year averages from 2007 to 2009 and from 1982 to 1984 for incidence SRRs, and 5-year averages from 2007 to 2011 and from 1982 to 1986 for mortality SRRs, to yield measures of changes in rates from 1982 to 2009 for incidence and from 1982 to 2011 for mortality. For incidence, we also calculated the annual percent change (APC) using Joinpoint analysis, restricting analyses to a maximum of 1 Joinpoint over the period.[12,13] We could not calculate APCs for mortality because data were supplied in 5-year aggregated blocks.

RESULTS

Incidence

From 1982 to 2009, 5715 cases of invasive vulvar cancer were diagnosed in Australia. Figure 1A shows the incidence rates for vulvar cancer over time in Australia in specific decile age groups, and Figure 1B shows rates stratified by age groups <60 years and 60+ years. As shown in Tables 1 and 2, age-standardized incidence rates in women across all ages did not significantly change from 1982–1984 to 2007–2009 (from 2.1 to 2.5 per 100,000 women; SRR from the later to the earlier period, 1.13 [95% CI 1.00–1.27]), although there was a statistically significant increase noted in the average APC over the period (APC, 0.5%; $P = 0.02$). During the same period, there was a significant 84% increase in the incidence rates in women younger than 60 years (0.7–1.2 per 100,000 women; SRR, 1.84 [95% CI 1.49–2.26]; APC, 2.5%; $P < 0.001$) but no change in incidence for women 60+ years (9.4–9.0 per 100,000 women; SRR, 0.90 [95% CI, 0.79–1.04]; APC, −0.4%; $P = 0.1$). Results were broadly similar when we stratified by less than 70- and 70+-year age groups (data not shown).

Mortality

From 1982 to 2011, 1586 deaths from invasive vulvar cancer occurred in Australian women. Figure 2A shows the mortality rates for vulvar cancer over time in Australia for women younger than 60 years and for each decile age group in older women, and Figure 2B shows the rates stratified by ages <60 years and 60+ years. As shown in Tables 1 and 2, age-standardized mortality in women across all ages significantly decreased by 22% from 1982–1986 to 2007–2011 (from 0.7 to 0.5 per 100,000 women; SRR, 0.78 [95% CI

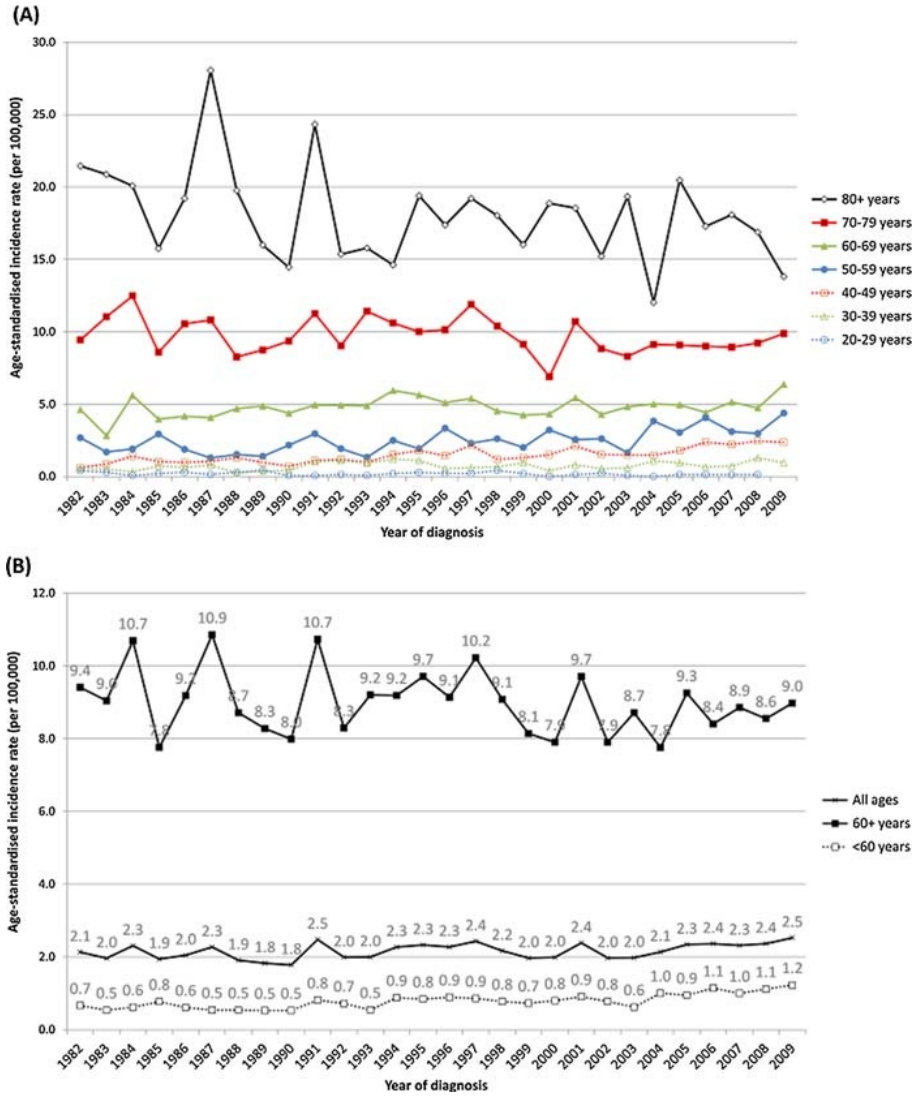

FIGURE 1. Age-standardized incidence of vulvar cancer in Australia, 1982 to 2009*. A, For each decile age group. B, Age-standardized rates for all ages, and stratified by women younger than 60 years and women 60+ years old.

0.66–0.93]). However, this was driven by declines in older women—rates in women 60+ years decreased by 24% (SRR, 0.76 [95% CI 0.63–0.91]), whereas rates in women younger than 60 years were stable (SRR, 1.05 [95% CI 0.62–1.79]).

DISCUSSION

We identified distinctive and differing trends in incidence and mortality rates for invasive vulvar cancer in women of different age groups in Australia. In women younger than 60 years, we found an 84% increase in the incidence of invasive vulvar cancer for 3 decades, whereas in older women, no change in incidence rates was observed. By contrast, mortality rates have remained stable in younger women and decreased by 24% in older women. The major strengths of our analysis are the use of national population-based data and the relatively long reporting period (covering 27 years). The detailed analysis by age group, for both incidence and mortality, is, to our knowledge, the first such comprehensive study of trends in vulvar cancer in Australia. A limitation of this study is that detailed information on histologic type, stage, treatment, and hospital is not routinely available in cancer registries in Australia. Because this study examined national trends using cancer registry data, we were unable to analyse according to these factors at a national, population-based level.

Our results for trends in incidence are broadly comparable to the few available long-term analyses of population-based trends in other countries. Our findings of a 2.5% average increase per year in Australian women younger than 60 years and stable incidence in older women over the last 3 decades are comparable, for example, to those from a review of vulvar squamous cell carcinoma registrations from the Danish Cancer Registry. For the period 1978 to 2007, they found an average 1.6% (95% CI, 0.5%–2.7%) annual increase in incidence in women younger than 60 years, but stable incidence in women 60+ years.[14] A Dutch study of

TABLE 1. SRRs for changes in incidence and mortality in vulvar cancer in Australia*

Age Group	SRR Incidence (95% CI) 2007–2009 Compared With 1982–1984	SRR Mortality (95% CI) 2007–2011 Compared With 1982–1986
All ages	1.13 (1.00–1.27)	0.78 (0.66–0.93)
<60 y	1.84 (1.49–2.26)	1.05 (0.62–1.79)
60+ y	0.90 (0.79–1.04)	0.76 (0.63–0.91)

*Age-standardized rates were determined using the Australian 2001 Standard Population.

trends in squamous vulvar cancer over the period 1989 to 2010 also identified a comparable average annual increase in incidence in women younger than 60 years of 3.5% (95% CI, 2.0–4.9)[15]; this study also identified increasing incidence in women 60+ years, but only from 2004 onward. A recent study in England reported an increase in the incidence of invasive vulvar cancer in women aged up to 70 years since 1990; in this study, a decrease in incidence for women 80 years or older was identified over the same period.[16]

Analysis of in situ and invasive vulvar carcinomas from the US Surveillance Epidemiology and End Results database, which considered cases diagnosed over the period 1973 to 2004, also identified an increase in vulvar cancer incidence.[17] An annual increase in invasive vulvar cancer of 1.0% (95% CI, 0.6%–1.4%) across all age groups was identified (as was an increase in in situ cancers of 3.5% [95% CI, 2.9%–4.1%]); the authors concluded that the effects were seen across all ages, although detailed analysis for specific age groups was not presented. A more recent analysis of population-based data from the United States and Canada showed an increased incidence of invasive squamous cell vulvar cancer over the period 1973 to 2010 in the United States and 1992 to 2008 in Canada.[18]

Although not population based, a number of single institutional reviews have also identified an increasing presentation of younger women with vulvar cancer.[19,20] For example, a retrospective chart review of patients with vulvar cancer presenting to a university hospital in Germany from 1980 to 1989 compared with 1998 to 2007 found that the number of women treated for vulvar squamous cell carcinoma had nearly doubled, and the percentage of women aged 50 years or younger had increased 4-fold (11.3% vs 41.2%).[19]

Our findings of an increased vulvar cancer incidence in younger women are consistent with data from other developed countries but diverge somewhat from international trends in elderly women (>70 years). If cohort effects and HPV exposure underlie the observed findings, some of the differences in the findings for elderly women are likely to, at least partly, reflect differences in population behavior in the past and the timing of the analysis in relation to cohort age and HPV exposure. More recent analyses are more likely to identify changes in rates in older women because these will reflect birth cohorts more likely to have been exposed to HPV infection. In addition, there may be geographic differences in the HPV-attributable fraction in vulvar cancers.

Recent analyses have identified changing trends in other HPV-related cancers. For example, in the United States, the incidence of oral squamous cell carcinoma has increased over time,[8] and the HPV-attributable fraction in cancers of the oropharynx has increased from 16% in 1984 to 1989 to 72% from 2000 to 2004.[9] Rates of oropharyngeal cancers have also increased in Australia over the period 1982 to 2005,[10] with a corresponding increase in the HPV-attributable fraction in these cancers in both sexes.[21] Rates of anal cancers in both males and females have also increased in the United Kingdom between 1960 and 2004[22] and in Australia from 1982 to 2005.[10]

Our results for vulvar cancer incidence are consistent with, but build upon, a previous analysis of trends in vulvar cancer incidence in Australia, which identified no change in rates of vulvar cancer incidence overall.[10] When we used the SRR as a measure of the change in incidence, we also identified no significant change when trends were considered across women of all ages. When the APC was used as a measure and given that we had access to an additional four years of data compared with the previous study, we did identify a significant trend in the APC. Our findings extend those from the earlier Australian study by identifying distinct trends by age group and by also identifying trends in mortality rates.

It is not possible to predict the future trends in incidence rates of vulvar cancer because these depend on a range

TABLE 2. Incidence and mortality of vulvar cancer at the beginning and end of the period

Age Group	Incidence Per 100,000 Women*			Mortality Per 100,000 Women*†	
	1982	2009	Average APC in the ASR (1982–2009) (95% CI)‡	1982–1986	2007–2011
All ages	2.1	2.5	0.5% (0.1% to 0.9%) ($P = 0.02$)	0.7	0.5
<60 y	0.7	1.2	2.5% (1.8% to 3.3%) ($P < 0.001$)	0.1	0.1
60+ y	9.4	9.0	−0.4% (−0.8% to 0.1%) ($P = 0.1$)	3.5	2.7

*Age-standardized rates were determined using the Australian 2001 Standard Population.
†The APC could not be calculated because mortality data were provided in 5-year aggregated blocks.
‡Negative signs indicate decrease in the ASR over time.

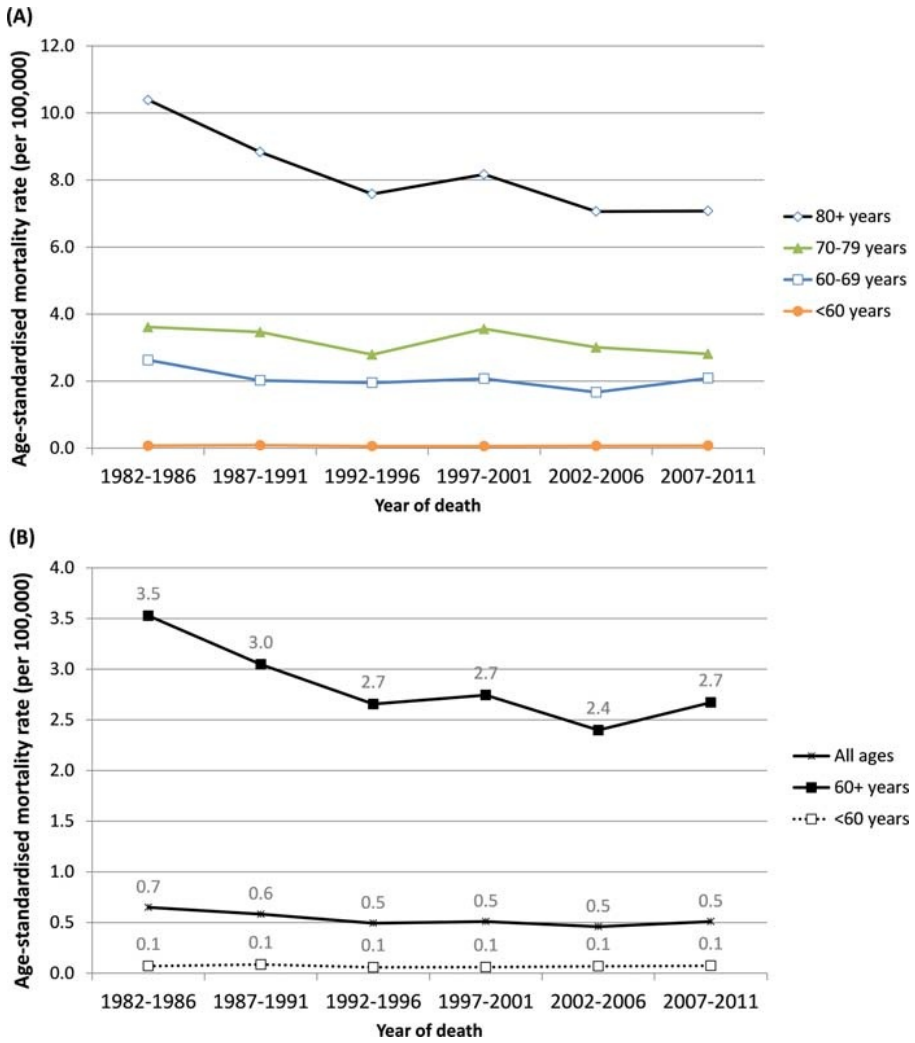

FIGURE 2. Age-standardized mortality of vulvar cancer in Australia, 1982–1986 to 2007–2011. A, For women younger than 60 years and for each decile age group in older women. B, Age-standardized rates for all ages, and stratified by women younger than 60 years and women 60+ years old.

of underlying factors including past and future exposure to risk factors and the uptake of the HPV vaccine by the community. However, assuming rates stay at those identified in the current study (ie, at around the rates observed in 2009–2011), population growth and aging are expected to drive increases in the overall case numbers for vulvar cancer. Using projections for population aging from the Australian Bureau of Statistics, if current rates are maintained, the burden of disease is expected to grow by 42% to 413 cases in 2030, and the number of deaths is expected to grow by 39% to 97 deaths in 2030. Over the longer term, HPV vaccination is expected to somewhat counteract this effect.

Our findings for trends in vulvar cancer mortality are broadly consistent with other studies internationally. We observed a significant decrease in mortality for women 60+ years, which drove an overall observed decrease in mortality across all ages. A population-based study in England also reported a significant decrease in mortality for women aged 60+ years between 1990–1994 and 2006–2010.[16] Consistent with this, recent analyses of vulvar cancer data from the United States (for the period 1973–2010) and Canada (1992–2008) have demonstrated an increase in 2- and 5-year relative survival ratios for all age groups, and particularly for women 80 years and older.[18] However, other population-based studies from the Netherlands have reported stable mortality rates for vulvar cancer.[15,23]

Over the past 30 years, surgery for vulvar cancer has become less radical, with more emphasis on vulvar conservation for the primary lesion and unilateral groin dissection for unilateral malignancies.[24–26] An institutional review of 175 American patients treated between 1990 and 2005 found survival among the lower-risk group was preserved, despite the less radical surgery, and the 5-year survival rates for patients with advanced vulvar cancer improved. The authors concluded that younger women presenting with less advanced disease and the widespread introduction of adjuvant (chemo)radiation were likely to have been critical in reducing recurrence rates and improving overall survival from vulvar cancer.[24]

The decreasing mortality rates that we observed in older women may also reflect earlier diagnosis. Although past generations of women have been reluctant to present with vulvar problems, better access to health information on the Internet and changing social mores have meant that most women present with relatively small lesions that are amenable to surgical resection. In addition, the treatment for women in specialized gynecologic oncology centres, as is advocated by Australian national guidelines, has likely contributed to the improvements in mortality.[23] The stable mortality rates that we observed in women younger than 60 years may reflect the fact that younger women are more likely to present with early-stage disease, which has a more favorable prognosis, and most recurrences are localized to the vulva and have a high cure rate, usually with further excision.[25]

In conclusion, we found that the incidence of vulvar cancer has substantially increased in Australian women younger than 60 years. Although an ecologic analysis of the type presented here cannot demonstrate causality, this increase is consistent with findings from other developed countries and also with the likely timing of increasing levels of population exposure to HPV due to changing sexual mores in women born from the 1950s onward. Because most HPV-related vulvar cancers are caused by HPV types 16 and 18, which are included in first-generation HPV vaccines, the introduction of HPV vaccination in Australia in 2007 is expected to provide current generations of young women with a level of protection against developing vulvar cancer as they age. We have also demonstrated that the mortality rate for older women has significantly decreased over the same period, despite a more conservative approach to management. This is presumably related to earlier stage at diagnosis and possibly better centralization of care.

ACKNOWLEDGMENTS

We thank the Australian Institute of Health and Welfare National Cancer Statistics Database for providing data for this study. K.C. receives salary support from the National Health and Medical Research Council Australia and from Cancer Institute NSW. We thank Megan Smith for repeating and checking the statistical calculations presented here.

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CHAPTER FOUR

THE CLINICAL RELEVANCE OF P16 AND P53 STATUS IN PATIENTS WITH SQUAMOUS CELL CARCINOMA OF THE VULVA

4.1 | Precis

This chapter comprises the second study of my thesis which examined the clinical relevance of the immuno-histochemical biomarkers p16, p53 and the presence of HPV in a cohort of 119 patients with squamous vulvar cancer treated between 2002 and 2014. A particular focus of this research was whether these markers could be used to modify the radicality of the surgery for the primary lesion.

This chapter addresses the following research questions:

- Is Human papillomavirus (HPV) status prognostically meaningful in vulvar squamous cell carcinoma, and is pre-operative determination of p16 or p53 status by immunohistochemistry clinically relevant?
- What are the clinicopathological variables associated with the immunohistochemical expression of p16 and p53?

Hindawi
Journal of Oncology
Volume 2020, Article ID 3739075, 8 pages
https://doi.org/10.1155/2020/3739075

Research Article

The Clinical Relevance of p16 and p53 Status in Patients with Squamous Cell Carcinoma of the Vulva

Ellen L. Barlow,[1] Neil Lambie,[2,3] Mark W. Donoghoe,[4] Zin Naing,[5] and Neville F. Hacker[1,6]

[1]*Gynaecological Cancer Centre, The Royal Hospital for Women, Sydney, NSW, Australia*
[2]*Anatomical Pathology, NSW Health Pathology, Prince of Wales Hospital Sydney, Sydney, NSW, Australia*
[3]*Anatomical Pathology, Canterbury Health Laboratories, Christchurch, New Zealand*
[4]*Mark Wainwright Analytical Centre, University of New South Wales, Sydney, NSW, Australia*
[5]*Serology and Virology Division, Microbiology Department, NSW Health Pathology, Prince of Wales Hospital, Sydney, NSW, Australia*
[6]*School of Women's & Children's Health, University of New South Wales, Sydney, NSW, Australia*

Correspondence should be addressed to Ellen L. Barlow; ellen.barlow@health.nsw.gov.au

Received 17 September 2019; Revised 31 January 2020; Accepted 13 February 2020; Published 24 March 2020

Academic Editor: Ozkan Kanat

Objective. To investigate the prognostic significance of HPV status in vulvar squamous cell carcinomas (VSCC) and to determine whether preoperative determination of p16 or p53 status would have clinical relevance. *Methods*. Patients treated for VSCC at a tertiary hospital in Sydney, Australia, from 2002 to 2014, were retrospectively evaluated ($n = 119$). Histological specimens were stained for p53 and p16 expression, and HPV status was determined by PCR detection of HPV DNA. *Results*. HPV DNA was detected in 19%, p16 expression in 53%, and p53 expression in 37% of patients. Kaplan–Meier survival estimates indicated that p16/HPV-positive patients had superior five-year disease-free survival (76% versus 42%, resp., $p = 0.004$) and disease-specific survival (DSS) (89% versus 75% resp., $p = 0.05$) than p53-positive patients. In univariate analysis, nodal metastases ($p < 0.001$), tumor size >4 cm ($p = 0.03$), and perineural invasion ($p = 0.05$) were associated with an increased risk of disease progression and p16 expression with a decreased risk ($p = 0.03$). In multivariable analysis, only nodal metastases remained independent for risk of disease progression ($p = 0.01$). For DSS, lymph node metastases ($p < 0.001$) and tumor size ($p = 0.008$) remained independently prognostic. *Conclusion*. The p16/HPV and p53 status of VSCC allows separation of patients into two distinct clinicopathological groups, although 10% of patients fall into a third group which is HPV, p16, and p53 negative. p16 status was not independently prognostic in multivariable analysis. Treatment decisions should continue to be based on clinical indicators rather than p16 or p53 status.

1. Introduction

Two subtypes of VSCC have previously been defined. The more common keratinising type typically occurs in older women, is generally associated with lichen sclerosus and/or differentiated vulvar intraepithelial neoplasia (dVIN) [1], and is often associated with p53 tumor suppressor gene mutations [2, 3]. The other subtype is more common in younger women and primarily associated with human papilloma virus (HPV) infection, and a common precursor is usual-type vulvar intraepithelial neoplasia (uVIN) of the basaloid or warty type [4, 5].

p53 is a tumor suppressor gene which is involved in maintaining genomic integrity by controlling cell cycle progression or inducing apoptosis. About 50% of primary human cancers carry mutations in this gene [6]. The tumor-suppressive activity of p53 has been attributed to its ability to regulate the transcription of many different genes in response to a range of stress signals [7]. Some viral oncogenes, such as the HPV viral oncogene E6, have been shown to cause p53 to be functionally inactive. This causes deregulated expression of many genes which p53 orchestrates, such as those involved in apoptosis, DNA stability, and cell proliferation [8].

Expression of the cyclin-dependent kinase inhibitor p16INK4A (p16) correlates closely with the presence of high-risk HPV types, and overexpression of p16 is a surrogate marker for HPV-driven neoplasia [9, 10]. The increase in p16 protein production is mainly related to elevated transcription, which is mediated by the high-risk HPV-encoded oncoprotein E7. The latter functionally inactivates the retinoblastoma protein (RB), releasing p16 from negative feedback control [11].

The prognostic significance of HPV DNA, p16 expression, and p53 expression in patients with squamous vulvar carcinomas is controversial. Some authors have suggested that these markers are not independent prognostic factors [12–15], while others have postulated that surgical aggressiveness could be modified depending on the presence or absence of HPV DNA and/or p16 immunohistochemistry [16, 17].

In oropharyngeal squamous cancers, there is a consensus that HPV-positive cancers are associated with a better prognosis and are more sensitive to radiation therapy [18]. This is true also for anal cancers [19].

The main aims of the current study were to further investigate the independent prognostic significance of HPV status in vulvar squamous cell carcinomas and to clarify whether preoperative determination of p16 or p53 status by immunohistochemistry would have any clinical relevance. A secondary aim was to evaluate clinicopathological variables associated with p16 and p53 status.

2. Materials and Methods

Ethics approval was obtained from the South Eastern Sydney Local Health District Human Research Ethics Committee (Reference number: 15/151(LNR/POWH/311)). Consecutive patients treated primarily for squamous cell carcinoma of the vulva at the Royal Hospital for Women, Sydney, between February 2002 and February 2014, were included in the study ($n = 119$). Demographic, clinical, surgical, histopathological, 2009 FIGO staging, and outcome data were retrospectively extracted from the medical records. The patients were followed up until death, or until 30/4/2019. All hematoxylin and eosin slides were reviewed by one of the authors (NL), and PCR detection of HPV DNA was performed by another author (ZN).

3. Immunohistochemistry

Each invasive carcinoma was stained for p53 (Leica Microsystems, Novocastra reagents) and p16 (Ventana Medical Systems, Roche Diagnostics) on a Leica Bond 111 platform. The staining was interpreted by a gynecologic pathologist (NL) as "positive" or "negative." To be interpreted as "positive" (indicating a p53 mutation), p53 staining needed to show definite, usually strong, staining in almost all tumor cell nuclei, with a good positive control. A variable, patchy positive pattern of staining was interpreted as the wild-type pattern ("negative"). For p16, a positive pattern was block-like positive nuclear, ± cytoplasmic staining in virtually all tumor cells. Variable and/or patchy positive staining was interpreted as negative.

In almost all cases, the staining pattern for p53 and p16 was clearly positive or negative. There were no cases with a complete negative (null staining) pattern of p53 staining (which would also be indicative of a p53 mutation) in this series.

