THE RISK OF MISCARRIAGE FOLLOWING IMMUNIZATION OF THE BIVALENT HUMAN PAPILLOMAVIRUS (HPV) - 16/18 VACCINE: A BAYESIAN APPROACH

by

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Abstract

Background: Cervarix is a prophylactic vaccine used in preventing cervical cancer associated with human papillomavirus types 16/18. A previous study that investigated the risk of miscarriage associated with this bivalent vaccine by pooled analysis of data from two clinical trials, showed a numerically higher but statistically insignificant miscarriage rate in the HPV arm for pregnancies that began within three months following the vaccination. We explored this issue using an alternative statistical approach.

Objectives: To develop a hierarchical Bayes model to identify the potential time-dependent risk window of miscarriage rate associated with the bivalent HPV vaccine.

Methods: This study comprised the development of a hierarchical Bayes Model with its model inference and the application of this model to a real-world question. A multivariate logistic model was proposed that involved an indicator variable to accommodate a risk window with lower and higher cut-off points. Gibbs Sampling algorithms were used for the inference on the parameters of interest. Over ninety sets of simulation studies were conducted to evaluate the performance of the proposed method and estimate the power in detecting the risk effect. The Bayesian approach was then compared to the existing traditional approaches (e.g. the permutation test). The proposed model was applied to the subpopulation of pregnant women from the Costa Rica Vaccine Trial.

Results: In simulation studies, the Bayesian model demonstrated a better performance over the traditional approaches. It showed higher power than the traditional hypothesis testing in detecting the risk effect; it was more informative than the permutation test because it provided both the point estimates and the corresponding credible intervals for the cut-points and the ratio of odds ratios. In the analysis of the CVT data, we observed an effect of 1.13 (95% credible interval: 0.49 to 2.75), implying no significant evidence to support the hypothesis that HPV is associated with a higher miscarriage rate.

Conclusions: The hierarchical Bayes model can be applied to investigate the time-dependent risk of adverse events in clinical trials. Using the new Bayesian method, no significant risk of miscarriage in the Costa Rica Vaccine Trial was established, which is consistent with previous report.

To my family

Co-Authorship

This thesis represents the work of Tian Fang in collaboration with her thesis supervisors Dr. Bingshu Chen and Dr. William J. Mackillop.

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Abbreviations

ADC	adenocarcinomas
AS04	adjuvant system 04
CGIN	cervical glandular intra-epithelial neoplasia
CIN	cervical intraepithelial neoplasia
CVT	Costa Rica Vaccine Trial
GSK	GlaxoSmithKline
HPV	human papillomaviruses
ICC	invasive cervical cancers
MCMC	Markov Chain Monte Carlo (algorithms)
MPL	3-O-desacyl-4' monophosphoryl lipid A
NCI	National Cancer Institute in the U.S.
Pap test	Papanicolaou test
PATRICIA	Papilloma TRIal against Cancer In young Adults
SCC	squamous cell carcinoma

Glossary

APC	antigen-presenting cell, a cell that displays foreign antigen complexes with major histocompatibility complex on their surfaces, which process antigens and present them to T-cells.
CIN	cervical intraepithelial neoplasia, early stage of squamous cell carcinomas
cone biopsy	a cone-shaped wedge of tissue is removed from the cervix, being used to diagnose and sometimes to treat abnormal cervical tissue
cytokines	small cell-signaling protein molecules that are secreted by numerous cells of the immune system and are a category of signaling molecules used in intercellular communications.
IL-4, 5, 6	interleukin-4, interleukin-5, interleukin-6, Th2 cytokines.
IFN-γ	interferon-γ, Th1 cytokine.
PBMCs	peripheral blood mononuclear cells, a critical component in the immune system to fight infection and adapt to intruders and widely used in research every day
Pap smear	a screening test aims to detect potentially pre-cancerous changes. A speculum is used to gather cells from the outer opening of the cervix of the uterus and the endocervix, then the cells are examined under a microscope to look for abnormalities.
PMA	phorbol-12-myristate-13-acetate, often used in biomedical research to activate the signal transduction enzyme protein kinase C
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells, a protein complex that controls the transcription of DNA. NF-κB is involved in cellular responses to cytokines and etc, and plays a key role in regulating the immune response to infection.

TLR4	Toll-like receptor 4, important in the activation of the innate immune system. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity.
TNF-α	tumor necrosis factor –alpha, Th1 cytokine.
Th1, Th2	T-helper cells, major immune cells involved in cytokine production.

Chapter 1

Introduction

1.1 Background and Rationale

Cervical cancer is recognized as a global public health problem. Worldwide, invasive cervical cancer (ICC) is the second most common type of cancer and the third leading cause of cancer death in women (Parkin & Bray, 2006; Willmott & Monk, 2009). Each year, an estimated 529,000 women worldwide develop cervical cancer and 275,000 die of this disease (Ferlay, 2010). The wide application of the Pap smear screening test in developed countries contributes to the reduction in cervical cancer incidence and mortality. In contrast, the screening programs are not effective in developing countries due to cultural and economic factors. Since persistent infection with one of approximately 15 carcinogenic human papillomaviruses (HPV) types is a necessary cause of cervical cancer (Munoz et al., 2003; Walboomers et al., 1999), prophylactic vaccinations (Gardasil^{TM} manufactured by Merck & Co. Inc and Cervirax^{TM} manufactured by GSK Inc.) against high risk types of HPV appear to be promising and of-value, particularly in countries with low resources where screening programs are less available. The World Health Organization (WHO) recommends that routine HPV vaccinations should be included in national immunization programs provided that it is feasible and cost-effective (World Health Organization, 2009).

Currently, the two HPV vaccines available in the market have demonstrated high efficacy, good tolerability and safety based on results from phase II or phase III clinical studies. In those studies, the most frequently reported adverse events after vaccination included pain at the injection site, fatigue, headache and myalgia. No increase in onset of chronic disease,

autoimmune disease or other medically significant conditions was seen in the HPV groups as compared to the control groups. Our focus in this study is the bivalent HPV vaccine — Cervarix. It was designed to protect against HPV types 16 and 18, which are responsible for 70% of cervical cancer (Keam & Harper, 2008). One study of the risk of miscarriage with the bivalent HPV vaccine caught our attention on the safety of Cervarix. It showed that there could be potential differences of miscarriage rates between the HPV vaccine arm and the hepatitis A vaccine arm in pregnancies that started within certain time periods after the vaccination, in spite of the fact that no overall statistical significance was established (Wacholder et al., 2010). The underlying biological mechanism for the relationship between miscarriage and the bivalent HPV vaccine is unclear, but literature reviews suggest that the unique adjuvant system AS04 in Cervarix may be related to the occurrences of miscarriage as a result of its alternations in maternal immune functions (Didierlaurent et al., 2009; Giannini et al., 2006; Laird et al., 2003). Our key research question is whether or not there is a risk window that is associated with an increase in the rates of miscarriage in pregnancies following the immunization of the bivalent HPV vaccine. We believe that it is worthwhile to investigate this issue using an advanced statistical approach.

This study seeks to identify the period of risk for miscarriage following the immunization with the bivalent HPV vaccine. The Bayes' theorem and Gibbs Sampling are the main mathematical approaches used for the model inference. We expect to add to our knowledge about the risk of miscarriage associated with the bivalent HPV vaccine, and provide insight on the safety of this bivalent HPV vaccine in pregnant women. Consequently, the results of this study could have important clinical and public health implications.

1.2 Objectives

1. To develop a Bayesian approach for the statistical inference of a multivariate logistic model that involves evaluation of a risk window of adverse events in clinical trials, and compare this approach with existing traditional approaches.

2. To apply the Bayesian logistic model to a Phase III HPV vaccine trial and explore the relationship between the occurrence of miscarriage and the bivalent HPV vaccine (Cervarix) with regard to the number of days between the vaccination and the onset of pregnancy.

(1) to identify the risk window associated with an increase in the rates of miscarriage.

(2) to estimate the size of the risk.

1.3 Ethics

The participants are anonymous in this work. The data transferred from the US National Cancer Institute provided only IDs corresponding to each subject, which does not allow the investigators to identify any individuals in real life. Data is kept confidentially in password-protected computers in the NCIC Clinical Trial Group at Queen's University. Only the student investigator, Tian Fang, and her supervisor, Dr. Bingshu Chen, have access to the data. At the end of the study, the original data will be securely removed from the hard drive and will not be used for any other purpose. This study was approved by Queen's University Health Science & Affiliated Teaching Hospitals Research Ethics Board in August, 2011.

1.4 Thesis Organization

The remainder of this thesis is organized into four sections. Chapter 2 presents a summary background about the epidemiology of cervical cancer, the bivalent HPV vaccine and its relevant clinical trials, the underlying biological hypothesis, the Bayes' theorem, and Gibbs Sampling. Applied mathematical methodology develops new models to address questions that arise from the real world, and applies the proposed mathematical technique to obtain results, adding more insight into real-world questions. Our key research question is whether or not there is a risk window that is associated with an increase of the risk of miscarriage in pregnancies following the immunization of the bivalent HPV vaccine. The other two main chapters of this thesis make efforts to answer this question. In Chapter 3, we propose a logistic regression model that resolves the direct estimation of risk window, we conceptually develop the mathematical procedures on the inference of the parameters in the model based on the Bayes' theorem and Gibbs Sampling, and we assess the performance of the proposed model with simulation studies. Following this, Chapter 4 provides the methods and results from analyzing phase III clinical trial data using the proposed Bayesian-inference model. Finally, Chapter 5 offers a discussion of the major findings, strengths and limitations of this study and future directions for research. Additional results of the simulation studies are present in the Appendices.

Chapter 2

Literature Review

2.1 Cervical Cancer

2.1.1 Pathology and Natural History

The cervix is the lower narrow part of uterus where it joins the top end of the vagina (Figure 2.1). Columnar epithelium covers the uterine cavity and extends downwards into the cervical canal, where it comes into contact with the squamous epithelium from the vagina. The boundary between the columnar and squamous epithelium is called the squamocolumnar junction (Figure 2.2) (Singer & Jordan, 2006). Invasive cervical cancer (ICC) arises either in the squamous or the columnar/glandular epithelial cells, or both, that line in the cervix (Buckley, 1994). Eighty percent of invasive cervical cancers (ICC) are squamous cell carcinoma (SCC), while glandular malignancies (adenocarcinomas (ADC)) account for the remainder. Cervical intraepithelial neoplasia (CIN) is known as the early stage of squamous cell carcinoma (SCC). CIN divides into three grades according to the degree of epithelial dysplasia and differentiation. Low-grade CIN is not precancerous, will often regress spontaneously, and can be treated. If left untreated, it will potentially develop into *in situ* cervical cancer or CIN3, which may subsequently become invasive (Koushik & Franco, 2006). Similarly, precursors to ADC exist, which are termed as cervical glandular intraepithelial neoplasia (CGIN). However, the grading of CGIN is less well established. CIGN1 and CIGN2 refer to lesser degrees of cytological and glandular morphological abnormalities, and CGIN3 correspond to adenocarcinoma *in situ* which is regarded as the immediate precursor of invasive adenocarcinoma (Buckley, 1994).

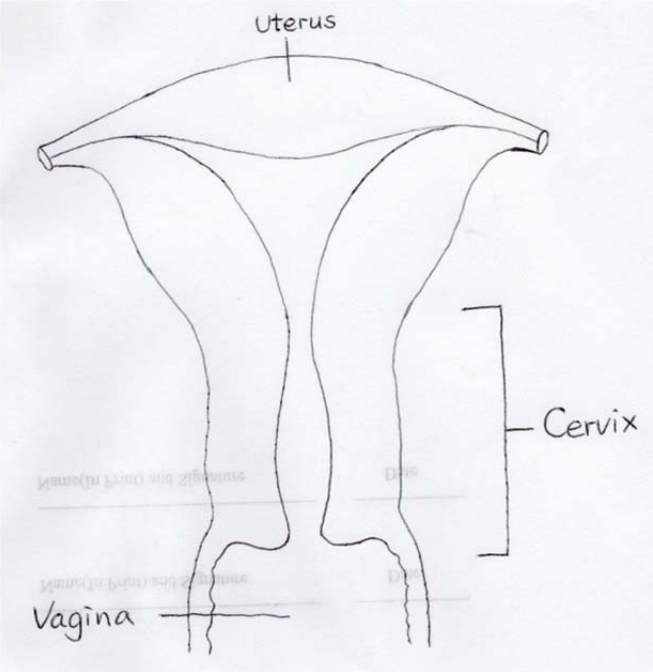


Figure 2.1 Schematic view of the position of the cervix (Simplified from Fig 5.52 in Gray's Anatomy, (Richard et al., 2009))

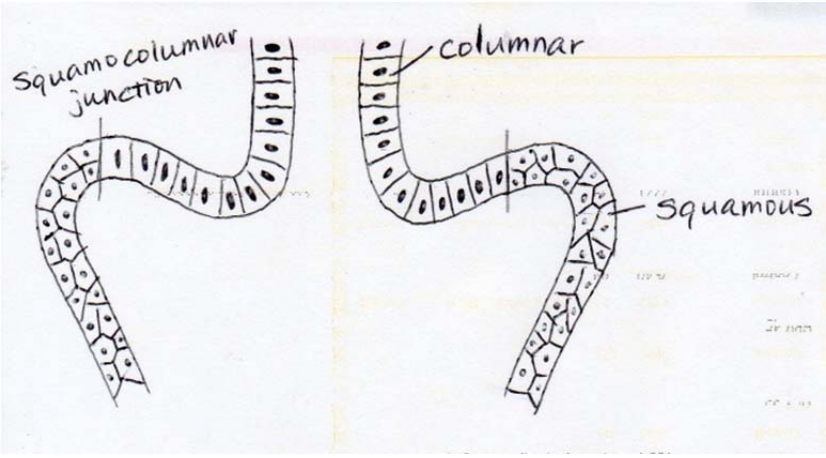


Figure 2.2 Types of cervical epithelium (Singer & Jordan, 2006).

2.1.2 Incidence and Mortality

The World Health Organization estimates there were over 529 000 new cases of cervical cancer in the world in 2008, and almost 275 000 deaths from this disease, of which about 90% occurred in developing countries (Ferlay, 2010). The incidence and mortality from cervical cancer in developing countries were more than double the rates in developed countries. Central and South America, eastern Africa, South and South-East Asia, and Melanesia were found to have the highest incidence rates of cervical cancer among the world (World Health Organization, 2006b). Most cases among women in underprivileged communities were diagnosed at advanced stage, resulting in low rates of survival (Ansink, 2007). The disparities between developed and developing countries have been attributed to cultural and economic factors affecting access to screening and treatment (Schorge et al., 2011). In Canada, statistics showed that the incidence rate for cervical cancer was 8 cases per 100 000 women in 2007 and the mortality rate was 2 deaths per 100 000 women in 2006, which were much lower than the worldwide data (Canadian Cancer Society's Steering Committee on Cancer Statistics, 2011).

2.1.3 Etiology

It is well established that human papillomaviruses (HPV) is a necessary cause of all cervical cancers (Chaturvedi, 2010; Walboomers et al., 1999). There are over 100 types of HPV, among which fifteen types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56,58, 59, 68, 73, and 82) are identified as carcinogenic and termed as high-risk (Munoz et al., 2003). It is acknowledged that HPV type 16 and 18 account for approximately 70% of squamous cell carcinoma (SCC) of the cervix while HPV type 16, 18, and 45 account for 90% of adenocarcinomas (ADC) (Clifford, Smith, Aguado, & Franceschi, 2003). HPV infections are also associated with squamous cancers of the penis, the

anus, and the upper aerodigestive tract. In addition, HPV infection can cause warts on the hands, and feet and in the genital area (Krishnan, 2008; World Health Organization, 2006b). Most HPV infections resolve spontaneously within 30 months (Moscicki et al., 1998). However, persistent infection may progress to cancer. An infected person may not show any visible symptoms for years after infection but could always be an active infector (Krishnan, 2008).

Infection of HPV is the most significant risk factor for cervical cancer. Since HPV is predominantly transmitted through sexual activities, sexual behaviour may increase one's chance of acquiring an HPV virus, including early age at sexual contact, having multiple sexual partners or having a sexual relationship with a partner who has had multiple partners, sexual contact with a partner who is infected, unprotected sexual intercourse, etc. (Krishnan, 2008). Other biological factors that contribute to the development of cervical cancer are human immunodeficiency virus (HIV), obesity, and smoking (Bekkers, Massuger, Bulten, & Melchers, 2004). Socio-cultural factors that contribute to increased incidence and mortality of cervical cancer include but are not limited to low educational attainment and awareness, neighbourhood poverty, and low priority of women's health, which result in lower rates of cervical cancer screening (Ansink, 2007; Datta et al., 2006; Jemal et al., 2006).

2.1.4 Treatment

Cervical cancer is staged clinically, and stage of the disease is related to the effectiveness of the treatment as well as long-term survival (Willmott & Monk, 2009). Early stages can be treated with cone biopsy or hysterectomy. Radical trachelectomy is a conservative alternative to radical hysterectomy for those who wish to maintain fertility (Zarchi et al., 2010). Those with advanced-stage tumors are given radiation therapy and cisplatin-based chemotherapy (Schorge et al., 2011).

Nevertheless, in developing countries, poor health care facilities, lack of access to health services, the lack of treatment options as well as inadequate skills have made the curative treatment as a solution to cervical cancer a difficult task (Ansink, 2007; Krishnan, 2008; World Health Organization, 2006b).

2.1.5 Prevention

Current secondary preventive strategies consist mainly of Papanicolaou (Pap) smear and HPV DNA testing (Chaturvedi, 2010). The Pap smear is a cytological screening test for early detection of cervical abnormalities (Patnick, 2006). This technique has been widely used since its first publication in mid-twentieth century. It is widely believed that effective screening programs in developed countries contributed to their overall decline in the incidence of cervical cancer (Willmott & Monk, 2009). For example, the 2011 Canadian Cancer Statistics reported that the annual incidence rates (1998-2007) and mortality rates (1997-2006) for cervical cancer continued declining by 1.4% and 3.4% per year, respectively (Canadian Cancer Society's Steering Committee on Cancer Statistics, 2011). A single Pap smear test may have limited sensitivity but the cumulative sensitivity of three successive smears is over 90%. The HPV test, for evaluation of the presence of high-risk HPV types, is usually used in conjunction with Pap smear given a positive Pap test result (Patnick, 2006). However, the cost-effectiveness of HPV DNA testing is still unclear (Patnick, 2006).

On the other hand, in many developing countries, access to health services is limited, and screening for cervical cancer either is non-existent or reaches few women. It was estimated that 95% of women in developing countries have never been screened for cervical cancer (World Health Organization, 2006b). In spite of the effectiveness of screening programs in early

detection of precursor lesions and prevention of cervical cancer, its implementation in developing countries is challenged by inadequate political support, lack of awareness among the population, health care providers and policy-makers, absence or poorly organized outreach programs, unsatisfactory professional training, lack of access to health services, substantial needs of financial, technical and human resources, etc. (Krishnan, 2008; World Health Organization, 2006b).

