Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

Vaccination as a means of Disease Prevention in HIV-infected Individuals; Successes, Challenges and Opportunities

By

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A thesis submitted to the University of Dublin in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

September 2016

Declaration

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Summary

Vaccines are one of the greatest advancements of modern medicine having achieved unprecedented success in preventing infectious diseases and preserving health at both individual and population level. Vaccine programmes have eliminated infectious diseases including smallpox and have significantly decreased the incidence of others such as polio, diphtheria and measles.

The success of immunisation programmes depend on a number of inter-related factors including baseline incidence and susceptibility of a population to vaccine-preventable infections, widespread availability of safe and effective vaccines, development of robust systems and infrastructures to deliver vaccine as well factors that may influence vaccination uptake at both individual and population level. Success is required at each of these levels to achieve the threshold coverage required for disease eradication or to provide protection at a population level.

Vaccines function by stimulating a protective immune response which can prevent development of acute infections caused by viruses and bacteria, for example influenza virus or Streptococcus pneumoniae as well as chronic infections such as human papillomavirus (HPV) and hepatitis B virus (HBV) which are causally associated with cancer.

Immunisation recommendations, on which international vaccine programmes are based, are determined by weighing the benefits of vaccination against the risks and costs. Due to the high burden of vaccine preventable infections observed in immunocompromised populations such as HIV-infected individuals, consensus immunisation guidelines frequently have a lower threshold for recommending certain vaccines in these at-risk groups relative to the general population.

HIV infection causes defects in T cell-mediated immunity, B cell dysfunction as well as sub-optimal humoral immune response. Thus, HIV-infected individuals are more susceptible to a variety of vaccine preventable infections and are at increased risk of morbidity and mortality associated with these infections. Immune response to vaccination in HIV-infected individuals is frequently impaired. Research, including historical work undertaken in the Department of Genito Urinary medicine and Infectious Diseases (GUIDE), St James's Hospital, Dublin has demonstrated that antibody response to the polysaccharide pneumococcal vaccine is suboptimal and decreases more rapidly in HIV-infected individuals.

Non-replicating vaccines (e.g. whole inactivated, polysaccharide, conjugated, and subunit vaccines, or virus-like particles) can be used safely in HIV-infected individuals. Replicating (live) vaccines such as measles, mumps and rubella (MMR) vaccine, varicella (VZV) vaccine and yellow fever vaccine had previously been contraindicated for use in HIV-infected individuals, however immune restoration with highly active antiretroviral therapy (HAART) reduces the risk of adverse events relating to live vaccines shifting the risk-benefit ratio in favour of vaccination.

Despite a significant body of evidence supporting the protective benefits of vaccination in HIV-infected individuals, reported vaccine uptake remains poor. Additionally, the utility of vaccination as a means of disease prevention in HIV-infected individuals, a subgroup at high-risk of infection and infection related complications, is poorly researched.

The overarching aim of this thesis is to examine vaccination as a means of disease prevention in HIV-infected individuals. To address this aim, I will investigate baseline susceptibility to vaccine preventable infections in HIV-infected adults, a novel model of care for delivery of vaccines to HIV-infected individuals, the burden of vaccine preventable infections in HIV-infected adults and immunologic response to the conjugate pneumococcal vaccine (PCV13) and the human papillomavirus (HPV) vaccine.

The structure of this thesis is such that retrospective seroepidemiological data describing susceptibility to vaccine preventable infections and vaccine uptake in HIV-infected individuals attending a single tertiary referral HIV centre are reported in earlier Chapters (Chapters 2 and 3). Results from this work highlight the importance and feasibility of vaccination as a means of disease prevention in this patient group and set the context for the main body of research undertaken as part of this thesis. Prospective experimental research presented in Chapters 4 and 5 represents the main body of work of this thesis and investigates immunological responses to the pneumococcal vaccine and to the HPV vaccine in HIV-infected individuals.

Chapter 2 of this thesis provides the first Irish seroepidemiological data regarding susceptibility to common vaccine preventable infections including hepatitis A virus (HAV), hepatitis B virus (HBV), varicella virus (VZV) and measles, mumps and rubella (MMR) in a cohort of HIV-infected individuals. Data regarding susceptibility to such infections is sparse in the international literature with geographical variations reported, highlighting the importance of generating local data to inform national immunisation policy for HIV-infected individuals.

I have identified high levels of susceptibility to vaccine preventable infections (6% susceptible to VZV, 68% susceptible to HBV, 33% susceptible to any component of the MMR vaccine) and thus an opportunity to prevent infection though vaccination.

HIV-infected individuals are at increased risk of morbidity and mortality relating to vaccine preventable infections. In Chapters 4 and 5, I have demonstrated the high burden of invasive pneumococcal disease (IPD) and anal cancer (potentially vaccine preventable infections) currently observed in HIV-infected individuals. Thus, Chapter 2 underscores the need for pro-active screening and delivery of appropriate vaccination to reduce the burden of preventable infectious diseases in this at-risk patient group.

In Chapter 3, I examine the effectiveness of an integrated vaccine programme, a novel model of care for delivery of vaccination to HIV-infected individuals. Despite a significant body of evidence that vaccination is safe and effective in HIV-infected individuals, the burden of vaccine preventable infections remains high and documented vaccination coverage in HIV-infected populations remains poor.

An integrated vaccine programme was established in GUIDE, St James's Hospital, Dublin, the largest ambulatory specialist HIV clinic in Ireland in 2003. This programme sought to improve vaccine coverage and impact the burden of vaccine preventable infection observed in the attending HIV-infected cohort. This is the first study to investigate the effectiveness of an integrated vaccine programme as a model of care for delivery of recommended vaccines to HIV-infected individuals.

I report a significant and sustainable increase in documented seasonal influenza vaccination coverage in the active attending HIV-infected cohort over a 10-year period (53% in 2003 versus 75% in 2015, $p<0.001$). The seasonal influenza vaccine uptake reported in 2014/2015 (76%) is significantly greater than that reported elsewhere in the literature in HIV-infected cohorts.

Additionally, I report high rates of HBV vaccine series completion and response in HIV-infected individuals in the setting of an integrated vaccine programme strengthening the hypothesis that the integrated vaccine programme can improve vaccination coverage and outcomes in at-risk patient groups.

In Chapter 4, I report on the burden of IPD in HIV-infected individuals over a 10-year period. While the incidence of IPD has decreased significantly in recent years, incidence (283 cases/100,000) remains higher than that reported in the general population.

Efficacy of pneumococcal vaccine in HIV-infected individuals remains debated in the medical literature. Failures of the 23-valent polysaccharide pneumococcal vaccine (PPV23) vaccine are well documented and indeed I have reported failures of PPV23 in the IPD incidence study undertaken as part of this thesis. We hypothesised that combining the 13-valent conjugate pneumococcal vaccine (PCV13) with the PPV23 would improve immunogenicity and thus protection against pneumococcal infection in HIV-infected adults.

To interrogate this hypothesis, I led a randomised controlled trial in which I examined a prime-boost pneumococcal immunisation strategy combining PCV13 with PPV23 versus PPV23 alone in HIV-infected adults. This work pre-dated changes to pneumococcal immunisation guidelines which now recommend the prime-boost immunisation strategy combining PCV13 and PPV23 as standard of care for HIV-infected individuals.

I report a trend towards a greater magnitude and duration of serotype specific IgG and killing opsonophagocytic assay (OPA) response to the prime-boost immunisation strategy. Additional factors which I examined as markers of immunological response to pneumococcal vaccine included IgG2 response and uptake OPA assay response. I did not observe significant differences in IgG2 and in-house phagocytic OPA response between groups.

In Chapter 5, I report a high burden of anal cancer in HIV-infected individuals in Ireland (44 cases/100,000). I have generated the first data relating to prevalence of HPV infection and high-risk (hr) or oncogenic HPV types 16 and 18 infection in HIV-infected and HIV negative men who have sex with men (MSM) in Ireland. I report high levels of persistence of anal HPV infection and hr anal HPV infection in a subgroup of HIV-infected MSM and high rates of HPV infection at multiple sites (oral and genital).

HPV infection and associated disease is vaccine preventable. Success of HPV immunisation programmes will depend on high levels of vaccine uptake. I report high levels of vaccine acceptability in MSM and HIV-infected MSM in Ireland.

To further interrogate the potential benefits of HPV vaccine in HIV-infected MSM <26 years of age in whom the HPV vaccine is indicated. We investigated baseline anti-HPV 16 and 18 antibody levels in serum, and using next generation sequencing (NGS) we investigated baseline prevalence and diversity of HPV infection at multiple site. We then measured immunogenicity of the quadrivalent HPV vaccine (HPV 4v) in a longitudinal study with serum samples collected up to 52 weeks post first HPV vaccine dose.

We observed high levels of HPV 16 and 18 antibodies at baseline (44% and 26% respectively) We observed a high prevalence (68%) and diversity (52 HPV genotypes identified on next generation sequencing (NGS)) of HPV infection at multiple sites on swabs including hr HPV types. Additionally, we observed high levels of seroconversion for hr HPV 16 (100%) and HPV 18 (86%) in those who were seronegative at baseline. Significant increases in HPV antibody titres were observed in those who were seropositive for HPV 16 and 18 at baseline.

Considering findings from this thesis, it is evident that vaccine preventable infections continue to impact the health of HIV-infected individuals and that vaccination has potential to further reduce morbidity and mortality in HIV-infected individuals. Vaccination has yet to achieve its potential in HIV-infected individuals primarily due to underutilisation of vaccination.

While resourcing and investment should continue to focus on developing newer and more effective vaccines, particularly in high-risk groups such as HIV-infected individuals, similar focus and investment should be directed at further developing infrastructures and supports (such as integrated vaccine units) to optimise delivery and fully realise protective benefits from available vaccines.

Funding to undertake this research was provided from the research fund in the Department of Genito Urinary Medicine and Infectious Diseases (GUIDE), St James's Hospital, Dublin, Ireland.

Acknowledgements

Work for this thesis was undertaken in the Department of Genito Urinary Medicine and Infectious Diseases (GUIDE), St James's Hospital, Dublin. There are several people to whom I owe a great deal of gratitude.

First and foremost, I must thank Professor Colm Bergin, my primary PhD supervisor. His outstanding mentorship, supervision and encouragement has made the experience productive and stimulating. I greatly admire his leadership, work ethic, enthusiasm and dedication as a mentor. I cannot thank him enough for the opportunities he has given me.

I am immensely grateful also to co-supervisors of this work, Professor Orla Sheils and Dr Niall Conlon. I thank them both for their generous advice and collaboration. I must also give a special mention to Dr Jean Dunne and Dr Paul Smyth in the Departments of Immunology and Histopathology respectively. Both contributed enormously to the laboratory aspects of this thesis, showing great patience in their supervision.

I was supported in statistical/analysis aspects of this research by Dr Kathleen Bennett (pneumococcal vaccine work) and Dr Gordon Blacksheilds (bio-informatics, HPV work). I am very grateful for their expertise and assistance.

I thank all my colleagues in GUIDE for supporting this work and for contributing to what is a wonderful work environment. I must particularly thank Ms Siobhan O'Dea, research nurse in GUIDE during the time this work was undertaken for her assistance with regulatory issues and recruitment and for her general input to the work. I thank Ms Ann Keegan and Ms June Craig, data managers of the vaccine unit and GUIDE cohort respectively for contribution of data

I must mention the patients who enthusiastically and generously gave their time to participate in this research. Without them this thesis would not have been possible and I am very grateful.

Finally, I would like to acknowledge my family and friends. To my Mum and Dad who have always shown great generosity and interest. To my girls, Laura, Kate and Mary, a source of great inspiration and motivation, but most of all providers of great amusement and happiness. To Eric, for his encouragement and support: I am very grateful, far beyond the limits of this page to specify.

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List of Abbreviations

µg/ml	micrograms per millilitre
ACIP	Advisory Committee on Immunisation Practices
AIDS	Acquired Immunodeficiency Syndrome
AIN	anal intraepithelial neoplasia
AMR	Antimicrobial resistant
Anti-HB cAb	Anti Hepatitis B core antibody
APR	Abdomino-peritoneal resection
ASCC	Anal squamous cell carcionoma
BHIVA	The British HIV Association
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CRT	Chemo-radiotherapy
CSF	Cerebro-spinal fluid
DAART	Dual acting anti-retroviral therapy
DD	Double dose
DNA	Deoxy ribose nucleic acid
ELISA	Enzyme linked immunosorbent assay
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUDRA CT	European Clinical Trials Database
FDA	Food and Drug Administration
GMC	Geometric Mean Concentration
GMCi	Geometric mean concentration increase
GMHS	Gay Men's Health service
GMT	Geometric Mean Titre
GMTi	Geometric Mean Titre increase
GP	General Practitioner
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
GUIDE	Department of Genito Urinary Medicine and Infectious Diseases
GU	Genito Urinary
HAART	Highly active antiretroviral therapy
HAV	Hepatitis A virus
HB sAb	Hepatitis B surface antibody

HB sAg	Hepatitis B virus surface antigen
HBSS	Hanks balances salt solution
HBV	Hepatitis B virus
Hetero	Heterosexual
Hib	*Haemophilus Influenza B*
HIPE	Hospital inpatient enquiry system
HIV	Human Immunodeficiency Virus
HPRA	The Health Products Regulatory Authority
HPSC	Health Protection Surveillance Centre
HPV 2v	Bivalent HPV vaccine
HPV 4v	Quadrivalent HPV vaccine
HPV 9v	Nonavalent HPV vaccine
HPV	Human Papillomavirus
HR	Hazard ratio
Hr	High-risk
HRA	High resolution anoscopy
IAI	Insertive anal intercourse
IDSA	Infectious Diseases Society of America
IDU	Injecting drug user
IgG	Immunoglobulin
IPD	Invasive pneumococcal disease
IQR	Interquartile range
IU	International units
JCVI	Joint committee on vaccination and immunisation
L	Litre
Liu	Luminex units
LR	Low risk
Ma	Multivariate analysis
MDR	Multi-drug resistant
MIC	Minimum inhibiting concentration
Ml	Millilitres
MMR	Measles, mumps, rubella
MS	Microsoft
MSM	Men who have sex with men
N	Number
ND	Not detected
NGS	Next Generation Sequencing

NIAC	National Immunisation Advisory Committee
NIH	National Institute of Health
NR	Non-responder
OPA	Opsonophagocytic
OR	Odds ratio
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV13	13-valent conjugate pneumococcal vaccine
PLWH	People living with HIV
PNSP	Penicillin non-susceptible streptococcus pneumoniae
PPS	Pneumococcal polysaccharide serotype
PPV23	23-valent polysaccharide pneumococcal vaccine
RAI	Receptive anal intercourse
RCF	Relative centrifugal force
RNA	Ribose nucleic acid
S. Pneumoniae	*Streptococcus Pneumoniae*
SD	Standard deviation
SMS	Short message service
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan Africa
SSSTDI	Society for the Study of Sexually Transmitted Diseases in Ireland
STI	Sexually transmitted infection
TMB	Tetramethylbenzidine
Ua	Univariate analysis
UAI	Unprotected anal intercourse
UK	United Kingdom
US	United States
VL	Viral Load
VLP	Virus like particle
VZV	Varicella zoster virus
WHO	World Health Organisation

Research outputs to date

Peer Reviewed Publications

Influenza vaccine coverage and factors associated with vaccine coverage in patients living with HIV (PLWH) over a decade in the setting of an integrated vaccine unit; a retrospective cohort study. (Accepted). **C Sadlier,** A O'Rourke, A Carr, C Bergin. **International Journal of STD and AIDs**. May 2017.

Seroepidemiology of hepatitis A, hepatitis B and varicella virus in people living with HIV in Ireland. **C Sadlier**, A O'Rourke, A Carr. **J Infect Public Health.** 2017 Mar 13. pii: S1876-0341(17)30054-0. doi: 10.1016/j.jiph.2017.01.019.

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Impact of a new vaccine clinic on hepatitis B vaccine completion and immunological response rates in an HIV-infected cohort. Rock C, de Barra E, **Sadlier C**, Kelly S, Dowling C, McNally C, Bergin C. **J Infect Public Health.** 2013 Jun;6(3):173-8. doi: 10.1016/j.jiph.2012.11.001.

In Preparation

Immunogenicity of HPV vaccine and natural history of HPV infection in young HIV positive MSM.
C Sadlier, S O'Dea, S Delamere, J Dunne, O Sheils, Bergin C.

Invasive pneumococcal disease (IPD) incidence and risk factors in HIV-infected individuals.
C Sadlier, C Rock, S O'Connell, M Kelleher, C Bergin

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Oral Presentations

Prevalence and genotype of HPV infection at multiple sites in young HIV-infected MSM.
Oral presentation.
British HIV association (BHIVA), Spring meeting, Manchester, April 2016.

Immunological efficacy of a prime-boost immunisations strategy combining PCV13 and PPV23 versus PPV23 alone in HIV-infected adults.
ID week, San Francisco, 2013.
British HIV association (BHIVA), Autumn meeting, Liverpool, United Kingdom, 2014.
Best poster presentation, Post graduate medical research meeting, Trinity College Dublin, 2014.

Invasive pneumococcal disease (IPD) incidence and risk factors in HIV-infected individuals. Oral presentation.
Infectious Diseases Society of Ireland (IDSI) annual meeting. Croke Park, Dublin, 2015.
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1. Introduction

1.1 Background and Rationale

HIV infection causes defects in T cell-mediated immunity, B cell dysfunction as well as sub-optimal humoral immune response (Moir and Fauci, 2009). As a result HIV-infected individuals are more susceptible to a variety of infections (many of which are vaccine preventable) and are at increased risk of morbidity and mortality associated with these infections (Sheth et al., 2010).

Vaccine guidelines are determined by weighing the benefits of vaccination against the risks. Due to the high burden of vaccine preventable infection observed in HIV-infected individuals, consensus immunisation guidelines have a lower threshold for recommending certain vaccines in HIV-infected individuals relative to the general population (Rubin et al., 2013, Geretti, 2015).

However, HIV-infected individuals may not elicit sufficient immunologic response to some vaccines due to associated immune dysfunctions and thus vaccines may not confer the same degree of protection gained by immunocompetent persons.

Clinical and immunological efficacy of many vaccines, including the recently licensed conjugated pneumococcal vaccine (PCV13) and the human papillomavirus (HPV) vaccine remain debated in HIV-infected individuals (Pedersen RH, 2011). Further research is warranted in this area to guide vaccine development and inform policy makers so that optimum protection can be afforded to this high-risk patient group. I seek to address many of these issues in this thesis.

1.2 Aims and Objectives

The overarching aim of this thesis is to examine vaccination as a means of disease prevention in HIV-infected individuals.

Specifically, the aims of this research are:

- To investigate the seroepidemiology of vaccine preventable infections in a HIV-infected cohort.

- To examine an integrated vaccine unit as a model of care for delivery of immunisations to HIV-infected individuals.

- To investigate the incidence of invasive pneumococcal disease (IPD) in HIV-infected adults and the immunological response to pneumococcal vaccination strategies in HIV-infected adults.

- To investigate the burden of human papillomavirus (HPV) infection and associated disease in HIV-infected individuals, acceptability of HPV vaccine in MSM and immunological response to HPV vaccine in young HIV-infected men who have sex with men (MSM) in whom the vaccine is indicated.

1.3 Structure of Thesis

This thesis is structured to systemically assess susceptibility to vaccine preventable infections in HIV-infected adults, to investigate effectiveness of an integrated vaccine programme as a model of care for vaccine delivery to a HIV-infected cohort, to investigate the burden of vaccine preventable infections (pneumococcal and HPV infection) in HIV-infected adults and to assess immunognenicity of a prime-boost pneumococcal vaccine strategy and HPV vaccine in HIV-infected adults.

Chapter 2 investigates seroepidemiology of common vaccine preventable infections (hepatitis A virus (HAV), hepatitis B virus (HBV), varicella zoster virus (VZV), measles, mumps and rubella (MMR) in HIV-infected adults attending the department of Genito Urinary Medicine and Infectious Diseases clinic (GUIDE), the largest tertiary referral HIV specialist centre in Ireland.

Chapter 3 investigates the effectiveness of an integrated vaccine unit as a model of care for delivery of routine immunisations including seasonal influenza vaccine, pneumococcal vaccine and HBV vaccine to a cohort of HIV-infected adults.

Chapter 4 examines the burden of invasive pneumococcal disease (IPD) in HIV-infected adults attending a tertiary referral hospital (St James's Hospital, Dublin). We then investigate immunogenicity of pneumococcal vaccine strategies in HIV-infected adults.

Chapter 5 examines the burden of anal cancer in HIV-infected adults attending the GUIDE clinic. Additionally, we investigate prevalence and persistence of HPV infection and infection with high-risk (hr) or oncogenic HPV types in HIV-infected MSM. We examine acceptability of HPV vaccine in this patient group and immunogenicity of the quadrivalent HPV vaccine in young HIV-infected MSM in whom the HPV vaccine is indicated.

Chapter 6 extends the context surrounding the research questions, discusses the main findings of the PhD, draws conclusions and alludes to future directions of research to build on findings of this thesis.

1.4 Role of the Candidate

My PhD advisory panel consists of my primary supervisor, Prof Colm Bergin and secondary supervisors of the pneumococcal vaccine immunogenicity research (Dr. Niall Conlon) and the HPV research (Prof Orla Sheils).

I directed and carried out all work presented here within as outlined below unless otherwise stated. I was involved in the seroepidemiology study (Chapter 2) from its conception and was responsible for study design and development, project management, applications to ethics committee and St James's Hospital institutional review bodies as appropriate, data management and analyses, and drafting manuscripts for publication. Ms. Ann Keegan (Data Manager) recorded vaccination status of patients and provided the preliminary vaccination data-base on which this study was based. I was supported in the study conception, drafting and review by my primary PhD supervisor Professor Colm Bergin.

I was involved in the study assessing the effectiveness of the integrated vaccine unit as model of care for delivery of influenza and Hepatitis B vaccine (Chapter 3) from conception. I was responsible for study design and development, project management, applications to ethics committee and St James's Hospital, Dublin institutional review bodies, data management, cleaning and analyses and drafting manuscripts for publication. Ms. Ann Keegan (Data Manager) recorded vaccination status of patients and provided the preliminary vaccination data on which this study was based. I was supported in the study conception, drafting and review by my primary PhD supervisor Professor Colm Bergin.

This invasive pneumococcal disease study (Chapter 4) built on previous published research which I collaborated on with Dr. Clare Rock examining invasive pneumococcal disease presenting to St James's Hospital (Rock et al., 2013b). I was responsible for study design and development, project management, applications to St James's Hospital institutional review bodies, data management, cleaning and analyses and writing the first drafts of publications. Data relating to pneumococcal disease cases and serotypes were provided by Ms. Mary Kelleher, surveillance scientist, Department of Microbiology, St James's Hospital. I was supported in the study conception, drafting and review by my primary PhD supervisor Professor Colm Bergin. In relation to the Pneumococcal vaccine randomised controlled trial, I was responsible for ethics submissions and approvals, Irish Medicines Board (The Health Products Regulatory Authority, (HPRA)) submissions and approvals, registering of the randomised controlled trial with the European Clinical Trials Database (EudraCT). I developed the study protocol. Prof Colm Bergin was the principal investigator and he met with the study team

regularly to monitor the progress of the study. Ms. Siobhan O'Dea, departmental research nurse and I recruited all participants to the study, spun blood samples before storing them as serum.

I attended the Vaccine Evaluation Laboratory in Manchester, UK to observe the serotype specific IgG ELISA assay being performed to investigate serotype specific IgG response post pneumococcal vaccination. This assay was performed by scientists in the vaccine evaluation unit.

I developed collaborative links with the Pfizer Vaccine Research Laboratory, Pearl River, New York. I arranged for samples to be shipped and the killing OPA assay performed by scientists in the research laboratory. I was responsible for collection, correlation and analysis of all results relating to the pneumococcal vaccine and for drafting of the manuscript.

I carried out the IgG2 assay under the supervision of Dr. Jean Dunne (Chief Medical Scientist) and Dr. Niall Conlon (Consultant Immunologist) in the immunology laboratory in St James's Hospital. I trained in laboratory techniques such as ELISA, neutrophil isolation and flow cytometry. I developed and validated the multiplex OPA assay under the supervision of Dr. Niall Conlon. This work was undertaken between the research immunology laboratory in the Trinity Centre for Health Sciences and the clinical immunology laboratory in the Central Pathology Laboratory in St James's Hospital.

I undertook all the data analyses relating to the pneumococcal immunogenicity study in this thesis with statistical support from Prof Kathleen Bennett (Professor of Biostatics), Trinity College Dublin.

I designed and undertook all data collection relating to incidence of anal cancer in HIV-infected individuals (Chapter 5). I was responsible for all ethics submissions and approvals relating to the HPV work. I developed the study protocol. Prof Colm Bergin was the principal investigator and he met with the study team regularly to monitor the progress of the study. Ms. Siobhan O'Dea (Research nurse) and I recruited all participants to the study from the GUIDE clinic and the Gay Men's Health service (GMHS), Baggot Street, Dublin.

I attended the Department of Histopathology research laboratory, St James's Hospital to observe HPV DNA extraction, PCR and next generation sequencing (NGS) methods. Prof Orla Sheils supervised this work and Dr. Paul Smyth carried out sample analysis with assistance from David Higgins and Nora Myers (MSc students, Department of Histopathology, St James's Hospital, Trinity College Dublin).

Dr. Gordon Blacksheils (Consultant in Bioinformatice), Trinity College Dublin, was responsible for HPV genotyping undertaken on the National Institute of Health (NIH) bioinformatics pipeline (https://pave.niaid.nih.gov/#home). I was responsible for analyzing results and drafting of the manuscript.

In relation to the vaccine acceptability survey, I was responsible for design of the survey and drafting of the manuscript. Statistical support was sought for multivariate regression analysis in this work from C-Star Statistics University College Dublin.

HPV vaccine immunogenicity work was undertaken in collaboration with Dr. Fiona van der Klis, Head of Immunesurveillance, Department of Public Health, Bilthoven, Netherlands. I was responsible for data analysis, interpretation of results and drafting of manuscripts for publication. Manuscripts were reviewed by Prof Colm Bergin and Prof Orla Sheils.

2. Seroepidemiology of common vaccine preventable infections in people living with HIV in Ireland

2.1 Research outputs to date

Peer Reviewed Publications

High levels of susceptibility to measles, mumps and rubella (MMR) in HIV-infected individuals in Ireland. **C Sadlier,** A Carr, C Bergin. **The Journal of infection** (April 2016) DOI: 10.1016/j.jinf.2016.04.013

Seroepidemiology of hepatitis A, hepatitis B and varicella virus in people living with HIV in Ireland. **Sadlier C**, O'Rourke A, Carr A, Bergin C. J Infect Public Health. 2017 Mar 13. pii: S1876-0341(17)30054-0. doi: 10.1016/j.jiph.2017.01.019.

Research Presentations

Seroepidemiology of hepatitis A, hepatitis B and varicella virus in newly-diagnosed HIV-infected adults in Ireland: an opportunity to vaccinate. Poster presentation. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) 2013, Germany, April 2013.

2.2 Introduction

Seroepidemiology is the use of data on the prevalence of bio-markers of infection or vaccination. It is a potentially powerful tool, providing information on the epidemiology of infection before vaccination and allowing monitoring the effectiveness of vaccination programmes (Cutts and Hanson, 2016).

Seroepidemiological studies are useful for determining groups at risk for a specific disease; evaluating transmission mechanisms; and determining population groups that are critical in maintaining the transmission of infectious agents (de Ory Manchon, 2009). Seroepidemiology is important also in planning and examining the impact of vaccine programmes.

HIV-infected individuals are at increased risk of morbidity and mortality relating to a number of vaccine preventable infections. International immunisation guidelines recommend proactive screening and vaccination in this patient group (ACIP, 2013). There is little data, and no Irish data on seroepidemiology of vaccine preventable infections in HIV-infected individuals. Such information is relevant in the planning and implementation of vaccine programmes, particularly at a national level.

Study Hypothesis

We hypothesised that a significant proportion of HIV-infected individuals attending for HIV care in Ireland were susceptible to vaccine preventable infections and thus could derive some protective benefit from vaccination.

Study aim

The aim of this study was to examine the seroepidemiology of common vaccine preventable infections and demographic factors associated with susceptibility to these infections in in a cohort of HIV-infected individuals in Ireland.

2.3 Specific aims of the chapter

The specific aims of this Chapter were:

- To examine seroepidemiology of hepatitis A (HAV), hepatitis B virus (HBV) and varicella zoster virus (VZV) in HIV-infected individuals attending the GUIDE clinic in 2013.

- To examine susceptibility to measles, mumps and rubella, and factors associated with susceptibility to MMR in newly diagnosed HIV-infected individuals attending an ambulatory HIV clinic in Dublin in 2013.

2.4 Seroepidemiology of common vaccine preventable infections in HIV-infected individuals in Ireland

2.4.1 Abstract

Epidemiological data on seroprevalence of vaccine preventable infections at both individual and population level is important in guiding screening and vaccination practices. International guidelines recommend screening for immunity to common vaccine preventable infections including varicella zoster virus (VZV), hepatitis A virus (HAV), and hepatitis B virus (HBV) and vaccination as appropriate in HIV-infected individuals. The aim of this study was to document baseline immunity to VZV, HAV and HBV infections in a HIV-infected cohort.

A retrospective study was undertaken in a cohort of HIV-infected individuals attending the Department of GU Medicine and Infectious Diseases (GUIDE) HIV clinic from January 2013-2014. Basic demographic data including risk of acquisition of HIV and region of origin was recorded. Between-group prevalence was compared using the Chi^2 test and Wilcoxon signed rank test. Univariate variables with $p<0.2$ were included in a multivariate logistic regression model.

Of 2035 patients who attended the GUIDE clinic in 2013—2014, 1287 patients were included in the study generating a 99% confidence interval +/-2.5%. At baseline, 75% were HAV IgG positive, 94% were VZV IgG positive, 3% were HBV surface antigen (sAg) positive and 29% were HBV core antibody (cAb) positive.

This is the first study to report seroprevalence of common vaccine preventable infections in HIV-infected individuals in Ireland. We found that a significant proportion of patients were non-immune to HAV and HBV. This study supports current proactive screening and immunisation recommendations for HIV-infected individuals to optimally protect against vaccine preventable disease.

2.4.2 Background

HIV-infected individuals are at increased risk of infection with, and frequently experience more severe morbidity from vaccine preventable infections. Consensus international guidelines exist for immunisation in HIV-infected individuals and have a lower threshold to recommend vaccines compared to the general population (Geretti, 2015, NIAC, 2015, Rubin et al., 2013). As there is a lack of large randomised controlled trials on vaccination in HIV-infected adults, most of these recommendations are based on expert opinion.

Recent changes to immunisation guidelines for HIV-infected individuals reflect the emergence of new vaccines (including HPV, Herpes zoster vaccine and pneumococcus), emerging data regarding optimal dosing of vaccination and the altered natural history of HIV-infection in the setting of guidelines recommending earlier initiation of highly active antiretroviral therapy (HAART).

The British HIV Association (BHIVA) guidelines and the National Immunisation Advisory Committee (NIAC) in Ireland both recommend a proactive approach to screening and vaccination of susceptible HIV-infected adults. Routinely recommended vaccines include hepatitis B (HBV) vaccination for all susceptible patients, targeted hepatitis A (HAV) vaccination for susceptible individuals from high-risk groups, and the use of the previously contra-indicated live attenuated vaccines including varicella-zoster virus (VZV) and measles, mumps and rubella (MMR) in patients with ART-induced immune restoration (CD4 T cell count >200 cells/mm3).

Data on the proportion of the population that is immune to, or has been infected with a specific virus has many important epidemiologic applications which are important at both local and national level. These include the identification of susceptible groups in a population and the planning and provision of universal or targeted screening and vaccination programmes. The most accurate way to monitor population immunity is through serologic surveillance.

Previous surveillance work in the general Irish adult population estimated the prevalence of past exposure to hepatitis B virus (anti-core antibody, HBV cAb) to be 0.51%. Prevalence of HBV cAb in Irish prisoners was reported at 8.7% with a prevalence in injecting drug-using prisoners of 18.5% (Long et al., 2001). Similar population based prevalence studies reported a population level immunity of 67% to HAV in Ireland (Rajan et al., 1998).

There is no data available from the general adult population in Ireland regarding susceptibility to VZV, however a study in 1996 reported 95.7% of healthcare workers (HCWs) in an Irish hospital had varicella immunity (Gallagher et al., 1996). A further study undertaken in patients attending a dermatology outpatient clinic in an Irish hospital reported seropositivity rate of 98.7% for VZV (Hackett et al., 2011).

Seroprevalence data for HAV, HBV or VZV in HIV-infected adults is lacking and there is no data available regarding HIV-infected patients in Ireland.

Study Aim

The aim of this study was to investigate the proportion of HIV-infected individuals lacking serological evidence of immunity to HAV, HBV and VZV. This information will help to guide pre-vaccination screening policies and help formulate targeted vaccine strategies at a local and national level.

2.4.3 Methods

All patients attending the GUIDE clinic have VZV, HAV and HBV serologies checked on first presentation to the clinic as standard of care. Results are entered into the vaccine unit database for all patients.

A total of 1726 of 2035 (85%) patients in the active 2013/2014 cohort had varicella serology documented in the vaccine unit database. We calculated that 1287 patients would generate a 99% confidence interval +/-2.5%. 1287 sequential patients with documented varicella serology from the 2013-2014 were included in the study.

Demographic data including age, gender, region of origin, risk of acquisition of HIV were obtained from laboratory and patient records.

Serology

Serum samples were screened for the presence of IgG antibodies against VZV, and HAV (measured as total antibody) using the Vidas (bioMérieux, France) analyser. The Vitros EciQ (OrthoClinical, UK) analyser was used for HBV surface antigen (HB sAg), and anti-core antibody total (anti-HB cAb). All assays were performed and interpreted per manufacturers' instructions.

Statistical analysis

The characteristics of patients with a positive serostatus for VZV or HAV were compared to those with negative serostatus. For HBV, the characteristics of those who tested seronegative for all markers were compared to those who tested HBV sAg or anti-HBV cAb positive using Wilcoxon or chi-squared tests. Factors with a p value <0.2 were entered into multivariate regression analysis model.

The study was approved by the St James's Hospital Institutional Review Board.

2.4.4 Results

In 1287 patients included in the study, mean age [SD] was 39 [10] years, 68% male were male, 46% Irish.

In total 75% were HAV IgG positive, 94% were VZV IgG positive, 32% were HBV sAg/anti-HBV cAb positive. Baseline characteristics of the cohort and serostatus are demonstrated in Table 2.4.1.

Table 2.4.1 Baseline characteristics of study participants grouped by serostatus to HAV, HBV and VZV

		VZV (n=1287)		HBV (n=1188)		HAV (n=1107)	
	Total Cohort	IgG negative	IgG positive	cAb/sAg negative	cAb/sAg positive	IgG negative	IgG positive
	n (%)	74 (6)	1213 (94)	804 (68)	384 (32)	273 (25)	834 (75)
Mean age [SD]	39 [10]	37 [9]	40 [10]	42 [9]	38 [10]	36 [10]	40 [9]
Male	877 (68)	42 (57)	835 (69)	524 (65)	258 (67)	54 (20)	219 (26)
Region of origin							
Ireland	594 (46)	27 (36)	567 (47)	383 (48)	131 (34)	146 (53)	358 (43)
SSA	356 (28)	29 (39)	327 (27)	159 (20)	148 (39)	15 (5)	286 (34)
South America	64 (5)	8 (11)	56 (5)	46 (6)	16 (4)	23 (8)	39 (5)
Europe	139 (11)	1 (1)	138 (11)	84 (10)	45 (12)	47 (17)	79 (9)
Other	134 (10)	9 (12)	125 (10)	72 (9)	4 (1)	42 (15)	47 (6)
Acquisition risk for HIV							
Heterosexual	559 (43)	35 (47)	524 (43)	299 (37)	185 (48)	80 (29)	399 (48)
MSM	521 (40)	23 (31)	498 (41)	341 (42)	127 (33)	163 (60)	296 (35)
IDU	181 (14)	13 (18)	168 (14)	84 (10)	68 (18)	27 (10)	121 (15)
Other	26 (2)	3 (4)	23 (2)	20 (2)	4 (1)	3 (1)	18 (2)

Abbreviations: VZV varicella zoster virus, HAV Hepatitis A virus, HBV Hepatitis B virus, SD standard deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug users, cAb core antibody, sAg surface antigen

When we examined factors associated with susceptibility to VZV. We found that age [OR, (95% CI) 0.96 (0.94-0.99), p=0.01], female gender [OR (95% CI), 0.6 (0.37-0.96), p=0.03], Sub Saharan ethnicity [OR (95% CI), 1.75 (1.10 -2.8), p=0.02] or South American ethnicity [OR (95% CI), 2.50 (1.18-5.38), p=0.02] were associated with seronegativity to VZV on univariate analysis.

On multivariate analysis, South American origin was the only factor that remained a significant predictor of seronegativity (Table 2.4.2).

Table 2.4.2 Characteristics associated with sero-negativity to VZV

Study Cohort	VZV negative	VZV positive	Univariate analysis	Multivariate analysis
	N=74	N=1213	OR (95% CI), p value	OR (95% CI), p value
Mean age [SD]	37 [9.3]	40 [9.7]	0.96 (0.94-0.99), 0.01	0.97 (0.94-1.0), 0.09
Male	42 (57)	835 (69)	0.6 (0.37-0.96), 0.03	1.57 (0.75 - 3.26) 0.23
Region of origin n (%)				
Ireland	27 (36)	567 (47)	1.53 (0.95-2.53), 0.09	(referent)
SSA	29 (39)	327 (27)	1.75 (1.10 -2.8), 0.02	1.65 (0.68 - 4.07), 0.27
South America	8 (11)	56 (5)	2.50 (1.18-5.38), 0.02	2.59 (1.00 - 6.71), 0.05
Europe	1 (1)	138 (11)	1.50 (0.14-8.51), 0.70	0 (0 - 1), 0.99
Acquisition risk of HIV				
MSM	23 (31)	498 (41)	1.54 (0.94-2.61), 0.09	(referent)
Heterosexual	35 (47)	524 (43)	1.18 (0.73-1.88), 0.49	0.96 (0.37-2.47), 0.92
IDU	13 (18)	168 (14)	1.33 (0.72-2.41), 0.37	1.64 (0.65-4.18) 0.3
Positive HAV IgG	44 (59)	790 (65)	1.23 (0.72-2.23), 0.41	1.52 (0.80-2.89), 0.2
Positive HBV cAb / sAg	24 (32)	360 (30)	0.88 (0.52-1.47), 0.61	0.90 (0.49-1.56), 0.64

Abbreviations: VZV varicella zoster virus, HAV Hepatitis A virus, HBV Hepatitis B virus, SD standard deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug user, c Ab core antibody, sAg surface antigen

We examined factors associated with susceptibility to HBV (HBV sAg negative and cAb negative). Age [OR (95% CI)], 3.47 (2.29-4.65), p<0.01], Irish origin [OR (95% CI), 1.75, (1.36-2.26), p<0.01], MSM as risk of acquisition of HIV [OR (95% CI), 0.49 (1.15 - 1.93), <0.01] were significantly associated with sero-negativity to HBV on univariate analysis.

SSA origin [OR (95% CI), 0.39 (0.30 - 0.52), p<0.01], positive VZV IgG [OR (95% CI), 0.46 (0.29-0.73), p<0.01] and positive HAV IgG [OR (95% CI), 0.54 (0.2 - 0.7), p<0.01] were more likely to have a marker of HBV exposure.

On multivariate analysis age, ethnicity and IDU remained significant predictors of susceptibility to HBV (Table 2.4.3).

Table 2.4.3 Characteristics associated with sero-negativity to HBV

Study Cohort	HBV cAb/sAg negative	HBV cAb/sAg positive	Univariate analysis	Multivariate analysis
	804 (68)	384 (32)	OR (95% CI), p value	OR (95% CI), p value
Mean age [SD]	42 [8.8]	38[9.9]	3.47 (2.29-4.65), <0.01	0.96 (0.94-0.97), <0.01
Male	524 (65)	258 (67)	0.91 (0.71 - 1.18), 0.51	1.41 (0.98 - 2.04),0.07
Region of origin n (%)				
Ireland	383 (48)	131 (34)	1.75 (1.36 - 2.26), <0.01	(referent)
SSA	159 (20)	148 (39)	0.39 (0.30 - 0.52), <0.01	0.16 (0.10 - 0.26), <0.01
South America	46 (6)	16 (4)	1.40 (0.77 - 2.45), 0.33	0.46 (0.24 - 0.89), 0.02
Europe	84 (10)	45 (12)	0.88 (0.60 - 1.30), 0.55	0.44 (0.28-0.69), <0.01
Acquisition risk of HIV				
MSM	341 (42)	127 (33)	1.49 (1.15 - 1.93), <0.01	(referent)
Heterosexual	299 (37)	185 (48)	0.64 (0.50 - 0.81), <0.01	1.24 (0.80 - 1.93), 0.34
IDU	84 (10)	68 (18)	1.84 (0.39 - 0.77), <0.01	0.33 (0.21 -0.51), <0.01
VZV IgG positive	703 (87)	360 (94)	0.46 (0.29-0.73), <0.01	0.87 (0.49 -1.53), 0.63
Hep A IgG positive	514 (64)	293 (76)	0.54 (0.2 - 0.7), <0.01	0.99 (0.70 - 1.40), 0.97

Abbreviations: VZV varicella zoster virus, HAV Hepatitis A virus, HBV Hepatitis B virus, SD standard deviation, SSA sub Saharan Africa, MSM men who have sex with men, IDU injecting drug user, cAb core antibody, sAg surface antigen

We examined factors associated with susceptibility to HAV. Age, [OR, (95% CI), 0.66, (2.86 - 5.43), p<0.01] gender [OR (95% CI), 2.15, (1.54 - 3.01), p<0.01], ethnicity, risk of acquisition of HIV and HBV immune status [OR (95% CI), 0.66, (0.49 - 0.89), p<0.01] were significant predictors of susceptibility to HAV (Table 2.4.4).

On multivariate analysis, younger age was predictive of sero-negativity to HAV, while IDU as risk of acquisition of HIV and originating from Sub Saharan Africa were predicative of immunity to HAV (Table 2.4.4).

Table 2.4.4 Characteristics associated with sero-negativity to HAV

Study Cohort	HAV negative	HAV positive	Univariate analysis	Multivariate analysis
	273 (25)	834 (75)	OR (95% CI), p value	OR (95% CI), p value
Mean age [SD]	36 [9.9]	40 [9.2]	0.66, (2.86 - 5.43) <0.01	0.96 (0.94 - 0.97), <0.01
Male n (%)	219 (20)	545 (26)	2.15, (1.54 - 3.01), <0.01	1.03, (0.64-1.66), 0.90
Region of origin n (%)				
Ireland	146 (53)	358 (43)	1.53, (1.16 - 2.01), <0.01	(referent)
SSA	15 (5)	286 (34)	0.11, (0.07 - 0.19), <0.01	0.08, (0.4 – 0.16), <0.01
South America	23 (8)	39 (5)	1.9, (1.1 - 3.2), 0.02	0.54, (0.3 – 1.14), 0.11
Europe	47 (17)	79 (10)	1.99, (1.34 - 2.92), <0.01	0.80, (0.52 – 1.22), 0.31
Acquisition risk for HIV				
MSM	163 (60)	296 (36)	2.69 (2.03 - 3.57), <0.01	(referent)
Heterosexual	80 (29)	399 (50)	0.45, (0.33 - 0.61), <0.01	0.97, (0.62 - 1.52), 0.91
IDU	27 (9)	121 (15)	0.65, (0.42 – 1.0), 0.05	0.44 (0.27 - 0.72), <0.01
VZV IgG Positive	255 (93)	790 (95)	0.79, (0.45 - 1.4), 0.45	0.98 (0.70-1.39), 0.93
Hep B cAb / sAg positive	72 (27)	293 (36)	0.66, (0.49 - 0.89), <0.01	1.55, (0.83 - 2.92), 0.15

Abbreviations: VZV varicella zoster virus, HAV Hepatitis A virus, HBV Hepatitis B virus, SD standard deviation, SSA sub Saharan Africa, MSM men who have sex with men, IDU injecting drug users, cAb core antibody, sAg surface antigen

2.4.5 Discussion

This study is the first to report seroepidemiology of HAV, HBV and VZV in an adult HIV-infected cohort at point of entry to specialist HIV-care in Ireland. We identify a significant proportion (6-68%) of HIV-infected individuals who are susceptible to these infections at baseline. This finding highlights the need for proactive screening and immunisation to prevent potential infection and complications relating to these infections in an at-risk patient group.

VZV IgG seropositivity in the study cohort was 94%. This is lower than the level of seropositivity for VZV IgG observed in the general population in Ireland (98-99%) (Gallagher et al., 1996). The reason for this finding may reflect the diversity of our cohort in terms of epidemiological exposure and geographic origin. Female gender, younger age and origin outside Ireland were associated with sero-negativity to VZV on univariate analysis. Nine percent of patients from SSA (n=356) included in our study were seronegative for VZV while 20% of patients from South America (n=64) were seronegative for VZV. Other studies have reported a lower level of seroprotection to VZV in individuals from Sub Saharan Africa (SSA) (Lenas-Garcia et al., 2013).