4. HPV DNA Sample Processing and Nucleic Acid Extraction

Formalin-fixed paraffin-embedded tissue specimens were processed for total nucleic acid extraction using the MagNA Pure 96 System (Roche). Firstly, paraffin-embedded tissue blocks were cut into 10×3-micron sections (30 microns total). A new microtome blade was used each time to section a new tissue block to avoid cross-contamination between different samples. Tissue sections were then subjected to xylene treatment (800 μl xylene, Sigma-Aldrich) to dissolve paraffin from the tissue. Tissue sections were pelleted by centrifugation at 16,000 ×g to remove xylene waste and then washed using 800 μl of 100% ethanol (Sigma-Aldrich). Following centrifugation and removal of ethanol supernatant, tissue pellets were air-dried for 10 minutes and then digested using 160 μl MagNA Pure 96 DNA Tissue Lysis Buffer (Roche) and 40 μl Proteinase K (Siemens), with an overnight incubation at 55°C. Subsequently, total nucleic acid was extracted from digested tissue μl preparations (200 μl) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche), with an elution volume of 100 μl. Extracts were stored at −20°C before testing for HPV DNA.

5. PCR Detection of Human Papillomavirus (HPV)

PCR detection of HPV DNA was performed using My11 (5′-GCACAGGGYCAYAAYAATGG-3′) and GP6+ (5′-AATCATATTCCTCMMCATGTC-3′) primers, targeting the conserved L1 region of the HPV genome [20, 21]. These primers were kindly provided by Noel Whitaker (School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney), and they can detect high risk HPV, as well as low-risk subtypes as described previously [20, 21]. Template nucleic acid (10.5 μl) was added to a 14.5 μl reaction mixture containing 12.5 μl of 2 × MyTaq™ Red Mix (Bioline) and 0.4 μM of each primer (My11 and GP6+). Cycling conditions include initial denaturation at 94°C for 3 min; 50 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, followed by a final extension at 72°C for 3 min. PCR products of 169 bp were expected for HPV-positive specimens and were visualised by gel electrophoresis.

The validity of the entire process (sample processing, total nucleic acid extraction, and HPV PCR amplification) was confirmed by testing known HPV-positive paraffin-embedded tissues ($n = 2$), along with the study samples.

6. Statistical Analysis

Descriptive analysis was performed using IBM SPSS Statistics for Windows (version 25) including frequencies and medians to compare p16/HPV and p53 status with

clinicopathological variables. Cross tabulations were performed to examine associations between the two groups using Pearson's χ^2 test. If there were less than five observations per cell, a two-tailed Fisher exact test was used. A p value of 0.05 or less was considered statistically significant.

Disease-free survival (DFS) was calculated from the date of treatment until the date of disease recurrence. Disease-specific survival (DSS) was calculated from the date of treatment to the date of death from VSCC. All other patients were censored at date of last follow-up, or date of death from another cause, without documented progression of VSCC. Kaplan–Meier estimates of DFS and DSS were calculated within groups determined by p16 and p53 status. Survival comparisons between the groups were performed using the two-sided log-rank test. To determine five-year survival for the Kaplan–Meier analysis, patients' follow-up was censored after 5 years.

Cox proportional hazards models were used in univariate and multivariable analyses to investigate potential prognostic factors for DFS and DSS. These models included eight prognostic variables in addition to p16 and p53 status. Hazard ratios (HR) with 95% confidence intervals (CI) are presented.

7. Results

There were 196 patients with vulvar cancer on our database between 2002 and 2014, of whom 119 were included in the study. The remaining 77 patients were excluded because they had nonsquamous histology ($n = 26$), were referred after primary treatment elsewhere ($n = 12$), presented with recurrent disease ($n = 21$), or had insufficient invasive tissue in the pathology blocks to perform immunohistochemical staining ($n = 18$).

HPV testing was performed on 117 samples (2 could not be evaluated) and 22 were HPV positive (19%). p16 and p53 immunohistochemistry were performed on all 119 tissue samples; 63 (53%) were p16 positive and 44 (37%) were p53 positive.

Twelve of the 119 cases (10%) stained negative for p16 and p53 and were HPV negative. In Kaplan–Meier analysis, this group had a disease-specific survival intermediate between the p16 and p53 groups. However, we have excluded them from further analysis as no distinction could be made based on their HPV, p16, or p53 status.

The remaining 107 patients with positive immunohistochemistry were divided into two groups based on their being p16/HPV positive or p53 positive. Of the 22 HPV-positive tumors, 21 were also p16 positive, and one was p53 positive. The HPV/p53-positive tumor was considered more likely not to be HPV-related because of the patient's age (87 years) and the tumor's association with lichen sclerosus. Five cases stained positive for both p16 and p53. Three of these were associated within a background of lichen sclerosus and dVIN and were therefore considered to be HPV-negative cancers. The remaining two were associated with uVIN and were therefore considered to be HPV-positive cancers.

The clinicopathological features of the 107 patients with positive immunohistochemistry are shown in Table 1. There were 101 Caucasian patients, and 6 were of aboriginal descent. Primary surgery was performed on 101 patients (94%), including radical local excision in 87 patients and radical vulvectomy in 14.

Patients with p16-associated tumors were younger ($p < 0.001$) and were more commonly past or present smokers ($p < 0.001$) than those with p53-associated tumors. The p53-associated group had a higher number of patients with perineural invasion (PNI) ($p = 0.001$), depth of invasion ≥ 5 mm ($p = 0.004$), positive nodes ($p = 0.011$), and higher FIGO stage ($p = 0.02$). Patients with p53-positive tumors had a slightly higher incidence of tumor recurrence than the p16-positive group (53% versus 47%, resp.), but this was not statistically significant ($p = 0.07$). They were also much more likely to have two or more local recurrences than the p16-associated group (78% versus 22%, resp., $p = 0.03$). No significant differences were observed between the groups for tumor size, tumor differentiation, or lymphovascular space invasion (LVSI).

Regarding the primary site of disease, tumors located on the clitoris were more frequently p53-associated (83% versus 17%, resp., $p = 0.003$), whereas tumors located on the vulvar vestibule were more often p16-associated (90% versus 10%, $p = 0.04$).

Eighty-six patients (80%) had a unilateral or bilateral inguinofemoral lymphadenectomy, or groin node debulking. Of the 20 patients who did not have a groin lymphadenectomy, 11 had Stage 1A, and 8 had early Stage 1B disease. None of these 19 patients developed a groin recurrence with a minimum follow-up of 30 months and were regarded as node-negative for analysis. The one remaining patient received primary radiotherapy. She had no palpable nodes and did not have a groin lymphadenectomy. She died of progressive disease within 6 months of diagnosis and her nodal status was recorded as unknown.

Six patients (6%) received primary vulvar radiotherapy, five combined with chemotherapy. Twenty patients (19%) had adjuvant radiotherapy, 2 to the vulva only, 9 to the vulva, groins, and pelvis, and 9 to the groins and pelvis.

The patients were followed up for a median of 72 months (range 3–198 months). At the completion of the study, 64 patients (60%) were without evidence of disease and 43 patients (40%) had died. Of the 43 deaths, 20 patients (19%) died of disease and 23 of other causes (21%). There were 38 recurrences (36%), of which 29 (27%) were local (four concurrent with a groin recurrence), five (5%) isolated groin, and four (4%) distant. Of the 29 vulvar recurrences, 14 (48%) were at a remote site and 15 (52%) were at the primary site ($p = 1.0$). Remote site vulvar recurrences occurred in 8/63 patients (13%) with p16-associated cancers and 6/44 (14%) with p53-associated cancers ($p = 0.9$). For both primary and remote vulvar sites, the earliest first recurrence in p16 and p53 cancers occurred at 6 and 3 months, respectively, while the latest first recurrences occurred at 118 months and 53 months, respectively.

8. Survival Analysis

Based on Kaplan–Meier estimates, p16-positive patients had a better five-year DFS than p53-positive patients (76% versus

42%, resp., $p = 0.004$) and a better five-year DSS (89% versus 75%, resp., $p = 0.05$) (Figures 1(a) and 1(b)).

In the univariate Cox regression analysis for DFS, nodal metastases ($p < 0.001$), PNI ($p = 0.05$), and tumor size >4 cm ($p = 0.03$) were significantly associated with an increased risk of disease progression, while p16 expression (compared to p53 expression) was associated with a decreased risk ($p = 0.03$) (Table 2).

For DSS, tumor size >4 cm ($p < 0.001$), depth of invasion >5 mm ($p = 0.008$), nodal metastases ($p < 0.001$), PNI ($p = 0.02$), and having had adjuvant radiotherapy ($p = 0.005$) were all associated with an increased risk of death (Table 2).

Table 3 shows the multivariable Cox regression model for DFS and DSS. Lymph node metastasis was the only statistically significant independent prognostic factor associated with disease progression ($p = 0.01$). For DSS, only tumor size >4 cm ($p = 0.008$) and lymph node metastases ($p = 0.001$) remained independent prognostic factors in the full model.

9. Discussion

Over the last ten years, the reported incidence of HPV DNA in VSCCs has varied between 17% [22] and 59% [4, 5, 23]. In our series, the HPV DNA prevalence rate was 19%, but the prevalence of the surrogate marker p16 was 53%.

Variation in the incidence of HPV infection rates is sometimes attributed to geographical differences [24], differences in the HPV detection methods across studies, and the number of HPV types detected [25]. Our method for detecting HPV DNA, using PCR assays on formalin-fixed paraffin-embedded (FFPE) tissue specimens, is considered the most sensitive, but it has been reported to be potentially impeded by the formalin fixation and paraffin embedding [26]. A recent German study showed a 53% decrease in DNA quantity following a second DNA extraction from 46 FFPE tissue blocks stored for a median time of 5.5 years [27]. As our FFPE samples were stored for a median of 7.6 years (range 2–14 years), this prolonged storage presumably contributed to the relatively low incidence of HPV DNA in our specimens. It also suggests that using PCR assays on FFPE tissue blocks to determine HPV DNA status lacks sensitivity, unless performed on relatively recent tissue blocks.

We used p16 expression to more accurately classify our HPV-related cancers because p16 is strongly overexpressed (without tumor suppressive action) in the presence of high-risk HPV infection due to the functional inactivation of the retinoblastoma protein RB by the HPV-encoded E7 oncoprotein [9, 11]. It is considered an effective surrogate for determining HPV-associated squamous abnormalities of the lower genital tract [9, 10] and squamous vulvar cancers [28].

Our prevalence of p53 expression was 37%, which is within the published range of 28–78% [15, 17, 23, 29, 30]. Like some other studies [29, 31], we found an inverse association between p53 expression and p16 expression, with only five exceptions, and between p53 and HPV DNA, with only one exception. This was not surprising, because mutation of the p53 gene is mostly seen in vulvar cancers which are unrelated to HPV infection [32].

In our study, 10% of the VSCCs were not associated with either p16/HPV DNA or p53 expression. The mechanism of carcinogenesis in this group is unknown, but several other molecular markers have been identified and correlated with clinical outcome in subsets of patients with VSCC. These include epidermal growth factor receptor (EGFR) [29, 33], the c-KIT proto-oncogene, also known as SCFR or CD117 [34], and NOTCH1 and HRAS mutations [17]. Nooij postulated a third molecular subtype of vulvar cancer which was HPV and p53 negative, but p53 wild type with frequent NOTCH1 mutations. In their series, the recurrence rate for this group was intermediate between their HPV-positive and -negative groups [17]. In our series, the p16- and p53-negative tumors also had an intermediate 5-year DSS of 83%, between the p16- (89%) and p53-positive cases (75%).

Univariate analysis showed distinct clinical and pathological differences between patients with p16- and p53-positive cancers. In accordance with previous studies, patients with p16-positive cancers were significantly younger [15, 16, 22, 29, 31], more commonly smokers [22], had earlier-stage disease [17, 22], had tumors which invaded less deeply [22], and had fewer lymph node metastases [17, 22, 31].

In our study, tumors located on the vulvar vestibule were more commonly p16-associated tumors (90% versus 10%, resp., $p = 0.04$). Hinten et al. reported that HPV-related cancers were more commonly located on the perineum. They attributed this to the perineum being potentially more susceptible to microtrauma during sexual intercourse, facilitating entry of HPV into the basal cell layer [22]. Should this hypothesis be true, the vulvar vestibule would also be susceptible to such microtrauma.

Our findings for univariate survival confirmed several long-established clinicopathological factors related to DFS and DSS. When adjusted for all other factors in multivariable analysis, only tumor diameter >4 cm and lymph node metastases remained significantly poor prognostic indicators for DSS, and only the latter for DFS. Lymph node metastases [35] and greater tumor diameter [36] are widely recognised as factors associated with negative outcomes for patients with VSCCs.

Previous studies on the influence of HPV/p16 expression on prognosis for VSCC's have reported contradictory results. Tringler et al. also found that patients with p16-positive vulvar cancers had significantly longer DFS and overall survival (OS) in univariate but not multivariable analysis [37]. Two other studies reported no survival advantage for patients with p16/HPV-positive tumors in either unadjusted or adjusted analysis [14, 15]. By contrast, two recent retrospective series have reported p16/HPV-associated tumors to have better DFS and DSS [16], as well as OS [22] when compared to p16/HPV-independent tumors in both univariate and multivariable analyses.

The reported correlation between p53 expression and prognosis in patients with squamous vulvar cancers is also inconsistent. One early study found p53 overexpression to be significantly associated with a poorer prognosis, but only

Table 1: Cohort characteristics and the association of clinicopathological variables with p16 and p53 expression.

Variable	Total no. (%) $N = 107$	p16-positive (%) $N = 63$	p53-positive (%) $N = 44$	p value
Follow-up (months, median)	72 (range 3–198)	72 (range 5–189)	71 (range 3–198)	
Median age in years	71 (range 36–93)	62 (range 39–89)	76 (range 36–93)	
Age groups				
(i) ≤65 years	47 (43.9%)	37 (79%)	10 (21%)	<0.001
(ii) >65 years	60 (56.1%)	26 (43%)	34 (57%)	
Smoking status				
(i) Never	63 (58.9%)	26 (41%)	37 (59%)	<0.001
(ii) Former/current	44 (41.1%)	37 (84%)	7 (16%)	
FIGO stage, *n* (%)				
(i) I	63 (58.9%)	43 (68%)	20 (32%)	
(ii) II	5 (4.7%)	3 (60%)	2 (40%)	
(iii) III	37 (35.6%)	16 (43%)	21 (57%)	
(iv) IV	2 (1.8%)	1 (50%)	1 (50%)	
(v) Stage I/II versus III/IV				0.024
Nodal status†				
(i) Positive	39 (36.4%)	17 (44%)	22 (56%)	0.011
(ii) Negative	67 (63.6%)	46 (67%)	21 (31%)	
LVSI				
(i) Yes	21 (19.6%)	11 (52%)	10 (48%)	0.500
PNI				
(i) Yes	15 (14%)	3 (20%)	12 (80%)	0.001^{β}
Tumor differentiation				
(i) Well	39 (36.5%)	23 (59%)	16 (41%)	0.988
(ii) Moderate/poor	68 (63.5%)	40 (59%)	28 (41%)	
Depth of invasion—mm				
(i) ≤5 mm	59 (55%)	42 (71%)	17 (29%)	0.004
(ii) >5 mm	48 (45%)	21 (44%)	27 (56%)	
Tumor size—cm				
(i) ≤4 cm	73 (68.2%)	46 (63%)	27 (37%)	0.203
(ii) >4 cm	34 (31.8%)	17 (50%)	17 (50%)	
Lesion location				
(i) Clitoris	12 (11.2%)	2 (17%)	10 (83%)	0.003
(ii) Labium minus	22 (20.6%)	14 (64%)	8 (36%)	0.611
(iii) Labium majus	41 (38.3%)	25 (61%)	16 (39%)	0.728
(iv) Perineum	7 (6.5%)	4 (57%)	3 (43%)	1.000^{β}
(v) Vulvar vestibule	10 (9.3%)	9 (90%)	1 (10%)	0.044^{β}
(vi) Multifocal	15 (14%)	9 (60%)	6 (40%)	0.924
Adjuvant radiotherapy‡				
(i) Yes	20 (19.8%)	8 (40%)	12 (60%)	0.048
(ii) No	81 (80.2%)	52 (64%)	29 (36%)	
Recurrence				
(i) Any	38 (35.5%)	18 (47%)	20 (53%)	0.073
(ii) Local	29 (27.1%)	14 (48%)	15 (52%)	0.174
(iii) Regional/distant	13 (12.1%)	5 (38.5%)	8 (61.5%)	0.110
(iv) ≥2 local	9 (8.4%)	2 (22%)	7 (78%)	0.031^{β}

FIGO: International Federation of Gynecology and Obstetrics; LVSI: lymphovascular space invasion; PNI: perineural invasion. †One p53-positive patient nodal status unknown, ‡6 patients were excluded who had primary radiotherapy. Statistically significant value ($p < 0.05$)—Pearson's Chi-square. $^{\beta}$Fisher's exact test for cell counts <5.

for patients with Stage III disease [38], while others reported no association [12, 13]. More recent studies have reported patients with HPV-positive cancers to have superior survival compared to patients with p53-positive cancers [17, 29]. A recent meta-analysis reported that patients with p16-positive tumors had a significantly better 5-year OS compared to those with p16-negative tumors and that patients with p53-positive tumors had a significantly lower 5-year OS when compared to those with p53-negative tumors [39].

Recently published data support the concept that p16 positivity may be a good prognostic indicator for radiotherapy response in patients with vulvar cancer [40, 41], as has been shown earlier for HPV-positive oropharyngeal cancers [18]. Our study was not designed to make a definitive comment regarding radiotherapy, and our number of patients receiving radiotherapy was small. However, we observed no advantage in DFS or DSS for patients with p16-positive tumors who had adjuvant radiotherapy in multivariable analysis.

Some authors have postulated that the HPV DNA, p16, and/or p53 status of squamous vulvar cancers could be used to change clinical management. In 2016, Hay et al. initially

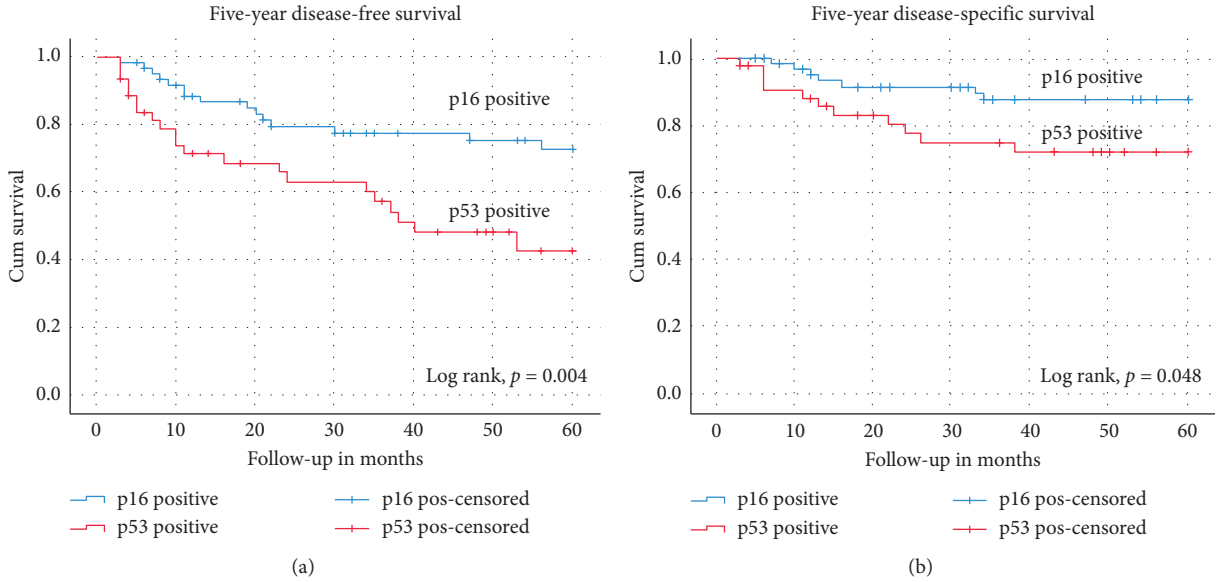

Figure 1: Kaplan–Meier curves for (a) five-year disease-free survival and (b) five-year disease-specific survival stratified by p16-positive and p53-positive groups.

Table 2: Univariate outcome analysis by Cox regression for disease-free survival and disease-specific survival.

Variable	Disease-free survival			Disease-specific survival		
	HR	95% CI	p value	HR	95% CI	p value
Age >65 years (ref—age ≤65 yrs)	1.62	(0.84–3.10)	0.15	2.32	(0.90–6.05)	0.09
Lesion size >4 cms (ref—≤4 cm)	2.05	(1.07–3.92)	0.03	8.05	(3.10–21.05)	<0.001
Depth of invasion >5 mm (ref—≤5 mm)	1.16	(0.62–2.20)	0.64	3.65	(1.40–9.51)	0.008
Lymph node metastases	3.30	(1.73–6.22)	<0.001	23.34	(5.40–101.12)	<0.001
Perineural invasion	2.23	(1.02–5.00)	0.05	3.20	(1.21–8.26)	0.02
LVSI	1.43	(0.70–3.02)	0.35	1.54	(0.56–4.23)	0.41
Differentiation—mod/poor (ref—well-differentiated)	1.20	(0.61–2.31)	0.62	2.62	(0.90–7.85)	0.09
Adjuvant radiotherapy	2.00	(0.92–3.91)	0.08	3.60	(1.45–8.73)	0.005
P16 positive (ref—p53 positive)	0.50	(0.30–0.95)	0.03	0.51	(0.21–1.24)	0.14

HR: hazard ratio; CI: confidence interval; ref: reference group; LVSI: lymphovascular space invasion.

Table 3: Multivariable outcome analysis by Cox regression for disease-free survival and disease-specific survival.

Variable	Disease-free survival			Disease-specific survival		
	HR	95% CI	p value	HR	95% CI	p value
Age >65 years (ref—age ≤ 65 yrs)	1.20	(0.54–2.50)	0.70	2.12	(0.68–6.61)	0.20
Lesion size >4 cm (ref—≤4 cm)	2.05	(0.91–4.63)	0.08	4.90	(1.52–15.80)	0.008
Depth of invasion >5 mm (ref—≤5 mm)	0.53	(0.24–1.15)	0.11	1.01	(0.34–3.03)	0.98
Lymph node metastases	3.03	(1.25–7.35)	0.01	14.83	(2.92–75.20)	<0.001
Perineural invasion	1.72	(0.70–4.25)	0.24	1.80	(0.64–5.00)	0.27
LVSI	0.84	(0.40–2.00)	0.70	0.61	(0.20–1.83)	0.38
Differentiation—mod/poor (ref—well-differentiated)	1.16	(0.60–2.40)	0.70	1.76	(0.47–6.54)	0.40
Adjuvant radiotherapy	0.64	(0.25–1.65)	0.36	0.53	(0.20–1.55)	0.25
p16 positive (ref—p53-positive)	0.68	(0.31–1.50)	0.33	0.90	(0.31–2.50)	0.80

HR: hazard ratio; CI: confidence interval; ref: reference group; LVSI: lymphovascular space invasion.

proposed that p53-positive VSCCs may require more aggressive surgery and adjuvant treatment [23]. McAlpine et al. noted a worse outcome for patients with HPV-negative cancers after the introduction of a more conservative surgical approach and postulated that more conservative surgery may be appropriate for younger patients with HPV-positive VSCCs, while patients with HPV-negative cancers may warrant more radical surgery with wider margins and more frequent surveillance [16]. Nooij et al. also suggested the possibility of more aggressive surgery and more stringent follow-up for patients with HPV-negative tumors [17].

Our results would not support any changes to clinical management based on HPV DNA, p16, or p53 status unless it could be shown to be justified in a prospective, randomised clinical trial. Only tumor diameter >4 cm and lymph node metastases were shown to be independent prognostic factors. In addition, remote site vulvar recurrences occurred with a similar frequency to primary site recurrences, as has been reported previously [42, 43], and will occur regardless of the margin status. With regular surveillance for life, preferably done in conjunction with self-inspection of the vulva with a mirror, recurrences can be diagnosed early and resected or radiated with excellent results [43]. One patient with a p16-positive cancer recurred for the first time at 118 months, although such a "recurrence" would have to be regarded as a new primary.

Our study has the limitations of a retrospective design and the inherent restriction in most vulvar cancer studies of limited patient numbers. The study strengths include the combined determination of HPV DNA, together with immunohistochemistry for the biomarkers p16 and p53, and the consistent patient management over the period of the study. Additionally, the long duration of follow-up (median of 72 months) allowed for accurate recurrence and survival outcomes to be assessed.

10. Conclusion

The p16 and p53 status of vulvar squamous carcinomas, as determined by immunohistochemistry, allows separation of patients into two distinct clinicopathological groups, although there is a third group which is both p16 and p53 negative. Univariate analysis demonstrated a lower recurrence rate and better survival for patients with p16-positive tumors, but multivariable analysis did not find evidence to suggest that differentiating between HPV/p16 and p53 status provided independent prognostic information. This may be related to the small number of events for recurrence and death from vulvar cancer, but the status of the groin lymph nodes was the only independent prognostic factor for disease-free survival in this study. In view of these results, clinical management should continue to be based on clinical indicators rather than p16 or p53 status.

Data Availability

The data used to support the findings of this study have not been made available because they are restricted by the Ethics Committee in order to protect patient confidentiality.

Disclosure

The funding sources had no influence on the study design, the data analysis, or the writing of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Acknowledgments

This research was supported, in part, by a grant from the GO Research Fund of the Royal Hospital for Women Foundation. EB is supported by an Australian Government Research Training Program Scholarship.

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CHAPTER FIVE

MORBIDITY RELATED TO THE GROIN LYMPH NODE DISSECTION FOR VULVAR CANCER.

5.1 | Precis

The research described in this chapter focused on answering the surgical management controversies related to the acute and chronic morbidity associated with the removal of the inguino-femoral lymph nodes, which are a principal concern for patients and clinicians. This chapter reports the incidence of short and long-term postoperative complications of groin node dissection from a cohort of 333 patients (525 groins) and identifies some causal factors.