In terms of primary prevention by vaccination, there are two licensed HPV vaccines against high-risk HPV types (Gardasil™ manufactured by Merck & Co. Inc and Cervirax™ manufactured by GSK Inc.). The incentive for developing vaccines against HPV derives from the serious nature of the associated diseases – cancer, pre-cancer and sexually transmitted infections, and the high costs of pre-cancer screening and treatment (Bornestein, 2010). The quadrivalent vaccine Gardasil is for prevention of infection caused by HPV type 6,11, 16 and 18, indicated in girls and women 9-26 years of age as well as boys and men of the same age range (Siddiqui & Perry, 2006). The bivalent vaccine Cervarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) targets HPV 16 and 18 only. According to existing large-scale phase III studies, both vaccines are safe and effective against cervical cancer and high-grade CIN (Bornestein, 2010). It is deemed that HPV vaccines have a promising future in primary prevention of cervical cancer for multiple reasons as discussed below.

Antibody concentrations after clearance of natural infection are low and perhaps insufficient to provide protection. Therefore, women with a naturally acquired HPV infection remain at risk for re-infection with the same HPV type throughout their life (Viscidi et al., 2004). In a vaccine efficacy study, antibody concentrations over 60 months after the bivalent HPV vaccination

remained at least 12-fold higher than after natural infection (Romanowski et al., 2009), suggesting that the HPV vaccine can maintain high antibody concentrations in the long-term (Francesca Paolini & Aldo Venuti, 2010).

Also, vaccination requires less expense and complexity in delivery, as it is administered in three doses over six-month period by intramuscular injection (Keam & Harper, 2008). Compared to the annual or triennial screening, the immunization for HPV is less demanding in resources and techniques. Therefore, it is possibly more feasible, especially in poor societies of the world where Pap smear screening programs are less or not available (Sabin Vaccine Institute, 2008). However, HPV vaccination cannot eliminate the need for screening later in life, because HPV types other than 16 and 18 cause up to 30% of all cases of cervical cancer (World Health Organization, 2009).

Further, considering the histological types of cervical cancer, adenocarcinomas are less easy to detect by screening methods and have poorer survival compared with squamous cell carcinoma (Davy, Dodd, Luke, & Roder, 2003). The overall decrease of cervical cancer incidence is largely attributed to the decline in squamous cell carcinoma. In contrast, the proportion of adenocarcinoma relative to all cervical cancer has been increasing (Smith, Tiffany, Qualls, & Key, 2000). In phase III clinical studies, the bivalent HPV vaccine demonstrated not only high efficacy in prevention against HPV 16 and 18, but also potential cross-protection to other types of HPV, including types 31 and 45. This is particularly important for the prevention of adenocarcinoma, as about 90% of adenocarcinomas result from HPV type 16, 18, and 45. In this study, we focused on the bivalent HPV vaccine which will be discussed in more detail in later sections.

2.2 The Bivalent Vaccine against HPV Type 16/18 - Cervarix

2.2.1 Indication and Vaccine Composition

Cervarix (Human Papilomavirus Bivalent (Types 16 and 18) Vaccine, Recombinant) is a prophylactic vaccine with a novel adjuvant system, designed for the prevention of cervical intraepithelial neopasia "CIN2+" [i.e. CIN2, CIN3, adenocarcinoma *in situ* (AIS) and invasive cervical cancer (ICC)] associated with HPV types 16 and 18 (Keam & Harper, 2008). It was developed by researchers of the US National Cancer Institute and other institutions, and manufactured by GlaxoSmithKline (GSK) Biologicals (Herrero et al., 2008).

This non-infectious vaccine, formulated with Adjuvant System 04 (AS04), is comprised of virus-like particles (VLPs) that are prepared from capsid L1 proteins from HPV types 16 and 18 (20 μg respectively per dose) (Vaccines and Related Biological Products Advisory Committee, 2009). AS04 contains 50 μg of 3-O-desacyl-4' monophosphoryl lipid A (MPL) and 500 μg of aluminum hydroxide ($Al(OH)_3$) per dose. Aluminum salts are the most widely used classical adjuvants contained in over 80% of current vaccines. MPL, acting as a TLR4 (toll-like receptor 4) agonist, is a very powerful stimulator to the immune system with reduced toxicity while retaining its adjuvant effect. (GarçOn, Chomez, & Van Mechelen, 2007) Compared to traditional vaccines adjuvanted with aluminum salt only, AS04 system demonstrates higher effectiveness and longer persistency in animal and human experiments (Didierlaurent et al., 2009; Giannini et al., 2006).

2.2.2 Dosage and Administration

Cervarix has been licensed in more than 100 countries worldwide, including member countries of the European Union, Mexico, Brazil, Australia, India, South Africa, Singapore, etc. It

was first approved in Australia in May 2007 for use in girls and women 10-45 years of age, and Health Canada approved this vaccine in February 2010. In Canada, it is recommended to girls and women between the age of 10 and 25. It is administered in a three-dose schedule at 0, 1, 6 months by intramuscular injection. (GlaxoSmithKline, 2010; Vaccines and Related Biological Products Advisory Committee, 2009)

2.2.3 Efficacy and Safety

The efficacy of Cervarix was evaluated in two randomized double-blind trials in healthy young women 15-25 years of age. One is a placebo-controlled phase II study starting in 2001 which initially enrolled 1113 women from Brazil, Canada and the USA, with extended follow-up study between 2003 and 2007. In the total vaccinated cohort from this study, Cervarix showed protective effects against both HPV 16 and 18 infection for up to 6.4 years after first vaccination, together with sustained antibody levels against HPV 16/18 (Romanowski et al., 2009). Moreover, the protection is expected to last more than 20 years as predicted by a mathematical model (David et al., 2009). Compared to the other licensed HPV vaccine, this is the longest reported duration, indicating no need for booster injections (Francesca Paolini & Aldo Venuti, 2010). This is important, particularly for low-resource countries, because it may decrease the cost of HPV vaccination programs. The other study is a large phase III PApilloma TRIal against Cancer In young Adults (PATRICIA). In this trial, over 18,000 women were initially enrolled between 2004 and 2005 from 14 countries in Asia, Europe, Latin America, and North America (Paavonen et al., 2009). The participants received either the bivalent HPV vaccine or hepatitis A vaccine (based on Havri*x*™ manufactured by GlaxoSmithKline Biologicals, Rixensart, Belgium) as control. In the interim analysis of this study when the average follow-up was 14.8 months, Cervarix demonstrated 90.4% efficacy against cervical intraepithelial neoplasla (CIN) 2+ and 89.2%

efficacy in preventing CIN 1+ associated with HPV 16 or HPV 18 (Paavonen, Jenkins, & Bosch, 2007). A higher vaccine efficacy (92.9%) against CIN2+ containing HPV 16/18 DNA was observed in the final analysis (mean length of follow-up was 34.9 months) (Paavonen et al., 2009). Further analysis of secondary endpoints in this trial showed cross-protection of Cervarix against new incident or 6-month persistent infection of HPV type 45 or 31 which are the third and fourth most prevalent carcinogenic types of HPV (Paavonen et al., 2009).

The findings about safety outcomes in the interim and final analysis of both trials were consistent. Similar numbers of adverse events were reported in both the HPV vaccine groups and the control groups, and none of the serious adverse events were judged related to the vaccine (Paavonen et al., 2007; Paavonen et al., 2009; Romanowski et al., 2009). In another pooled analysis of two randomized controlled trials (PATRICIA & CVT), which specifically looked into the risk of miscarriage among healthy non-pregnant women who were recruited into the clinical trials but became pregnant after enrollment, no significant differences in the miscarriage rates were found between study groups. It was notable that miscarriage rates in the bivalent HPV vaccine group were approximately 1.5 times the rate in the control group in pregnancies that began within a 3-month time frame following the vaccination, although this increase was not statistically significant (Wacholder et al., 2010). This potentially important finding triggered our interest in further investigating the risk of miscarriage associated with Cervarix by improving the mathematical model to provide a higher level of precision on the risk estimate.

2.3 Underlying Biological Hypothesis

MPL (3-O-desacyl-4' monophosphoryl lipid A) is a detoxified form of the endotoxin lipopolysaccharide with promising application as vaccine adjuvants (C. R. Casella & Mitchell, 2008). The induction of adaptive immune responses by AS04 is primarily attributed to MPL (Didierlaurent et al., 2009). MPL stimulates the expression of pro-inflammatory cytokines by signaling via toll-like receptor 4(TLR4) and activating NF-κB activity which subsequently induces production of cytokines (Didierlaurent et al., 2009). These cytokines can stimulate the maturation of antigen-presenting cells (APCs) and subsequently enhance the adaptive immune response. Tumor necrosis factor –alpha (TNF-α) and interferon-γ (IFN-γ) are Th1 (type 1 T-helper cells) cytokines with pro-inflammatory effects, while interleukin -4 (IL-4) , interleukin-5 (IL-5), interleukin-6 (IL-6) are Th2 (type 2 T-helper cells) cytokines with anti-inflammatory effects(Calleja-Agius, Schembri-Wismayer, Calleja, Brincat, & Spiteri, 2011). Th1-type immunity is deleterious to pregnancy while Th2-type immune response plays a protective role during pregnancy (Raghupathy, 2001). The production of IFN-γ was promoted by MPL and its level was higher in AS04 system compared to aluminum salt only (C. R. Casella & Mitchell, 2008; Didierlaurent et al., 2009). Didierlaurent et al. found over 10-fold increase in the levels of TNF-α in mice muscle after injection of MPL containing formulation (2009). On the other hand, the aluminum hydroxide in AS04 appeared to prolong the MPL-mediated cytokine response and stimulate the production of Th2 cytokines, such as IL-4 and IL-5 (Brewer, 2006; Didierlaurent et al., 2009). Overall, AS04 biased the immune response towards Th1 compared to aluminum salt alone.

The production of pro-inflammatory cytokines appeared to be abnormal in the situation of miscarriage. For example, studies showed that the production of IL-6 decreased but IFN-γ and TNF-α increased in phorbol-12-myristate-13-acetate (PMA)-stimulated peripheral blood mononuclear cells (PBMCs) obtained from women with recurrent miscarriage at the time of miscarriage compared with stimulated PBMCs obtained during the first trimester of ongoing pregnancies in women with a normal reproductive history (Raghupathy et al., 2000). Another study found that TNF-α/IL-6 ratio was significantly higher in women presenting with threatened miscarriage who subsequently miscarried, compared with the women with normal outcomes (Calleja-Agius, Muttukrishna, Pizzey, & Jauniaux, 2011). That being said, change in levels of pro-inflammatory cytokines, corresponding to change in Th1/Th2 balance, was associated with increased risk of adverse pregnancy outcome (Laird et al., 2003).

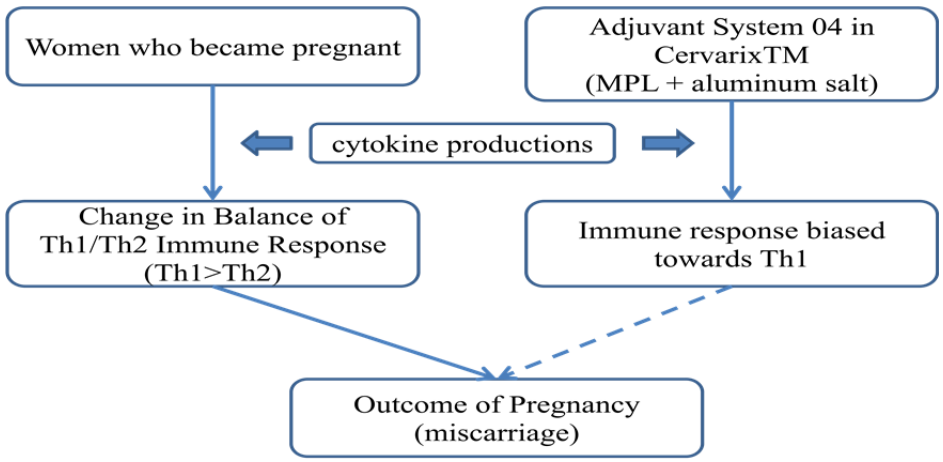


Figure 2.3 Diagram illustrating the biological hypothesis of the plausibility of AS04 system affecting the outcome of pregnancy among women after they have had Cervarix vaccination.

Altogether, it is hypothesized that it is the AS04 with MPL that skews the immune response towards Th1 while aluminum salt promotes Th2 response, which perhaps in turn result in

imbalance of Th1/Th2 cells, as a result, the imbalance of Th1/Th2 cells affect the outcome of pregnancies (Figure 2.3). Though quantitative data on the Th1/Th2 cytokine performance in pregnant women with Cervarix vaccine is not available, it is biologically plausible that Cervarix might be associated with increased risk of miscarriage because the AS04 system may alter maternal immune functions in early pregnancy.

2.4 Review of the Statistical Method

2.4.1 The Bayes' Theorem

The Bayesian approach, as distinguished from the traditional frequentists approach, is the other main philosophical approach to statistics which was defined by Thomas Bayes in the 1700s (Bolstad, 2008). This theory is based on the idea that the parameters of interest, such as the population mean, median, standard deviation, etc., are random variables with probability distributions rather than unknown but fixed constants. We could understand the Bayes theorem as a restatement of conditional probability and the multiplication rule of probability. Here we will use the following example to explain how it works.

In a random experiment, suppose *A* and *B* are two individual events with positive probabilities ($P(A) > 0$, $P(B) > 0$). The occurrence of event *A* is observable, but event *B* is unobservable. The conditional probability of *B* given *A* is defined as below,

$$P(B|A) = \frac{P(A \cap B)}{P(A)} \cdots\cdots\cdots (2.1)$$

The probability that event *B* occurs given event *A* occurs is proportional to the joint probability of both event *A* and *B*, and is rescaled by the scale factor $\frac{1}{P(A)}$ so that the reduced universe ($U_r = A$) equals 1 (Figure 2.4).

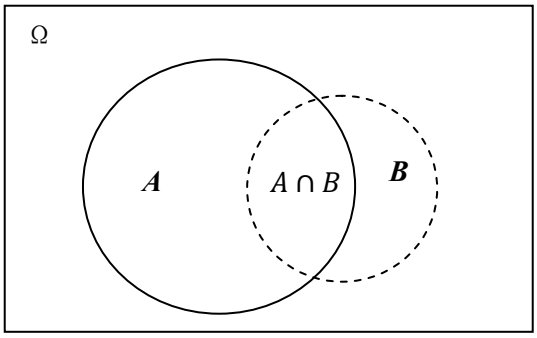


Figure 2.4 Venn Diagram of two independent events *A* and *B*. Given A has occurred, the reduced universe is event *A*.

If we reverse event *A* and event *B*, we have $P(A|B) = \frac{P(A \cap B)}{P(B)}$. By moving $P(B)$ to the left of the equation, we have

$$P(A \cap B) = P(A|B) \times P(B) \cdots\cdots\cdots (2.2)$$

Similarly, $P(A \cap \bar{B}) = P(A|\bar{B}) \times P(\bar{B}) \cdots\cdots\cdots (2.3)$. This is known as the multiplication rule of probability.

Since the marginal probability of event *A* is found as a sum of the probabilities of its all disjoint parts,

$$P(A) = P(A \cap B) + P(A \cap \bar{B})$$

By substituting this into the definition of conditional probability (equation 2.1), we get

$$P(B|A) = \frac{P(A \cap B)}{P(A \cap B) + P(A \cap \bar{B})} \cdots\cdots\cdots (2.4)$$

Then we apply the multiplication rule (equation 2.2 and 2.3) to equation 2.4, we obtain Bayes' theorem for a single event

$$P(B|A) = \frac{P(A|B) \times P(B)}{P(A|B) \times P(B) + P(A|\bar{B}) \times P(\bar{B})}$$

This can be extended to *n* events, $B_1, \cdots, B_n$, partitioning the universe *A*

$$P(B_i|A) = \frac{P(A|B_i) \times P(B_i)}{\sum_{j=1}^{n} P(A|B_j) \times P(B_j)} \; for\; i = 1, \cdots n$$

Here $P(B_i)$ is the prior probability for event B_i; the conditional probability of event *A* given B_i, $P(A|B_i)$, is the likelihood of event B_i; $P(B_i|A)$ is the posterior probability of event B_i given the occurrence of event *A*; $\sum_{j=1}^{n} P(A|B_j) \times P(B_j)$ is the marginal probability of event *A* and $\frac{1}{\sum_{j=1}^{n} P(A|B_j) \times P(B_j)}$ is the scale factor used to rescale the reduced universe *A* to 1.

To apply Bayes' theorem in statistical inference, we start with the following definitions. Let π be the parameter of interest and y indicate the observed data. $g(\pi)$ denotes the prior distribution, $f(y|\pi)$ is the likelihood function of π given fixed y (observed data), $g(\pi|y)$ is the conditional posterior distribution of π, $g^*(\pi)$ denotes the true distribution (marginal posterior distribution) of π. Prior distributions $g(\pi)$ are usually specified using prior knowledge before observing the data. We then modify the prior distribution to obtain the conditional posterior

distributions $g(\pi|y)$ by Bayes' theorem after obtaining the data. (Bolstad, 2008). This can be summarized as '*posterior is proportional to the prior times the likelihood*':

$$g(\pi|y) \propto g(\pi) \times f(y|\pi)$$

2.4.2 MCMC approach and Gibbs Sampling

Researchers often face challenges of intractable computational problems when using Bayesian approach, but the development of Markov Chain Monte Carlo (MCMC) algorithms and the advancement of computational techniques have impelled this approach and make Bayesian computation feasible (Bland & Altman, 1998). The MCMC method is an approach to iteratively generate samples of the parameters in a statistical model so that these samples approximate to the marginal distribution by taking a large enough number of random samples. This is an example.

> Suppose we have a probability distribution of π given actual data y $[g(\pi|y)]$ from the Bayes' theorem, but we are in fact interested in obtaining the marginal distribution $g^*(\pi)$. If draw $\pi_1, \cdots \pi_n$ from $g(\pi|y)$, then posterior $g^*(\pi) \sim \pi_1, \cdots \pi_n$

This shows that the MCMC process enables us to obtain the marginal posterior distribution of the parameter of interest from its conditional distribution. If $g(\pi|y)$ is in a simple form, such as a normal distribution, drawing can be easily done by software. However, if $g(\pi|y)$ is complicated and direct drawing is difficult, we need to introduce Metropolis-Hastings algorithm (Metropolis & Ulam, 1949; Metropolis et al., 1953; Hasting, 1970).

> Suppose we want to generate a Markov Chain $\pi_0, \pi_1, \cdots, \pi_t, \cdots \pi_n$ from $g(\pi|y)$, we start with an initial value π_0 $[g(\pi_0|y) > 0]$, and use the current value π_{t-1} to

sample a candidate point π^*. By computing $\alpha = \frac{g(\pi^*|y)}{g(\pi_{t-1}|y)}$, we accept a candidate point with transition probability$\alpha = min(\frac{g(\pi^*|y)}{g(\pi_{t-1}|y)}, 1)$.