In Ireland, VZV vaccine is not included as part of the routine immunisation schedule, therefore the risk of exposure to VZV in non-immune adults in Ireland is relatively high. While chickenpox is considered a mild self-limiting infection in children, serious complications do occur (Yousif et al., 2016). Varicella infection in adults results in more frequent complications and can be a fatal infection in immunocompromised individuals (Danovaro-Holliday et al., 2004).

As VZV vaccine is a live vaccine it is recommended only for those with CD4 T cell count >200 cells/mm^3 (Geretti, 2015). In our study, 93% of those who were VZV IgG negative had a CD4 T cell counts >200 cells /mm^3 and thus could receive VZV immunisation. Those with a CD4 T cell count <200 cells/mm^3 should be monitored closely and immunised as soon as immune recovery is established. In the interim, they should be educated regarding the requirement for secondary prophylaxis with VZV immunoglobulin should significant exposure occur. In the absence of immune recovery consideration should be given to providing VZV vaccination to household contacts, particularly children to limit exposure (Konkle-Parker, 2014).

HAV seroprevalence in the cohort was 75% which is higher than seroprevalence described in the general population in Ireland in 1998 (67%) (Rajan et al., 1998). HAV vaccine is recommended for individuals considered to be at risk of HAV infection including MSM, IDU's,

individuals who will travel to areas endemic for HAV. Given the common risks of acquisition, current practice in our HIV out-patients clinic is to check baseline serostatus to HAV in all comers to our HIV clinic and to vaccinate those who are seronegative. One in four individuals attending our clinic are susceptible to HAV and vaccine is indicated.

Over two thirds (68%) of individuals in our cohort were both HBV cAb and sAg negative indicating susceptibility to infection. HIV-infected individuals are at increased risk of HBV infection and of developing chronic HBV infection (Colin et al., 1999). Chronic HBV infection is found in up to 10% of HIV-infected individuals worldwide and co-infected individuals are more likely to progress to liver cancer, cirrhosis and have a higher mortality than those with HIV-infection alone (Martin-Carbonero and Poveda, 2012).

Optimising protection against HBV is important in HIV-infected individuals given that both risk of infection and subsequent chronicity of infection is increased in HIV-infected individuals exposed to HBV. Co-infection with HIV and HBV is associated with a significant increase in the risk of liver-related mortality (Nikolopoulos et al., 2009).

This study has several limitations. This was a retrospective study and we did not record immunisation status of patients. There is no adult immunisation registry in Ireland apart from a HPV vaccination register. Our sample size was large; however, it was selected from a group within the overall cohort with serologies documented in the vaccine unit database which may represent a selection bias. Additionally, the GUIDE cohort may not be representative of the general HIV-infected population in Ireland. Finally, it should be acknowledged that lack of humoral immunity does not necessarily indicate lack of protection, as cellular immunity may be present.

This study presents the first Irish data regarding susceptibility to common vaccine preventable infections in HIV-infected individuals. We identified a significant proportion of HIV-infected adults who are potentially at risk of vaccine preventable infections and who could derive protective benefit from vaccination. Our data support pro-active screening as advocated by current national guidelines to identify patients who should be vaccinated.

2.5 High levels of susceptibility to Measles, mumps and rubella (MMR) in HIV-infected individuals in Ireland

2.5.1 Abstract

Immunisation guidelines recommend proactive screening and vaccination of HIV-infected individuals given the increased risk of infection and infection related complications. The MMR vaccine is recommended for HIV-infected individuals who are susceptible to any component of the vaccine with a CD4 T cell count >200 cells/mm^3. The aim of this study was to investigate levels of susceptibility and factors associated with susceptibility to MMR in HIV-infected individuals in Ireland.

A single centre retrospective cohort study was undertaken. All new comers to the HIV-outpatient's clinic had serum tested for MMR antibodies from January 2013. Factors associated with sero-negativity to MMR were identified using univariate and multivariate regression (SPSSv23). The study was approved by the St James's Hospital, research ethics committee.

213 patients were included in the study. 30% were susceptible to at least one component of the MMR vaccine, 15% to measles, 14% to mumps and 9% to rubella. 57 of 60 individuals who were susceptible to at least one component of the vaccine had a CD4 T cell counts greater than 200 cells/mm^3 and thus would be suitable to receive MMR vaccine.

A high proportion of HIV-infected individuals (30%) remain susceptible to at least one component of the MMR vaccine. These patients are at increased risk of infection on exposure and complications should infection occur. Our findings highlight the need for proactive screening and vaccination of this high-risk patient group.

2.5.2 Background

In recent years, outbreaks of measles, mumps and rubella (MMR) have been observed even in highly vaccinated populations where coverage has fallen below the threshold required to contain spread.

HIV-infected individuals are more susceptible to such infections and on exposure frequently experience more severe illness. Immunisation guidelines recommend proactive screening and have a lower threshold to recommend immunisation of HIV-infected individuals. The MMR vaccine is recommended for HIV-infected individuals susceptible to any component of the vaccine with CD4 T cell count greater than 200 cells/mm^3 (ACIP, 2013).

Studies investigating response to the MMR vaccine in HIV-infected adults report variable levels of seroconversion (25-90%) and a more rapid waning of detectable antibody following a single dose of MMR vaccine (Belaunzaran-Zamudio et al., 2009). While immunologic response to MMR vaccine may be less than that observed in immunocompetent hosts, even an attenuated vaccine response will confer some degree of protection against potentially serious infections.

Study Aim

The aim of this study was to examine frequency of sero-negativity and factors associated with sero-negativity to MMR in HIV-infected adults attending a tertiary referral HIV clinic in Ireland.

2.5.3 Methods

All new attendances who had MMR serology checked from January 2013 – January 2014 were included in the study. Data was collected using the GUIDE clinic cohort database, the vaccine unit database along with electronic patient records (EPR).

MMR IgG status was analysed using enzyme linked immunosorbent assay (ELISA) and reported as IgG positive, equivocal or negative. For analysis purposes, IgG negative serologies were classified as non-immune, IgG positive and equivocal categories were combined and reported as seropositive.

Data was analysed using MS Excel 2011 for Windows and SPSS(v22). Continuous data is reported as mean ± standard deviation, categorical data as count and relative frequency. Chi ^{2} or Fishers exact tests were used to evaluate the association between categorical variables. Paired or unpaired t tests were used to compare means between groups.

2.5.4 Results

A total of 213 patients were included in the study. Mean age was 35 years. 78% were male, 36% were Irish, 23% were from Western Europe, 22% from South America, 36% from Africa. Risk of acquisition of HIV was MSM in 67%, heterosexual in 28%, IDU in 3%. Mean CD4 T cell count was 480 cells/mm^3. 30% (n=63) were susceptible to at least component of the MMR vaccine (Table 2.5.1).

Table 2.5.1 Characteristics of patients according to serostatus for MMR

		Mumps (n=213)				Measles (n=209)				Rubella (n=202)			
	Total n (%) 213	**IgG Negative 29 (14%)**		**IgG Positive 184 (86%)**		**IgG Negative 31 (15%)**		**IgG Positive 178 (85%)**		**IgG Negative 19 (9%)**		**IgG Positive 183 (91%)**	
Male	165 (78)	25	(86)	140	(76)	31	100	133	(75)	15	(79)	142	(78)
Mean age [SD]	35 [10]	31	[8]	36	[10]	29	[6]	36	[11]	37	[12]	35	[10]
Region of Origin													
Ireland	76 (36)	11	(38)	65	(35)	5	(16)	69	(39)	8	(42)	61	(33)
Eastern Europe	15 (7)	0	0	15	(8)	2	(7)	12	(7)	2	(11)	13	(7)
Western Europe	23 (11)	3	(10)	20	(11)	6	(19)	17	(10)	1	(5)	20	(11)
South America	48 (23)	10	(35)	38	(21)	15	(48)	33	(19)	3	(16)	45	(25)
SSA	17 (36)	3	(10)	33	(18)	1	(3)	34	(19)	3	(16)	31	(17)
Other	15 (7)	2	(7)	13	(7)	2	(7)	13	(7)	2	(11)	13	(7)
Acquisition risk for HIV													
MSM	143 (67)	24	(83)	119	(65)	29	(94)	113	(64)	13	(68)	121	(66)
Heterosexual	60 (28)	2	(7)	58	(32)	0	(0)	57	(32)	5	(26)	53	(29)
IDU	6 (3)	1	(3)	5	(3)	0	(0)	6	(3)	0	(0)	6	(3)
Vertical	4 (2)	2	(7)	2	(1)	2	(7)	2	(1)	1	(5)	3	(2)
Mean CD4 [SD]	480 [250]	445	[187]	485	[259]	529	[268]	461	[236]	414	[239]	483	[248]

Variables reported as number (%) unless otherwise stated.
Abbreviations: SD Standard Deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug use, CD4 T cell count mm^3

When we examined factors associated with susceptibility to MMR vaccine we found that heterosexual acquisition of HIV and Irish origin were negatively associated although both were of borderline significance (Table 2.5.2).

Table 2.5.2 Factors associated with susceptibility to MMR

Total Cohort n=213	Susceptibility to MMR vaccine N=64 (%)	Immunity to MMR N=149 (%)	Univariate analysis OR (95% CI), p value	Multivariate analysis OR (95% CI), p value
Male	55 (86)	110 (74)	2.2 (1-4.) 0.07	0.6 (0.16-2.3), 0.46
Mean age [SD]	33 [10]	36 [11]	3.18 (0.16-6.2), 0.04	0.98 (0.95-1.0), 0.29
Region of Origin				
Ireland	19 (30)	57 (38)	0.68 (37 - 1.3), 0.28	0.55 (0.28-1.1), 0.09
Acquisition risk for HIV				
MSM	52 (81)	91 (61)	2.8 (4-5.5), <0.01	(referent)
Heterosexual	7 (11)	53 (36)	0.22 (0.09 - 0.52), <0.01	6.7 (1.7-25.8), 0.06
IDU	1 (2)	1 (0.7)	2.3 (0.12 - 45), 0.5	2.16 (0.2-21), 0.5
Vertical	3 (5)	1 (0.7)	2.3 (0.12 to 45), 0.08	0.26 (0.02-2.79), 0.27

Variables reported as number (%) unless otherwise stated.
Abbreviations: SD Standard Deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug use, CD4 T cell count mm^3

2.5.5 Discussion

This study identifies a high level (up to 30%) of susceptibility to at least one component of the MMR vaccine in HIV-infected individuals. This data is comparable to similar studies undertaken in Europe (Molton et al., 2010, Grabmeier-Pfistershammer et al., 2014, Lenas-Garcia et al., 2013).

The identified level of susceptibly to MMR is of particular concern given the recent outbreaks of measles and mumps in the Irish context (O Riordan et al., 2014). HIV-infected individuals are at increased risk of complications should infection occur but may also facilitate onward transmission of such infections.

We did not record vaccine history in our study so it not possible to infer whether lack of sero-immunity is due to non-receipt of vaccine, poor response to vaccine or waning of vaccine titres over time. It is worth noting however that other studies have shown considerable variability between self-reported vaccine history and observed sero-status (Molton et al., 2010, O'Riordan et al., 2014).

Our study identifies migrants as a group who were more likely to be susceptible to MMR. This may be due to differences in international immunisation policies and immunisation coverage achieved. It is notable that a significant proportion of native Irish individuals were susceptible also indicating a need for testing of all comers for susceptibility.

Our study findings support screening and immunisation guidelines which recommend checking sero-status of all HIV-infected individuals, particularly for measles given the potential severity of infection in this patient group and providing vaccination as appropriate.

In our study, 57 of 60 individuals who were susceptible to one or more component of the MMR vaccine had a CD4 T cell count greater >200 cells/mm^3 indicating a high level of suitability for MMR vaccine.

The increasing requirements for screening and provision of vaccines in HIV-infected individuals is challenging for providers in the context of rapidly evolving immunisation guidelines and indications. Despite strong evidence for protective effect of vaccination in HIV-infected individuals, vaccine coverage remains suboptimal.

To address poor vaccination coverage and the significant burden of vaccine preventable infections observed in our HIV-infected cohort an integrated vaccine clinic was established in the GUIDE clinic in 2003. This unit runs alongside the HIV clinic and incorporates a specialist vaccine nurse, a pharmacist, a data manager as well as support from an Infectious Disease Consultant. Vaccination coverage and outcomes in the attending HIV-infected cohort have improved significantly since the establishment of the unit (Rock et al., 2013). Such a unit may be considered as a successful model of care and could be employed in other settings to improve vaccination coverage to ensure optimal protection for our patients.

Screening and provision of appropriate vaccinations such as MMR in HIV-infected individuals is a worthwhile endeavour and should be seen as an opportunity for disease prevention in a vulnerable patient group.

2.6 Summary of Findings

The seroepidemiological data reported in this chapter highlights the high rates of susceptibility to common vaccine preventable infections in HIV-infected individuals, on first attendance to a large tertiary referral HIV specialist centre in Ireland. This is the first Irish data investigating seroprevelance of these common vaccine preventable infections. Findings from this study support both national and international immunisation guidelines for HIV-infected individuals which recommend a proactive approach to screening for and vaccination in this at-risk patient group.

Little is known currently about delivery of vaccinations to HIV-infected cohorts and the limited available data would suggest that immunisation recommendations are poorly adhered to. The reasons for this are likely to be multifactorial, including increasingly complex clinics, limitations relating to staff resources along with demand capacity issues.

In contrast to the successful structured infant immunisation programmes that are in place internationally, formal immunisation programmes for at risk adult groups do not exist. While immunisation guidelines exist, vaccines are administered in an ad-hoc manner with the majority of specialists depending on primary care physicians to administer vaccinations. In Ireland, no centralised immunisation or medical record exist for adults which compounds the issue of estimating accurate and appropriate immunisation coverage in the general population as well as in at-risk populations such as HIV-infected adult. A central HPV vaccine registry has been established allowing for accurate monitoring of HPV vaccine coverage in eligible females.

In the next chapter of this thesis, I will examine vaccine coverage and delivery in HIV-infected adults by investigating an integrated vaccine unit as a model of care for vaccine delivery in HIV-infected individuals.

3. Effectiveness of an integrated vaccine unit as a model of care for delivery of vaccination to a HIV-infected cohort

3.1 Research outputs to date

Peer Reviewed Publications

Development of Chronic Hepatitis B Infection in a Hepatitis B Vaccine Responder.
C Sadlier, K Madden, S O'Gorman, B Crowley, C Bergin. **International Journal of STD and AIDs.** 2017 Apr;28(5):526-528. doi: 10.1177/0956462416674835. Epub 2016 Nov

Strategies to address poor influenza vaccine compliance in healthcare workers.
Sadlier C, Carr A, Kelly S, Bergin C. **Ir Med J.** 2015 May; 108 (5):157.

Poor adherence to vaccination guidelines in dermatology patients on immunosuppressive therapies: an issue that needs addressing. Sadlier M, **Sadlier C,** Alani A, Ahmad K, Bergin C, Ramsay B. **Br J Dermatol.** 2015 Jul;173 (1):288-9. doi: 10.1111/bjd.13543. Epub 2015 Mar 11

Impact of a new vaccine clinic on hepatitis B vaccine completion and immunological response rates in an HIV-infected cohort. Rock C, de Barra E, **Sadlier C**, Kelly S, Dowling C, McNally C, Bergin C. **J Infect Public Health**. 2013 Jun;6(3):173-8. doi: 10.1016/j.jiph.2012.11.001.

In Preparation

Influenza vaccine coverage and factors associated with vaccine coverage in HIV-infected individuals over a decade in the setting of an integrated vaccine unit; a retrospective cohort study. Accepted April 2017 **International Journal of STD and AIDS**. **C Sadlier**, A Carr, S Kelly, C Bergin.

Research Presentations

Strategies to improve poor influenza vaccine compliance in Healthcare workers.
Oral Presentation, Croke park **National Patient Safety Conference** November 2014.

Prevalence and predictors of seasonal influenza vaccination among HIV-infected persons receiving care in Ireland 2011-2012. Poster presentation. **European Society of Clinical Microbiology and Infectious Diseases** 2013.

3.2 Introduction

Vaccines are one of the greatest achievements of modern medicine and have saved countless lives worldwide (Nabel 2013). Vaccines stimulate protective immune responses against acute and chronic infectious diseases as well as infectious diseases which cause cancer such as Hepatitis B virus (HBV) and Human papillomavirus (HPV) (Mast, 2006, Dunne et al., 2007).

To date, over 70 vaccines have been licensed for use against approximately 30 microbes. Diseases such as poliomyelitis, measles, mumps, and rubella caused over 35 million infections in the United States alone in the 20th century, however development of effective vaccines against these pathogens have since rendered them uncommon (Roush et al., 2007).

The success of vaccines as a public health intervention is multifactorial, emanating from a combination of identifying effective vaccines, a robust infrastructure for vaccine manufacturing, regulatory and safety oversight, and organised approaches to delivery. At a societal level, economic benefits are derived by preventing hospitalisation, avoiding long-term disability, and reducing absence from work (O'Brien et al., 2004). In brief, vaccines provide the most cost and clinically effective means to preserve health, protect against epidemics and save lives (Nabel et al., 2013).

In Ireland, the National Immunisation Advisory Committee (NIAC) recommendations provide guidance for use of vaccines in clinical practice. Vaccines included in the national immunisation programme include diphtheria, pertussis, tetanus, poliomyelitis, measles, mumps, rubella, influenza, invasive *Haemophilus influenzae* type b (Hib), hepatitis A, hepatitis B, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and human papillomavirus (HPV) (for girls only) (NIAC, 2015). In the United States (US), additional recommendation exist for rotavirus, varicella and herpes zoster (shingles) and the HPV vaccine indication has been extended to include boys and MSM up to the age of 26 years (Robinson, 2016). HIV-infected individuals are at increased risk of morbidity and mortality relating to many common vaccine preventable infections. Therefore, immunisation guidelines recommend proactive screening and immunisation of these high-risk patient groups.

Despite a substantial body of evidence supporting the safety and efficacy of vaccinations for the prevention of infection in HIV-infected individuals, reported immunisation coverage in HIV-infected individuals remains poor (Thornhill et al., 2015, Valour et al., 2014, Durham et al., 2011).

In Ireland, access to highly active anti-retroviral therapy (HAART) and clinically indicated immunisations is universal, with no associated cost for HIV-infected patients. Despite this,

vaccine uptake remains poor (Giese et al., 2016, Mereckiene et al., 2007). While the burden of vaccine preventable infections is not quantified in HIV-infected individuals at a national level, we know from the literature and from work reported in subsequent chapters of this thesis that the incidence of invasive pneumococcal disease (a vaccine preventable infection) is significantly greater than that of the general population. This causes significant morbidity and mortality (Barry et al., 2006).

To address high levels of vaccine preventable infections observed in HIV-infected patients attending the Department of Genito Urinary medicine and Infectious Diseases (GUIDE), St James's Hospital, a dedicated vaccine programme was established in 2003. This novel model of care incorporates an on-site vaccine unit with a specialised multi-disciplinary team including a dedicated vaccine nurse, a data manager and a pharmacist specifically appointed to the unit. The programme is overseen by a senior Infectious Diseases physician.

The vaccine programme routinely provides recommended immunisations for all attending HIV-infected patients including seasonal influenza vaccine, pneumococcal vaccine, hepatitis A and hepatitis B vaccination, measles, mumps, rubella (MMR) and varicella vaccine to the non-immune as well as travel vaccines as appropriate.

The integrated vaccine unit has been in operation for over a decade and has introduced several strategies during this time to improve vaccine coverage and outcomes such as individual patient vaccine passports, real time monitoring of vaccine uptake and short message service (SMS) text reminders.

Study Hypothesis

We hypothesised that the introduction of the integrated vaccine programme model of care has resulted in improved vaccine coverage and outcomes in the attending HIV-infected cohort.

Study Aims

The aim of this chapter was to examine the effectiveness of an integrated vaccine unit as a model of care for delivery of seasonal influenza vaccine and hepatitis B vaccine in HIV-infected individuals.

3.3 Specific aims of this Chapter

The specific aims of this chapter are:

- To examine seasonal influenza vaccine coverage and factors associated with vaccine uptake in HIV-infected individuals over a 10-year period in the setting of an integrated vaccine unit

- To examine hepatitis B virus vaccine coverage, completion and immunological response and factors associated with response to HBV vaccine in HIV-infected individuals attending the GUIDE clinic in the setting of an integrated vaccine programme

3.4 Influenza vaccine coverage in HIV-infected adults over a decade in the setting of an integrated vaccine unit; a retrospective cohort study

3.4.1 Abstract

As cohorts of HIV-infected individuals continue to age successfully, the issue of vaccination coverage in this population continues to grow in importance. Despite the benefits of vaccination, documented coverage with recommended vaccines remains poor. We hypothesised that an integrated vaccine programme could achieve high levels of seasonal influenza vaccine coverage in a HIV-infected cohort. I considered influenza vaccine as the most reliable marker of success of the vaccine programme given that it has been recommended for all HIV-infected patients, regardless of CD4 T cell count since the vaccine unit was established.

The aim of this study was to examine seasonal influenza vaccine coverage and factors associated with influenza vaccine coverage in a HIV-infected cohort from 2003-2016 using data derived from the vaccine unit database and the GUIDE clinic database.

We observed a significant improvement in seasonal influenza vaccine coverage in the active HIV-infected cohort attending during the study period from 50% in 2004 to 76% in 2014 ($p<0.01$). Factors that were significantly associated with non-receipt of vaccine included age [OR, (95% CI), 0.98, (0.97- 0.99), $p<0.01$], IDU as risk of acquisition of HIV [OR (95% CI), 1.51 (1.07-2.12), $p=0.02$] and pneumococcal vaccine [OR (95% CI), 0.73 (0.56-0.97), $p=0.03$].

The documented seasonal influenza vaccine coverage in our cohort increased in the setting of an integrated immunisation programme. While the seasonal influenza and pneumococcal vaccine coverage rates observed are higher than those reported in other HIV-infected cohorts they remain suboptimal. Future challenges in maintaining and further improving immunisation coverage in HIV-infected individuals must be addressed by improving vaccine accessibility as well as improving patient and provider awareness of vaccine recommendations.

3.4.2 Background

In the era of highly active antiretroviral therapy (HAART), HIV-infection has come to be regarded as a chronic treatable illness. Life expectancy is projected as normal and care now focuses on optimisation of health through education, screening and increasingly, vaccination.

HIV-infected individuals are more susceptible to and frequently experience more complications from common vaccine preventable infections (Long et al., 2001). Thus, disease prevention through vaccination is of particular importance in this patient group.

Given the burden of vaccine preventable infections observed in HIV-infected individuals, immunisation guidelines recommend proactive vaccination of HIV-infected individuals and frequently have a lower threshold to recommend vaccines (Rubin et al., 2013, NIAC, 2015, Geretti, 2015). However, despite evidence-based recommendations, reported vaccination coverage in HIV-infected individuals remains poor (Lim et al., 2013).

HIV care providers are increasingly challenged in busy, complex outpatient clinics and may not have the time or resources (for staffing or vaccine) to integrate routine vaccination into clinical care.

To address poor vaccination uptake in the attending HIV-infected cohort in the Department of GU medicine and Infectious Diseases (GUIDE), St. James's Hospital, Dublin, Ireland, an integrate vaccine programme was established. This novel model of care incorporates an on-site vaccine unit with a specialised multi-disciplinary team including a dedicated vaccine nurse, a data manager and a pharmacist specifically appointed to the unit. The programme is overseen by a senior Infectious Diseases physician.

The vaccine programme offers universal access to recommended immunisations for all patients and routinely provides seasonal influenza vaccine, pneumococcal vaccine, HAV and HBV vaccination, measles, mumps, rubella (MMR) and VZV vaccine to the non-immune as well as travel vaccines as appropriate.

Locating the vaccine unit on-site in the HIV-clinic facilitates ease of access to vaccination for patients attending for routine HIV care. Other interventions employed to improve vaccine uptake since establishment of the programme include use of short message service (SMS) text notification and reminders to announce availability of seasonal influenza vaccine. Text

reminders have also facilitated improved adherence to hepatitis B vaccine programmes (Rock et al., 2013).

HIV-infected individuals are at increased risk of influenza infection and more frequently experience complications relating to influenza infection (Radwan et al., 2000). Seasonal influenza vaccine is safe (Sullivan et al., 2000) and is recommended for all HIV-infected individuals, regardless of their CD4 T cell count and HIV viral load level (Aberg et al., 2009). Two prospective non-randomised studies identified substantially fewer episodes of laboratory-confirmed influenza in vaccinated patients with HIV (6.1%) versus unvaccinated patients (21.2%) (Yamanaka et al., 2005) and a lower frequency of influenza infection in patients who were simultaneously vaccinated for influenza and pneumococcus than in those who declined vaccination (Ranieri et al., 2005).

The World Health Organization (WHO), and European Agencies recommend influenza vaccination for individuals at-risk due to age (≥65 years), underlying diseases including HIV, pregnancy and for health care workers (HCWs) in Europe. In Ireland, there is no comprehensive information system with which to estimate uptake of influenza or pneumococcal vaccines among at-risk adults. Currently the Health Protection Surveillance Centre (HPSC) uses administrative data (payment claims from GP or pharmacies) to estimate vaccine uptake among elderly individuals entitled to free vaccination. Two studies have estimated the influenza vaccine coverage in individuals in Ireland in whom vaccine is reported at between 28% in individuals less than 65 years with an indication for vaccination and 60% in patients over the age of 65 years (Giese et al., 2016, Mereckiene et al., 2007).

Seasonal influenza vaccine has been recommended in HIV-infected adults regardless of CD4 T cell count since the mid 1980's. There is limited international data and no Irish data reporting influenza vaccine coverage in HIV-infected individuals.

Study Aim

The primary aim of this study was to examine temporal trends in seasonal influenza vaccine coverage in a HIV-infected cohort in the setting of an active integrated vaccine programme (a novel model of care).

The secondary aim of this study was to assess factors associated with non-receipt of influenza vaccine in the attending HIV-infected cohort from September 2014-April 2015.

3.4.3 Methods

A retrospective cohort study was undertaken to investigate annual documented influenza vaccine coverage in the attending HIV-infected cohort from 2003-2015.

Study subjects

The total number of HIV-infected patients attending the HIV-outpatient clinic annually from 2003-2015 were identified from the GUIDE clinic cohort database. The annual active attending cohort includes all patients that attended clinic on at least one occasion during a 12-month period.

The total number of influenza vaccines administered annually in the HIV-infected cohort was retrieved directly from the vaccine unit data base. Proportion of HIV-infected patients who were in receipt of vaccine was calculated annually by dividing the total number of vaccines recorded by the documented active attending cohort for that year.

Demographic Data

To assess factors associated with influenza vaccine uptake, demographic data was prospectively collected for all individuals who attended the GUIDE clinic on at least one occasion from 2014-2015.

Variables collected included age, gender, region of origin, risk of acquisition of HIV, CD4 T cell count, HAART, previous receipt of pneumococcal vaccine (Table 3.4.1).

Statistical analysis

Variables were reported as numbers and percentages (%) for categorical variables, and medians and interquartile ranges [IQR] for continuous values. For the percentage calculations, missing values were excluded from the denominator. Parametric and non-parametric statistical tests were used to compare groups: the chi squared test was used to compare categorical variables and the Mann–Whitney *U* test for continuous variables.

Poisson regression was used to examine temporal trends in vaccination coverage over time. Multivariate regression analysis including all variables was used to identify factors associated with influenza vaccine uptake in 2014/2015.

St James's Hospital Institutional board review approval was obtained to undertake this study.

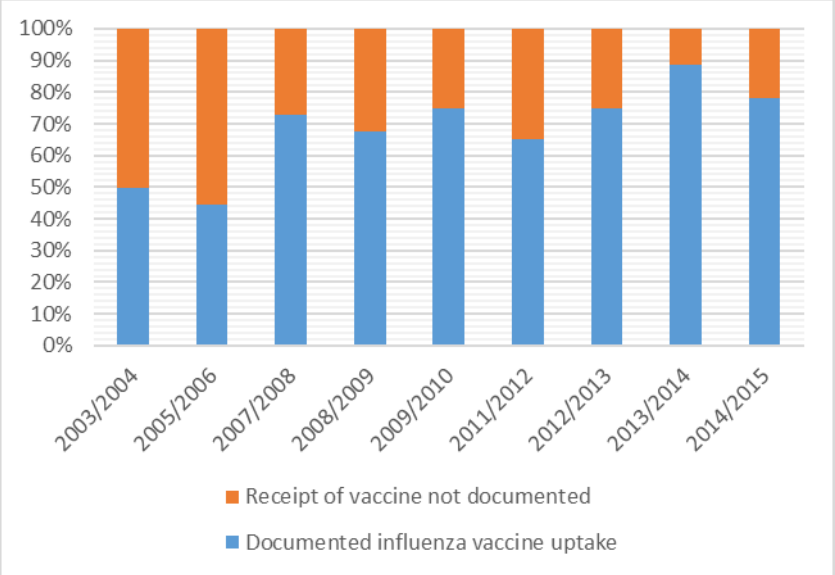
3.4.4 Results

A total of 3419 individual HIV-infected patients were identified as having attended the GUIDE clinic on at least one occasion from 2003-2015. An average of 218 new patients attended the clinic annually during the study period.

Trends in Influenza vaccine coverage

Seasonal influenza vaccine coverage in the cohort was documented at 50% in 2003. Seasonal influenza vaccine uptake in the cohort increased significantly during the study period and was documented at 76% in 2014/2015 versus 50% in 2003/2004 ($p<0.01$) (Figure 3.4).

Figure 3.4 Influenza vaccination coverage in a HIV-infected cohort (% uptake) September 2003-April 2014



Seasonal Influenza vaccine coverage 2014/2015

From January 2014-January 2015, 2035 patients were recorded as having attended the GUIDE clinic on at least one occasion. Sixty-nine percent of patients were male, mean [SD] age 40 [9.3] years, 53% Irish, 40% MSM. The median [IQR] duration of HIV infection was 6 [2-11] years, 87% were virally suppressed on HAART and mean [SD] CD4 T cell count was 504 [367-684] cells/mm^3 (Table 3.4.1).

Table 3.4.1 Characteristics of active attending cohort 2014-2015

Total Cohort who attended on one occasion	N=2035 (%)
Mean age [SD]	40 [9.3]
Male	1403 (69%)
Region of origin n (%)	
Ireland	1069 (53)
SSA	468 (23)
South America	113 (6)
Western Europe	120 (6)
Eastern Europe	79 (4)
Other	186 (9)
Median duration of HIV diagnosis (years) [IQR]	6 [2-11]
Acquisition risk of HIV	
Heterosexual	795 (39)
MSM	823 (40)
IDU	379 (19)
Other	38 (2)
Died	13 (0.6)
Transferred care	38 (2)
Median CD4 T cell count (cells/mm3) [IQR]	504 [367-684]
Viral load ND on HAART	1768 (87)

Data presented as number (percent) unless otherwise stated.

Abbreviations: SD, standard deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug user, IQR interquartile range, ND not detected, HAART highly active antiretroviral therapy

In calculating influenza vaccine coverage, patients who transferred care (n=27) or died (n=8) prior to availability of influenza vaccine in September 2014 were excluded from the denominator. In total, 1523 influenza vaccines were documented in the remaining 2000 patients giving an influenza vaccine coverage rate of 76%. Factors associated with receipt of influenza vaccine are outlined in Table 3.4.2.

Table 3.4.2 Factors associated with non-receipt of Influenza vaccine 2014/2015

	Influenza vaccine documented n (%) 1523 (76)	Influenza vaccine not documented n (%) 477 (24)	Univariate analysis (95% CI) p value	Multivariate analysis OR (95% CI) p value
Mean age [SD]	40 [10]	38 [10]	1.9 (0.92-2.96), <0.01	0.98 (0.97- 0.99), <0.01
Male	1059 (70)	320 (67)	0.85 (0.68-1.06), 0.15	0.83 (0.62-1.12), 0.22
Region of origin				
Ireland	787 (52)	257 (54)	0.81 (0.62-1.06), 0.12	0.78 (0.55-1.21), 0.18
SSA	368 (54)	96 (20)		
South America	84 (6)	27 (6)		
Europe	142 (9)	54 (12)		
Other	138 (9)	47 (10)		
Acquisition risk of HIV				
IDU	250 (17)	112 (23)	1.54 (1.17-2.03), 0.01	1.51 (1.07-2.12) 0.02
MSM	635 (42)	181 (38)		
Heterosexual	610 (40)	177 (37)		
Other	24 (2)	11 (2)		
Mean duration of HIV infection (years) [SD]	7 [6]	6.5 [6]	0.05 (-0.56-0.67), 0.87	1.02 (0.99-1.04), 0.18
Mean CD4 T cell count [SD]	547 [290]	523 [292]	28 (2.7-59), 0.07	1 (0.99-1.0), 0.45
Viral load ND on HAART	1329 (86)	411 (85)	0.88 (0.65-1.84), 0.39	0.95 (0.69-1.30), 0.74
Pneumococcal vaccine	1229 (81)	357 (75)	0.71 (0.56-0.91), <0.01	0.73 (0.56-0.97), 0.03

Abbreviations: SD, standard deviation, SSA Sub Saharan Africa, MSM men who have sex with men, IDU injecting drug user, IQR interquartile range, ND not detected, HAART highly active antiretroviral therapy, OR Odds Ratio

Factors that were significantly associated with non-receipt of seasonal influenza vaccine on multivariate regression analysis included age [OR (95% CI), 0.98 (0.97- 0.99), p<0.01], IDU as risk of acquisition of HIV [OR (95% CI), 1.51 (1.07-2.12), p=0.02] and pneumococcal vaccine [OR (95% CI), 0.73 (0.56-0.97), p=0.03] (Table 3.4.2).

3.4.5 Discussion

Seasonal influenza vaccine coverage in our study was documented at 76% in 2014/2015. This is higher than coverage reported in other published studies in HIV-infected cohorts (19-53%) (Valour et al., 2014).

We observed a significant increase in influenza vaccine coverage during the study period from 50% in 2003 to 76% in 2014 ($p<0.01$). I hypothesise that the observed increase in immunisation coverage relates to successes of the integrated vaccine programme which facilitates ease of access to vaccine, pro-actively educates, engages and reminds both patients and healthcare providers regarding immunisation requirements and benefits.

In our study, factors associated with receipt of seasonal influenza vaccine included older age and previous receipt of pneumococcal vaccine. It is possible that older individuals are more conscious regarding their health and have a greater perceived risk of infection and thus are more amenable to vaccination. Individuals who previously received pneumococcal vaccine were also more likely to attend for seasonal influenza vaccine indicating that prior engagement with the vaccine programme may have positively influenced acceptability for subsequent vaccines. Injecting drug use (IDU) as a risk of acquisition of HIV was negatively associated with receipt of influenza vaccine on univariate analysis. This is of concern as IDU's are a vulnerable risk group who we have identified in subsequent chapters as the group at highest risk of admission with invasive pneumococcal disease.

Following completion of this research, additional interventions employed by the vaccine subgroup committee to address suboptimal vaccination coverage in IDUs was to liaise with drug treatment centres and encourage vaccine to be offered at satellite drug treatment centres where these patients attend regularly for methadone maintenance therapy.

This study has a number of limitations including limitations inherent in a retrospective cohort study. Additionally, reported coverage represents only those with vaccine documented in the vaccine unit database. This may represent a relative underestimation if patients received vaccine elsewhere or did not report receipt of vaccination. This study was quantitative and did not explore barriers to or reasons for non-receipt of influenza vaccination in patients or providers and future work will assess this. Such information would be important in guiding targeted interventions to improve influenza vaccination uptake in the future. Additionally, this study did not investigate prior receipt of influenza vaccine as a factor that may influence receipt of vaccine in subsequent years.

HIV-infected patients should be educated about their vulnerability to influenza and pneumococcal infections and the potential serious complications. Increased training and educational efforts are needed to ensure that both providers and patients are aware that influenza vaccination is safe and is recommended for HIV-infected adults regardless of CD4 T cell count. The decreased likelihood of vaccination among injecting drug users in our study supports focused efforts to reduce these disparities.

Better characterisation of the barriers to immunisation at both patient-level and healthcare provider is needed. Additionally, cost effectiveness of the vaccine unit as a model of care should be examined to assess the feasibility of introducing a similar model of care in other clinic settings and this will be a future direction of research in the vaccine subgroup committee in St James's Hospital.

Ancillary research undertaken with collaborators nationally which builds on this model of care indicates that strategies employed in the vaccine unit could be used to improve vaccine coverage in a diverse range of cohorts including healthcare workers (Sadlier et al., 2015a) and patients commencing on immunomodulatory therapies (Sadlier et al., 2015b).

The integrated vaccination-unit model and targeted strategies employed including use of SMS texting, personal vaccine passports, patient and provider education and reminders to announce availability of seasonal influenza vaccine have facilitated ongoing improvement in vaccination coverage and outcomes.

Future interventions will target influenza vaccination coverage in IDUs through drug treatment centres. This simple but effective model of care could be used in other health care settings to facilitate improvements in vaccine coverage in other immunosuppressed patient groups.

In the future, services should plan to apply knowledge gained from this study of the vaccine unit initiative to develop a pan-hospital chronic inflammatory diseases assessment clinic and thus improve patient safety and quality in other "at-risk" patient groups.

3.5 Sustained high Hepatitis B vaccine completion and immunological response rates in an HIV-infected cohort in the setting of an integrated vaccination unit

3.5.1 Abstract

Screening for serological markers of hepatitis B virus (HBV) immunity is recommended for all HIV-infected individuals. HBV and HIV share common modes of transmission and co-infection is associated with an increased risk of chronic HBV infection, liver damage, and mortality. HBV vaccine is the mainstay of HBV prevention however immunogenicity of HBV vaccine is lower in HIV-infected adults compared to the general population.

The primary aim of this study was to examine completion rates and immunological response to standard three dose HBV vaccine course (Engerix B® 20μg) in HIV-infected adults. Secondary aims were to investigate factors associated with response to HBV vaccine and response to double dose vaccine course in study participants who did not respond to the primary HBV vaccine series.

A 5-year retrospective review of HBV vaccine completion and immunologic response was undertaken. All patients recorded as having received a first dose of HBV vaccine from January 2008-January 2013 were included. Completion rates of 3 dose HBV vaccine series and immunological response to standard three dose series of HBV vaccine were calculated. Proportion of non-responders to standard series of HBV vaccine who complete double dose (DD) HBV vaccine series and immunological response to DD HBV vaccine was calculated.
Factors associated with HBV vaccine response in individuals who completed standard 3 dose series of HBV vaccine were assessed using univariate and multivariate regression analysis.

A total of 257 patients were included in the study. Sixty-eight percent completed three standard dose HBV vaccines series of whom 67% achieved sero-protective HBV sAb titres. We did not identify any demographic or clinical factor as being significantly associated with protective antibody response post completion of 3 dose HBV vaccine series. Of the 58 individuals who did not respond to primary HBV vaccine series, 48 (83%) completed a DD HBV vaccine series and 69% achieved a protective anti-HB sAb titer.

Overall, 83% of the 257 individuals who were included in the study had an anti-HB sAb response >10IU/L following HBV vaccine (either single or DD series).

This study demonstrates high HBV vaccine series completion and high rates of seroprotection following HBV vaccine in a HIV-infected cohort. The establishment of an integrated vaccine programme may have facilitated high completion and response rates.

3.5.2 Background

Hepatitis B virus (HBV) is a preventable infection, however it continues to cause significant morbidity and mortality worldwide (Puoti et al., 2002). HIV-infected individuals are at increased risk of acute and chronic HBV infection given their common modes of acquisition. Chronic HBV infection affects approximately 10% of the 34 million people living with HIV worldwide (Puoti et al., 2002).

The prevalence of co-infection with HBV and HIV varies by geographical region and risk factors for transmission. In the US, the prevalence of HBV infection in HIV-infected individuals has been reported at between 20 and 300 times greater than that of the general population (Kellerman et al., 2003, Spradling et al., 2010). In the US and Europe, prevalence of chronic hepatitis B infection is highest in men who have sex with men (MSM) and injection drug users (IDUs) (Spradling et al., 2010).

Epidemiological research that I have undertaken as part of my thesis has established the seroprevalence of HBV core antibody or surface antigen at 32% in HIV-infected individuals in Ireland (Table 2.4.1, page 18).

HBV vaccine is highly effective and remains the mainstay of prevention of HBV infection. HBV vaccine has been shown to significantly decrease the incidence of HBV infection in HIV-infected individuals and to decrease the risk of development of chronic HBV infection (Kellerman et al., 2003).

Consensus international immunisation guidelines from the United States (Masur et al., 2014) and Europe (Rockstroh et al., 2008) recommended that HIV-infected individuals are proactively screened for HBV infection and markers of HBV immunity and all susceptible individuals vaccinated.

HBV vaccine series typically consists of a three dose vaccine series administered at 0, 1 and 6 months with a double dose series recommended for individuals who do not achieve an anti- HB surface antibody (sAb) response >10 IU/mL (Geretti et al., 2015).

More than 90% of immunocompetent adults achieve protective anti-HB sAb titers after completion of the recommended primary vaccine series (Assad and Francis, 1999) although rare cases of HBV infection have been observed in immunocompetent individuals who have a documented vaccine response (O'Halloran et al., 2011). Recent studies have indicated detection

of protective antibody levels and evidence of long-lasting cellular immunity regardless of anti-HB sAb levels up to 30 years post primary HBV vaccine series (Bruce et al., 2016).

Immunocompromising illnesses including chronic kidney disease (Hashemi et al., 2011, Fabrizi et al., 2006, Khan et al., 1996), diabetes mellitus (Fabrizi et al., 2011, Alavian and Tabatabaei, 2010), male sex, older age, (Fisman et al., 2002) obesity, and some human leukocyte antigen (HLA) types (Alper et al., 1989) and HIV-infection are associated with low HBV vaccine responses (Hollinger, 1989, Wood et al., 1993).

Only 18–71% of HIV-infected individuals achieve protective anti-HB sAb titers on completion of the standard-dose (SD) primary vaccine series and anti-HB sAb titers wane more rapidly (Rock et al., 2013a). The wide range in reported seroconversion range may be attributable to differences in methodological design for example vaccine type, administration schedule and route, characteristics of the population studied, and the relatively small sample size in prospective studies.

Anti-HB sAb response in HIV-infected individuals has been shown in some studies to correlate with CD4 T cell count and HIV viral load (Biggar et al., 1987), however this has not been a consistent finding .

Strategies to improve response to HBV vaccine in HIV-infected individuals such as augmented dosing regimens (Launay et al., 2011), intradermal injection (Rey et al., 2015) , and the use of adjuvants (Cooper et al., 2005) to improve immunogenicity in this group have been studied.

Recently published British HIV Association (BHIVA) immunisation guidelines (2015) recommend a 4 double dose HBV vaccine series as the primary immunisation strategy for all non-immune HIV-infected individuals (Geretti, 2015). This recommendation is based on randomised controlled trial data (Launay et al., 2011) and meta-analysis data indicating significantly better immunological response to double dose HBV vaccine (Munier et al., 2013).

Despite the proven preventative benefits of HBV vaccine and the high burden of related disease in HIV-infected individuals, HBV vaccine coverage and completion in HIV-infected individuals is poor (from 49.6 to 75.5%) (Bailey et al., 2008, Rock et al., 2013a).

To address challenges around completion rate and immunological response rate for HBV vaccination, an integrated vaccine programme was established in our HIV ambulatory care

center in the Department of GU Medicine and Infectious Diseases (GUIDE) in Dublin, Ireland. We previously demonstrated successes of this model of care in achieving high rates of completion of the 3 dose vaccine series (75%) (Rock et al., 2013a) and in research presented as part of this thesis, high levels of coverage with seasonal influenza vaccine (Chapter 3.4).

Interventions employed to increase the rate of completion of the vaccine series included the appointment of a full-time vaccination nurse, patient education and the implementation of patient reminder systems, including short message service (SMS) texts sent to mobile phones, message card reminders, nurse phone calls and "vaccine passports" (where patients keep a record of their appointments).

The intervention used to increase the immunological response rate was automatic enrollment into a double-dose HBV vaccination series for those who did not respond to standard-dose HBV vaccination.

The primary aim of this study was to assess completion rates of standard three dose HBV vaccine series and assessing antibody response in individuals who completed recommended primary course in the setting of an integrated vaccine unit over a 5-year period.

The secondary aim was to examine factors associated with response to standard HBV vaccine, to examine immunological response to DD HBV vaccine in individuals who failed to respond to standard HBV series and to compare outcomes to previous published results in the attending cohort.

3.5.3 Methods

Study population

A retrospective cohort study including all patients who were recorded as having received a first standard dose HBV vaccine (Engerix B®) in the GUIDE clinic from January 2008-January 2013 was undertaken. Baseline demographics and clinical characteristics were recorded for participants using the vaccine unit data base and electronic patient records.