This chapter addresses the following research questions:

- What is the incidence of short and long-term postoperative morbidity of the groin lymph node dissection in a large cohort of patients treated for invasive vulvar cancer?
- What clinical factors are associated with post-operative morbidity following groin node dissection?

Supplementary research questions:

- What is the incidence of lymphocyst formation in patients with and without a groin drain following groin node dissection?
- Is short term post-operative morbidity increased in patients with a groin drain?

Barlow et al., Int J Gynecol Clin Pract 2019, 6: 149
https://doi.org/10.15344/2394-4986/2019/149

International Journal of Gynecology & Clinical Practices

Research Article | Open Access

Special Issue: Gynecology & Surgical Oncology

Morbidity Related to the Groin Lymph Node Dissection for Vulvar Cancer

Ellen L Barlow[1*], Mark W Donoghoe[2] and Neville F Hacker[1,3]

[1]*Gynaecological Cancer Centre, the Royal Hospital for Women, Sydney, NSW, Australia*
[2]*Mark Wainwright Analytical Centre, University of New South Wales, Sydney, Australia*
[3]*School of Women's & Children's Health, University of New South Wales, Sydney, Australia*

Abstract

Objective: To determine the incidence of morbidity following groin lymphadenectomy for vulvar cancer, to explore causal factors, and examine strategies to reduce morbidity.

Method: A retrospective analysis of clinical and histopathological data was conducted on patients treated for invasive cancer of the vulva at a tertiary hospital in Sydney, Australia, from 1987 to 2016.

Results: Some type of groin dissection was performed on 525 groins in 333 patients. Lymphocysts occurred in 36.6% of groins and was higher in patients having an inguino-femorallymph node dissection compared to those having groin node debulking, or a sentinel node procedure (42.5% versus 14.6% versus 0% respectively: $p < 0.0001$). In multivariable analysis, no significant difference in lymphocyst incidence was observed between patients with or without a groin drain. Wound breakdown occurred in 8.2% and wound infection in 10.7% of groins. Lymphedema occurred in 31.6% of lower limbs. The number of nodes resected was the only factor significantly associated with all complications, but current smoking and increasing age also increased the risk of wound breakdown.

Conclusion: A more extensive lymph node dissection is a significant risk factor for lymphocyst formation, groin wound infection, groin wound breakdown, and lower limb lymphedema. Debulking of bulky positive lymph nodes rather than complete inguino-femorallymphadenectomy reduces the risk of all post-operative complications. Our incidence of groin wound breakdown was less than 10% despite resection of the saphenous vein in all cases. Preservation of all subcutaneous fat above Camper's fascia appears to be the most critical factor in wound healing.

Publication History:

Received: September 04, 2019
Accepted: November 20, 2019
Published: November 22, 2019

Keywords:

Groin drain, Groin node debulking, Groin wound breakdown, Inguino-femoral lymph node dissection, Lymphocyst, Lymphedema, Vulvar cancer

Introduction

The status of the groin lymph nodes is the most important prognostic factor for patients with vulvar cancer. Selected early vulvar cancers may be amenable to sentinel node biopsy, but many patients will require an inguino-femoral lymphadenectomyin order to adequately treat the groin nodes.

The use of a separate incision approach significantly improved wound healing and decreased post-operative hospital stay, but the long-term problem of lower limb lymphedema remained. Several attempts have been made to try to reduce the risk of lymphedema, including elimination of groin dissection in patients with 'micro-invasive' vulvar cancer [1], the performance of a superficial inguinal lymphadenectomy [2] and the use of primary groin irradiation. These approaches were shown to increase the incidence of groin recurrence [3-5].

The purpose of this study was to determine the incidence of short and long-term postoperative morbidity of groin node dissection in a large cohort of patients, to investigate causal factors, and to postulate possible strategies to further reduce this morbidity.

Materials and Methods

Study design

A retrospective observational single institutional study.

Following ethics approval obtained from the South Eastern Sydney Local Health District Human Research Ethics Committee (Reference Number 15/151), the medical records of 429 consecutive patients treated for primary invasive vulvar cancer at the Royal Hospital for Women in Sydney, between February 1987 and June 2016 were reviewed. Ninety-six patients were excluded as their groins were not surgically treated. The remaining 333 patients underwent either unilateral or bilateral inguino-femoral lymphadenectomy, groin node debulking, or a sentinel node procedure and were included in the analysis. Data retrieved from the medical records included age at diagnosis, body mass index (BMI), smoking status, co-morbidities, disease stage, tumour diameter, histologic type, histologic grade, primary treatment, adjuvant treatment, type of lymph node dissection, number of lymph nodes removed, intra-operative insertion of a groin drain, duration of drain use, post-operative groin wound infection, groin wound dehiscence/breakdown, lymphocyst formation, length of stay and hospital readmission. Follow up data on lymphedema and patient disease status was retrieved from the outpatient clinical files. All patients were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system [6].

Lymphocyst formation was recorded if confirmed by an ultrasonic scan, or if fluid was drained from the groin. Groin wound breakdown

***Corresponding Author:** Ellen L Barlow, Gynaecological Cancer Centre, The Royal Hospital for Women Barker Street, Randwick, NSW 2031, Australia, Tel: 61 2 93826184, Fax: 61 2 93826200; E-mail: ellen.barlow@health.nsw.gov.au

Citation: Barlow EL, Donoghoe MW, Hacker NF (2019) Morbidity Related to the Groin Lymph Node Dissection for Vulvar Cancer. Int J Gynecol Clin Pract 6: 149. https://doi.org/10.15344/2394-4986/2019/149

was defined as opening of the wound requiring either wound packing, or a negative pressure dressing. Groin wound infection was defined as erythema or a purulent exudate necessitating the use of antibiotics. Chronic lower limb lymphedema was recorded if documented as clinically obvious (mild, moderate, severe) during routine follow up, or patient reported as requiring compression garments and lymphatic massage to manage.

Three forms of groin node resection were performed; (1) complete inguino-femoral lymphadenectomy (2) resection of bulky positive nodes and (3) sentinel node biopsy.

The technique for inguino-femoral lymphadenectomy was to make a linear incision down to Camper's fascia, 1 cm above the groin crease, extending from a line perpendicular to the pubic tubercle medially to about 2 cm medial to the anterior superior iliac spine laterally. Camper's fascia was incised, and the fat in the femoral triangle deep to the fascia was removed as inguinal lymph nodes. All subcutaneous fat was preserved. The femoral nodes were obtained by removing the fat beneath the cribriform fascia in the fossa ovalis, medial to the femoral vein. After 1991, the fascia lata was left intact, but previously it was removed, and a sartorius muscle transposition performed to protect the femoral vessels. The saphenous vein was removed routinely.

Patients with palpable groin nodes were treated by resection of bulky nodes and frozen section diagnosis. If metastatic disease was confirmed, only palpably enlarged nodes were removed. When sentinel node biopsy was performed, pre-operative lymphoscintigraphy was combined with intraoperative blue dye injection for nodal identification.

Groin suction drains were routinely used up until 2002, and then variably over subsequent years. They were removed when fluid production was less than 50 millilitres over 24 hours. All patients received one dose of prophylactic antibiotics pre-operatively and thrombotic prophylaxis post-operatively.

Statistical Analysis

Risk factors for short and long-term complications were assessed with univariate analysis. Descriptive analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25 software (IBM Corp., Armonk, New York, USA) including frequencies and medians. Cross tabulations were performed to examine associations between two variables using Pearson's χ^2 test (SPSS), or the Cochran-Armitage trend test to assess linear trends using Stata Statistical Software 15 [7]. A p value of <0.05 was considered statistically significant.

To investigate the factors associated with groin morbidity in multivariable models, the lme4 package [8] in R [9] was used to fit a mixed-effects logistic regression model for each outcome. Patient factors (age, BMI, diabetic and smoking status) and treatment factors (number of nodes removed, groin drain insertion, radiotherapy) were included as fixed effects, with random intercepts to account for within-patient correlation. Odds ratios and their 95% confidence intervals for each fixed effect were calculated by exponentiating the parameter estimates and Wald confidence intervals produced by the model.

Results

We included 333 eligible patients. Table 1 shows the characteristics of the study group. Among the 333 patients, 525 groins were dissected, 192 patients (57.7%) undergoing a bilateral procedure and 141 (42.3%) a unilateral procedure. Inguino-femoral lymphadenectomy was performed in 278 patients (79.7%) (416 groins), a nodal debulking in 65 patients (18.6%) (103 groins), and a sentinel node biopsy in 6 patients (1.7%) (6 groins). The median number of nodes removed per groin was 9 for patients having an inguino-femoral lymphadenectomy, 3 for a nodal debulking and 2.3 for a sentinel node procedure.

Sixty-nine patients (20.7%) received adjuvant radiotherapy to the groins and pelvis, while 12 patients (3.6%) received primary radiotherapy to the vulva and both groins. All 12 patients underwent some form of groin node procedure prior to their radiotherapy.

Groin wound drains were used in 211 patients (63.4%) and 348 groins (66.3%), with the drain left in-situ for a median of 6 days (range 2 - 16). Overall median length of post-operative hospital stay was 13

Patient Characteristic	Study Group (n = 333)
Age, years (Range 20 – 96)	
Mean	67
Median	71
Smoking status	
Current	77 (23.1%)
Former	45 (13.5%)
Never	211 (63.2%)
BMI (Range 14.6 – 54.7)	
< 20	13 (3.9%)
20 - < 30	230 (69.1%)
30 – 35	66 (19.8%)
> 35	24 (7.2%)
Diabetic	
Insulin dependent	16 (4.8%)
Non-Insulin dependent	31 (9.3%)
Histopathological sub-type	
Squamous cell carcinoma	302 (90.7%)
Melanoma	10 (3%)
Adenocarcinoma	8 (2.4%)
Sarcoma	6 (1.8%)
Other	7 (2%)
FIGO Stage 2009†	
1B	182 (54.6%)
11	12 (3.6%)
111A (1)	39 (11.7%)
111A (11)	15 (4.5%)
111B (1)	5 (1.5%)
111B (11)	11 (3.3%)
111C	42 (12.6%)
1VA (1)	3 (0.9%)
1VA (11)	7 (2.1%)
1VB	5 (1.5%)
Groin Radiotherapy	
Neoadjuvant	12 (3.6%)
Adjuvant	69 (20.7%)

Table 1: Patient Characteristics
BMI = Body mass index, FIGO = International Federation of Gynecology and Obstetrics.
† FIGO Staging not done on Melanoma (n = 10), or Neuro-endocrine tumours (n = 2).

days (range 2 - 65) and was significantly longer when a groin drain was used (14 days versus 10 days respectively, p = 0.005). The median follow-up was 49 months (range 6 - 366 months). Twenty-two patients (6.6%) were excluded from the analysis for long term complications (lymphedema and recurrent lower limb cellulitis) due to follow up of less than 6 months. Eleven of these patients died within five months of surgery (4 of progressive disease), and 11 were lost to follow up.

Short-term complications of the groin dissection

The commonest immediate post-operative complication was lymphocyst formation which occurred in 36.6% of the groins dissected (Table 2). There was no difference in lymphocyst incidence in groins having an inguino-femoral lymphadenectomy before 1991when the fascia lata was resected compared to after 1991 when the fascia lata was preserved (39.4% vs 42.7% respectively, P = 0.4). Lymphocyst formation was most strongly associated with a greater number of nodes removed (p = 0.0001) (Table 3). When adjusted for other risk factors, the number of nodes removed remained statistically significant for lymphocyst formation (p = 0.0001; OR 1.24 [95% CI 1.12-1.36] per node) (Table 4).

Univariate analysis indicated no difference in the incidence of lymphocyst formation when a groin drain was used. There was a bias in the indication for the use of drains, as they were more commonly used following an inguino-femoral lymphadenectomy (72.4%) than following nodal debulking, (43.8%) (p < 0.001). Use of a drain compared to no drain resulted in no significant difference in the incidence of lymphocyst formation for either an inguino-femoral lymphadenectomy (39.7% vs 48.7% respectively, p = 0.121) or nodal debulking (17.4% vs 13.5% respectively, p = 0.647) on univariate analysis. After adjusting for the number of nodes removed, patients having more nodes removed had a lower rate of lymphocyst formation with a groin drain, but this failed to reach statistical significance (p = 0.06) (Table 4).

The next most common short-term complication was groin wound infection, which occurred in 10.7% of the groins dissected. This was more common in the groins having an inguino-femoral lymphadenectomy (11.3%) than a nodal debulking (7.8%), but the difference was not significant in univariate analysis (p= 0.4) (Table 2). However, in multivariable analysis, increasing number of nodes removed was associated with an increased incidence of groin wound infection (p = 0.02) (Table 4).

The least common short-term complication was groin wound breakdown, which occurred in 8.2% of groins dissected (Table 2). In univariate analysis, the factors significantly associated with a higher rate of groin wound breakdown were increasing number of nodes removed (p = 0.005), current smoking (p = 0.02) and obesity (p < 0.001) (Table 3). On multivariable analysis, increasing age was also associated with groin wound breakdown (p = 0.02; OR 1.74, [95% CI

Total Number of Groins 525 Total No of Patients = 333	**No of groins (no of patients)**	**Complication per groin (per patient)**	**% per groin (% per patient)**	**P value†**
Lymphocyst				
Inguino-femoral LND	416 (278)	177 (150)	42.5% (54.3%)	<.0001
Nodal debulking	103 (65)	15 (14)	14.6% (21.5%)	(<.0001)
Sentinel node	6 (6)	0 (0)		
Incidence per patient	*333*	*164*	*49.2 %*	
Groin wound breakdown				
Inguino-Femoral LND	416 (278)	39 (33)	9.4% (11.9)	0.1570
Nodal debulking	103 (65)	4 (4)	3.9% (3.8%)	(0.3946)
Sentinel node	6 (6)	0 (0)		
Incidence per patient	*333*	*37*	*13%*	
Groin wound infection				
Inguino-Femoral LND	416 (278)	47 (45)	11.3% (16.1%)	0.3713
Nodal debulking	103 (65)	8 (6)	7.8% (9.2%)	(0.3773)
Sentinel node	6 (6)	1 (1)	16.6%	
Incidence per patient	*333*	*52*	*15.6%*	
Lymphedema				
Inguino-Femoral LND	392 (262)	137 (113)	35% (43.1%)	0.0032
Nodal debulking	92 (58)	18 (13)	19.6% (22.4%)	(0.0025)
Sentinel node	6	0	0%	
Incidence per patient	*311*	*126*	*40.5%*	
Recurrent cellulitis per patient†				
Inguino-Femoral LND	262	17	6.5%	1.000
Nodal debulking	58	4	6.9%	
Sentinel node	6	0	0%	
Incidence per patient	*311*	*21*	*6.8%*	

Table 2: Incidence of short and long-term complications to the type of groin dissection.
For lymphedema 35 Groins (22 patients) excluded due to follow up < 6 months.
† Cochran-Armitage trend test.
‡ Recurrent cellulitis data only available per patient, 22 patients excluded due to follow up < 6 months.

1.11 – 2.74] per 10 years), along with current smoking (p = 0.02) and number of nodes removed (p = 0.04) (Table 4).

Long-term complications of groin node dissection

Lymphedema was the major long-term complication occurring in 31.6% of the groins dissected. Lymphedema was more common in groins having an inguino-femoral lymphadenectomy (35%) compared to those having a nodal debulking (19.6%) or a sentinel node procedure (0%) (p = 0.003) (Table 2). An increasing number of nodes removed was significantly associated with an increasing incidence of lymphedema (p = 0.003) (Table 5).

On univariate analysis, there was evidence that obesity was associated with an increased incidence of lymphedema (p =0.01)(Table 5). However, no significant association was found in the multivariable analysis, where BMI was included as a continuous variable (Table 4).

When radiotherapy to the groin was included in our multivariable analysis, the wide 95% confidence interval did not allow a strong conclusion to be drawn about its association with lymphedema (p = 0.4; OR 1.61 [95% CI 0.53 - 4.90]) Table 4.

Recurrent lower limb cellulitis was documented in 6.8% of patients (Table 2), but this was probably substantially under-reported because over 50% of our patient population were referred from regional and rural areas and would have been treated for this complication locally. For this reason, recurrent cellulitis was excluded from further analysis.

Discussion

This is one of the largest series in the literature reporting on groin morbidity following groin node dissection for vulvar cancer. The principal findings were the relatively high incidence of lymphocyst formation and lymphedema, and the relatively low incidence of groin wound breakdown and infection, despite routine resection of the saphenous vein.

Our lymphocyst incidence of 36.6% per groin falls within the reported range of 13% to 60% [10-15]. The incidence of lymphocysts increased significantly as the number of nodes resected increased.

The issue of drains is controversial. In view of the relatively high incidence of lymphocysts despite drain usage, the senior author began to omit the insertion of a drain in 2002. Instead, Camper's fascia was firmly sutured to the underlying fascia lata. Lymphocysts continued to be a problem but another recent Australian study has also reported no statistically significant difference in lymphocyst formation between patients with and without groin drains [15]. In a prospective Dutch study where drains were routinely used, the incidence of lymphocyst formation was reported to be lower when the drain was left in situ until drainage was < 30mls (range 2-40 days), compared to routine removal on the 5th post-operative day (16 % versus 60% respectively) [14].

The post-operative drain management after axillary lymphadenectomy for breast cancer has been studied more extensively. Two

Total groins 528 (Total patients 333)	**No of Groins** (No patients)	**Lymphocyst incidence** per groin (per patient)			**Groin Wound Breakdown incidence** per groin (per patient)			**Groin Wound Infection incidence** per groin (per patient)		
Variable		**Number**	**% (%)**	**p value**	**Number**	**% (%)**	**p value**	**Number**	**% (%)**	**p value**
Age in years										
≤ 50	76 (51)	27 (23)	35.5% (45%)	0.4773†	6 (5)	7.9% (9.8%)	0.4129†	12 (11)	15.8% (21.5%)	0.2701†
51 – 70	179 (112)	64 (55)	35.6% (49%)	(0.8274)	11 (11)	6.1% (9.8%)	(0.7625)	21 (19)	11.7% (16.9%)	(0.4278)
> 70	270 (170)	101 (86)	37.4% (50.5%)		26 (21)	9.6% (12.3%)		23 (22)	8.5% (12.9%)	
Smokers										
Current	119 (77)	50 (42)	42% (54.5%)	0.2489†	16 (15)	13.4% (19.5%)	0.020‡	18 (17)	15.1% (22%)	0.2731†
Past	74 (45)	23 (20)	31% (44.4%)	(0.4040)	8 (6)	10.8% (13.3%)	(0.023)	7 (7)	9.4% (15.5%)	(0.2930)
Never	332 (211)	119 (102)	35.8% (48.3%)		19 (16)	5.7% (7.6%)		31 (28)	9.3% (13.3%)	
Diabetic										
No	451 (286)	159 (138)	35.3% (48.2%)	0.1766†	38 (32)	8.4% (11.2%)	0.3784†	46 (44)	10.2% (15.4%)	0.4600†
Non-Insulin	51 (31)	20 (16)	39.2% (51.6%)	(0.5202)	2 (2)	3.9% (6.4%)	(0.4657)	8 (6)	15.7% (19.3%)	(0.7920)
Insulin	23 (16)	13 (10)	56.5% (62.5%)		3 (3)	13% (18.7%)		2 (2)	8.7% (12.5%)	
Nodes removed										
≤ 4 nodes	103 (83)	18 (16)	17.5% (19.3%)	0.0001†	2 (2)	1.9% (2.4%)	0.0054†	6 (5)	5.8% (6%)	0.1994†
5 – 8 nodes	168 (148)	56 (57)	33.3% (38.5%)	(< 0.0001)	11 (10)	6.5% (6.7%)	(0.0047)	22 (22)	13% (14.8%)	(0.1645)
9 + nodes	254 (195)	118 (102)	46.4% (52.3%)		30 (27)	11.8% (13.8%)		28 (25)	11% (12.8%)	
BMI										
< 30	388 (243)	136 (115)	35 (47.3%)	0.191‡	20 (17)	5.2% (7%)	< 0.001‡)	40 (38)	10.3% (15.6%)	0.725‡
≥ 30	137 (90)	56 (49)	40.9% (54.4%)	(0.248)	23 (20)	16.8% (22.2%)	(< 0.001	16 (14)	11.7% (15.5%)	(0.985)
Groin Drain										
Yes	348 (211)	123 (99)	35.3% (46.9%)	0.854‡	34 (28)	9.8% (13.3%)	0.064‡	42 (39)	12.1% (18.6%)	0.155‡
No	177 (122)	69 (65)	39% (52.8%)	(0.669)	9 (9)	5.1% (7.4%)	(0.094)	14 (13)	7.9% (10.7%)	(0.152)

Table 3: The incidence of short-term complications of the groin node dissection and their association to study variables.

† Cochran Armitage Trend Test, ‡ Pearson Chi Square Test.

	P value	Odds Ratio (95% CI)
Lymphocyst		
Age (+ 10 years)	0.4510	1.09 (0.87 – 1.36)
BMI (+ 5 points)	0.6103	0.93 (0.71 – 1.22)
Diabetes		
Non-insulin dependant	0.8496	1.10 (0.40 – 3.06)
Insulin dependant	0.2840	2.17 (0.52 – 9.01)
Smoker		
Past	0.4780	0.72 (0.30 – 1.77)
Current	0.1670	1.74 (0.79 – 3.84)
Number of Nodes (+)	0.0001	1.24 (1.12 – 1.36)
Groin drain (Yes)	0.0578	0.53 (0.27 – 1.02)
Groin Wound Breakdown		
Age (+ 10 years)	0.0166	1.74 (1.11 – 2.74)
BMI (+ 5 points)	0.0724	1.47 (0.96 – 2.23)
Diabetes		
Non-insulin dependant	0.3448	0.41 (0.65 – 2.60)
Insulin dependant	0.5352	1.82 (2.74 – 12.1)
Smoker		
Past	0.1730	2.44 (6.76 – 8.80)
Current	0.0237	4.83 (1.23 – 18.9)
Number of Nodes (+)	0.0360	1.12 (1.01 – 1.24)
Groin drain (Yes)	0.1476	2.32 (0.74 – 7.28)
Groin Wound Infection		
Age (+10 years)	0.1767	0.83 (0.64 – 1.08)
BMI (+ 5 points)	0.2302	0.80 (0.60 – 1.15)
Diabetes		
Non-insulin dependant	0.1157	2.51 (0.80 – 7.93)
Insulin dependant	0.7432	1.36 (0.21 – 8.70)
Smoker		
Past	0.7162	1.21 (0.43 – 3.50)
Current	0.4544	1.41 (0.60 – 3.50)
Number of Nodes (+)	0.0164	1.11 (1.02 – 2.80)
Groin drain (Yes)	0.5839	1.25 (0.56 – 2.80)
Lymphedema		
Age (+ 10 years)	0.6315	0.92 (0.67 - 1.28)
BMI (+ 5 points)	0.4210	1.18 (0.80 - 1.80)
Diabetes		
Non-insulin dependant	0.6899	0.74 (0.17 – 3.30)
Insulin dependant	0.9183	1.11 (0.14 – 8.75)
Smoker		
Past	0.8533	1.11 (0.32 – 3.90)
Current	0.9348	1.05 (0.34 – 3.24)
Radiotherapy	0.4013	1.61 (0.53 – 4.90)
Number of Nodes (+)	0.0109	1.16 (1.03 – 1.30)
Groin drain (Yes)	0.4234	0.68 (0.30 – 1.73)
Groin Infection	0.7785	0.85 (0.30 – 2.60)
Groin Breakdown	0.4700	1.70 (0.40 – 7.13)
Lymphocyst	0.0578	2.17 (0.97 – 4.81)

Table 4: Odds ratios with 95% confidence intervals (CI) for short and long-term complications of the groin dissection associated in multivariable models with patient-specific random effects.

recent studies of breast cancer patients have also concluded that the use of an axillary drain did not significantly affect symptomatic seroma rates, or any other wound complication rates [16,17]. In both these studies, post-operative hospital stay was significantly longer in the drainage groups, which was also our experience.

Our wound infection rate of 10.7% per groin is low when compared to other studies, where incidences are reported to range from21% to 59% [10,12,14,15,18]. The only risk factor we identified was an increasing number of nodes removed. One recent study found that the incidence of post-operative groin cellulitis was lower in patients without a groin drain [15].

Our 8.2% incidence of groin wound breakdown is one of the lowest rates reported [10,11,13,14,18-20]. In addition to increasing number of nodes removed, increasing age was also a significant risk factor. This association has been noted in some studies [11,20], but not in others [10,12].

A Gynecologic Oncology Group study reported that the presence of a drain significantly increased the risk of groin wound breakdown [21]. Our groin breakdown rate was also higher in patients having a drain (9.8% versus 5.1%), but this was not significant on either univariate or multivariable analysis.

As expected, we found that current smokers were at a higher risk for groin wound breakdown. To our knowledge, only one other vulvar cancer study has reported this association [12]. However, two recent studies from the United States have reported significantly increased wound dehiscence rates in smoking cohorts. One study involved plastic and general surgical patients [22], and the other patients undergoing radical cystectomy [23].

The issue of saphenous vein preservation versus resection is controversial [19,20,24]. Two reports have suggested that saphenous vein preservation decreases groin wound breakdown, but the incidence of groin breakdown in these papers (13% and 16% respectively) was higher than our 8% incidence with vein resection [19,20]. Other studies have reported no correlation between saphenous vein ligation and complication rates for the groin dissection [10,13]. We believe that the most important aspect of preventing groin wound breakdown is the preservation of all the subcutaneous fat above Camper's fascia.