This is known as the Metropolis sampling process. Since the early values in the Markov Chain can be greatly affected by the choice of starting value (π_0), a number of samples (k) are usually thrown out from the chain, which is called burn-in period. The basic strategy to choose starting value properly is to take a value close to the center of the distribution as possible, though multimodal prior distribution can be more complicated. There are a variety of ways to determine an appropriate number of burn-in samples, one of which is to draw time-series of sampling values over time. After a sufficient burn-in period, the chain $\pi_{k+1}, \cdots, \pi_{k+n}$ approaches a stationary distribution and represents the marginal posterior distribution $g^*(\pi)$. Summaries of the parameters in the model including point estimates of the posterior mean and 95% credible intervals of the posterior distribution can be easily obtained from $\pi_{k+1}, \cdots, \pi_{k+n}$.

Except for the choice of starting value, choice of prior distribution is another factor that often affects the effectiveness of the sampling process. Poor choice may increase the burn-in period, that is, increase of computational demand in time (it will take longer time for the Markov chain to become stationary). To optimize, incorporating knowledge about the parameters from previous studies is sometimes appealing by using informative prior distributions. When prior knowledge is not available, an objective non-informative prior can be used, for example, by assigning equal probability to a set of possible values. Nevertheless, the point estimate and the credible interval will be driven more and more by the actual data with the increase of sample size but less by the prior, regardless how the prior is chosen (Dunson, 2001).

Gibbs Sampling is a special case of the Metropolis-Hastings algorithm. When we have a large number of parameters, say $\gamma_1, \gamma_2, \gamma_3$with their joint conditional distribution $g(\gamma_1, \gamma_2, \gamma_3|y)$, Gibbs sampling is useful to generate a sequence of samples for a large set of variables by sampling each variable in turn (G. Casella & George, 1992). The Gibbs Sampling proceeds as follows,

$$\begin{cases} \gamma_1^{(i)} \sim g(\gamma_1|y, \gamma_2 = \gamma_2^{(i-1)}, \gamma_3 = \gamma_3^{(i-1)}) \\ \gamma_2^{(i)} \sim g(\gamma_2|y, \gamma_1 = \gamma_1^{(i)}, \gamma_3 = \gamma_3^{(i-1)}) \\ \gamma_3^{(i)} \sim g(\gamma_1|y, \gamma_1 = \gamma_1^{(i)}, \gamma_2 = \gamma_2^{(i)}) \end{cases}$$

Repeating the above process n times generates the following Gibbs sequence.

$$\gamma_1^{(0)}, \gamma_2^{(0)}, \gamma_3^{(0)}, \gamma_1^{(1)}, \gamma_2^{(1)}, \gamma_3^{(1)}, \cdots \gamma_1^{(n)}, \gamma_2^{(n)}, \gamma_3^{(n)}$$

Then, the characteristics of $g(\gamma_1), g(\gamma_2)$, and $g(\gamma_3)$, such as the means and variances, can be computed from $\gamma_1^{(0)}, \cdots \gamma_1^{(n)}, \gamma_2^{(0)}, \cdots \gamma_2^{(n)}$, and $\gamma_3^{(0)} \cdots \gamma_3^{(n)}$, respectively.

2.4.3 Bayesian vs. Traditional

Bayesian dominated statistical practices in the nineteenth century while the twentieth century was more frequentists (Greenland, 2006). The frequentists regard the parameter of interest as fixed but unknown quantity, its inference is based on hypothetical infinite random samplings, that is, all possible data sets that might have occurred but did not. In contrast, Bayesian method makes inference given the actually occurring data (Bolstad, 2008) . The

credible interval about the parameters allowed by Bayesian approach has a clearer and more direct interpretation than the confidence statement by frequentist statistics. For example, the 95% Bayesian credible interval can be intuitively interpreted as "*the interval containing the true parameter with 95% probability*", while the 95% confidence interval is "*the range of values containing the true parameters 95% of the time in repeated sampling*" (Dunson, 2001). Bayesian also allows predictions of future data which is not easy in frequentist (Bolstad, 2008). Other advantages of Bayesian over frequentist are discussed in the literature (Bland & Altman, 1998; Bolstad, 2008; Dunson, 2001; Kruschke, 2010). Though frequentist statistics seem to be easier and more effective, it is believed that Bayesian methods or combination of Bayesian-frequentist outperform traditional frequentist approach alone when solving bigger problems with huge datasets and perhaps thousands of parameters (Bland & Altman, 1998). In the past two decades, Bayesian statistics have become more commonly used in the design and analysis of clinical trials (Spiegelhalter, Abrams, & Myles, 2004; Spiegelhalter, Freedman, & Parmar, 1994) and in a wide range of other epidemiological studies (Craig, Fryback, Klein, & Klein, 1999; Chen et al., *under revision*; Dunson, 2001; Efron, 2005).

Chapter 3

A Hierarchical Bayes Model for Logistic Regression

3.1 Overview

Vaccination produces immunity against pathogens, such as viruses and bacteria, via the introduction of live, killed, or altered pathogens. Since its first application in smallpox, it has become the most cost-effective strategy in protecting animals and humans from many infectious diseases (Public Health Agency of Canada, 2012). Currently, the toxicity and safety of novel vaccines are well studied in each stage of the randomized clinical trials before the approval for its implementation. In most vaccine clinical research, the safety was commonly reported with respect to the absolute numbers or percentages of adverse events between treatment arms, or sometimes the relative risk (e.g., J. Paavonen et al., 2009; J. Paavonen et al., 2007; Romanowski et al., 2009; Verstraeten et al., 2008). The numbers could be defined either in a specified time frame or the whole follow-up period. One study noticed that the occurrence of adverse events may be elevated only within a certain period of time (Wacholder et al., 2010). However, it reported the numbers of adverse events using an arbitrarily-defined time frame. In this study, we attempt to find out the true risk window via direct estimation without using empirical knowledge.

Specifically, we are interested in identifying the risk window associated with an increase in the risk of miscarriage among women who became pregnant after having had the bivalent HPV vaccinations. Most of the statistical inference used in clinical trials was based on the frequentist theory of hypothesis testing. This study will use an alternative, the Bayes' theorem. In this chapter, we investigate the above the research question through mathematical modeling. We ask three main questions: 1) How do we model the risk window? 2) How can we estimate the risk

window? 3) How is our method comparable to the existing methods? We start by proposing a model to include the risk window and addressing the inference theoretically with The Bayes' theorem and Gibbs Sampling algorithms. Simulation studies are then carried out to evaluate the performance of the model under the Bayesian approach.

Considering the traditional method, we have to pre-specify the risk window for comparison. Mis-specification is quite possible as the size and location of the risk window is uncertain, which may result in an increase of type II error. Also, this method contains multiple comparisons as multiple risk windows need to be specified, which will lead to an increase of false positives by inflating type I error. When the investigator is only interested in hypothesis testing, permutation test may be used to make correction of type I error (Blair, 2008). In contrast, the Bayesian approach will allow us to estimate the risk window directly, rather than making arbitrary specifications. Estimation of the risk window will be very close to the true values, implying that we may have higher power to detect the risk window as long as it truly exists. In this chapter we will also make an effort to compare the Bayesian inference and traditional approaches by simulation studies.

3.2 Development of the Logistic Regression Model with Bayes' theorem

3.2.1 The Variables

3.2.1.1 The Dependent Variable

We start by introducing some notation and variables that are commonly used in the logistic regression model. Suppose that we take a random sample of size n from the population. The

outcome of interest in this study is the occurrence of a miscarriage (a event), which is usually described as a binary variable. Let Y_i indicate the observed outcome of interest ($Y_i = 1$ if event, $Y_i = 0$ if no event). We assume that $Y_1, \dots, Y_n$ are random variables with Bernoulli distributions with $P(Y_i = 1) = \pi_i$. The probability distribution function of y_i is given by

$$f(y_i|\pi_i) = {\pi_i}^{y_i}(1-\pi_i)^{1-y_i}$$

Let $\boldsymbol{y} = \sum y_i$, the conditional probability function for $\boldsymbol{y}$ given $\boldsymbol{\pi}$ is the joint distribution $P(Y_1 = y_1, \dots, Y_n = y_n|\boldsymbol{\pi})$:

$$f(\boldsymbol{y}|\boldsymbol{\pi}) = P(Y_1 = y_1, \dots, Y_n = y_n|\boldsymbol{\pi}) = P(Y_1 = y_1, \dots, Y_n = y_n|\pi_1, \dots, \pi_n)$$
$$= \prod_{i=1}^{n}[{\pi_i}^{y_i}(1-\pi_i)^{1-y_i}]$$

For the observed outcome $y_1, \dots, y_n$, the probability distribution above can be viewed as a function of the parameter $\boldsymbol{\pi}$. Therefore, we have the likelihood function of $\boldsymbol{\pi}$ given by

$$L(\boldsymbol{\pi}) = \prod_{i=1}^{n}[{\pi_i}^{y_i}(1-\pi_i)^{1-y_i}] \text{ for } 0 \le \pi_i \le 1 \cdots (1)$$

And the log-likelihood function is

$$l(\boldsymbol{\pi}) = \sum_{\mathrm{i=1}}^{\mathrm{n}}[y_i \log \pi_i + (1-y_i)\log(1-\pi_i)]$$

3.2.1.2 The Covariates

The major predicting variables we are interested in comprise the treatment and a factor with regard to the timing between vaccination and onset of pregnancy. Let x_{1i} be the individual treatment status that takes binary values ($x_{1i} = 1$ if HPV vaccine, $x_{1i} = 0$ if hepatitis A vaccine).

Next, we define 'time interval' as the period of time between the vaccination and onset of pregnancy for each individual. We also want to introduce the definition of risk window that refers to a period of time after treatment, defined by lower and upper cut-off points c_1 and c_2. The time interval is a continuous variable and can be easily included in any regression models. However, considering the overall goal of this study — to find out the risk window possibly associated with elevated risk of adverse events, this continuous variable cannot answer the question. On the other hand, the usage of cut-off points for risk window cannot be directly incorporated into a regression model, but this can be solved by using an indicator variable that shows whether the individual time interval falls into the risk window or not (Figure 3.1). Define X_{2i} to be the indicator for the timing factor and x_{2i}^* to be time interval. Their relationship is defined by

$$x_{2i} = \begin{cases} 1 \text{ if } c_1 < x_{2i}^* < c_2 \\ 0 \text{ if otherwise} \end{cases}$$

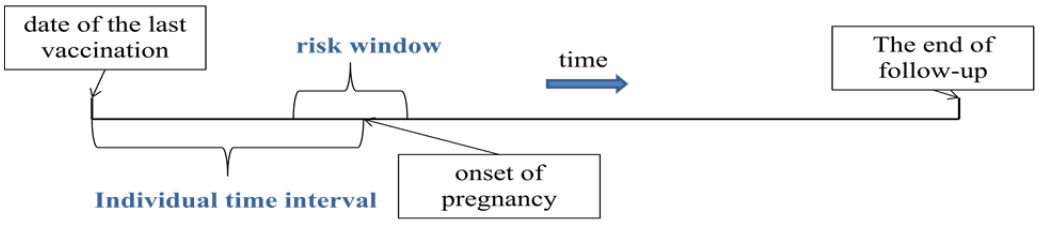


Figure 3.1 Illustration of the definitions of risk window and time interval with regard to the date of last vaccination and onset of pregnancy.

We set c_1, c_2 and x_{2i}^* to be values between 0 and 1. When analyzing real data, the actual values of x_{2i}^* can be converted to a value of (0, 1) using the empirical cumulative distribution function. After conversion, the value of x_{2i}^* for one subject represents the proportion of the population whose value is less than x_{2i}^*. Thus, this model can be generalizable to studies with different length of follow-up, and the estimation of the risk window will not be influenced by extreme values of x_{2i}^* in the dataset.

3.2.2 The Logistic Model

3.2.2.1 The Likelihood Functions

The relationship between the outcome variable and predicting variables is modeled with a logistic regression. This is because that logistic regression is a standard approach to analyze binary outcome data, and the regression coefficients from logistic models have straightforward interpretations in terms of odds ratio (O'Brien & Dunson, 2004). The logistic regression model was proposed as

$$logit\ \pi_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} = \boldsymbol{x_i^T \beta}\ , \text{where } \boldsymbol{\beta} = \{\beta_0, \beta_1, \beta_2, \beta_3\} \cdots (2)$$

The regression coefficients in this model can be interpreted as below:

(1) e^{β_0} = the rates of the event occurring in the control arm outside the risk window;

(2) e^{β_1} = odds ratio of the event occurring in the treatment arm versus in the control arm outside the risk window;

(3) e^{β_2} = the odds ratio of the event occurring within the risk window versus outside the risk window in the control arm;

(4) $e^{\beta_1+\beta_3}$ = odds ratio of the event occurring in the treatment arm versus in the control arm within the risk window;

(5) e^{β_3} = the ratio of the odds ratio of the event occurring in the treatment arm versus in the control arm in the risk window relative to the odds ratio outside the risk window. In this study, we will focus on the estimation of e^{β_3} and the risk window (c_1, c_2).

Next, we continue to work on the likelihood function of the logistic regression model. Taking exponential of both sides in Formula (2) gives

$$\frac{\pi_i}{1-\pi_i} = e^{\boldsymbol{x_i^T\beta}} \text{ , } \pi_i = \frac{e^{\boldsymbol{x_i^T\beta}}}{1+e^{\boldsymbol{x_i^T\beta}}} \text{ and } (1-\pi_i) = \frac{1}{1+e^{\boldsymbol{x_i^T\beta}}}$$

Plugging the above results into the likelihood function (1) gives

$$f(\boldsymbol{y}, \boldsymbol{\beta}|c_1, c_2) = L(\boldsymbol{\pi}) = \prod_{i=1}^{n}[(\frac{\pi_i}{1-\pi_i})^{y_i}(1-\pi_i)] = \prod_{i=1}^{n}[(e^{\boldsymbol{x_i^T\beta}})^{y_i}(\frac{1}{1+e^{\boldsymbol{x_i^T\beta}}})] \cdots (7)$$

and $l(\boldsymbol{\pi}) = \sum_{\mathrm{i}=1}^{\mathrm{n}}\left[y_i \log\left(\frac{\pi_i}{1-\pi_i}\right) + \log(1-\pi_i)\right] = \sum_{\mathrm{i}=1}^{\mathrm{n}}\left[y_i\left(\boldsymbol{x_i^T\beta}\right) - \log\left(1+e^{\boldsymbol{x_i^T\beta}}\right)\right]$

3.2.2.2 The Priors

There is limited information about the values of model parameters and the risk window before analyzing the data, for flexibility, we propose hierarchical priors for c_1 and c_2. And for

simplicity, uniform priors are used for $\boldsymbol{\beta}$. Figure 3.2 illustrates the choices of prior distributions for model parameters.

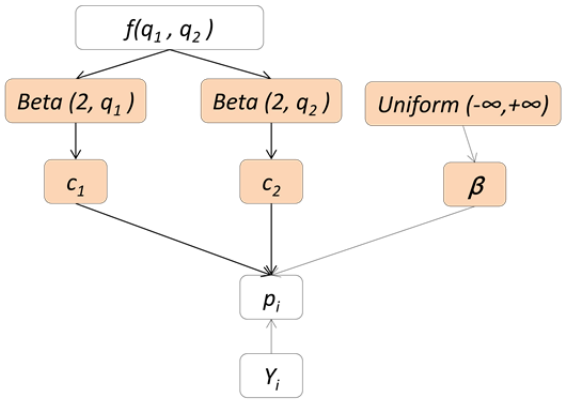


Figure 3.2 Illustration of prior distributions for parameters in the proposed model.

We assume c_1 and c_2 are unknown random variables following beta distributions. Suppose a $beta\ (2, q_2)$ prior density is used for c_2. q_2 is a random variable with a certain distribution. The probability density function of c_2 given q_2 is

$$f(c_2|q_2) = \frac{\Gamma(2+q_2)}{\Gamma(2)\Gamma(q_2)} c_2(1-c_2)^{q_2-1} \text{ for } 0 < c_2 < 1, \text{and } 1 < q_2 < \infty \cdots (3)$$

Since a gamma function has the property that $\Gamma(2+q_2) = (1+q_2)\cdot\Gamma(1+q_2) = (1+q_2)\cdot q_2 \cdot \Gamma(q_2)$ and $\Gamma(2) = 1\times\Gamma(1) = 1$, Formula (3) can be rewritten as

$$f(c_2|q_2) = \frac{\Gamma(q_2)(1+q_2)q_2}{\Gamma(2)\Gamma(q_2)} c_2(1-c_2)^{q_2-1} = \frac{(1+q_2)q_2}{1} c_2(1-c_2)^{q_2-1}$$

$$= (1+q_2)q_2c_2(1-c_2)^{q_2-1}$$

To ensure $c_1 < c_2$, we use another parameter c to define the relationship between c_1 and c_2. Let $c_1 = c \cdot c_2$, where c $(0 < c < 1)$ follows a $beta$ $(2, q_1)$ distribution

$$f_c(c|q_1) = \frac{\Gamma(2+q_1)}{\Gamma(2)\Gamma(q_1)} c(1-c)^{q_1-1} = \frac{(1+q_1)q_1}{1} c(1-c)^{q_1-1}$$

$$= (1+q_1)q_1 c(1-c)^{q_1-1} \text{ for } 0 \le c \le 1 \cdots (4)$$

The conditional probability function of $f(c_1|c_2)$ is found by its cumulative probability function.

$$F(c_1|c_2) = Pr(C_1 < c_1|c_2) = Pr(C \cdot c_2 < c_1) = Pr(C < \frac{c_1}{c_2})$$

then

$$f(c_1|c_2\,) = F_c{}'\left(\frac{c_1}{c_2}\right) = \frac{1}{c_2} f_c\left(\frac{c_1}{c_2}\right) \cdots (5)$$

Based on Formula (4) and (5), the conditional probability $f(c_1|c_2)$ given q_1 is

$$f(c_1|c_2\,;q_1) = \frac{1}{c_2} \cdot (1+q_1)q_1 \left(\frac{c_1}{c_2}\right)\left(1-\frac{c_1}{c_2}\right)^{q_1-1}$$

$$\text{for } 0 \le c_1 \le 1, 0 \le c_2 \le 1 \text{ and } c_1 < c_2 \cdots (6)$$

When q_1 and q_2 take different values between $(1, \infty)$, the prior distributions for c_1 and c_2 can be of a variety of different forms (Figure 3.3).