Vaccine schedule and reminder systems

The HBV vaccine series was administered in accordance with the recommendations of the 2008 British HIV Association (BHIVA) (Geretti et al., 2008) and the National Immunisation Advisory Committee (NIAC) guidelines (NIAC, 2015).

The single-dose recombinant HBV vaccine (Engerix® 20 µg) was administered intramuscularly at 0, 1 and 6 months. The patients were given a "vaccine passport" at the first visit that recorded the vaccine administered and listed the future dates for the second and third doses and the anti-HBV sAb titre evaluation. In addition, SMS text message reminders were sent by the vaccine clinic via mobile telephones prior to each scheduled clinic visit for vaccination.

The HBV vaccine administered and the anti-HBV sAb results were entered into a clinical database. HIV-infected patients who completed the vaccine series but who had HBV sAb levels ≤10 IU/L were offered a DD HBV vaccine (Engerix®40µg) series that followed the same schedule: 0, 1 and 6 months. These patients were also asked to return for HBV sAb evaluation 6–8 weeks after the third vaccine.

Patients were considered to have completed the vaccine series if they attended all three vaccination appointments and returned for the anti-HB sAb evaluation 4-12 weeks after the third vaccine.

Determination of the hepatitis B vaccination completion and response rates

Demographic data, including age, gender, region of origin, CD4 T cell count and use of highly active anti-retroviral therapy (HAART) was recorded for each patient. The CD4 T cell count used in this study was the most recent recorded level prior to receiving the first dose of HBV vaccine.

Responders were defined as individuals having HBV sAb levels >10 IU/L 4–12 weeks after the 3rd HBV vaccine. Non-responders were defined as individuals having hepatitis B sAb levels ≤10 IU/L at 4–12 weeks after the third HBV vaccine.

Non-responders were routinely offered DD HBV vaccine, and the HBV sAb level was evaluated 4–12 weeks after the third DD HBV vaccine. The HBV sAb titre was used to define responders and non-responders after double dosing.

Statistical analyses

Descriptive analysis was performed using percentages for categorical data and medians [interquartile range] for continuous variables. Factors associated with HBV vaccine response were assessed using univariate and multivariate regression models. Statistical significance was assumed at $p < 0.05$, and the analysis was performed using SPSS (version 23).

3.5.4 Results

In total, 257 patients were recorded as having received standard HBV vaccine during the study period. Baseline demographics of patients included in the study are outlined in Table 3.5.1.

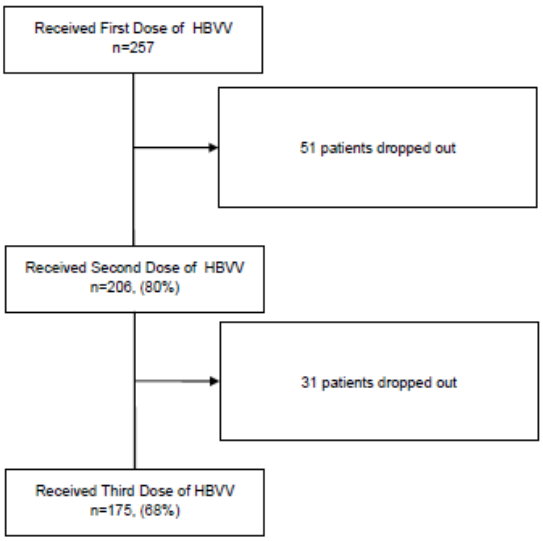
Table 3.5.1 Baseline characteristics of HBV vaccine recipients

Total Cohort	**N=257**	**(%)**
Male	160	(63)
Median age [IQR]	36	[30-42]
Region of Origin		
Ireland	142	(55)
SSA	73	(28)
Other	42	(17)
Group of Acquisition of HIV		
Heterosexual	122	(47)
MSM	76	(30)
IDU	55	(21)
Other	4	(2)
Median CD4 T cell count (cells/mm3) [IQR]	473	[382-678]
Median CD4 % [IQR]	27	[20-33]
On HAART	177	(69)
VL ND	169	(95)

Abbreviations: IQR interquartile range, SSA Sub-Saharan Africa, MSM men who have sex with men, IDU Injecting drug user, HAART Highly active antiretroviral therapy, VL ND viral load not detected.

Of the 257 individuals who received a first dose of HBV vaccine during the study period 206 (80%) completed two doses and 175 (68%) completed three doses of the vaccine series. The flow of participants included in the study is outlined in Figure 3.6.

Figure 3.5 Hepatitis B vaccination standard dose completion rates



Of those who completed the recommended three standard dose HBV vaccine series, 117 (67%) had a HBV surface Ab level >10 IU/L 4-12 weeks post completion of vaccine series and were deemed to be responders.

HBV sAb response rates observed by vaccine doses recorded as administered in the vaccine unit are outlined in Table 3.5.2.

Table 3.5.2 Completion rates of primary HBV vaccine series

Total n 257 (%)	**Completed 3 doses of HBV vaccine series n=175 (68)**	**Completed 2 doses of HBV vaccine series n=31 (12)**	**Completed 1 dose of HBV vaccine series n=51 (20)**
HBV sAb >100 IU/L	70 (40)	9 (29)	13 (25)
HBV sAb 10-100 IU/L	47 (27)	12 (39)	25 (49)
HBV sAb <10 IU/L	58 (33)	10 (32)	13 (25)

Abbreviations: HBV sAb anti-Hepatitis B surface antibody, HBV Hepatitis B virus, iu/L international units per Litre

MSM [OR (95% CI), 2.67 (1.28-5.90), p=0.01] and heterosexual [OR (95% CI), 1.97 (1.05 - 3.81), p=0.05] as risk of acquisition of HIV were associated with HIV vaccine response on univariate analysis (Table 3.5.3).

Table 3.5.3 Factors associated with response to standard dose HBV vaccine series

	Overall cohort n=175 (%)		HBV sAb ≥10 IU/L n=117 (67)		HB sAb <10 IU/L n=58 (33)		Univariate analysis OR (95% CI), p
Male	108	(62)	77	(66)	31	(53)	0.6 (0.31-1.13), 0.1
Median age [IQR]	35	[30-41]	35	[30-41]	35	[29-42]	1
Region of Origin							
Ireland	88	(50)	61	(52)	27	(47)	1.25 (0.67-2.36), 0.5
SSA	52	(30)	31	(27)	21	(36)	
Other	35	(20)	25	(21)	10	(17)	
Acquisition risk of HIV							
Heterosexual	83	(47)	49	(42)	34	(59)	1.97 (1.05 - 3.81), 0.053
MSM	56	(32)	45	(39)	11	(19)	2.67 (1.28-5.90), 0.01
IDU	32	(18)	20	(17)	12	(21)	1.27 (0.55 - 2.81), 0.68
Other	4	(2)	3	(2)	1	(2)	
Median CD4 [IQR] (n=171)	473	[380-655]	469	[380-647]	484	[383-683]	0.74
On HAART (data available, 167)	116	(70)	80	(71)	36	(66)	0.76 (0.38-1.51), 0.4
VL ND (data available, 169)	117	(69)	80	(71)	37	(66)	0.80 (0.41-1.60), 0.5

Abbreviations: IQR interquartile range, SSA Sub Saharan Africa, MSM men who have sex with men, IDU Injecting drug user, HAART Highly active antiretroviral therapy, VL ND viral load not detected. Responders- HBV sAb >10 IU/L, NR Non-responders – HBV sAb <10iu/L. Ua univariate analysis.

Of the 51 individuals who were non-responders (HBV sAb <10 IU/L) to standard 3 course HBV vaccine series, 48 completed a 3 course DD series of HBV vaccine. Sixty-nine percent (33/48) of non-responders had a HBV sAb level >10 IU/L following completion of DD series of HBV vaccine.

Overall 83% of individuals included in the study had a documented HBV sAb >10 IU/L following course of single or double doses of HBV vaccine.

3.5.5 Discussion

We observed high levels of HBV vaccine series completion and HBV sAb response (83%) following HBV vaccination in a cohort of HIV-infected adults attending the GUIDE clinic in St James's Hospital in the setting of an integrated vaccine programme.

The documented level of completion of the standard three dose HBV vaccine series from 2008-2012 was 68%. This documented completion rate for the three-dose vaccine schedule may however represent an underestimate. It is possible that a proportion of study participants received HBV vaccine prior to attending the vaccine unit. This is suggested by results which showed that 75% of participants who received a single HBV vaccine and 68% of participants who were documented as receiving two HBV vaccine doses, achieved a HBV sAb titre >10IU/L. Documentation practices in the vaccine unit will now aim to capture prior receipt of vaccination as a variable to further investigate this hypothesis.

Of those who completed the standard 3 dose vaccine series, 67% achieved a HBV sAb response >10 IU/L. This response rate is comparable to response rates reported in other similar cohorts (Rock et al., 2013a).

CD4 T cell count and HIV viral load have been reported to be associated with HBV sAb response, however this has not been a consistent finding (Wilson et al., 2001). We did not identify an association between immunological or demographic variables and HBV vaccine response in our cohort. The majority of our study cohort had a CD4 T cell count >350 (81%) cells/mm^3 and only four participants had a CD4 <200 cells/mm^3 at the time of commencing HBV vaccine series. This may have limited our ability to detect significant differences. Similarly, the majority of participants were on HAART with undetectable HIV VL.

Of the 58 patients who did not respond to standard 3 dose HBV vaccine series, 48 completed a DD HBV vaccine series. Of those who completed DD HBV vaccine, 33/48 (69%) achieved an HBV sAb response >10IU/L. Several studies have demonstrated a primary DD HBV vaccine regimen in HIV-infected individuals who are HBV vaccine naïve results in greater magnitude and durability of HBV sAb response (Flynn et al., 2011, Chaiklang et al., 2013). In the main randomised trial investigating response to DD HBV vaccine (Launay et al., 2011), a four double-dose regimen was found to be more effective than a standard three-dose regimen in obtaining a protective HBV sAb response (82% vs 65%). A meta-analysis (Munier et al., 2013) concluded that an increased-dose vaccination schedule in previously unvaccinated patients improved anti-HBs response rates compared with standard-dose vaccination (odds ratio 1·96,

95% CI 1·47–2·61) (Madhi et al., 2013). BHIVA guidelines now recommend 4 double dose HBV vaccines for all HIV-infected individuals without serological evidence of immunity to HBV given the greater magnitude of response observed (Geretti et al, 2015). Our study further supports these recommendations.

Non-response to single dose or double dose HBV vaccine is likely to be multifactorial and studies have identified factors other than immunosuppression that can contribute to vaccine non-response including genetic predisposition, chronic disease, and immunomodulatory medications. The human leukocyte antigen (HLA) along with MHC-II plays an important role in presentation of the viral peptides to CD-4 T-helper cells and subjects who fail to respond may have a defect in the antigen presentation or the stimulation of T-helper cells. Studies have shown that patients who are homozygous for HLA DRB1*0301, HLA-B8, SC01, DR-3, HLAB44, FC-31, DR-7 have an increased predisposition to non-responsiveness (Godkin et al., 2005). It is possible that such factors influenced vaccine non-response in our cohort.

Our study has several limitations. We cannot account for individuals who had HBV vaccine prior to attendance at our clinic. We hypothesise that rates of completion of the three dose primary series of vaccine are underestimated as a result. HBV sAb response is measured at 4-12 weeks following completion of HBV vaccine series and thus we cannot comment on durability of HBV sAb response in our cohort. In addition, we cannot account for other variables that may influence response to HBV vaccine.

Overall 83% of individuals included in our study had a HBV sAb response >10IU/L documented following completion of primary single dose or DD HBV vaccine series. HBV sAb titres are known to wane more rapidly in HIV-infected individuals compared to the general population. BHIVA immunisation guidelines now recommend 4 DD HBV vaccines as standard of care for all HIV-infected individuals without serological markers of HBV infection.

Additionally, given that duration of HBV protection in HIV-infected individuals is unknown, it is recommended that HBV sAb is checked regularly and boosters provided once HBV sAb <10 IU/L. An interval of 2-4 years is suggested for individuals with and HBV sAb >100 IU/L, CD4 T cell count >350 cells/mm3 and undetectable viral load. Annual screening is recommended for other subjects.

With increasing strains on the demand capacity ratio in busy outpatient HIV-clinics, implementing HBV infection screening, immunisation and surveillance recommendations will become increasingly challenging for HIV-care providers. Prevention of HBV infection must

remain a priority and strategies to augment protection afforded by immunisation such as inclusion of a dual active antiretroviral therapy (DAART) (tenofovir, lamivudine, and emtricitabine) as a component of HAART must be considered by physician.

Recent data from the Swiss cohort suggests that DAART has an additional important role to play in pre-exposure prophylaxis of HBV co-infection in HIV-infected individuals, independent of CD4 cell count and risky behaviour (Shilaih et al., 2016).

3.6 Summary of Findings

Despite widespread availability of vaccines in Ireland and other developed countries, vaccination coverage in adults and at risk patient groups for many vaccine-preventable infections remains low. In this setting, significant morbidity and mortality due to vaccine-preventable illnesses continues to occur.

It is well established that immunisation is one of the most effective methods of disease prevention. As such, vaccination has great potential to reduce morbidity and mortality relating to vaccine preventable infections particularly in high-risk populations including HIV-infected individuals.

Generally, HIV care providers recommend immunisations and refer patients to primary care physicians for administration of these immunisations. This is a fragmented method of care and is currently not achieving desired outcomes.

In this Chapter we examine vaccination uptake and outcomes in a HIV-infected individuals in the setting of an integrated onsite vaccination programme, a novel model of care. Reported coverage and immunological outcomes in terms of HBV vaccine response are higher than those reported elsewhere in the literature.

Thus I would conclude that an integrated immunisation programme represents a successful model of care for delivery of recommended vaccinations to HIV-infected individuals This model of care has improved patient access to, and knowledge of vaccines and has facilitated improved vaccine coverage and outcomes in our patients.

Future work should assesss cost effectiveness of such a model in terms of disease prevention and assess feasability of introducing a similar model of care for other immunocompromised pateint groups such as patients commencing immunomodulatory therapies.

While endeavours must continue to develop new and superior vaccines, so too must we endeavour to improve the performance of existing vaccination programmes. Only when vaccination coverage targets are achieved will we see the desired decrease in vaccine-preventable disease-associated morbidity and mortality.

4. *Streptococcus pneumoniae* infection and prevention in HIV-infected adults.

4.1 Research outputs to date

Peer reviewed publications

Immunological efficacy of pneumococcal vaccine strategies in HIV-infected adults; a randomized clinical trial.

C Sadlier, S O'Dea, K Bennett, J Dunne, N Conlon, C Bergin.

Nature Scientific Reports. DOI: 10.1038/srep32076. 2016.

http://www.nature.com/articles/srep32076#f1.

Research Presentations

Invasive pneumococcal disease (IPD) incidence and risk factors in HIV-infected individuals.

Oral presentation. Infectious Diseases Society of Ireland annual meeting. Croke Park, Dublin, 2015.

Poster presentation. ID week, New Orleans, LA. October 2016.

Immunological efficacy of a prime-boost immunisations strategy combining PCV13 and PPV23 versus PPV23 alone in HIV-infected adults.

Oral presentation, ID week, San Francisco, 2013.

Oral Presentation, British HIV association Autumn meeting, Liverpool, United Kingdom, 2014.

Best poster presentation, Post graduate medical research meeting, Trinity College Dublin, 2014.

IgG2 response to pneumococcal vaccine

Poster presentation European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Barcelona, Spain, 2014.

In preparation

Invasive pneumococcal disease (IPD) incidence and risk factors in HIV-infected individuals.

C Sadlier, A O'Rourke, S O'Connell, M Kelleher, C Bergin.

4.2 Introduction

In 1881 two microbiologists, George M. Sternberg (Sternberg, 1881) in the United States and Louis Pasteur in France (Pasteur, 1881), independently described roughly lancet-shaped pairs of coccoid bacteria in human saliva. Each injected human saliva into rabbits: Pasteur used saliva from a child who had died of rabies, while Sternberg used his own saliva. Both researchers subsequently recovered diplococci from the blood of these rabbits. Sternberg and Pasteur were first to demonstrate the pathogenic potential of the organism we now refer to as *Streptococcus pneumoniae (S. pneumoniae)* in animals.

S. pneumoniae was identified as the causative agent in human lobar pneumonia in the early 1880s and later in that same decade, was identified as the causative agent in meningitis (Netter, 1887) and otitis media (Zaufal, 1887). Over a century later and despite major advances in medicine, *S. pneumoniae* continues to cause significant morbidity and mortality worldwide accounting for 1-2 million deaths per year. The elderly, the very young as well as immunosuppressed patient groups including those with HIV infection are at particular high-risk (Hausdorff et al., 2005). Mortality associated with invasive pneumococcal disease has remained unchanged over the past 60 years at 20% (Harboe et al., 2009, Rello et al., 2010).

Pneumococcal vaccination has impacted significantly on the burden of pneumococcal infection, particularly since the introduction of the conjugate pneumococcal vaccine (PCV) to infant immunisation programmes (Hausdorff et al., 2005). Efficacy of pneumococcal vaccine has been demonstrated in the general adult population (Moberley et al., 2013). However efficacy of pneumococcal vaccine in HIV-infected individuals remains to be established (Pedersen et al., 2011a). The incidence of invasive pneumococcal disease remains up to 40 times greater in HIV-infected individuals despite widespread availability of highly active antiretroviral therapy (HAART) and pneumococcal vaccination (Harboe et al., 2014b).

Study Hypothesis

We hypothesised that *S. Pneumoniae* causes a significant burden of morbidity and mortality in HIV-infected individuals in Ireland and that improved pneumococcal vaccine could impact the burden of disease observed.

Study Aims

The overarching aims of this chapter are to further knowledge and our understanding of S. pneumoniae infection and prevention of pneumococcal infection in HIV-infected adults.

4.3 Specific aims of this chapter

The specific aims of this chapter are:

- To determine incidence and risk-factors for invasive pneumococcal disease in HIV-infected individuals attending a single tertiary referral center in Dublin, Ireland.

- To determine serotype specific IgG response to a novel pneumococcal vaccine strategy combining the 13-valent conjugate pneumococcal (PCV13) vaccine and the 23-valent polysaccharide pneumococcal vaccine (PPV23) versus PPV23 alone in HIV-infected individuals.

- To determine killing opsonphagocytic assay (OPA) response to a novel pneumococcal vaccine strategy combining PCV13 vaccine and PPV23 alone in HIV-infected individuals.

- To determine IgG2 response to a novel pneumococcal vaccine strategy combining PCV13 vaccine and PPV23 versus PPV23 alone in HIV-infected individuals.

- To establish a multiplex flow cytometric assay to measure phagocytosis of pneumococcal targets from isolated donor neutrophils from a healthy control.

- To determine serotype phagocytic response to a novel pneumococcal vaccine strategy combining PCV13 and PPV23 versus PPV23 alone in HIV-infected individuals using a multiplex flow cytometric assay.

4.4 Incidence and risk factors for invasive pneumococcal disease in HIV-infected individuals

4.4.1 Abstract

Invasive pneumococcal diseases (IPD) remain a significant cause of morbidity and mortality in HIV-infected individuals despite widespread use of highly active antiretroviral therapy (HAART) and availability of pneumococcal vaccines. The aim of our study was to measure incidence and risk factors for IPD (defined as culture of *Streptococcus pneumoniae* from blood, CSF or both) in a cohort of 3160 HIV-infected patients who attended a single ambulatory HIV care centre in Dublin, Ireland from 2006-2015.

Incidence of IPD was determined as events per 100,000 person-years of follow-up. Poisson regression was used to assess linear trend in incidence over time. A nested case-control study (four controls per case) assessed risk factors for IPD.

There were 47 episodes of IPD recorded in 42 HIV-infected individuals (median [IQR] age 38 [33 - 43], 69% male, 86% IDU, median CD4 T-cell count 213 cells/mm^3) over 16,008 person-years follow-up (overall incidence rate of IPD, 293/100 000 person-years). Three patients had 2 episodes and one patient had 3 episodes of IPD during the study period. The overall case fatality rate was 15% (95% confidence interval (CI), 4 - 24).

The incidence of IPD per 100 000 person-years decreased significantly over time from 728 [95% CI, 455 – 1002], to 242 (95% CI, 120 – 365) to 82 (95% CI, 40 - 154) in calendar periods 2006 - 2008, 2009 – 2012, and 2013 – 2015, respectively ($P < 0.01$ for linear trend).

The decrease in incidence of IPD observed is likely multifactorial. This relates to improved vaccination coverage in our cohort in the setting of an integrated vaccination programme, changes to HIV care guidelines driving earlier initiation of HAART, herd immunity conferred following introduction of conjugate pneumococcal vaccine (PCV) to the infant immunisation programme in 2008 and the changing demographics of individuals presenting with HIV infection.

Despite decreases observed, HIV-infected individuals remain at higher risk of IPD compared to the general population. PCV may confer greater protection in HIV-infected individuals and should be seen as a priority to ensure best protection for our patients.

4.4.2 Background

Invasive pneumococcal diseases (IPD) remains a significant cause of morbidity and mortality in HIV-infected individuals in the era of highly active antiretroviral therapy (HAART) (Yin Z, 2012). The incidence of IPD in HIV-infected individuals has been reported at up to 100 times greater than that of the general population (Janoff et al., 1992, Jones et al., 1998). In addition, HIV-infected individuals are at increased risk of recurrent episodes of IPD with up to one in four experiencing a recurrence during a 12-month period (French et al., 2000, McEllistrem et al., 2002).

HIV infection results in defects in cell-mediated immunity, B cell dysfunction, loss of memory B cell subset, and suboptimal humoral immune responses. These defects increase the susceptibility of HIV-infected patients to *Streptococcus pneumoniae* infection (Titanji et al., 2006, Hart et al., 2007). The level of immunocompromise is considered the most important risk factor for IPD in HIV-infected individuals. Numerous studies have reported a decrease in incidence of IPD following the introduction of HAART likely due to resultant immune reconstitution (Feikin et al., 2004). However, this has not been a consistent finding with other studies reporting no significant change in incidence of IPD relating to use of HAART (Nunes et al., 2011, Jordano et al., 2004).

Other factors including older age, uncontrolled viral replication, alcohol consumption, injecting drug use and smoking have been shown to be associated with IPD in HIV-infected individuals (Harboe et al., 2014a, Rock et al., 2013b).

Factors such as vaccination with the 23-valent polysaccharide pneumococcal vaccine (PPV23) and antimicrobial prophylaxis for opportunistic infections such as co-trimoxazole have been identified in some studies as protective factors against IPD (Grau et al., 2005).

PPV23 has been shown to be safe and effective for prevention of pneumococcal infection in the general adult population (Moberley et al., 2013) and covers the majority of pneumococcal serotypes implicated in IPD (Hausdorff et al., 2000b, Hausdorff et al., 2000a). Given the burden of pneumococcal infection observed in HIV-infected individuals, PPV23 has been recommended in consensus immunistation guidelines in the United States (US) since 1996. However, clinical and immunological efficacy of PPV23 in HIV-infected adults remains debated (Pedersen RH, 2011).

More recently, conjugate pneumococcal vaccine (PCV) has been added to immunisation recommendations for HIV-infected individuals (Geretti, 2008, Konkle-Parker, 2014). PCV has been shown to prevent pneumococcal pneumonia in HIV-infected children (Black et al., 2000), to prevent recurrent IPD in HIV-infected adults (French et al., 2010b), and to elicit a greater immunological response in HIV-infected individuals when compared to polysaccharide pneumococcal vaccine (Lesprit et al., 2007b).

PCV was introduced to the national infant immunisation programme in Ireland in 2008. A 78% decline in IPD in all age groups due to serotypes covered by PCV7 has been observed due to both direct and indirect effect of the vaccine (HPSC, 2016). It is unknown whether these changes similarly have affected HIV-infected individuals.

Study Aims

The aim of this study was to examine temporal trends in the incidence of IPD in a cohort of HIV-infected individuals attending a single tertiary referral centre in Dublin Ireland between 2006 and 2015.

4.4.3 Methods

Study population and setting

This study was undertaken in St James's Hospital, Dublin. St James's hospital is a 1000 bed acute tertiary referral adult hospital which serves a catchment area of approximately 270,000 adults in a socially deprived area of Dublin city. The majority of patients admitted through the emergency department are in the highest quintile of deprivation index (Conway et al., 2013). Compared with other parts of the country, it is also an area of high HIV prevalence, with a crude prevalence rate of 2/1,000 population (Tuite et al., 2015).

The Department of GU Medicine and Infectious Diseases (GUIDE) clinic is the largest HIV specialist centre in Ireland with an active attending cohort of 2191 persons in 2015. Attending patients have universal access to HAART and vaccination. All patients attending GUIDE are recorded in a clinic database. Every patient who has attended within a 12-month period is counted in the active attending cohort for that year.

In 2002 an integrated vaccine unit with support from a data manager, specialist nurse and pharmacist was established in the GUIDE clinic to address poor vaccination uptake within the attending cohort. Vaccination records for all patients are maintained in a database within the unit.

Loss to follow-up in the cohort, defined as more than 12 months without contact, is approximately 10% (O'Connell et al., 2016). Approximately 90% of the attending cohort are on HAART while 89% have been vaccinated with PPV23 (O'Connell et al., 2016).

This study was approved by the St James's Hospital Intuitional Review Board.

Definitions

For the purpose of the study, IPD was defined as isolation of *Streptococcus pneumoniae* from blood, cerebrospinal fluid (CSF) or both. Blood cultures were performed routinely in the evaluation of HIV-infected patients with fever or pulmonary infiltrates on chest radiography throughout the study period.

Blood culture processing and detection techniques at the institution were not substantively altered during the study period.

A recurrent episode of IPD was considered to have occurred when more than 30 days separated isolation of *S. pneumoniae* from blood.

HAART was defined as the use of at least three antiretroviral agents from two or more classes (nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, or protease inhibitors).

Individuals who identified themselves as being an Injecting Drug User (IDU) was determined by self-report at the time of initial enrolment in the cohort.

Microbiology

Isolates of *S. pneumoniae* were identified by standard laboratory methods. Isolates were tested for susceptibility to penicillin, cefotaxime, tetracycline, erythromycin, clindamycin and levofloxacin using the E-test method (BioMerieux, Solna, Sweden). Minimum inhibitory concentration (MIC) to penicillin and cefotaxime were routinely performed on all sterile site isolates by the E-test, used in accordance with the manufacturer's instructions (AB BioMérieux, Solna, Sweden) and were interpreted according to the 2013 Clinical and Laboratory Standards Institute (CLSI) guidelines (Jackson and Janoff, 2008).

Isolates were considered antimicrobial resistant (AMR) if they were non-susceptible or had intermediate susceptibility to at least one of the antimicrobials. Multi-drug resistant isolates (MDR) were defined as resistant to three antimicrobials or more.

Isolates were serotyped using a combination of multiplex PCR and serological co-agglutination with pneumococcal antisera (Statens Serum Institut, Copenhagen, Denmark).

Statistical analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) for Windows Version 23 (SPSS, Chicago, IL, USA). Incidence rates of IPD were calculated as events per 100,000 person-years of follow-up and were compared during three calendar periods: 2006 - 2008, 2009 - 2012 and 2013 - 2015.

These calendar intervals approximately correspond with the period prior to introduction of conjugate pneumococcal vaccine to the infant immunisation program (2006 - 2008), introduction of the 7-valent conjugate program to the national infant immunisation program (2009 - 2012) and introduction of the 13-valent conjugate pneumococcal vaccine to the infant immunisation program (2013 - 2015).

Poisson regression was used to assess the linear trend in incidence rates over calendar time. To assess risk factors for IPD, four controls per case were selected from the cohort population under observation during the calendar period in which IPD occurred in the case (incidence–density sampling).

To assess risk factors for IPD, four controls per case were selected from the active cohort under observation during the time interval in which the case occurred. Controls were not matched with cases in any other characteristic. Variables including age, gender, risk of acquisition of HIV, smoking status, hepatitis C PCR status, pneumococcal vaccine status, CD4 T cell count and HIV viral load were recorded for cases and controls and included in univariate and multivariate analysis. Measures for cases were taken from most recent labs which were not greater than 6 months or not less than 1 week before the IPD episode. This avoided an acute illness effect on these laboratory markers in the cases.

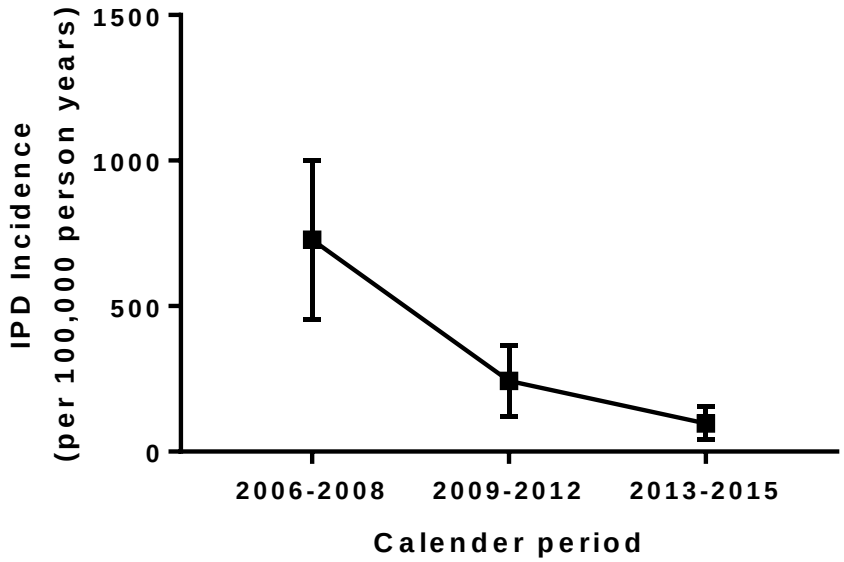
A bivariate analysis using the chi square and fisher exact test was conducted with a significance level of 0.05. A multivariate analysis was performed using binary logistic regression that incorporated variables associated with pneumococcal infection in the bivariate analysis.

4.4.4 Results

Overall 212 cases of IPD were identified from the microbiology surveillance system over the study period. Forty-seven (22%) cases of IPD occurred in 42 HIV-infected individuals over 16,008 person-years of follow-up (overall crude incidence rate of IPD in HIV-infected individuals, 293/100 000 person-years). Three patients had 2 episodes of IPD and one patient had 3 episodes of IPD during the study period. The overall case fatality rate was 15% (95% confidence interval (CI), 4-24).

The crude incidence of IPD per 100 000 person-years decreased from 728 [95% CI, 455 – 1002], to 242 (95% CI, 120–365) to 82 (95% CI, 40-154) in calendar periods 2006 - 2008, 2009 – 2012, and 2013 – 2015, respectively (P <0.01 for linear trend) (Figure 4.1).

Figure 4.1 Incidence of IPD 2006-2015 per 100,000 person years follow up



Factors associated with IPD

There were 168 controls selected for the 42 individuals who presented with IPD during the study period. Baseline demographics for cases and controls are outlined in Table 4.4.1.

On univariate analysis, Caucasian race, IDU as risk of acquisition of HIV, CD4 T cell count <200 cells/mm^3, hepatitis C virus infection, not taking HAART, smoking and non-receipt of PPV23 vaccine were significantly associated with IPD (Table 4.4.1).

On multivariate analysis, age, male gender, detectable HIV VL, non-receipt of pneumococcal vaccine remained significantly associated with IPD while IDU was of borderline significance (Table 4.4.1).

Table 4.4.1 Risk factors associated with invasive pneumococcal disease

Characteristic		Case (n = 42)	Control (n=168)	Bivariate analysis OR (95% CI) P value	Multivariate analysis OR (95% CI) P value
Mean Age [years (SD)]		38 (6)	35 (9)	1.05 (1.01 - 1.09), 0.02	1.10 (1.01 - 1.20), 0.02
Race [No. (%)]					
Caucasian		38 (90)	121 (72)	3.69 (1.25 - 10.91), 0.02	0.34 (0.04 - 3.15), 0.34
Non-Caucasian		4 (10)	47 (28)		
Sex [No. (%)]					
Male		29 (69)	108 (64)	1.24 (0.60 - 2.56), 0.56	4.00 (1.03 - 15.54), 0.05
Female		13 (31)	60 (36)		
Risk of acquisition of HIV					
IDU		36 (86)	59 (35)	13.38 (5.31 - 33.72), < 0.01	9.67 (0.90 - 104.30), 0.06
Other	Heterosexual	4 (10)	61 (36)		
	MSM	0	42 (25)		
	Other	2 (5)	6 (4)		
CD4 T cell count <200 cells/mm^3		20 (48)	32 (19)	3.99 (1.93 - 8.22), < 0.01	1.23 (0.35 - 4.35), 0.75
Hepatitis C RNA positive		32 (76)	39 (23)	10.26 (4.63 - 22.73), < 0.01	3.65 (0.78 - 17.13), 0.10
Undetectable HIV VL		3 (7)	110 (65)	52.96 (15.21 - 184.39), < 0.01	47.39 (8.85 - 253.83), < 0.01
Smoker		34 (81)	62 (37)	6.99 (3.04 - 16.07), < 0.01	1.19 (0.20 - 7.07), 0.85
PPV23 vaccine		13 (31)	121 (72)	6.28 (2.99 - 13.17), < 0.01	4.20 (1.18 - 14.98), 0.03

Abbreviations: HAART defined as the use of at least three antiretroviral agents from two or more antiretroviral classes, IQR interquartile range, IDU injecting drug user, MSM men who have sex with men, PPV23 23-valent polysaccharide pneumococcal vaccine, HIV VL, HIV viral load.

Vaccination with PPV23 was documented in 30% of cases prior to presentation with IPD (8 of 13 who had received PPV23 were infected with a vaccine serotype).

Microbiology

In 46 (98%) of cases of IPD, *S. Pneumoniae* was isolated from blood alone. In 1 case *S. Pneumoniae* was isolated from both blood and CSF.

Serotype Distribution of Pneumococcal Isolates causing IPD

Serotype was available for 34 (72%) pneumococcal isolates identified during the study period. There were 21 different serotypes identified (Table 4.4.2).

Table 4.4.2 Frequency and diversity of pneumococcal serotypes identified

Pneumococcal Serotype	Frequency Observed
1	1
4	3
6	1
6C	2
7F	1
8	2
9N	1
9v	3
11A	1
12F	1
15A	1
18C	1
19a	3
19f	1
20	3
22F	1
23F	1
28A	2
33f	3
34	1
35F	1

Seventy-six percent of serotypes isolated were contained in the PPV23 vaccine, 26% were contained in PCV7 while 41% of serotypes isolated were contained in PCV13.

Penicillin non-susceptible *S. Pneumoniae*

Six of forty-seven (13%) pneumococcal isolates from HIV-infected individuals were resistant to at least one antimicrobial. Four of forty-seven (9%) isolates were penicillin non-susceptible (all with intermediate level resistance).

4.4.5 Discussion

While the incidence of IPD in our cohort has decreased in recent years it remains significantly greater than that of the general population (9/100,000 in Ireland 2012, (HPSC 2016). The incidence of IPD reported in our study is similar to that observed in other studies examining IPD in HIV-infected individuals (Grau et al., 2005). However not all studies have reported such significant decreases in incidence of IPD (Barry et al., 2006). This may relate to differing demographic, social, economic and geographical factors between cohorts.

Over 85% of patients presenting with IPD in our study were injecting drug users (IDUs), the majority were not engaged in HIV care and not on HAART. Other studies have similarly identified persistent high-risk of IPD in IDUs (Harboe et al., 2014a). Other risk factors for IPD identified such as smoking, non-receipt of PPV23 and lower CD4 T cell count is consistent with previous studies (Grau et al., 2005).

The decrease in incidence of IPD observed in our HIV-infected cohort mirrors the decrease in IPD observed in the general population following introduction of PCV7 to the Irish childhood in 2008 (HPSC, 2016) due to the effects of herd immunity. It is likely that herd immunity has impacted on the epidemiology of IPD in our attending cohort however additional factors will have also contributed.

Patients attending the GUIDE clinic have universal access to pneumococcal vaccine and HAART. An integrated vaccine unit was established in the GUIDE clinic in 2002 to address poor vaccination coverage in the attending HIV-infected cohort. Research previously presented in this thesis has documented influenza vaccine coverage at 76% in the attending cohort (n=2035) and pneumococcal vaccine coverage of 75% in 2013 (section 3.4).

In this study, 72% of controls and 31% of cases had been vaccinated with PPV23. Of the IPD cases who had received PPV23 vaccine, 8 of 13 were infected with a vaccine serotype and median [IQR] time in years from receipt of vaccine to IPD episode was 3 [2-5].

Suboptimal protection with polysaccharide pneumococcal vaccine in HIV patients is well recognised (French et al., 2000, Watera et al., 2004). The conjugate pneumococcal vaccine has been shown to elicit a greater and more durable immunologic response and has been added to recent immunisation guidelines for HIV-infected individuals (Geretti, 2008).

Of the serotypes identified in our study, PCV7 would have covered only 26%, PCV13 would have covered 41% while PPV23 would have covered 76% of disease causing serotypes. While our study sample size is too small to reliably comment on serotype shifts, the last PCV 13 serotype causing IPD in our cohort occurred in early 2011 shortly following introduction of PCV13 to the national infant immunisation programme. Our findings indicate that PPV23 may cover more relevant serotypes than PCV13 in the future given the shift in disease causing serotypes observed due to direct and indirect effects of introduction PCV to the infant immunisation programme.

It will be important for policy makers to continue to monitor epidemiology of IPD serotypes at both national level and within risk groups such as HIV-infected individuals so appropriate immunisation recommendations can be made.

The benefit of PCV13 alone (as recommended in BHIVA guidelines 2015) may be limited if non-PCV13 serotypes become the main circulating serotypes and the main serotypes responsible for pneumococcal infections in HIV-infected individuals. It may be that the combination of PCV13 and PPV23 (as recommended in the IDSA guidelines 2014) or development of extended valency conjugate pneumococcal vaccines offers the broadest and most appropriate coverage.

Next generation pneumococcal vaccines directed toward common pneumococcal virulence factors are promising and may have the potential to offer serotype-independent coverage against pneumococcal diseases (Lujan and Gallego, 2016).

Ireland had one of the highest proportions of penicillin non-susceptible streptococci in Europe (17.5% in 2014), ranked fifth out of 29 countries. The proportion of antimicrobial resistance (AMR) in our cohort (13%) is high and reflects findings in the general population. Higher antimicrobial resistance rates have been reported in other HIV-infected cohorts and is likely promoted by antibiotic use for other infections and selective pressure from chronic antimicrobial use as may be the case with prophylaxis for opportunistic infections in individuals with low CD4 T cell counts (Munier et al., 2013).

This study has a number of limitations. This was a single centre study undertaken in Dublin, Ireland. Risk factors for IPD, circulating pneumococcal serotypes as well as the baseline characteristics and general health of our HIV-infected population may differ from other populations or areas. However, risk factors were similar to other reports. As IPD is a rare occurrence, the case number over the study period was relatively small. Controls were matched

to cases based on HIV diagnosis and attendance during interval in which IPD episode occurred. We cannot account for all factors that may have contributed to risk of IPD in the cohort.

The burden of IPD has decreased significantly in our HIV-infected cohort from 2006-2015. However, incidence remains significantly greater than that of the general population.

The observed decrease is likely multifactorial relating to improved vaccination coverage in the setting of an integrated vaccination programme, changes to HIV care guidelines driving earlier initiation of ART, herd immunity conferred following introduction of PCV to the infant immunisation programme and the changing demographics of individuals presenting with HIV infection.

Individuals with additional risk factors beyond HIV such as IDU as identified in our study are at greater risk of IPD and should be targeted with preventative interventions such as vaccine.

The ongoing burden of pneumococcal disease in HIV-infected individuals highlights the need for reduction of modifiable risk factors and development of more effective vaccines to further reduce pneumococcal-related morbidity and mortality in this high-risk group.

4.5 Immunological response to the 13-valent conjugate pneumococcal vaccine (PCV13) combined with the 23-valent polysaccharide pneumococcal vaccine (PPV23) versus PPV23 alone in HIV-infected adults

4.5.1 Abstract

The incidence of invasive pneumococcal disease (IPD) in HIV-infected individuals remains significantly greater than that of the general population despite the widespread availability of highly active antiretroviral therapy (HAART) and pneumococcal vaccines. The polysaccharide pneumococcal vaccine has been recommended for HIV-infected adults for since the early 1990's, however efficacy of the vaccine remains debated. Conjugate pneumococcal vaccine elicits a memory T cell response and it has been hypothesised that a prime-boost immunisation strategy combining a conjugate and polysaccharide pneumococcal vaccine may elicit a greater magnitude and duration of immunological response in HIV-infected individuals and thus offer better protection against pneumococcal infection.

This single-centre randomised clinical trial examined immunological efficacy of a prime-boost immunisation strategy combining 13-valent conjugate pneumococcal vaccine (PCV13) with 23-valent polysaccharide pneumococcal vaccine (PPV23) versus PPV23 alone in HIV-infected adults.

Quantitative serotype specific IgG antibody geometric mean concentrations (GMC) and killing opsonophagocytic assay (OPA) geometric mean titres (GMT) were greater in the prime-boost vaccine group versus the un-primed vaccine group at week 8 and week 28. This study adds to evidence supporting current pneumococcal vaccination recommendations in the United States and Europe for HIV-infected individuals.

4.5.2 Background

Pneumococcal infection remains a significant threat to HIV-infected individuals in the era of highly active antiretroviral therapy (Harboe et al., 2014b). Despite longstanding recommendations for the 23-valent polysaccharide pneumococcal vaccine (PPV23) in HIV-infected individuals in developed countries (Jain et al., 1995) , efficacy of the vaccine remains debated (Pedersen et al., 2010).

Efficacy of the conjugate pneumococcal vaccine (PCV) has been demonstrated for the prevention of pneumonia in HIV-infected children (Pavia et al., 2009) and for reduction of recurrent pneumococcal disease in HIV-infected adults (French et al., 2010a). Immunogenicity studies with PCV or combination regimens of PCV7 and PPV23 have been conducted in HIV-infected adults, yielding variable results (Lesprit et al., 2007a).

PPV23 covers serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F. PCV13 covers serotypes 1, 3, 5,6A, 6B, 7F, 9V, 14, 18, 19A, 19F, 23F.

There are no validated correlates of pneumococcal vaccine protection in adults. Historically, the most commonly recommended criteria for an adequate vaccination response in adults have been either an absolute level of >1μg/ml or a 4-fold increase in antibody concentration at a time point greater than 4 weeks following vaccination, usually in 70% of serotypes (Paris and Sorensen, 2007).

Clinical data on the protective effects of these thresholds are limited, particularly in immunocompromised patient groups and as a result, a universal consensus does not exist. Lower thresholds have been suggested as correlates of response for the percentage of serotypes (50% versus 70%) and the fold change (2-fold versus 4-fold) required (Orange et al., 2012) for a response to be documented.

Study Aims

The primary aim of this study was to compare serotype specific IgG GMC and fold increase in GMC for the 12 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) shared by both vaccines in the prime-boost versus the un-primed vaccine groups. OPA GMTs and fold increase in OPA titres for 13 serotypes including 12 shared by both vaccines as outlined above with an additional serotype 6A (present in PCV13 only) were compared between groups.

Secondary objectives were to compare the proportion of patient responders to the twelve shared pneumococcal polysaccharide serotypes (PPS) at week 8 and week 28 in the primed versus the un-primed vaccine groups.

Since this study was undertaken and given the potential benefits of conjugate pneumococcal vaccine in HIV-infected individuals and the high burden of pneumococcal infection, PCV13 has been added to existing recommendations for pneumococcal vaccination of immunocompromised populations (ACIP, 2013).

4.5.3 Methods

Pneumococcal vaccine naïve HIV-infected adults ≥18 years of age were randomized to PCV13 (Prevnar; Wyeth Pharmaceuticals) at week 0 followed by PPV23 at week 4 or PPV23 (Pneumovax; Merck) alone at week 4.

Inclusion criteria were HIV positive individuals ⩾18 years who were pneumococcal vaccine naïve with CD4 T cell count ⩾200 cells/mm^3, with absolute neutrophil count >750 cells/μL, haemoglobin level ⩾9.0 g/dL, platelet count ⩾100,000 platelets/μL, and aspartate and alanine aminotransferase levels ⩽3 times the upper limit of normal; total or conjugated bilirubin <2.5 times the upper limit of normal; calculated creatinine clearance by Cockcroft-Gault equation ⩾60 mL/min; and a Karnofsky performance score ⩾70.

All subjects provided written informed consent. Participants were stratified by computer generated randomization allocation sequence with equal probability of being randomised to either arm of the study. Vaccines were supplied in pre-filled single-dose syringes without preservatives and stored as directed at 2°C – 8°C. Vaccines were injected intramuscularly.

Immunogenicity

Serum concentrations of anti-capsular IgG for 12 shared PPS (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) were determined by a validated enzyme-linked immunosorbent assay and expressed as micrograms per millilitre (μg/ml) (Balmer et al., 2003).