The reported incidence of lymphedema ranges from 10.9% [25] to 67% [21]. Our overall incidence of 31.6% per groin is in the mid-range of those reported [13,15,19,20,24,26,27]. The incidence was strongly correlated with the number of nodes resected and most studies concur with this finding [12,18,20,25,28]. Some authors have suggested that preservation of the saphenous vein may decrease the incidence of lymphedema [19,20], but as the problem is related to lymphatic obstruction, not venous congestion, this hypothesis lacks biologic credibility.

We have previously reported that nodal debulking for patients with bulky positive groin nodes followed by post-operative groin and pelvic radiation does not compromise survival [29], and the procedure is applicable to all patients with bulky positive nodes. The safety of the procedure was recently confirmed in a study from Leiden University [30]. In our experience, only 3.9% of groins experienced a wound breakdown after a lymph node debulking, 7.8% a wound infection and 14.6% developed a lymphocyst. These data support the earlier initiation of post-operative groin and pelvic radiation and would suggest that nodal debulking rather than an inguino-femoral lymphadenectomy should be considered the treatment of choice for patients with bulky positive nodes.

Total patients 311 Total groins 490	**No of groins (no of patients)**	**Complication per groin (per patient)**	**% per groin (% per patient)**	**P value**
Lymphedema				
No of nodes removed				
≤ 4 nodes	94 (76)	16 (13)	17% (17.1%)	0.0026†
5 – 8 nodes	160 (140)	52 (47)	32.5% (33.5%)	(0.0003)
9 + nodes	236 (180)	87 (78)	36.9% (43.3%)	
Lymphedema				
Age groups				
≤ 50 years	72 (48)	19 (18)	26.4%(37.5%)	0.217‡
51 – 70 years	174(109)	64 (48)	36.8% (44%)	(0.707)
> 70 years	244 (154)	72 (60)	29.5% (39.1%)	
Lymphedema				
With drain	323 (193)	100 (82)	31% (42.5%)	0.576‡
Without drain	167 (118)	55 (44)	32.9% (37.3%)	(0.821)
Lymphedema				
Current Smokers	115 (74)	38 (31)	33% (41.2%)	0.799‡
Past smokers	72 (43)	25 (18)	34.7% (41.8%)	(0.955)
Non-smokers	303 (194)	93 (77)	30.7% (39.7%)	
Lymphedema				
BMI < 30	359 (224)	102 (81)	28.4% (36.1%)	0.011‡
BMI ≥ 30	131 (87)	53 (46)	40.5% (52.9%)	(0.015)

Table 5: Incidence of lymphedema and its association to study variables.
35 Groins (22 patients) excluded due to follow up < 6 months.
† Cochran-Armitage trend test, ‡ Pearson Chi Square Test.

In accordance with two previous studies [10,25,28], and in contrast to two other studies [20,28] we found no significant association between the incidence of lymphedema and the addition of groinradiotherapy, although the relatively small numbers having radiation therapy may have not provided sufficient power to detect important differences.

Like some earlier studies [10,11], we found evidence of an association between the development of a lymphocyst and the subsequent development of lymphedema in univariate analysis (p = 0.04), although the evidence was weaker after accounting for other factors (p = 0.06). Obesity was also found to be a risk factor on univariate but not multivariable analysis, possibly due to the small number of patients in the higher BMI range. To our knowledge, higher BMI as a risk factor for developing lymphedema has only been reported in two other studies [28,31].

The major limitation of this study is the retrospective nature of the review. The incidence of long-term complications, particularly recurrent cellulitis, may have been under-reported because over 50% of the patients came from rural areas, and some were only seen annually. The strengths of the study are its large sample size, its per groin analysis, and the management of all patients in one specialised unit with a common treatment protocol.

Conclusions

Appropriate groin node dissection is a critical part of the treatment for all patients with vulvar cancer, except those with stage IA disease. Lymphocyst formation in the immediate post-operative period and lymphedema after several months are the major morbidities, and both are associated with the number of lymph nodes removed. In this study, the use of groin drains did not significantly decrease the incidence of lymphocyst formation. Groin node debulking for all patients with bulky positive nodes, and sentinel node biopsy for patients with small primary tumours are the only legitimate ways to reduce the number of resected groin nodes. Groin wound breakdown should occur in less than 10% of groins if care is taken to preserve the subcutaneous fat above Camper's fascia, regardless of resection of the saphenous vein.

Author Contribution

The authors declare their responsibility for the content of this publication. EB and NH developed the concept of the article. EB conducted the data collection and analysis and drafted the manuscript. NH edited all drafts. MD provided statistical guidance and assistance with interpretation and reporting of results. All authors approved the final version for submission.

Funding

This research was supported, in part by a grant from the GO Research Fund of the Royal Hospital for Women Foundation. EB is supported by an Australian Government Research Training Program Scholarship. These funding sources had no influence on the study design, the data analysis, or in the writing of the manuscript.

Competing Interests

The authors declare no conflicts of interest.

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This article was originally published in a special issue:

Gynecology & Surgical Oncology

Handled by Editor(s):

Dr. Maria Teresa Gomez Garcia
Department of Medical Sciences
University of Castile-La Mancha
Spain

CHAPTER SIX

THE PROGNOSTIC ROLE OF THE SURGICAL MARGINS IN SQUAMOUS VULVAR CANCER: A RETROSPECTIVE AUSTRALIAN STUDY

6.1 | Precis

This chapter comprises the final area of investigation of my thesis. The research was focused on determining the long-term survival of 345 patients with primary vulvar cancer who were treated with a conservative approach to the primary cancer whenever possible. Particular attention was paid to determining the significance of the surgical margins in the treatment of the primary vulvar lesion in relation to the incidence of local recurrence.

This chapter addresses the following research questions:

- What is the long-term survival of patients with squamous vulvar cancer treated in the era of conservative management?
- Is there a relationship between the extent of the surgical excision margin and local vulvar recurrences in women treated with primary surgery for squamous vulvar cancer?
- Is treatment of close or positive surgical excision margins beneficial in reducing local recurrence?

cancers

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Article

The Prognostic Role of the Surgical Margins in Squamous Vulvar Cancer: A Retrospective Australian Study

Ellen L Barlow [1,*], Michael Jackson [2,3] and Neville F Hacker [1,4]

[1] Gynaecological Cancer Centre, Royal Hospital for Women, Sydney 2031, Australia; n.hacker@unsw.edu.au
[2] Radiation Oncology Department, Prince of Wales Hospital, Sydney 2031, Australia; Michael.Jackson@health.nsw.gov.au
[3] Prince of Wales Clinical School, University of New South Wales, Sydney 2052, Australia
[4] School of Women's & Children's Health, University of New South Wales, Sydney 2052, Australia
* Correspondence: ellen.barlow@health.nsw.gov.au; Tel.: +61-2-93826184

Received: 30 September 2020; Accepted: 9 November 2020; Published: 14 November 2020

Simple Summary: Squamous cell carcinoma of the vulva is a rare disease, but cure rates are good if managed appropriately. The need for radical vulvectomy was initially challenged about 40 years ago for lesions 1−2 cm diameter. Since then, there has been progressive acceptance of radical local excision for most unifocal squamous vulvar cancers. Originally, a surgical margin of 3 cm around the primary cancer was considered appropriate. Subsequently, a 1 cm margin was generally accepted, but this has become the subject of recent debate. The aims of this study were to determine survival following conservative vulvar resection, and to determine the clinicopathological predictors associated with vulvar recurrence, focusing on the surgical margin. In multivariable analysis, primary site recurrences were increased in patients with margins <8 mm, and all vulvar and primary site recurrences in patients with margins <5 mm. Treatment of close or positive margins decreased the risk of recurrence.

Abstract: For the last 30 years at the Royal Hospital for Women, unifocal vulvar squamous cancers have been treated by radical local excision, aiming to achieve a histopathological margin of ≥8 mm, equating to a surgical margin of 1 cm. The need for a margin of this width has recently been challenged. We aimed to determine the long-term outcome following this conservative approach, and the relationship between vulvar recurrences and surgical margins. Data were obtained retrospectively on 345 patients treated primarily with surgery for squamous vulvar cancer between 1987 and 2017. Median follow-up was 93 months. Five-year disease-specific survival was 86%. Of 78 vulvar recurrences, 33 (42.3%) were at the primary site and 45 (57.7%) at a remote site. In multivariable analysis, a margin <5 mm showed a higher risk of all vulvar (Hazard ratio (HR), 2.29; CI, 1.12−4.70), and primary site recurrences (subdistribution hazard ratio (SHR), 15.20; CI, 5.21−44.26), while those with a margin of 5 to <8 mm had a higher risk of a primary site recurrence (SHR, 8.92; CI, 3.26−24.43), and a lower risk of remote site recurrence. Excision margins <8 mm treated by re-excision or radiation therapy had a significantly decreased risk of recurrence. Guidelines should continue to recommend a surgical margin of 1 cm.

Keywords: squamous vulvar carcinoma; vulvar conservation; resection margin; local recurrence; primary site recurrence; remote site recurrence

1. Introduction

Squamous cell carcinoma of the vulva (VSCC) is a rare disease, but cure rates are good if the primary tumour and groin lymph nodes are managed appropriately [1]. In the mid-20th century, radical vulvectomy was the standard of care for the primary tumour, although it caused serious psychosexual morbidity [2]. The main argument against performing a more conservative vulvar operation was that multicentricity occurred in 20–30% of cases, and more conservative procedures would result in more vulvar recurrences [3]. This concern has been explained by the concept of field cancerization [4].

The need for radical vulvectomy in all patients was initially challenged about 40 years ago for lesions up to 1 cm [5] and 2 cm [6] in diameter. Since that time, there has been a slow acceptance of radical local excision for most unifocal squamous vulvar cancers [7,8], although the change in management has never been subjected to a prospective, randomized trial.

The width of the surgical margin has remained controversial. In the original papers, a 3 cm margin of normal skin on all sides of the primary cancer was considered appropriate [5–7]. In a 1990 study of 135 patients, 81.5% of whom had early stage disease, Heaps et al. reported that an 8 mm histological tumour-free surgical margin resulted in a high rate of local control, whereas a margin of <8 mm was associated with a 50% chance of local recurrence [9]. With tissue shrinkage after formalin fixation, an 8 mm histological margin equated to a surgical margin of 1 cm. Subsequently, a 1 cm disease-free margin was generally accepted as being appropriate, although in 2002, deHullu et al. recommended that the margin should be increased to 2 cm [10].

An important new concept regarding local vulvar relapse was introduced by Rouzier and colleagues in 2002 [11]. They proposed that vulvar relapses should be divided into (i) primary site and (ii) remote site recurrences. They defined primary site recurrences as those involving the skin within 2 cm of the vulvectomy scar, and remote site recurrences as those occurring further away. On multivariable analysis, primary site recurrences were significantly related to surgical margins less than 1 cm and had a mean time to recurrence of 13 months. Remote site recurrences often arose from epithelial disorders, such as lichen sclerosis, and the mean disease-free interval was 33 months. They considered remote site recurrences to be new primary cancers.

Recently, the relevance of 1cm surgical margins has been questioned [12–18]. We have had a consistent policy of vulvar conservation for unifocal vulvar cancer at the Royal Hospital for Women in Sydney since 1987, so we decided to review our experience. Our primary aims were to determine the relationship between local vulvar recurrence and the extent of the histopathological surgical margin, and to explore patterns of local recurrence. A secondary aim was to determine the long-term survival of patients treated with a conservative approach to the primary lesion.

2. Results

2.1. Primary Treatment and Staging

The study population included 345 patients treated primarily with surgery for VSCC. Table 1 shows a summary of the clinicopathological and treatment characteristics for these patients. The patients were followed for a median of 93 months (range 1–367 months). All FIGO stages were represented, except FIGO stage IVA(i).

Of the 63 patients who did not have a groin lymphadenectomy, 31 had Stage IA disease and no lymphadenectomy was offered. A further 31 patients had stage IB disease. Twenty-one of these patients had tumours 15 mm or less in diameter, their groin nodes were followed with ultrasound for 12 months post-operatively on a research protocol, and all remained negative. Of the remaining 10 patients, lymphadenectomy was contraindicated in six because of poor performance status and four refused any lymphadenectomy. None of these 31 patients developed a groin recurrence with a minimum follow-up of 30 months and they were regarded as node negative for analysis. One patient with stage II disease refused a lymphadenectomy. She was treated with radiotherapy to the groin and pelvis and her nodal status was recorded as unknown.

Table 1. Clinical, surgical, and pathological characteristics of the vulvar. Squamous cell carcinoma cohort (n = 345).

Variable	N (%)
Median follow-up	93 months (range 1–367)
Age, years (range 29–96)	
Mean	66.7
Median	70
FIGO Stage 2009	
IA	37 (10.7%)
IB	194 (56.2%)
II	12 (3.5%)
IIIA (1)	35 (10.1%)
IIIA (11)	14 (4.1%)
IIIB (1)	5 (1.5%)
IIIB (11)	11 (3.2%)
IIIC	32 (9.3%)
IVA (11)	2 (0.6%)
IVB	3 (0.9%)
Smoking	
Current	91 (26.4%)
Past	53 (15.3%)
Never	201 (58.3%)
Groin node status	
Unknown	1 (0.3%)
Negative	242 (70.1%)
Positive	102 (29.6%)
Lesion Location	
Clitoris	39 (11.3%)
Labium minus	95 (27.5%)
Labium majus	118 (34.2%)
Perineum	15 (4.3%)
Vulvar vestibule	35 (10.1%)
Multifocal	43 (12.5%)
Primary surgery	
Radical local excision	275 (79.7%)
Radical vulvectomy	70 (20.3%)
Primary groin treatment	
Bilateral IGFLND	123 (35.6%)
Unilateral IGFLND[a]	122
Nodal debulking[a]	47
Sentinel node[a]	5
No groin node dissection	63 (18.3%)
Adjuvant radiotherapy	
vulva/groins/pelvis[b]	39 (11%)
groins/pelvis	22 (6.1%)
vulva	7 (1.2%)
Epithelial disorder	
uVIN	115 (33.3%)
dVIN	77 (22.3%)
Lichen sclerosus (LS)	77 (22.3%)
LS + dVIN	26 (7.5%)
uVIN + dVIN	1 (0.3%)
None	49 (14.2%)
Margin distance[c]	
Positive	11 (3.2%)
0.1 mm – 7.9 mm	111 (32.5%)
≥8 mm	219 (64.2%)

Tumour Size	
≤4 cm	286 (82.9%)
>4 cm	59 (17.1%)
LVSI	
Yes	53 (15.4%)
No	291 (84.3%)
Unknown	1 (0.3%)
Perineural invasion	
Yes	24 (6.9%)
No	318 (92.2%)
Unknown	3 (0.9%)
Depth of invasion	
≤5 mm	222 (64.3%)
>5 mm	123 (35.7%)
Differentiation	
Well	153 (44.3%)
Moderate	152 (44.1%)
Poor	39 (11.3%)
Unknown	1 (0.3%)

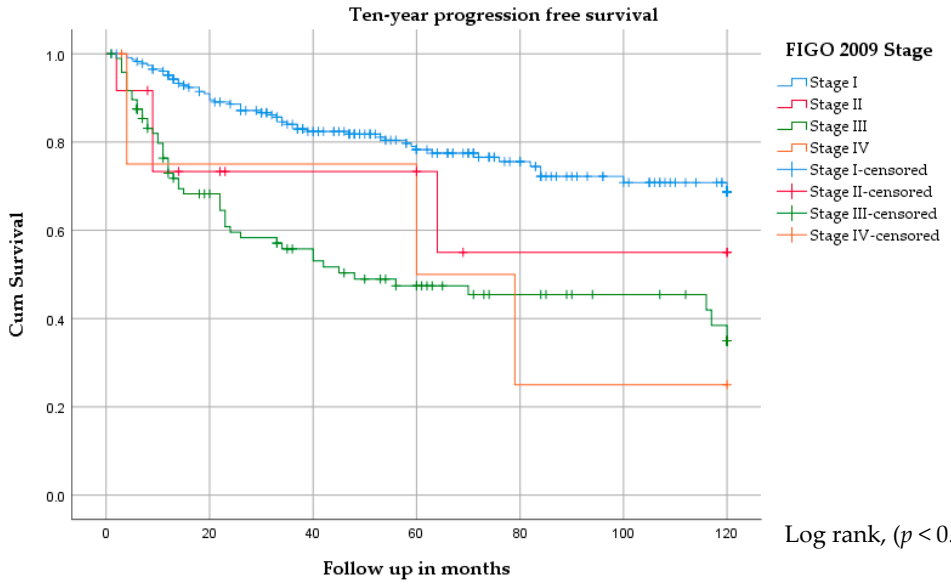
FIGO, International Federation of Gynecology and Oncology; IGFLND, inguino-femoral lymph node dissection; uVIN, usual-type vulvar intraepithelial neoplasia; dVIN, differentiated vulvar intraepithelial neoplasia; LVSI, lymphovascular space invasion. [a] Different groin procedure in alternate groins are included in each group (% not calculated). [b] Four patients had chemoradiation, [c] four patients excluded due to follow-up < 6 months.

2.2. Survival

At the completion of the study, of the 345 patients treated primarily with surgery with curative intent, 209 patients (60.6%) were alive without evidence of disease, and 136 patients (39.4%) had died. Of the 136 deaths, 55 patients (15.9%) died of disease, two died from peri-operative complications (0.6%) and one died of sepsis during adjuvant radiotherapy (0.3%). The remaining 78 patients died of unrelated causes (22.6%).

Kaplan–Meier estimates for five and ten-year progression-free survivals (PFS) were 78% and 70% for stage I, 75% and 55% for stage II, 47% and 35% for stage III, and 50% and 26% for stage IV, respectively (Figure 1). Five and ten-year disease-specific survivals (DSS) were 95% and 92% for stage I, 82% and 82% for stage II, 66% and 58% for stage III, and 75% and 25% for stage IV, respectively (Figure 2).

The five and ten-year DSS for all 345 patients was 86% and 80%, respectively (Figure S1), and for the 102 patients with positive lymph nodes it was 66% and 55%, respectively (Figure S2). The five and ten-year DSS was 94% and 80%, for the 69 patients with an isolated vulvar recurrence, and 8% and 4% for the 42 patients with a regional or distant recurrence (Figure S3). The five and ten-year overall survival (OS) for all 345 patients was 73.6% and 64.3%, respectively (Figure S4.).

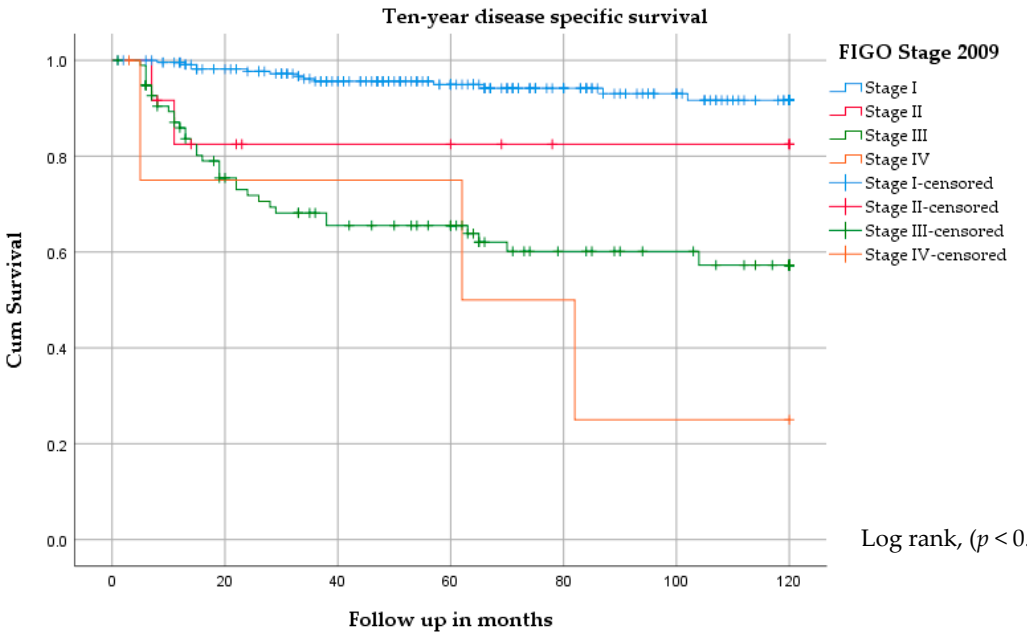


Log rank, ($p < 0.001$)

Months	0	20	40	60	80	100	120
Stage I	231	192	146	109	73	52	35
Stage II	12	7	5	5	2	2	2
Stage III	97	56	41	30	20	15	11
Stage IV	5	3	3	3	1	1	1

Number at risk

Figure 1. Kaplan–Meier curve for ten-year progression-free survival stratified by FIGO stage.



Log rank, ($p < 0.001$)

Months	0	20	40	60	80	100	120
Stage I	231	207	173	137	96	72	52
Stage II	12	8	6	6	3	3	3
Stage III	97	63	50	44	27	22	16
Stage IV	5	3	3	3	2	1	1

Number at risk

Figure 2. Kaplan–Meier curve for ten-year disease-specific survival stratified by FIGO stage.

2.3. Surgical Margins

To determine the relationship between peripheral margin distance and vulvar recurrence, patients with less than six months follow-up, none of whom had a vulvar recurrence, were excluded (*n* = 4). Of the remaining 341 patients, 219 (64.2%) had a histopathological margin distance ≥8 mm, 111 (32.5%) had a margin between 0.1 mm and 8 mm (close margins) and 11 (3.2%) had positive margins. Of the 111 patients with close margins, 48 patients had a margin between 0.1 mm and <4.9 mm, and 63 between 5 mm and 7.9 mm (Table S1). Of the 122 patients with close or positive margins, 46 (37.7%) were treated by re-excision or adjuvant radiation therapy and 76 (62.3%) were observed. The relationship between surgical margins and subsequent management is shown in Figure 3 and Table S1. All 'surgical margins' or 'margins' referred to in this paper are the histopathological surgical margin distance as measured by the pathologist and recorded in the histopathology report.

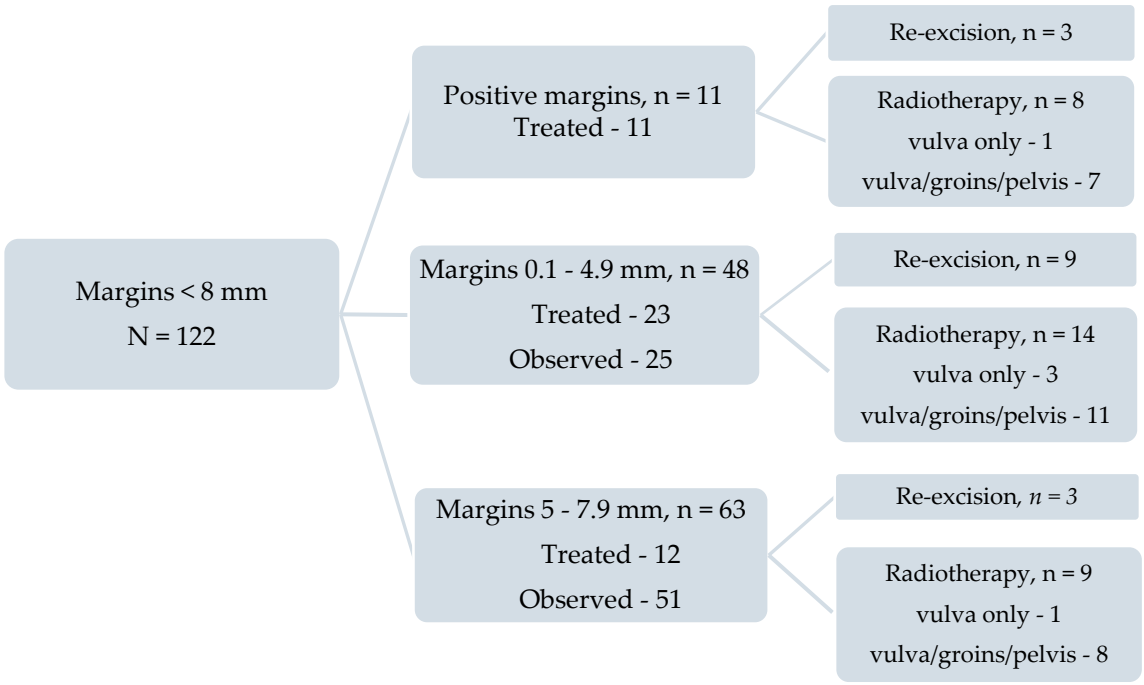


Figure 3. The relationship between surgical margins <8 mm and subsequent management.

2.4. Recurrences

There were 111 recurrences (32.2%). Of these, 78 (70.3%) were on the vulva (seven concurrent with a groin recurrence, one concurrent with a vaginal recurrence, and one concurrent with a distant recurrence). Thirty-four of the 78 vulvar recurrences (43.6%) developed two or more vulvar recurrences. Of the remaining 33 recurrences, 14 were distant, 11 isolated groin, two isolated vagina, one isolated skin bridge, three concurrent groin and distant, one concurrent groin and vagina, and one concurrent groin and skin. Among those with a groin or distant recurrence, the median interval to a groin recurrence was 8 months, (range 3–22 months) and the median interval to a distant recurrence was 11 months, (range 2–79 months). Of the 78 vulvar recurrences, 33 (42.3%) were at the primary site and 45 (57.7%) were at a remote site. The median interval from initial treatment to a primary site recurrence was 20 months compared to 39 months for a remote site recurrence. For both vulvar sites, the earliest first recurrence occurred at two and seven months, respectively. Two primary and 14 remote site recurrences developed more than five years after the initial treatment.