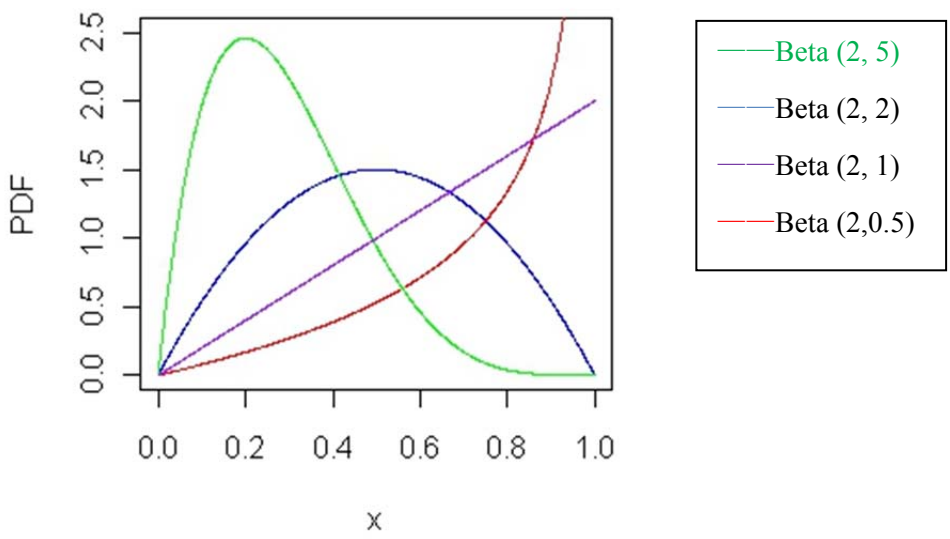


Figure 3.3 Probability Density Functions of Beta Distributions.

3.2.2.3 The Posterior Distributions

Bayes' theorem is summarized as 'the *posterior is proportional to the prior times the likelihood*'. Thus, we could obtain the shape of the joint posterior distribution of $y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2$

$$f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2) \propto f(y, \boldsymbol{\beta}, c_1, c_2 | q_1, q_2) \times f(q_1, q_2) = f(y, \boldsymbol{\beta} | c_1, c_2) \times f(c_1, c_2) \times f(q_1, q_2)$$
$$= f(y, \boldsymbol{\beta} | c_1, c_2) \times f(c_1 | c_2) \times f(c_2) \times f(q_1, q_2) \cdots (8)$$

where $f(y, \boldsymbol{\beta} | c_1, c_2)$, $f(c_1 | c_2)$, $f(c_2)$ suppress the overall dependence on q_1, q_2.

Plugging Formula (3), (6) and (7) into (8) gives

$$f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)$$

$$\propto \left\{\prod_{i=1}^{n}[(e^{x_i^T\boldsymbol{\beta}})^{y_i}(\frac{1}{1+e^{x_i^T\boldsymbol{\beta}}})]\right\} \cdot \frac{1}{c_2} \cdot (1+q_1)q_1\left(\frac{c_1}{c_2}\right)\left(1-\frac{c_1}{c_2}\right)^{q_1-1} \cdot (1$$

$$+ q_2)q_2c_2(1-c_2)^{q_2-1} \cdot f(q_1, q_2)$$

$$= \left\{\prod_{i=1}^{n}[(e^{x_i^T\boldsymbol{\beta}})^{y_i}(\frac{1}{1+e^{x_i^T\boldsymbol{\beta}}})]\right\}(1+q_1)q_1(1+q_2)q_2$$

$$\cdot c_1(c_2-c_1)^{q_1-1}{c_2}^{-q_1}(1-c_2)^{q_2-1} \cdot f(q_1, q_2) \cdots (9)$$

Let $f(q_1, q_2) = 1/[(1+q_1)q_1(1+q_2)q_2]$, then distribution (9) becomes

$$f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2) \propto \left\{\prod_{i=1}^{n}[(e^{x_i^T\boldsymbol{\beta}})^{y_i}(\frac{1}{1+e^{x_i^T\boldsymbol{\beta}}})]\right\} c_1(c_2-c_1)^{q_1-1}{c_2}^{-q_1}(1-c_2)^{q_2-1} \cdots (10)$$

This gives us the shape of the posterior density. To obtain the exact posterior probability density function (p.d.f), we need to divide this by the area under the curve. This is expressed as

$$f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)$$

$$= \frac{f(y, \boldsymbol{\beta}|c_1, c_2) \times f(c_1|c_2) \times f(c_2) \times f(q_1, q_2)}{\int \cdots \int f(y, \boldsymbol{\beta}|c_1, c_2) \times f(c_1|c_2) \times f(c_2) \times f(q_1, q_2) d(q_1, q_2) d\boldsymbol{\beta} dc_1 dc_2 dq_1 dq_2}$$

The above integration is difficult and we do not use the exact posterior distribution in future analysis. In the following, we will work with the function which is proportional to the posterior [Formula (10)] and use Gibbs Sampling algorithm to obtain random samples from the posterior p.d.f. Eventually, we are interested in obtaining the marginal distributions of $f_1(c_1)$, $f_2(c_2)$, $f_3(\boldsymbol{\beta})$, $f_4(q_1)$, $f_5(q_2)$. Their marginal p.d.f. can be found by

$$\begin{cases} f_1(c_1) = \int \cdots \int f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)\, d\boldsymbol{\beta} dc_2 dq_1 dq_2 \\ f_2(c_2) = \int \cdots \int f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)\, d\boldsymbol{\beta} dc_1 dq_1 dq_2 \\ f_3(\boldsymbol{\beta}) = \int \cdots \int f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)\, dc_1 dc_2 dq_1 dq_2 \\ f_4(q_1) = \int \cdots \int f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)\, d\boldsymbol{\beta} dc_1 dc_2 dq_2 \\ f_5(q_2) = \int \cdots \int f(y, \boldsymbol{\beta}, c_1, c_2, q_1, q_2)\, d\boldsymbol{\beta} dc_1 dc_2 dq_1 \end{cases}$$

Again, the marginal distributions involve complicated integration that are extremely difficult to perform because there is no closed form expression for these integrations. In this case, Gibbs sampling provides an alternative way to obtain the marginal distributions, for example, by generating a sample $c_1^{(0)}, c_1^{(1)}, \cdots, c_1^{(m)} \sim f_1(c_1)$ without requiring exact expression of $f_1(c_1)$. In the next section, we will develop a Gibbs Sampling algorithm to solve this problem.

3.2.2.4 Finding the Marginal Distributions of the Parameters of Interest (The Gibbs Sampling Algorithms)

Suppose that we want to obtain m random samples of $\emptyset = \{\boldsymbol{\beta}, c_1, c_2, q_1, q_2\}$. Denote the jth sample by $\emptyset^{(j)} = \left\{ \boldsymbol{\beta}^{(j)}, c_1^{(j)}, c_2^{(j)}, q_1^{(j)}, q_2^{(j)} \right\}$ for $(0 \leq j \leq m)$. We begin with initial values $c_1^{(0)}$, $c_2^{(0)}$. The initial values for $c_2^{(0)}$ and $c_1^{(0)}$ are generated using the profile likelihood method (Davison, 2003). That is to select the initial values from fifty pairs of c_2 and c_1 that has the maximum likelihood of function (3).

Given $c_1^{(0)}$ and $c_2^{(0)}$, the regression coefficients $\boldsymbol{\beta}^{(0)} = \left\{\beta_0^{(0)}, \beta_1^{(0)}, \beta_2^{(0)}, \beta_3^{(0)}\right\}$ in the logistic model can be easily obtained using the classical method with Formula (2). Subsequently, $q_1^{(0)}, q_2^{(0)}$ can be generated alternately from

$$q_1^{(0)} \sim f(q_1 | y, \boldsymbol{\beta}^{(0)}, c_1^{(0)}, c_2^{(0)}, q_2^{(0)})$$

$$q_2^{(0)} \sim f(q_2 | y, \boldsymbol{\beta}^{(0)}, c_1^{(0)}, c_2^{(0)}, q_1^{(1)})$$

Next, we can iteratively generate random samples for the parameters $\boldsymbol{\beta}, c_1, c_2, q_1, q_2$ through the following cycle.

Step 1: conditional on $\boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)}$ from previous iteration and the observed data, the conditional distribution of $c_1^{(j+1)}, c_2^{(j+1)}$ is

$$c_1^{(j+1)}, c_2^{(j+1)} \sim f\left(c_1, c_2 \middle| y, \boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)}\right)$$

$$\propto \left\{\prod_{i=1}^{n}[(e^{x_i^T\boldsymbol{\beta}})^{y_i}(\frac{1}{1+e^{x_i^T\boldsymbol{\beta}}})]\right\} c_1(c_2-c_1)^{q_1^{(j)}-1} c_2^{-q_1^{(j)}} (1-c_2)^{q_2^{(j)}-1} \cdots (11)$$

Random sampling of $c_1^{(j+1)}, c_2^{(j+1)}$ from distribution (11) can be obtained using the Metropolis-Hasting (MH) algorithm (Metropolis et al., 1953). Using current values of $c_1^{(j)}, c_2^{(j)}$, we sample a candidate point u_2^* from a uniform distribution $u_2^* \sim Unif(0,1)$. To ensure $c_1 \leq c_2$, sample the other candidate point u_1^* from a uniform distribution $u_1^* \sim Unif(0, u_1^*)$. Given the candidate point u_1^* and u_2^*, calculate the ratio of the density at the candidate and current points:

$$\alpha = \frac{f(u_1^*, u_2^* | y, \boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)})}{f(c_1^{(j)}, c_2^{(j)} | y, \boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)})}$$

If $\alpha > 1$, accept the candidate points$c_1^{(j+1)} = u_1^*,\ c_2^{(j+1)} = u_2^*$.

Else $(\alpha < 1)$, sample a uniformly distributed random variable $U \sim Unif(0,1)$,

$$\begin{cases} c_1^{(j+1)} = u_1^*,\ c_2^{(j+1)} = u_2^* \text{ if } U < \alpha \\ c_1^{(j+1)} = c_1^{(j)},\ c_2^{(j+1)} = c_2^{(j)} \text{ if } U > \alpha \end{cases}$$

The above process can be summarized as computing $\alpha = min(\frac{f(u_1^*, u_2^* | y, \boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)})}{f(c_1^{(j)}, c_2^{(j)} | y, \boldsymbol{\beta}^{(j)}, q_1^{(j)}, q_2^{(j)})}, 1)$ and accepting a candidate point with probability α. Markov Chain Monte Carlo theory (Robert and Casella, 1999) will ensure that random samples from the MH algorithm follow the posterior distribution $f(c_1, c_2 | y, \boldsymbol{\beta}, q_1, q_2)$ when the iteration are repeated large enough number of times.

Step 2: conditional on $c_1^{(j+1)}, c_2^{(j+1)}, q_1^{(j)}, q_2^{(j)}$ and the observed data, the conditional distribution of $\boldsymbol{\beta}^{(j+1)}$ is the likelihood function of the logistic regression model

$$\boldsymbol{\beta}^{(j+1)} \sim f(\boldsymbol{\beta} | y, c_1^{(j+1)}, c_2^{(j+1)}, q_1^{(j)}, q_2^{(j)}) \propto \left(e^{x_i^T \boldsymbol{\beta}}\right)^y \left(1 + e^{x_i^T \boldsymbol{\beta}}\right)^{-n}$$

Estimators of the regression coefficients $\widehat{\boldsymbol{\beta}}^{(j+1)}$ can be calculated through the logistic regression model (Formula (2)). At the same time, the variance of $\widehat{\boldsymbol{\beta}}^{(j+1)}$ will also be obtained from the variance-covariance matrix $\widehat{\sum}^{(j+1)}$ of the regression model. On top of this, the random

samples of $\boldsymbol{\beta}^{(j+1)}$ can be generated from a multivariate normal distribution $\boldsymbol{\beta}^{(j+1)} \sim N(\widehat{\boldsymbol{\beta}}^{(j+1)}, \widehat{\sum}^{(j+1)})$.

Step 3: conditional on $\boldsymbol{\beta}^{(j+1)}, c_1^{(j+1)}, c_2^{(j+1)}, q_2^{(j)}$ and the observed data, the conditional p.d.f of $q_1^{(j+1)}$ is given by

$$q_1^{(j+1)} \sim f(q_1|y, \boldsymbol{\beta}^{(j+1)}, c_1^{(j+1)}, c_2^{(j+1)}, q_2^{(j)}) \propto \left(\frac{c_2^{(j+1)} - c_1^{(j+1)}}{c_2^{(j+1)}}\right)^{q_1}$$

This p.d.f. has a form of *Gamma* distribution with a shape parameter of 1 and a scale parameter of $log(\frac{1}{\lambda_1})$ where $\lambda_1 = \frac{c_2^{(j+1)} - c_1^{(j+1)}}{c_2^{(j+1)}}$. A random sample of $q_1^{(j+1)}$ can be obtained using *rgamma* function in R.

Step 4: conditional on $\boldsymbol{\beta}^{(j+1)}, c_1^{(j+1)}, c_2^{(j+1)}, q_1^{(j+1)}$ and the observed data, the conditional p.d.f. of $q_2^{(j+1)}$ is expressed as

$$q_2^{(j+1)} \sim f(q_2|y, \boldsymbol{\beta}^{(j+1)}, c_1^{(j+1)}, c_2^{(j+1)}, q_1^{(j+1)}) \propto (1 - c_2^{(j+1)})^{q_2}$$

This distribution is also a *Gamma* distribution with a shape parameter of 1 and a scale parameter of $log(\frac{1}{\lambda_2})$ where $\lambda_2 = 1 - c_2^{(j+1)}$. A random sample of $q_2^{(j+1)}$ can be obtained using *rgamma* function in R.

By repeating Step1-4 for $(m-1)$ times, we will obtain the following Gibbs sequence of m random samples for each parameter of interest. If we take m large enough, the random samples will represent the posterior distributions of each parameter. However, the first $(0, \dots, B)$ samples

may not have stationary distribution, so that these samples are usually discarded, which is usually called burn-in period. A key issue in the successful implementation of Gibbs Sampling or any other MCMC methods is choosing an appropriate length of burn-in samples. The length of burn-in samples refers to the number of runs that are discarded before the random samples (or Markov chain) approach stationarity. Several tests are available to determine whether or not the random samples have reached its stationary distribution, such as the Geweke test (Geweke, 1992) and Raftery-Lewis test (Raftery, 1992). An easier approach is to look at the time series trace, that is, the plot of the random samples being generated from Gibbs Sampling versus the number of iterations. This type of plot can easily visualize the mixing of random samples, as well as suggest a minimum burn-in period. In practice, typically we exclude the first 1000 to 5000 samples and then conduct convergence test to assess whether the Markov Chain is indeed stationary. Alternatively, we could plot the generated values over time to see how the chain performs and then choose an appropriate B.

The Gibbs sequence :

$$c_1^{(0)}, c_2^{(0)}, \boldsymbol{\beta}^{(0)}, q_1^{(0)}, q_2^{(0)}, \cdots\cdots, c_1^{(B)}, c_2^{(B)}, \boldsymbol{\beta}^{(B)}, q_1^{(B)}, q_2^{(B)}, \cdots\cdots c_1^{(B+m)}, c_2^{(B+m)}, \boldsymbol{\beta}^{(B+m)}, q_1^{(B+m)}, q_2^{(B+m)}$$

3.2.3 Statistical Inferences

3.2.3.1 Estimation

The random samples for each parameter (posterior distribution) approximate the marginal distribution of the parameter after convergence.

$$\begin{cases} c_1^{(B+1)}, c_1^{(B+2)}, \cdots\cdots c_1^{(B+m)} \sim f_1(c_1) \\ c_2^{(B+1)}, c_2^{(B+2)}, \cdots\cdots c_2^{(B+m)} \sim f_2(c_2) \\ \boldsymbol{\beta}^{(B+1)}, \boldsymbol{\beta}^{(B+2)}, \cdots\cdots \boldsymbol{\beta}^{(B+m)} \sim f_3(\boldsymbol{\beta}) \\ q_1^{(B+1)}, q_1^{(B+2)}, \cdots\cdots q_1^{(B+m)} \sim f_4(q_1) \\ q_2^{(B+1)}, q_2^{(B+2)}, \cdots\cdots q_2^{(B+m)} \sim f_5(c_2) \end{cases}$$

By the probability Law of Large Numbers (LLN), the posterior mean of any random variable can be approximated by averaging the values over the random samples. That is, for example, by taking m large enough, we could use $1/_{(m)} \sum_{B+1}^{B+m} c_1^{(j)}$ to calculate the posterior mean of random variable c_1, where B is the number of burn-in samples. The $(1-\alpha)\%$ credible interval for any parameter can be obtained easily through the empirical quantiles of posterior samples.

$$\begin{cases} \hat{c}_1 = 1/_{(m)} \sum_{B+1}^{B+m} c_1^{(j)} \\ \hat{c}_2 = 1/_{(m)} \sum_{B+1}^{B+m} c_2^{(j)} \\ \widehat{\boldsymbol{\beta}} = 1/_{(m)} \sum_{B+1}^{B+m} \boldsymbol{\beta}^{(j)} \\ \hat{q}_1 = 1/_{(m)} \sum_{B+1}^{B+m} q_1^{(j)} \\ \hat{q}_2 = 1/_{(m)} \sum_{B+1}^{B+m} q_2^{(j)} \end{cases}$$

3.2.3.2 Hypothesis Testing

We would be interested to hypothesize that $H_0: \beta_j = 0$ versus $H_1: \beta_j \neq 0$, for $j = 1, 2, 3$, at the α level of significance before we use the logistic regression model to make predictions. In terms of marginal $\widehat{\boldsymbol{\beta}}$, we look at where 0 lies in relation to the credible interval obtained in the previous step. If it lies outside the interval, we reject H_0; otherwise, accept H_0. This test assesses the significance of $\widehat{\boldsymbol{\beta}}$ over all possible c_1 and c_2.

3.3 Simulation Studies

3.3.1 Data Generation

To assess the performance of the proposed model, a series of simulations studies were conducted. We attempted to determine how the estimators of the parameters of interest perform in approximation to the actual values that were used to generate the data. We considered sample sizes n =1000, 2000, and 4000 and generated data using the proposed logistic model. The risk window takes values of the following pairs: $c_1 = 0.1, c_2 = 0.2$; $c_1 = 0.2, c_2 = 0.3$; $c_1 = 0.3, c_2 = 0.4$; $c_1 = 0.1, c_2 = 0.3$; $c_1 = 0.2, c_2 = 0.5$, representing different sizes and locations of the risk window. The baseline probabilities of miscarriage were set at $\beta_0 = \log(0.1), \beta_1 = 0$. The effect of the time indicator β_2 took values $\log(1.0)$, $\log(1.5)$, and the interaction term β_3 between treatment and the time indicator took values $\log(1.5)$, $\log(2.5)$, and $\log(3.5)$. These values were considered as the actual values of the model parameters. In total, thirty datasets were simulated, respectively, for each designated sample size. For each simulation setting, the response variable and covariates were generated with the following methods:

(1) One binary covariate (0 and 1) representing the treatment arms was generated assuming equal probabilities for each treatment arm, given the randomization of treatment allocation in clinical trials.

(2) Without loss of generality, one continuous covariate representing the time interval was generated from a uniform distribution between 0 and 1.

(3) As assumed previously, the response variable was following the Bernoulli distribution. The response variable was generated individually from Bernoulli distributions with probabilities computed based on the logistic model [Formula (2)] in section 1.2.2.