Serum levels of functional antibacterial opsonophagocytic activity were measured using 13 serotype-specific validated assays. The OPA measures complement mediated phagocytic killing and is generally accepted as a key measure of vaccine response since opsonophagocytosis is considered to be the main protective response against (Cooper et al., 2011).

Titres were defined as the interpolated reciprocal serum dilution that resulted in complement-mediated killing of 50% of assay input bacteria. IgG concentrations and opsonophagocytic titres were measured in blood samples obtained before (week 0) vaccination, and at week 8 and week 28 post vaccination.

Serotype specific IgG responders were defined as patients who had a 2-fold increase from baseline of serotype specific IgG antibody levels (μg/ml). A second more stringent definition of responders was applied, where responders were defined as patients who met the first criterion and achieved serotype-specific IgG levels at least 1μg/ml.

OPA responders were defined as individuals who had a 4-fold or greater increase in OPA titre. The laboratory measurements were performed blinded to case-control status.

The criteria selected in our study to define responders was selected to provide comparison with defined responder parameters outlined in another study comparing a prime-boost vaccine strategy combining the 7-valent conjugate pneumococcal vaccine and PPV23 versus PPV23 alone in HIV-infected individuals (Lesprit et al., 2007a).

Statistical analysis

We calculated that a sample size of 100 participants, 50 in the prime-boost vaccine group versus 50 in the un-primed vaccine group would allow detection of a pre/post IgG difference of 0.87 with a significance level of 0.05 and a power of 10%. Descriptive statistics are presented as *N* (%) for categorical values, means with standard deviations (SDs) for numerical values. Total IgG values (reported as μg/ml) and OPA titres (reported as μg/ml) are expressed as geometric means with 95% confidence interval (CI) using logarithmically transformed assay results.

The Wilcoxon or Mann-Whitney U test were used to assess the increase in serotype-specific IgG and OPA titres from pre- to post-vaccination within study groups and for comparisons of post-vaccination IgG and OPA titres as appropriate. To correct for differences in pre-vaccination IgG concentration and OPA titres, we also compared the ratios of baseline IgG and OPA and post vaccine time points (week 8 and week 28) between vaccine groups.

We compared proportion of 'vaccine responders' between vaccine groups using Fishers exact test. Data analyses were performed using SPSS (version 22). No imputations were performed for missing data.

Ethical considerations

This was a single centre study undertaken in an ambulatory HIV outpatient clinic in the Department of GU Medicine and Infectious Diseases (GUIDE), St James's Hospital, Dublin, Ireland from July 2011-January 2013**.** As this study was commenced at a time when PCV13 was unlicensed for use in adults, a significant number of regulatory approvals were required. The study was approved by the St James's Hospital/Tallaght Hospital Research Ethics Committee (approval number 10102010) and the Irish Medicines Board (approval number 2095901). This study was registered on the European Clinical Trials Database,
(EudraCT number 2011-000260-99).

4.5.4 Results

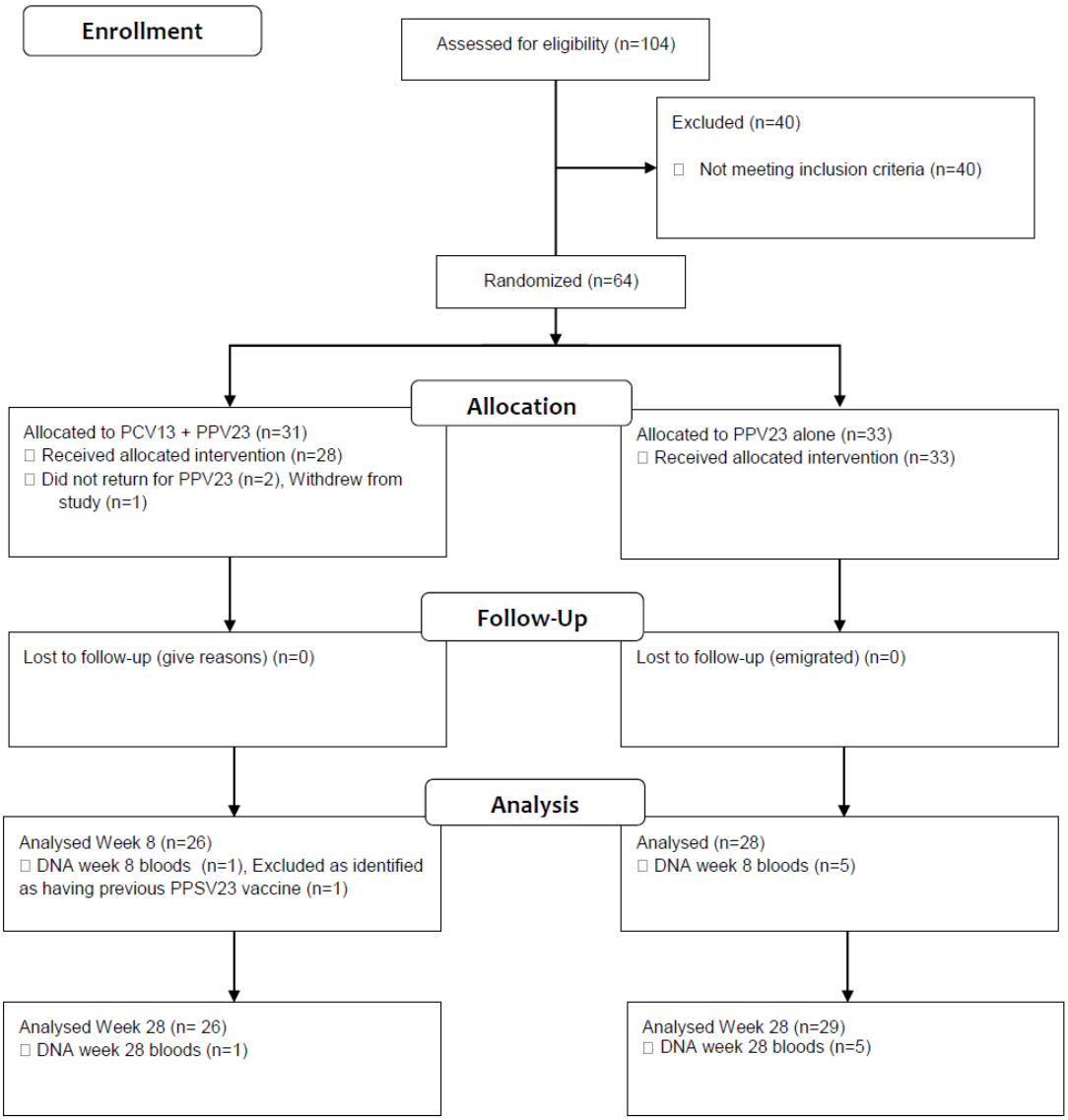
Study Flow

Of 104 eligible patients, 64 were agreeable to participate in the study. A total of 60 completed the vaccination series and contributed time point samples to the analysis as outlined in Figure 4.2. Overall 31 participants were randomised to receive the prime-boost vaccine strategy and 33 to receive PPV23 alone (figure 4.2).

Two patients who were randomised to the prime-boost group did not return for PPV23 within the allocated time frame and were thus excluded from the analysis. One patient randomized to receive PCV13 followed by PPV23 was subsequently identified as having received PPV23 outside the study and thus was excluded from further analysis. One who was randomised to the prime-boost vaccine withdrew from the study. A total of 26 samples were analysed at week 8 and week 28 in the prime-boost vaccine group.

All participants allocated to PPV23 contributed data for analysis. Five patients did not attend for bloods at week 8 and four failed to attend at week 28 as outlined in Figure 4.2. A total of 28 samples were analysed at week 8 and 29 at week 28 in the PPV23 alone vaccine group.

Figure 4.2 Study flow of vaccination with PCV13 and PPV23 or PPV23 alone



Abbreviations: PCV13, 13-valent conjugate pneumococcal vaccine, PPV23, 23-valent polysaccharide pneumococcal vaccine.

Patient Characteristics

Baseline characteristic of patients who participated in this study are outlined in Table 4.5.1. Mean age [SD] was 37 [10] years, 92% were male, mean CD4 T cell count was 503 [219] cells/mm^3. Prior to randomization 47% of patients were on highly active antiretroviral therapy. Mean HIV RNA was 2.37 $\log_{10}$ copies/ml.

Table 4.5.1 Baseline characteristics of patients by vaccine group

	TOTAL (n=60)	PPV 23 group (n=33)	PCV13+PPV23 (n=27)
Male n, (%)	55 (92)	29 (88)	26 (96)
Age (mean)[SD]	37 [10]	37 [10]	36 [10]
Race n, (%)			
Caucasian	48 (80)	25 (76)	23 (85)
Hispanic	7 (12)	4 (12)	3 (11)
Black	5 (8)	4 (12)	1 (4)
Risk group of HIV Acquisitions n, (%)			
MSM	49 (82)	25 (76)	24 (89)
Hetero	8 (13)	6 (18)	2 (7)
IDU	3 (5)	2 (6)	0
Mean CD4 T cell count [SD]	503 [219]	447 [181]	572 [243]
On HAART n, (%)	28 (47)	17 (52)	11 (41)
HIV $\log_{10}$ (mean) [SD]	2.37 [2.1]	2.16 [2]	3 [2]
Smoker n, (%)	20 (33)	11 (33)	9 (33)
Co-infection Hepatitis C (RNA positive)	5 (8)	3 (9)	2 (7)

Abbreviations: HAART, highly active antiretroviral therapy; SD, standard deviation; MSM, Men who have sex with men; Hetero, Heterosexual, IDU, injecting drug user.

Serotype specific IgG response

At week 8, GMCs in the prime-boost group were higher than in the un-primed group for all serotypes except for serotype 14. However, a statistically significant difference was reached for only one serotype, serotype 23F (3.20 vs. 0.52 μg/mL, $p<0.01$) (Table 4.5.2). Mean fold increase in GMC was greater in the prime-boost group (mean [SD] 8.69 [4.61] vs. 4.49 [1.24]) (Table 4.5.3).

Table 4.5.2 Geometric mean antibody concentration (µg/mL) for pneumococcal serotypes

	W0 IgG GMC[b]			W8 IgG GMC[b]			W 28 IgG GMC[b]		
	PCV13+PPV23	PPV23		PCV13+PPV23	PPV23	p[a]	PCV13+PPV23	PPV23	
Serotype	n=27	n=33	p[a]	n=26	n=28		n=26	n=29	p[a]
1	0.17	0.14		0.86	0.73		0.48	0.30	
	(0.05-0.37)	(0.09-0.19)	0.59	(0.51-1.94)	(0.45-1.17)	0.45	(0.25-0.77)	(0.21-0.43)	0.05
3	0.38	0.47		2.16	1.18		1.21	0.56	
	(0.11-1.07)	(0.26-0.87)	0.77	(1.21-3.42)	(0.60-2.30)	0.06	(0.84-1.98)	(0.33-0.95)	0.02
4	0.12	0.11		0.82	0.35		0.41	0.17	
	(0.05-0.15)	(0.08-0.15)	0.77	(0.33-1.26)	(0.19-0.64)	0.08	(0.23-0.57)	(0.12-0.24)	0.01
5	0.18	0.15		1.68	1.01		0.83	0.42	
	(0.05-0.40)	(0.10-0.24)	0.77	(0.84-4.77)	(0.49-2.09)	0.35	(0.44-1.97)	(0.21-0.81)	0.09
6B	0.18	0.18		1.56	0.81		0.82	0.49	
	(0.05-0.44)	(0.11-0.27)	0.84	(0.84-3.67)	(0.33-1.99)	0.20	(0.33-2.03)	(0.25-0.96)	0.18
7F	0.51	0.49		1.97	2.14		1.34	1.21	
	(0.24-0.29)	(0.33-0.71)	0.61	(0.80-4.38)	(1.29-3.54)	0.89	(0.72-2.19)	(0.80-1.86)	0.77
9V	0.29	0.27		1.97	1.17		0.99	0.70	
	(0.16-0.63)	(0.17-0.44)	0.7524	(0.90-4.38)	(0.63-2.18)	0.19	(0.52-2.09)	(0.38-1.25)	0.44
14	0.44	1.04		4.61	5.71		2.91	3.44	
	(0.16-0.63)	(0.57-1.90)	0.07	(1.88-20.96)	(3.15-10.36)	0.93	(1.24-8.15)	(2.03-5.81)	0.91
18C	0.17	0.32		3.67	1.81		1.44	1.08	
	(0.05-0.27)	(0.20-0.52)	0.06	(0.85-13.36)	(0.98-3.36)	0.20	(0.42-5.39)	(0.58-2.01)	0.73
19A	0.52	0.66		3.69	3.48		1.94	2.11	
	(0.32-0.27)	(0.40-1.10)	0.61	(1.65-10.80)	(1.63-7.40)	0.72	(0.84-4.11)	(1.10-4.02)	0.92
19F	0.43	0.45		2.90	1.54		1.51	0.88	
	(0.24-0.64)	(0.28-0.71)	0.45	(1.64-4.7)	(0.86-2.76)	0.18	(1.05-1.99)	(0.57-1.36)	0.04
23F	0.25	0.17		3.20	0.55		1.55	0.42	
	(0.05-0.92)	(0.11-0.28)	0.45	(0.86-14.44)	(0.28-1.07)	<0.01	(0.63-4.78)	(0.24-0.75)	0.01

Abbreviations: W, week; PCV13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; number of patients with valid assay results for the specified serotype;

[a] Student T test or Wilcoxon test

[b] GMCs were calculated using all subjects with available data for the specified blood collection. GMCs expressed as micrograms per millilitre (μg/mL).

PCV13 vaccine serotypes 1, 3, 5, 6A, 6B, 7F, 9V, 14, 18, 19A, 19F, 23F

PPV23 vaccine serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F

At week 28 GMC in the prime-boost group was significantly greater for 5 serotypes; 1 (0.48 vs 0.3 μg/ml, p=0.05), 3 (1.21-0.56 μg/ml, p=0.02), 4 (0.17 vs. 0.42 μg/ml, p=0.02), 19F (1.51 vs. 0.88 μg/ml, p=0.04), 23F (1.54 vs. 0.42 μg/ml., p=0.01) (Table 4.5.2). Mean fold increase in GMC remained greater in the prime-boost group (mean [SD] 4.39 [1.77] vs. 2.47 [0.67]).

Table 4.5.3 Fold increase in Serotype Specific IgG Geometric Mean

	W8		W 28	
Serotype	**GMFi PCV13+PPV23**	**GMFi PPV23**	**GMFi PCV13+PPV23**	**GMFi PPV23**
1	4.98	5.44	2.77	2.24
3	5.64	2.48	3.15	1.18
4	6.97	3.16	3.48	1.54
5	9.21	6.6	4.52	2.73
6B	8.57	4.6	4.5	2.8
7F	3.89	4.37	2.65	2.47
9V	6.7	4.26	3.18	2.52
14	10.42	5.47	6.57	3.29
18C	21.08	5.69	8.32	3.38
19A	7.14	5.23	3.76	3.17
19F	6.78	3.44	3.53	1.96
23F	12.97	3.19	6.3	2.43

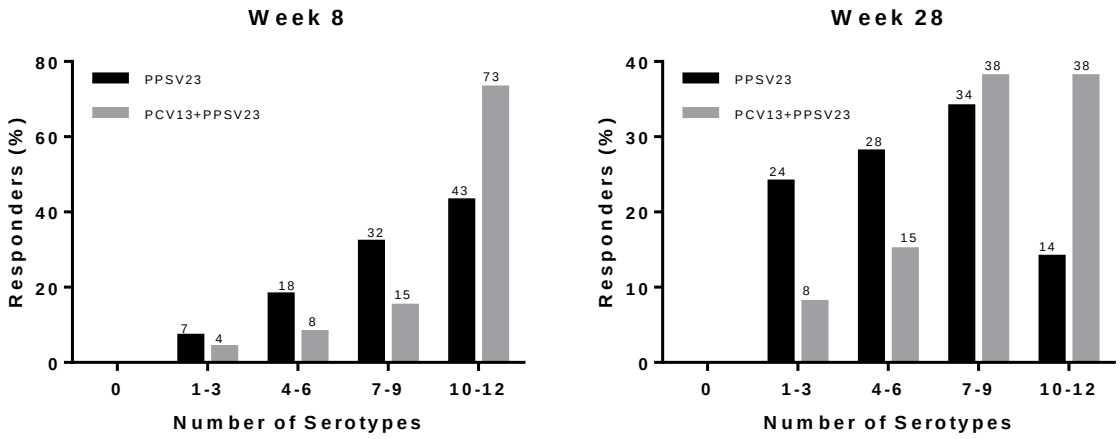
Abbreviations: W, week; GMFi, geometric mean fold increase; PCV 13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine

Proportion of IgG vaccine responders

At week 8, responders with a two-fold increase in serotype specific IgG levels to 0, 1-3, 4-6, 7-9 and 10-12 PPS were 0, 2, 5, 9, 12 in the PPV23 group versus 0, 1, 2, 4, 19 in the prime-boost group (Figure 4.3). The overall proportion of PPS responses in the prime-boost group was significantly greater than responses in the PPV23 group alone (80% versus 70% respectively, OR 2.00, 95% CI 1.38 – 2.92, p< 0.01).

At week 28, the frequency of responses to 0, 1-3, 4-6, 7-9 and 10-12 PPS were 0, 7, 8, 10, 4 (Figure 4.3) in the PPV23 group versus 0, 2, 4, 10, 10 in the prime-boost group. The overall proportion of PPS responses in the prime-boost group remained significantly greater than responses on the PPV23 group (70% versus 52%, OR 2.19, 95% CI 1.59-3.03 p<0.01).

Figure 4.3 Proportion of responders (patients with twofold increase in serotype specific IgG) at week 8 and week 28

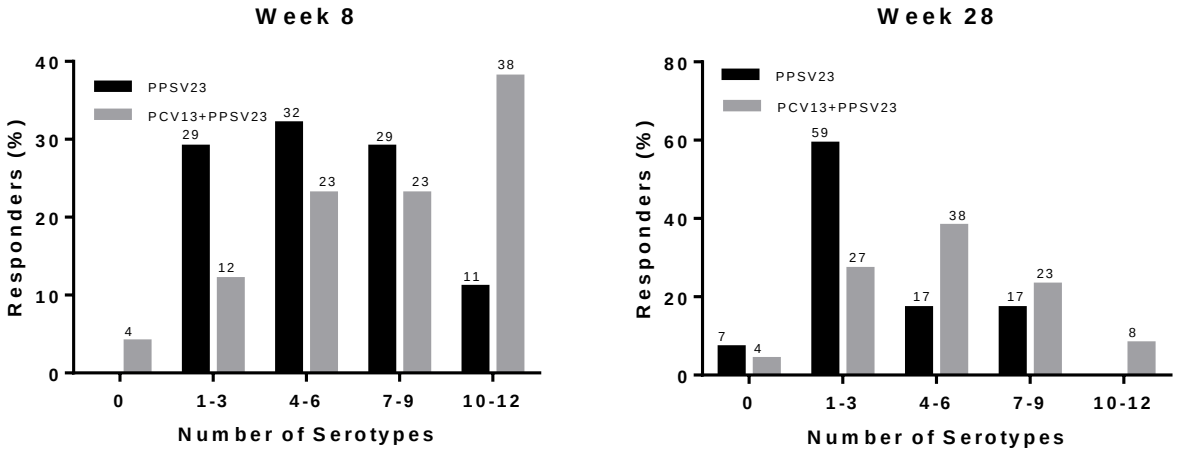


Applying the more stringent criteria where responders had both a twofold increase in PPS-specific antibody levels and IgG concentrations >1 μg/ml, the prime-boost vaccine strategy generated a higher proportion of serotype responders (Figure 4.3).

At week 8 number of responders to 0, 1-3, 4-6, 7-9 and 10-12 PPS were 0, 8, 9, 8, 3 in the PPV23 group versus 1, 3, 6, 6, 10 in the prime-boost group. Greater frequency of response was observed in the prime-boost group versus the PPV23-alone group (63% versus 46%, OR 2.00, 95% CI 1.46–2.74, $p < 0.01$).

At week 28, the frequency of responses to 0, 1-3, 4-6, 7-9 and 10-12 PPS were 2, 17, 5, 5, 0 in the PPV23-alone group versus 1,7, 10, 6, 2 in the prime-boost group. A greater frequency of response was observed in the prime-boost group versus the PPV23-alone group (43% versus 27%, OR 1.95, 95% CI 1.40–2.70, $p < 0.01$).

Figure 4.4 Proportion of responders (patients with both a twofold increase in serotype specific IgG and a IgG level >1 μg/ml) at week 8 and week 28



Opsonophagocytic Assay Response

At week 8 geometric mean titre (GMT) in the prime-boost group was significantly greater for serotype 6A (618 versus 38.55, p<0.01) and serotype 23F (330.9 versus 31.56, p=0.06), although this was of borderline significance (Table 4.5.4). Mean fold increase in GMT was greater in the prime-boost group also (mean [SD] 39.51 [37.46] versus 17.99 [21.47]) (Table 4.5.5).

At week 28 GMT in the prime-boost group was greater for serotype 6A (209 versus 23.0 μg/ml., p=0.05), serotype 14 (1010 versus 375.4 μg/ml., p=0.03) and serotype 23F (99.0 versus 18.3 μg/ml, p=0.03) (Table 4.5.4, Supplementary material). Mean fold increase in GMT in the prime-boost group remained greater (mean [SD] 12.67 [10.30] versus 4.97 [4.22]) (Table 4.5.5).

Table 4.5.4 Geometric mean antibody titres (*μ*g/ml) for pneumococcal OPA

	W0 GMT (95% CI)			Week 8 GMT (95% CI)			Week 28 GMT (95% CI)		
Serotype	**PCV13+PPV23**	**PPV23**	**P[a]**	**PCV13+PPV23**	**PPV23**	**P[a]**	**PCV13+PPV23**	**PPV23**	**P[a]**
1	4.98 (4.07-6.08)	4.80 (4.02-5.73)	0.89	27.35 (13.97-53.54)	33.42 (18.11-61.66)	0.85	12.20 (7.31-20.37)	12.34 8.51 17.91	0.29
3	5.28 (3.63-7.69)	5.28 (3.80-7.32)	0.91	19.53 (11.78-32.37)	13.36 (7.46-23.93)	0.66	6.91 (4.50-10.61)	5.95 (4.33-8.18)	0.36
4	10.19 (4.48-23.17)	6.77 (3.66-12.53)	0.67	938 (397-2216)	431 (153-1218)	0.26	281 (95.08-828)	50.05 (15.96-157)	0.26
5	5.39 (4.10-7.09)	4.42 (3.79-5.16)	0.24	55.19 (27.21-112)	24.60 (12.12-49.93)	0.80	22.02 (11.73-41.34)	10.36 (5.94-18.09)	0.19
6A	8.14 (3.98-16.66)	9.89 (4.83-20.25)	0.66	618 (238-1607)	38.55 (13.44-111)	<0.01	209 (72.90-598)	23.02 (7.95-66.66)	0.05
6B	68.12 (16.63-279)	81.54 (22.04-302)	0.82	738 (250-2178)	717 (253-2028)	0.40	522 (184-1479)	643 (266-1553)	0.68
7F	11.87 (5.10-27.64)	10.19 (4.97-20.91)	0.81	1354 (809-2267)	613 (295-1277)	0.21	395 (191-816)	142 (47.69-424)	0.88
9V	94.74 (26.67-337)	73.21 (24.02-223)	0.66	1324 (701-2502)	955 (386-2363)	0.65	1193 (772-1844)	292 (95.85-892)	0.31
14	190 (57.69-624.9)	414 (222-773)	0.24	2544 (1721-3762)	1266 (827-1938)	0.11	1010 (541-1886)	375 (185 762)	0.03
18C	13.69 (4.84-38.74)	7.92 (3.87-16.21)	0.81	1090 (466-2551)	295 (108-806)	0.23	225 (78.75-645)	97.45 (34.98 272)	0.20
19A	8.84 (5.44-14.38)	17.64 (9.78-31.81)	0.30	239 (127-452)	228 (127-408)	0.55	88.37 (48.72-160)	85.80 (46.80-157)	0.76
19F	10.41 (6.22-17.42)	13.24 (7.12-24.60)	0.31	274 (134-560)	157 (80.43-305)	0.47	57.10 (30.79-106)	30.66 (16.42-57.25)	0.50
23F	7.68 (3.90-15.10)	7.16 (4.03-12.74)	0.81	331 (128-856)	31.65 (11.79-85.00)	0.06	99.02 (37.64-261)	18.32 (8.50-39.52)	0.03

Abbreviations: W, week; PCV 13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; number of patients with valid assay results for the specified serotype;

[a]Student test or Wilcoxon test

GMTs were calculated using all subjects with available data for the specified blood collection.

PCV13 vaccine serotypes 1, 3, 5, 6A, 6B, 7F, 9V, 14, 18, 19A, 19F, 23F

PPV23 vaccine serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F

At week 8 the proportion of serotypes with a 4-fold increase in GMT was 64% in the prime-boost group versus 51% in the PPV23-alone group (OR 1.71, 95% CI, 1.22–2.39, p < 0.01).

At week 28, the proportion of serotypes with a 4-fold increase in GMT remained greater in the prime-boost group 48% vs. 36% in the PPV23-alone group (OR 1.6, 95% CI, 1.15–2.3, p < 0.01). There were no serious adverse events or adverse events observed or reported in vaccine recipients.

Table 4.5.5 Serotype Specific IgG Geometric Mean Titre Increase for OPA

	W8		W 28	
Serotype	**GMTi PCV13+PPV23**	**GMTi PPV23**	**GMTi PCV13+PPV23**	**GMTi PPV23**
1	5.50	6.96	2.45	2.57
3	3.70	2.53	1.31	1.13
4	90.0	63.62	27.54	7.39
5	10.24	5.56	4.09	2.34
6A	75.90	3.98	25.64	2.32
6B	10.83	8.79	7.67	7.89
7F	114.06	60.20	33.27	13.95
9V	13.98	13.04	12.59	3.99
14	13.40	3.06	5.32	0.91
18C	79.62	37.25	16.46	12.31
19A	27.07	12.91	9.99	4.86
19F	26.27	11.83	5.49	2.32
23F	43.11	4.15	12.90	2.56

Abbreviations: W, week; GMFi, geometric mean fold increase; PCV 13, 13-valent pneumococcal conjugate vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine

4.5.5 Discussion

This study demonstrated a high level of immunological response to both PPV23 alone and PCV13 followed by PPV23 in HIV-infected adults indicating that both vaccines will confer some protection against pneumococcal infection in HIV-infected adults.

This study suggests that combining PCV13 with PPV23 elicits a greater magnitude and durability of immunological response compared to PPV23 alone and represents the optimal approach for protecting HIV-infected persons from pneumococcal illness. At week 28 the proportion of individual serotype responses in the prime-boost group was significantly greater for both serotype specific IgG and OPA responses. This strengthens current pneumococcal vaccine recommendations which recommend combining PCV13 followed by PPV23 in pneumococcal vaccine naïve HIV-infected adults (Geretti et al., 2015, ACIP 2015).

We did not observe a direct correlation between serotype specific IgG and killing OPA response. Discrepancies between serotype specific and OPA assays have been reported in several adult populations including the elderly and immunocompromised. The OPA assay is generally regarded as a better measure of protection compared to antibody concentrations as it mimics the host phagocytic response (Song et al., 2013). Serological criteria for evaluation of pneumococcal vaccines in infants have been established, but correlates of protective pneumococcal immunity in adult populations and in immunocompromised groups are lacking (Westerink et al., 2012).

Immunological markers of immune response examined in this study included serotype specific IgG response and killing OPA response using validated assays. These markers represent immunologic surrogates of vaccine response. Currently there are no validated correlates of pneumococcal vaccine protection in adults. Historically, the most commonly recommended criteria for an adequate vaccination response in adults have been either an absolute IgG level of >1 μg/ml or a 4-fold increase in antibody concentration at a time point greater than 4 weeks following vaccination, usually in 70% of serotypes (Paris and Sorensen, 2007).

Clinical data on the protective effects of these thresholds are limited, particularly in immunocompromised patient groups and thus, a universal consensus does not exist. Lower thresholds have been suggested as correlates of response for the percentage of serotypes (50% versus 70%) and the fold change (2-fold versus 4-fold) required for a response to be documented (Orange et al., 2012).

Efficacy of the conjugate pneumococcal vaccine (PCV) has been demonstrated for the prevention of pneumonia in HIV-infected children (Pavia et al., 2009) and for reduction of recurrent pneumococcal disease in HIV-infected adults (French et al., 2010a). Immunogenicity studies with PCV or combination regimens of PCV7 and PPV23 have been conducted in HIV-infected adults, yielding variable results (Lesprit et al., 2007a).

A prime-boost immunisation strategy has been recommended as standard of care for pneumococcal vaccine naïve HIV-infected adults in the USA by the Food and Drug Administration (FDA) (ACIP, 2013) and in Ireland from 2014 (NIAC, 2012). The recently published British HIV association guidelines recommend a single dose of PCV13 for HIV-infected adults with the addition of PPV23 recommended for those identified as being at increased risk for pneumococcal infection (Geretti, 2008).

Previous pneumococcal vaccine recommendations suggested waiting for immunological recovery (i.e. CD4 T cell count >200) prior to administering pneumococcal vaccine to ensure optimal immunological response. While our study was not powered to detect differences in vaccine response based on CD4 T cell count, HIV viral load or HAART, other studies have indicated that response in the prime-boost group remained significantly greater when adjusted for these factors (Lesprit et al., 2007a). Immunisation guidelines now suggest proceeding with pneumococcal vaccination regardless of CD4 T cell count and to consider revaccination when immune recovery occurs on initiation of HAART.

Our study has limitations. Sample size was small and we did not recruit the desired number to power the study or to examine covariates associated with vaccine response. More subjects were lost to follow-up than expected further limiting the power of the study. In addition, whether the increase in antibody response observed for some serotypes after the conjugate vaccine in our study will translate into improved clinical efficacy is unknown.

Further studies addressing clinical end points are warranted as HIV-infected individuals will continue to be a group who will benefit greatly from protection against pneumococcal disease. Our study adds to evidence supporting the current pneumococcal vaccination recommendations in the United States and Europe for HIV-infected individuals.

4.6 IgG2 responses to pneumococcal vaccination

4.6.1 Abstract

IgG2 plays a critical role in immune response to pneumococcal vaccine. Previously in this Chapter we compared serotype specific IgG response and serotype specific OPA response to pneumococcal vaccine in HIV-infected individuals.

The aim of this study was to examine total IgG2 response to 23 pneumococcal serotypes contained in PPV23 in HIV-infected individuals randomised to a prime-boost vaccine strategy or un-primed vaccine strategy described in chapter 4.5.

We observed a high level of IgG2 response in the prime boost (92%) and un-primed vaccine groups (93%) at week 8. At week 28 68% of the prime boost group and 76% of the unprimed vaccine group had a >2-fold response to vaccine.to both pneumococcal vaccine strategies in HIV-infected individuals.

Our results are interesting in that no significant difference in IgG2 response was observed between vaccine groups. It is notable however that direct comparisons cannot be made as IgG2 level reported represents response to all 23 pneumococcal serotypes contained in PPV23.

4.6.2 Background

Conjugate pneumococcal vaccine studies rely on immunological surrogates of vaccine response to infer vaccine efficacy. Serotype specific IgG and killing opsonophagocytic assay (OPA) levels correlate well and have been shown to be associated with protection in infants (Black et al., 2000, Black et al., 2002). OPA assays are widely regarded as gold standard in assessing non-inferiority of new pneumococcal vaccines in infants (Jodar et al., 2003). However, IgG and OPA have not been shown to correlate well with OPA antibody response in older children and adults (Romero-Steiner et al., 1999).

Despite this, and in the absence of other appropriate end-points, OPA and IgG are currently used as surrogate endpoint to predict vaccine response and infer clinical benefit of pneumococcal vaccine in adults.

IgG is the main immunoglobulin in human blood. IgG is made up of a combination of four specific IgG subclasses: IgG1 (60-70%), IgG2 (20-30%), IgG3 (5-8%) and IgG4 (1-3%) and contains long-term protective antibodies against many infectious agents. Each IgG subclass serves a slightly different function in protection of the body against infection (Vidarsson et al., 2014). IgG1 and IgG3 subclasses are rich in antibodies against proteins such as the toxins produced by the diphtheria and tetanus bacteria, as well as antibodies against viral proteins (Ferrante et al., 1990). IgG2 antibodies are predominantly active against the polysaccharide capsule of certain disease-producing bacteria including *S. pneumoniae* (Siber et al., 1980).

Absolute or functional deficiency of IgG2 at any age is associated with susceptibility to infection with encapsulated organisms such as *S. pneumoniae;* thus IgG2 response to pneumococcal vaccination is of specific importance (Jefferis and Kumararatne, 1990).

Despite its critical role in eliciting protection against pneumococcal infection, IgG2 response to pneumococcal vaccine is HIV-infected individuals is poorly described in the literature with only two published studies measuring the IgG2 subclass response in HIV-infected individuals (Unsworth DJ, 1993, Ballet JJ, 1987). Both studies reported suboptimal IgG2 response to pneumococcal vaccine in HIV-infected patients.

Study Aims

The primary aim of this study was to compare IgG2 response to a prime-boost immunisation strategy combining PCV13 and PPV23 versus PPV23 alone in HIV-infected individuals.

4.6.3 Methods

In brief, pneumococcal vaccine naïve HIV-infected adults ≥18 years with CD4 T cell count > 200 cells/mm^3 were randomised to receive PCV13 at week 0 followed by PPV23 at week 4 or PPV23 alone at week 4.

IgG2 antibody response against all 23 pneumococcal capsular polysaccharides (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33) contained in PPV23 were measured at week 0, 8 and 28.

The Binding Site® anti-pneumococcal capsular polysaccharide IgG2 enzyme immunoassay kit (MK13) was used and performed in-house in collaboration with the Immunology department, St James's Hospital.

Assay Description

Microwells were pre-coated with pneumococcal capsular polysaccharides (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33). Samples were diluted in a diluent containing c-polysaccharide antibodies. Calibrators, controls and diluted patient samples were added to the wells and antibodies recognising pneumococcal capsular polysaccharide antigen bound during the first incubation.

After washing the wells to remove all unbound proteins, purified peroxidase sheep labelled anti-human IgG2 (y chain specific) conjugate was added. The conjugate bound to the captured human antibody and excess unbound conjugate was removed by a further wash step. The bound conjugate was visualised with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of antibody in the sample. Phosphoric acid was added to each well to stop the reaction producing a yellow end-point colour. Pre-and post-vaccine samples were run simultaneously. Calibrators and controls were included in each run. The measuring range of the assay was 1.1-90mg/L.

No level of protective immunity has been established for a collective response to multiple pneumococcal serotypes. A threshold value was therefore not used, but results were compared to published age-based ranges using the same assay.

Fold increase in IgG2 geometric mean concentration (GMC) and proportion of "responders" (≥2-fold increase in GMC and IgG2 GMC >0.35µg/mL) post vaccination were measured.

Statistical Analysis

Several of the parameters studied were not normally distributed. We therefore used non-parametric methods of statistical analysis where appropriate.

Geometric means are quoted in the figures and throughout the text. Paired data were analysed using the Wilcoxon's signed-rank test. Non-paired data were analysed using the Mann-Whitney U Test. Correlation between sets of non-parametric data used Spearman's rank analysis.

4.6.4 Results

IgG2 levels were checked on samples from patients included in the randomised controlled trial described in 4.5. In brief, 60 patients (mean age [SD] 37 [10] years, 92% male, mean CD4 T cell count 503 [209] cells/mm^3, 47% on HAART) as previously described (Table 4.5.1).

A wide range of pre-immunisation IgG2 concentrations were observed in the cohort pre-vaccination (Figure 4.5). Normal range baseline IgG2 is reported at 17-63 mg/l. A below normal baseline IgG 2 level (<17mg/l) was observed in 63% (38/60) of study participants.

Pre-vaccine IgG2 GMC was GMC (12.08 μg/ml, 95% CI 9.82-14.86 µg/ml). A significant increase in IgG2 GMC was observed at week 8 (63.60 μg/ml, 95% confidence interval (CI), 51.84 - 77.52 μg/ml, p<0.01) and week 28 (33.08 μg/ml, 95% CI 26.22 - 41.73 μg/ml, p<0.01) (Table 4.6.1).

Table 4.6.1 IgG2 GMC pre and post vaccination

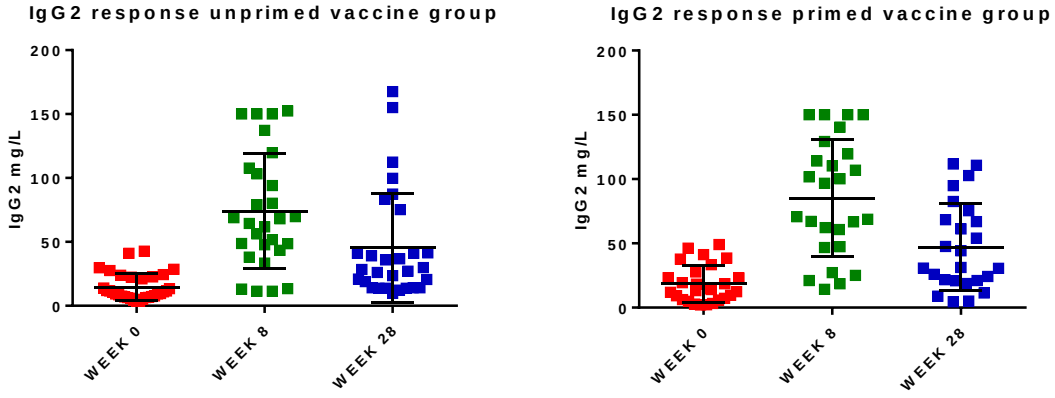
Total Cohort n=60	Week 0 mg/L (95% CI)	Week 8 mg/L (95% CI)	Week 28 mg/L (95% CI)
Geometric mean IgG2	12.08 (9.82-14.86)	63.88 (52.13-78.29)	33.08 (26.22 - 41.72)

Abbreviations: mg/L milligrams per Litre, (95% CI) 95% Confidence Interval

At week 8, 92% of the prime-boost and 93% of the un-primed group had a ≥2-fold increase in IgG2. No significant difference was observed in proportion of responders in the prime-boost vaccine group compared to the un-primed vaccine group (92% versus 93% respectively). Fold increase in mean (standard deviation SD) IgG2 GMC was comparable between the two groups

At week 28, 68% of the prime-boost group and 76% of the un-primed group had ≥2-fold increase in IgG2 (p=0.56). Fold increase in IgG2 GMC remained comparable between groups (mean [SD] 3.55 [2.87] versus 3.56 [2.84], p=0.99).

Figure 4.6 IgG 2 response post vaccination in study subgroups



4.6.5 Discussion

This study examined IgG2 response to pneumococcal vaccine. At baseline we found that 63% (38/60) of study participants had an IgG2 level <17 mg/L (normal IgG2 range 17-63 ml/L) (Agarwal, 2007). Abnormal IgG subclass levels have been reported in HIV-infected individuals, with studies reporting increased IgG1 and IgG3 levels and decreased IgG2 levels (Aucouturier , 1986) although these findings are not consistently observed (McGowan et al., 2006).

Low level of IgG2 may predispose HIV-infected individuals to infection with bacteria and encapsulated organisms early in HIV-infection. This may in part explain why high levels of pneumococcal infection have persisted in the era of HAART while more significant decreases in rates of other opportunistic infections have been observed.

At week 8 post pneumococcal vaccination, 93% of study participants had $\geq$2-fold increase in IgG2 response. At week 28 only 72% maintained a $\geq$2 fold IgG2 response. Rapid decline in antibody following pneumococcal vaccine is well described and has been reported in previous research undertaken in HIV-infected individuals attending the GUIDE department (Brown, 2010).

Studies examining IgG2 response to pneumococcal vaccine in HIV-infected individuals are limited and there are have been no studies examining IgG2 response to pneumococcal vaccine in HIV-infected individuals in recent years. The level of IgG2 response observed in our study (93% at week 8, 76% at week 28) are significantly greater than those reported in other of other published studies of PPV23 response (<50%) in HIV-infected adults (Unsworth, 1993;). The reason for the higher proportion of response observed in our study is likely multifactorial. Almost half of our study participants were on HAART at study entry and all had CD4 T cell counts >200 cells/mm^3. The referenced studies examined IgG2 response in HIV-infected and AIDS patients in the pre-HAART era which may have impacted vaccine response. In addition, increased sensitivity and specificity of the IgG2 assay over time may also account for the difference observed.

No significant difference in proportion of responders was observed in proportion of $\geq$2 fold IgG2 responders or fold increase in IgG2 response between the prime-boost versus the un-primed vaccine groups. Earlier in this Chapter, we reported high levels of serotype specific IgG and killing OPA response to PPV23 alone as well as the prime-boost immunisation strategy combining PPV23 and PCV13. We reported a greater magnitude and durability of serotype specific IgG and OPA response in the prime-boost group versus the un-primed group in HIV-infected individuals. IgG2 in this study was measured as response to all 23 serotypes in PPV23

versus the 13 serotypes included in the previous study which may account for the difference in response observed between studies.

Some immunisation guidelines are moving to recommend PCV13 alone in HIV-infected individuals given the limited data supporting efficacy of PPV23 (Geretti, 2015b). PPV23 contains 10 additional serotypes and will offer broader protection against pneumococcal infection. This may be of increasing importance as circulating PCV13 serotypes decline in the community in the context of infant immunisation programmes. Our study indicates that PPV23 elicits a comparable IgG2 response to the prime-boost immunisation strategy which again suggests that it may provide additional protection compared to PCV13 alone.

This study has a number of limitations including small study number. No protective level of IgG2 has been established and reported findings must be interpreted in this context. The IgG2 levels reported represent total IgG2 response to all 23 pneumococcal serotypes contained in the PPV23 vaccine thus direct correlation with serotype specific IgG response and OPA response is not possible. Total IgG2 may represent functional and non-functional IgG2 antibody and future studies examining correlation of serotype specific IgG2 and OPA response are warranted.

Protective immunological surrogates for pneumococcal vaccines remain to be defined in the general adult population or in HIV-infected adults. IgG2 is known to be an essential component of immune defences to *S. pneumoniae* infection. Further research is required to clarify the role of IgG2 antibody levels in conferring protection to both conjugate and polysaccharide pneumococcal vaccines.

4.7 Phagocytic response to a prime-boost immunisation strategy combining PCV13 and PPV23 versus PPV23 alone in HIV-infected individuals

4.7.1 Abstract

Phagocytosis is a complex and universally important component of the host defence to infection with encapsulated organisms such as *Streptococcus pneumoniae*. Here we describe the development of a multiplex in-house opsonophagocytic assay (OPA) to assess phagocytic or uptake response to three pneumococcal serotypes (7F, 14, 19A) in paired patient samples pre- and post-pneumococcal vaccination. This work was undertaken prior to securing access to the validated killing opsonophagocytic assay (OPA) (described in Chapter 4.5), which is now widely regarded as the gold standard measure of pneumococcal vaccine response.

Functional OPA assays including phagocytic and killing OPA's closely mimic host defences by measuring the ability of antibodies to effectively opsonise bacteria, leading to killing.

We developed a trivalent phagocytic OPA to measure uptake response to three pneumococcal vaccine serotypes (7F, 14, 19A) in a subgroup of patients. This assay was conducted on 12 participants in the pneumococcal vaccine study (6 who received prime boost vaccine and 6 who received unprimed vaccine) as outlined in Chapter 4.5.

We identified a significant increase in phagocytic uptake of fluorescent labelled pneumococcal beads for two of the three serotypes assessed at week 28 (7F, p=0.05 and 14, p=0.05).

4.7.2 Background

Host response to *S. Pneumoniae* infection is mediated by a combination of innate and adaptive immune responses including opsonising antibodies, complement, and phagocytic effector cells. (Guckian et al., 1980).

Phagocytosis is a key component of the innate immune response to *S. Pneumoniae* infection. It is responsible for the internalisation of bacteria by the cell membrane which facilitates lysis of bacteria. Phagocytosis is a complex process which can stimulate a diverse range of inflammatory mediators and cytokines resulting in responses of varying magnitudes and efficacy (Underhill and Ozinsky, 2002). Since the conjugate pneumococcal vaccine (Prevnar) was introduced for use in children in the United States (US) in 2000 and in Ireland in 2008 a significant decrease in pneumococcal infections has been observed.

The conjugate pneumococcal vaccine elicits antibodies to pneumococcal capsular polysaccharide, and these antibodies protect the host by opsonizing pneumococci and thus facilitating phagocytosis. The ability of a serum sample to opsonise bacteria can be measured by various in-vitro opsonophagocytosis assays (OPAs) including phagocytic or uptake assays and killing assays. Phagocytic assays measure uptake of fluorescent bacteria by granulocytes while killing assays measure actual killing of bacteria by enumerating surviving bacteria. OPAs have been shown to be the best functional correlate of protection in various studies (Romero-Steiner et al., 2006).