2.5. Relationship Between Vulvar Recurrence and Various Histopathological and Other Factors

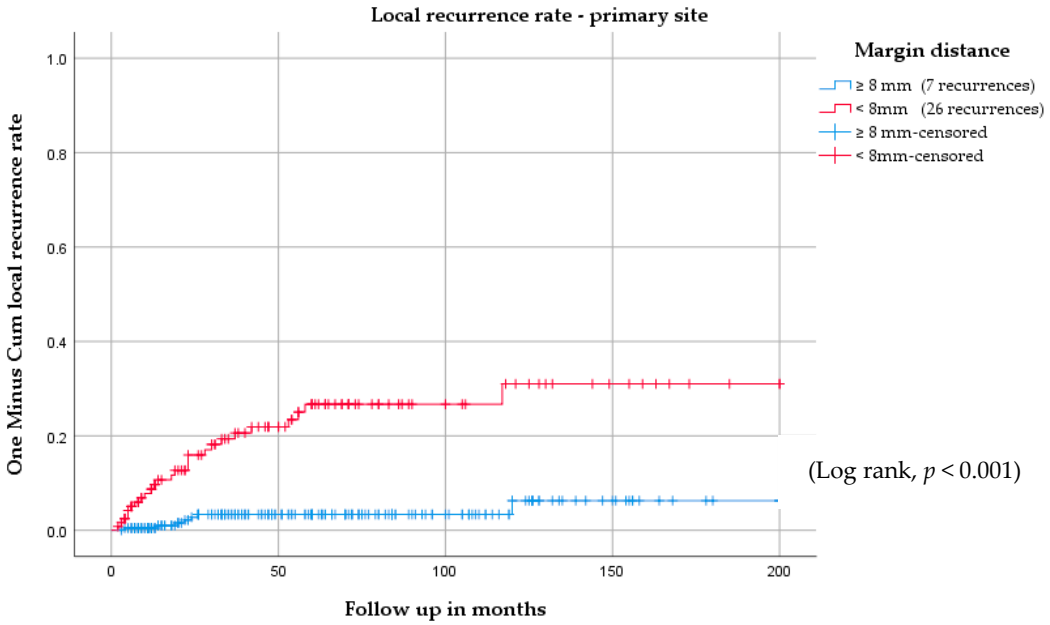
2.5.1. Surgical Margins

Vulvar recurrences occurred in 51 of 219 patients (23.3%) with pathological margins ≥8 mm, compared to 27 of 122 patients (22.1%) with pathological margins <8 mm (p = 0.65) (Figure S5). However, vulvar recurrences occurred in 23 of 76 patients with untreated margins <8 mm (30.2%) compared to four of 46 patients with treated close or positive margins (8.7%) (p = 0.005) (Table S1).

For patients with margins 0.1–4.9 mm, treatment with either radiotherapy or re-excision decreased the rate of primary site recurrence from 40 to 4.3% (p = 0.003). For each individual modality, re-excision decreased the recurrence rate from 40 to 11.1% (p = 0.09), and radiation from 40% to 0% (p = 0.009) (Table S1). A per mm breakdown of the incidence of recurrence in untreated patients whose margins were <5 mm is shown in supplementary Table S2. Treatment for patients with margins 5–7.9 mm was less beneficial, although treatment with either modality was associated with a decreased rate of recurrence from 25.5 to 11.1% (p = 0.165) (Table S1).

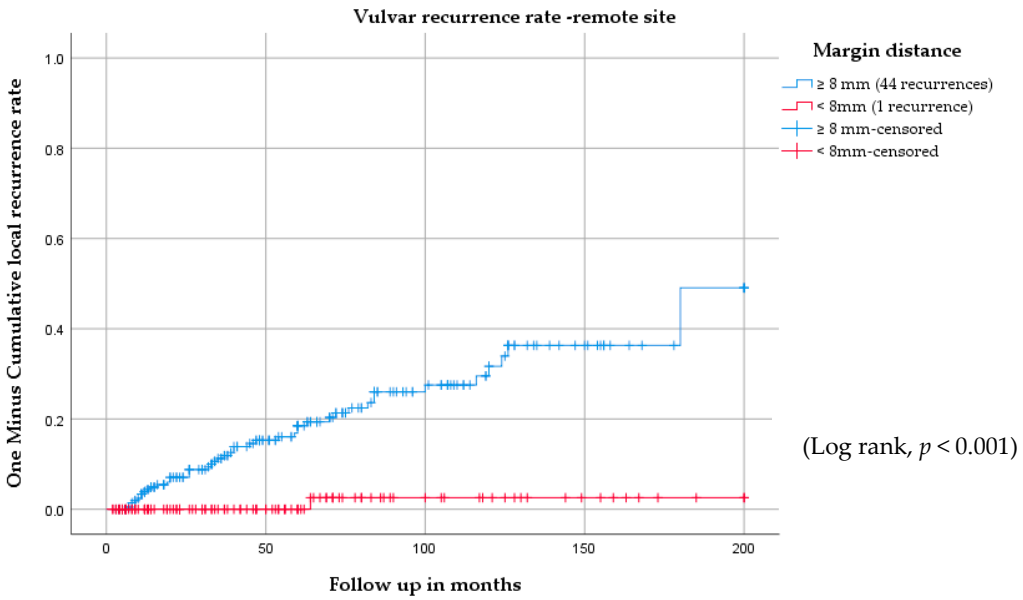
There were 27 vulvar recurrences in patients with margins <8 mm, of which 26 were at the primary site, and one was at a remote site (p < 0.001). Of the 51 recurrences in patients with margins ≥8 mm, 44 were at a remote site and seven were at the primary site (p < 0.001) (Figure 4, Table S1).

(a)



(Log rank, p < 0.001)

(b)



(Log rank, $p < 0.001$)

Months	0	20	40	60	80	100	120	180
<8mm margin	122	85	62	44	27	20	15	3
≥8mm margin	219	172	131	102	68	49	33	5

Number at risk

Figure 4. Vulvar recurrence rate for (a) the primary site and (b) the remote site stratified by surgical margins of <8 mm and ≥8 mm.

For univariable (Table 2) and multivariable (Table 3) Cox and Fine–Gray regression analyses, patients were categorised using the margin cut offs <5 mm, and 5 to <8 mm, compared to ≥8 mm. We identified significant differences in local recurrence rates related to pathological margin distance and the location of a recurrence on the vulva (primary or remote site). In univariable analysis, although we found no increased risk for all vulvar recurrences in patients with margins <8 mm, primary site vulvar recurrences occurred with increased risk in both close margin groups; <5 mm (Sub-distribution hazard ratio [SHR], 8.82; CI, 3.54–21.99), and 5 mm to <8 mm (SHR, 7.59; CI, 3.04–18.95). This result was consistent in multivariable analysis, where both margin groups <8mm maintained a significantly increased risk for primary site recurrence. In addition, in the pathological margin group <5 mm, there was evidence of an increased risk for all vulvar recurrences (Hazard ratio (HR), 2.29; CI, 1.12–4.70). There was a decreased risk for remote vulvar recurrence in patients with margins 5mm to <8 mm in the univariable (SHR, 0.07; CI, 0.01–0.54) and multivariable analysis (SHR, 0.08; CI, 0.01–0.71). There were no remote vulvar recurrences in the <5 mm margin group.

For patients with positive or close margins <8 mm, those treated by surgical re-excision or radiation therapy had a significantly lower risk of all vulvar recurrences in univariable analysis (HR, 0.34; CI, 0.01–0.54). However, in multivariable analysis, treatment was strongly associated with a decreased risk for all vulvar recurrence (HR, 0.15; CI, 0.05–0.44), and for a primary site recurrence (SHR, 0.14; 0.05–0.41).

2.5.2. Epithelial Abnormality

In univariable analysis, the presence of an epithelial abnormality (compared to no epithelial abnormality) was not associated with a significantly increased risk for a vulvar recurrence at any site. For patients with differentiated vulvar intraepithelial neoplasia (dVIN) in the specimen, we observed

no significantly increased rate of any vulvar recurrence in univariable or multivariable analyses. In multivariable analysis, dVIN at the excision margin showed a significantly increased risk for recurrence at the primary vulvar site (SHR, 5.35; CI, 1.05- 27.34). In univariable analysis, the presence of usual-type vulvar intraepithelial neoplasia (uVIN) in the specimen was associated with a lower rate of all vulvar recurrences (HR, 0.43; CI, 0.20−0.96), but not primary or remote vulvar site recurrences compared to no epithelial abnormality. However, this was not significant in multivariable analysis ($p = 0.06$).

2.5.3. Positive Nodes

In both the univariable and multivariable analysis, patients with one or more positive lymph nodes had an increased risk of all vulvar recurrences, and primary site vulvar recurrence.

2.5.4. Lymphovascular and Perineural Space Invasion

In univariable analysis, lymphovascular space invasion (LVSI) and perineural invasion (PNI) were associated with an increased risk of recurrence, but only at the primary vulvar site; LVSI, (SHR, 2.67; CI, 1.21−5.91) and PNI, (SHR, 3.12; CI, 1.08−9.06). This was not confirmed in the multivariable analysis.

2.5.5. Other Factors

In univariable analysis, current smokers had a significantly lower risk of vulvar recurrence at all vulvar sites, and this remained in multivariable analysis (HR, 0.42; 0.20−0.87). The evidence of lower risk was not as strong when primary or remote site recurrences were considered separately. Tumour diameter, grade of differentiation, and depth of invasion had no significant influence on local recurrence rates in either univariable or multivariable analysis. Multivariable analysis also identified a decreased risk for all vulvar recurrences in women older than 65 years (HR, 0.58; CI, 0.33–1.00).

Table 2. Univariable Cox and Fine-Gray regression analyses comparing all vulvar recurrences, and primary and remote site vulvar recurrences with clinico-pathological variables.

Variable Title	All vulvar recurrence	Primary vulvar recurrence	Remote vulvar recurrence
	HR (95% CI) *p*-value	SHR (95% CI) *p*-value	SHR (95% CI) *p*-value
Age > 65 years (Ref: ≤65 years)	1.16 (0.74–1.82) 0.52	1.38 (0.69–2.77) 0.36	0.98 (0.54–1.77) 0.94
Current smoker - Yes (Ref: No)	0.33 (0.17–0.64) 0.001	0.34 (0.12–0.96) 0.04	0.37 (0.15–0.86) 0.02
Tumour size >4 cm (Ref: ≤4 cm)	1.22 (0.64–2.32) 0.54	1.79 (0.77–4.15) 0.17	0.69 (0.24–1.98) 0.49
Margin distance (Ref: ≥8 mm)			
- <5mm margins	1.17 (0.64–2.16) 0.61	8.82 (3.54–21.99) < 0.001	0.00 †
5 mm to < 8 mm margins	1.06 (0.59–1.92) 0.84	7.59 (3.04–18.95) < 0.001	0.07 (0.01–0.54) 0.01
Margin treatment			
< 8mm treated margins (Ref: all other margins)	0.34 (0.12–0.93) 0.04	0.95 (0.34–2.70) 0.93	0.00 †
Nodal status [a] - Positive (Ref: negative)	1.82 (1.15–2.89) 0.01	3.77 (1.91–7.46) < 0.001	0.81 (0.40–1.65) 0.57
Tumour differentiation			
Mod/Poor (Ref: well differentiated)	1.29 (0.82–2.03) 0.27	1.92 (0.92–4.03) 0.08	0.93 (0.52–1.66) 0.81
Lymphovascular invasion - Yes (Ref: No)	1.67 (0.90–3.09) 0.10	2.67 (1.21–5.91) 0.02	0.79 (0.27–2.30) 0.67
Perineural invasion - Yes (Ref: No)	1.95 (0.78–4.86) 0.15	3.12 (1.08–9.06) 0.04	0.56 (0.07–4.33) 0.58
Depth of invasion - >5 mm (Ref: ≤5 mm)	1.23 (0.77–1.97) 0.39	1.51 (0.76–2.99) 0.24	0.97 (0.51–1.82) 0.92
Epithelial abnormality (Ref: No abnormality) [b]			
Lichen sclerosus (LS) +/- SH	1.63 (0.80–3.29) 0.18	2.40 (0.66–8.77) 0.19	1.20 (0.51–2.83) 0.68
dVIN present, not at margin	0.98 (0.44–2.19) 0.96	2.18 (0.57–8.35) 0.25	0.50 (0.17–1.51) 0.22
dVIN at margin	2.07 (0.88–4.89) 0.10	3.73 (0.87–15.95) 0.08	1.21 (0.41–3.60) 0.73
uVIN present	0.43 (0.20–0.96) 0.04	0.60 (0.14–2.55) 0.49	0.40 (0.15–1.05) 0.06
LS + dVIN	0.78 (0.25–2.47) 0.68	0.73 (0.08–6.81) 0.78	0.84 (0.24–2.95) 0.79

HR indicates hazard ratio; SHR, subdistribution hazard ratio; CI, confidence interval; Ref, Reference group; LS, lichen sclerosus; SH, squamous hyperplasia; dVIN, differentiated vulvar intraepithelial neoplasia; uVIN, usual-type vulvar intraepithelial neoplasia; [a] 1 patient nodal status unknown, [b] 1 uVIN + dVIN excluded. † The SHR could not be estimated as no remote recurrences were observed in the <5 mm and treated margin groups.

Table 3. Multivariable Cox and Fine–Gray regression analyses comparing all vulvar recurrences, and primary and remote vulvar recurrences to clinico-pathological variables.

Variable Title	All vulvar recurrence	Primary vulvar recurrence	Remote vulvar recurrence
	HR (95% CI) *p*-value	SHR (95% CI) *p*-value	SHR (95% CI) *p*-value
Age >65 years (Ref: ≤65 years)	0.58 (0.33–1.00) 0.05	0.49 (0.18–1.33) 0.16	0.62 (0.31–1.23) 0.17
Current smoker - Yes (Ref: No)	0.42 (0.20–0.87) 0.02	0.51 (0.16–1.62) 0.26	0.41 (0.16–1.05) 0.06
Tumour >4 cm (Ref: ≤4 cm)	1.34 (0.64–2.82) 0.44	2.41 (0.57–10.13) 0.23	0.84 (0.24–2.94) 0.79

Margin distance (Ref: ≥8 mm)			
<5 mm margins	2.29 (1.12–4.70) 0.02	15.19 (5.21–44.26) < 0.001	0.00 †
5 mm to <8 mm margins	1.31 (0.69–2.49) 0.41	8.92 (3.26–24.43) < 0.001	0.08 (0.01–0.71) 0.02
Margin treatment			
<8 mm treated margins (Ref: all other margins)	0.15 (0.05–0.44) < 0.001	0.14 (0.05–0.41) < 0.001	0.00 †
Nodal status [a] - Positive (Ref: negative)	1.77 (1.05–3.01) 0.03	3.05 (1.18–7.89) 0.02	1.07 (0.49–2.32) 0.86
Tumour differentiation			
Mod/Poor (Ref: well differentiated)	1.17 (0.70–1.96) 0.54	1.28 (0.50–3.27) 0.61	1.25 (0.65–2.40) 0.50
LVSI present (Ref: not present)	1.62 (0.77–3.42) 0.21	1.07 (0.33–3.47) 0.91	2.74 (0.73–10.29) 0.14
PNI present (Ref: not present)	1.72 (0.61–4.88) 0.31	2.06 (0.51–8.30) 0.31	0.50 (0.03–7.11) 0.61
Depth of invasion - >5 mm (Ref: ≤5 mm)	0.77 (0.44–1.36) 0.37	0.66 (0.26–1.68) 0.38	0.96 (0.47–1.95) 0.91
Epithelial abnormality (Ref: No abnormality) [b]			
Lichen sclerosus (LS) +/- SH	1.72 (0.81–3.65) 0.16	2.34 (0.55–9.92) 0.25	1.46 (0.60–3.53) 0.40
dVIN present, not at margin	0.96 (0.41–2.27) 0.93	1.59 (0.40–6.31) 0.51	0.70 (0.19–2.60) 0.59
dVIN at margin	2.37 (0.94–5.99) 0.07	5.35 (1.05–27.34) 0.04	1.65 (0.56–4.87) 0.36
uVIN present	0.42 (0.17–1.02) 0.06	0.47 (0.09–2.45) 0.37	0.46 (0.17–1.27) 0.13
LS + dVIN	0.59 (0.18–1.97) 0.39	0.52 (0.05–5.58) 0.59	0.96 (0.24–3.90) 0.96

HR indicates hazard ratio; SHR, subdistribution hazard ratio; CI, confidence interval; Ref, Reference group; LVSI, lymphovascular space invasion; PNI, perineural invasion; SH, squamous hyperplasia; dVIN, differentiated vulvar intraepithelial neoplasia; uVIN, usual-type vulvar intraepithelial neoplasia.

[a] One patient nodal status unknown, [b] One patient with both dVIN and uVIN excluded from analysis.

† The SHR could not be estimated as no remote recurrences were observed in the <5 mm and treated margin groups.

3. Discussion

This study demonstrates that a conservative surgical approach to vulvar cancer is associated with excellent rates of survival. The old standard treatment was radical vulvectomy and bilateral groin dissection, with or without pelvic node dissection in all patients. A report of 96 patients with T1 and T2 lesions from the United States in 2007 reported radical vulvectomy in 49.2% of cases and bilateral groin dissection in 51% [19]. A Surveillance, Epidemiology and End Results (SEER) study of 141 patients with FIGO stages I/II disease treated in 1999 reported that 47% of patients were treated with a radical vulvectomy [20]. More recently, a 2020 study, which included a cohort of 1535 patients treated for all stages of squamous vulvar cancer between 2001 and 2005 across 100 European centres reported radical vulvectomy in 76.5% and bilateral groin dissection in 45.2% of patients [21].

By contrast, only 20% of patients with all stages of disease in this study had radical vulvectomy, and only 36% had bilateral groin dissection. Radical vulvectomy was reserved for patients with multifocal disease, while bilateral groin dissection was reserved for midline lesions, or those within 1–2 cm of an imaginary line drawn from the clitoris to the anus. Despite this more conservative surgical approach, the five-year DSS for all 345 patients was 86% and the five-year DSS for the 102 patients with positive lymph nodes was 66%.

Seventy-eight of the 111 recurrences (70.3%) were on the vulva, 69 of which were isolated vulvar recurrences. With further surgical excision and/or radiation therapy, 94% of patients with an isolated vulvar recurrence, including the 31 patients in this group who had more than one vulvar recurrence, were free of disease at five years. By contrast, of the 42 patients who had a groin, vaginal or distant recurrence, only 8% were alive at five years.

Although this study confirms the benefit of a more conservative approach to vulvar resection, the actual extent of the surgical margin has been controversial. Several recent studies have reported that the histopathological margin distance was not predictive of vulvar recurrence [12–14,18,22–25]. However, there have been two recent exceptions, A meta-analysis of 10 studies reported by Nooij et al. showed that a tumour-free margin of <8 mm was associated with a higher risk of local recurrence compared to a tumour-free margin of ≥ 8 mm (HR, 1.99; 95% CI: 1.13–3.51) [15], but in a cohort study of their own patients, they were unable to confirm this finding. Similarly, Yang et al., in a multicentre study of 335 patients, also reported that patients with surgical margins <8 mm had a higher rate of local recurrences [26].

None of these studies was as large as the present study, and none subdivided the recurrences into those at the primary or at a remote site. In our univariable analysis, margin distance was not predictive of a vulvar recurrence per se, but when subdivided into primary and remote site recurrences, there was a significantly increased risk of primary site recurrence in patients with margins <8 mm. In multivariable analysis, patients with a pathological margin <5 mm had a significantly increased risk of all vulvar and primary site recurrences, while those with a margin of 5 to <8 mm had a significantly higher risk of a primary site recurrence and lower risk of a remote site recurrence.

In a review of the literature, te Grootenhuis et al. concluded that the division into primary versus remote site recurrences was arbitrary and not reproducible [17]. Our results demonstrate that with large enough numbers, this division is reproducible if accurate clinical records of tumour locations have been kept. We agree that distinguishing a "true" local recurrence from a second primary cancer would require molecular profiling of both cancers, and two of our primary site recurrences occurred more than five years post-treatment. These were almost certainly second primary cancers. However, the division is important because it allows clear guidelines to be given regarding primary surgical treatment.

Due to the confusion in the literature regarding the significance of surgical margins, there has also been confusion regarding surgical recommendations. Woelber et al. recommended that the main goal should be to achieve "complete tumour resection, irrespective of tumour-free margin" [22], and te Grootenhuis et al. concluded from their systematic review that there seemed to be "no lower limit (apart from involved margins) below which further treatment to the vulva should be recommended"

[17]. Nooij et al. and Preti et al. also suggested that "tumour positive margins" were the only risk factor [15,27]. Groenen et al. recommended removing "no more than sufficient surrounding tissue" [12], while others have recommended that margins should be at least 2 mm [23], 5 mm [28,29] or ≥8 mm [30].

In 1953, Slaughter et al. undertook a histopathological study of 783 squamous oropharyngeal cancers to better understand their natural history [31]. They concluded that these carcinomas arose from multiple areas which had been preconditioned by some carcinogenic agent, rather than from a single cell. They coined the term "field cancerization" and believed that such a concept would in part explain the high local recurrence rate of oral cancers. Over 50 years later, and with current knowledge of the molecular basis of cancer, Dakubo et al. explored the clinical implications of this concept in multiple cancers, including those of the head and neck, lung and vulva [32]. They concluded that there were two types of local recurrence—those that occurred at the primary site and those that occurred at a distant site. They called recurrences that were genetically similar to the primary "second field tumours" and those that were genetically dissimilar "second primary tumours".

In the future, genetic sequencing of the primary tumour and histologically normal, but genetically transformed, surgical margins should be able to better identify patients at risk for primary site recurrences. Treatment of such patients by re-excision or radiation therapy should decrease the incidence of these recurrences and allow closer surgical margins in selected cases. Another possible approach in the future may be the use of electrochemotherapy [33]. However, until a reliable alternative becomes routine, we believe that surgical margins should ideally be 1 cm of macroscopic skin, which translates to a histopathological margin of 8 mm. This is consistent with current guidelines from the National Comprehensive Cancer Network (NCCN) [34] and the European Society of Gynaecological Oncology (ESGO) [35], which suggest that the surgical margin should be at least 1 cm.

Unlike several studies that questioned the benefit of treating close margins [12,13,18,24], we found treatment with radiotherapy or vulvar re-excision for margins <8 mm to be associated with a lower rate of all vulvar and primary site recurrences in multivariable analysis. The beneficial effect of treatment was most apparent in those patients with margins <5 mm.

Based on these findings, we believe that patients whose surgical margins are <5 mm should undergo surgical re-excision if feasible. Treatment to prevent recurrence merely requires re-excision of the scar, whereas observation until a recurrence occurs will inevitably mean a wider excision, even if the recurrence is diagnosed early. If proximity to the clitoris, anus or distal urethra makes surgical resection inappropriate, or if radiation is required because of positive lymph nodes, a local field of radiation should be given. Patients whose margins are 5–7.9 mm could be followed closely if surgical re-excision were not appropriate. Follow-up should be for life and should include teaching the patient techniques for self-examination. The risk of recurrence in this group of patients, 22.2%, is not different from the risk of recurrence with margins ≥8 mm (23.3%), although over 90% of recurrences will be at the primary site in patients with close margins, while for patients with margins ≥8 mm, 86% of recurrences will be at a remote site.

In addition to having close or positive surgical margins, primary site recurrences occurred earlier than remote site recurrences, 66.7% occurring within two years compared to 31% of remote site recurrences. They were also significantly associated with positive groin nodes in multivariable analysis.

In multivariable analysis, differentiated VIN at the margin was significantly associated with a primary site recurrence, as has been previously reported for local recurrence [18,26]. Others have found LS to be associated with an increased risk of local recurrence in multivariable analysis [16,25]. We could not confirm the latter finding.

An unexpected finding in this study was that current smokers had a lower rate of all vulvar recurrences than non-smokers, with a hazard ratio on multivariable analysis of 0.42. The level of evidence for this association was weaker when primary and remote site recurrences were examined individually. Yap et al. reported smoking to be protective against remote, but not primary site recurrences [16]. This may be related to the fact that smokers are generally younger and have more

uVIN, and the latter was associated with a lower rate of recurrences in univariable analysis. Age over 65 years was also associated with a significantly lower rate of all vulvar recurrences on multivariable analysis, (HR 0.58), possibly related to the fact that local recurrences often occur many years post-treatment, and these elderly patients die of other causes first.

3.1. Strengths and Limitations

To our knowledge, this study is the largest monocentric series in the literature focusing primarily on margin distance and site of local recurrence. All patients were treated in a high-volume tertiary referral centre and 70% of the patients were treated by the one surgeon (NFH). Surgical management and indications for adjuvant radiation were consistent throughout. Other strengths include the long duration of follow up (median 93 months), with all patients being followed at least annually for life. The main limitation is that it is a retrospective study, but sites of primary and recurrent disease were carefully recorded prospectively. The histopathology was not retrospectively reviewed. All slides were reported by a specialist gynaecological pathologist, although there were several different pathologists involved over the 29-year period.

4. Materials and Methods

4.1. Study Design

This study was a retrospective review of patients with squamous cell carcinoma of the vulva, treated primarily with surgery with curative intent, at the Royal Hospital for Women, Sydney. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the South Eastern Sydney Local Health District Human Research Ethics Committee (Reference number: 15/151(LNR/POWH/311). The departmental database was reviewed for consecutive patients treated for primary carcinoma of the vulva between February 1987 and December 2016 (n = 438). Figure 5 describes the inclusion and exclusion criteria for the study group. Demographic, clinical, surgical, histopathological, 2009 FIGO staging and outcome data were extracted from the hospital's medical records. Patients were followed until death from any cause, or until the end of data extraction on 31 July 2019.

4.2. Histopathological Margins and Recurrence

The closest histopathological invasive cancer-free skin margin measured in millimetres on haematoxylin and eosin-stained slides was retrospectively retrieved from the histopathology report. The margin was measured by a specialist gynaecological pathologist from the peripheral margin of the invasive cancer to the inked skin margin of the specimen. Positive margins were defined as invasive carcinoma at any surgical skin edge. The presence of any associated lichen sclerosus in the specimen was also retrieved from the pathology report, as was the presence of any vulvar intraepithelial neoplasia (VIN). Different terminologies were used for VIN over the course of the study, but we classified all lesions as either usual VIN, or differentiated VIN. For this study, a vulvar recurrence was defined as any invasive recurrence located on the vulva. Vulvar recurrences were subdivided into primary site recurrences (when they recurred within 2 cm of the primary resection scar) and remote site recurrences (when they recurred more than 2 cm from the primary scar).