3.3.2 The Bayesian Analysis

With regard to the Bayesian inference, each Gibbs Sampling consisted of 500 burn-in samples and a 5000-length chain for each parameter. Sample mean and credible intervals were computed from those 5000 random samples. Then the entire estimation procedures was repeated 500 times. Eventually, bias, coverage probability and power for the parameters of interest were derived from the 500 replications. The procedures were run in R (R development team, 2010). Corresponding R code can be found in the Appendix.

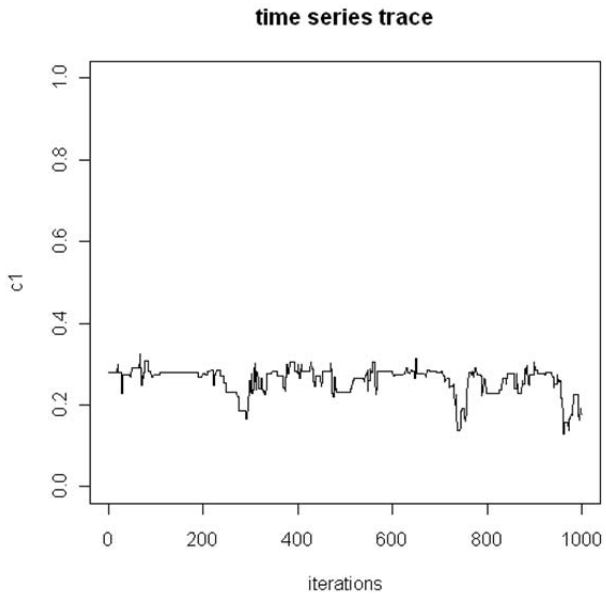
Massive computation was expected in the simulation studies. The R programs were performed on an Ontario-wide network named SHARCNET. SHARCNET stands for Shared Hierarchical Academic Research Computing Network. It is a platform with high-performance clusters across 17 Ontario universities with close to 20,000 CPUs specifically designed for massive computations.

3.3.2.1 Convergence Diagnostics

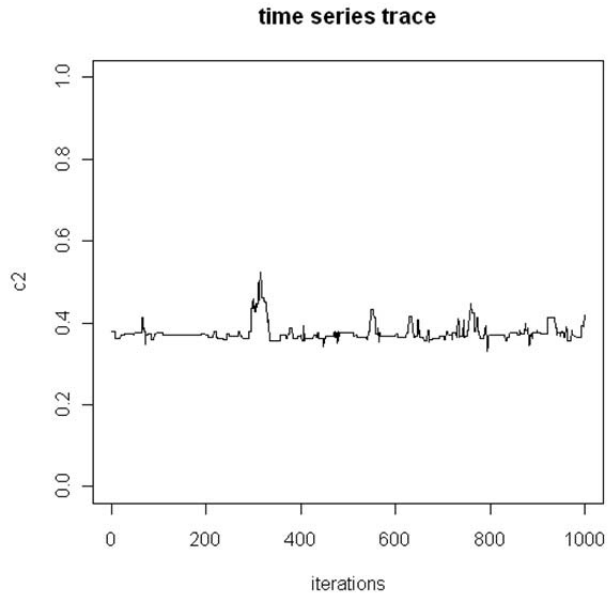
Here we used time series trace to determine the number of burn-in samples. We set the following values and generated a dataset

$$c_1 = 0.3, c_2 = 0.4,\ exp(\beta_0) = 0.1, exp(\beta_1) = 1, \exp(\beta_2) = 1.5, \exp(\beta_3) = 1.5$$

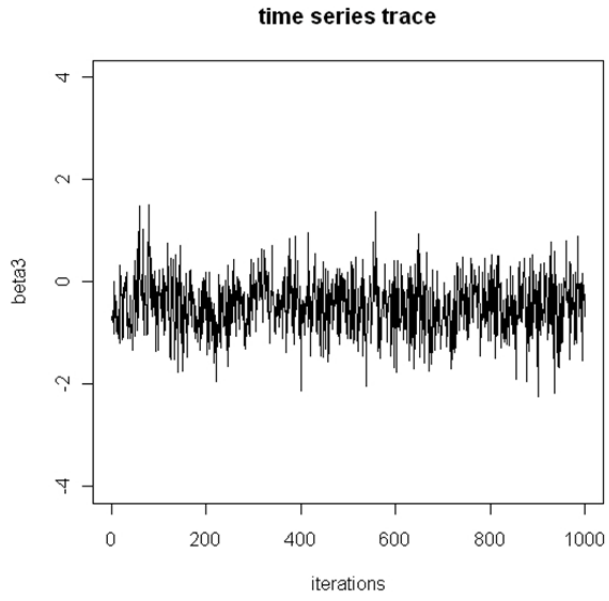
The first 1000 random samples of $\hat{c}_1, \hat{c}_2$ and $\hat{\beta}_3$ from the Gibbs Sequence were plotted over time, respectively (Figure 3.4). It was observed that the traces of $\hat{c}_1, \hat{c}_2$ and $\hat{\beta}_3$ seemed to explore a range of values well and appeared to be fairly stable. This plot indicated that 500 iterations were sufficient in this case, which was consistent with plots under other combinations of the risk window and effect sizes (datasets generated with different pre-specified values of c_1 , c_2 and $\beta_0, \beta_1, \beta_2, \beta_3$) (figures not shown).



(a)



(b)



(c)

Figure 3.4 Time series traces of random samples generated from one Gibbs Sampling over time (1000 iterations): (a) c_1; (b) c_2; (c) $\boldsymbol{\beta_3}$. The true values used to generate the data were set at $\boldsymbol{c_1 = 0.3, c_2 = 0.4, \beta_3 = log(1.5)}$.

3.3.2.2 Testing Procedures

The parameters of primary interest include c_1, c_2, and β_3 because they are the most relevant to the research question in this study. We compared the testing procedures among different simulations in terms of bias, coverage probability for $\hat{c}_1$, $\hat{c}_2$, and $\hat{\beta}_3$, and power for $\hat{\beta}_3$ (Table 3.1). Bias is computed as the difference between an estimator's expectation and the true value of the parameter being estimated. Coverage Probability (C.P.) is the proportion of the time that the credible interval contains the true value of interest. Power for $\hat{\beta}_3$ is defined as the probability of rejecting a false null hypothesis, where the null hypothesis is $\beta_3 = 0$. All calculations were based on 500 replications. Additional results regarding the inference on other parameters ($\hat{\beta}_0, \hat{\beta}_1, \hat{\beta}_2$) are presented in the Appendix E. When presenting the results in Table 3.1 and the Appendix E, the simulations were grouped by the values that were given to the risk window in data simulation, so that, in other words, 'a group' refers to the generated datasets that have the same actual values of c_1 and c_2.

The estimates of $\hat{c}_1$, $\hat{c}_2$, and $\hat{\beta}_3$ appeared to be fairly concordant over each sample size. The biases of $\hat{c}_1$, $\hat{c}_2$, and $\hat{\beta}_3$ diminished with the increase of the effect size ($exp(\beta_3)$ & $exp(\beta_2)$) in each group (when holding the risk window c_1 and c_2 fixed). Specifically, when the effect sizes were small, for example, $exp(\beta_2) = 1.0, exp(\beta_3) = 1.5$, the biases for $\hat{c}_1$, $\hat{c}_2$, and $\hat{\beta}_3$ were relatively large, as compared with estimates from other datasets in the same group. When $exp(\beta_2) = 1.5, exp(\beta_3) = 3.5$, we observed the smallest biases for $\hat{c}_1$, $\hat{c}_2$, and $\hat{\beta}_3$ in each group and each sample size, with a few exceptions for $\hat{\beta}_3$, presumably due to random error.

Regarding the coverage probabilities, C.P. for $\hat{\beta}_3$ was always above 80%, even when C. P. for $\hat{c}_1$and/or $\hat{c}_2$ was low.There were a few cases of low coverage probabilities observed for $\hat{c}_1$ and

$\hat{c}_2$. For example, when $c_1 = 0.1$, $c_2 = 0.2$, $exp(\beta_2) = 1.0, exp(\beta_3) = 1.5$ under sample size n =1000,2000, and 4000, C.P. for $\hat{c}_2$ were 39.2%, 52.8%, and 56.4%, respectively; and when $n = 1000$, $c_1 = 0.2$, $c_2 = 0.3$, $exp(\beta_2) = 1.0, exp(\beta_3) = 1.5$, C.P. for $\hat{c}_2$ was 67.4%, but they all improved when sample size increased. Other than that, the coverage probabilities for $\hat{c}_1$ and $\hat{c}_2$were mostly close to the nominal level (95%).

In terms of power of $\hat{\beta}_3$, it seemed as if it tend to increase dramatically with the increase of either the sample size n or the effect size $exp(\beta_3)$ (Figure 3.5). When n = 1000, $\hat{\beta}_3$'s power rarely reached 80% except for $c_1 = 0.2$, $c_2 = 0.5$, $exp(\beta_2) = 1.5, exp(\beta_3) = 3.5$. When n = 2000, if the size of the risk window is $c_2 - c_1 = 0.1$, $\hat{\beta}_3$'s power was close to or higher than 80% when the effect size is large enough [$exp(\beta_3) = 3.5$], if the size of the risk window is wider $c_2 - c_1 \geq 0.2$, $\hat{\beta}_3$'s power was close to or higher than 80% when $exp(\beta_3) \geq 2.5$. When n = 4000, it was noticeable that 80% power for $\hat{\beta}_3$ could be achieved when $exp(\beta_2) = 1.5, exp(\beta_3) \geq 2.5$ no matter what sizes or locations the risk windows were.

Table 3.1 Summary Results of estimation of selected parameters in simulation studies under three levels of sample size. Each Gibbs sampling included 500 burn-in samples and 5000 random samples. The procedure was repeated 500 times. (1) n=1000; (2) n=2000; (3) n=4000.

(1) $n = 1000, exp(\beta_0) = 0.1, exp(\beta_1) = 1$

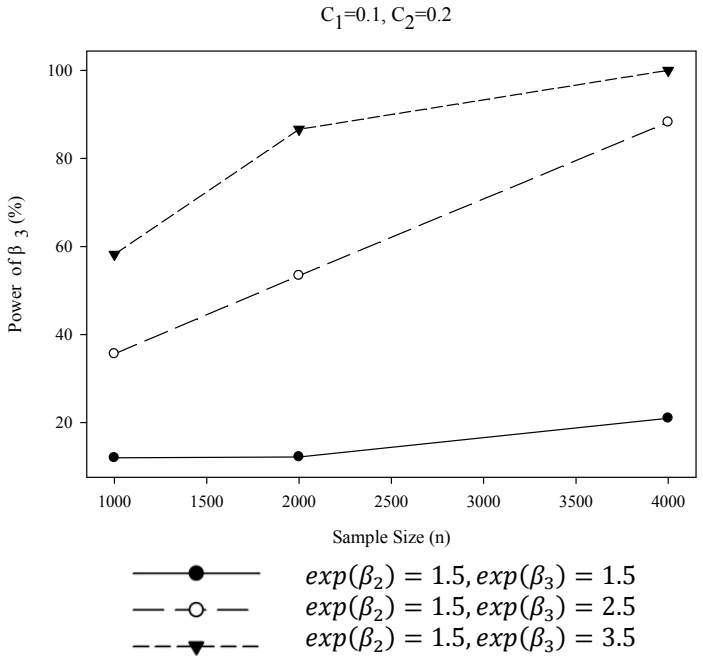
c_1	c_2	e^{β_2}	e^{β_3}	$\hat{c}_1$		$\hat{c}_2$		$\hat{q}_1$	$\hat{q}_2$	Marginal $\hat{\beta}_3$		
				Bias	C.P.	Bias	C.P.			Bias	C.P	Power
0.1	0.2	1	1.5	0.14	79	0.28	39	1.76	1.76	-0.37	85	14
0.1	0.2	1	2.5	0.06	90	0.16	70	1.67	1.52	-0.28	87	29
0.1	0.2	1.5	1.5	0.06	93	0.15	73	1.67	1.50	-0.06	91	12
0.1	0.2	1.5	2.5	0.01	96	0.05	91	1.65	1.32	0.02	94	36
0.1	0.2	1.5	3.5	0.00	95	0.03	93	1.67	1.27	0.01	95	58
0.2	0.3	1	1.5	0.06	90	0.20	67	1.80	1.80	-0.25	90	11
0.2	0.3	1	2.5	0.01	92	0.11	83	1.80	1.58	-0.20	93	26
0.2	0.3	1.5	1.5	0.01	92	0.12	81	1.79	1.60	-0.05	92	13
0.2	0.3	1.5	2.5	-0.01	94	0.05	90	1.89	1.44	0.01	96	35
0.2	0.3	1.5	3.5	-0.01	95	0.02	94	1.96	1.40	-0.03	93	52
0.3	0.4	1	1.5	-0.03	87	0.14	79	1.80	1.87	-0.21	88	14
0.3	0.4	1	2.5	-0.04	92	0.09	86	1.89	1.73	-0.19	93	25
0.3	0.4	1.5	1.5	-0.03	92	0.09	84	1.91	1.72	-0.02	92	14
0.3	0.4	1.5	2.5	-0.03	91	0.04	93	2.07	1.60	-0.02	96	31
0.3	0.4	1.5	3.5	-0.02	92	0.02	94	2.18	1.56	-0.05	95	55
0.1	0.3	1	1.5	0.13	82	0.17	75	1.74	1.73	-0.16	89	13
0.1	0.3	1	2.5	0.04	92	0.06	90	1.57	1.49	0.01	95	40
0.1	0.3	1.5	1.5	0.05	94	0.06	91	1.58	1.48	-0.02	95	13
0.1	0.3	1.5	2.5	0.01	95	0.01	93	1.49	1.38	0.08	94	53
0.1	0.3	1.5	3.5	0.01	96	0.00	94	1.45	1.36	0.05	95	79
0.2	0.4	1	1.5	0.04	94	0.09	85	1.76	1.75	-0.09	93	13
0.2	0.4	1	2.5	0.01	94	0.04	93	1.72	1.61	0.02	95	38
0.2	0.4	1.5	1.5	0.01	96	0.04	93	1.73	1.61	0.07	94	15
0.2	0.4	1.5	2.5	0.00	96	0.01	94	1.71	1.54	0.05	96	49
0.2	0.4	1.5	3.5	0.00	94	0.00	94	1.71	1.52	0.02	96	77
0.2	0.5	1	1.5	0.05	92	0.02	86	1.76	1.81	0.05	91	17
0.2	0.5	1	2.5	0.02	94	0.00	94	1.63	1.73	0.13	95	53
0.2	0.5	1.5	1.5	0.02	96	0.00	94	1.66	1.73	0.03	94	14
0.2	0.5	1.5	2.5	0.01	95	0.00	95	1.57	1.70	0.05	95	57
0.2	0.5	1.5	3.5	0.00	94	0.00	94	1.53	1.70	0.05	96	87

(2)$n = 2000, exp(\beta_0) = 0.1, exp(\beta_1) = 1$

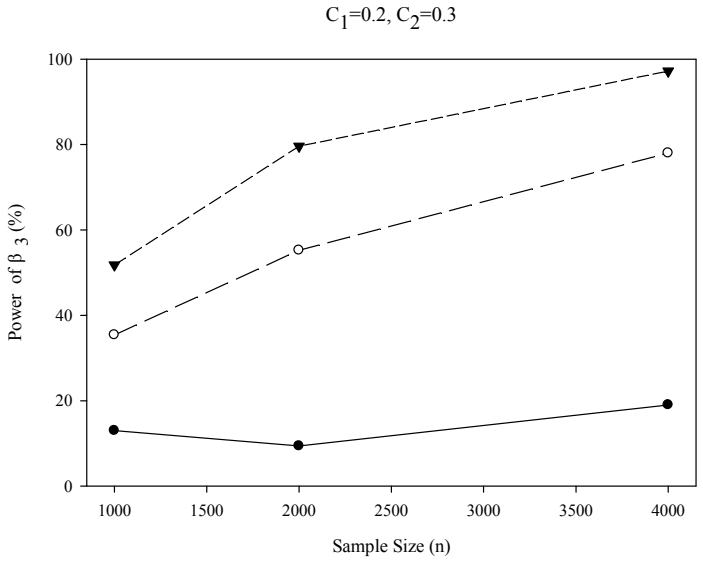
c_1	c_2	e^{β_2}	e^{β_3}	$\hat{c}_1$		$\hat{c}_2$		$\hat{q}_1$	$\hat{q}_2$	Marginal $\hat{\beta}_3$		
				Bias	C.P.	Bias	C.P.			Bias	C.P.	Power
0.1	0.2	1	1.5	0.16	87	0.29	53	1.83	1.77	-0.31	96	4
0.1	0.2	1	2.5	0.07	92	0.15	82	1.71	1.51	-0.33	92	31
0.1	0.2	1.5	1.5	0.08	91	0.16	79	1.73	1.52	-0.17	92	12
0.1	0.2	1.5	2.5	0.01	98	0.03	95	1.68	1.27	-0.03	95	53
0.1	0.2	1.5	3.5	0.00	96	0.01	95	1.68	1.23	0.01	94	87
0.2	0.3	1	1.5	0.07	96	0.21	85	1.86	1.81	-0.26	95	7
0.2	0.3	1	2.5	0.01	97	0.10	91	1.85	1.57	-0.22	95	31
0.2	0.3	1.5	1.5	0.02	95	0.12	88	1.86	1.60	-0.13	95	9
0.2	0.3	1.5	2.5	-0.01	95	0.02	95	1.99	1.40	0.01	93	55
0.2	0.3	1.5	3.5	0.01	89	0.05	91	2.01	1.47	-0.12	89	80
0.3	0.4	1	1.5	-0.02	95	0.11	92	1.87	1.81	-0.27	97	4
0.3	0.4	1	2.5	-0.02	95	0.07	90	1.99	1.69	-0.28	95	30
0.3	0.4	1.5	1.5	-0.03	95	0.07	91	1.97	1.69	-0.12	97	8
0.3	0.4	1.5	2.5	-0.02	94	0.02	95	2.19	1.56	-0.09	95	48
0.3	0.4	1.5	3.5	-0.01	95	0.01	95	2.32	1.52	0.02	96	88
0.1	0.3	1	1.5	0.14	88	0.17	84	1.79	1.74	-0.21	95	9
0.1	0.3	1	2.5	0.04	93	0.04	92	1.55	1.45	-0.03	95	61
0.1	0.3	1.5	1.5	0.04	94	0.04	91	1.57	1.45	-0.01	95	18
0.1	0.3	1.5	2.5	0.00	94	0.00	92	1.44	1.36	0.04	94	79
0.1	0.3	1.5	3.5	0.00	92	0.01	94	1.42	1.37	0.00	93	97
0.2	0.4	1	1.5	0.07	97	0.10	95	1.84	1.79	-0.16	96	8
0.2	0.4	1	2.5	0.01	94	0.02	93	1.73	1.57	0.00	94	67
0.2	0.4	1.5	1.5	0.01	97	0.03	95	1.75	1.58	0.00	97	19
0.2	0.4	1.5	2.5	0.00	96	0.00	95	1.70	1.51	0.04	96	80
0.2	0.4	1.5	3.5	0.00	93	0.00	94	1.70	1.51	0.04	96	98
0.2	0.5	1	1.5	0.06	96	0.01	93	1.80	1.77	-0.07	94	12
0.2	0.5	1	2.5	0.01	95	0.00	95	1.59	1.71	0.03	93	76
0.2	0.5	1.5	1.5	0.02	95	0.00	95	1.62	1.72	0.00	95	20
0.2	0.5	1.5	2.5	0.02	91	0.02	91	1.55	1.75	-0.07	92	87
0.2	0.5	1.5	3.5	0.00	95	0.01	91	1.52	1.72	-0.02	94	100