Evaluation of pneumococcal vaccine response is now heavily dependent on surrogates or immunological markers of clinical response. Clinical trial assessments of pneumococcal vaccine response generally measure the ability of a vaccine strategy to both stimulate antibody and elicit opsonic response. Total antibody levels and level of opsonic or functional response to pneumococcal vaccine has been shown to correlate well in infants, however a similar correlation has not been observed in older children, adults or groups with immune deficiency (Romero-Steiner et al., 1999). The reason for this may be that total antibody response measurement may record both functional and non-functional antibody. Additionally, emerging evidence suggests that additional components of the immune system such as IgM, which were not routinely measured as markers of response may play an important role, particularly in vaccine response in elderly patients (Park and Nahm, 2011). Currently, opsonisation assays are widely regarded as gold standard measurements of pneumococcal vaccine response.

Two major formats of opsonisation assays exist: the killing OPA and the phagocytic uptake OPA. Previously in this Chapter we have described results from our randomised controlled trial which measured killing OPA assay response using a validated assay from an accredited laboratory (Cooper et al., 2011). Phagocytic uptake assays have also been described and reported as measures of phagocytic uptake pre-and post-pneumococcal vaccination (Jodar et al., 2003).

The basic principles of these assays involved the incubation of targets with an opsonising mix of complement and serum, followed by endocytosis of opsonised targets to phagocytic cells. Phagocytosis is then stopped by rapid temperature reduction and cells which have phagocytosed fluorescent targets are identified by flow cytometry (Lehmann et al., 2000).

The aim of this study was to develop an in-house flow cytometric assay to measure phagocytic response to three common circulating pneumococcal serotypes (7F, 14, 19A) pre-and post-completion of pneumococcal vaccine strategy as previously described in HIV-infected adults. This phagocytic OPA assay work was undertaken prior to availability of the killing OPA described earlier in this chapter. The killing OPA has since superseded the phagocytic OPA and is now widely regarded as the gold standard measure of response to pneumococcal vaccine.

4.7.3 Methods

Study participants

A subgroup of 12 HIV-infected individuals were selected from the original recruited cohort (Chapter 4.5), six from the prime-boost vaccine group and six from the un-primed vaccine group.

Neutrophil isolation

Neutrophil isolation was carried out using a previously described technique (Conlon, 2013).

Briefly, 40 mls of EDTA blood was obtained from a healthy volunteer and mixed gently (Stuarr STR9 roller mixer, staffordt tube, UK).

A volume of 40 mls of whole blood (in EDTA) was placed in a 50ml Sarstedt tube (Sarstedt, Drinagh, Ireland).

Plasma was removed after spinning at 210rcf (Beckman Coulter Allegra X 17R, Beckman Coulter, High Wycombe, UK) for 20 minutes (no brake).

Ten mls of 6% Dextran (6g Dextran in 100mls of HBSS, without calcium or magnesium or phenol red, pH 7.0-74.) (Life Technologies, Paisley, UK) was added to the pelleted blood and made up to 40mls with 0.9% saline.

The suspension was placed at a 30º angle for 30 minutes and then placed upright for a further 10 minutes, allowing gentle sedimentation of red cells.

The neutrophil rich upper layer was removed and spun at 210 rcf for 10 minutes with no break
A 90% Percoll solution (Sigma Aldrich, UK) in 10X PBS was initially made up. From this a series od dilutions in 1XPBS were made resulting in 79%, 68% and 55% solutions.

The solution was then centrifuged gently at 201 rcf with no break for 10 minutes. The pellet was then re-suspended in 55% Percoll solution.

A gradient was set up in a 15mls Sarstedt tube (55%, over 68% over 79%). The gradient was placed in a centrifuge and spun at 210 rcf for 20 minutes.

Following centrifugation, the separated neutrophil layer was carefully removed using a 21-gauge needle. The neutrophils were washed (twice in HBSS) and re-suspended in HBSS (with calcium and magnesium, without phenol red, pH7.0-7.4) (Life Technologies, Paisley, UK).

Cell count was performed by light microscopy and cell suspension diluted to give a final concentration of 1000 cells/µl.

Bead based phagocytosis assay

A phagocytosis assay using pneumococcal coated polystyrene beads (Flow Apps Ltd, Illinois, USA) was established. This assay was based on the assay developed by Martinez *et al* (Martinez, 1999) with modifications included as outlined by Conlon *et al* (Conlon, 2013) .

OPA Buffer

OPA buffer was made up in 20 ml aliquots. 0.04g of powdered bovine serum albumin (BSA) was added to 20mls of HBSS with calcium and magnesium but without phenol red, under sterile conditions.

Complement

Six-week old baby rabbit sera was used as a complement source (Pel-Freeze, AK, USA). Lyophilised aliquots were stored at 4ºC. They were reconstituted as directed using 1ml of sterile water, aliquoted and stored at -80º until use.

Pneumococcal bead dilution

Three ul of each concentrated bead solution was diluted in 100ul of HBSS (with calcium and magnesium, pH 7.0-7.4) according to manufacturer's instructions to give a final concentration of 10,000 beads/µl.

In addition, a combination of 3ul (3ul x 3 = 9ul) of each bead type was diluted in 100ul of HBSS.

Fresh diluted bead solutions were made up for each assay with the volumes calculated dependent on the number of experimental wells used.

Antibodies

Serum from 12 HIV-infected individuals, 6 of whom were vaccinated with PCV13 followed by PPV23 and 6 of whom were vaccinated by PPV23 alone was used in the analysis. Individual

patient serum was tested separately and pre-and post-vaccine serums were analysed in the same experiment.

Polysaccharide conjugate polystyrene beads

Polystyrene beads conjugated to different pneumococcal polysaccharides were obtained from FlowApps (FlowAppsLtd, Illionois, USA). Three serotypes were obtained for assessment 7F (yellow), 14 (blue), 19A (pink).

The concentrated bead solution was diluted in HBSS (with calcium and magnesium) according to manufacturer's instructions to give a final concentration of 10,000 beads/µl. Each opsonophagocytic reaction mix required 20µl of the bead solution amounting to $2x10^5$ beads.

Fresh diluted bead solutions were made up for each assay with the volumes calculated dependent on the number of experimental wells used.

Opsonophagocytic assay

The OPA was carried out with 100ul reaction mixes in sterile 96 well round bottomed tissue plates (Corning Life Sciences, USA).

Volumes of all reagents were made up specific to the number of wells required to limit wastage.

A cell: target ratio of 1:4 was used.
Reactions were carried out at 37 degrees C.

Control wells were laid out as follows:
Positive control – 50 µl cells, 50ul serum, bead mix x 3 serotypes, OPA buffer, complement
Negative control – 50 µl cells, bead mix x 3 serotypes, OPA buffer, complement

Experimental wells contained:
50µl cell suspension
10µl study specific sera
20µl complement source
20µl bead suspension of varying polysaccharide serotype

A 100µl reaction mix was used in all wells; in those where reagents were omitted for experimental or control reasons volume was replaced with OPA buffer.

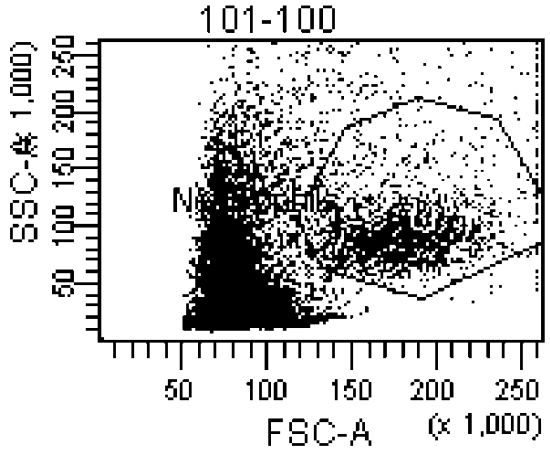
- STEP 1: Initially 20µl bead mix and 10µl opsonising serum was mixed in selected wells for 1 hour at 37ºC with slow agitation on a plate shaker 50 rpm (Stuart SSM1, BibbyScientific, Staffordchire UK).
- STEP 2: After antibody opsonisation, in appropriate wells, 20µl of the complement source was added and incubated at 37ºC for 20 minutes with slow agitation on a plate shaker.
- STEP 3: Finally, following pre-opsonisation and complement activation phases, 50µl of cell suspension was added.

The final OPA mixture was returned to the 37ºC incubator and agitates gently on a plate shaker. On completion reaction mixes were removed from the 96 well plates, placed in falcon tubes. Samples were analysed using a BD FACS Calibre Flow Cytometer (BD Biosciences, Oxford, UK).

Flow Cytometry

Samples were analysed immediately. The isolated neutrophil population was gated by simple forward and side scatter to exclude debris observed (Figure 4.7). Phagocytosis was measured as the percentage of total gated cells with an increase in fluorescence above the gated negative population.

Figure 4.7 Neutrophils gated by forward and side scatter.



Analysis

Data was analysed and graphed using Graph Pad Prism 5.0 (Graph pad Software, CA, USA). As results observed were not normally distributed non-parametric tests including the Wilcoxon and Man Whitney U test were used for comparisons.

4.7.4 Results

Baseline characteristics of subgroup participants included in this study are outlined in Table 4.7.1.

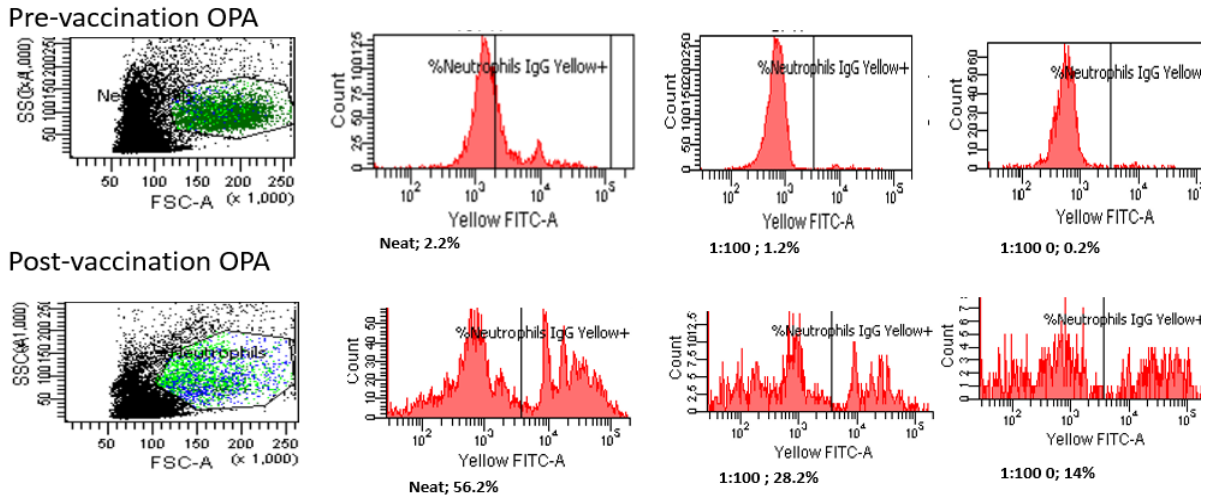
Table 4.7.1 Baseline characteristics of phagocytic OPA subgroup

	TOTAL (n=12)	PPV23 group (n=6)	PCV13+PPV23 (n=6)
Male n, (%)	10 (83)	5	5
Age (mean)[SD]	37 [10]	40 [12]	34 [9]
Race n, (%)			
Caucasian	9 (75)	6	3
Hispanic	2 (17)	0	2
Black	1 (8)	0	1
Group of Acquisition of HIV n, (%)			
MSM	9 (75)	4	5
Heterosexual	3 (25)	2	1
IDU	0	0	0
Mean CD4 T cell count [SD]	533 [254]	427 [148]	638 [384]
On HAART n, (%)	5 (42)		2
Undetectable HIV viral load	5 (42)	3	2
Smoker n, (%)	3 (25)	1	2
Hepatitis C RNA positive	1 (8)	1	0

Abbreviations, n number, MSM men who have sex with men, IDU injecting drug user, HAART highly active antiretroviral therapy, RNA ribonucleic acid

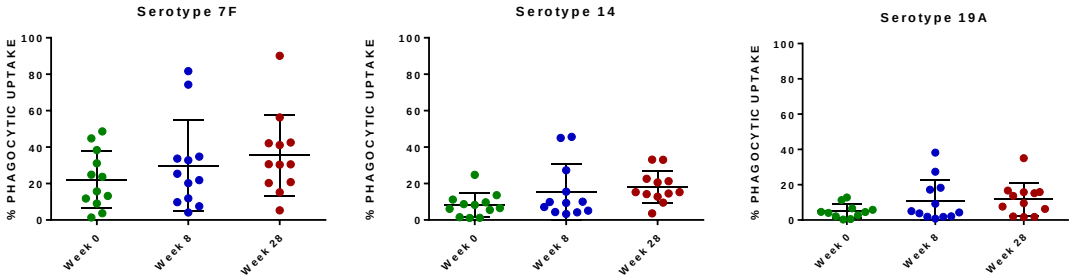
The flow cytometric OPA measured the change in fluorescence observed as effector cells phagocytose fluorescently labelled targets (percent of effector cells with fluorescent target uptake). Figure 4.8 illustrates fluorescence histograms for an individual patient pre-and post-vaccine (over a series of serum dilutions, 1/10, 1/100, 1/1000).

Figure 4.8 Opsonophagocytic fluorescence histogram for serotype 7F (yellow) over a series of dilutions in a single patient pre-and post-vaccination.



Percentage of phagocytic uptake observed in neat (undiluted) samples at time point analysis is shown in Figure 4.9.

Figure 4.9 Percentage phagocytic uptake observed at time point analysis



Percentage phagocytic uptake in neat (undiluted) samples were compared pre-and post-vaccination.

Linear trend in phagocytic uptake from week 0 to week 28 was calculated using a repeat measure ANOVA. A significant increase in the percentage of phagocytosed beads was observed at week 28 for serotype 7F (p=0.05) and serotype 14 (p=0.03) while the change in percentage of uptake was observed for serotype 19 was of borderline significance (p=0.06). (Table 4.7.2).

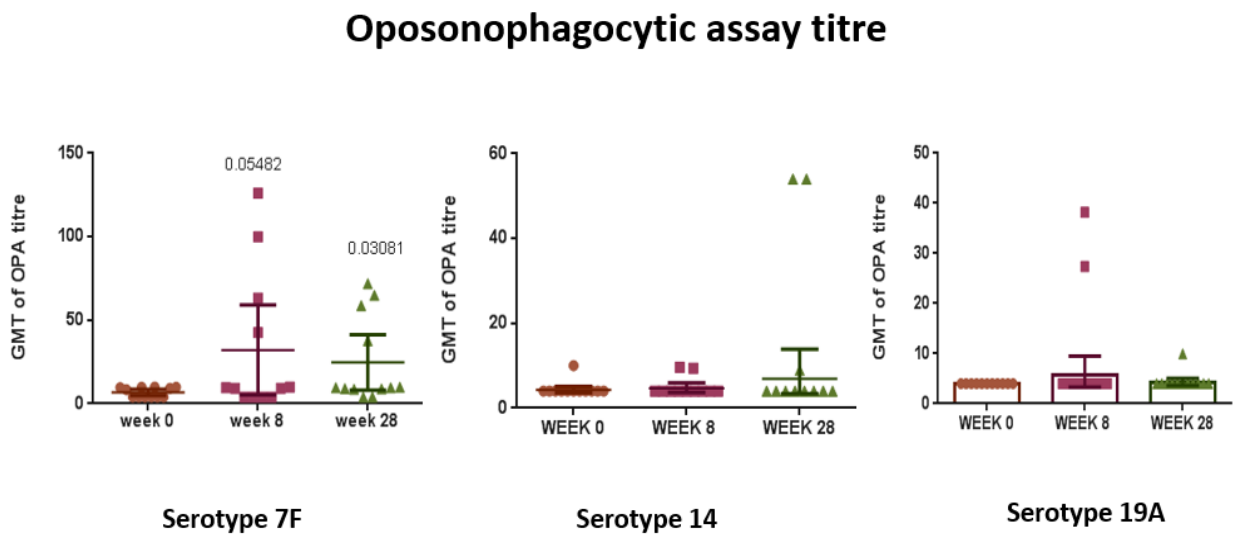
Table 4.7.2 Percentage of fluorescent phagocytic uptake in patients at time point assessment pre-and post-vaccination

% Phagocytosis	Week 0	Week 8	Week 28	P-values
Serotype 7F (yellow)	27% [16]	37% [24]	45% [24]	0.05
Serotype 14 (pink)	12% [9]	15% [18]	21% [12]	0.03
Serotype 19A (blue)	8% [7]	13% [11]	14% [10]	0.06

Data presented as mean [SD],
Linear trend between paired samples at week 0, 8 and week 28 (repeated measure ANOVA)

The reciprocal dilution at which 50% of maximal uptake for a given sample was reported as the titre for antibody reacting to a serotype-specific polysaccharide (Martinez, 1999). A significant increase in OPA titre was observed for a single serotype only, serotype 7F at week 8 and week 28 (Figure 4.10).

Figure 4.10 Phagocytic OPA titre response



Case specific IgG, killing OPA and phagocytic OPA responses (defined as >2-fold increase in titre post vaccine) are compared in Table 4.3.7.

Table 4.7.3 Comparison of IgG, Killing OPA and Phagocytic OPA response at week 28 post vaccine

	Serotype 7F response			Serotype 14F			Serotype 19A		
Case	IgG	Killing OPA	Phagocytic OPA	IgG	Killing OPA	Phagocytic OPA	IgG	Killing OPA	Phagocytic OPA
1	R	R	R	R	R	R	R	R	NR
2	R	R	R	R	R	R	R	R	R
3	R	R	NR	NR	R	R	NR	R	NR
4	R	R	NR	R	R	NR	R	R	NR
5	NR	NR	R	NR	NR	R	R	NA	R
6	NR	R	NR	R	R	R	NR	R	NA
7	R	R	NR	NR	NA	R	NR	R	NR
8	R	NR	NR	R	NR	NR	NR	R	R
9	R	R	NR	NR	NR	NR	R	R	NR
10	R	NR	R	R	NR	R	R	NR	R
11	NR	R	NR	NR	NR	NR	NR	R	NR
12	R	R	R	NR	NR	R	R	R	R

Abbreviation: R (Responder) defined as individuals with >2-fold increase in titre at week 28 post-vaccination
NR (Non-responder) defined as individual with <2-fold increase in titre at week 28 post-vaccination

4.7.5 Discussion

In this study, we have developed a trivalent fluorescent uptake pneumococcal OPA to assess pre-and post-serotype specific pneumococcal phagocytic uptake response to three pneumococcal serotypes (7F, 14, 19A). Multiplex uptake bead based flow OPAs have been demonstrated to be reliable measures of pneumococcal vaccine response without interference among different antigenic targets (Martinez, 1999). Uptake OPA assays however have significant labour requirements and are an extremely sensitive assay. Each experiment run undertaken for this study lasted 9-11 hours from neutrophil extraction stage to flow cytometry read stage. Additionally, given the delicate nature of neutrophils and the requirement for prompt use to avoid degradation artefact, each run had to be progressed through to completion without interruption once started. The significant labour requirements attached and sensitive nature of such assays has prompted the development and validation of other OPA assays and replacement of neutrophils with HL-60 cells (Fleck et al., 2005).

A study comparing four different OPA methodologies: (i) the killing-type OPA method determined the titer by measuring the number of surviving bacteria, (ii) two phagocytosis-type OPA methods measured the phagocytosis of fluorescent bacteria by flow cytometry, and (iii) one method measured the uptake of radiolabeled bacteria found the killing-type OPA to be the most sensitive method of measurement (Vakevainen et al., 2001).

OPA assays measurements are of particular importance in immunocompromised patient groups such as those with HIV infection. ELISA and OPA assays responses to pneumococcal vaccine have been demonstrated to correlate poorly in HIV-infected children suggesting that a proportion of measured antibodies may be dysfunctional thus highlighting the importance of functional assays in assessing response to pneumococcal vaccine (Madhi et al., 2005).

Our study has a number of limitations including small number of study participants which limits the power of this study to detect significant differences or factors associated with pneumococcal vaccine response. Our study was not powered to detect significant differences in assay response. Our assay is experimental and is not a validated assay for assessment of pneumococcal vaccine response.

While pneumococcal vaccine antibody response has a role in clinical assessment of immune deficiencies, OPA response is primarily used as a tool in clinical trials for assessment of response to pneumococcal vaccines. In this study, we demonstrate successful development of an in-house multiplex uptake OPA. While our assay provides important insights into

pneumococcal vaccine response in HIV-infected individuals, it has largely been superseded in recent years by the killing OPA assay as outlined and reported in our randomised controlled trial.

The killing OPA assay, which provides a measure a step beyond phagocytosis, is now regarded as the gold standard for assessment of pneumococcal vaccine response. The killing-type OP is thought to be more biologically relevant and there is more robust information regarding the performance of the killing-type OPA as well as the validation parameters needed available. The killing-type OPA that was originally published in 1997 by Romero-Steiner et al. and is posted on the internet (www.vaccine.uab.edu) should be the reference assay and can be used to validate other OPAs.

A final important issue to consider in the context of development and reporting of an in-house assay is assay validation. Although OPA techniques have been refined to become faster and more reproducible, inter-laboratory variability is still significant and is an important issue to be resolved and must be considered when interpreting pneumococcal vaccine study results (Romero-Steiner et al., 2003). Standardisation of OPA methods and measures used must be seen as a priority as new pneumococcal vaccines are developed and trialed.

4.8 Summary of Findings

HIV-infected individuals remain at increased risk of invasive pneumococcal disease. Ongoing efforts are required to improve vaccines and afford the best possible protection to our patients from this potentially life-threatening infection.

Both clinical and immunological efficacy of pneumococcal vaccine in HIV-infected individuals remains debated (Pedersen et al., 2011b). Indeed, we observed seven cases of pneumococcal vaccine failure in our IPD incidence study. Additionally, previous research undertaken in the GUIDE department identified that only a minority of HIV-infected adults had "protective" levels of antibody following vaccination with the PPV23 vaccine (Brown, 2010).

The prime-boost pneumococcal immunisation strategy investigated in our study indicates that the conjugate vaccine (PCV13) combined with the polysaccharide pneumococcal vaccine (PPV23) confers a greater magnitude of response (both serotype specific and OPA); however a statistically significant difference in response was not observed. Our study was undertaken when PPV23 alone was recommended as standard of care for HIV-infected adults and prior to licensure of PCV13 in adults. It represents the first randomised controlled trial examining PCV13 combined with PPV23 versus PPV23 alone in HIV-infected adults.

Although, there has been little research undertaken in the area, most recent immunisation guidelines recommend the prime-boost immunisation strategy PCV13 followed by PPV23 as standard of care for HIV-infected adults.

Recent BHIVA immunisation guidelines for HIV-infected adults recommend PCV13 alone for HIV-infected adults with addition of PPV23 recommended only in individuals with additional risks. Data supporting efficacy of PPV23 in HIV-infected adults is limited and the only RCT data available showed an unexpected adverse effect of the vaccine (French et al., 2000). Decisions regarding pneumococcal vaccine strategies must be take into account circulating serotypes and national infant immunisation strategies given the potential for herd immunity and serotype replacement.

In our cohort, no PCV13 serotypes caused IPD in HIV-infected individuals in recent years, likely due to herd immunity conferred from the PCV13 infant immunisation programme. This trend has been mirrored in the general adult population. PPV23 provides potential protection for 10 additional serotypes and this must be considered by policy makers when guidelines are being developed.

As extended valency pneumococcal vaccines are being tailed serotype protection may become less relevant. In the interim, efficacy of available pneumococcal vaccines must be clarified and to this end, I am currently funded by the Health research Board of Ireland to lead a Cochrane review examining pneumococcal vaccine for prevention of Streptococcus pneumoniae infection in HIV-infected adults (Sadlier et al., 2016).

5. Human papillomavirus infection and prevention in men who have sex with men (MSM) with a focus on HIV-infected MSM

5.1 Research outputs to date

Peer Reviewed Publications

Human papillomavirus infection and prevention in MSM. (Book Chapter)
C Sadlier, O Sheils, C Bergin.
Human papillomavirus. Research in a global perspective. ISBN 978-953-51-4776-3. 2016.
http://www.intechopen.com/books/human-papillomavirus-research-in-a-global-perspective

HPV vaccine acceptability in HIV-infected and HIV negative men who have sex with men (MSM) in Ireland.
C Sadlier, A Lynam, S O'Dea, S Delamere, M Quinlan, S Clarke, C Bergin.
Human Vaccines & Immunotherapeutics. DOI:10.1080/21645515.2016.1151588. 2016

Limitations of human papillomavirus DNA testing in measuring previous exposure and vaccine protection. (Response to letter)
C Sadlier, O Sheils, C Bergin; HPV MAPS research group (Human Papilloma Virus in Men, Awareness, Prevention Surveillance).
HIV Med. 2016 Feb 9. doi: 10.1111/hiv.12353.

Human papillomavirus (HPV) and the usefulness of the HPV vaccine for men who have sex with men (MSM).
C Sadlier, D Rowley, D Higgins, O Sheils, C Bergin.
J Infect Dis. 2014 Nov 15;210(10):1679. doi: 10.1093/infdis/jiu266.

Prevalence of human papillomavirus in men who have sex with men in the era of an effective vaccine; a call to act.
C Sadlier, D Rowley, D Morley, S Surah, S O'Dea, S Delamere, J O'Leary, P Smyth, S Clarke, O Sheils, C Bergin.
HIV Med. 2014 Sep;15(8):499-504. doi: 10.1111/hiv.12150.

In Preparation
Immunogenicity of HPV vaccine and natural history of HPV infection in young HIV positive MSM.
C Sadlier, S O'Dea, S Delamere, J Dunne, O Sheils, Bergin C.

Research Presentations

Prevalence and genotype of HPV infection at multiple sites in young HIV-infected MSM.
Oral presentation.
British HIV association (BHIVA), Spring meeting, Manchester, April 2016.

HPV vaccine acceptability in HIV-infected and HIV negative men who have sex with men (MSM) in Ireland.
Oral Presentation.
Society for the Study of Sexually Transmitted Diseases in Ireland (SSSTDI), Winter meeting Dublin, 2015.

Prevalence of HPV infection in MSM in Ireland.
Oral presentation.
5 Nations Health Conference, Dublin 2014.

Prevalence of HPV infection in MSM in Ireland.
Best research poster presentation.
Infectious Diseases Society of Ireland (IDSI) annual meeting, Cork, 2014.

5.2 Introduction

The epidemiological association between cervical cancer and sexual contact dates to the 1800's when an Italian physician observed high rates of cervical cancer on death certificates of prostitutes, widows and married women and relatively low rates in nuns and virgins. In the 1960's, studies proposed Herpes simplex virus type 2 (HSV2) as the etiological agent for cervical cancer (Rawls et al., 1968). A large scale prospective study subsequently discredited this theory (Vonka et al., 1984).

Dr Harold Zur Hausen was first to make the causal association between human papillomavirus (HPV) 16 and cervical cancer in the 1970's (zur Hausen et al., 1975). Dr Zur Hausen has been awarded the Nobel Prize in Medicine for his discovery. The landscape in relation to HPV infection has evolved rapidly in the intervening years.

HPV is causally associated with anal cancer, penile cancer, vulval cancer and oropharyngeal cancers. HPV associated cancers now account for over 5% of all cancers worldwide (Pankin and Bray, 2006).

The first effective HPV vaccine was licensed in 2006. Most developed countries have implemented national HPV immunisation programmes for young females, with an increasing number of countries calling for extension of the HPV vaccine to males given the burden of extra-cervical HPV associated disease observed.

Certain groups including men who have sex with men (MSM) and in particular HIV-infected MSM are disproportionately affected by HPV-infection and HPV associated anal cancer. Following implementation of HPV immunisation programmes in young females, significant decreases in incidence of HPV infection in young males were observed due to herd immunity. No protective effect was observed in MSM (Donovan et al., 2011).

Factors other than sexual behavior and immunosuppression have been demonstrated to impact on the prevalence and persistence of HPV infection. An association between smoking and cervical squamous cell cancer has been demonstrated (Appleby et al., 2006).

Currently in Ireland, the HPV vaccine is provided through the national immunisation programme for girls only. A pervasive recommendation has been made by the National Immunisaiton Advisory Committee (NIAC) stating that 'HPV vaccine should be considered in MSM'.

From December 2014, NIAC guidelines recommend the quadrivalent HPV vaccine for HIV-infected individuals (males and females) aged 26 years or younger however the HPV vaccine is currently not funded by the Department of Health. The cost for the HPV vaccine in Ireland ranges from €150-200 per vaccine amounting to up to €600 euro for three doses.

Despite NIAC recommendations and the potential protective benefits of the HPV vaccine in this high-risk group, cost remains a prohibitive factor for most. Cost effectiveness of HPV vaccine has only been investigated in MSM up to the age of 26 years (Kim, 2010). Research underway in the UK indicates that HPV vaccine may be effective in older MSM prompting the recent British HIV Association (BHIVA) recommendation for HPV vaccine in HIV positive individuals up to the age of 45 years (Geretti et al, 2015).

Study Hypothesis

I hypothesised that HPV infection causes a significant burden of potentially vaccine preventable disease in high-risk groups including MSM and HIV-infected MSM in Ireland.

Study Aim

The overarching aim this Chapter is to further knowledge and understanding of HPV infection and prevention of HPV infection in MSM and in particular HIV-infected MSM. This work will provide a basis for future policymaking on the use of targeted HPV vaccine in immunisation programmes for high-risk groups

5.3 Specific aims of this chapter

The specific aims of this chapter were:

- To document the incidence, risk factors and outcomes of anal cancer presentations in HIV-infected patients attending a single tertiary referral center.

- To determine the prevalence of anal HPV infection and high-risk (hr) anal HPV (HPV 16 and 18) infection in MSM and HIV-infected MSM in Ireland.

- To determine rates of persistence of anal HPV infection in HIV-infected MSM.

- To determine HPV vaccine acceptability in HIV-infected and HIV negative MSM in Ireland.

- To measure seroprevalence of hr HPV types 16 and 18 in young HIV-infected MSM.

- To examine natural history of HPV infection at multiple sites in HIV-infected MSM.

- To measure immunologic response to the quadrivalent HPV vaccine (HPV 4v) in young HIV-infected MSM.

5.4 Incidence, risk factors and presentation of anal cancer in a HIV-infected cohort in the era of highly active antiretroviral therapy (HAART): experience of a single centre

5.4.1 Abstract

The incidence of acquired immune deficiency syndrome (AIDS) defining malignancies including cervical cancer, Kaposi's sarcoma and lymphoma have decreased significantly in the era of highly active antiretroviral therapy (HAART). As HIV-infected patients live longer, they are at increased risk of non-AIDs defining malignancies (NADM). Anal cancer is now reported as the most common NADM observed in HIV-infected individuals in developed counties.

The aim of this study was to estimate incidence, risk factors and outcomes of anal cancer in a HIV-infected cohort attending an ambulatory HIV care centre in the absence of a routine anal cancer screening programme.

A retrospective cohort study was undertaken. All episodes of anal cancer presenting to St James's Hospital, Dublin, Ireland over a decade (2006-2015) were identified through the hospital inpatient enquiry system (HIPE). Basic demographic data, clinical characteristics along with information on staging at anal cancer at diagnosis, histological type and clinical outcome was recorded. Categorical data was described as totals and percentages, continuous data was reported as medians and interquartile range [IQR].

A total of 51 cases of anal cancer presented to St James's Hospital during the study period. Seven cases (14%) of anal cancer occurred in HIV-infected individuals. Estimated incidence of anal cancer in HIV-infected individuals was 44 cases per 100,000 person years follow up (95% confidence interval (CI), 20-90). Estimated incidence of anal cancer was 49 cases per 100,000 (95% CI, 1-97) in MSM, 34 cases per 100,000 (95% CI 13-81) in heterosexual males and 16 cases per 100,000 (95% CI 15-47) in heterosexual females. Both heterosexual males diagnosed with anal cancer had injecting drug use (IDU) as risk of acquisition of HIV and were co-infected with Hepatitis C. All cases of anal caner identified in this study occurred in individuals who had low nadir CD4 T cell count (median [IQR] 18 [6-19]).

HIV-infected individuals are at high-risk of anal cancer compared to the general population. HPV vaccine represents a promising opportunity to prevent HPV associated anal cancer in HIV-infected individuals in the future. Further research is required to identify effective screening tools for early detection and prevention of anal cancer in HIV-infected individuals in who remain at increased risk.

5.4.2 Background

Anal cancer accounts for approximately 2.5% of all cancers of the gastrointestinal tract (Siegel et al., 2013). Anal squamous cell carcinomas (ASCC) account for 85% of anal cancers (Myerson et al., 1997).

Although relatively rare in the general population with a reported incidence of 1-2 cases per 100,000 (van der Zee et al., 2013), the incidence of ASCC has increased steadily in the past three decades. In contrast, the incidence of adenocarcinoma of the anus has remained stable over the same time interval (Nelson et al., 2013).

While ASCC is classified as a cancer of the gastrointestinal tract, it shares more similarities with cancers of the genital tract, in particularly cervical cancer. Both cancers occur at transitional zones in the squamo-columnar epithelium, are associated with HPV infection (Palefsky et al., 1991) and both are associated with greater number of sexual partners (Frisch et al., 1997), smoking (Frisch et al., 1997) and HIV infection (Goldstone et al., Mooij et al.).

The incidence of anal cancer in the general population in Ireland is reported at 1.04 cases per 100,000 from 2006-2013 with a higher incidence observed in females compared to males (NCRI, 2016).

Men who have sex with men (MSM) and in particular HIV-infected MSM are disproportionately affected by anal cancer. The incidence in MSM, reported at up to 35 cases per 100,000, is greater than the incidence of cervical cancer pre-introduction of cervical screening programs (Machalek et al., 2012, Gustafsson et al., 1997). The incidence of anal cancer in HIV-infected MSM is reported in the range of 42-135 cases per 100,000 (Patel et al., 2008, D'Souza et al., 2008).

The majority of AIDS defining malignancies have decreased since the advent of HAART, however the incidence of anal cancer has increased steadily (Piketty et al., 2008, Brickman and Palefsky, 2015). As HIV-infected individuals live longer, the survival benefits associated with HAART have revealed a cumulative risk of anal cancer that had not been observed previously due premature mortality relating to HIV.

HPV infection is causally associated with over 80% of anal cancers. HPV type 16 causes 66% of anal cancers while HPV type 18 is responsible for an additional 6% of cases (De Vuyst et al., 2009). The prevalence of anal HPV infection in MSM is higher than that observed in

heterosexual men (47.2% versus 12.2%) (Nyitray et al., 2011a). The prevalence of high-risk (hr) or oncogenic anal HPV infection is documented at 26-73% in HIV negative MSM (van der Snoek et al., 2003, Chin-Hong et al., 2004). Prevalence of hr HPV has been shown to be significantly higher in HIV-infected MSM compared to HIV negative MSM with prevalence reported at up to 93% (Sadlier et al., 2014b).

Persistent HPV infection is thought to promote changes to cellular DNA resulting in cellular dysplasia (Darragh et al., 2012). Dysplastic changes observed in the anal canal are termed anal intra-epithelial neoplasia (AIN). AIN is graded as AIN-1 (often referred to as low-grade AIN), AIN-2 and AIN-3 (often referred to as high-grade HGAIN). AIN grades represent increasing severity similar to the classification of cervical intraepithelial neoplasia (CIN-1, CIN-2, CIN-3). HGAIN is regarded as a precursor for ASCC although progression from HGAIN to anal cancer remains poorly understood.

While some experts advocate screening for anal cancer in at risk populations including immunosuppressed patients or MSM (Palefsky, 2009, Barroso, 2012), current screening modalities have not demonstrated improvements in morbidity or mortality and no consensus recommendations exist.

Study Aims

The primary aim of this study was to estimate the incidence of anal cancer in a HIV-infected cohort from 2006-2015 attending a single tertiary referral ambulatory HIV care centre in the absence of a formal anal cancer screening programme.

Secondary aims were to identify baseline and clinical characteristics associated with anal cancer and to describe symptoms present at diagnosis, tumour staging at diagnosis and clinical outcome.

5.4.3 Methods

A retrospective cohort study was undertaken. All episodes of anal cancer diagnosed in St James's Hospital were identified using the hospital inpatient enquiry (HIPE) system in association with histopathological surveillance records. Anal cancer cases occurring in HIV-infected individuals were identified by cross referencing anal cancer cases with the GUIDE clinic data base. This database records all patients with HIV that have ever attended St James's Hospital.

Basic demographic data including risk of acquisition of HIV, nadir CD4 T cell count, duration of HIV diagnosis and anti-retroviral therapy was collected from electronic patient records and/or patient charts. Characteristics of anal cancer at diagnosis including staging, histological type, intervention and outcome were recorded.

5.4.4 Results

Fifty-one cases of anal cancer were diagnosed in St James Hospital during the study period. Seven cases (14%) occurred in HIV-infected individuals. An additional 7 cases of AIN III (Tis or anal carcinoma in-situ) were identified in HIV-infected individuals during the study period.

A total of 3161 HIV-infected patients were identified as having attended St James's Hospital on at least one occasion from 2006-2015 with 16,008 patient years of follow up recorded. Estimated incidence of anal cancer in HIV-infected individuals was 44 cases per 100,000 person years of follow up (95% confidence interval CI, 20-90).

Estimated incidence of anal squamous cell cancer (ASCC) was 49 cases per 100,000 (95% CI, 1-97) in individuals with MSM documented as risk of acquisition of HIV, 34 cases per 100,000 (95% CI 13-81) in injecting drug users (IDU) and 16 cases per 100,000 (95% CI 15-47) in heterosexual females.

Baseline characteristics of patients presenting with anal cancer are outlined in Table 5.4.1.

Table 5.4.1 Baseline characteristics of patients presenting with ASCC and AIN3 from 2006-2015.

	ASCC n (%)	**AIN 3 n (%)**
Total	**7**	**7**
Median [IQR] age at diagnosis	38 [35-49]	48 [29-54]
Male	6 (86)	7 (100)
Region of Origin		
Ireland	7 (100)	7 (100)
Risk of acquisition of HIV		
Heterosexual	1 (14)	0
MSM	4 (57)	5 (71)
IDU	2 (29)	2 (29)
Median [IQR] duration of HIV infection at diagnosis	3 [2-5]	5 [2-8]
Median [IQR] CD4 T cell count (cells/mm^3)	239 [135-401]	395[274-651]
Nadir CD4 T count (cells/mm^3)	18[6-19]	147[102-301]
On HAART	6 (86)	7 (100)
HIV VL ND	6 (86)	7 (100)
Smoker	6 (86)	4 (57)
Hepatitis C RNA positive	2 (29)	1 (14)
Hepatitis B sAg positive	1 (14)	0

Abbreviations: n number, IQR Interquartile range, MSM Men who have sex with men, IDU Injecting Drug User, HAART highly active antiretroviral therapy, VL viral load. RNA ribonucleic acid, sAg surface antigen

All HIV-infected patients in our cohort who were diagnosed with anal cancer were symptomatic at ASCC diagnosis. The most common symptoms prompting referral for proctoscopy and colorectal review were passage of bright red bloods PR (71%), awareness of a mass in the anal canal 57% and proctalgia (43%). Perianal HPV was present in 57% of patients presenting with anal cancer. Symptoms in patients presenting with anal cancer are outlined in Table 5.4.2.

Table 5.4.2 Presenting symptoms in HIV-infected individuals with anal cancer

Total number of cases n	7 (%)
PR Bleeding	5 (71)
Awareness of a mass PR	4 (57)
Proctalgia	3 (43)
Change in bowel habit	2 (29)
Perianal HPV	4 (57)

Abbreviations: PR per rectum, HPV human papillomavirus

Anal cancer is staged according to size. T1 lesions are less than 2 cm, T2 lesions are 2-5 cm and T3 lesions are greater than 5 cm. Lesions that invade vagina, urethra or bladder are classified as T4 regardless of size. Invasion to the rectum, skin, or sphincter muscles is considered a T4 lesion. Lymph node metastasis is divided into perirectal, iliac and inguinal lymph node (Edge et al., 2009).

Histological type and staging of anal cancers presenting in HIV-infected individuals in our case series are outlined in Table 5.4.3. The majority of patients had a good performance status on presentation. A single patient had nodal extension at diagnosis and no patients had evidence of metastatic disease beyond the pelvis.

Table 5.4.3 Characteristics of anal cancers

Total	**N=7**
Histologic subtype	
ASCC	7
WHO performance status	
0	6
1	1
2	0
Size of primary tumour at diagnosis	
T0	0
Tis	7
T1	0
T2	4
T3	3
Lymph node involvement %	
N0	6
N1	0
N2	1
N3	0
Extra-pelvic metastases (%) Stage	
0	7
I	0
II	0
III	0
Management	
Abdomino-peritoneal resection	3
Chemotherapy	7
Radiotherapy	7

Abbreviations: ASCC Anal squamous cell carcinoma, Tis squamous carcinoma in situ, WHO World Health Organisation

Three patients (43%) underwent abdomino-peritoneal resection (APR) with defunctioning colostomy and ileostomy. One patient had reversal of the colostomy. All seven patients received combination chemo-radiotherapy (CRT).

A single patient who had undergone AP resection with adjunctive CRT had a recurrence of ASCC during the study period. Two patients (29%) died during the study period, neither death was directly related to the anal cancer diagnosis.

5.4.5 Discussion

The incidence of anal cancer reported in the general population in Ireland from 2006-2013 ranges from 0.8 per 100,000 to 1.2 cases per 100,000 (NCRI, 2016).

We report a crude incidence of 44 cases per 100,000 patient years of follow up occurring in a HIV-infected cohort in the era of HAART (2006-2015).

The incidence of anal cancer in other HIV-infected cohorts in the era of HAART has been reported at 65-109 cases per 100,000 (Piketty et al., 2012, D'Souza et al., 2008, Chaturvedi et al., 2009, Machalek et al., 2012). The majority of these studies were undertaken in institutions which implement proactive anal cancer screening programmes for at risk HIV-infected individuals. It is possible that the lower incidence of anal cancer observed in our cohort represents a relative underdiagnoses of anal cancer in our cohort in the absence of any anal cancer screening programme or protocol.

Anal cancer has been shown to occur in HIV-infected individuals at a younger age (Oehler-Janne et al., 2008). This finding is reflected in our cohort where median age at diagnosis was 38 years. Median age at diagnosis in the general population has been reported at 60 years (http://www.cancer.gov/cancertopics/types/anal., Accessed March 2016).

Six of the seven cases of anal cancer identified in this study occurred in males. The male preponderance observed in our study reflects findings in other HIV-infected cohorts however is at odds with findings in the general population where the incidence of anal cancer is reported at 3-5 times greater in females (http://www.cancer.gov/cancertopics/types/anal., Accessed March 2016).

HIV-infected MSM have been identified as the highest risk group for anal cancer (Sadlier et al., 2014b) and this is thought to account for the male preponderance of anal cancer in HIV-infected cohorts. Four of 6 HIV-infected males with anal cancer had MSM documented as risk of acquisition of HIV, 2 were IDUs (heterosexual). Estimated incidence of anal cancer in HIV-infected MSM in our study was comparable to that observed in IDUs (heterosexual) (p=1).

Median [IQR] CD4 T-cell count at anal cancer diagnosis was 239 [135-401]. Two patients had CD4 T cell counts less than 200 cells/mm^3 at anal cancer diagnosis, both were IDUs. Median nadir CD4 T cell count in our study was 18 cells/mm3 [6-19]. All patients had CDC stage B3 or C3 at HIV diagnosis. This finding supports other large cohort studies which have suggested that

individuals with lower nadir CD4 T cell count are at increased risk of anal cancer (Piketty et al., 2012).

Six of the seven patients who had anal cancer in our study were virally suppressed on HAART at anal cancer diagnosis and the majority had achieved significant immune reconstitution. No correlation between HIV viral load and anal cancer has been observed (Bertisch et al., 2013). Six of seven cases in our study were smokers. Smoking is a well described factor associated with persistence of hr HPV and a risk factor for anal cancer (Frisch et al., 1997).

All patients in our study had symptoms at anal cancer diagnosis prompting referral for investigation leading to diagnosis (Table 5.4.2). It has been estimated that 20% of anal cancers are asymptomatic highlighting the potential for screening. Screening is further supported by evidence that earlier stage at diagnosis correlates to improved long-term prognosis (Osborne et al., 2014).

Anal cancer stage at diagnosis has been reported as similar in HIV-infected and HIV negative individuals (Osborne et al., 2014). All anal cancers in our study were stage T2 (2-5 cm) at diagnosis indicating that the lesions could potentially have be palpable on digital rectal exam (DRE). DRE has been advocated by some experts as a basic screening tool for anal cancer however it is not routinely used in clinical practice (Landstra et al., 2012).

Three patients in our study underwent abdominoperineal (AP) resection of anal cancer. All seven patients underwent chemoradiotherapy (CRT). We did not examine treatment related toxicities in our study however HIV-infected individuals have been reported to experience more treatment toxicities and are more likely to have local relapse of anal cancer compared to HIV negative individuals (Oehler-Janne et al., 2008).