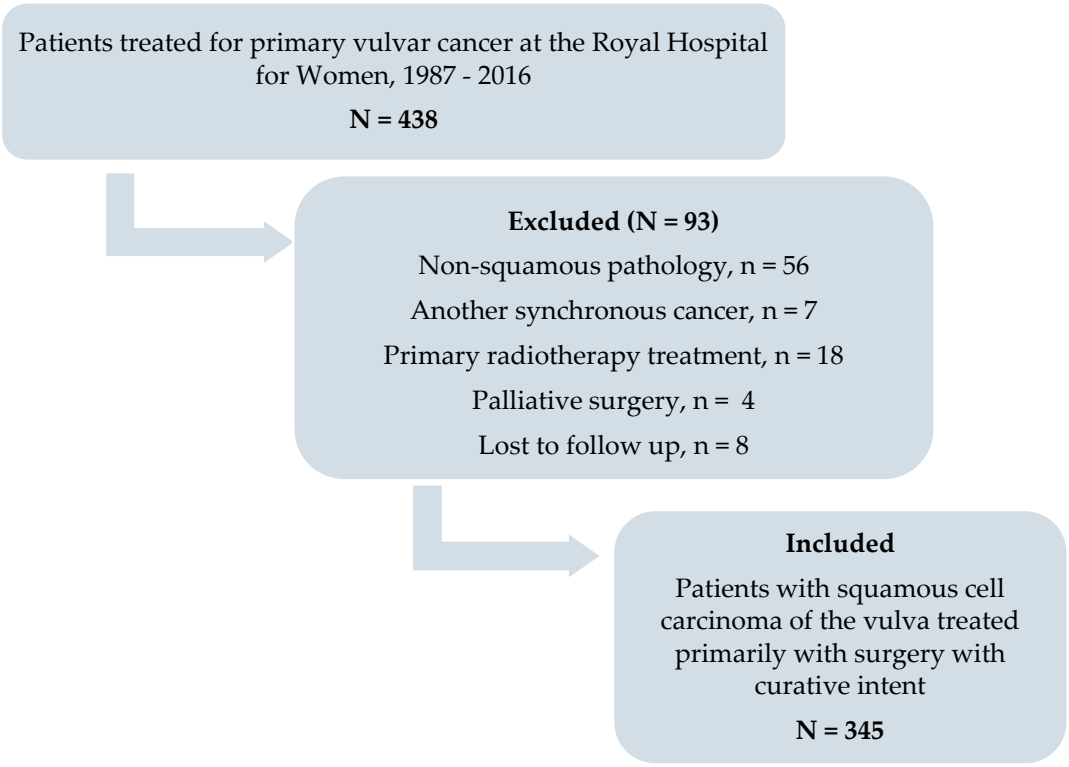


Figure 5. Flow diagram to illustrate patients included and excluded from the study.

4.3. Surgical Treatment

Over the study period, we performed radical local excision or modified radical vulvectomy [6], initially aiming for a skin margin of 2–3 cm, with the deep margin being the fascia overlying the urogenital diaphragm. After the publication of the paper by Heaps et al. [9] in 1990, we aimed for a skin margin of 1 cm. This margin was drawn with a marking pen before stretching the skin for excision. Any clinically apparent usual or differentiated VIN was superficially resected with 5 mm margins, but no attempt was made to excise lichen sclerosus. If the cancer was within 5 mm of the clitoris or anus, primary radiation was used, and those patients were excluded from this study. If it were adjacent to, or encroaching on, the distal urethra, up to 1.5 cm of urethra was resected if that would provide a 1 cm margin. All patients with stages IB and above had some type of groin node evaluation. Most had at least a unilateral inguino-femoral lymphadectomy, but if there were palpably enlarged nodes, these were resected and sent for frozen section. If positive, complete groin dissection was not performed, and post-operative groin and pelvic radiation was given [36,37]. In recent times, some patients underwent sentinel node biopsy, or ultrasonic groin surveillance for early lesions, on a research protocol, if the tumours were 15 mm or less in diameter with a depth of invasion of 3 mm or less.

4.4. Radiotherapy Treatment

Sixty-eight patients received some form of post-operative radiation. Of these, 54 (79%) were treated at the adjacent Prince of Wales Hospital and 14 (21%) were treated at various other city or regional cancer centres, usually in consultation with a radiation oncologist from our team. Of the 68 patients receiving radiation, 22 patients received radiation to the groins and pelvis for positive nodes, and 39 received radiation to the vulva, groins and pelvis for close surgical margins and positive nodes (n = 31), or multifocal disease and positive nodes (n = 8). Seven patients received radiation to the vulva only. Of these, five had close or positive surgical margins and negative or 1–2 microscopically positive groin nodes, one had dVIN at the margin, and another had multifocal disease, a positive

margin for uVIN and immunosuppression following an organ transplant. A small, direct electron beam field was used, based on a margin of 2–3 cm around the scar. The dose ranged from 54 to 60 Gy, with up to 65 Gy given for gross residual disease. Patients with multifocal vulvar disease received a similar dosage via a direct electron field to the whole vulva.

Various techniques and dosages were used over the 29-year period to treat the groins and pelvis. Since 2010, intensity modulated radiotherapy (IMRT) or volumetric modulated arc therapy (VMAT) were used to give a more uniform dose to the vulva (if incorporated into the field), groins or lower pelvic lymph nodes as required. Bolus was used to increase skin dose if needed. If the vulva required a higher dose than the nodes, this was incorporated into the VMAT plan or given as a separate electron field at the end of treatment. Patients were treated with legs together for comfort or slightly apart (frog leg position) to reduce unnecessary dose to the inside of the thigh. Concurrent chemotherapy was not normally used in this older population.

Any lymph nodes greater than 1.5 cm were resected unless fixed, so treatment for positive nodes ($n = 61$) usually involved a dose of 45–50 Gy to the nodal bed. Patients were planned with a computed tomographic (CT) scan, using intravenous contrast to outline the vessels. Patients with extracapsular nodal spread were boosted to a dose of 54 Gy, and higher for any macroscopic residual disease.

4.5. Statistical Analysis

All analyses were performed using IBM SPSS Statistics for Windows (version 26) and R (version 4.0) [38,39]. Descriptive statistics were used to summarize patient demographics and clinicopathological variables and are presented as frequencies or medians. Median follow-up was calculated using the reverse Kaplan–Meier method [40].

For the primary analysis, the outcome of interest, time to first local recurrence, was calculated from the date of surgery until the date of disease recurrence on the vulva, or date of last follow-up. The local recurrence rate was calculated using the Kaplan–Meier method, and associations between various margin groups and treatment factors with local recurrence were assessed using the log rank test. A p value of 0.05 or less was considered statistically significant.

Cox proportional hazard models [41] were used in univariable and multivariable analyses to estimate the associations between all vulvar recurrences, and potential clinicopathological risk factors. Three margin sub-groups were used in these models: <5mm (including positive margins), 5 mm to <8 mm, and ≥8 mm, but only a sub-group of <8 mm was used to determine association with treatment. To examine the competing risks of primary and remote site vulvar recurrence, we fit univariable and multivariable proportional subdistribution hazards models [42] to each outcome, using the same covariates as for the main analysis. Hazard ratios (HR), or subdistribution hazard ratios (SHRs) as appropriate and the corresponding 95% confidence intervals (CI) are presented.

A secondary analysis, using the Kaplan–Meier method, was conducted to compare five and ten-year progression-free survival (PFS) and disease specific survival (DSS) with 2009 FIGO stage for the entire study group. Five and ten-year DSS was also calculated to determine potential survival differences between patients with an isolated vulvar recurrence and with a regional or distant recurrence. Five and ten-year DSS and overall survival (OS) were estimated for the whole study cohort. PFS was defined from the date of surgery until the date of disease recurrence or last follow-up. DSS was defined from date of surgery to the date of death due to vulvar cancer. All other patients were censored at date of last follow-up, or date of death from another cause, without a vulvar cancer recurrence. OS was determined from date of surgery to the date of death from any cause, or last follow-up. The two-sided log-rank test was used to calculate survival comparisons.

5. Conclusions

In summary, our study has demonstrated that conservative vulvar resection (radical local excision) in 80% and bilateral inguino-femoral lymphadenectomy in 35.6% of patients with squamous cell carcinoma of the vulva is associated with an excellent survival. Although surgical margins <8 mm were not associated with an increased risk of all vulvar recurrences in univariable analysis, when broken down into primary and remote site recurrences, margins <8 mm were significantly associated

with an increased risk of primary site vulvar recurrence. In multivariable analysis, primary site recurrences were significantly increased in patients with margins <8 mm, and all vulvar and primary site recurrences in patients with margins <5 mm. Primary site recurrences were also significantly increased in patients with one or more positive nodes, or with differentiated VIN at the excision margin. Patients with close or positive margins who were treated with either surgical re-excision or radiotherapy had a significantly decreased risk of recurrence. Our results support the recommendation that excision margins should be at least 1 cm, which equates to a histopathological margin of 8 mm. Surgical re-excision or radiation therapy should be recommended if the histopathological margin is <5 mm. Future genetic analysis of the skin adjacent to the cancer may allow closer margins in selected cases.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6694/12/11/3375/s1, Figure S1. Kaplan–Meier curve for ten-year disease specific survival for the whole study cohort; Figure S2. Kaplan–Meier curve for ten-year disease-specific survival for patients with positive nodes; Figure S3. Kaplan–Meier curve for ten-year disease-specific survival stratified by site of first recurrence; Figure S4. Kaplan–Meier curve for ten-year overall survival for the total study cohort; Figure S5. Local recurrence rate in margins ≥8 mm compared to margins <8 mm; Table S1. margin distance and treatment of positive or close margins to site of vulvar recurrence; Table S2. Breakdown of vulvar recurrence per mm margin distance in 25 patients with untreated margins <5 mm.

Author Contributions: Conceptualization: E.B. and N.F.H.; methodology: E.B and N.F.H.; software: E.B.; validation: E.B, M.J. and N.F.H.; formal analysis: E.B.; investigation: E.B. and N.F.H; resources: E.B.; data curation: E.B.; writing—original draft preparation: E.B.; writing—review and editing: E.B., M.J. and N.F.H; supervision: N.F.H.; project administration: E.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to acknowledge Mark Donoghoe (Statistical Consultant, Mark Wainwright Analytical Centre, University of New South Wales) for his support with the statistical analysis. He checked the Cox-regression analysis and completed the Fine–Gray analysis. EB was supported by an Australian Government Research Training Scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Material: The Prognostic Role of the Surgical Margins in Squamous Vulvar Cancer: a Retrospective Australian Study

Ellen L Barlow [1]*, Michael Jackson [2,3] and Neville F Hacker [1,4]

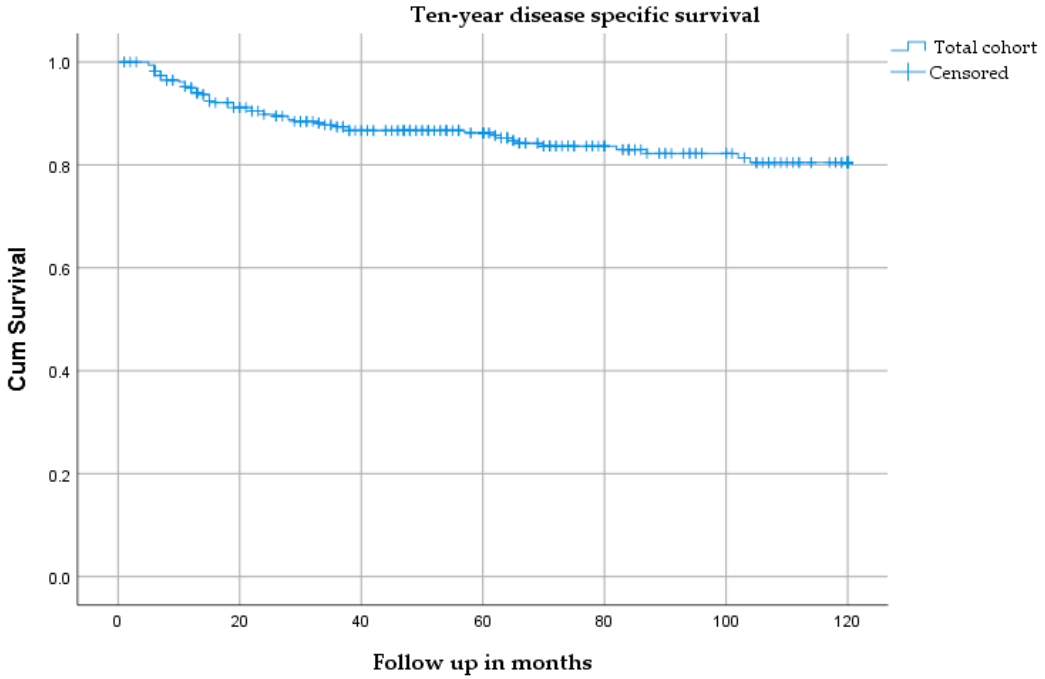


Figure S1. Kaplan-Meier curve for ten-year disease specific survival for the whole study cohort (n = 345).

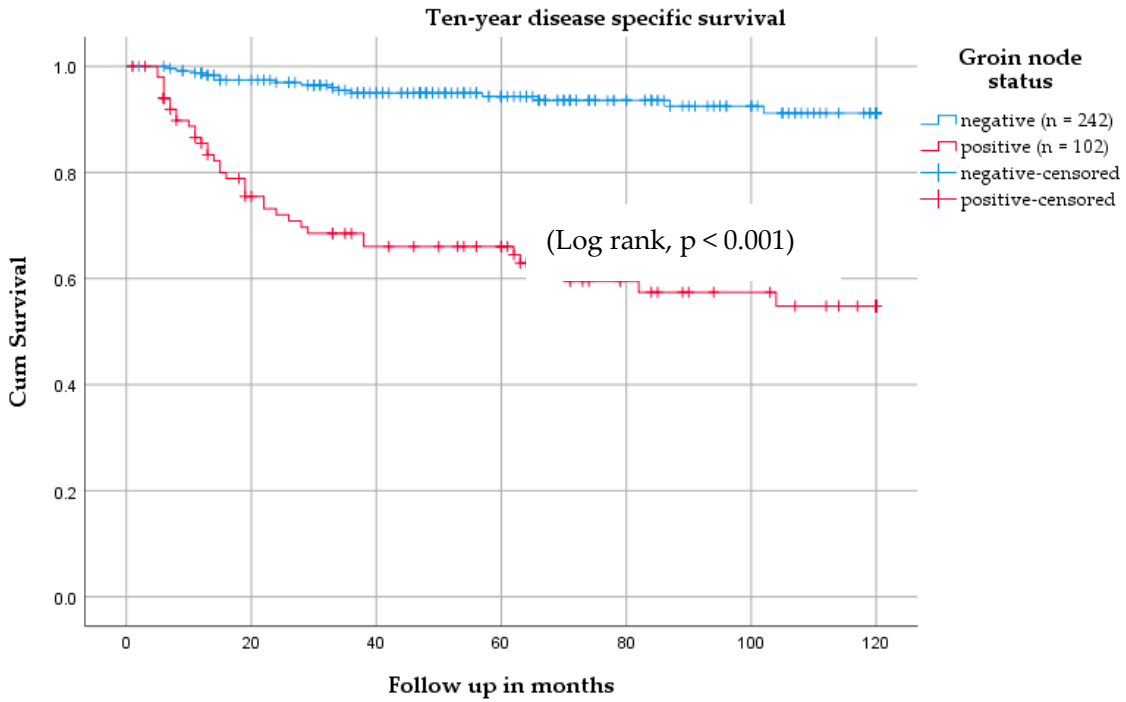


Figure S2. Kaplan-Meier curve for ten-year disease-specific survival stratified by nodal status.

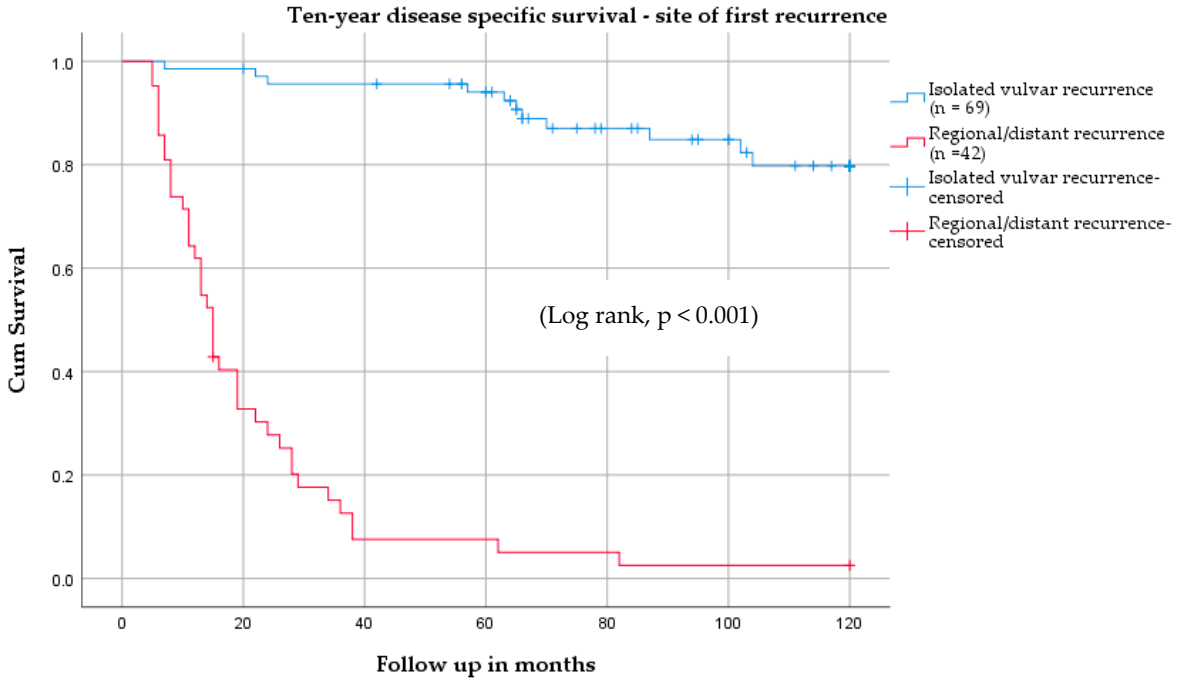


Figure S3. Kaplan-Meier curve for ten-year disease specific survival stratified by site of first recurrence.

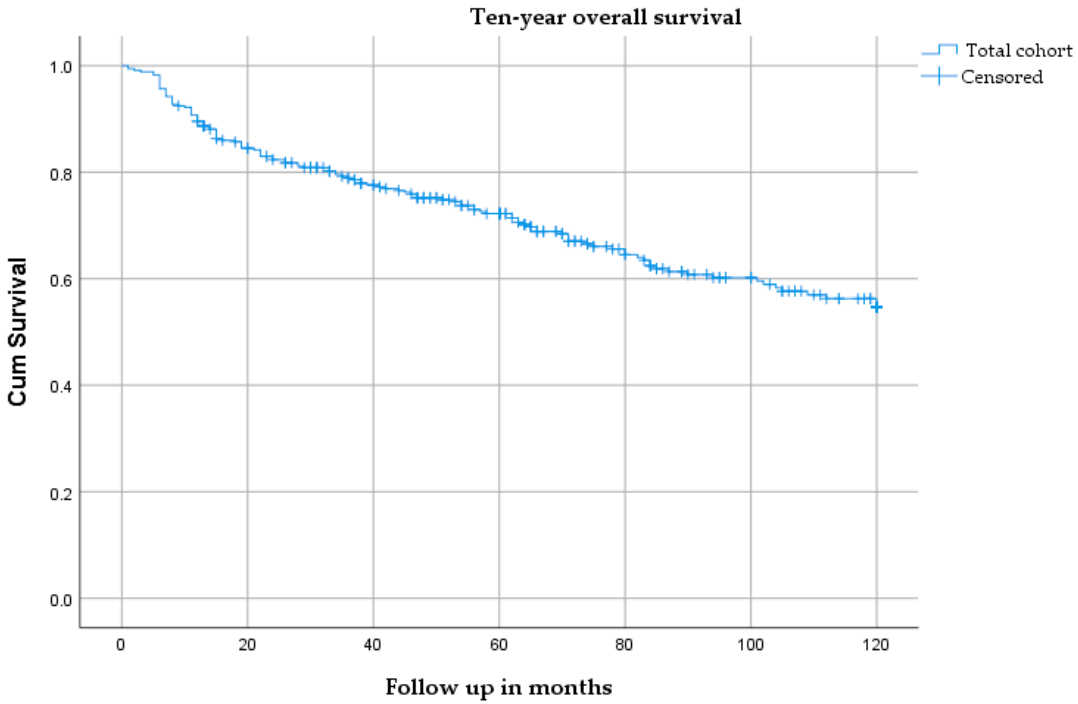
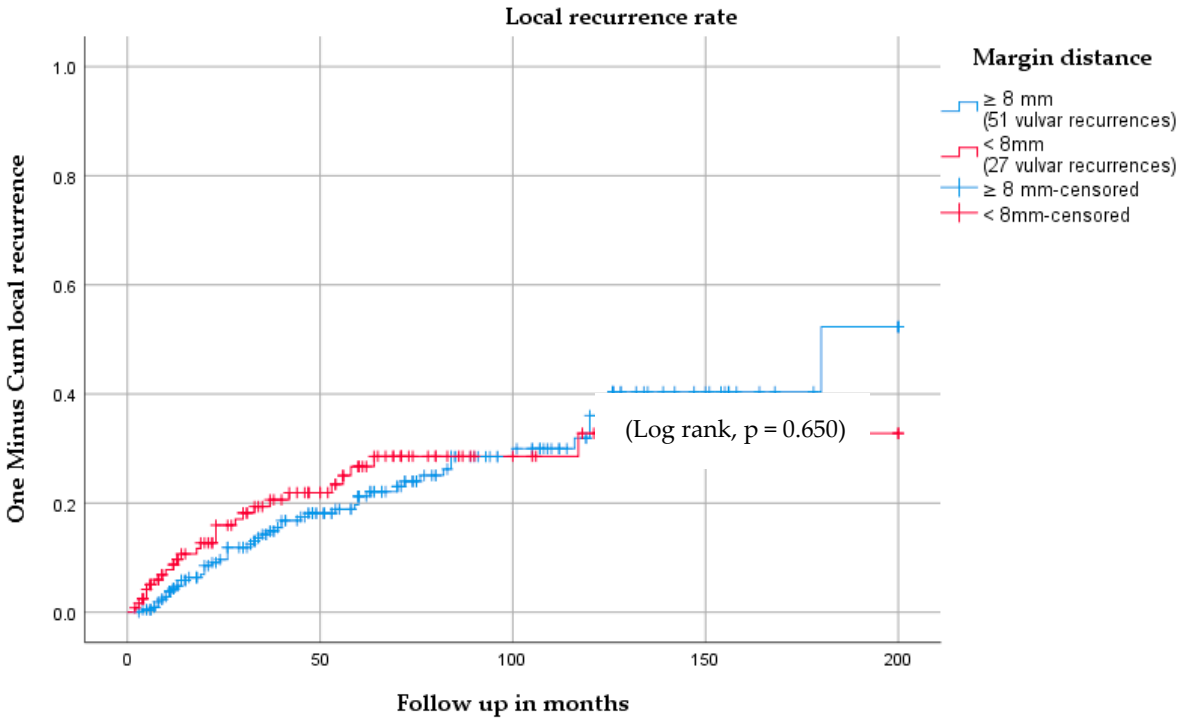


Figure S4. Kaplan-Meier curve for ten-year overall survival for the total study cohort (n = 345).



Months	0	20	40	60	80	100	120	180
< 8 mm margin	122	85	62	44	27	20	15	3
≥ 8 mm margin	219	172	131	102	68	49	33	5

Number at risk

Figure S5. Local recurrence rate in 219 patients with margins ≥ 8 mm and in 122 patients with margins < 8 mm.

Table S1. Margin distance and treatment of positive or close margins to incidence and site of vulvar recurrence.

Margin distance	Total n	Vulvar recurrence n (%)	P value[a]	Primary site recurrence (n =33)	Remote site[b] recurrence (n = 45)
*Positive margin	11	2 (18.2%)			
- treated with radiotherapy	8	2 (25%)		2	0
- treated with re-excision	3	0 (0%)	0.157		
Close margins					
0.1 mm – 4.9 mm treated margins	23	1 (4.3%)			
0.1 mm – 4.9 mm untreated margins	25	10 (40%)	0.003		
- treated with radiotherapy	14	0 (0%)			
- untreated margins	25	10 (40%)	0.009	11	0
- treated with re-excision	9	1 (11.1%)			
- untreated margins	25	10 (40%)	0.095		
5 mm – 7.9 mm treated margins					
5 mm – 7.9 mm untreated margins	12	1 (8.3%)			
	51	13 (25.5%)	0.165		
- treated with radiotherapy					
- untreated margins	9	1 (11.1%)			
	51	13 (25.5%)	0.352		
- treated with re-excision					
- untreated margins	3	0 (0%)		13	1
	51	13 (25.5%)	0.252		
< 8 mm treated margins					
< 8 mm untreated margins	76	23 (30.2%)			
	46	4 (8.7%)	0.005		
Wide margins					
≥ 8 mm margins	219	51 (23.3%)		7	44

*All positive margins treated with either radiotherapy or re-excision. [a] Log-rank test. [b] no remote site recurrences in positive, or 0.1 - 4.9 mm margins.

Table S2. Breakdown of vulvar recurrence per mm margin distance in 25 patients with untreated margins < 5 mm.