(3)$n = 4000, exp(\beta_0) = 0.1, exp(\beta_1) = 1$

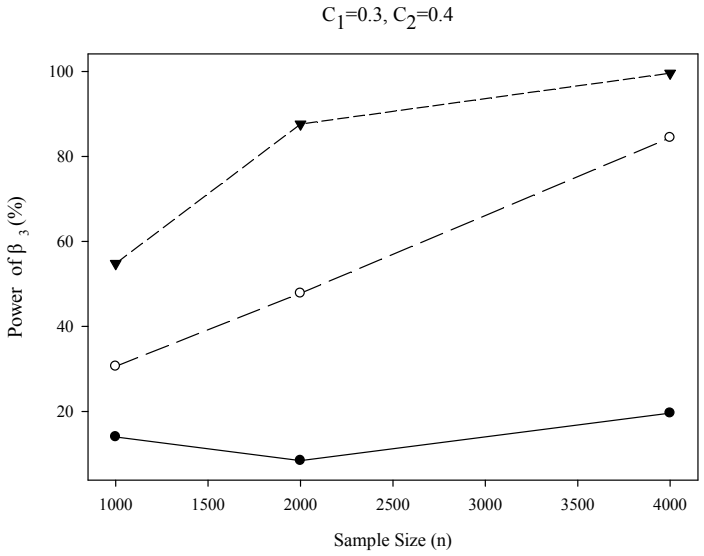
c_1	c_2	e^{β_2}	e^{β_3}	$\hat{c}_1$		$\hat{c}_2$		$\hat{q}_1$	$\hat{q}_2$	Marginal $\hat{\beta}_3$		
				Bias	C.P.	Bias	C.P.			Bias	C.P.	Power
0.1	0.2	1	1.5	0.16	84	0.27	56	1.84	1.75	-0.3	93	7
0.1	0.2	1	2.5	0.02	95	0.06	92	1.69	1.34	-0.1	92	64
0.1	0.2	1.5	1.5	0.03	95	0.07	89	1.68	1.35	-0.1	90	21
0.1	0.2	1.5	2.5	0.00	95	0.00	95	1.69	1.23	0.0	94	88
0.1	0.2	1.5	3.5	0.00	94	0.00	93	1.69	1.22	0.0	96	100
0.2	0.3	1	1.5	0.05	95	0.17	84	1.85	1.72	-0.2	94	8
0.2	0.3	1	2.5	0.00	94	0.05	93	1.94	1.46	-0.1	90	62
0.2	0.3	1.5	1.5	0.00	95	0.05	93	1.92	1.46	-0.1	95	19
0.2	0.3	1.5	2.5	0.01	86	0.05	86	2.02	1.48	-0.1	87	78
0.2	0.3	1.5	3.5	0.00	88	0.02	89	2.07	1.41	-0.1	92	97
0.3	0.4	1	1.5	-0.02	96	0.11	92	1.89	1.80	-0.2	96	7
0.3	0.4	1	2.5	-0.02	94	0.04	95	2.12	1.60	-0.1	92	60
0.3	0.4	1.5	1.5	-0.03	93	0.04	94	2.09	1.61	-0.1	96	20
0.3	0.4	1.5	2.5	0.00	95	0.00	93	2.34	1.52	0.0	94	84
0.3	0.4	1.5	3.5	0.00	91	0.00	91	2.38	1.51	0.0	96	100
0.1	0.3	1	1.5	0.12	88	0.14	85	1.76	1.66	-0.2	93	18
0.1	0.3	1	2.5	0.01	93	0.01	95	1.46	1.37	0.0	96	96
0.1	0.3	1.5	1.5	0.01	96	0.01	95	1.47	1.38	0.0	95	41
0.1	0.3	1.5	2.5	0.00	93	0.00	91	1.41	1.36	0.0	96	98
0.1	0.3	1.5	3.5	0.00	90	0.01	93	1.41	1.38	0.0	93	100
0.2	0.4	1	1.5	0.05	96	0.07	94	1.81	1.71	-0.1	96	12
0.2	0.4	1	2.5	0.00	94	0.01	96	1.70	1.53	0.0	96	92
0.2	0.4	1.5	1.5	0.00	95	0.01	94	1.71	1.53	0.0	94	38
0.2	0.4	1.5	2.5	0.00	94	0.00	93	1.69	1.51	0.0	95	98
0.2	0.4	1.5	3.5	0.00	92	0.00	92	1.69	1.51	0.0	95	100
0.2	0.5	1	1.5	0.05	97	0.00	95	1.76	1.76	0.0	95	23
0.2	0.5	1	2.5	0.00	94	0.00	94	1.54	1.70	0.0	94	97
0.2	0.5	1.5	1.5	0.00	95	0.00	94	1.54	1.70	0.0	94	50
0.2	0.5	1.5	2.5	0.01	91	0.01	90	1.52	1.74	0.0	91	99
0.2	0.5	1.5	3.5	0.00	90	0.00	89	1.51	1.70	0.0	94	100



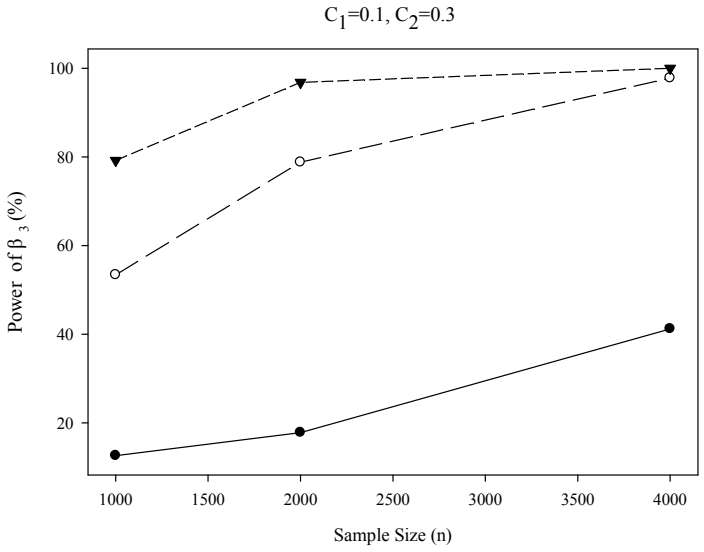
(a) The pre-specified values for risk windows: c_1=0.1, c_2=0.2



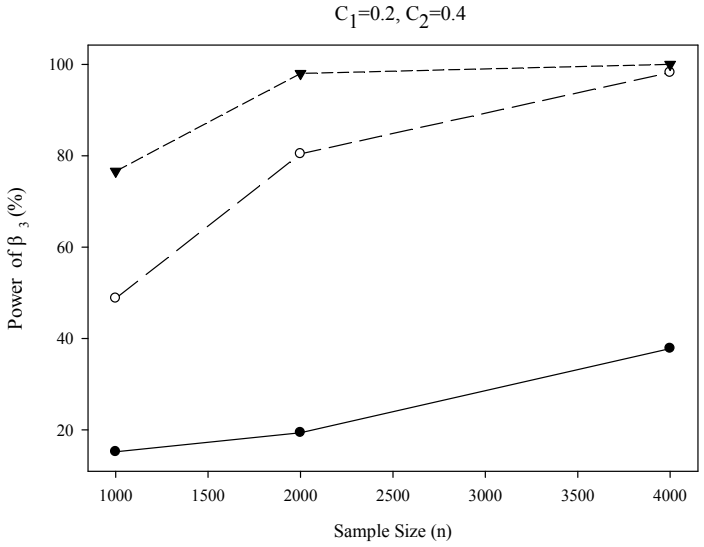
(b) The pre-specified values for risk windows: c_1=0.2, c_2=0.3



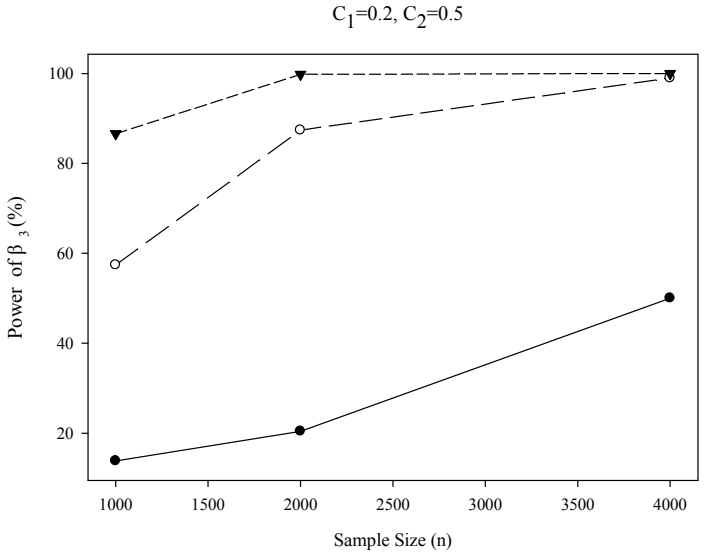
(c) The pre-specified values for risk windows: c_1=0.3, c_2=0.4



(d) The pre-specified values for risk windows: c_1=0.1, c_2=0.3



(e) The pre-specified values for risk windows: c_1=0.2, c_2=0.4



(f) The pre-specified values for risk windows: c_1=0.2, c_2=0.5

Figure 3.5 Comparisons of the powers for marginal β_3 estimation against sample size (n=1000, 2000, and 4000) with respect to each combination of pre-specified β_2 and β_3.

3.3.3 Standard Analysis and Permutation Test

In the previous sections, we developed a Bayesian approach to address a research question related to the timing of conception during which the bivalent HPV vaccine might confer a miscarriage risk. Alternative approaches under the frequentist statistics are sometimes more commonly used. Typically by specifying a risk window, a standard hypothesis testing procedure can be applied using the same logistic regression model [formula (2)]. For example, a risk window that represents conceptions that begins between 2 weeks and 12 weeks following the vaccination date can be pre-defined, followed by use of the maximum likelihood method for the inference on model parameters. Another approach used by previous research is the permutation test. The test statistic is the lowest P value among multiple tests of the same hypothesis in overlapping subsets of pregnancies defined by the time interval between vaccination and conception (Wacholder et al., 2010). In this section, one group of simulated data ($c_1 = 0.2, c_2 = 0.4, \beta_0 = \log(0.1), \beta_1 = \log(0.1)$, β_2= $\log(1.0), \log(1.5)$, and β_3 = $\log(1.5), \log(2.5)$, $\log(1.0), \log(3.5)$) were analyzed with the standard test and the permutation test, in order to compare them with the results from Bayesian inference.

To compare the three approaches, we looked at the study power for the risk estimation (Table 3.2). The Bayesian approach and standard test were testing the null hypothesis that $\beta_3 = 0$. Since the permutation test does not apply to an interaction term in multivariate regression model (Anderson & Robinson, 2001), we hereby applied the permutation test for the odds ratio of the rates of miscarriage in the treatment arm versus the control arm . As the three approaches were concerning the same underlying hypothesis, their powers were still comparable. In the standard tests, when the risk window was correctly specified, the power for β_3's estimation was slightly

higher than the Bayesian approach, but, in reality, the chance of making a correct guess of the risk window is low. Any specification of a wrong risk window substantially reduced the power to detect the effect, as compared with the analysis based on the correct risk window. In the permutation test, a P value of 0.05 was considered statistically significant. Overall, the powers to detect difference in occurrence of adverse events between the two treatment arms by the permutation test were equal or close to the Bayesian approach. The power of permutation test was over 80% only when $\beta_2 = \log(1.5)$ and $\beta_3 \geq \log(2.5)$.

Table 3.2 Comparisons of the power for testing the null hypothesis that the treatment confers no additional risk under Bayesian Inferences, standard hypothesis testing, and permutation test (***n***=2000, results were based on 500 replications). The Numbers are percentages.

Parameters used for data generation $\beta_0 = \log(0.1)$ $\beta_1 = \log(1)$ $c_1 = 0.2, c_2 = 0.4$		Bayesian	Standard Hypothesis testing when the risk window for testing was specified					Permutation test
$\beta_2 =$	$\beta_3 =$		exactly the same as the true risk window (0.2, 0.4)	partially overlapped with the true risk window (0.3, 0.5)	narrower than the true risk window (0.25, 0.35)	wider than the true risk window (0.1, 0.6)	no overlap with the true risk window (0.6, 0.8)	
log(1)	log(1.5)	8.2	18.8	6.8	13.8	10	6.4	12.8
log(1)	log(2.5)	66.6	76	20	40.4	30.6	13.6	66.6
log(1.5)	log(1.5)	19.4	22	8.2	13.4	9.6	6.2	16.6
log(1.5)	log(2.5)	80.4	82.8	22.8	45.4	40.6	14.6	80.4
log(1.5)	log(3.5)	98	99	33.6	78.6	68.6	28.6	99.2

3.4 Discussion

In this chapter, we introduced a Bayesian approach with Gibbs Sampling algorithms for the inference on the risk window of adverse events and the magnitude of the effect. The relationship between the dependent variable and covariates was modeled by a multivariate logistic regression model. The dependent variable (e.g., occurrence of miscarriage) was dichotomous and had

Bernoulli distributions, while there were no other distributional assumptions regarding the covariates. Hierarchical priors were used for model parameters that represented the risk window while uniform priors were used for $\boldsymbol{\beta}$ for simplicity. The joint posterior distribution of all parameters was in a complex form. Given that the conditional posterior distributions of each parameter were complicated and integration was difficult to perform, the posterior marginal distributions for the risk window (c_1 and c_2) were obtained through Metropolis-Hasting algorithms. Then, random sampling was performed from normal and gamma distributions, respectively, to obtain the posterior marginal distributions for the betas ($\boldsymbol{\beta}$) and the two parameters of second-stage prior (q_1 and q_2). No calculus was involved in the process of generating samples of the posterior distributions of each parameter.

Regarding the choices of priors, the priors for the lower and higher cut-off points of the risk window (c_1 and c_2) were following beta distributions that involved two random variables q_1 and q_2. The shape of the Beta priors was not fixed because a second-stage "prior" using q_1 and q_2 was added to the Beta priors. Thus, the shape of the beta priors could be flexible and the use of hierarchical priors was less subjective than ordinary priors (e.g., fixed beta prior or uniform prior) (Chen et al., *under revision*). We chose *Beta* (2, q) because we did not expect c_1 and c_2 to be very large. Since the mean of Beta (a,b) is *a/(a+b)*, for example, if we have prior knowledge that c_2 is around 0.5, we can choose the start value for q_2 as $q_2 = 2$ as the mean of *Beta* (2,2) is 0.5. In other applications, if it is reasonable to assume large values for c_2, one can choose Beta(3, q), Beta(4, q), etc., as the prior for c_2. In terms of the priors for $\boldsymbol{\beta}$, there are other possible choices other than the uniform priors as used in this study. For example, in the case that the investigators do have some knowledge about the prevalence of the disease, or odds ratio, normal priors could be considered for $\boldsymbol{\beta}$.

The proposed logistic model involved a time indicator to incorporate the effect of the risk window into the modeling process. As compared to modeling the time interval as a continuous variable, the time indicator could be more clinically meaningful because, from a public health perspective, being able to identify the risk window can provide more tangible implications for pregnancies in women of childbearing age who have received the bivalent HPV vaccine. The interpretation of the interaction effect between the treatment and the time indicator in the model is of clinical importance because it indicates the risk of adverse events in the treatment arm versus the control arm within the risk window relative to outside the risk window. This model can be applicable to trial data investigating the occurrence of adverse events associated with treatment and a risk window.

When developing the Bayesian inference procedure of the proposed logistic model, we took only three predicting variables (treatment, time indicator, and their interaction) into consideration in the simulation studies. However, this model is flexible if the researchers are interested in additional predicting variables. For example, factors such as age and BMI are usually considered in real data. This model can be easily extended to more covariates by modifying the *GLM* function that corresponds to the proposed logistic model in R programming.

The ninety sets of simulation studies added knowledge on sample size determination and power calculation for future design of clinical trials. According to the simulation studies, if the sample size is equal to or less than 2000, the effect is detectable only when the actual effect size is large, for example, $\beta_2 = \log(1.5)$ and $\beta_3 \geq \log(2.5)$. Otherwise, we may expect to have very a large sample size (>>4000) in order to obtain a satisfactory power when the effect size is small. On the other hand, the approach can be used to determine the sample size at the trial design stage;

or alternatively, to estimate the power to detect a certain size of effect when the sample size is already known.

According to the comparisons between the Bayesian inference, the standard hypothesis testing, and the permutation test in stimulation studies (Table 3.2), the Bayesian approach demonstrated better performance over the traditional approaches. The Bayesian approach controls the Type I error, and have a higher power to detect the risk window as compared with the standard hypothesis testing. Although the permutation test showed similar power to the Bayesian approach, it is not as informative as the Bayesian approach because it provides neither the estimates for the risk window nor the magnitude of the effect other than the p-value. On the other hand, both the estimation of cut-off points of the risk window and the corresponding 95% credible intervals can be easily obtained by the Bayesian approach. The Bayesian approach to estimate the risk window, therefore, can serve as an alternative when the size of the effect is of interest and the traditional hypothesis testing does not have sufficient power to detect the effect.

In the next chapter, we will use the proposed logistic model with the Bayesian inference approach to analyze the Costa Rica Vaccine Trial data.