One of seven cases (14%) in our cohort experienced a relapse of anal cancer during the study period. Three-year overall survival rate in our study was 5/7 (71%) which is comparable to survival reported in the general population. Three-year survival rate in other HIV-infected cohorts has been reported as significantly worse compared to the general population (42% versus 76%, p = 0.037; HR, 2.335 (95% CI, 1.032-5.283).

We observed a 3-year colostomy-free survival rate of 71% in our case series which reflects findings from other studies. Three-year colostomy-free survival is worse in HIV-infected individuals compared to HIV negative individuals (67% versus 88%, p = 0.036; HR, 3.231, 95% CI, 1.014-10.299) (Grew et al., 2015).

A number of anal cancer screening tools other than DRE have been advocated in high-risk populations including surveillance for HPV infection, anal cytology and high resolution anoscopy however no consensus recommendations exist. To date no screening strategy has been shown to decrease morbidity and mortality relating to anal cancer. Despite this, many large institutions have implemented screening programmes for HIV-infected individuals.

Dysplastic changes of the anal canal can be detected on cytological examination however sensitivity to detect HGAIN is poor compared to cervical cytology (Mathews et al., 2011).

High resolution anoscopy (HRA) allows visualisation of the transitional zone (where the squamocolumnar junction of the rectum changes into non-keratising squamous epithelium of the anus) and biopsies can be taken from suspect areas. HRA has a high sensitivity and specificity for detecting HGAIN however it is not widely available. Additionally, there is evidence that a high proportion of HGAIN lesions (19-43 per 100 patient years follow up) may regress spontaneously (Tong et al., 2013). Currently no markers have been identified to distinguish lesions that may progress to anal cancer versus those that will regress spontaneously.

Preventative efforts relating to anal cancer now focus around HPV vaccination. Three vaccines are currently licensed for the prevention of HPV. The bivalent HPV vaccine (HPV-2v) (Cervarix™, GlaxoSmithKline) protects against oncogenic HPV types 16 and 18. The quadrivalent HPV vaccine (HPV 4v) (Gardasil™, Merck and Co., Inc.) offers additional protection against HPV types 6 and 11, commonly associated with genital warts. The recently licensed nonavalent HPV vaccine (HPV 9v) (Gardasil 9™, Merck and Co., Inc.) provides protection against 5 additional oncogenic HPV types (31, 33, 45, 52, and 58).

National immunisation programs delivering HPV vaccine to females have been established in the majority of developed countries. In recent years there has been a move by countries including the US, Canada and Australia to recommend HPV 4v for boys also, given the broader benefits of the vaccine.

The majority of European countries do not recommend HPV vaccine for boys due to lack of cost-effectiveness data in the setting of high vaccine coverage in girls. Where high levels of HPV vaccine coverage have been achieved in females, heterosexual men have been observed to benefit from herd immunity, however no protective effect has been observed in MSM, the highest group for HPV infection and associated disease (Ali et al., 2013).

HPV vaccine has been shown to be most effective prior to exposure to HPV (Medeiros et al., 2009). Gender neutral immunisation programmes providing vaccine for boys and girls would offer the greatest preventative potential, however such programmes will not address the existing increased risk of HPV associated disease in high-risk groups such as HIV-infected individuals. In addition, universal immunisation programmes are unlikely to be implemented in the short term given cost implication.

HPV vaccine has been demonstrated to be cost effective in MSM up to the age of 26 years over a range of assumptions (Kim, 2010). Emerging evidence suggests that the vaccine may offer additional protective benefits in older MSM and that the vaccine may be cost effective in this group (Deshmukh et al., 2014) however this has not been a consistent finding (Cranston, 2016).

The incidence of anal cancer is high in HIV-infected individuals compared to the general population. Almost 80% of anal cancer is caused by persistent infection with hr HPV type 16 which is preventable through vaccination. While many experts advocate routine screening for anal cancer in high-risk groups such as MSM and HIV-infected individuals, it has not been demonstrated to impact anal cancer related morbidity or mortality to date.

While there is likely to be high levels of awareness among HIV clinicians of risk of anal cancer in HIV-infected MSM our paper highlights the increased risk in non-traditional groups such as IDU. Low nadir CD4 T cell count must be considered as a risk factor for development of anal cancer even in individuals who have achieved immune reconstitution.

While targeted HPV vaccine has potential to greatly reduce the burden of HPV associated anal cancer in HIV-infected individuals in the future, further research is needed to improve screening modalities to impact the burden of anal cancer currently observed in HIV-infected individuals.

5.5 Prevalence and risk factors for HPV infection in HIV-infected and HIV negative MSM in Ireland

5.5.1 Abstract

The incidence of human papillomavirus (HPV)-associated anal cancer is increasing. Men who have sex with men (MSM), particularly those co-infected with HIV, are disproportionately affected. Documenting the molecular epidemiology of HPV infection is important in guiding policy makers in formulating universal and/or targeted vaccine guidelines.

A prospective cohort study was conducted. HIV-infected and HIV-negative MSM > 18 years old were invited to participate. Provider-performed anal swabs were collected and anal HPV infection was detected using consensus primer solution phase polymerase chain reaction (PCR) followed by type-specific PCR for high-risk (hr)-HPV types 16, 18 and 31. Between-group differences were analysed using χ^2 tests and Wilcoxon rank tests.

One hundred and ninety-four MSM [mean, standard deviation (SD) age 36 (10) years; 51% HIV-infected] were recruited. The median [IQR] number of reported sexual contacts in the preceding 12 months was 4 [2-10]. HIV-infected subjects had a mean (SD) CD4 T cell count of 557 (217) cells/mm^3, and 84% were on highly active antiretroviral therapy (HAART). Thirty-one samples were *B*-globin negative and thus excluded from further analysis.

A total of 113 subjects (69%) had detectable HPV DNA. Sixty-eight subjects (42%) had a hr HPV type detected. hr HPV type 16 was detected in 44 samples (27%), hr HPV type 18 in 26 samples (16%) and hr HPV type 31 in 14 samples (23%). Twenty-eight subjects (17%) had more than one type of hr HPV type detected. When HPV and hr HPV were stratified by age, those > 35 years had a higher prevalence ($P = 0.001$ and $P = 0.028$, respectively). HIV-infected subjects were more likely than HIV-negative subjects to have any detectable HPV (77% *vs.* 61%, respectively; $P = 0.04$), to have hr HPV type 18 or 31 ($P = 0.05$ and $P = 0.006$, respectively) and to be infected with more than one hr HPV type (31% *vs.* 3%, respectively; $P < 0.01$). Within the HIV-infected group, the prevalence of HPV was higher in those not on HAART ($P = 0.04$), although it did not differ when stratified by CD4 T cell count.

The identified prevalence of anal HPV infection was high. Emerging patterns of HPV-related disease strengthen the call for universal vaccination of boys and girls with consideration of catch-up and targeted vaccination of high-risk groups such as MSM and those with HIV infection.

5.5.2 Background

Human papillomavirus (HPV) infection is the most common sexually transmitted infection (STI) worldwide; it is highly prevalent in the sexually active population and is rapidly acquired after sexual debut (Dunne et al., 2007). Over 90 serotypes of HPV have been identified; low-risk (lr)-HPV types (predominantly 6 and 11) can cause genital warts, while high-risk (hr)-HPV types (predominantly 16 and 18) can cause cancer of the cervix, anus and oropharynx (de Villiers et al., 2004).

Following the introduction of screening programmes in the developed world, the incidence of cervical cancer has decreased significantly; however, the incidences of HPV-associated anal and oropharyngeal cancers have increased dramatically in the past decade (Jemal et al., 2013). Non-cervical HPV-associated cancers, while individually relatively rare, now collectively parallel the burden of cervical cancers in developed countries. Hr HPV is now thought to cause over 5% of all cancers world-wide (Parkin and Bray, 2006).

Certain 'at-risk' groups such as men who have sex with men (MSM), particularly those with HIV infection, are disproportionately affected. The incidence of anal cancer is 1−2/100 000 in the general population, 35/100 000 in HIV-negative MSM (Daling et al., 2004) and up to 70/100 000 in HIV-infected MSM (Patel, 2008).

Since the advent of highly active antiretroviral therapy (HAART), HIV-infected individuals are living longer. In the Department of GU Medicine and Infectious Diseases (GUIDE), St James's Hospital, Dublin, the largest HIV specialist center in Ireland, HIV-related mortality has fallen over tenfold from 16.8 deaths per 100 active patient-years in 1995 to 1.4 deaths per 100 active patient-years in 2012 (M.. 2001). With increased survival, there are an increasing number of non-AIDS defining illnesses contributing to morbidity and mortality. Malignancy and in particularly anal cancer is a major driver of this trend (Palella et al., 2006).

Strategies to curtail the increase in anal cancer include screening, the benefits of which remain to be determined, and HPV vaccination.

Three vaccines have been developed to protect against HPV infection, a quadrivalent vaccine HPV 4v protects against HPV 6, 11, 16 and 18 (Gardasil®, HPV-6/11/16/18, Merck, Whitehouse Station, NJ, USA) and the bivalent vaccine (HPV-2v) protects against HPV 16 and 18 (Cervarix®, HPV 16/18, GlaxoSmithKline Biologicals, GSK, Brentford, London, UK). The nonavalent HPV vaccine (HPV 9v) (Gardasil 9™, Merck and Co., Inc.) was approved by the

Food and Drug Administration (FDA) US in December 2014 and provides protection against five additional oncogenic HPV types (31, 33, 45, 52, and 58) (Petrosky et al., 2015) .

All HPV vaccines have demonstrated efficacy against the development of cervical intraepithelial neoplasia (CIN) 2 or 3, adenocarcinoma in situ or cervical cancer among HPV-naïve women (Brown et al., 2009, De Carvalho et al., 2009). HPV 4v has in addition demonstrated efficacy against genital warts in male and female individuals and anal intraepithelial neoplasia related to HPV 6, 11, 16 and 18 in male individuals (Giuliano et al., 2011). HPV 9v is approved for the same indications as HPV 4v.

Despite the potential clinical benefit of the HPV vaccine in males, mathematical models suggest that vaccinating males would exceed acceptable cost-effectiveness thresholds particularly in a setting where female vaccine coverage is high (Kim and Goldie, 2009). However, in models looking at specific 'at-risk' male populations such as MSM and those infected with HIV cost effectiveness has been established over a range of assumptions (Kim, 2010).

Although no therapeutic benefit of the HPV vaccine has been demonstrated for the treatment of active disease present at the time of vaccination, there are early data suggesting a possible benefit of HPV vaccination in the setting of previous disease which could represent an important opportunity for intervention in older high-risk patient groups such as HIV-infected MSM (Swedish et al., 2012). In addition, if the HPV vaccine proved efficacious in the HIV-infected population against vaccine subtypes, the potential reduction in anal cancer rates could be upwards of 60% (Sahasrabuddhe et al., 2013).

Study Aims

The primary aim of this study was to document the prevalence of anal HPV infection and the prevalence of infection with hr HPV types 16, 18 and 31 in HIV-infected and HIV-negative MSM. Secondary objectives were to identify factors associated with HPV and hr HPV infection.

Understanding the prevalence of HPV and HR HPV infection in the MSM population will help inform strategies for primary and secondary prevention of HPV-associated anal cancer in this at-risk group.

5.5.3 Methods

HIV-infected and HIV-negative MSM were recruited from the GUIDE clinic in St James's Hospital, Dublin, Ireland, a dedicated HIV clinic, and from the GMHS (Gay Men's Health Service), a community-based gay men's sexual health clinic. Participants were recruited from April 2012 to May 2012.

Basic demographic data along with information regarding sexual behaviour were collected by means of a self-completed questionnaire. Trained medical providers at each site collected anal samples by rotating a Dacron swab in the anal canal without direct visualization. DNA was extracted using the QIA amp Mini kit (Qiagen, Hilden, Germany). HPV was detected using consensus primer solution phase polymerase chain reaction (PCR) followed by type-specific PCR for hr HPV types 16, 18 and 31. Between-group differences were analysed using χ^2 tests and the Wilcoxon rank test. At enrolment, each participant provided written informed consent. The study received approval from the St James's Hospital Research Ethics Committee.

5.5.4 Results

One hundred and ninety-four MSM participated in the study (51% HIV-infected; 77% Irish; mean age 36 years) (Table 5.5.1). The mean number of reported sexual contacts in the preceding 12 months was 8; 75% of individuals reported both oral and anal intercourse, 42% reported always using condoms. Fifty-six per cent reported a previous diagnosis of an STI, 27% reported a history of anogenital warts and 38% were smokers.

Table 5.5.1 Baseline characteristics of HPV study cohort

	Total cohort	**HIV positive**	**HIV negative**	**P-value**
N (%)	194	99 (51)	95 (49)	
Age (years) [mean (SD)]	36 (10)	40 (10)	32 (8)	< 0.001
Region of birth [n (%)]				
Ireland	149 (77)	74 (75)	75 (79)	
Western Europe	13 (7)	9 (9)	4 (4)	
Eastern Europe	13 (7)	7 (7)	6 (6)	
Other	19 (10)	9 (9)	10 (10)	
Number of partners in past 12 months [mean (SD)]	8 (11)	7 (10)	8 (12)	
Type of intercourse [n (%)]				
Anal intercourse only	6 (3)	4 (4)	2 (2)	
Oral intercourse only	26 (13)	12 (12)	15 (15)	
Both oral and anal intercourse	146 (75)	69 (70)	77 (81)	
No sexual contacts in past 12 months [n (%)]	14 (7)	12 (12)	2 (2)	0.012
Condom use in past 12 months [n (%)]				
Always	81 (42)	43 (43)	38 (40)	
Sometimes	96 (49)	44 (44)	52 (55)	
Never	11 (6)	7 (7)	4 (4)	
Previous STI [n (%)]	109 (56)	67 (70)	42 (44)	0.001
History of anogenital warts [n (%)]	52 (27)	39 (39)	13 (14)	< 0.001
Smoker [n (%)]	74 (38)	45 (45)	29 (30)	0.038
B-globin [n (%)]	**163 (84)**	**83 (84)**	**80 (84)**	
HPV DNA detected [n (%)]	113 (69)	64 (77)	49 (61)	0.04
HR HPV 16	44 (27)	26 (31)	18 (23)	0.23
HR HPV 18	26 (16)	18 (22)	8 (10)	0.05
HR HPV 31	23 (14)	18 (22)	5 (6)	0.006
Any HR HPV type	68 (42)	39 (47)	29 (36)	0.146
Individuals with > 1 HR HPV type [n (%)]	28 (17)	26 (31)	2 (3)	< 0.001
HR HPV 16 and 18	10 (6)	10 (12)	0 (0)	0.0016
HR HPV 16 and 31	11 (7)	10 (12)	1 (1)	0.009
HR HPV 18 and 31	7 (4)	6 (7)	1 (1)	0.1
HR HPV 16, 18 and 31	3 (2)	3 (4)	0 (0)	0.2

Abbreviations: HR, high-risk; SD, standard deviation; STI, sexually transmitted infection

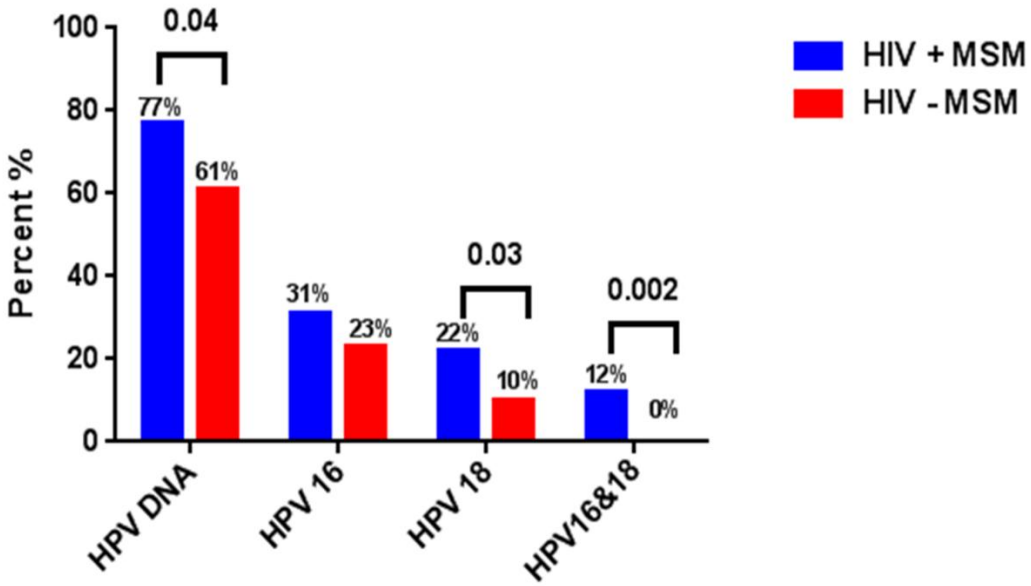
HIV-infected participants were older (mean age 40 vs. 32 years, respectively; $P < 0.01$), were more likely to report no sexual contacts in the preceding 12 months (12% vs. 2%, respectively; $P = 0.01$), to report prior history of an STI (70% vs. 44%, respectively; $p<0.01$), and to be current smokers (49% vs. 25%, respectively; $P = 0.04$).

Of the 194 MSM recruited, 163 contributed interpretable HPV data (*B*-globin positive), with no significant differences in *B*-globin detection rates between the HIV-infected and HIV-negative groups. Only those with detectable *B*-globin were included in further analysis to calculate prevalence of HPV DNA and hr HPV.

One hundred and thirteen participants (69%) had detectable HPV DNA, while 68 subjects (42%) had any hr HPV type detected. Hr HPV 16 was detected in 44 samples (27%), hr HPV 18 in 26 samples (16%) and hr HPV 31 in 23 samples (14%). Ten subjects (6%) had both hr HPV types 16 and 18 detected, 11 (7%) had both hr HPV types 16 and 31 detected, and seven (4%) had both hr HPV types 18 and 31 detected. Three subjects (2%) had hr HPV types 16, 18 and 31 detected.

HIV-infected participants were significantly more likely than HIV-negative participants to have HPV DNA detected (64% vs. 49%, respectively; P = 0.04) and to be infected with hr HPV type 18 (22% vs. 10%, respectively; P = 0.05) or hr HPV type 31 (22% vs. 6%, respectively; P < 0.01 (Figure 5.1). In addition, those with HIV infection were more likely to be infected with multiple types of hr HPV (31% vs. 3%, respectively; P < 0.01) (Table 5.5.2). All three subjects who tested positive for hr HPV types 16, 18 and 31 were HIV-infected.

Figure 5.1 Prevalence of HPV infection and hr HPV infection in HIV-infected and HIV negative MSM



HPV DNA and hr HPV types were more likely to be detected in subjects over 35 years of age (*P* < 0.01 and 0.03, respectively) (Table 5.5.2). In addition, subjects who reported a previous STI were more likely to have HPV DNA and hr HPV detected (*P* = 0.01). A reported history of

anogenital warts was positively associated with detectable HPV DNA, although this was of borderline significance ($P = 0.09$).

Within the HIV-infected group, the prevalence of HPV was higher in those not on HAART ($P = 0.04$), although it did not differ when stratified by CD4 T cell count.

Table 5.5.2 Factors associated with human papillomavirus (HPV) DNA positivity

	B-Globin detected	HPV DNA	p	HR HPV DNA	P
Total [n (%)]	163	113 (69)		68 (42)	
Age [n (%)]					
18–24 years	17	14 (82)	< 0.01	4(24)	0.03
25–29 years	32	13(41)		8(25)	
30–35 years 32	32	22 (69)		14 (44)	
> 35 years	82	64 (78)		42 (51)	
Region of birth [n (%)]					
Ireland	117	79 (68)	0.46	50 (43)	0.53
Western Europe	13	10 (77)		7 (54)	
Eastern Europe	11	8(73)		3(27)	
Other	22	16(73)		8(36)	
Sexual partners in past 12 months [n (%)]					
≤5	93	60 (65)	0.18	42 (45)	0.52
> 5	64	48 (75)		25 (39)	
Unknown	6	6 (83)		1 (8)	
Type of intercourse [n (%)]					
Anal intercourse only	5	3 (60)	0.69	1 (20)	0.32
Oral intercourse only	21	17 (81)		6 (29)	
Both oral and anal intercourse	124	83 (67)		56 (45)	
Unknown	13	10 (77)		5 (38)	
Condom use in past 12 months [n (%)]					
Always	69	44 (64)	0.24	25 (36)	0.37
Sometimes	73	51 (70)		32 (44)	
Never	9	8 (89)		5 (56)	
Unknown	12	10 (83)		6 (50)	
Previous STI [n (%)]	94	70 (74)	0.01	48 (51)	0.01
History of anogenital warts [n (%)]	47	36 (77)	0.09	23 (49)	0.29
Smoker [n (%)]	63	44 (69)	0.84	24 (38)	0.59
Nonsmoker [n (%)]	76	44 (58)		35 (46)	
Past smoker [n (%)]	22	14 (64)		9 (41)	
HIV-infected [n (%)]	83	64 (77)	0.04	39 (47)	0.15
HIV-negative [n (%)]	80	49 (61)		29 (36)	

Abbreviations: n number, HR high-risk, STI, sexually transmitted infection

5.5.5 Discussion

The prevalence of HPV infection in MSM included in this study is high (69% of participants had detectable HPV DNA and 42% had hr HPV detected). Hr HPV type 16, which was found in 27% of subjects participating in our study, has been shown to be associated with over 80% of anal cancers (Hoots et al., 2009).

While this study did not investigate the persistence of HPV infection, 14 participants reported no sexual contacts in the preceding 12 months. Nine of these 14 (64%) were found to have detectable anal HPV DNA, while seven of the 14 (50%) had a hr HPV type detected. International studies have reported a similar high prevalence of hr HPV infection, with persistence of hr HPV infection in 50−70% of MSM and a higher persistence rate in HIV-infected subjects. (Sirera et al., 2006).

HIV infection was positively associated with detection of HPV DNA ($P = 0.04$) and hr HPV DNA (47% vs. 36% in HIV-negative individuals; $p = 0.15$) in this study. The high prevalence of HPV infection in HIV-infected subjects may be driven by an increased persistence of HPV infection as a consequence of compromised immunity and/or by a high incidence of new infections as a consequence of sexual behaviour.

We identified a higher prevalence of HPV infection in subjects reporting more than five sexual partners in the preceding 12 months (75% vs. 65% in those reporting five or fewer sexual partners; $p = 0.18$). In addition, individuals with a self-reported history of a previous STI were more likely to have HPV or hr HPV detected ($P = 0.01$ and 0.01, respectively). Sexual behaviours, including a greater number of sexual partners, receptive anal intercourse and smoking have been shown to be positively associated with anal HPV infection and anal cancer in a number of studies (Palefsky et al., 1998, Nyitray AG, 2011, Poynten et al., 2012).

In our study, rates of infection with HPV in those reporting not using condoms consistently in the preceding 12 months were greater than in those who reported always using condoms: 73% vs. 60% for HPV DNA and 36% vs. 43% for hr HPV, respectively. A number of studies have shown that consistent use of condoms prevents or decreases the risk of HPV infection (Baldwin et al., 2004).

Our study has a number of limitations. Study participants were recruited from a sexual health clinic and an HIV clinic, and so MSM recruited may not be representative of the general MSM population. As this was a point prevalence study, anal swabs were taken at one time-point only

and we cannot thus comment reliably on the duration or persistence of HPV infection. Planned follow-up of this cohort will clarify this issue. Data on sexual behaviour and history were collected by means of a self-completed questionnaire and therefore may be subject to recall bias.

This study highlights the burden of anal HPV infection in MSM and those with HIV co-infection. A significant proportion (73%) of participants in our study did not have evidence of infection with hr HPV type 16 or 18. This indicates that a large proportion of sexually active MSM could potentially benefit from HPV vaccination. There is a growing body of knowledge supporting targeted vaccination of MSM and HIV-infected individuals.

STI and HIV clinics would be well placed to facilitate HPV vaccination of risk groups such as MSM given previous experience and successes with hepatitis B virus vaccination (Rock et al., 2013a). In addition, provision of HPV vaccination in STI clinics may encourage young MSM to access sexual health services earlier, which may provide an opportunity for education interventions targeting HIV prevention.

5.6 Six-month persistence of HPV infection and HPV infection at multiple sites in a subgroup of HIV-infected MSM

5.6.1 Abstract

Persistence of hr HPV infection, rather than transient infection is associated with development of anal cancer. The aim of this study was to investigate the persistence of HPV infection in a subgroup of HIV-infected MSM who were identified as having HPV DNA detected at baseline in our study examining prevalence of anal HPV infection. We also examined point prevalence of HPV infection at multiple sites (oropharyngeal and genital) in this subgroup.

HIV-infected MSM identified as having HPV DNA detected in the original HPV prevalence study were invited to participate. Anal swabs were repeated on one occasion at >6-8 months following initial sampling. Oropharyngeal and genital swabs were also taken. HPV was detected using consensus primer solution phase polymerase chain reaction (PCR) followed by type-specific PCR for hr HPV types 16, 18.

In total, 45 of 64 (70%) individuals who had HPV DNA detected in the HPV prevalence study provided samples. Mean [standard deviation (SD)] age was 42 [12] years, 69% were Irish. Thirty-five (78%) repeat anal samples had *B*-globin detected, 49% (17/35) had persistence of HPV DNA, 33% (5/15) had persistence of hr HPV 16, 36% (4/11) had hr HPV 18 detected at 6-month sampling.

Twenty percent (8/41) of those with B-globin detected on oropharyngeal swabs had HPV DNA detected on oropharyngeal swabs, 12%(5/41) had HPV 16 and 10% (4/41) had HPV 18 detected.

Sixteen percent (6/45) of those with B globin detected on genital swab had HPV DNA detected (11% (4/45) had HPV 16 detected, 5% (2/45) had HPV 18 detected).

We identified a high level of persistence of anal HPV and hr HPV infection in HIV-infected MSM. The poor clearance of anal HPV infection in HIV-infected MSM likely contributes to the high burden of anal cancer in HIV-infected MSM.

While the prevalence of oropharyngeal and genital HPV infection identified is less than that observed in the anal canal it is still significant and may contribute to the increased risk of oropharyngeal and penile cancer observed in HIV-infected individuals.

5.6.2 Background

Prevalence of ano-genital HPV infection in men in the general population has been reported as between 1-84% (Dunne et al., 2006). The wide range in prevalence observed is likely multifactorial relating to differences in study populations, sampling methods including the anatomical sites of sampling, and analysis methods used.

The prevalence of anal HPV infection in MSM is higher than that observed in heterosexual men (47.2% versus 12.2%) (Nyitray et al., 2011a). The prevalence of high-risk (hr) or oncogenic anal HPV infection is documented at 26-73% in HIV negative MSM (van der Snoek et al., 2003, Chin-Hong et al., 2004). Prevalence of hr HPV has been shown to be significantly higher in HIV-infected MSM compared to HIV negative MSM with prevalence reported at up to 93% (Sadlier et al., 2014b).

Receptive anal intercourse, number of sexual partners in the preceding 6 months and HIV infection have been identified as independent predictors of anal HPV infection (Goldstone et al., Mooij et al.).

Prevalence of oropharyngeal and genital HPV infection has also been reported at rates of up to 45% in MSM and HIV-infected MSM (Beachler and D'Souza, 2013).

Studies examining point prevalence of HPV infection provide important epidemiological insights however, persistence of hr HPV infection is the critical factor associated with development of cancer.

The most oncogenic hr HPV type 16 has been identified as the most likely HPV type to persist over time (Sahasrabuddhe et al., 2013).

Given that prevalence of hr HPV is more common in HIV-infected MSM and rates of clearance are decreased it is unsurprising that persistence of anal HPV in HIV-infected individuals is higher compared to HIV negative individuals (Geskus et al., 2016).

Neither CD4 T cell count nor nadir CD4 T cell were shown to influence clearance of HPV infection (Beachler et al., 2013, Machalek et al., 2012, Mooij et al., 2016, del Amo et al., 2013). This may partly explain high incidence of anal cancer observed in HIV-infected individuals despite immune reconstitution in the setting of highly active antiretroviral therapy (HAART).

Because persistence of hr HPV infection increases the risk of AIN and anal cancer it is important to understand the molecular epidemiology of persistent HPV infection.

Study Aim

The aim of this study was to examine persistence of HPV DNA in a subgroup of HIV-infected MSM who had HPV DNA detected at baseline. An additional aim was to examine the point prevalence of HPV infections at multiple sites (oropharyngeal, anal, genital).

5.6.3 Methods

HIV-infected MSM identified as having HPV DNA detected in the original HPV prevalence study were invited to participate. For the purpose of the study persistence of HPV infection was defined as detection of HPV DNA at > 6 months following original sampling. Incident hr HPV 16 and 18 infections were defined as absence of hr HPV on baseline sampling and detection on interval sampling.

Anal swabs were repeated on one occasion at >6 months following initial sampling. Oropharyngeal and genital swabs were also performed.

Healthcare provider collected samples from each site using Dacron swabs. DNA was extracted using the QIA amp Mini kit (Qiagen, Hilden, Germany). HPV was detected using consensus primer solution phase polymerase chain reaction (PCR) followed by type-specific PCR for HR HPV types 16, 18. Between-group differences were analysed using $\chi 2$ tests and the Wilcoxon rank test. At enrolment, each participant provided written informed consent.

The study received approval from the St James's Hospital Research Ethics Committee.

5.6.4 Results

Forty-five of 64 (70%) individuals who had HPV DNA detected in the HPV prevalence study provided samples for this study. Baseline characteristics are outlined in Table 5.6.1. Mean [SD] age was 42 [12] years, 69% were Irish.

Table 5.6.1 Baseline characteristics of subgroup of HIV positive MSM

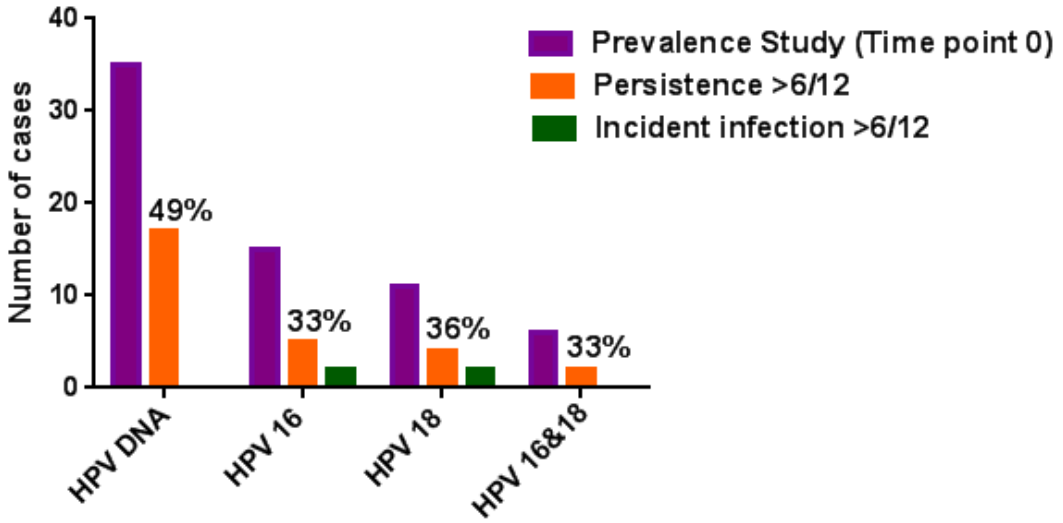
Baseline Characteristics	N=45	(%)
Mean age years [SD]	42	[12]
Region of Birth		
Ireland	31	(69)
Mean number of partners [SD]	8	[11]
Type of Intercourse		
Anal	32	(71)
Oral	38	(84)
No sexual contacts	5	(11)
Always used condoms	15	(33)
Ano-genital warts	18	(40)
Smoker	18	(40)
On HAART	36	(80)
CD4 T cell count >350cells/mm3	40	(89)

Abbreviations: SD standard deviation, N number, HAART highly active antiretroviral therapy

Six-month persistence of anal HPV infection

Thirty-five (78%) of repeat anal samples had *B*-globin detected, 49% (17/35) persistence of HPV DNA at > 6 months. 33% (5/15) had persistence of hr HPV 16. 36% (4/11) had hr HPV 18 detected. 2 patients had persistence of both HPV 16 and 18. 2 (10%) patients had incident hr HPV 16 infection. 2 (8%) patient had incident HPV 18 infection on interval sampling (Figure 5.3).

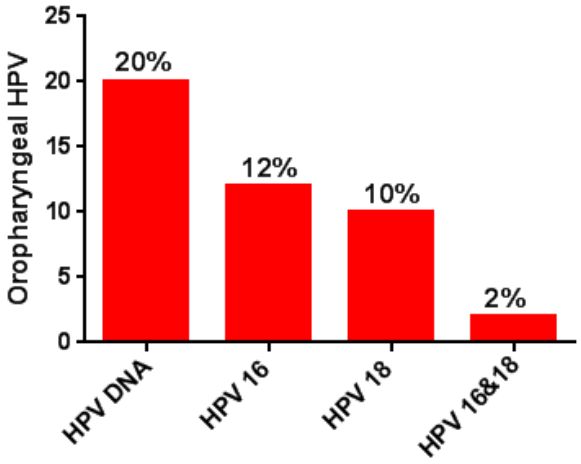
Figure 5.2 Prevalence, persistence and incidence of HPV infection in HIV-infected MSM



Prevalence of oropharyngeal HPV infection in HIV-infected MSM

Forty-one (91%) of oropharyngeal swabs had *B*-globin detected. 8/41 (20%) had HPV DNA detected, 5/41 (12%) had hr HPV 16, 4/41 (10%) had hr HPV 18 while 1 individual (2%) had both hr HPV 16 and 18 detected (Figure 5.4).

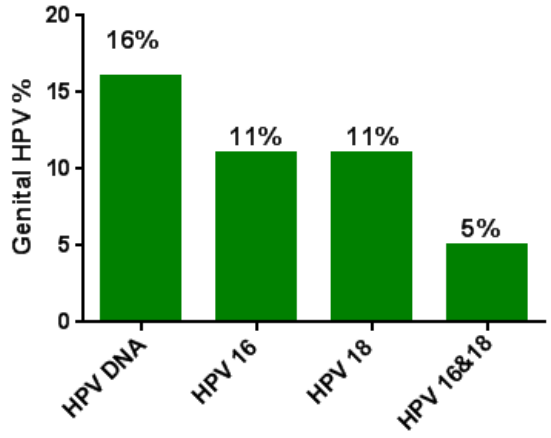
Figure 5.3 Prevalence of oropharyngeal HPV infection in HIV-infected MSM



Prevalence of genital HPV infection in HIV-infected MSM

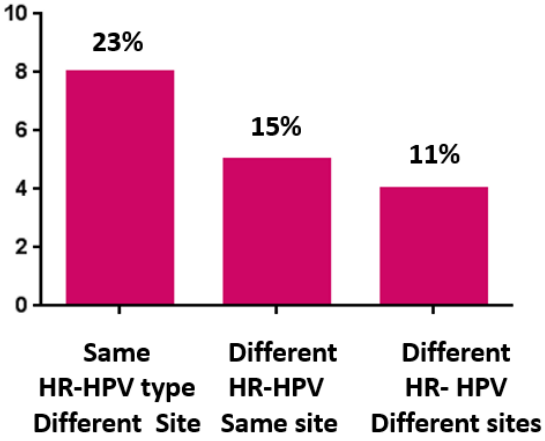
Thirty-seven (82%) of genital swabs had *B*-globin detected. 6 (16%) had HPV DNA detected. 4 (11%) had hr HPV 16 detected, 4 (11%) had hr HPV 18 detected and 2 (5%) had both hr HPV 16 and 18 detected (Figure 5.5).

Figure 5.4 Prevalence of genital HPV infection in HIV-infected MSM



Eight (23%) had concordant HPV types detected at more than one site, 5 (15%) had both HPV 16 and 18 detected at the same site and 4 (11%) had different hr HPV types detected at different sites (Figure 5.6).

Figure 5.6 Concordance of hr HPV infection at multiple sites in HIV-infected MSM



5.6.5 Discussion

Persistence of HPV and hr HPV infection at 6 months occurred in approximately 50% and 30% respectively of HIV-infected MSMs who participated in our study. While this has a small study, results are reflective of those reported in other larger cohorts of HIV-infected MSM (Nyitray et al., 2011b, Parisi et al., 2011, Phanuphak et al., 2013).

HIV-infected MSM are the highest risk group for anal squamous cell carcinoma and infection hr HPV 16 accounts almost 70% of anal cancers (De Vuyst et al., 2009).

Screening for anal cancer is a topic of much international debate. Some experts advocate for screening of high-risk populations such as MSM (Abramowitz et al., 2016), however no screening tool has been shown to impact morbidity or mortality of anal cancer.

Screening for presence or persistence of hr anal HPV infection had been proposed as a tool for screening, however its prevalence of hr HPV infection and infection with multiple hr genotypes was found to be too high for it to be a discriminatory screening tool (Garbuglia et al., 2015). In addition, detection of hr anal HPV did not correlate well with abnormal histology (Schofield et al., 2016).

Similar to HPV detection, anal cytology has been shown to be a poor predictor of HGAIN (Botes et al., 2013).

High resolution anoscopy (HRA) and biopsy of suspect lesions is by considered to be the gold standard for detection of AIN in high-risk groups, although there are several important challenges including high cost, intra and inter-observer variability and varying acceptability rates for HRA in patients (Hillman et al., 2016). In addition, the optimal treatment for HGAIN is yet to be established. Rates of recurrence of HGAIN after treatment is relatively high (Schofield et al., 2016).

Three vaccines have been licensed for the prevention of persistent HPV infection. All are subunit vaccines which use a recombinant form of the L1 major capsid protein of HPV as an antigen. L1 proteins self-assemble into non-infectious, nononcogenic units called virus-like particles (VLP).

HPV vaccines are highly immunogenic. More than 99% of recipients develop an antibody response to HPV types included in the respective vaccines 1 month after completing the three-

dose series with comparable levels of antibody response following 2 doses (Sankaranarayanan et al., 2016). However, there is no known serologic correlate of immunity and no known minimal titre has been determined to be protective.

HPV 4v has been demonstrated to be highly efficacious in preventing infection with HPV vaccine types related to external genital lesions, and anal intraepithelial neoplasia (AIN) in men (Giuliano et al., 2011). The HPV 9v has been demonstrated to be highly efficacious in preventing infection with HPV vaccine types and disease related to the additional 5 types HPV-31, 33, 45, 52, and 58 in a susceptible population. Antibody response generated to HPV-6, 11, 16, and 18 were non-inferior to that generated by the HPV 4v vaccine and thus the same indication as HPV 4v was applied (Joura et al., 2015). No HPV vaccine has demonstrated protection beyond type covered in the vaccine.

HPV vaccine has great potential to decrease the burden of HPV infection and associated disease in the future however it will have little effect on the increased burden of HPV infection currently observed.

This study has several limitations including small sample size. The study was not powered to investigate factors associated with persistence of HPV infection or HPV infection at multiple sites. Persistence of HPV infection in our study was defined as detection of HPV at > 6 months. There are inherent difficulties with this definition in that we cannot account for transient HPV infection that may have occurred and cleared during the study period. Similarity we cannot account for individuals who cleared HPV infection and were re-infected during the study period.

Some experts would argue that sequential sampling at a six month interval is too short to reflect persistence of HPV infection, however there is evidence that this interval is a reasonable correlate (Fakhry et al., 2010).

Only hr HPV 16 and 18 were tested for in this study so we cannot account for other hr HPV types that may have been present. We do not have a seroprevalencee correlate of HPV infection. Neither do we have cytological correlates for HPV types detected.

This study contributes to existing data examining the natural history of HPV infection in HIV-infected MSM. It provides an important insight into the persistence of HPV infection and hr HPV infection in HIV-infected MSM. This data can inform policy makers and assist on developing preventative strategies.

5.7 HPV vaccine acceptability in HIV-infected and HIV negative MSM in Ireland

5.7.1 Abstract

Men who have sex with men (MSM), particularly HIV-infected MSM are disproportionately affected by HPV infection and associated disease. The HPV vaccine has potential to greatly reduce the burden of HPV-associated disease including anal cancer in MSM. The efficacy of the HPV vaccine is dependent on high levels of vaccine uptake. The aim of this study was to examine HPV vaccine acceptability and factors influencing vaccine acceptability in MSM in Ireland.

A self-administered survey was distributed to HIV-infected and HIV negative MSM examining HPV vaccine acceptability and factors associated with vaccine acceptability. Logistic regression was used to identify key variables and predictors of HPV vaccine acceptability. 302 MSM participated in the study. Acceptability of HPV vaccine was 31% (unconditional), 51% (conditional on stated efficacy and a cost of €300), 65% (conditional on stated efficacy and a cost of €100) and 78% (conditional on stated efficacy and no cost). Cost was negatively associated with HPV vaccine acceptability ($p<0.01$) while knowledge of HPV vaccine efficacy was significantly associated with vaccine acceptability, even in the context of associated cost ($p<0.01$).

Acceptability of HPV vaccine in MSM in Ireland is high, based on no cost vaccine and on stated vaccine efficacy (78%). Cost is negatively associated with vaccine acceptability. Understanding levels of knowledge of HPV infection, HPV associated disease and attitudes towards HPV vaccination are important as they will contribute to HPV vaccine acceptability among MSM and will help guide effective preventive programmes.

5.7.2 Background

HPV is the most common sexually transmitted infection (STI) worldwide (Baseman et al., 2008). It is causally associated with cervical cancer, penile cancer, vulval cancer, oropharyngeal cancer and anal cancer (Hartwig et al., 2012).

The prevalence of HPV infection is greater in MSM, particularly in HIV-infected MSM compared to the general population (Sadlier et al., 2014a).The reported incidence of anal cancer in MSM is up to 35 cases per 100,000 (Jemal et al., 2003) and up to 131 cases per 100,000 in HIV-infected MSM (Patel, 2008).

The quadrivalent HPV vaccine (HPV 4v) (Gardasil™, Merck and Co., Inc.) is highly efficacious in preventing infection with HPV vaccine types 6/11/16/18, related external genital lesions and anal intraepithelial neoplasia (AIN) in males and females (Giuliano et al., 2011). The 9-valent HPV vaccine (HPV 9v) (Gardasil 9™, Merck and Co., Inc.), has recently been approved for the same indications, offering additional protection against HPV types 31, 33, 45, 52, and 58 (Petrosky et al., 2015).

While gender neutral vaccination programmes offer the best preventive opportunities, such programmes are unlikely to be implemented where levels of female vaccination coverage is high due to lack of cost-effectiveness evidence (Kim and Goldie, 2009). HPV vaccination of boys and male adolescents is not yet recommended in Ireland or in the majority of European countries that provide HPV vaccination for girls through national immunisation programs due to lack of cost effectiveness data.

High levels of female vaccination coverage have been shown to decrease genital warts in both females and unvaccinated heterosexual males through herd immunity, however no protection has been observed in MSM (Ali et al., 2013). Targeted vaccination of MSM has been shown to be cost-effective up to and beyond the age of 26 years (Deshmukh et al., 2014, Deshmukh et al., 2015).

Emerging evidence suggests that HPV 4v may provide additional benefits in older sexually experienced MSM who have previous exposure to HPV by reducing high grade anal lesions (HGAIN) progression and recurrence among MSM (Swedish et al., 2012). The HPV 4v has also shown to be effective for the prevention of anal condyloma of older MSM (Swedish and Goldstone, 2014).

The Joint Commission on Vaccination and Immunisation (JCVI) in the UK are reviewing the cost effectiveness of universal vaccination of boys and girls through the national immunisation programme as well as targeted vaccination of MSM aged 16–40 years in sexual health and HIV clinics (2014).

When considering the feasibility of targeted HPV vaccination, it is important to examine vaccine acceptability and factors influencing acceptability as low vaccine uptake will fail to reduce HPV-related diseases.

There is no data available on acceptability of HPV vaccine in MSM in Ireland and only two published studies examining HPV vaccine acceptability in Europe. A meta-analysis of HPV vaccine acceptability in MSM including data from North America and Australia (where HPV vaccine is offered to boys and MSM up to the age of 26 years through national programmes) found that approximately 50% of MSM would accept HPV vaccine (Newman et al., 2013).

Success of targeted vaccination programmes for preventing HPV associated disease such as anal cancer in MSM will greatly depend on high levels of uptake of HPV vaccine.

Study Aim

The aim of this study was to examine HPV vaccine acceptability and factors associated with HPV vaccine acceptability in MSM.

5.7.3 Methods

Study Population

MSM aged 18 years and over were invited to complete an anonymous self-administered survey from January 2014 to April 2014. The survey was developed in partnership with researchers and community-based associations in Dublin, and was piloted among 20 participants prior to implementation. The survey was based on previous published studies examining HPV vaccine acceptability in MSM (Simatherai et al., 2009). Participants provided verbal informed consent. Recruitment was from two sites, a walk in sexual health clinic (the Gay Men's Health Service, GMHS) and a HIV outpatient clinic (The Department of Genitourinary medicine and Infectious Diseases, St James's Hospital, GUIDE) in Dublin Ireland. Convenience sampling was performed. Refusal to participate in the study was not recorded.