Margin distance	Number	Local recurrence
1 mm	2	0 (0%)
2 mm	4	1 (4%)
3 mm	5	3 (12%)
4 mm	14	6 (24%)
Total	25	10 (40%)

CHAPTER SEVEN

DISCUSSION

The objectives of this body of work were two-fold: firstly, to determine trends in the incidence and mortality of vulvar cancer in Australia, by interrogating the population-based data from the Australian Institute of Health and Welfare from 1982 to 2011; and secondly, to investigate some of the controversial issues in the current management of invasive squamous cell carcinoma of the vulva, by reviewing a large cohort of patients treated with a common philosophy of management in the Gynaecological Cancer Centre of the Royal Hospital for Women over a 29 year period (1987 to 2016). Specific questions addressed in this review were: (i) is determination of p16 and p53 status by immunohistochemistry of any value in determining management or prognosis for these patients; (ii) what is the post-operative morbidity related to groin dissection, and what can be done to reduce it; and (iii) what are the risk factors for local recurrence in patients with vulvar cancer, and what preventive measures can be taken?

In this chapter, I provide a summary of the methods and key findings of Chapters 3 through 6 in the form of a table (Table 7.1). I then discuss our research outcomes in relation to the literature and in accordance with the thesis research questions. Recommendations for future research are suggested. This chapter ends with a conclusion and implications for practice incorporating all four research projects.

Table 7.1. Summary of the methods and key findings from Chapters 3 to 6.

Chapter	Methods	Key Findings
Chapter 3 [10]	Case numbers for invasive carcinoma of the vulva (1982-2009) and vulvar cancer deaths (1982-2011) were obtained from the National Cancer Statistics database. Standardised rate ratios (SRRs) were used to assess changes in age-standardized incidence (ASI) and mortality rates (ASM), for all ages and for women younger than 60 years and 60+ years	ASI rates in women across all ages did not significantly change from 1982-84 to 2007-09. SRR from the later to the earlier period were, 1.13 (95% CI: 1.00-1.27). There was an 84% increase in incidence in women younger than 60 years (SRR, 1.84; 95% CI: 1.49-2.26), with no change for women 60+ years (SRR, 0.90; 95% CI: 0.79-1.04). All ages ASM decreased by 22% (1982-86) to (2007-11) (0.7 to 0.5 per 100,000 women: SRR, 0.78; 95% CI: 0.66-0.93). This was driven by declining mortality rates in older women, with stable rates in women younger than 60 years (SRR, 1.05; 95% CI: 0.62-1.79); rates in 60+ years decreased by 24% (SRR, 0.76; 95% CI: 0.63-0.91).
Chapter 4 [260]	A retrospective analysis of patients treated for VSCC at a tertiary hospital in Sydney, Australia, from 2002 to 2014, (n = 119). Histological specimens were stained for p53 and p16 expression, and HPV status was determined by PCR detection of HPV DNA	HPV DNA was detected in 19%, p16 expression in 53%, and p53 expression in 37% of cancers. Kaplan-Meier analysis showed patients with p16/HPV-positive cancers had superior five-year disease-free survival (76% vs 42%, resp., $p = 0.004$) and disease-specific survival (DSS) (89% vs 75% resp., $p = 0.05$) when compared to patients with p53-positive cancers. In univariate analysis, nodal metastases ($p < 0.001$), tumour size > 4 cm ($p = 0.03$), and perineural invasion ($p = 0.05$) were associated with an increased risk of disease progression, and p16 expression with a decreased risk ($p = 0.03$). In multivariable analysis, only nodal metastases remained independent for risk of disease progression ($p = 0.01$). For DSS, lymph node metastases ($p < 0.001$) and tumour size ($p = 0.008$) remained independently prognostic.

Chapter	Methods	Key Findings
Chapter 5 [261]	A retrospective analysis of clinical and histopathological data for 333 patients (525 groins) treated with all types of groin node dissection for invasive cancer of the vulva from 1987 to 2016.	The incidence of complications per groin were, wound breakdown 8.2%, wound infection,10.7%, lymphoedema, 31.6%, and lymphocysts, 36.6%. Lymphocysts were higher in patients having an IGFLND* compared to those having nodal debulking, or a sentinel node procedure (42.5% vs 14.6% versus 0% respectively: $p < 0.0001$). In multivariable analysis, no significant difference in lymphocyst incidence was observed between patients with or without a groin drain. Lymphocyst formation was most strongly associated with a greater number of nodes removed ($p < 0.0001$). The number of nodes resected was the only factor significantly associated with all complications, but current smoking and increasing age also increased the risk of wound breakdown
Chapter 6 [262]	A retrospective analysis of 345 patients treated primarily with surgery for squamous vulvar cancer between 1987 and 2016, looking specifically at-risk factors for local recurrence, and means of preventing a recurrence.	Five-year disease-specific survival was 86%. Local (vulvar) recurrences occurred in 78 patients (22.6%) Of the 78 local recurrences, 33 (42.3%) were at the primary site and 45 (57.7%) were at a remote site. In multivariable analysis, a surgical margin < 5 mm was associated with a higher risk of all vulvar (Hazard ratio (HR) 2.29; CI: 1.12−4.70), and primary site recurrences (subdistribution hazard ratio (SHR) 15.20; CI: 5.21−44.26) Margins of 5 to < 8mm had a higher risk of a primary site recurrence (SHR 8.92; CI: 3.26−24.43), and a lower risk of remote site recurrence. Treatment of margins < 8mm by re-excision or radiation therapy significantly decreased the risk of recurrence.

* **SRRs**, Standardised rate ratios; **ASI**, Age standardised incidence rates; **ASM**, Age standardised mortality rates; **PCR**, Polymerase chain reaction; **HPV DNA**, Human Papilloma virus deoxyribonucleic acid; **VSCC**, Vulvar squamous cell carcinoma; **IGFLND**, Inguino-femoral lymph node dissection; **HR**, Hazard ratio; **SHR**, Subdistribution hazard ratio.

7.1 | Australian Incidence and Mortality Trends

The first study of my thesis (Chapter 3) aimed to address the following research questions by analysing vulvar cancer incidence and mortality data obtained from the Australian Institute of Health and Welfare National Cancer Statistics database [268].

Research Questions:

- What are the temporal trends in the incidence and mortality of vulvar cancer in Australian women?
- Is there evidence of increasing incidence of vulvar cancer in younger cohorts of Australian women born after 1950?

The main findings were that although the age-standardised incidence rates of vulvar cancer in women across all ages did not change significantly between the time periods 1982 - 1984 and 2007 - 2009, there was a significant 84% increase in incidence in women younger than 60 years, with no change for women 60 + years. For mortality incidence rates, we identified an overall 22% decrease over two four-year time periods (1982-1986 and 2007–2011), but this was driven by a 24% decrease in mortality for older women, with stable rates in younger women [10].

When the standardised rate ratio was used to measure vulvar cancer incidence trends in women of all ages, our findings are similar to several earlier population-based analyses where stable incidence rates were reported in women from Australia [71], the United States [69], Norway [79], and Sweden [80]. However, our study built on the earlier Australian analysis [71] by (i) including an additional four years of Australian data, (ii) comparing incidence trends in women of different ages, and (iii) using the annual percent change (APC) as a measure of trends. From this analysis, we estimated there was a 2.5% average APC in women younger than 60 years, but stable rates in women aged 60 years and older.

In Chapter 3, we reported that our findings of an increased incidence of vulvar cancer in younger women were broadly comparable to recent population-based trends in other countries where incidence trends were analysed in relation to various age groups [9,12,76,78]. Since the publication of our findings, there has been further evidence of an increasing incidence of vulvar cancer among women younger than 60 years [11,72,73]. However, several studies have reported their greatest increase in incidence to be in elderly women [36,74,75].

In Chapter 3, we postulated [10] that this increased incidence in younger Australian

women may be related to an increase in human papillomavirus infections, which would correlate with the changes in sexual mores from the 1960's onward. Other HPV-related cancers, such as anal and oro-pharyngeal, have also increased in incidence in the same general time frame [71,269,270], and a recent (2018) paper reported an increase in the incidence of squamous anal cancer was most evident in men and women under 60 years of age [271].

With respect to mortality trends, we observed a significant decrease in mortality in women aged 60 years and older, which directed an overall decrease in all women [10]. Two population-based studies from the United Kingdom have also shown decreased mortality, particularly in women aged 60 years and older [12,70], whereas five-year relative survival rates have remained stable in the Netherlands [78] and in Norway [79].

In contrast, squamous vulvar cancer mortality rates have increased with advancing age in Canada [75], and the United States [74,75], and in women of all ages in South Africa [72].

In Chapter 3 we suggested that the decreasing mortality rates we observed in older women may be due to earlier diagnosis. We attributed this to women having better access to health information on the Internet, and to changing social mores. The latter may have meant that older women presented with smaller lesions that were more amenable to surgical resection [10] and would carry a better prognosis.

The lower relative survival in older women in some studies could be related to adverse effects of treatment related to co-morbidities which are more predominant in elderly women [75]. Co-morbidities and access to the Internet are unlikely to vary significantly among developed countries, and we believe the increasing mortality in some countries may reflect different philosophies regarding management of the elderly patient, particularly in relation to performance status. This may be related to either the clinician's beliefs [272,273], or to patient preferences.

There are some limitations associated with population-based cancer data, and these have been discussed in detail in Chapter 2 (section 2.1.4). Our data obtained from the Australian Institute of Health and Welfare's National Cancer Statistics Database [268], included cancer incidence and mortality data from the cancer registries of all Australian states and territories. Although not completely comprehensive, it provides coverage of approximately 81% of the Australian female population [11].

7.2 | HPV DNA, p16 and p53 in Squamous Cell Carcinoma of the Vulva

Moving on from our population-based trends and survival analyses, our research focused on the more clinical aspects of vulvar cancer treatment. In Chapter 4 we aimed to answer two further research questions by retrospectively analysing 119 consecutive patients treated for VSCC in our institution between 2002 and 2014.

Research Questions:

- Is Human Papillomavirus (HPV) status prognostically meaningful in vulvar squamous cell carcinoma, and is pre-operative determination of p16 or p53 status by immunohistochemistry clinically relevant?
- What are the clinicopathological variables associated with the immunohistochemical expression of p16 and p53?

All histological specimens were stained for p53 and p16 expression, and HPV status was determined by PCR detection of HPV DNA. Our results suggested that p16 expression was a more accurate reflection of HPV positive cancers than HPV DNA determination, with a p16 prevalence of 53% compared to a HPV DNA prevalence of 19% in our cohort [265].

We identified significant clinicopathological differences between patients with p16 and p53 positive tumours (Chapter 4, Table 1.). In relation to our research question regarding the prognostic relevance of these markers, in our univariate analysis we found that patients with p16-positive vulvar tumours (compared to patients with p16-negative, or p53-positive tumours) displayed several histopathological characteristics that are commonly associated with a better prognosis (Chapter 4, Table 1). This is consistent with other reports in the literature [27,152,274].

Accordingly, our Kaplan-Meier analysis estimated that patients with p16-positive tumours had a better 5-year disease-free, and disease-specific survival than patients with p53-positive tumours (Chapter 4, Figure 1). Our subsequent univariate Cox-regression analysis for disease-free and disease-specific survival found that patients with p16-positive tumours (compared to p53-positive tumours) had a significantly decreased risk for disease progression, but not for disease-specific survival (Chapter 4, Table 2). However, we were not able to confirm these findings in our multivariable analysis. (Table 3.) [265]. Whether or not p16 status is an independently significant prognostic marker remains controversial [26,27,140,145-147,151,152].

Recently published retrospective studies have indicated that p16 positivity may be a prognostic indicator for response to radiotherapy in squamous vulvar cancers, as has been shown in patients with oro-pharyngeal cancers [156-158]. Women with p16-positive tumours have been reported to have a significantly lower rate of in-field relapse [162,164], as well as better overall [162,165] and progression free survival [165]. One study also reported that women with p16-positive tumours were more likely to achieve a complete clinical response compared to those with p16-negative tumours [163]. We were unable to confirm these results. However, our study was not designed to make a conclusive comment regarding response to radiotherapy, as there were only 20 patients who received adjuvant radiotherapy and only eight had p16-positive tumours.

Our failure to confirm improved progression and disease-specific survival for patients with p16-positive tumours (compared to those with p53-positive tumours) in multivariable analysis, despite strong evidence for both in our Kaplan-Meier analysis, and for disease-free survival in our univariate analysis, may reflect the number of participants and the small number of vulvar cancer recurrences and deaths in our series.

There are also inconsistentcies between early published studies [126,133,150,275] and recent studies [134,153] with respect to the prognostic significance of p53 expression. In our study we found that p53 expression was associated with several poor prognostic factors (Chapter 4, Table 1), and our Kaplan-Meier survival analysis determined that patients with p53-positive tumours had inferior 5-year disease-free and disease specific survival compared to patients with p16-positive tumours. However, again we were unable to confirm this in the multivariable analysis.

We described a third subgroup of patients in our study (n =12) who were HPV-negative and stained negative for p16 and p53 using immunohistochemistry. Because no distinction could be made based on their HPV, p16 or p53 status they were subsequently excluded from further analysis. However, we did include this subgroup in a Kaplan-Meier survival analysis and determined that they had survival intermediate between the patients with p16-positive, and p53-positive tumours [265]. Similar survival rates were reported for a third subgroup of patients in a 2017 Dutch study [152]. A 2020 multi-institutional Dutch study [153] also identified three clinically distinct squamous vulvar cancer subtypes with similar survival outcomes to the earlier Dutch study, and in line with our own.

7.3 | Morbidity Related to the Groin Lymph Node Dissection for Vulvar Cancer

In Chapter 5, the third study of my thesis focused on evaluating the short and long-term complications associated with the removal of the inguino-femoral lymph nodes. This

study retrospectively analysed clinical and histopathological data for 333 patients (525 groins) treated with all types of groin node dissection for invasive cancer of the vulva from 1987 to 2016. We aimed to answer two further research questions:

Research Questions:

- What is the incidence of short and long-term postoperative morbidity of the groin lymph node dissection in a large cohort of patients treated for invasive vulvar cancer?
- What clinical factors are associated with post-operative morbidity following groin node dissection?

Our data for short term post-operative complications demonstrated that groin lymphocyst formation was the most common short-term complication, with lymphocysts occurring in 192 of 525 groins (36.6%) dissected [266].

Several clinical variables were examined to determine their association with the risk of lymphocyst formation, but the only factor we found to be associated was the number of groin lymph nodes removed. In our univariate analysis, lymphocysts were more common in patients having an inguino-femoral lymhadenectomy (IGFLND) compared to those having nodal debulking, or a sentinel node procedure. This correlated with the strong association we found between the greater number of nodes removed and the increased incidence of lymphocyst formation in multivariable analysis (Chapter 5, Table 4).

Inguinal suction drains became a particular focus of this study as they are routinely used in most centres worldwide to reduce lymphocyst formation, despite there being no conclusive evidence of their benefit. In our study we aimed to answer two additional research questions related to their use.

Supplementary Research Questions:

- What is the incidence of lymphocyst formation in patients with and without a groin drain following groin node dissection?
- Is short term post-operative morbidity increased in patients with a groin drain?

We reported inguinal drains were used in 348 groins (211 patients), and 177 groins (122 patients) were not drained. We found that lymphocyst incidence rates were similar between the drain and no drain groups (35.3% vs. 39%, respectively; $p = 0.85$). Two retrospective studies also reported no significant difference in lymphocyst formation rates depending on whether or not an inguinal drain was used [170,188], and drains have

not been shown to significantly affect the incidence of seromas in patients after axillary lymphadenectomy [203,204,209].

In Chapter 5, we reported groin wound infection to be the next most common short-term complication, with an incidence of 10.7%. There is limited information in the literature on the risk factors for post-operative groin wound infection. The only other Australian study [188] reported post-operative groin cellulitis occurred less frequently in patients without an inguinal drain, but we identified no significant difference between the drain and no drain groups for groin wound infection. Obesity has been associated with increased rates of wound cellulitis [166], while older age and diabetes mellitus have been reported to be significantly associated with an increased risk for any short-term complication in a recent Dutch study [186].

In our multivariable analysis, the only risk factor we found to be associated with an increased risk for groin wound infection was an increasing number of nodes removed (Chapter 5, Table 4). Consistent with this finding, van der Zee et al. [185] reported groin cellulitis rates of 4.5% in patients who had a sentinel node biopsy compared to 21.3% in patients who had a sentinel node biopsy + inguino-femoral lymphadenectomy.

Our data identified groin wound breakdowns as the least common short-term complication, occurring in only 8.2% of groins. In our study, groin wound breakdowns were more common in patients who had nine or more nodes removed compared to those who had four or less removed, and this remained significant in our multivariable analysis. One earlier study has confirmed this association [185], while others have not [186,192].

We also found increasing age to be significantly associated with groin wound breakdown in multivariable analysis. This has been reported in two other retrospective studies [183,186].

Obesity has been reported to be associated with an increased risk for groin wound breakdown [183], but we found that neither diabetes mellitus nor obesity were significantly associated with groin wound breakdown in our multivariable analysis.

Cigarette smoke has been shown to contain toxins associated with impaired wound healing, principally nicotine and the gases carbon monoxide and hydrogen cyanide [276,277]. These have all been shown to impair oxygen supply to tissues [278]. Not surprisingly, we found that current smoking was associated with an increased risk of groin wound breakdown. This association has only been reported in one other retrospective vulvar cancer study [192], but it has been reported to be associated with acute wound dehiscence rates in several other surgical disciplines [279-281]. We have subsequently improved the

pre-operative smoking cessation information, and the post-operative nicotine replacement management we provide to the patients treated in our department.

Two studies have reported an association between inguinal suction drains and groin wound breakdown rates, but these studies looked at different types of drains [170], or different durations of use [192]. In our study, we examined two groups of patients – one who had a drain and another who did not. We found a higher wound breakdown rate in groins where a drain was used compared to those without a drain (9.8% vs. 5.1%, respectively), but this was not statistically significant.

In Chapter 5, we reported lower limb lymphoedema (LLL) to be the major long-term complication, occurring in 31.6 % of groins dissected. In our multivariable analysis, increasing number of nodes removed was the only factor we found to be significantly associated with an increased risk of developing LLL (OR 1.16, p = 0.01), (see Chapter 5, Table 4). This finding is in line with other reports in the literature [168,192,215].

One way that has been reported to decrease the number of nodes removed without compromising survival is to resect only bulky positive lymph nodes, rather than performing a complete inguino-femoral lymphadenectomy, and follow this with groin and pelvic radiotherapy [282,283]. In our current study, significantly less lymphoedema developed in patients having this approach, compared to those having a complete inguino-femoral dissection (19.6% per groin vs. 35% respectively). In addition, only 3.9% of such patients experienced a groin wound breakdown (compared to 9.4% after IGFLND) and only 14.6% developed a lymphocyst (compared to 42.5%) (see Chapter 5, Table 2).

Adjuvant radiotherapy was not found to confer a significantly increased risk for the development of LLL in our study, although this remains controversial [168,169,183,215]. In our cohort, radiation was mainly given after nodal debulking, where the median number of nodes removed was 3 compared to 9 for an IGFLND. This is clearly a confounding factor, but it suggests that adjuvant radiation is not as important a risk factor as number of nodes removed in the induction of lower limb lymphoedema.

We found a low rate of recurrent lower limb cellulitis (6.8%) but consider this to be most likely related to under-reporting. As discussed in Chapter 5, a large proportion of our patients came from rural and regional areas and would have been treated for lower limb cellulitis in their local area.

7.4 | The Prognostic Role of the Surgical Margins in Squamous Vulvar Cancer

Following on from our investigation of the morbidity associated with groin lymph node

dissection our remaining three research questions focused on the surgical management of the primary vulvar tumour. Traditionally, radical vulvectomy has been the standard of care, but it is associated with significant physical [171-173] and psycho-sexual morbidity [174-176]. Since the early 1980s, radical local excision for most unifocal vulvar cancers, along with unilateral groin dissection for unilateral tumours, has progressively been practised. Our next research question focused on determining the survival of patients whose vulvar cancer had been treated conservatively whenever possible.

Research Question:

- What is the long-term survival of patients with squamous vulvar cancer treated in the era of conservative management?

In Chapter 6, the findings from our cohort of 345 patients treated surgically with curative intent over a 29-year period were reported [267]. We showed that in patients with all stages of disease, radical vulvectomy was performed in only 20%, and only 36% had bilateral groin dissection. This conservative surgical management resulted in five and ten-year disease-specific survivals of 86 and 80% respectively for the total study group, and 66 and 55% respectively, for the 102 patients with positive nodes (see Chapter 6, Figures S1 and S2).

In contrast to our findings it is apparent from the literature that radical local excision is still not universally undertaken for patients with unifocal vulvar tumours. A 2020 retrospective study [284] of 1535 patients with vulvar cancer treated between 2001 and 2005 for all stages of disease across 100 European centres, reported radical vulvectomies in 76.5%, and bilateral groin node dissection in 45.2% of patients. The same year, another retrospective study of 335 patients treated between 2000 and 2018 in the three Mayo Clinic centres, reported 57.9% of patients had a radical vulvectomy [252]. Recent single centre studies from Turkey [237], the United Kingdom [248], and a 2019 dual-centre Dutch study [235] have reported much lower rates of radical vulvectomy, which are more in keeping with our own low rate.

Vulvar squamous cell carcinoma has high local recurrence rates, regardless of the type of surgery performed. We identified an overall local recurrence rate of 22.9% within a median follow-up time of 93 months (range 1-367). Of these recurrences, 69 were isolated vulvar recurrences, and with additional surgery or radiotherapy, 94% were free of disease five years later [267] (Chapter 6, Figure S3).

A recent retrospective study from the United States reported a comparable local recurrence rate of 23.3% (median follow-up of 73 months), but almost 60% of their patients had a

radical vulvectomy [252]. Other studies have reported overall local recurrence rates as low as 14%, but in patients where the mean follow up time was only 37.6 months [236], and as high as 43.5% where the median follow-up was 80 months [235].

The width of the surgical margin has been considered an important prognostic factor for vulvar recurrence. As discussed in Chapter 2, since the study by Heaps et al. [238] in 1990, a surgical margin width of at least 1cm, (pathological margin of 8mm) has generally been accepted. Several recent studies have challenged the need for an 8mm pathological surgical margin [235,237,244-250], so our next research question was directed towards addressing this controversy:

Research Question:

- Is there a relationship between the extent of the surgical excision margin and local vulvar recurrences in women treated with primary surgery for squamous vulvar cancer?

Vulvar recurrences may occur at the site of the primary cancer or at a remote site, such as the contralateral side. These two sites of recurrence were not considered in the initial report by Heaps et al. [238], and were only recognized after the report by Rouzier et al. in 2002 [94].

During the conceptualisation of our study in Chapter 2, we hypothesised that patients with a histologic excision margin of less than 8 mm would be at a higher risk for local recurrence at the primary tumour site but would not be at increased risk for a remote site recurrence. These hypotheses were confirmed in our analysis (see Chapter 6, Tables 2 and 3).

We are concerned that several authors are now proposing that a pathological excision margin of 8mm need no longer be a surgical objective to decrease the risk of local recurrence [235,237,244-247,249,250]. These papers have failed to account for these two different sites of local recurrences..

A number of these studies did report other pathological factors to be associated with an increased local recurrence risk. Two of them [248,250] reported lichen sclerosus, but we could not confirm this finding. te Grootenhuis et al. [235] reported that patients with dVIN alone, or dVIN and lichen sclerosus at the margin, had higher local recurrence rates. In our multivariable analysis differentiated VIN at the excision margin was associated with an increased risk for local recurrence, but only at the primary site (SHR 5.35) [267].

Given that many vulvar cancers develop in a background of dysplastic surrounding skin

such as usual VIN (now termed vulvar high grade intaepithelial lesions (HSIL), dVIN or lichen sclerosus [256], it is not surprising that such patients may develop a 'recurrence' on the vulva but in an area remote from the primary tumour. It has been suggested that these 'recurrences' may be second primary tumours which arise in a 'field of cancerization' [256].

In Chapter 2 (section 2.3.4), we described the theory of field cancerization, which is based on the premise than an area of epithelium contains epigenetic and genetic changes in numerous cells, which may ultimately manifest as cancer [258-261]. This concept was first proposed by Slaughter et al. [259] in 1953 for oropharyngeal squamous cancers, and Dakubo et al. [258] have more recently examined its relevance for patients with squamous vulvar cancer. They described two types of recurrences: (i) those that occurred at the primary site and were genetically related to the primary tumour, which they called 'second field tumours', and (ii) those that occurred at a distant site, and were genetically different to the primary tumour, which they called true 'second primary tumours' [258].

As we highlighted in Chapter 6 [267], te Grootenhuis et al. [249] proposed that defining vulvar recurrences as local recurrences or second primary cancers based on the site of recurrence was too subjective and could not be replicated. Our results indicate that with sufficient patient numbers and accurate, prospective documentation of the original tumour location, this division is reproducible. We agree with te Grootenhuis et al. [249] that genetic profiling and molecular sequencing of primary and recurrent tumours is the only way to distinguish with certainty a 'true' local recurrence from a new primary cancer. However, we believe that distinguishing between these two types of local recurrence is important because it permits clear guidelines to be given concerning surgical management [267].

Surgical re-excision has generally been recommended for those patients whose minimum histopathological resection margin was less than 8mm. If there was proximity to the clitoris or anus, vulvar radiation was usually recommended. However, there is limited evidence in the literature of the benefit of surgical re-excision or adjuvant radiotherapy for close margins. The final question of my thesis aimed to address this issue.

Research Question:

- Is treatment of close or positive surgical excision margins beneficial in reducing local recurrences?

Our results have demonstrated a significant benefit in treating close or positive margins. In our multivariable analysis radiotherapy or vulvar re-excision for patients with margins

< 8mm was significantly associated with a lower risk of all vulvar and primary site recurrences. This was most evident in the < 5mm margin group (Chapter 6, Table 2 and 3) [267].