Chapter 4

Application of the Bayes Model to the Costa Rica Vaccine Trial

4.1 Overview

Worldwide, cervical cancer (ICC) is the second most common type of cancer and the third leading cause of cancer death in women. There were an estimated 529,000 new cases of cervical cancer and 275,000 deaths from it in 2008. At least 85% of global cervical cancer cases and deaths occur in developing countries (Ferlay, 2010). It is well established that human papillomavirus (HPV) is a necessary cause of cervical cancer because of its presence in virtually all cases of cervical cancer (Walboomers et al., 1999). Currently, two prophylactic vaccines (Gardasil^{TM} manufactured by Merck & Co. Inc and Cervirax^{TM} manufactured by GSK Inc.) against a few high-risk types of HPV are licensed for use in many countries around the world. Gardasil is designed for cervical cancer associated with HPV type 16/18 and genital warts associated with HPV type 6/11. Cervarix targets HPV 16 and 18. Vaccination is deemed to be promising in prevention of cervical cancer because it covers the HPV types that are predominantly responsible for the majority of cervical cancers. Also, in comparison with screening programs such as the Pap smear and HPV DNA testing that are highly demanding in techniques, manpower, and finance, vaccination can potentially bring tangible benefits for developing countries due to its low-cost and easy administration. Additionally, screening techniques are not effective in detecting adenocarcinoma, the incidence of which are increasing in contrast to the overall decline of cervical cancer incidence. HPV type 16, 18 and 45 are responsible for about 90% of adenocarcinomas. Cervarix may mitigate the burden of this disease as a result of its protection effect against HPV 16/18 plus its cross-protection potential against

HPV type 45. Given the prominent role of HPV vaccination in the future prevention of cervical cancer worldwide, examination of the safety of the vaccine at the population level will be particularly important in the enhancement of the primary prevention efforts in the long run. In terms of the efficacy of Cervarix, the results from several double-blind, controlled, randomized phase II or III clinical studies on vaccination against oncogenic HPV types are encouraging, as it was shown that this bivalent HPV vaccine demonstrated high efficacy in providing long-term protection against cervical intraepithelial neoplasia 2+ associated with HPV 16/18 (J. Paavonen et al., 2007; J. Paavonen et al., 2009; Romanowski et al., 2009). This vaccine is well tolerated in general (Keam & Harper, 2008). Injection-site reactions, fatigue, headache and myalgia were the most common vaccine-related adverse events, no other serious adverse events were reported differentiating between treatment group and control group (Vaccines and Related Biological Products Advisory Committee, 2009). In an independent evaluation of miscarriage rates based on a pooled analysis of data from two randomized controlled trials (PATRICIA & CVT), the analysis failed to establish an overall relationship between Cervarix and miscarriage but, the miscarriage rates in the bivalent HPV vaccine group were approximately 1.5 times of the rates in control group within a 3-month time frame after the vaccination (Wacholder et al., 2010) . Since the majority of the target population of Cervarix were adolescent females or young women of child-bearing potential, the pregnancy outcomes were important regarding the safety of the vaccine in pregnancy. Theoretically, it is biologically plausible that Cervarix might be associated with increased risk of miscarriage because its novel AS04 adjuvant system may alter maternal immune functions in early pregnancy (Chapter 2).This triggered our interest to further investigate the risk of miscarriage associated with Cervarix through alternative mathematical methods. We hypothesized that there may be a risk window that is associated with increased risk of miscarriage

in pregnancies following the immunization of the bivalent HPV vaccine. In this chapter, we analyzed the data from Costa Rica Vaccine Trial using a logistic regression model with the Bayesian approach that was developed in the previous chapter.

4.2 Methods

4.2.1 The Costa Rica Vaccine Trial

Data on the occurrence of miscarriage among pregnant women were extracted from the Costa Rica Vaccine Trial (CVT) sponsored by the National Cancer Institute (NCI). It is a community-based double-blind randomized controlled phase Ⅲ trial of the efficacy of the prophylactic VLP-based vaccine in the prevention of advanced cervical intraepithelial neoplasia (CIN2, CIN3, adenocarcinoma in situ and invasive cervical cancer) associated with HPV 16 or 18 cervical infection among healthy young adult women 18-25 years of age residing in Guanacaste Province, Costa Rica (National Cancer Institute, 2010). More than 24,000 women between 18 and 25 years of age were invited to undergo preliminary screening for eligibility between 28 Jun 2004 and 21 December 2005. To be eligible, women had to be non-pregnant and had negative pregnancy test right before the administration of each vaccine and, if they reported having been sexually active, had to be using contraception at least one month before vaccination and be willing to use it until 2 months after the last dose. Additional details on eligibility criteria are available elsewhere (Herrero et al., 2008). A total of 7466 women were recruited. Enrolled women were randomized in a 1:1 ratio to receive either human papillomavirus 16/18 L1 virus-like particle/AS 04 vaccine or hepatitis A vaccine (Havrix^{TM}) intramuscularly once at month 0, 1, and 6. After vaccination,

the participants were revisited at least on a yearly base and followed up for at least 4 years. (Herrero et al., 2008; Wacholder et al., 2010)

In this study, we focused on the outcome of pregnancies, specifically, the occurrence of miscarriage. Pregnancies were self-reported to any member of the study team and documented by staff. All pregnant women were followed until resolution of their pregnancies. Wacholder et al. described in his article how the information on pregnancy was collected (Herrero et al., 2008). Briefly, women were instructed to report serious adverse events related to pregnancies at any time during follow-up. Miscarriage events were self-reported and confirmed by clinical judgment of the investigator.

4.2.2 Exclusion Criteria in the Analysis

Pregnancies were excluded from the study if they were marked as ongoing at the end of follow-up, were conceived before the first vaccination, had missing data of the last menstrual period, had missing record of pregnancy outcomes, or were lost to follow-up. Ongoing pregnancies were determined based on their recorded pregnancy outcome status.

4.2.3 Collection of Information

We requested data on pregnant women enrolled in CVT through the Special Studies Institutional Review Board (SSIRB) at National Cancer Institute. We obtained information on the dates of each vaccination, order of pregnancy, date of last menstrual period, gestational age, delivery outcome, and mode of delivery. Ectopic and molar pregnancies were excluded before data transfer. Miscarriage was defined as loss of pregnancy within 20 weeks after the last period. Pregnancies that began before the first dose of vaccination were identified as if the estimated date

of conception was ahead of the date of first dose, where the date of conception was estimated as two weeks after the last menstrual period. Other information with respect to demographic characteristics and socio-economic status of the pregnant women included age, BMI, monthly income, education, marital status, and smoking status at enrollment.

4.2.4 Statistical Analysis

After the removal of ongoing pregnancies and all pregnancies with estimated date of conception before the first vaccination, we calculated the individual time interval as the number of days between the estimated date of conception and the date of the last vaccination before the onset of pregnancy. Then the time interval was converted to a value between 0 and 1 using empirical cumulative distribution function (c.d.f.). That is a process starting by reordering n individual observations from lowest to highest and then computing the empirical c.d.f. value of the r th observation of the ordered sample $x_1 \leq x_2 \leq \cdots \leq x_n$ as the probability that $r-1$ observations are less than r.

Data was analyzed by the logistic Bayesian model (details can be found in Chapter 3) via the computer program R (R development core team, 2010). The estimation were based on 50,000 random samples from the Gibbs Sampling procedure. The first 500 random samples in the Gibbs sequence were discarded from estimation. Point estimators and 95% credible intervals were obtained for all parameters of interest. We used forward selection process to identify potential predicting variables to include in the final model. The starting point was the smallest model consisting of three main variables — treatment, time indicator, and their interaction. These variables were always retained in the model regardless of their significance. Time indicator was a dichotomous variable implying whether the individual time interval fell into the risk window or

not. Bayesian information criterion (BIC) was the measurement used for model selection. BIC was calculated as [-2*loglikehood+k*ln(n)], where k is the number of parameters in the model, including the intercept, and n is the sample size (Blair, 2008). Then one factor (age, BMI, monthly income, education, marital status, and smoking status) at a time was added to the smallest model in a stepwise fashion, at each step adding the covariate with a smaller BIC as compared with the BIC from the baseline model (Table 4.5). Categorical variables, such as monthly income, education, marital status, and smoking status, were converted to dummy variables before analysis. Those pregnancies with unknown data of a certain categorical variable were excluded only when that variable was in use.

To avoid the potential correlations of miscarriages among multiple pregnancies of the same woman, primary analysis was done on first-time pregnancy data only. To explore the possible effects of selection bias, choices of priors, and different models on the results, the following sensitivity analysis was also conducted:

1) all pregnancy data including multiple pregnancies were analyzed with the full final Bayesian model, assuming no correlations of multiple miscarriages that occurred in the same person;

2) the full final model was re-analyzed using uniform priors for the risk window c_1 and c_2;

3) A reduced model with the interaction effect between treatment and time indicator as the only predicting variable in the model $logit\ \pi_i = \beta_0 + \beta_3' x_{1i} x_{2i}$ (x_1: treatment; x_2: time indicator) was used analyzing the 1st time pregnancy data with both the hierarchical priors and uniform priors.

4.3 Results

The reproductive characteristics of all pregnant women are presented in Table 4.1. Of a total 4252 pregnancies, 3433 were first-time pregnancies and 819 were second or higher order pregnancies. Approximately 80% of the women had only one pregnancy. One woman was removed from analysis because she received both HPV vaccine and hepatitis A vaccine. Among first-time pregnancies, we excluded 68 (2.0%) ongoing pregnancies, 37 (1.1%) pregnancies with missing data on the last menstrual period, and 18 (0.5%) pregnancies due to the onset of conception which occurred prior to the first dose of vaccination (Figure 4.1). In Table 4.2, we summarized the outcome status of first-time pregnancies recorded by the end of follow-up. Of the remaining 3309 pregnancies, 228 cases of miscarriages were identified in the HPV vaccine arm and 205 in the hepatitis A vaccine arm. Table 4.3 showed the compliance with vaccination in each arm. All pregnant women had completed at least one dose of vaccination with over 75% of them completing all three doses. Selected demographic, physical and socioeconomic characteristics for the 3309 women were presented in Table 4.4. The median age was 23 years ranging between 15 and 30. About 60% of the women had a normal weight according to the WHO classification using the body mass index (BMI), 23% were overweight and 12% were obese (WHO, 2006a). Nearly half the women were single at the time of enrollment. In terms of education, about 80% had 11 years of education or less. About 15% of the women ever smoked.

Regarding the identification of potential confounders, Table 4.5 lists the results from the forward selection process. Each of these confounders had only 1 to 3 unknown data points except for monthly income. There were 187 pregnancies with unknown income status. Considering that 187 was relatively large as compared to the amount of missing data in any other categorical variable, these 187 data points were retained in the primary analysis, and they were treated as a

separate category under income status. In a sensitivity analysis by excluding the unknown data points of income status, the results did not show any significant change in terms of the estimation of the interaction term in the logistic model. Only age appeared to be significant when it was added to the smallest model. The parameter estimate for the category of "separated/widowed/divorced" under marital status had a 95% credible interval excluding zero, but overall inclusion of marital status into the model did not improve the model fit (no decrease in BIC as compared to the baseline model, as shown in Table 4.5). Therefore, the final model was determined to include the three must-have variables and age.

Looking at the inference on the interaction between treatment and time indicator in the final model, the point estimation $\hat{\beta}_3$ was 1.13 with a 95% credible interval (0.49, 2.75) which was considerably wide. Also, both cut-off points of the risk window had extremely wide 95% credible intervals. These indicated that the interaction effect was not statistically significant, and the point estimates for the risk window were meaningless.

Table 4.6 shows the comparisons of the inference on the risk window and the interaction term β_3 between the final model and four other sensitivity analyses. Overall, conclusions from all sensitivity analyses were consistent with the primary analysis of 1st-pregnancy data that no significant interaction effect was observed. Neither the analysis of multiple pregnancy data nor the use of uniform prior had a change of over 10% in terms of the estimates of the risk window and the interaction effect $[exp(\hat{\beta}_3)]$. Analyses of the reduced model, using either hierarchical priors or uniform priors, indicated the same conclusion. However, the point estimates for the interaction term were negative in the reduced model, as opposed to positive in the full final model. This was because of the absence of the two terms (treatment and time indicator) from the reduced model, so that the interpretation of the interaction term $[\beta_3']$ in the reduced model was actually

different from the interaction term [β_3] in the full model. The interaction term β_3' from the reduced model meant the ratio of the rates of miscarriage in the HPV vaccine arm within the risk window relative to the total rates of miscarriage that occurred in the HPV vaccine arm outside the risk window and that occurred at any time in the control arm.

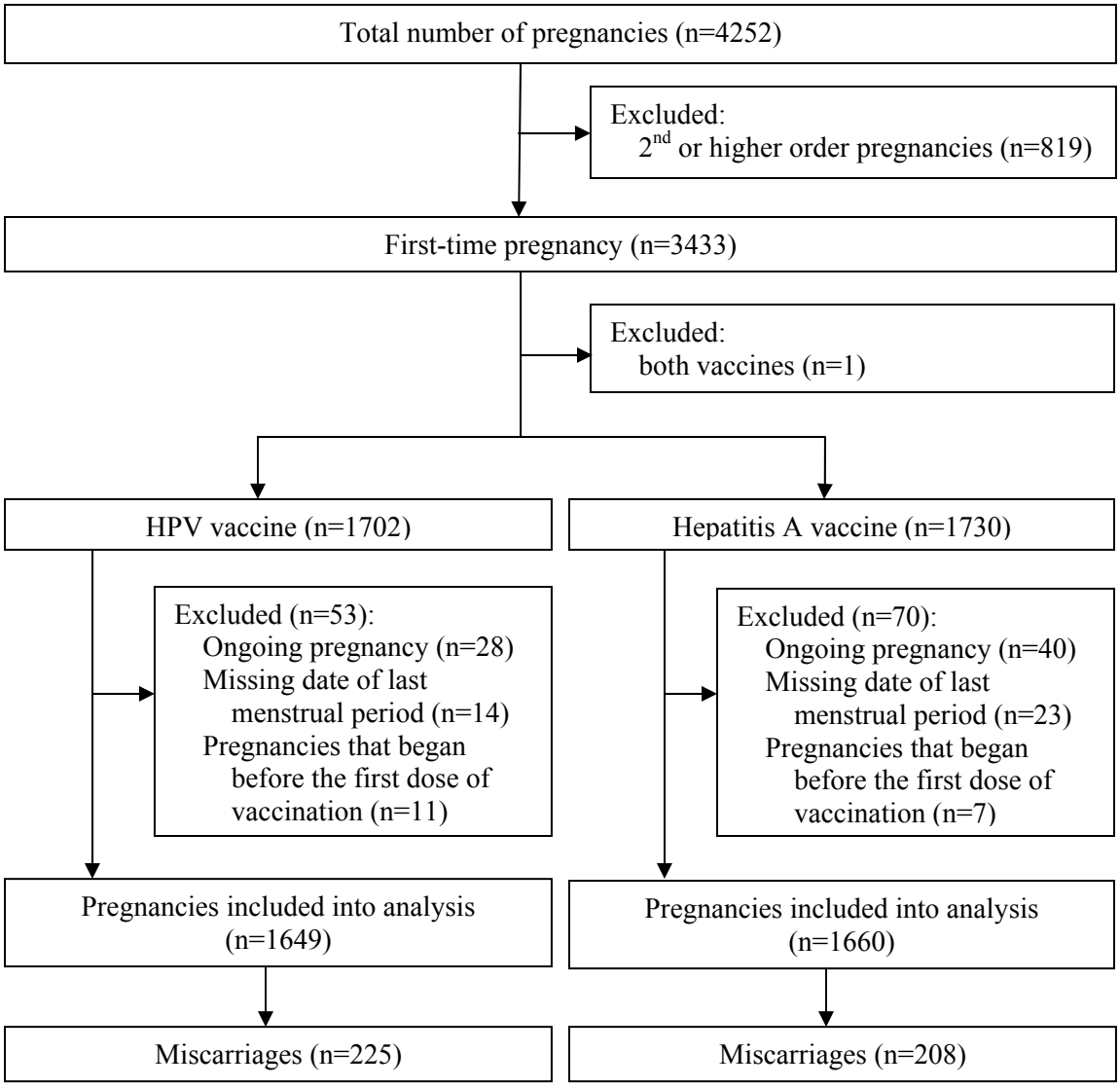


Figure 4.1 Inclusion of pregnancies in the primary analysis (first-time pregnancy data).

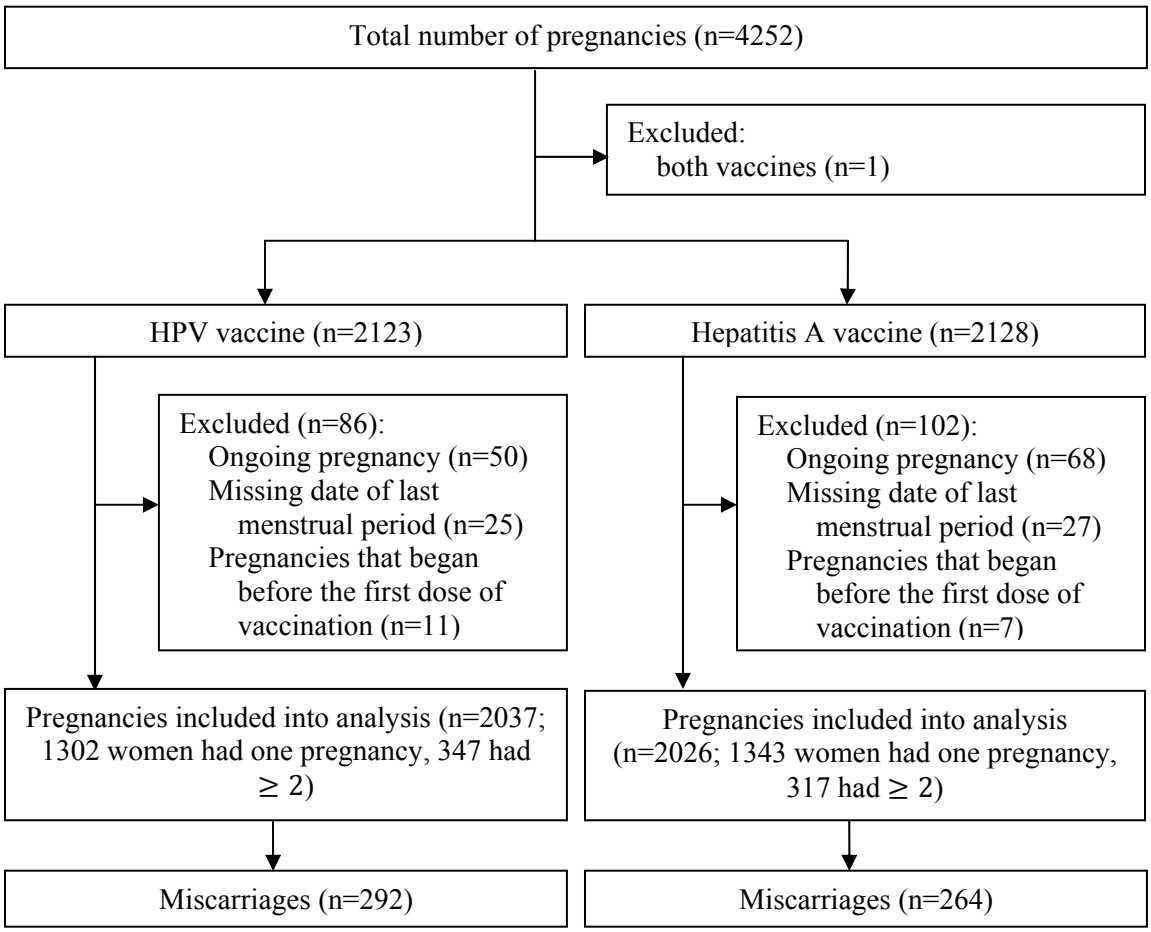


Figure 4.2 Inclusion of pregnancies in the secondary analysis (data on all pregnancies).