Measures

Socio-demographic information including highest education level attained, history of STI, unprotected anal intercourse (UAI) and number of male sex partners in the last year was recorded (Table 5.7.1).

Questions explored knowledge and cognitions on HPV infection and vaccine (Table 5.7.2, Table 5.7.3).

Intention to take the HPV vaccine in the next 6 months and acceptability of HPV vaccine was assessed based on modified scenarios including unconditional, relating to stated HPV vaccine efficacy (HPV vaccine is 90% effective for prevention of genital warts and 75% effective for prevention of HPV induced anal/penile cancer) and vaccine cost (based on cost of €300 per vaccine series, a discounted cost of €100 per vaccine series or no cost vaccine) (Table 5.7.4).

Statistical Analysis

Basic demographics were reported as totals and percentages. Missing data was excluded from analysis. To examine factors associated with HPV vaccine acceptability for no cost vaccine univariate odds ratios were calculated. Corresponding 95% confidence intervals (95% CI) of odds ratios were presented (Table 5.7.5). SPSS version 22.0 was used for data analysis, with p values of <0.05 taken as statistically significant.

Ethics Approval

Ethics approval was obtained from the St James's Hospital Research Ethics Committee.

5.7.4 Results

Background Characteristic

302 MSM participated in the study. Baseline characteristics are outlined in Table 5.7.1. 39% were aged 18-30 years, 79% were educated to third level or above. 77% reported unprotected anal intercourse (UAI) with a male partner, 30% reported a sexually transmitted infection (STI) in the previous 12 months while 62% reported multiple male sex partners in the previous 12 months. 156 (52%) were HIV-infected.

Table 5.7.1 Demographic and behavioural characteristics of participants (n=302)

Background Characteristics	N	(%)a
Age group		
18-30	117	39
31-40	87	29
41-60	97	32
Highest education level attained		
Secondary School/equivalent or lower	60	21
College or above	223	79
Diagnosed as having a sexually transmitted infection in the past 12 months[b]		
No	209	70
Yes	90	30
Ever had unprotected anal intercourse with a male partner		
No	68	23
Yes	231	77
No. of male partners that you have had anal intercourse with in the past 12 months		
0	44	15
1	69	23
2-5	120	40
>6	67	22
HIV-infection	156	52

a Proportion of respondents for each characteristic. Missing data not shown.
b Based on self-reporting, includes chlamydia, gonorrhoea, syphilis, genital warts and genital herpes.

Knowledge and perceived severity of HPV infection

Appropriate knowledge response relating to HPV infection ranged from 16% to 58% (Table 5.7.2). 58% recognised that HPV could affect men. 22% recognised that HPV could not be treated with antibiotics. 16% recognised that HPV infection does not commonly lead to death.

24% perceived that prevalence of HPV infection in MSM in Ireland was high, 44% perceived the infectivity of HPV infection to be high, 33% believed their chance of catching HPV was high. 28% perceived that HPV infection was highly likely to damages physical health, 46% perceived that HPV was highly likely to cause genital warts while 26% perceived that HPV was highly likely to cause penile or anal cancer (Table 5.7.2).

Table 5.7.2 Knowledge and perceived severity of HPV infection

Knowledge of HPV Infection	N	(%) a
HPV can affect men		
No	15	5
Yes *	172	58
Don't know	110	37
HPV can be treated with antibiotics		
No*	65	22
Yes	51	17
Don't know	177	60
HPV infection can commonly lead to death		
No*	48	16
Yes	64	22
Don't know	180	62
Perceived Susceptibility to HPV		
The chance of you catching HPV infection is		
Low	56	22
Moderate	112	44
High	84	33
Prevalence of HPV infection in MSM in Ireland is		
Low	52	22
Moderate	129	54
High	58	24
The infectivity of HPV is		
Low	36	15
Moderate	99	41
High	105	44
Perceived Severity of HPV Infection		
The chance of HPV infection impacting on physical health is		
Low	62	26
Moderate	113	46
High	60	28
The chance of HPV infection causing genital warts is		
Low	41	17
Moderate	87	36
High	111	46
The chance of HPV infection causing penile/anal cancer is		
Low	62	26
Moderate	113	48
High	60	26

a Proportion of respondents for each characteristic. Missing data not shown.
* Appropriate response

Knowledge and perceptions relating to HPV vaccine

Appropriate knowledge response relating to HPV vaccine ranged from 21% to 56% (Table 5.7.3). 29% recognised that HPV vaccine was effective in men. 21% recognised that HPV vaccine series consisted of three doses. 56% recognised that it would be optimal to receive HPV vaccine at a younger age.

Thirty percent perceived that HPV vaccine would prevent genital warts while 26% perceived that HPV vaccine would prevent penile and anal cancers.

Ten percent believed HPV vaccines were expensive. 25% believed HPV vaccine may have side-effects Table 5.7.3.

Table 5.7.3 HPV vaccine knowledge/perceived efficacy of HPV vaccine

HPV vaccine knowledge	N	(%)a
An effective HPV vaccine is available for men		
No	30	11
Yes *	87	31
Don't know	167	59
The number of shots of HPV vaccine required is		
1-2	108	54
3*	64	32
4 or above	29	14
The best age to receive HPV vaccine is		
30 years or above	36	18
Below 30 years*	170	82
Perceptions relating to HPV vaccine		
The HPV vaccine is effective in preventing genital warts		
Not effective	12	4
Effective	82	30
Don't know	180	66
The HPV vaccine is effective in preventing penile and anal cancer		
Not effective	18	7
Effective	71	26
Don't know	189	68
The HPV vaccine is effective in preventing sexually transmitted infections other than genital warts		
Not effective	59	21
Effective	40	14
Don't know	178	64
The HPV vaccine is too expensive		
No	36	12
Yes	29	10
Don't know	212	70
The HPV vaccine could have side effects		
No	19	7
Yes	69	25
Don't know	189	68

a Proportion of respondents for each characteristic. Missing data not shown.

* Appropriate response

HPV Vaccines Acceptability

Acceptability of HPV vaccine was 31% (unconditional), 51% (conditional on stated efficacy (90% for prevention of genital warts and 75% for prevention of HPV induced cancers) and a cost of €300) 65% (conditional on stated efficacies and a discounted cost of €100) and 78% (conditional on stated efficacies and free price) (Table 5.7.4).

Table 5.7.4 Intention to take up HPV vaccine

Intention to take up HPV vaccine	N	(%)a
Do you plan to take up HPV vaccine in the next 6 months?		
Yes	87	31
No	42	15
Don't know	152	54
Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer and cost €300 in total?		
Yes	144	51
No	48	17
Don't know	87	31
Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer at a discounted price of €100 in total?		
Yes	179	65
No	21	8
Don't know	76	28
Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer and free?		
Yes	217	78
No	3	1
Don't know	57	21

a Proportion of respondents for each characteristic. Missing data not shown.

Factors associated with vaccine acceptability based on stated efficacies and free vaccine

Individual factors identified as being significantly associated with HPV vaccine acceptability based on no cost vaccine on univariate analysis are reported in Table 5.7.5. There was no significant difference in HPV vaccine acceptability identified in HIV-infected MSM versus HIV negative MSM based on no cost vaccine (p=0.9).

Table 5.7.5 Characteristics associated with HPV vaccine acceptability (n=277)

Demographic Data	P value	OR(u) (95%CI)	
Higher education level attained	0.01	3.01	1.57-5.75
Correct knowledge items relating to HPV infection/ vaccine			
HPV can affect men	0.01	4.87	2.62-9.05
HPV can be treated with antibiotics	0.03	2.57	1.10-5.99
Best age to receive vaccine	0.01	1.29	0.15-11.26
Perceptions regarding HPV infection/ vaccine			
The chance of HPV impacting on health is moderate/high	0.01	2.65	1.32-5.35
HPV vaccine effective preventing genital warts	0.02	2.54	1.17-5.48
HPV vaccine effective preventing anal/penile cancer	0.01	4.08	1.55-10.72
Not effective preventing other STI	0.01	3.96	1.36-11.49
HPV vaccine is expensive	0.07	6.45	0.85-49.07
Vaccine has side effects	0.01	3.67	1.50-8.97

Abbreviations: STI, Sexually Transmitted Infection, OR, Odds ratio, CI, confidence interval

5.7.5 Discussion

MSM who participated in this study were at high-risk of HPV infection and associated disease.

HPV vaccine acceptability (unconditional) observed in our study (31%) is lower than that reported in similar international studies in MSM 47-74% (Reiter et al., 2010). This may be due to poor knowledge relating to HPV infection and sexual health in general, HPV associated disease and HPV vaccine in MSM in Ireland.

HPV vaccine acceptability increased from 31% (unconditional) to 51% when participants were informed of efficacy of HPV vaccine for prevention of genital warts and anal/penile cancer, even in the context of associated vaccine cost of €300. This highlights the important role of education interventions in raising awareness of HPV infection and highlighting the potential role of HPV vaccine in prevention and protection. Such interventions will be critical in ensuring the success of targeted HPV vaccine programmes.

Cost was found to be significantly associated with HPV vaccine acceptability. HPV vaccine acceptability increased from 51% based on a cost of €300 for the vaccine series to 78% based on no cost vaccine ($p<0.01$). Similar studies undertaken in MSM in Australia (Simatherai et al., 2009) and Hong Kong (Lau et al., 2013) also identified cost as a significant factor influencing HPV vaccine acceptability in MSM.

Cost effectiveness data currently supports vaccination of MSM <26 year (Kim, 2010) with recent research suggesting HPV vaccine would be cost effective regardless of age in HIV-infected MSM (Deshmukh et al., 2015, Deshmukh et al., 2014). Despite these findings HPV vaccine for MSM is currently not funded through the national programme in Ireland or in the majority of European countries. As cost is significantly associated with HPV vaccine acceptability provision of free HPV vaccine through national programmes will be an important factor to consider if targeted vaccination programmes are to be successful.

Participants in our study had a poor knowledge of HPV-infection and HPV vaccination. Appropriate HPV infection and HPV vaccine knowledge response rates ranged from 16-58% and 32-82% (Table 5.7.2) respectively. MSM who completed the survey had a low perceived susceptibility (33%) to HPV infection despite being from a high-risk group. Less than 50% of study participants were aware that HPV could cause genital warts while only 26% were aware that HPV could cause penile or anal cancer.

Factors associated with HPV vaccine acceptability were examined based on no cost vaccine, as this would best reflect the real-life scenario should a targeted HPV immunisation programme be introduced for MSM in Ireland.

Higher education level was significantly associated with HPV vaccine acceptability [odds ratio (OR) 3.01, (95% CI) 1.57- 5.75, p=0.01] based on stated efficacy (90% effective in preventing genital warts, 75% effective in preventing anal cancer). This may represent greater understanding or insight into the stated protective effects of the vaccine in more educated individuals. Awareness campaigns and education interventions to promote HPV vaccine must use simplified material with a clear message to ensure it is easily understandable regardless of level of education so it can reach the widest audience. This will help to ensure the highest level of vaccine coverage can be achieved.

Several individual knowledge items relating to HPV infection and HPV vaccine were significantly associated with HPV vaccine acceptability again highlighting the importance of awareness and education in promoting the HPV vaccine in MSM. The finding that individual knowledge items relating to HPV infection increases HPV vaccine acceptability supports findings from other similar surveys (Pitts et al., 2007). Educational interventions should be developed to improve knowledge of HPV infection and associated disease to optimise HPV vaccine acceptability.

Other studies of HPV vaccine acceptability have found that a recommendation from a health care provider is an important factor in acceptability of HPV vaccine. While this was not examined in our study, it should be considered when education programmes are being developed (Newman et al., 2013).

Perception that HPV vaccine will protect against genital warts, penile and anal cancers were associated with HPV vaccine acceptability on univariate analysis (Table 5.7.5). Interestingly, perception that HPV vaccine may have side effects [OR 3.67, (95% CI) 1.50-8.97, p=0.01] and that HPV vaccine is expensive [OR 6.45, (95% CI) 0.85-49.07, p=0.07] were positively associated with vaccine acceptability.

As factors reported as associated with HPV vaccine acceptability are based on the scenario of no cost vaccine, perception that market cost of the vaccine is high may attribute a value to the vaccine and represent an incentive to avail of no cost vaccine.

Acknowledgement that the HPV vaccine may have side effects (although specific side effects were not stated) was positively associated with HPV vaccine acceptability. This may indicate that despite perception of associated side effects relating to vaccine, the risk benefit profile would be such that individuals would choose to receive vaccinate.

This study included HIV-infected (n=156) and HIV negative (n=146) MSM aged 18 to 60 years. While current guidelines recommend HPV vaccine for MSM up to the age of 26 years, emerging evidence suggests that HPV vaccine may have efficacy beyond primary prevention and thus may be of benefit in older MSM (Swedish and Goldstone, 2014). In this context, investigating the acceptability of HPV vaccine in a group that includes MSM >26 years is of relevance.

This study has a number of limitations; convenience sampling was undertaken and results may be subject to selection bias. HIV-infected MSM are over-represented in the study as one sampling site was a HIV outpatient clinic and thus results are not applicable to the general MSM population. Interestingly there was no significant difference in HPV vaccine acceptability between HIV-infected and HIV negative MSM based on no cost vaccine.

In addition, as this was a self-administered questionnaire not all questions were completed and missing data was not included in the analysis thus some findings must be interpreted with caution. The ordering effect was not accounted for in the survey design which may represent a bias. Information on MSM who refused to participate in the study was not recorded although anecdotal reports indicate that participation rates in survey were high. Self-reported sexual behaviours are subject to recall bias. Finally, reported acceptability of a vaccine does not correlate with uptake of a vaccine. We do not know what proportion of those who indicated that they planned to receive the vaccine were subsequently vaccinated. At the time of the study none of the MSM surveyed had received the HPV vaccine.

This research provides important insights into acceptability of HPV vaccine in MSM in Ireland and adds to limited data available in this area. Immunisation programmes providing universal HPV vaccination to HPV infection naïve individuals have the greatest potential to prevent HPV-associated disease. Such programmes are unlikely to be implemented in Ireland or in the majority of European countries in the short term given lack of cost effectiveness data. Universal vaccine programmes, while offering the best preventative opportunities to those vaccinated, will have no effect on risk of HPV associated disease in current high-risk adult populations such as MSM.

Our study indicates that acceptability of HPV vaccination in MSM would be high and would be expected to increase following implementation of health education programmes outlining the risks of HPV associated disease and efficacies of the HPV vaccine. Much of this education could be delivered synergistically using existing infrastructure alongside HIV prevention programmes.

A growing body of evidence supports use of HPV vaccine to prevent development of HPV-associated disease in older MSM (Sahasrabuddhe et al., 2013). Although no therapeutic benefit of HPV vaccine has been demonstrated for the treatment of active disease present at the time of vaccination, emerging data suggests a possible benefit of HPV vaccination in the setting of previous disease (Deshmukh et al., 2014, Deshmukh et al., 2015).

Sexual health and HIV outpatient clinics are well placed to facilitate targeted/catch-up HPV vaccination for the high-risk groups including HIV-infected and HIV negative MSM, particularly in the setting of similar effective models for hepatitis B vaccination (Rock et al., 2013a). The landscape in relation to HPV infection, prevention and associated disease is evolving rapidly. The recently available nonavalent HPV vaccine (HPV 9v) offers protection against an additional 5 oncogenic HPV types (31, 33, 45, 52, and 58) associated anal cancers in MSM (Hoots et al., 2009).

Targeted HPV vaccine has potential to greatly reduce the increasing burden of HPV associated anal cancer in MSM and HIV-infected MSM in the future. Our study suggests that acceptability of HPV vaccine will be high and given the individual and population health benefits vaccine should not be withheld.

5.8 Anal, Penile, and Oral HPV Infection and HPV 16/18 seropositivity in young HIV-infected Men Who Have Sex with Men

5.8.1 Abstract

HIV-infected MSM are a high-risk group for HPV associated anal cancer and are at increased risk of HPV associated oropharyngeal cancer. High-risk (hr) HPV types, particularly HPV 16 and 18 are causally associated with anal and oropharyngael cancers. The aim of this study was to investigate seroprevalence of hr HPV types 16 and 18 and the prevalence and diversity of HPV infections at anal, genital and oropharyngeal sites in young HIV-infected MSM in whom HPV vaccine is indicated.

HIV-infected, HPV vaccine naïve MSM $\geq$18 and $\leq$26 years were recruited. Serum samples along with anal, penile and oropharyngeal swabs were collected. Serum antibodies against L1 virus like particles (VLPs) of hr HPV 16 and 18 were detected using a VLP multiplex immunoassay. HPV DNA was detected from swabs by multiplex PCR using PGMY 09/11 consensus primers. Positive samples were subtyped using next generation sequencing (NGS) on the Ion Torrent platform and coupled to a HPV genotype survey and bioinformatics platform to examine the diversity of HPV genotypes present. We investigated prevalence of International Agency for Research on Cancer (IARC) Group 1 (high-risk oncogenic), Group 2A (probably oncogenic), Group 2B (possibly oncogenic) HPV genotypes and Group 3 low risk (lr) HPV type 6 and 11 which are the main causative agent for genital warts.

In 50 HIV-infected MSM, median [IQR] age was 25 [23-26] years, median [IQR] CD4+T cell count was 580 [440-696] cells/mm3 and 76% had an undetectable viral load.

Seropositivity was 44% (n=22) for HPV 16, 26% (n=13) for HPV 18 and 24% (n=12) for both HPV 16 and 18.

HPV DNA was detected from one or more site by PCR in 68% (n=34). The prevalence of HPV DNA detection was higher in the anal canal (66%) versus genital (8%) and oropharyngeal sites (4%). One or more of the 12 Group 1 hr HPV types were detected in 60% of study participants. Quadrivalent HPV types 16 and 18 were detected (on swabs, in serum or both) in 52% and 30% of study participants respectively while HPV types 6 and 11 were detected in 5 (10%) and 6 (12%) of study participants respectively. Nonavalent vaccine hr HPV types 31, 33, 45 and 52 were detected in 2%, 4%, 22% and 8% of study participants. Non-vaccine Group 1 hr HPV types 51, 56 and 59 were detected in 4%, 10% and 16% of participants.

Surveillance data generated from NGS provides further insights into HPV genotype diversity within a host ecosystem. Such information will further clarify the role of HPV infection and

multiple HPV infections in disease causation and will inform optimal vaccine preventative programmes, particularly given the availability of extended valency HPV vaccines.

Findings from this study indicate that while HPV is highly prevalent in young HIV-infected MSM, a significant proportion may derive protective benefit from the HPV vaccine. Vaccine based preventative strategies for HPV infection should monitor HPV type distribution among HIV-infected MSM.

5.8.2 Background

Human papillomavirus (HPV) is the most common sexually transmitted infection (STI) worldwide. It is highly prevalent in the sexually active population and rapidly acquired after sexual debut (Einstein et al., 2009). The majority of HPV infections are subclinical and clear spontaneously, however HPV can result in a wide variety of presentations ranging from benign genital dermatoses to disseminated invasive malignancy.

HPV is causally associated with genital warts, cervical cancer, vulval cancer, anal cancer, penile cancer and head and neck cancers (Giuliano et al., 2008). HPV now accounts for approximately 5% of all cancers worldwide (Forman et al., 2012). Over 150 types of HPV have been identified. The International Agency for Research on Cancer (IACR) have identified 12 carcinogenic (Group 1) HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 1 probably carcinogenic (Group 2A) HPV type (68) and 12 possibly carcinogenic (Group 2B) HPV types (26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97) (Chen et al., 2015). HPV 6 and 11 are the most common types that cause genital warts and are included in the HPV 4v and HPV 9v vaccine.

Anal cancer is a relatively rare occurrence in the general population (1-2 cases per 100,000) (van der Zee et al., 2013), however certain risk groups including MSM (40 cases per 100,000) and in particular HIV-infected MSM are disproportionately affected (up to 135 cases per 100,000) (Patel et al., 2008, D'Souza et al., 2008). The incidence of anal cancer in MSM is now greater than the incidence of cervical cancer pre-introduction of cervical screening programs (Machalek et al., 2012, Gustafsson et al., 1997).

The prevalence of ano-genital HPV infection in men in the general population has been reported at 1-84% (Dunne et al., 2006). The wide range in prevalence observed is likely multifactorial relating to differences in study populations, sampling methods (including the anatomical sites of sampling), and analysis methods used.

The prevalence of anal HPV infection in MSM is higher than that observed in heterosexual men (47.2% versus 12.2%) (Nyitray et al., 2011a). The prevalence of hr anal HPV infection is reported at 26-73% in HIV negative MSM (van der Snoek et al., 2003, Chin-Hong et al., 2004). Prevalence of hr HPV has been shown to be significantly higher in HIV-infected MSM compared to HIV negative MSM with prevalence reported at up to 93% (Sadlier et al., 2014b). Receptive anal intercourse, number of sexual partners in the preceding 6 months and HIV infection have been identified as independent predictors of anal HPV infection (Goldstone et al., Mooij et al.).

Prevalence of oropharyngeal and genital HPV infection has also been reported at rates of up to 45% in MSM and HIV-infected MSM (Beachler and D'Souza, 2013).

Detection of HPV antibodies in serum is considered a poorly sensitive but highly specific marker of HPV infection. A number of studies have demonstrated association of HPV seropositivity with type-specific anal HPV infection, but not detection of HPV at other sites (Heiligenberg et al., 2013). Prevalence of anti HPV 16 antibodies have been shown to be higher among HIV-infected versus HIV-negative MSM (Poynten et al., 2012)

Studies on the association between hr HPV infections at various anatomical sites and type-specific HPV seropositivity in MSM with and without HIV infection are scarce (van Rijn et al., 2014, Poynten et al., 2012, Heiligenberg et al, 2013).

As HPV antibodies are generally regarded as a marker of lifetime HPV exposure, more insight into antibody responses will assist in the interpretation of sero-epidemiological studies and in targeting HPV prevention strategies.

Study Aims

The aim of this study was to investigate baseline seroprevalence of hr HPV 16 and 18, prevalence of IARC Group 1 hr HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), Group 2A IARC genotype (68) and group 2B HPV types (26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97) as well as lr HPV types 6 and 11 at multiple sites in HIV-infected MSM in whom HPV vaccine is indicated.

5.8.3 Methods

Study population

HPV vaccine naïve HIV-infected MSM ≥18 years ≤26 years were recruited from the Department of GU Medicine and Infectious Diseases (GUIDE) HIV clinic between July 2013 and July 2014. Eligibility criteria included age between 18 and 26 years, being male, HIV-infected, having had sex with men, and competence in English.

Sample and data collection

A self-administered questionnaire was used to assess socio-demographic characteristics and details of lifetime and recent sexual behaviour. Healthcare provider performed swabs were taken from the anal canal, penile shaft, and oropharyngeal cavity. Anal swabs were collected by inserting a dry Dacron swab 3-4cm into the anal canal and rotating it. Penile swabs were obtained by rubbing a dry swab firmly over the penile shaft. Oropharyngeal swabs were taken by rubbing a dry swab over the oroparyngeal cavity. Swabs were immediately placed in a liquid based preservative medium, Thin prep® (Hologic Inc., Marlborough, MA 01752). A serum sample was collected for HPV antibody testing. HIV-related data (CD4 cell count, HIV viral load, and use of (HAART)) was obtained from the electronic patient record.

HPV DNA detection and NGS library preparation and sequencing

Detection of HPV was carried out by multiplex PCR using PGMY09/11 consensus primers.
DNA was extracted from samples using QIAamp DNA Mini kit (Qiagen). Barcoded library preparation was performed according to the Ion AmpliSeq Library kit 2.0-96LV protocol (Thermofisher Scientific, Massachusetts, USA), in conjunction with HPV GP5+/6+ primers (de Roda Husman et al., 1995). Sequences of approximately 150 base pairs of DNA residing in the L1 region, a consensus area of the HPV sequence were amplified. Final DNA libraries were quantified by qPCR using the Ion Library Quantitation Kit and pooled together. Pooled DNA libraries were mixed with Ion Sphere Particles and clonally amplified in an emulsion PCR, performed in accordance with the Ion PGM™ Template OT2 200 Kit protocol and using the Ion OneTouch™ 2 instrument (Thermofisher, Carlsbad, USA). Template-positive Ion Sphere Particles (ISPs) were then enriched using the Ion OneTouch™ ES instrument (Thermofisher, Carlsbad, USA). Enriched samples were loaded onto Ion 318™ chips and sequenced by using the Ion PGM™ System, following the Ion PGM™ Sequencing 200 Kit v2 protocol. This work was undertaken by Dr Paul Smyth, Histopathology laboratory, St James's Hospital.

Reads generated were evaluated for quality and quantity with FastQC (Babraham Bioinformatics, Cambridge, UK). The average quality of reads from each sample varied

between 28 and 30 Phred scores and the length was 100 base pairs before trimming. A low percentage (<10%) of N's was observed at some positions in 27 sequences. Trimming was performed with Sickle (Joshi and Fass, 2011) to remove base calls with Phred quality score lower than 28 (base calling accuracy of approximately 99.9%) and reads with a length lower than 70bp. Trimmed reads from each sample were mapped against a set of reference sequences from 167 different HPV types available at the PAVE database (http://pave.niaid.nih.gov) with BWA(LiandDurbin,2009).

The number of reads mapped against each HPV reference sequence was calculated with SAM tools (Li etal.,2009) and HPV infection was defined by two means: the presence of at least one read aligned to the L1ORF (the most conserved and used to classify HPV types) of a given HPV reference; or at least a pair of reads properly mapped against a given HPV reference and one of the reads similar a that given HPV type when evaluated by a HPV specific blast (http://pave.niaid.nih.gov). The bioinformatics work was undertaken by Dr Gordon Blackshields, Trinity College, Dublin.

HPV serum antibody testing

Serum samples were stored at –80°C on-site in the GUIDE laboratory and transported on dry ice to the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands). Virus-like particle (VLP) based multiplex immunoassay was used to assess HPV-specific serum antibodies against L1 VLPs of genotypes 16, 18 as previously described (Scherpenisse et al., 2012b). Serum samples were reported to be HPV-seropositive at cut-offs previously determined using a 99% prediction interval method based on children aged 1–10 years (Frey et al., 1998). Cut-off values were determined at ≥9 and ≥13 Luminex Units/millilitre (LU/ml) for HPV 16 and 18 respectively.

Statistical analyses

Categorical data is reported as totals and percentages. Continuous data is reported as medians and interquartile range. Variables were compared using parametric and non-parametric tests as appropriate.

Ethics statement

The Research Ethics Committee of St James's Hospital, Dublin approved this study. Written informed consent was obtained from all participants prior to enrolment.

5.8.4 Results

Baseline characteristics including data on selected sexual behaviours are outlined in Table 5.8.1. In brief, 50 HPV vaccine naive HIV-infected MSM 26 years or younger were recruited. Median [IQR] age was 25 [23-26] years, median [IQR] CD4 T cell count was 580 [440-696] cells/mm^3 and 76% had an undetectable viral load.

Table 5.8.1 Baseline characteristics of the cohort

Baseline characteristics among young HIV-infected MSM	Total MSM N=50 (%)
Median age [IQR]	25 [23-26]
Race n (%)	
Caucasian	34 (68)
Other	16 (32)
Age at sexual debut	16.5 [15-17.3]
Male partner at sexual debut n (%)	41 (82)
Age at first RAI	17 [15.5-18]
Age at first IAI	17 [16-18]
Lifetime number of RAI partners	10 [3-20]
Lifetime number of IAI partners	10 [3-20]
Number of sexual partners past 3 months	
RAI	1 [0-1]
IAI	1 [0-2]
Current smoker n (%)	20 (40)
Duration of HIV infection (years)	2 [1-3]
Baseline CD4 T count cells/mm^3	580 [440-696]
Nadir CD4 T count cells/mm^3	409 [326-551]
On HAART n (%)	43 (86)
Viral load not detected n (%)	36 (72)

Data reported as Median [IQR] unless otherwise specified
Categorical data is reported as number (n) and percent (%).
Continuous variables are reported as median and [IQR].
Abbreviations: MSM men who have sex with men, HPV human papillomavirus, HAART highly active antiretroviral therapy, RAI receptive anal intercourse, IAI insertive anal intercourse.

HPV seropositivity

Seropositivity for hr HPV 16 was 44% (n=22). Seropositivity for hr HPV 18 was 26% (n=13). Seropositivity for both hr HPV 16 and hr HPV 18 in the cohort was 16% (n=8).

HPV DNA detection by PCR

HPV DNA was detected from one or more sites by PCR in 68% (n=34). The prevalence of HPV DNA detection was 66% (n=33) in the anal canal, 8%, (n=4) on genital swabs and 4%, (n=2) in the oropharyngeal cavity.

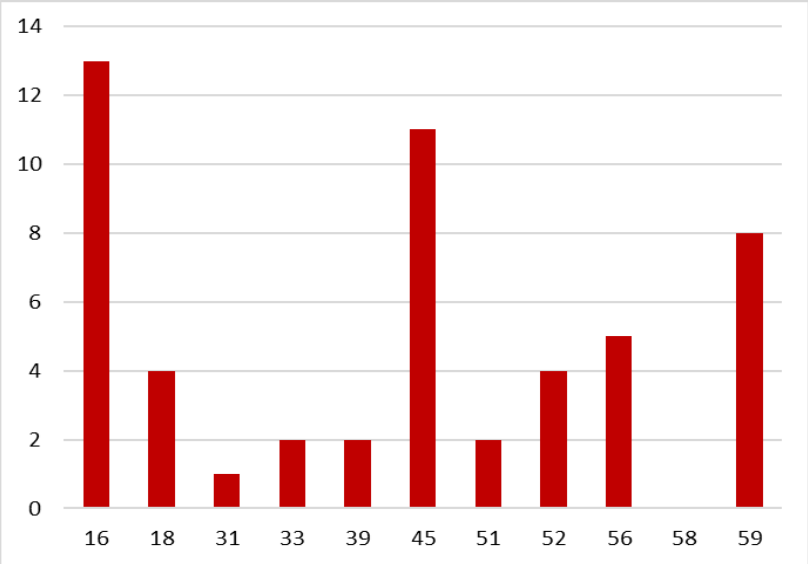
HPV genotypes detected by Next Generation Sequencing

Sequence reads mapping to 52 HPV genotypes were identified from swabs using NGS. Thirty individuals had multiple (two or more) HPV types detected. Median [IQR] number of HPV types detected was 2 [2-4].

Prevalence and distribution of Group 1 HPV genotypes

Group 1 hr HPV types with >1% mapped reads were detected in 54% (n=27) on anal swabs and 6% (n=3) on penile swabs. Oropharyngeal hr HPV was not detected. The relative distribution and percentage mapped reads of the 12 Group 1 hr HPV by type is outlined in Figure 5.6

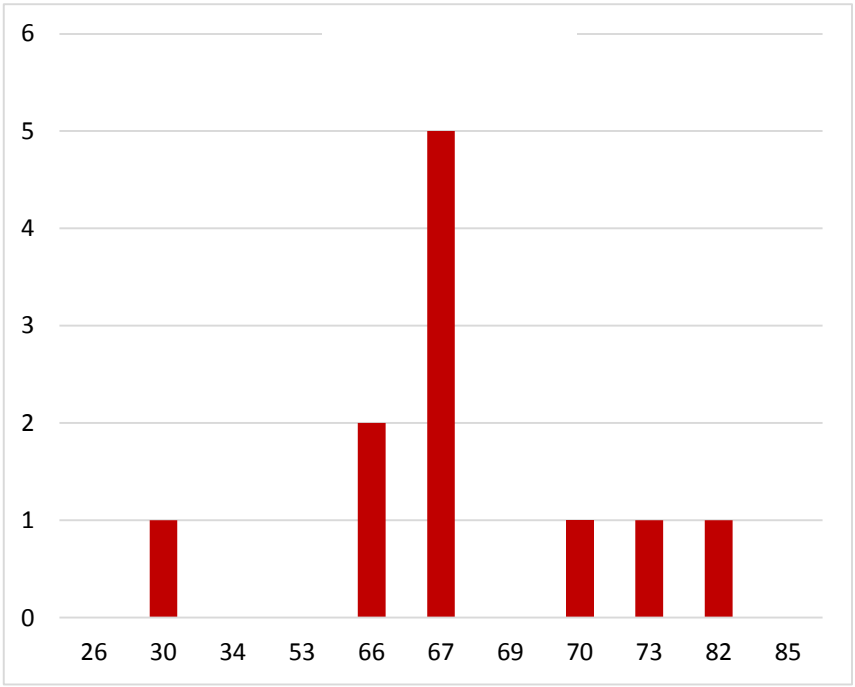
Figure 5.6 Frequency and diversity of Group 1 hr HPV types



Prevalence and distribution of Group 2 HPV genotypes

Group 2A HPV 68 was not detected. Group 2b HPV types with >1% mapped reads were detected in 11 (22%) individuals. The relative distribution and % mapped reads of the 12 Group 2b HPV by type is outlined in Figure 5.7.

Figure 5.7 Frequency and diversity of Group 2b HPV types



Prevalence and distribution of Group 3 lr HPV genotypes

Group 3 HPV 6 was detected in 5 participants (10%), three on anal swab and two on penile swab. HPV 11 was detected in 6 participants (12%), five were detected on anal swabs, one on pharyngeal swabs.

HPV vaccine types

Quadrivalent HPV vaccine types 16 and 18 were detected in 13 (26%) and 4 (8%) individuals respectively while HPV types 6 and 11 were detected in 5 (10%) and 6 (12%) respectively. Additional hr HPV types covered by the nonavalent HPV vaccine 31, 33, 45 and 52 were detected in 2%, 4%, 22% and 8% of study participants. Non-vaccine Group 1 hr HPV types 51, 56 and 59 were detected in 4%, 10% and 16% of participants.

Table 5.8.2 Proportion of participants with nonavalent HPV vaccine types detected

Nonavalent HPV serotype	6	11	16	18	31	33	45	52
Detected in n (%)	5 (10)	6 (12)	13 (26)	4 (8)	1 (2)	2 (4)	11 (22)	4 (8)

Abbreviations: HPV human papillomavirus, n number

Type specific HPV antibody and type specific HPV detection

Of 22 individuals with anti-HPV 16 antibodies detected, 9 (41%) had HPV 16 DNA detected, 8 on anal swabs and one on penile swab. Individuals with HPV 16 detected on swabs were significantly more likely to have anti-HPV 16 serum antibodies detectable (p=0.054).

Of the 13 individuals with anti-HPV 18 antibodies detected, 2 (15%) had hr HPV 18 detected on anal swabs. No significant association was observed between detection of anti-HPV 18 antibodies and detection of HPV 18 on peripheral swabs (p=0.25), although our ability to detect such differences may be limited by the small number identified with HPV 18 on swabs.

5.8.5 Discussion

Rates of hr HPV 16 and 18 seropositivity observed in this study are high (44% and 26% respectively). This finding supports those reported in other HIV-infected cohorts (Sharma et al., 2013) and indicates that a significant proportion of individuals are infected or have been previously exposed to these hr HPV types in the absence of previous HPV vaccination.

Seropositivity is a highly specific but poorly sensitive marker of prior exposure to HPV infection and thus may underestimate prior exposure to HPV infection. Anti-HPV specific antibodies are not always detectable in the setting of peripheral HPV infection and when present are observed to wane over time (Wang et al., 2004).

Group 1 hr HPV types were identified in 60% of study participants with a significant proportion of individuals having multiple HPV infections identified. Despite the high prevalence of HPV antibodies and HPV DNA detected in this study, a significant proportion of HIV-infected MSM in our study would be considered naïve to hr HPV serotypes covered by the quadrivalent or nonavalent HPV vaccines.

The reported incidence of HPV 16 (10.8 cases/1000 person-months) and HPV 18 (4.4 cases/1000 person-months) infection is high in HIV-infected MSM (de Pokomandy et al., 2009) suggesting that future risk of exposure remains high in this population.

In larger studies HPV 16 seropositivity has been demonstrated to be associated with number of recent sexual contacts rather than number of lifetime sexual contacts (Sharma et al., 2013). Participants in our study reported high-risk sexual behaviour as well as multiple sexual contacts thus it is possible that these individuals were previously exposed to hr HPV 16 and or 18, but that antibody titres decreased over time to undetectable or that HPV infection cleared spontaneously.

Individuals who were seropositive for hr HPV type 16 were more likely to have concurrent HPV 16 detected on anal swabs. This finding suggests a potential correlation between site of HPV infection and development of HPV antibodies.

It may be that anal hr HPV infection is more common in HIV-infected MSM (Sadlier et al., 2014b) or is more likely to persist in HIV-infected MSM (Sadlier et al., 2014a) compared to other groups. It has also been hypothesised that HPV infections in keratinised epithelium, which covers the glans and the shaft of the penis, may be less likely to elicit an immune response (and

subsequently induce antibody production) than infections in mucosal epithelium, which covers the anal canal and oral cavity (Lu et al., 2012) (Heiligenberg et al., 2013).

Next generation sequencing (NGS) is a relatively new technology and offers many advantages over older methods for HPV detection such as hybridisation and sequence based tests. NGS provides high sensitivity in capturing even low frequency HPV types (Arroyo et al., 2013). Deep sequencing technology allows detection of genotypes present at low frequency which may further clarify how diversity and sub-lineages of HPV contribute to development of disease and provide insights into the dynamics and competition among genotypes that frequently co-exist within a host ecosystem (Yin et al., 2016).

This study has a number of limitations. This study is a pilot study and lacks power due to limited number of oral and penile HPV infections to examine factors associated with HPV infection. From a HPV detection perspective, consensus primer systems all suffer from certain amplification bias towards HPV genotypes with greater priming efficiency (Chan et al., 2006); thus, the percent of sequences may not correlate with the actual amount of virus present and cannot serve as a quantitative measure of viral population structure (Arroyo et al., 2013).

In conclusion, a high proportion of participants in our study were seropositive for hr HPV types or had HPV detected on swabs. While rates of infection observed were high, a significant proportion of individuals would be considered naïve (undetectable HPV on swabs and serum antibodies negative) to more than one vaccine HPV type. Studies have shown that HPV vaccination is projected to be cost-effective in HIV-negative and HIV-infected MSM up to 26 years of age and cost effectiveness is currently being assessed in older HIV-infected individuals (Kim, 2010).

Vaccine based prevention strategies for HPV infection should monitor potential differences in HPV type distribution among HIV-infected MSM when designing interventions to optimise preventative potential of the vaccine.

5.9 Immunogenicity of the quadrivalent HPV vaccine in young HIV-infected MSM

5.9.1 Abstract

HIV-infected MSM are the highest risk group for human papillomavirus (HPV) infection, HPV associated disease and HPV associated anal cancer. HPV vaccine is safe, highly effective and prevents HPV infection, associated disease and has potential to greatly reduce the burden of anal cancer. The aim of this study was to examine immunogenicity of the quadrivalent HPV vaccine (HPV 4v) in young HIV-infected men who have sex with men (MSM) in whom the HPV vaccine is indicated.

A total of 50 HPV vaccine naïve MSM <26 years old who met inclusion criteria were recruited. We assessed baseline seroprevelance of high-risk (hr) HPV 16 and 18 as well as type specific HPV infection at multiple sites as presented in this thesis in section 5.8. MSM received 0.5 mL HPV 4v intramuscularly at study entry (week 0), week 8, and week 24. Serum samples were drawn at week 8, week 12, week 24, week 28 and week 52 to measure type specific antibody response to HPV vaccine. The primary end-point of this study was seroconversion to hr HPV vaccine types 16 and 18 at week 52 in MSM who were seronegative to the relevant HPV type at baseline.

At baseline (week 0), 56% (n=28) of study participants were seronegative for anti-HPV 16 antibody and 74% (n=37) were seronegative for anti-HPV 18 antibody. A total of 35 patients (70%) attended for week 52 bloods. Seroconversion rate at week 52 was 100% (18/18) for anti HPV 16 antibodies and 86% (24/28) for HPV 18. Peak GMT was observed at week 12 for anti-HPV 16 (1767 Liu/ml) and anti-HPV 18 (362 Liu/ml) following second dose of HPV vaccine. A decline in GMT was observed between week 28 and week 52 for HPV 16 (p=0.01) and HPV 18 (p=0.06).

A higher GMT was observed at week 52 in individuals who were seropositive at baseline for HPV 16 (504 versus 239 Liu/ml, p=0.16) and HPV 18 (411 versus 45 Liu/ml, p<0.01) versus those who were seronegative.

This study demonstrated that the quadrivalent HPV vaccine is highly immunogenic in young HIV-infected MSM. Type specific GMT observed at week 52 was greater in individuals who were seropositive versus those who were seronegative at baseline. Further studies examining immunogenicity over a longer time interval as well as clinical efficacy endpoints in HIV-infected MSM are warranted.

5.9.2 Background

As evidenced from international literature and data outlined in this chapter, HIV-infected MSM are at high-risk of HPV infection, HPV associated disease and HPV associated anal cancer (Giuliano et al., 2008, Patel et al., 2008, D'Souza et al., 2008).

The quadrivalent HPV vaccine (HPV 4v) (covering HPV serotypes 6,11,16,18) prevents persistent vaccine type HPV infection in females and prevents nearly 100% of vaccine type high-grade cervical, vaginal, and vulvar intraepithelial neoplasia as well as precursor lesions to invasive cancers at these sites in HPV naïve females at baseline (Garland et al., 2007). Efficacy of HPV 4v has also been demonstrated for prevention of vaccine type HPV infection and development of external genital lesions in males up to the age of 40 years (Giuliano et al., 2015).

There is only one published study that we are aware of examining immunogenicity of HPV vaccine in HIV-infected MSM. This study excludes MSM who are seropositive or HPV DNA positive on swabs for vaccine type HPV at baseline (Wilkin et al., 2010) and thus is poorly representative of the real world population.

Our study was designed as a pilot study to investigate the immunogenicity of HPV vaccine in young HIV-infected MSM in whom HPV vaccine is indicated regardless of HPV infection status at baseline. This data will inform policy makers on potential protective benefits of HPV vaccine in HIV-infected MSM.

5.9.3 Methods

A single centre prospective cohort study was undertaken to measure HPV 16 and HPV 18 antibody response post vaccination in young HIV-infected MSM in whom HPV vaccine is indicated.

Study population

HPV vaccine naïve HIV-infected MSM were recruited from the Department of GU Medicine and Infectious Diseases (GUIDE) clinic, St James's Hospital, Dublin, between July 2013 and July 2014. Inclusion criteria were as follows: MSM aged ⩾18 years; laboratory documentation of HIV infection, CD4 T cell count ⩾200 cells/μL, having an absolute neutrophil count >750 cells/μL, haemoglobin level ⩾9.0 g/dL, platelet count ⩾100,000 platelets/μL, and aspartate and alanine aminotransferase levels ⩽3 times the upper limit of normal; total or conjugated bilirubin <2.5 times the upper limit of normal; calculated creatinine clearance by Cockcroft-Gault equation ⩾60 mL/min; and a Karnofsky performance score ⩾70.

Sample and data collection and analysis

Samples and data were collected as analysed as outlined in chapter 5.8.3.

HPV vaccine

At week 0, week 8, and week 24, participants received the quadrivalent HPV (types 6, 11, 16, and 18) recombinant vaccine (0.5 mL) intramuscularly. The vaccine contains a L1 VLP and 20μg HPV 18 L1 virus like particle (VLP), along with 225μg of amorphous aluminum hydroxyphosphate sulfate adjuvant.

HPV serum antibody testing

Serum was drawn pre-vaccine (week 0), week 8, week 12, week 24, week 28, week 52.

Serum samples were stored at –80°C on-site in the GUIDE laboratory and transported on dry ice to the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands), where samples were stored at –80°C until processing. VLP based multiplex immunoassay was used to measure HPV-specific serum antibodies against L1 VLPs of genotypes 16, 18 as previously described (Scherpenisse et al., 2012a). Serum samples were reported to be HPV-seropositive at cut-offs previously determined using a 99% prediction interval method based on children aged 1–10 years (Frey et al., 1998). Cut-off values were determined at ≥9 and ≥13 Luminex Units/millilitre (LU/ml) for HPV 16 and 18 respectively.

Statistical analyses

Baseline characteristics of HIV-infected individuals are reported as totals and percentages for categorical variables and medians and interquartile range for continuous variables.

Separate analyses were conducted for HPV types 16, and 18 antibodies. The primary endpoint for the study was seroconversion at week 52 defined as being seronegative at entry (ie. having no detectable type-specific anti- HPV antibodies or detectable antibodies below the pre-specified cut-off for the assay) and seropositive at week 52 (ie. antibody concentrations above the assay cut-off).

Increase in type specific HPV antibody GMT was compared at time point analysis and between individuals who were HPR 16/18 seronegative at baseline and individuals who were seropositive for 16/18 at baseline.

Ethics statement

The Research Ethics Committee of St James's Hospital, Dublin approved this study. Written informed consent was obtained from all participants prior to enrolment.

5.9.4 Results

Baseline characteristics of study participants (n=50) are outlined in Table 5.9.1.

Three patients who were recruited to the study did not subsequently attend for vaccine. Of the remaining 47 of 50 patients completed 3 doses of HPV vaccine, 46, 41, 45, 31 and 35 attended to have serum samples taken at week 8, 12, 24, 28, 52 respectively.