In the literature, the benefit of adjuvant treatment on local recurrence is unclear. Arvas et al. [237] reported radiotherapy to be beneficial, but only in patients with pathological margins of 2mm or less, and te Grootenhuis et al. [235] reported no difference in local recurrence rates between patients that did or did not receive adjuvant radiotherapy. Bedell et al. [97] could not confirm that either surgical re-excision or vulvar radiotherapy significantly reduced local recurrence rates.

On the other hand, two studies specifically investigating the impact of adjuvant radiotherapy for close or positive margins have reported similar findings to ours [254,255]. In addition, Viswanathan et al. [255] reported that patients who received a total radiation dose of ≥ 56 Gray (Gy) had a lower risk of recurrence than those who received a dose ≤ 50.4 Gy. In our series, the adjuvant radiation dose ranged from 54 to 60 Gy, with up to 65 Gy given for gross residual disease [267]. The optimal radiation dose required to prevent or reduce the risk of a local recurrence is a question to be addressed in future research.

7.5 | Future Research Possibilities

- To continue to monitor geographic differences and vulvar cancer incidence trends, and to determine the impact of the HPV vaccination in future population-based incidence trend analyses. It is expected that younger cohorts of women receiving HPV vaccination wiil receive some protection against vulvar cancer in the future [11,73]. This impact has not yet been observed in our trends analysis, or in the analyses referenced in my thesis, as the time periods covered were either before or near the time the vaccination program commenced. Future analysis could be achieved by assessing trends in age-specific incidence in an individual country, and international levels in countries where suitable cancer registry data are available by utilising the analysis method recently undertaken by Kang et al. [11,271] and Bray et al.[73].
- To determine why the mortality in older women is decreasing in Australia but increasing in countries such as the United States and Canada. Is it related to access to adequate health care, or to attitudes to care of the elderly, either on the part of the patient herself, or on the part of the medical or nursing professionals? This could be achieved by utilising a collaborative multi-institutional retrospective research approach to examine treatment patterns of vulvar cancer in older women from single-institutions in countries where mortality has increased and comparing

these data with Australian data where mortality rates have decreased. The analysis would aim to determine co-morbidities, disease and treatment characteristics that are predictive of vulvar cancer mortality.

- To determine if there is equity in care for all women diagnosed with vulvar cancer in Australia, by investigating if there are disparities in vulvar cancer outcome for Australian women related to their geographical location, between indigenous and non-idigenous women, or in women from culturally and linguistically diverse backgrounds? As there is currently a paucity of data on stage and treatment of vulvar cancer in national Australian population-based datasets [285], future analysis could be achieved by undertaking a vulvar cancer patterns of care study utilising national population data sets, combined with hospital registry-data from across the states and territories. Over time, the potential to conduct a nation-wide pattterns of care study will be greatly enhanced by the National Gynae-Oncology Registry (NGOR) [286]. The NGOR is a clinical quality registry developed to systematically monitor patterns of care for Australian women diagnosed with gynaecological cancer (http://ngor.org.au). The NGOR obtains data from clinical databases currently held by clinicians and/or maintained by hospital gynaecological cancer units. In the future, as data on vulvar cancer are included in the NGOR, and as more sites across Australia are included, the potential to identify variations in vulvar cancer care [287], and to make comparisons between indigenous/non-indigenous, and other ethnic groups will be greatly enhanced

- To determine the true panorama of genetic and epigenetic changes in vulvar squamous cell carcinoma by using whole genome or next generation sequencing [288]. The less studied molecular markers may be important in understanding disease biology and determining future treatment possibilities [289]. In the future, acquiring further biomarker information may permit more personalised cancer treatments and surveillance plans [290].

- To determine in a large scale, prospective study if p16INK4a expression could be used as a predictive marker for radiotherapy sensitivity in patients with squamous cell vulvar cancer. To achieve this, the tumours of patients with vulvar cancer treated with radiotherapy would need to be analysed for immunohistochemical expression of p16. This could apply both to patients undergoing primary radiotherapy, and those undergoing adjuvant radiation. Survival comparisons between patients with p16 positive and p16 negative tumours would be undertaken to determine the predictive role of p16 expression in radiation response. If p16 status was found to be predictive of radiosensitivity, optimal radiation doses could be determined for patients with p16 positive and p16 negative tumours.

- To determine precise therapeutic options that selectively target TP53 mutations in patients with advanced or recurrent vulvar cancer, by developing opportunities for inclusion of vulvar cancer patients in clinical trials testing targeted therapies [290,291].
- To further examine the role of groin suction drains following groin lymph node dissection. This would require a multi-centre collaborative study with patients prospectively randomised to 'drain' or 'no drain' groups. A standardised drain management protocol, strict definition of the type of groin dissection performed, consistent definition of lymphocyst and other short-term post-operative complications, and uniform LLL measurement criterion would be necessary. This would provide a higher level of evidence regarding the value or otherwise of groin drains.
- To include health-related quality of life (QOL) and patient-reported outcomes in future vulvar cancer treatment research. This could be achieved by conducting a multi-institutional prospective, longitudinal mixed methods study, utilising a standardised quality of life tool specifically adjusted for cancer, such as the 30 item European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) [292], or the Functional Assessment of Cancer Therapy General (FACT-G) [293] questionnaire. Ideally, QOL would be measured pre-operatively (baseline), post-operatively (within 30 days), and at 3-, 6-, 9-, and 12-months post-treatment. In-depth interviews of a smaller number of women would be undertaken to understand the participants own interpretation of their experiences [294].
- To determine the effectiveness of serial ultrasonic examination of the groins to detect subclinical nodal metastases in patients who have a negative sentinel node biopsy or who have refused an inguino-femoral lymphadenectomy. The hypothesis would be that detection of subclinical metastases would allow early treatment and a better likelihood of cure, because once positive untreated groin nodes become palpable, they have a mortality of about 90%. This could be achieved by prospectively scanning these patients during two-monthly follow-up visits for 12 months after treatment, along with physical examination of the groin(s) and vulva. This approach has recently been reported from the Netherlands for patients following a negative sentinel node biopsy [229]. In my own institution, a cohort of patients who refused inguino-femoral lymphadenectomy, or who had very early stage IB vulvar cancers, has been followed in this way under a research protocol since 2016, with encouraging results (yet to be published).
- To determine the genetic profile of apparently normal skin between the vulvar

cancer and the surrounding surgical margin in patients with a pathological margin less than 8mm. If this skin was genetically normal, such patients could be prospectively observed on a research protocol, without re-excision or radiotherapy, to determine whether or not primary site recurrences occurred, and whether or not a histological margin closer than 8mm could be accepted as being safe.

- To determine the threshold dose of radiation required to reduce the risk of local recurrence in patients with vulvar cancer and close or positive pathological margins. This could be achieved by undertaking a multi-centre, longitudinal cohort study to determine the relationship between minimum and maximum radiotherapy dose and its effect on primary site vulvar recurrence and morbidity. Radiation Oncology Departments could use their own treatment protocols, with total doses and fraction sizes prospectively recorded.

7.6 | Conclusion and Implications for Practice

This body of work examined a broad range of controversial issues relating to vulvar cancer across four research projects. In my first study, I examined temporal trends in incidence and mortality of vulvar cancer in Australian women since the early 1980's. This was to my knowledge, the first Australian population-based study of both incidence and mortality trends, in addition to being the first to provide a detailed analysis of Australian vulvar cancer trends defined by age groups. I demonstrated that there was a significantly increasing incidence of the disease in women under the age of 60 years, and a significantly decreasing mortality in women over the age of 60 years.

It has been predicted that more women will be diagnosed with vulvar cancer in the future due to population growth, and an ageing population [73]. The predicted high coverage of the HPV vaccination program should eventually offset this increase [11,73,295], although this effect should be seen predominately in women under 60 years of age, where HPV associated vulvar carcinomas are more common [11,73].

In my second study, immunohistochemistry was used to identify patients with squamous cell carcinomas which were p16 positive, using this marker as a surrogate for HPV positivity. HPV status was also determined using PCR, but p16 status was demonstrated to be more reliable. I also determined the p53 status of the cancers in the cohort, and showed that patients with p16-positive cancers had a significantly better prognosis than those with p53-positive cancers. I also identified a smaller sub group of patients who were both p16 and p53 negative and had an intermediate prognosis between the two. In multivariable analysis, I demonstrated that neither p16 nor p53 were independent prognostic markers.

Some have suggested that clinical management should be based on p16 or p53 status [26,134,152] but I have recommended that management of squamous cancer vulvar cancer should continue to be based on clinical indicators until more information is available [265].

My third study examined the post-operative morbidity associated with groin node dissection and looked at ways of reducing it. I demonstrated that lymphocyst formation was the most common short-term complication and lower limb lymphoedema the major problem long-term. The incidence of all complications increased as the number of nodes removed increased. Sentinel node biopsy in selected patients is currently the most effective method to reduce the number of lymph nodes removed but carries a small but definite false-negative rate. If follow-up of patients with negative nodes is based on groin palpation, there is a high mortality in patients with a false-negative result. Most patients are not willing to accept this risk if properly informed. More recently, near-infrared (NIR) fluorescence imaging using indocyanine green (ICG)-99mTc-nanocolloid has been introduced to improve the intraoperative visual identification of sentinel nodes in squamous vulvar cancer. Comparative studies have shown it to be superior to the previous standard 99mTc-nanocolloid and blue dye SLN detection method [296,297]. There is also preliminary evidence that ultrasonic surveillance of the groin after a negative sentinel node biopsy may allow detection of false-negative nodes when they are still not palpable but may still be curable, and this should be a focus of future research.

Nodal debulking also reduces the number of lymph nodes removed. I confirmed that nodal debulking, followed by groin and pelvic radiation for patients with bulky positive nodes, is beneficial in reducing the incidence of both short-and long-term morbidity, and it carries a good prognosis. This should become the treatment of choice for this group of patients. I also demonstrated that the universal use of groin drains prolonged hospitalisation, without decreasing morbidity. This common practice should be subjected to a randomised, prospective study to provide level 1 evidence for the benefit(s) of the use of post-operative groin drains.

My final study examined the outcome of a large cohort of patients treated conservatively whenever possible i.e., by radical local excision rather than radical vulvectomy, and by unilateral rather than bilateral groin dissection. It also examined the controversial issues of the appropriate width of the surgical margin and the benefit of treating patients with close or positive surgical margins. I demonstrated that a conservative approach was associated with a 5-year disease-specific survival of 86% for the cohort of 345 patients treated with curative intent, and 66% for the 102 patients with positive nodes. I also demonstrated that when local recurrences are divided into those at the primary and those at a remote site, a pathological margin distance of 8mm or less is an important predictor of primary site vulvar recurrence.

Contrary to several recent opinions [235,237,244-247,249,250], I would recommend that vulvar cancer management guidelines continue to endorse a surgical margin of at least 1cm. I also demonstrated that patients with pathological margins less than 8mm benefit from treatment with surgical re-excision, if feasible. For tumours near the clitoris, anus, or distal urethra, or if radiotherapy is required for positive groin lymph nodes, local vulvar radiation is also effective. Patients with pathological margins between 5 and 8mm should be closely monitored if surgical re-excision is not feasible [267].

In the future, molecular assessment of the primary tumour and surrounding skin margins to detect genetic 'field effect' abnormalities may facilitate the identification of lesions that do not require treatment for close margins [258].

The objectives of my thesis have been achieved. The research undertaken has contributed to the body of evidence in the international literature, and has impacted on our own clinical practice. All research projects have generated new professional partnerships which should facilitate ongoing collaborative research. This research should include the projects proposed in this thesis, which would provide further benefits to women with vulvar cancer.

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APPENDIX 1.

LIST OF WORD COMBINATIONS AND SEARCH TERMS USED IN THE LITERATURE REVIEW.

Vulvar cancer

International classification of diseases and vulvar cancer

Squamous vulvar cancer

Vulvar cancer incidence

Vulvar cancer mortality

Australian vulvar cancer incidence

Australian vulvar cancer mortality

Vulvar cancer incidence and younger women

Pathways to squamous vulvar carcinoma

Vulvar cancer and older women

Vulvar cancer incidence

Vulvar cancer mortality

Worldwide incidence trends and vulvar cancer

Vulvar cancer and population-based data

Vulvar carcinoma and survival

Vulvar carcinoma and burden of disease

HPV and squamous vulvar carcinoma

Vulvar neoplasia

Human papilloma virus

Vulvar cancer and high-income countries

Vulvovaginal disease terminology

Epidemiology of vulvar neoplasia

HPV vaccination

HPV vaccination and prevention of vulvar cancer

Differentiated vulvar intraepithelial neoplasia

Usual vulvar intraepithelial neoplasia

High grade squamous intraepithelial neoplasia

Lichen sclerosus and pathogenesis

Lichen sclerosus management

Lichen sclerosus treatment

Lichen sclerosus and vulvar cancer

Lichen sclerosus and topical steroids

Paget's disease of vulva

In-situ vulvar disease

Molecular classification and vulvar squamous precursor lesions

Malignant progression and HSIL

Malignant progression and dVIN

Malignant progression and uVIN

HPV prevalence and squamous vulvar precursor lesions

HPV type distribution and squamous precursor lesions

Squamous hyperplasia

Limitations and population-based data

Cancer registry data

Anatomy of the vulva

Lymphatics of the vulva

Early-stage vulvar cancer

Advanced vulvar cancer

Vulvar squamous cell carcinoma and etiology

Staging of squamous vulvar cancer

Vulvar carcinoma and imaging

Vulvar cancer risk factors

Localisation of vulvar cancer

Inguino-femoral lymphadenectomy and squamous vulvar cancer

Staging for vulvar cancer

Vulvar cancer histology

Management of lymph nodes and vulvar carcinoma

Positive lymph nodes and vulvar carcinoma

Positive pelvic nodes and vulvar carcinoma

Bulky positive nodes and vulvar carcinoma

Local relapse and vulvar carcinoma

FIGO 2009 staging and vulvar cancer

Radiotherapy and vulvar cancer

Adjuvant radiotherapy and local vulvar carcinoma recurrence

Prognostic significance of groin node metastases and vulvar carcinoma

Pathological prognostic factors and vulvar carcinoma

Prognostic indicators and vulvar carcinoma

Perineural invasion and vulvar carcinoma

Exenteration and vulvar carcinoma

Cell cycle regulation and viral infection

The cell cycle

p16INK4A expression and HPV

p16 protein and HPV related neoplasia's

p16 and vulvar carcinoma

p16 expression and female genital tract malignancies

p16 immunostaining and vulvar squamous cell carcinoma

The p53 pathway and cancer

Cellular senescence and tumour suppressor genes

p53 expression and vulvar carcinoma

Carcinoma of the vulva and p53 mutations

Prognostic value of p53 and vulvar carcinoma

HPV detection and in-situ hybridisation

Prognostic significance of HPV and vulvar carcinoma

HPV and head and neck cancers

HPV and head and neck cancer survival

HPV and anal cancer

HPV and anal cancer survival

HPV positivity and radiotherapy and vulvar cancer

Radiotherapy response and vulvar carcinoma

Radical vulvectomy

Modified radical vulvectomy

Radical local excision

Vulvar carcinoma treatment and morbidity

Vulvar carcinoma and post-operative complications

Vulvar carcinoma and post-operative morbidity

Vulvar carcinoma and treatment related side effects

Vulvar carcinoma and surgical outcomes

Psychosexual outcomes and vulvar excision

Psychosexual functioning and vulvar carcinoma

Sexuality and body image and vulvar cancer surgery

Quality of life and vulvar carcinoma treatment

Wound complications and vulvar cancer surgery

Urinary incontinence and radical vulvar excision

Short-term wound complications and vulvar cancer treatment

Groin node dissection and short-term wound complications

Groin node dissection and long-term complications

Pathophysiology of lymphoedema

Lymphoedema and gynaecological cancer treatment

Chronic lymphoedema

Lower limb lymphoedema

Lymphoedema and groin node dissection

Groin drains and post-operative morbidity

Inguino-femoral lymphadenectomy and lymphatic drainage

Lymphatic drainage

Auxillary lymphadenectomy and auxillary drainage

Auxillary drains and breast cancer surgery

Sartorius transposition and vulvar carcinoma surgery

Saphenous vein preservation and inguino-femoral lymphadenectomy

Post-operative wound dehiscence

Lymphadenectomy and post-operative lymphoceles

Management of post-operative lymphoceles

Seroma formation and lymphadenectomy

Incidence of lower limb lymphoedema and vulvar carcinoma

Sentinel node biopsy

Sentinel node biopsy and vulvar carcinoma

Sentinel lymph node mapping and vulvar carcinoma

False-negative sentinel nodes and vulvar carcinoma

Ultrasound follow-up and negative sentinel nodes

Ultrasound and sentinel node detection

Sentinel lymph node detection and ICG and vulvar cancer

Pathological margin distance and vulvar carcinoma

Surgical margins and vulvar carcinoma

Histopathological measurement of surgical margins

Patterns of recurrence and vulvar carcinoma

Tumour-free margin distance and vulvar squamous cell carcinoma

Vulvar pre-invasive disease and local vulvar cancer recurrence

Adjuvant radiation and vulvar carcinoma

Margin status and radiotherapy and vulvar cancer recurrence

Remote site recurrence and vulvar carcinoma

Primary site recurrence and vulvar carcinoma

Field cancerization

Field cancerization and vulvar carcinoma

Second field tumours and vulvar carcinoma

Field cancerization and clinical implications

Cancer treatment and older female patients

Smoking and wound healing

Cigarette smoking and tissue oxygenation

Surgical complications and smoking

Genetic/epigenetic changes and vulvar carcinoma

Vulvar cancer and patterns of care

National Gynae-Oncology Registry

Quality of life evaluation and oncology

Patient perspectives and gynaecological cancer treatment

APPENDIX 2.

FIGURES 1 AND 2, AS SHOWN IN THE ARTICLE, 'CHANGING TRENDS IN VULVAR CANCER INCIDENCE AND MORTALITY RATES IN AUSTRALIA SINCE 1982' – CHAPTER 3.

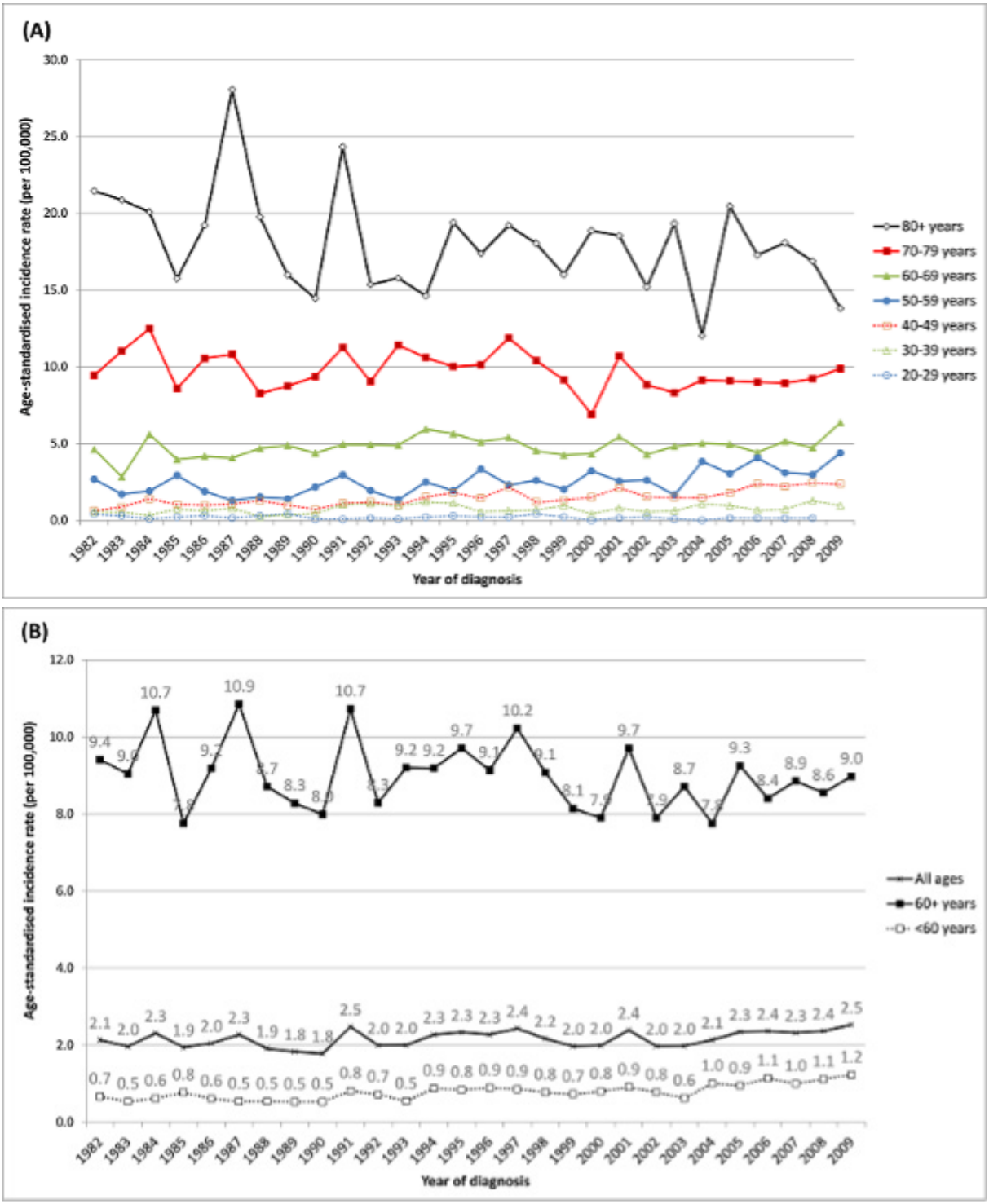

FIGURE 1. Age-standardised incidence of vulvar cancer in Australia, 1982 to 2009*. A, For each decile age group. B, Age-standardised rates for all ages, and stratified by women <60 years, 60+ years.

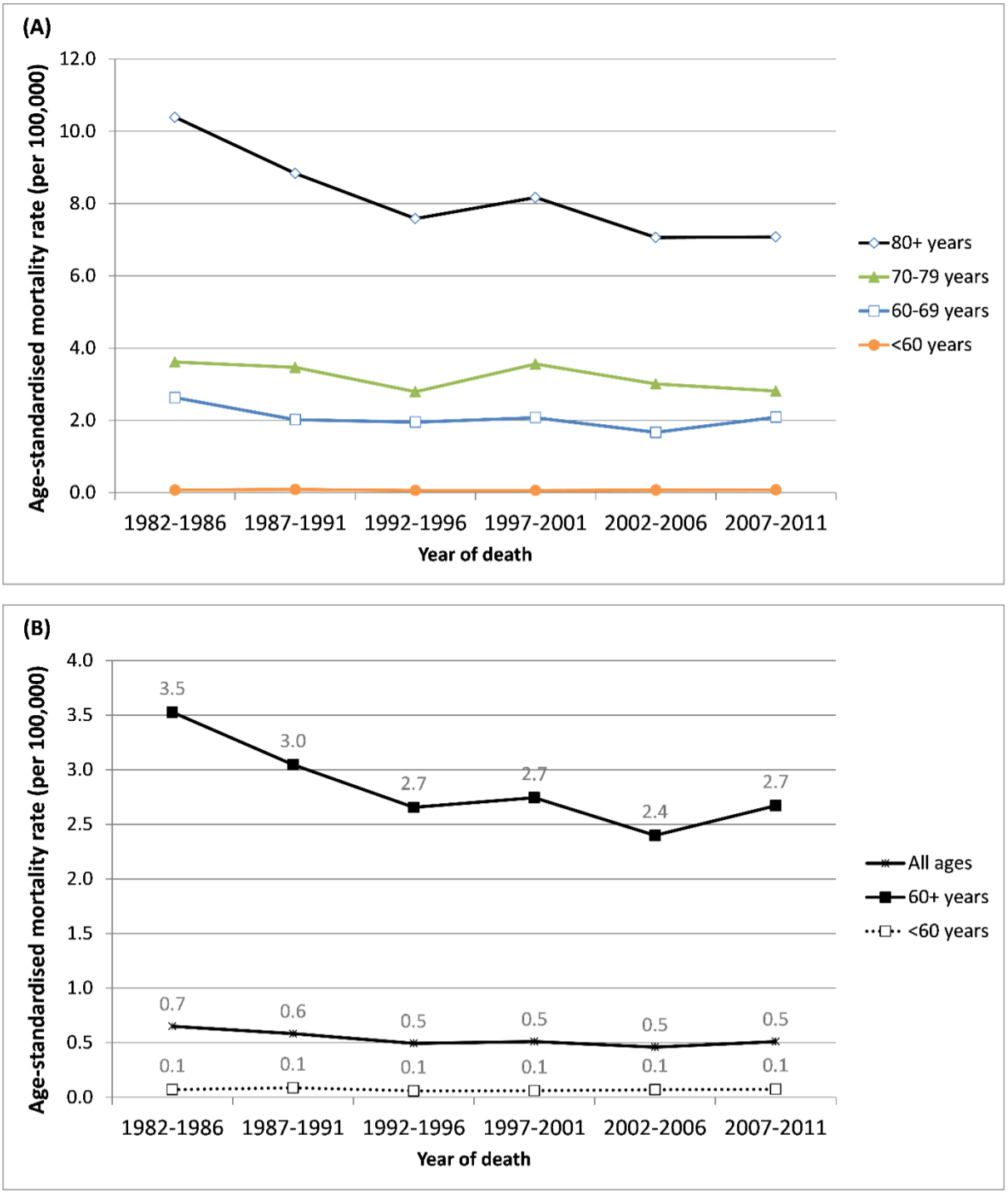

FIGURE 2. Age-standardised mortality of vulvar cancer in Australia, 1982-1986 to 2007-2011*†. A, For women <60 years and for each decile age group in older women. B, Age-standardised rates for all ages, and stratified by women <60 years, 60+ years