Table 5.9.1 Flow of subjects recruited to the study

Study Time point	Provided serum for analysis n=	HPV Vaccine 1 n=	HPV Vaccine 2 n=	HPV Vaccine 3 n=
Week 0	50	47		
Week 8	46		47	
Week 12	41			
Week 24	45			47
Week 28	31			
Week 52	35			

Seropositivity

At week 0, anti-HPV seropositivity was 44% (22/50) for anti HPV 16 antibodies and 26% (13/50) for anti-HPV 18 antibodies. At week 52, following completion of vaccine series, seropositivity was 100% (35/35) and 89% (31/35) seropositivity for genotypes 16 and 18 respectively.

Type specific HPV antibody titres at time point evaluation

Overall geometric mean titres (95% confidence interval (CI)) reported in Luminex international units/ml (Liu/ml) for HPV 16 and at week 0, 8, 12, 24, 28 and 52 are outlined in Table 5.9.1 /Figure 5.8.

Peak anti-HPV 16 GMT (1767 Liu/ml) were observed at week 12, 4 weeks following the second dose of HPV vaccine. A decline in anti-HPV 16 antibody titre was observed between week 28 and week 52 from 1492 Liu/ml to 343.5 Liu/ml.

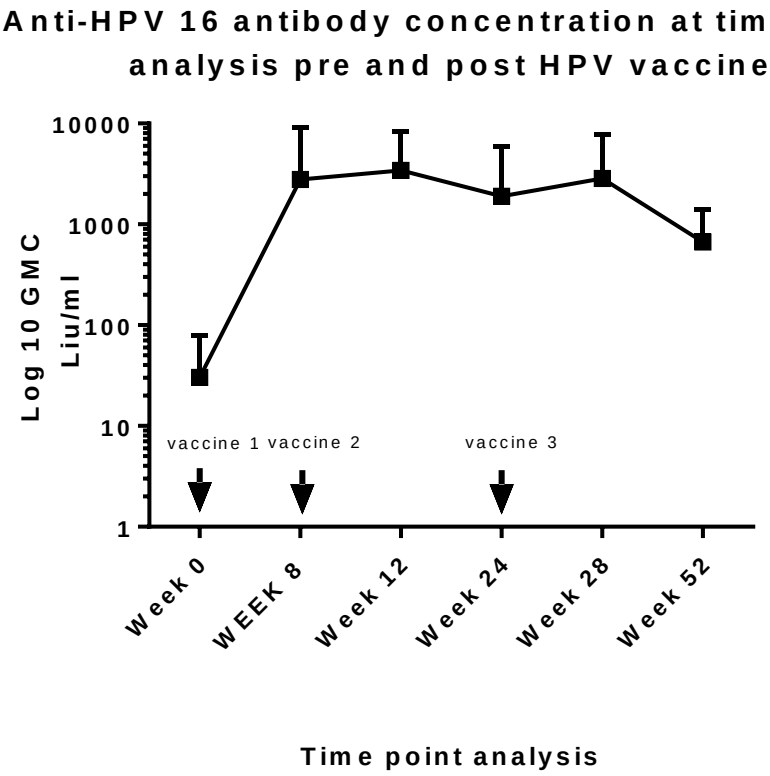
A significant increase in anti HPV 16 GMT was observed from 9.5 Liu/ml (95% CI, 5.8 - 15.6) at week 0 to 343.5 (95% CI 215.9 - 546.6) at week 52, $p<0.01$).

Table 5.9.2 HPV 16 GMT Liu/ml at Time point analysis

	HPV 16 GMT Liu/ml at Time point analysis					
	Week 0	**Week 8**	**Week 12**	**Week 24**	**Week 28**	**Week 52**
GMT LiU/ml	9.499	354.6	1767	723.2	1492	343.5
95% CI	5.79-15.6	179.8-699.4	1192-2617	469.5-1114	977.8-2276	215.9-546.6

Abbreviations GMT Geometric Mean Titre, Liu Luminex units, CI Confidence Interval

Figure 5.8 Anti HPV 16 antibody GMC at time point evaluation



Overall geometric mean titres (95% Confidence Interval) Liu/ml for HPV 18 and at week 0, 8, 12, 24, 28 and 52 are outlined in Table 5.9.2 /Figure 5.9.

Peak anti-HPV 18 GMT was observed at week 12 (360 Liu/ml), 4 weeks following second dose of HPV vaccine.

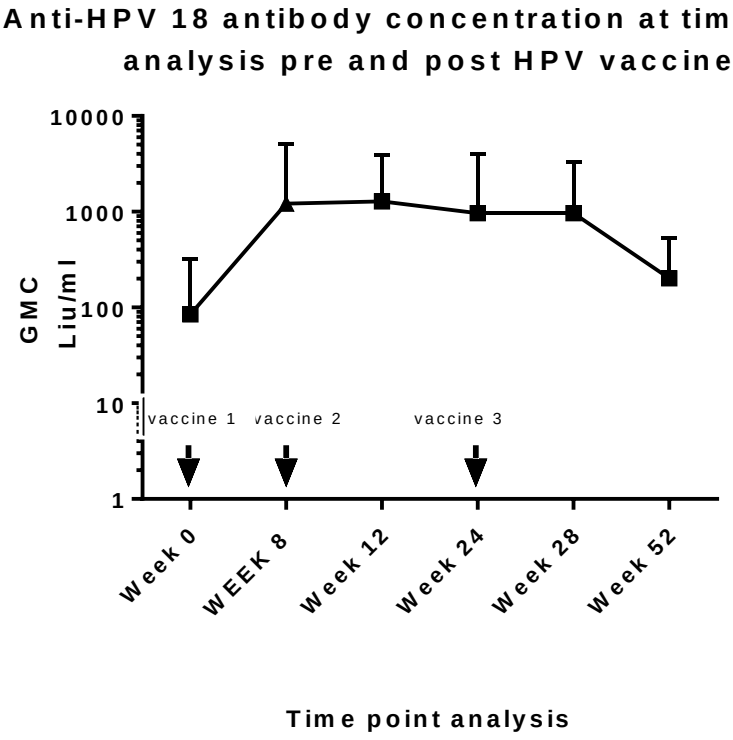
An increase in anti HPV 18 GMT was observed from 6.1 Liu/ml (95% CI, 3.1 - 12.2) at week 0 to 75.33 (95% CI 46.2 - 122.9) at week 52 (p=0.08). A decline in anti-HPV 18 antibody GMT was observed from week 28 to week 52 from 269.9 - 75.33 Liu.

Table 5.9.3 HPV 18 GMT Liu/ml at Time point analysis

	HPV 18 GMT Liu/ml at Time point analysis					
	Week 0	**Week 8**	**Week 12**	**Week 24**	**Week 28**	**Week 52**
GMT LiU/ml	6.14	60.46	360.2	118.4	269.9	75.33
95% CI	3.0-12.2	27.9-130.9	210.7-615.8	65.1-215.3	154.9-470.2	46.2-122.9

Abbreviations GMT Geometric Mean Titre, Luminex units, CI Confidence Interval

Figure 5.9 Anti HPV 16 antibody GMC at time point evaluation



As a secondary end-point we analysed baseline predictors of seropositivity at week 52. Individuals who were seropositive for type specific HPV and who had concurrent type specific HPV DNA detected on swabs were observed to have higher type specific antibodies at week 52 for HPV 16 (504 versus 239, p=0.16) and HPV 18 (411 versus 45, p=0.002) respectively.

No serious adverse events or adverse events were reported in the cohort following vaccine.

The study was not powered to measure the influence of CD4 T cell count or HIV viral load on vaccine efficacy.

5.9.5 Discussion

In this pilot study, HPV 4v elicits a highly immunogenic response in HIV-infected MSM with 100% and 82% of individuals who were seronegative for HPV 16 and HPV 18 at baseline seroconverted following vaccine.

GMTs observed in our study to vaccine types 16 and 18 were lower in HIV-infected MSM compared to those observed in studies of women and HIV negative MSM (Hillman et al., 2012). Week 28 MTs to vaccine HPV types 16 and 18 in study participants were 1492 mMU/ml for HPV 16 and 267 versus for HPV 18. In comparison, week 28 GMT in HIV negative males have been reported at 2,404 mMU/ml for HPV 16, and 402 mMU/ml for HPV 18 (Hillman et al., 2012) and at 3,870 mMU/ml, and 741 mMU/ml respectively for females aged 16 to 23 years (Olsson et al., 2007).

This suggests that HIV-infected individuals may have a less immunogenic response to vaccine, however it is not possible to make direct statistical comparisons across these study populations given differences with respect to countries included, populations enrolled, number of sexual partners, and other factors such as smoking and HIV infection status.

A protective threshold to prevent HPV infection from occurring has yet to be established. Additionally, it remains unknown if a correlation exists between frequency of L1 VLP-specific memory lymphocytes and serum antibody titres.

The increase in GMT observed in individuals who were seropositive and PCR positive for HPV 16 and/or HPV 18 at baseline was greater compared to those who were seronegative. This has been observed in other studies (Wilkin et al., 2010, Olsson et al., 2007, Hillman et al., 2012) supporting the theory of an amnestic response, however the significance of a higher type specific antibody titre remains to be determined. Further research is warranted to investigate whether higher antibody titres may impact on reinfection or reactivation of HPV in these patients.

This study has a number of limitations including small sample size. Retention of subjects in the study was less than anticipated with 10 of 47 (22%) participants transferring care abroad by week 52 reflecting a young highly mobile patient group. Our study was not powered to reliably examine the influence of confounding factors such as smoking or HAART on immune response.

We did not observe any adverse events attributable to vaccination during the study. Other HPV 4v studies undertaken in HIV-infected MSM have demonstrated vaccine safety in HIV-infected individuals and no effects on CD4 cell counts have been observed (Wilkin et al., 2010).

Our data suggests that the HPV 4v is highly immunogenic in young HIV-infected MSM. The observed responses were substantially higher than those seen during natural HPV infection and support with the established efficacy of the vaccine in the prevention of incident and persistent HPV infection, anogenital warts, and anal intraepithelial neoplasia in HIV-infected MSM.

We have previously demonstrated that acceptability of the HPV 4v would be high in MSM and HIV-infected MSM. A significant proportion of patients in this study demonstrated to be HPV vaccine naïve at baseline could derive potential protective benefits from the HPV 4v. Further studies examining clinical endpoints for HPV vaccine in HIV-infected MSM are warranted.

5.10 Summary of findings

We observed a high burden of anal cancer (44 cases/100,000) in the HIV positive cohort in the GUIDE clinic in St James's Hospital, Dublin.

We identified a high prevalence of HPV infection and hr HPV 16 and 18 infections in MSM. Prevalence of HPV infection and hr HPV 16/18 infection is higher in HIV-infected MSM which is likely an important contributory factor to the high incidence of anal cancer observed. Approximately 70% of anal cancers are caused by HPV 16 and 18 infections.

HPV vaccine is highly effective in preventing incident and persistent vaccine type HPV infection. A significant proportion of participants in this research were susceptible to one or more component of the quadrivalent or nonavalent HPV vaccines and thus could derive protective benefit from vaccination.

We identified a high level of vaccine acceptability in MSM and HIV-infected MSM indicating that targeted vaccine programmes would have high levels of uptake needed to impact the burden of HPV associated disease observed in this high-risk group.

To further investigate the natural history of HPV in young MSM in whom HPV vaccine is indicated we measured HPV 16 and 18 seropositivity at baseline. We also examined HPV genotype diversity in this group using next generation sequencing, a highly sensitivity technology allowing detection of multiple HPV types.

The HPV vaccine has been demonstrated to be highly immunogenic in the general population. Studies in HIV-infected individuals are limited. We examined baseline seropositivity to HPV 16 and 18 in HIV-infected MSM aged 18-26 years. We investigated HPV genotype diversity at baseline in this patient cohort. We found that 86-100% of participants who were seronegative for HPV 16/18 at baseline seroconverted on assessment at week 52 supporting evidence that HPV vaccine is highly immunogenic in HIV-infected individuals.

This research provides first Irish data on HPV infection and burden of HPV associated disease in high-risk groups including HIV-infected individuals and MSM. This research can help inform policy makers when considering implementation of targeted HPV vaccination programmes.
Future directions of research in this area should look to clarify HPV types underlying HPV associated anal cancer in the general population as well as high-risk groups given evidence for

geographical variability and the variable HPV genotypes observed in HIV-infected females presenting with cervical pathology.

Secondary preventative measures should be assessed to impact the burden of anal cancer which continues to increase in the era of HAART.

Studies with clinical endpoints are required to further assess potential benefits of HPV vaccine (including nonavalent vaccine) for primary prevention or as a therapeutic adjunct in individuals with HPV associated pathology (including AIN, VIN, CIN).

6. Overall Conclusions

6.1 Summarising discussion

This thesis adds significantly to literature in the area of vaccination and vaccine preventable infections in HIV-infected individuals.

The thesis is framed thematically, so that retrospective and seroepidemiological data describing susceptibility to vaccine preventable infections and vaccine uptake in HIV-infected individuals attending a single tertiary referral HIV specialist centre are outlined in Chapters 2 and 3. The sero-epidemiological data presented in Chapter 2 represents the first Irish data on susceptibility of HIV-infected individuals to vaccine preventable infections. The integrated vaccine unit described in Chapter 3 is a novel model of care for vaccine delivery to HIV-infected individuals and this is the first study examining trends in vaccine coverage in HIV-infected individuals in the setting of an integrated vaccine programme.

The findings presented in Chapters 2 and 3 set the context for and highlight the importance of the main body of work of this thesis, presented in Chapters 4 and 5. Chapter 4 investigates the burden of invasive pneumococcal disease in a HIV-infected cohort and assesses immunological response to pneumococcal vaccine strategies using a variety of measures in HIV-infected adults. The randomised controlled trial presented examines immunological response to a prime-boost immunisation strategy combining the 13-valent conjugate pneumococcal vaccine (PCV13) with the polysaccharide pneumococcal vaccine (PPV23) compared to PPV23 alone in HIV-infected adults. This work was undertaken at a time when PCV was unlicensed for use in adults. This field has evolved rapidly in the intervening years and the prime-boost immunisation strategy combining PCV13 with PPV23, which was investigated in our study, is now recommended as standard of care for HIV-infected adults. Additionally, the killing opsonophagocytic assay (OPA), one of the measures of vaccine response used in our study is now widely accepted as the gold standard measure of pneumococcal vaccine response, although an immunological threshold for clinical protection following pneumococcal vaccine remains to be determined.

Chapter 5 examines the burden of anal cancer in a HIV-infected cohort and investigates epidemiology of HPV infection focusing on HIV-infected MSM, the highest risk group for HPV associated disease. Immunogenicity of the HPV vaccine is also investigated in young HIV-infected MSM, a group who could potentially derive protective benefit from the vaccine. This research highlights the preventative potential of the HPV vaccines in this at-risk patient group.

Invasive pneumococcal disease (IPD) and HPV infection cause a significant burden of morbidity and mortality in HIV-infected individuals, and both have vaccines recently licensed

for use in HIV-infected individuals. While these vaccines are now recommended for HIV-infected adults in consensus international immunisation guidelines, there is limited published data on the efficacy and immunogenicity of these vaccines in HIV-infected individuals. This thesis adds to the limited literature published in this area.

This final chapter of my thesis will summarise the key findings reported and their implications for HIV care delivery. Results and future directions are discussed in the context of the current literature and most recent immunisation guidelines for HIV-infected individuals.

6.2 Summary of thesis findings

Chapter 2 of this thesis yields the first Irish seroepidemiologic data describing susceptibility to a number of common vaccine preventable infections including hepatitis A virus, hepatitis B virus, varicella zoster virus as well measles, mumps and rubella in a cohort of HIV-infected individuals. While limited data regarding susceptibility to these infections in HIV-infected individuals has been published, geographical variations occur and this highlights the importance of generating the first Irish dataset to inform national immunisation policy for HIV-infected individuals (Molton et al., 2010, Sticchi et al., 2014).

We identified high rates of susceptibility to vaccine preventable infections in the study population (6% susceptible to varicella, 68% susceptible to HBV) highlighting an opportunity for disease prevention though vaccination in this high-risk patient group. Supporting data in this thesis shows that HIV-infected individuals are at increased risk of morbidity and mortality relating to vaccine preventable infections. Thus, Chapter 2 underscores the need for pro-active screening and vaccination to ensure optimal protection is afforded to these at-risk patients.

In **Chapter 3**, I examine the integrated vaccine unit employed in the Department of Genito Urinary Medicine and Infectious Diseases (GUIDE) ambulatory HIV clinic, St James's Hospital as a model of care for delivery of vaccination to HIV-infected individuals. Despite a significant body of evidence that vaccination is safe and effective in HIV-infected individuals, the burden of vaccine preventable infections remains high and documented vaccination coverage in HIV-infected populations remains poor.

Although other studies in the international literature have examined influenza vaccination coverage in HIV-infected individuals, this is the first study to examine temporal trends in vaccination coverage in the setting of an integrated vaccine programme, a novel model of care. We observed a significant increase in documented seasonal influenza vaccination uptake in the attending HIV-infected cohort over a 10-year period (from 50% in 2003 to 76% in 2015, $p<0.01$), since establishment of the integrated vaccine programme. Furthermore, the seasonal influenza vaccine coverage that I report in 2014/2015 (76%) is significantly greater than that reported elsewhere in the literature in HIV-infected cohorts (Durham et al., 2015).

We also report improved outcomes in relation to HBV vaccine in attending HIV-infected individuals. These findings demonstrate sustainability of improvements that we previously reported in HBV vaccine series completion and response in the setting of an integrated immunisation unit (Rock et al., 2013b).

While the vaccine unit has delivered higher levels of vaccination coverage than have been documented in other clinical settings, further improvements are required to achieve the Healthy people 2020 vaccination coverage target of 90% (Health.gov, 2016). The disparity between immunisation recommendations and clinical practice are significant and I would suggest that education and awareness in both clinical providers and patients as well as funding and resourcing of systems and infrastructures such as the vaccine unit model, need to be employed to achieve target vaccination coverage. Internationally, infant immunisation programmes have been hugely successful as reliable infrastructures and systems deliver the result they set out to achieve.

In **Chapter 4**, I report on trends in the burden of invasive pneumococcal disease (IPD) in HIV-infected individuals. While the incidence of IPD has decreased significantly in recent years, the incidence reported in our study (283 cases/100,000) remains significantly greater than that reported in the general population. I identified injecting drug users (IDU) as a significant risk group for IPD. This information has facilitated a targeted immunisation programme within our vaccine unit where IDUs are prioritised within the cohort for PCV13.

Efficacy of pneumococcal vaccine in HIV-infected individuals remains debated. Failures of PPV23 vaccine are well documented and indeed we have reported failures of PPV23 in our IPD study.

The randomised controlled trial undertaken as part of this thesis examined a novel pneumococcal vaccination strategy combining PCV13 with PPV23 compared to PPV23 alone in HIV-infected individuals. This research pre-dated recent changes to pneumococcal immunisation guidelines which now recommend the prime-boost immunisation strategy combining PCV13 and PPV23 as standard of care for HIV-infected individuals and was undertaken at a time when PCV13 was unlicensed for use in adults in Ireland. This resulted in a significant number of submissions to achieve necessary ethical and regulatory approvals including a submission to the Health Products Regulatory Authority (HPRA) to undertake and register this clinical trial.

This research demonstrated a trend towards greater magnitude and duration of response to the prime-boost immunisation strategy supporting current immunisation guidelines. However, the IgG2 and in-house phagocytic OPA response between groups did not differ significantly. This highlights the need to standardise measures of vaccine efficacy and for correlates of clinical efficacy of these vaccines to be determined. This was a study with a small sample size and

findings must be interpreted in this context. The data presented do not provide sufficient evidence to strongly recommend a prime-boost vaccine strategy for all HIV-infected individuals and policy makers must consider all available data when making recommendations.

In **Chapter 5**, I report a significant burden of anal cancer in HIV-infected individuals attending a single tertiary referral centre in Ireland (44 cases/100,000). I have generated the first data relating to prevalence of HPV infection and high-risk or oncogenic HPV types 16 and 18 infections in HIV-infected and HIV negative MSM in Ireland. My findings are similar to those reported in the international literature where high rates of anal HPV infection and hr HPV infection are reported in MSM.

I report high levels of persistence of anal HPV infection and hr anal HPV infection in a subgroup of HIV-infected MSM and significant rates of HPV infection at multiple sites (oral and genital).

HPV infection and associated disease is potentially preventable with the HPV vaccine. Success of such an immunisation programme would depend on high levels of vaccine uptake. I report high levels of vaccine acceptability in MSM and HIV-infected MSM in Ireland. To further interrogate the potential benefits of HPV vaccine in young HIV-infected MSM in whom the HPV vaccine is indicated, I completed a pilot study where 50 young HIV-infected MSM were recruited. Using next generation sequencing I investigated baseline prevalence of HPV infection at multiple sites and measured immunogenicity of the quadrivalent HPV vaccine in a longitudinal study. I report a high prevalence and diversity of HPV including oncogenic or high-risk HPV types.

Additionally, I report high levels of seroconversion for HPV 16 (100%) and HPV 18 (86%) post HPV vaccine in those whom were seronegative at baseline. Significant increases in HPV antibody titres were observed in those who were seropositive for HPV 16 and 18 at baseline. Interestingly, the highest HPV titres were observed 1 month post the second HPV vaccine rather than the third dose. There has been a move in some countries to change HPV vaccine dosing to two rather than three doses. Further research is warranted regarding HPV vaccine dosing in HIV-infected individuals. These findings suggest that the HPV vaccine has the potential to impact the burden of HPV associated disease observed in this patient group.

6.3 Interpretation of results

Considering findings from this thesis, alongside published literature in the area, it is clear that vaccination has potential to greatly reduce morbidity and mortality in HIV-infected individuals. However, this valuable preventative modality has yet to achieve its full potential in this population due to both impaired immunological response to vaccines and suboptimal vaccine coverage.

While resourcing and investment continues to focus on developing newer and more effective vaccines, similar focus and investment should be directed at developing infrastructures and supports (such as integrated vaccine units) to optimise delivery and realise protective benefits from available vaccines.

6.4 Future directions

Results from this thesis have already informed and influenced practice in the integrated vaccine unit in St James's Hospital and work presented at both national and international meetings and published in the medical literature raises awareness of the importance of vaccination as an integral component of care of HIV-infected individuals.

Chapter 2 underscores the need for ongoing monitoring of seroepidemiology in newly diagnosed HIV-infected individuals at point of entry to care. This is of particular relevance given the changes in demographic in recent years with a significant increase in HIV diagnoses in young MSM and individuals from South America.

In relation to vaccination coverage outlined in Chapter 3, quarterly vaccine subgroup meetings will continue to review performance metrics in relation to vaccination coverage in patients attending the GUIDE clinic with more frequent meetings during the seasonal influenza vaccine period to allow real-time assessment of vaccination coverage in the cohort. Targeted interventions including liaising with drug treatment services and opportunistic vaccination of in-patients have been employed following identification of IDU as a group less likely to be vaccinated.

In relation to pneumococcal vaccination, the prime-boost immunisation strategy has been implemented as standard of care nationally. Future research will examine longevity of immunological response in the pneumococcal vaccine cohort. Additionally, to further investigate clinical efficacy of the pneumococcal vaccine in HIV-infected individuals, I have been awarded funding from the Health Research Board (HRB) in Ireland to lead a Cochrane review examining pneumococcal vaccination for disease prevention in HIV-infected individuals (Sadlier et al., 2016). This will inform clinical policy relating to pneumococcal vaccine in HIV-infected individuals at both a national and international level.

Following commencement of this thesis, the HPV vaccine has been recommended for HIV-infected males and females under the age of 26 years, however it has yet to be funded by the Department of Health in Ireland. Findings from my research in relation to prevalence and persistence of HPV infection, HPV vaccine acceptability and HPV vaccine response has been presented to key stakeholders at a national level including the National Immunisation Advisory Committee (NIAC), the National Cancer Control Programme (NCCP) and most recently has informed a proposal which I collaborated on, to introduce phased funding of targeted the HPV vaccine for HIV-infected individuals and MSM.

Building on HPV research undertaken to date, a future HRB funded collaboration with Ceviva, national and international leaders in HPV research, will look to further elucidate the natural history of HPV infection.

Appendix 1

Immunogenicity of pneumococcal vaccination after prime-boosting in HIV-Infected Adults: A Randomised Controlled Trial

Clinical Study Protocol, Study Code 10102010

Study Code:	10102010
Protocol Version:	Version 2
Number of pages:	22
CT Number	CT 900/508/1- Vaccines- PCV13/PPV23
Eudra CT number:	2011-000260-99

Confidentiality Statement
May not be used, divulged, published or otherwise disclosed without the written consent of Sponsor.

LIST OF ABBREVIATIONS

HIV – Human immunodeficiency virus

PPV 23 - 23-valent polysaccharide pneumococcal vaccine

PCV 13 - 13-valent conjugate pneumococcal vaccine

SPP - Streptococcus Pneumoniae Polysaccharides

HAART - Highly Active Antiretroviral Therapy

IM – Intramuscular

IPD – Invasive pneumococcal disease

IgG - Immunoglobulin

ELISA – Enzyme-linked Immunosorbent Assay

AIDS – Acquired Immunodeficiency Syndrome

FDA – Food and Drug Administration

IMP – Investigational Medicinal Product

PIL – Patient Information Leaflet

NIAC - National Immunisation Advisory Committee

STUDY SYNOPSIS

Title: Immunogenicity of pneumococcal vaccination after prime-boosting in HIV-Infected Adults: A Randomised Controlled Trial

Investigational Medicinal Products
Test and comparator:
13-valent pneumococcal conjugate vaccine (PCV 13) (Prevnar 13™)
23-valent pneumococcal polysaccharide vaccine (PPV 23) (Pneumovax® 23)

Study objectives

The primary objective of this study is to:

1. Evaluate whether a strategy combining a prime with the 13-valent conjugate pneumococcal vaccine (PCV) followed by a boost with the polysaccharide pneumococcal vaccine (PPV23) would improve immunogenicity against S*treptococcus Pneumoniae Polysaccharides* (SPP) in HIV-infected patients.

The secondary objectives of this study are to:

2. Determine the association if any between HIV RNA, CD4+T cell count, Highly active antiretroviral therapy (HAART) and immunological response to above pneumococcal vaccination strategies.

Study Design
Prospective open-label randomized controlled trial

Patient Population
HIV-infected patients with a CD4 T cell count >200 cells/ml are eligible for pneumococcal vaccination will be prospectively recruited.

Eligibility
Ages Eligible for Study: ≥ 18 Years, <60 years old
Genders Eligible for Study: Both
Accepts Healthy Volunteers: No

Inclusion Criteria

- Subjects must be HIV-1 infected adults with a CD4 T cell count >200cells/ml.
- Male or Female Patients Age≥ 18 years old, <60 years old.
- Females may be eligible for enrolment in the study if she is of:
    - Non-childbearing potential; or, Child-bearing potential females must have a negative pregnancy test at initial screening and agree to an acceptable barrier and/or hormonal method of contraception.
- Subjects must be free of current bacterial infection.
- Subjects must have no history of prior pneumococcal vaccination.
- Subject must be willing and able to understand and provide written, informed consent prior to participation in the study.

Exclusion Criteria

- Subject is pregnant or breast feeding.
- Subject has a history of allergy to any of the investigational medicinal products or any excipients therein
- Subject has an estimated creatinine clearance < 50 mL/min via the Cockcroft-Gault method [Cockcroft, 1976].
- Subjects with severe hepatic impairment (Child-Pugh score > 9).
- Subject has any acute laboratory abnormality at screening, which, in the opinion of the Investigator, would preclude the subject's participation in the study of an investigational compound. Any verified Grade 4 laboratory abnormality would exclude a subject from study participation.
- Subject has received treatment with radiation therapy or cytotoxic chemotherapeutic agents within 28 days prior to screening, or has an anticipated need for these agents within the study period.
- Splenectomy
- Temperature >38 degrees Celsius at time of vaccination
- Subject suffers from any serious medical condition which would compromise the safety of the subject.
- Patients in whom Intramuscular (IM) vaccination is not possible because of disease or medication. (e.g. haemophilia, warfarin therapy).

NAME AND DESCRIPTION OF INVESTIGATIONAL PRODUCTS:

(i) 23-valent pneumococcal polysaccharide vaccine (PPV 23) (Pneumovax® 23)

QUALITATIVE AND QUANTITATIVE COMPOSITION
The 0.5 mL dose of vaccine contains 25 micrograms of each of the following 23 pneumococcal serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F.

PHARMACEUTICAL FORM
Solution for injection in a vial

(ii) 13-valent pneumococcal conjugate vaccine (PCV 13) (Prevnar 13™)

QUALITATIVE AND QUANTITATIVE COMPOSITION
1 dose (0.5 ml) contains:
Pneumococcal polysaccharide serotype 1 2.2 ug
Pneumococcal polysaccharide serotype 3 2.2 ug
Pneumococcal polysaccharide serotype 4 2.2 ug
Pneumococcal polysaccharide serotype 5 2.2 ug
Pneumococcal polysaccharide serotype 6A 2.2 ug
Pneumococcal polysaccharide serotype 6B 4.4 ug
Pneumococcal polysaccharide serotype 7F 2.2 ug

Pneumococcal polysaccharide serotype 9V 2.2 ug
Pneumococcal polysaccharide serotype 14 2.2 ug
Pneumococcal polysaccharide serotype 18C 2.2 ug
Pneumococcal polysaccharide serotype 19A 2.2 ug
Pneumococcal polysaccharide serotype 19F 2.2 ug
Pneumococcal polysaccharide serotype 23F 2.2 ug

PHARMACEUTICAL FORM
Suspension for injection.
The vaccine is a homogeneous white suspension.

STUDY DESIGN

We propose to carry out a prospective randomised control trial where HIV positive subjects eligible for pneumococcal vaccination will be recruited over a two-year period (March 2011- March 2013). Participants will be randomised to standard vaccination with the 23-valent polysaccharide pneumococcal vaccine (PPV23) or a prime-boosting strategy where participants will be administered the recently approved 13-valent conjugate vaccine (PPV13) at week 0 followed by standard PPV23 at week 4. Serological response to each vaccine strategy will be determined immunologically by measuring serotype specific immunoglobulin levels at week 0 (pre-vaccination), week 4 post PPV vaccination, week 24 and week 52 post vaccination.

IgG-specific antibody levels will be used to measure response to each vaccination. Total anti-pneumococcal antibody vaccine responses will be measured using a commercial ELISA assay. This assay measures antibodies to all of the pneumococcal serotypes present in the polysaccharide vaccine. ELISA assays will also be established to measure antibody responses to the three most common pneumococcal serotypes (19A, 7F, 14) causing invasive pneumococcal disease in Ireland. These serotypes are represented in both PCV and PPV.
In addition, functional antibody response will be measured using a flow cytometric assay which evaluates the ability of antibody to induce neutrophil phagocytosis of serotype specific fluorescent beads. This novel assay will allow an evaluation of the biological effectiveness of the pneumococcal vaccination protocols.

The primary endpoint of this study is to compare vaccine response to each vaccine strategy 4 weeks after the administration of PPV (one group will receive PPV alone versus PCV followed by PPV at 4 weeks). Vaccine response is defined as a 2-fold increase from baseline of serotype specific IgG antibody levels (ug/ml). The study is designed to detect a mean fold pre/post vaccine IgG difference between groups of 0.61ug/ml with a significance level of 0.05 and power of 10%.

Secondary endpoints are to determine if there is an association between CD4 T cell count, HIV RNA, age, HAART and comorbid illness with vaccine response. Those factors found to be significant ($p<0.05$) in univariate analysis will be considered for inclusion in a multivariate logistic regression model to identify factors independently associated with vaccine response

Method of Randomisation

At enrolment, patients will be randomised to either intervention with use of a computerised random-number generator. Patients will be randomized to receive either one dose of PCV 13 vaccination at week 0 followed by a boost of PPV 23 at week 4 (prime-boost group) or PPV 23 alone at week 0 (PPV-alone group). Patients will have an equal probability of assignment to the two interventions.

Route of administration/dose of Investigational Medicinal Product

- 23-valent polysaccharide pneumococcal vaccine (PPV23) (Pneumovax® 23)(Merck Vaccines), an inactivated bacterial vaccine, 0.5 mL administered as a single dose intramuscularly to the deltoid region.
- 13-valent conjugate pneumococcal vaccine (PCV13) (Prevnar 13™)(Wyeth Vaccines), an inactivated bacterial vaccine, 0.5mL administered as a single dose intramuscularly to the deltoid region.

The National Immunisation Advisory Committee (NIAC) of the Royal College of Physicians of Ireland vaccination guidelines 2011 recommends PPV23 as standard of care in HIV-infected adults. PCV13 is recommended as standard of care for all infants <12 months. Immunisation with PCV13 followed by immunisation with polysaccharide vaccine (PPV23) is recommended to afford broader protection for children up to 5 years. Although only strictly licensed for use in children<5 years the NIAC of the Royal College of Physicians of Ireland and the Food and Drug Administration of the United States state that "PCV13 may be used in those aged 5 -18 years who are at significantly increased risk of IPD including HIV-infected patients".

Reference: Immunisation guidelines for Ireland, Chapter 12, Pneumococcal Infection, page 6 (updated December 2010).

For the purpose of our study PCV 13 will be administered as s single dose 0.5ml (paediatric dose). It is unlikely to have any additional adverse effects in an adult population.

Informed consent
A thorough verbal discussion regarding the details of the study will take place at first contact. The patient will be provided with a Patient Information Leaflet (PIL) and will be given ample time to read it thoroughly. Any queries that a patient has will be answered to the satisfaction of the patient prior to obtaining their written consent to participate in this study. Patients will be provided with their own copy of the signed PIL and Consent Form. An interval of 4 weeks will pass between point of first contact and entry to the study. Patients will have their baseline clinic bloods including CD4 T cell count, full blood count, renal and liver profile reviewed and if all eligibility criteria are satisfied they will be randomised to either vaccination strategy.

Subjects will be counselled on potential adverse effects of the vaccines. They will be monitored in the dedicated vaccine unit by the vaccine nurse for 15 minutes post vaccination to ensure there is no immediate adverse effects. Patients will be given a direct number to contact the vaccine unit during working hours (01-4103826) and a number to contact the study sponsor Professor Bergin directly after hours should they develop any later reaction or have any concerns relating to the study.
Patients will be monitored as part of the study for a period of 12 months and blood draw will be taken as an extra serum sample at their routine clinic visit.

ADVERSE EFFECTS

IMP 1 PPV 23
The 23-valent polysaccharide pneumococcal vaccine (PPV23) (Pneumovax® 23) is very safe. Administration is recommended as standard of care in at risk groups including HIV-infected patients. The most common side effect experienced is tenderness and redness at the injection site. This is usually transient. Less than 1% will develop fever, aches or more severe local reactions. A single dose of 0.5ml will be given subcutaneously or intramuscularly to the deltoid area.

Below is a comprehensive list of potential adverse reactions for PPV 23 as listed by the FDA:

- Central nervous system: Chills, Guillain-Barre syndrome, fever ≤102°F*, fever >102°F, headache, malaise, pain, radiculoneuropathy, seizure (febrile)
- Dermatologic: Angioneurotic oedema, cellulitis, rash, urticaria
- Gastrointestinal: Nausea, vomiting
- Hematologic: Haemolytic anaemia (in patients with other hematologic disorders), leucocytosis, thrombocytopenia (in patients with stabilized ITP)
- Local: Injection site reaction* (erythema, induration, swelling, soreness, warmth); peripheral edema in injected extremity
- Neuromuscular & skeletal: Arthralgia, arthritis, limb mobility decreased, myalgia, paresthesia, weakness
- Miscellaneous: Anaphylactoid reaction, C-reactive protein increased, lymphadenitis, lymphadenopathy, serum sickness

*Reactions most commonly reported in clinical trials.

IMP 2 PCV 13
13-valent conjugate pneumococcal vaccine (PCV13) (Prevnar 13™) has been licensed for use in children from February 2010. It has also been approved for use in all children under 2 years of age as standard of care and also use in children 6-18 years at high-risk for invasive pneumococcal disease including HIV positive children: 0.5 mL as a single dose. It has not been licensed for routine use in adults as yet but can be prescribed as per doctor discretion. PCV13 is to replace PCV 7 as a standard childhood vaccine. PCV7 (although not licensed for use in adults) has been used safely in a number of clinical trials involving HIV-infected adults.
The safety of PCV13 was assessed in 13 clinical trials in which 4,729 healthy infants and toddlers were administered at least 1 dose of PCV13 and 2,760 children received at least 1 dose of PCV7, concomitantly with other routine pediatric vaccines. The most commonly reported (more than 20% of subjects) solicited adverse reactions that occurred within 7 days after each dose of PCV13 were injection-site reactions, fever, decreased appetite, irritability, and increased or decreased sleep. The incidence and severity of solicited local reactions at the injection site (pain/tenderness, erythema, and induration/swelling) and solicited systemic reactions (irritability, drowsiness/increased sleep, decreased appetite, fever, and restless sleep/decreased sleep) were similar in the PCV13 and PCV7 groups.
Listed below are the adverse effects in infants from PCV13 as listed by the FDA. Adverse events although not studied are likely to be less in adults given same dose of vaccine will be administered.

- >10%: Central nervous system: Drowsiness, fever, insomnia, irritability,
- Gastrointestinal: Appetite decreased,
- Local: Erythema, swelling, tenderness
- 1% to 10%: Dermatologic: Rash, Gastrointestinal: Diarrhoea, vomiting
- <1% (Limited to important or life-threatening): Abnormal crying, breath holding, febrile seizures, hypersensitivity reaction (bronchospasm, dyspnoea, facial oedema), seizure, urticaria, urticaria-like rash
- Adverse reactions observed with PCV7 which may also be seen with PCV-13: Anaphylactic reaction, angioneurotic oedema, apnoea, breath holding, oedema, erythema multiforme, hypotonic hypo-responsive episode, injection site reaction (dermatitis, pruritus), lymphadenopathy, shock

In addition to the small risk of side effects associated with the pneumococcal vaccine, drawing blood from a vein may cause local pain, bruising, swelling at the needle site, occasional light-headedness, fainting and very occasionally infection at the site of blood draw. Subjects will be requested to have an additional serum sample drawn at week 0, week 4-6, week 24 and week 52. These samples will largely be taken at scheduled clinic visits and as such minimal extra visits for the purpose of the study alone will be required.

CONCOMITANT MEDICATION USE WITH INVESTIGATIONAL MEDICINAL PRODUCT (IMP):

IMP 1 PPV 23
PNEUMOVAX® and ZOSTAVAX should not be given concurrently because concomitant use in a clinical trial resulted in reduced immunogenicity of ZOSTAVAX.

Interaction with other medicinal products and other forms of interaction have not been documented.

IMP 2 PCV 13
PREVENAR 13 can be given with any of the following vaccine antigens, either as monovalent or combination vaccines: diphtheria, tetanus, acellular or whole cell pertussis, *Haemophilus influenzae* type b, inactivated poliomyelitis, hepatitis

B, meningococcal serogroup C, measles, mumps, rubella and varicella. Clinical studies demonstrated that the immune responses and the safety profiles of the administered vaccines were unaffected.
In clinical studies, where there was concomitant administration of Prevenar 13 and rotavirus vaccine, no change in the safety profiles of these vaccines was observed.

Interaction with other medicinal products and other forms of interaction have not been documented.

PREGNANCY AND LACTATION
Females may be eligible for enrolment in the study if she is of:
Non-childbearing potential; or, Child-bearing potential females must have a negative pregnancy test at initial screening and agree to an acceptable barrier and/or hormonal method of contraception.

Safety of the IMP's in pregnancy and lactation:
IMP 1- PPV 23

Use during pregnancy
Animal studies are insufficient with respect to effects on pregnancy. PNEUMOVAX® II should not be used during pregnancy unless clearly necessary (the potential benefit must justify any potential risk to the fetus).

Use during lactation
It is not known whether this vaccine is excreted in human milk. Caution should be exercised when PNEUMOVAX® II is administered to a nursing mother.

IMP 2 - PCV 13

Use during pregnancy/lactation
PCV 13 is not licenced for use in adults. Human data on the use during pregnancy or lactation and animal reproduction studies are not available.

SAFETY
Patients will be monitored in the vaccination unit for 15 minutes after vaccination for
immediate side effects such as anaphylaxis.
They will be given advised to contact one and short-term (within 5 days) side effects. will be recorded for safety evaluation.
Safety data will be collected according to the definitions of an adverse event and a serious adverse event as outlined in ICHE2A ref: CPMP/ICH/377/95

CRITERIA AND PROCEDURE FOR DEALING WITH WITHDRAWALS FROM STUDY.
Patients can withdraw from the study at any time.
They will continue to be followed up for their primary diagnosis at routine clinic visits.

ENDPOINTS/STASTISTICAL ANALYSES
The primary endpoint of this study is to compare vaccine response to each vaccine strategy 4 weeks after the administration of PPV (one group will receive PPV alone versus PCV followed by PPV at 4 weeks). Vaccine response is defined as a 2-fold increase from baseline of serotype specific IgG antibody levels (ug/ml). The study is designed to detect a mean fold pre/post vaccine IgG difference between groups of 0.61ug/ml with a significance level of 0.05 and power of 10%.
Sequential IgG levels in both groups will be monitored at 24 weeks and 52-week post PPV vaccination to measure trends in levels of antibody protection. Sequential opsonophagocytic antibody response will be measured at the same time intervals as a biological measure of antibody protection and compared between groups. Categorical variables will be analysed using Fisher's exact test while continuous variables will be analysed with Student's t test for normally distributed data and the Mann-Whitney U test for nonnormally distributed data.

Secondary endpoints are to determine if there is an association between CD4 T cell count, HIV RNA, age, HAART and comorbid illness with vaccine response. Those factors found to be significant ($p<0.05$) in univariate analysis will be considered for inclusion in a multivariate logistic regression model to identify factors independently associated with vaccine response.

DATA STORAGE AND PROTECTION
All study subjects will be allocated a study number and data will be collected on de-identified case report forms and no other patient identifiable information will be included. Case report forms will be forwarded to and stored in a locked filing cabinet in the Department of Infectious Diseases at St. James's Hospital, Dublin, and managed by the study team. Data linkage will be between the subjects MRN and study number on a password-protected file located on a secure network drive in the Department of Infectious Diseases at each center.

Data will be transferred from case report forms to an electronic database at the research office at the Guide Clinic, St. James's Hospital, Dublin and stored securely for final analysis. Final statistical analysis will take place once all consented patients have undergone all study assessments or withdrawn from the study. Access to the files will be restricted to those who need access. All centres will follow the principles of Departmental Policy on data security in line with the 1998 Data Protection Act. Specifically, for IT, this includes restricting access (through password protection) to the database to personnel working directly with the project and not permitting remote access to the database.

Individual results will be discussed with patients once available. On completion of the study and analysis study findings will be discussed and explained to patients and a written summary explaining study results will be made available to patients.

Appendix 2

Thank you for taking the time to complete this survey on the human papillomavirus (HPV) in men who have sex with men (MSM). The purpose of this study is to see what the awareness and acceptability of the HPV vaccine is. We hope this will help in planning for HPV vaccine provision in the future.

Please read the following questions and tick the most appropriate box

1. HPV can affect men

No	
Yes	
Never heard of HPV/Don't know	

2. HPV can be treated with antibiotics

No	
Yes	
Don't know	

3. HPV infection can lead to death

No	
Yes	
Don't know	

4. The chance of you catching HPV infection is

Low	
Moderate	
High	

5. The prevalence of HPV infection in MSM in Ireland is

Low	
Moderate	
High	

6. The infectivity of HPV is

Low	
Moderate	
High	

7. The chance of HPV infection impacting on physical health is

Low	
Moderate	
High	

8. The chance of HPV infection causing genital warts is

Low	
Moderate	
High	

9. The chance of HPV infection causing penile cancer or anal cancer is

Low	
Moderate	
High	

10. An effective HPV vaccine is available for men

No	
Yes	
Don't know	

11. The number of shots of HPV vaccine required is

1-2	
3	
4 or above	

12. The best age to receive HPV vaccine is

30 years or above	
Below 30 years	

13. The HPV vaccine is effective in preventing genital warts

Not effective	
Effective	
Don't know	

14. The HPV vaccine is effective in preventing penile and anal cancer

Not effective	
Effective	
Don't know	

15. The HPV vaccine is effective in preventing sexually transmitted infections other than genital warts

Not effective	
Effective	
Don't know	

16. The HPV vaccine is too expensive

No	
Yes	
Don't know	

17. The HPV vaccine could have side effects

No	
Yes	
Don't know	

18. Do you plan to take up HPV vaccine in the next 6 months

Yes	
No	
Don't know	

19. Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer and cost **€300** in total

Yes	
No	
Don't know	

20. Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer at a discounted price of **€100** in total

Yes	

No	
Don’t know	

21. Would you take the HPV vaccine if it was 90% effective in preventing genital warts, 75% effective in preventing anal cancer and **free**

Yes	
No	
Don’t know	

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