Table 4.1 Numbers of pregnancies from 3309 pregnant women in Costa Rica Trial of vaccine against human papillomavirus (HPV) types 16 and 18 (with hepatitis A vaccine as control). Pregnancies that began before the first vaccination, ongoing pregnancies and pregnancies with missing date of last menstrual period were excluded.

	HPV vaccine		Hepatitis A vaccine	
	n	(%)	n	(%)
Total number of pregnant women	1649	100	1660	100
Order of pregnancies				
1	1302	79.0	1343	80.9
2	299	18.1	273	16.4
3	36	2.2	40	2.4
4	1	0.06	3	0.2
5	1	0.06	1	0.06

Table 4.2 Pregnancy Outcomes from 3377 pregnant women in Costa Rica Trial of vaccine against human papillomavirus (HPV) types 16 and 18 (with hepatitis A vaccine as control). Pregnancies that began before the first vaccination and pregnancies with missing date of last menstrual period were excluded. Figures are numbers (percentages).`

	HPV vaccine	Hepatitis A vaccine	Total
Total pregnancies	1677	1700	3377
Miscarriages	225 (13.4)	208 (12.2)	433 (12.8)
Gestational age at miscarriage (weeks):			
0-6	35	37	72
7-12	132	138	270
13-20	58	33	91
Stillbirths*	13 (0.8)	18 (1.0)	31 (0.9)
Live births	1411 (84.1)	1434 (84.4)	2845 (84.2)
Gestational age at live birth (weeks):			
≤33	24	38	62
34-36	76	77	153
≥37	1311	1319	2630
Ongoing pregnancies	28 (1.7)	40 (2.3)	68 (2.0)
Gestational age at last contact in ongoing pregnancies (weeks):			
≤20	3	8	11
>20	25	32	57
*Stillbirths defined as lost of pregnancy after 20 weeks of pregnancy			

Table 4.3 Compliance with vaccination in both arms among the pregnancies included in primary analysis. Pregnancies that began before the first vaccination, ongoing pregnancies and pregnancies with missing date of last menstrual period were excluded. Figures are numbers (percentages).

	CERVRIX	HAVRIX	Total
at least one dose	1649 (100)	1660 (100)	3309 (100)
at least two doses	1550 (94.0)	1551 (93.4)	3101 (93.7)
all three doses	1264 (76.7)	1284 (77.3)	2548 (77.0)

Table 4.4 Demographic, marital, socioeconomic, physical characteristics and smoking behaviors of pregnant women included in the analysis by study arm.

	HPV vaccine		Hepatitis A vaccine	
	n	(%)	n	(%)
Total number of pregnancies	1649	100	1660	100
Maternal age at conception				
⩽20	333	20.2	341	20.5
21-23	611	37.1	636	38.3
⩾24	705	42.8	683	41.1
median [range]	23 [15-30]		23 [18-30]	
Body mass index				
＜18.5	102	6.2	119	7.2
18.5-25	960	58.2	973	58.6
25-30	385	23.3	380	22.9
⩾30	202	12.2	188	11.3
median[range]	23.3 [15.6-50.7]		23.03 [15.2-50.2]	
Marital status				
Married/common-law	786	47.7	758	45.7
Separated/divorced/widowed	48	2.9	58	3.5
Single	814	49.4	842	50.7
Unknown	1	0.06	2	0.1
Years of education (years)				
none	7	0.4	7	0.4
＜7	527	32.0	507	30.7
7-11	835	50.6	903	54.4
12-15 (vocational)	39	2.4	31	1.9
⩾16 (University and above)	243	14.7	211	12.7
Unknown	0	0	1	0.06
Monthly Income (in Canadian dollars)				
less than 200	595	36.1	572	34.5
200-400	596	36.1	613	36.9
more than 400	372	22.6	374	22.5
Unknown	86	5.2	101	6.1
Ever smoked at least one cigarette a week (at enrollment)				
Yes	253	15.3	246	14.8
No	1395	84.6	1412	85.1
Unknown	1	0.06	2	0.1

Table 4.5 Analysis of BIC in Forward Selection in primary analysis. Numbers are BIC (degrees of freedom). BIC= -2*loglikehood+k*ln(n).

Variables in the baseline model	BIC of the baseline model	BIC of the model that includes one of the following potential predicting variable					
		Age	BMI	Education	Marital Status	Income	Smoking Status
Treatment, time indicator, the interaction	2593.08(2)	2591.70 (3)	2600.37 (3)	2616.54 (6)	2602.67 (4)	2614.16(4)*	2599.14 (3)
Treatment, time indicator, the interaction, and age	2591.70 (3)		2601.95 (4)	2616.86 (7)	2602.49 (5)	2615.43 (5)	2600.71 (4)

*This number was reported as the 187 subjects without income information were included; If excluded, the corresponding BIC was 2490.782. The decline in BIC was not due to better fitness but explained by the large number of exclusion.

Table 4.6 Comparisons of the estimation of the risk window and the beta3 estimation and its credible interval in sensitivity analysis. Model (1) is the full final model $\boldsymbol{logit\,\pi_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \beta_4 age}$. Model (2) is the reduced model $\boldsymbol{logit\,\pi_i = \beta_0 + \beta_3' x_{1i} x_{2i}}$. The selection for priors was detailed in Chapter3.

Data analyzed	Model	Prior selection	the risk window		interaction effect	
			lower cut-point	higher cut-point	$\hat{\beta}_3$	$exp(\hat{\beta}_3)$
1st pregnancy	(1)	hierarchical	0.29 (0.10, 0.54)	0.49 (0.29, 0.81)	0.12 (-0.71, 1.01)	1.13 (0.49, 2.75)
multiple pregnancies	(1)	hierarchical	0.27 (0.07, 0.66)	0.47 (0.22, 0.86)	0.02 (-0.75, 0.86)	1.02 (0.47, 2.36)
1st pregnancy	(1)	uniform	0.29 (0.07, 0.62)	0.53 (0.25, 0.94)	0.09 (-0.80, 0.99)	1.09 (0.45, 2.69)
1st pregnancy	(2)	hierarchical	0.27 (0.07, 0.55)	0.50 (0.23, 0.82)	-0.12 (-0.77, 0.48)	0.89 (0.46, 1.62)
1st pregnancy	(2)	uniform	0.30 (0.05, 0.67)	0.57 (0.24, 0.93)	-0.07 (-0.75, 0.49)	0.93 (0.47, 1.64)

4.4 Discussion

In order to determine if the characteristics of women included in this study were similar to the population in the Costa Rica Vaccine Trial, we compared data on maternal age at conception, BMI, education, marital status, and smoking status at enrollment. The women in our study tended to be older (fewer women younger than 20 and more women older than 24), reflecting the fact that women became pregnant at an older age. The other characteristics in terms of BMI, education, and marital status were similar to the CVT population, indicating that, in general, it was a good representative of the original sample. However, it was notable that the CVT population was slightly younger, more educated and more likely to be single than women from the Costa Rican National Census of 2000 (Herrero, et al. 2008). Also, due to the large number of exclusions and relatively low participation rates in the CVT recruitment, the generalizability of the results from this study may be affected.

To our knowledge, this was the second study that exclusively explored the relationship between the HPV vaccination and the occurrence of miscarriage. Underlying biological hypotheses support the reasoning of this study. The adjuvant system (AS04) used in Cervarix was a new generation of vaccine adjuvants licensed for use in humans (GarçOn, Chomez, & Van Mechelen, 2007). MPL (3-O-desacyl-4' monophosphoryl lipid A) is the novel component in AS04, as compared with traditional adjuvants consisting only of aluminum. The capability of Cervarix for stimulating stronger and persistent effects was primarily attributed to MPL Didierlaurent et al., 2009). MPL could skew the immune response towards pro-inflammatory (Th1) cytokines while aluminum salt promotes the anti-inflammatory (Th2) response, which perhaps resulted in an imbalance of Th1/Th2 cells. The imbalance of Th1/Th2 cells may affect

the outcome of pregnancies. Though quantitative data on the Th1/Th2 cytokine performance in pregnant women with the Cervarix vaccine is not available, it is biologically plausible that Cervarix might be associated with an increased risk of miscarriage because the AS04 system may alter maternal immune functions in early pregnancy.

In the primary analysis of first-pregnancy data, we found no evidence of a risk window with significant increase of miscarriage rates in women who received Cervarix against the HPV before pregnancy compared with women from the hepatitis A vaccine group. This could be explained as either 1) A risk window with elevated risk of miscarriage does not exist, or 2) the power of this study is still insufficient to detect the risk window, perhaps due to the small sample size or small number of observed events. This finding was consistent with the results reported in Wacholder's study. However, there was a slight difference between these two studies. Wacholder's study focused on subjects with pregnancies around the time of vaccination while this study included pregnancies after vaccination only.

Previous exploratory analysis of clinical trial data indicated that no safety concerns have arisen following administration of the bivalent HPV vaccine because the numbers of observed adverse events were similar between treatment arms. So far no adverse events were causally associated with the vaccine. The Vaccine Adverse Event Reporting System (VAWRS) is continuing to monitor post-market adverse events. And, concerns over the safety of the HPV vaccines persist. Controversies arose partly due to the over 30 cases of Guillain-Barre syndrome (GBS) reported after the administration of Gardasil, the other quadratic HPV vaccine. GBS is a disorder in which the body's immune system affects part of the nervous system causing weakness and tingling of the extremities, and occasionally it can be lethal. But the causal relationship between the occurrence of GBS and Gardasil has not yet been fully established. Neither should

we overlook the fact that adverse reactions to Gardasil have been lower than average (Krishnan, 2008). Even though there is no solid evidence linking GBS to Gardasil, the potential side effects of HPV vaccine on the immune system may be possible. Cervarix produces a higher titer of immune responses due to its use of AS04 adjuvant system. This leaves us with the question of whether the stronger effects of AS04 would also cause side effects on the immune system. This question remains to be investigated further from both the biological and epidemiological perspectives. In the future, population-based epidemiological studies of Cervarix primarily targeting pregnant women could be considered.

Chapter 5

Discussion

5.1 Summary of The Study

This study was conducted to identify the time-dependent risk for miscarriage following immunization with the bivalent Human Papillomavirus (HPV) vaccine (Cervarix) among women at the childbearing age using a Bayesian approach. To address our research question, we proposed a logistic model that involved time-indicator representing the relationship between individual time interval and the risk window. We then developed the procedures for statistical inferences of the parameters in the logistic model using The Bayes' theorem and Gibbs Sampling algorithms. The performance of the proposed model was assessed with simulation studies. Then the research question was investigated by applying the model to the data from the Costa Rica Vaccine Trial (CVT). The study population included women who received the bivalent HPV vaccine in CVT and became pregnant after their vaccinations. Potential predicting variables, such as age, BMI, income, education, marital status, and smoking status were considered in the model selection.

The simulation studies provided information on sample size determination and power calculation when using the Bayesian approach for risk window estimation. Based on the comparison between the Bayesian inference, standard hypothesis testing, and the permutation test in simulation studies, the Bayesian approach performed better than traditional approaches in terms of the power to detect the risk window and the risk estimates.

In the analysis of the Costa Rica Vaccine Trial, we found that the point estimate of the ratio of the odds ratio of the event occurring in the treatment arm versus in the control arm in the risk window relative to the odds ratio outside the risk window [$\exp(\beta_3)$] was 1.13 with a 95%

credible interval (0.49, 2.75). Age appeared to be an important predicting factor, while the other factors, including BMI, education, marital status, income, and smoking, did not contribute to the improvement of model fitness. Since the point estimate for $\exp(\beta_3)$ was small and its credible interval was remarkably wide, we concluded that there was no evidence showing an existence of a risk window associated with an increase in miscarriage rate in women who became pregnant after the immunization of the bivalent HPV vaccine.

The Bayesian model is broadly applicable to other studies that attempt to model the time-dependent effect (e.g., effects that only exist in a certain time window) associated with binary outcomes. In literature where a risk has already been established, this approach can be considered to further identify the risk window. This work increased our understanding of the association between miscarriage rates and vaccination of Cervarix. From the public health point of view, this study shed light on providing the public, doctors and health system managers with complete and accurate information about the safety of Cervarix.

5.2 Strengths and limitations

5.2.1 Strengths

A major strength of this study was the way in which the risk window was evaluated and the use of Bayesian approach. This was the first study to use an advanced Bayesian approach to model the time-dependent effect of the bivalent HPV vaccine on the risk of miscarriage in pregnant women.

1) The design of a time-indicator variable in the logistic model, together with the use of Bayesian inference, allowed us to make direct estimations of the risk window, which is a challenge in traditional methods.

2)The use of hierarchical regression model attempted to improve standard regression model estimates by adding a second-stage "prior" to an ordinary model. The first-stage "priors" for the risk window had quite flexible forms by using a second-stage "prior". The use of hierarchical priors was more objective and superior to subjective choice, especially when there was little knowledge about the prior distributions.

3) The use of the Bayes' theorem and Gibbs Sampling algorithms for parameter estimation had several advantages over traditional approaches. Overall, the Bayesian approach theoretically required fewer assumptions about the data, and the credible interval in parameter estimation provided direct interpretation of estimates. Specifically in this study, the Bayesian approach in general showed higher power than the standard hypothesis testing when comparing the power in detecting the risk window. The only exception was when we had correctly guessed the risk window in hypothesis testing, but having a correct guess is unlikely when there is usually little knowledge about risk window. The permutation test showed comparable powers as the Bayesian approach in detecting the risk window. However, the Bayesian approach could provide a credible interval along with the point estimate for parameters of interest, while the permutation test provides only a p-value. The credible interval is more informative than the p-value because it indicated not only the statistical significance but also the possible range of the variable, which is more clinically relevant.

5.2.2 Limitations

The generalizability of the results from the analysis of clinical trial data may be affected due to the large number of exclusions and relatively low participation rates in the Costa Rica Vaccine Trial.

Though the Bayesian approach improves the study power to detect the risk window, the power of the study is inherently limited by the sample size and number of observed events in the trial data.

One other limitation of this work is its high computational demand. The computational demand was predominantly due to simulation studies in this project, in which the Gibbs Sampling procedure was repeated 500 times for each simulation. Even with the support of the super-computing facility SHARCNET, about three months were spent on simulations studies. One set of simulations took up to six days when the job was run on SHARCNET. One analysis of the clinical trial data took up to three hours, but it will be more time-consuming that a program may take days or months to run on one's personal computer. Therefore, when this approach is used in the future, the time and technical issues should be taken into consideration on top of the methodology.

5.3 Future Work

Given the insufficient biological knowledge with regard to the possible negative impacts of the bivalent HPV vaccine, particularly its use of the novel adjuvant system AS04, future studies to explore underlying biological theories may be worthwhile. The model in this study was designed for one risk window only, but multiple risk windows may exist. If more biological and immunological knowledge is available in the future to support the multiple risk window

hypotheses, more advanced mathematical models can be considered. Since a significant signal of the miscarriage risk was not detected by the clinical trial data, well-designed population-based outcome studies through post-market surveillance may increase the ability to identify the risk of rare adverse outcomes of pregnancy (e.g. miscarriage) associated with this bivalent HPV vaccine (Booth & Mackillop, 2008).

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Appendix A

R Coding for Bayesian analysis in Simulation Studies

(A) Library codes

```
#generate one dataset
datgen<-function(n,b,c1,c2){
  n2<-n/2
  x0<-rep(1,n) #intercept
  x1<-c(rep(0,n2),rep(1,n2))#create values for treatment

  t<-runif(n,0,1)#create values for time interval
  x2<-ifelse (c1<=t&t<=c2,1,0)#create values for time indicator

  x3=x1*x2 #create values for interaction
  X<-cbind(x0,x1,x2,x3)
  xbeta<-X%*%b

  p<-exp(xbeta)/(1+exp(xbeta))
    #calculate the probablity for the occurence of outcome event
  # y<-matrix(rbinom(n,size=1,prob=p),nrow=n)
  y = rbinom(n,size=1,prob=p)
    #create values for outcome

  dat<-data.frame(cbind(y,X,t))
  names(dat)=list("y","x0","x1","x2","x3","t")
  return(list(dat = dat, X = X))
}

#generate regression coefficients
logitfit <-function(c1,c2,dat){
 y = dat[, 1]
 x1 = dat[, 3]
 t<-dat[,6]
 x2 <-ifelse(c1<=t&t<=c2,1,0)
 x3 = x1 * x2

 fit<-glm(y~x1+x2+x3, family=binomial("logit"))
 sd = vcov(fit)
 # new: add loglike function as output
 out = list(bh = fit$coef, sd = sd, conv = fit$converged, lglk = logLik(fit))
 return(out)
}
```

```
#calculate the joint probability (log likelihood) of y
lj<-function(c1,c2,bh,X,t,y){
 X[,3]<-ifelse (c1<=t&t<=c2,1,0)
 X[,4] = X[,2]*X[,3]
 xbeta = X%*%bh
 exb = exp(xbeta)
 li = y*xbeta - log(1+exb)
 lj = sum(li)
 return(lj)
}

#calculate the (log) conditional probability of c1 & c2
pmh<-function(n,y,X,t,qj,cj,bh){
 c1<-cj[1]
 c2<-cj[2]
 q1<-qj[1]
 q2<-qj[2]
 p1<-lj(c1,c2,bh,X,t,y)
 p2<-log(c1)+(q1-1)*log(c2-c1)-q1*log(c2)+(q2-1)*log(1-c2)
 p<-p1 + p2
 #cat('p1=',p1,'p2=',p2,'\n')

 return(p)
}

#test whether the risk window has at least 5 subjects in each arm
testn = function(c1, c2, X, t) {
 threshold = 5
 X[,3]<-ifelse (c1<=t&t<=c2,1,0)
 trtn = sum(X[,2]*X[,3])
 ctln<-sum((1-X[,2])*X[,3])
 if( trtn >= threshold & ctln >= threshold & (c2-c1) >= 0.05) rpt = F else rpt = T
 return(rpt)
}

#one iteration of gibbs sampling
gibbs<-function(qj,cj, bh, dat, X){
 q1<-qj[1]
 q2<-qj[2]
 c1<-cj[1]
 c2<-cj[2]
 t = dat[, 6]
 y = dat[, 1]

 #generate c1 and c2
 rpt = T
```

```
 pcj = pmh(n,y,X,t,qj,cj,bh)

 while (rpt) {
   D  = min(0.05, (c2-c1)/3)
   D1 = min(0.05 ,c1/2)
   D2 = min(0.05, (1-c2)/2)

   u1 = runif(1, c1-D1, c1+D)
   u2 = runif(1, c2-D, c2+D2)
   uj<-c(u1,u2)
   rpt = testn(u1, u2, X, t)
 }
 alpha<-exp(pmh(n,y,X,t,qj,uj,bh)- pcj)
 #cat('alpha = ', alpha, '\n')
 U<-runif(1,0,1)
 if(U < alpha) {
    c1 = u1
    c2 = u2
 }

 #generate betas
 fit = logitfit(c1, c2, dat)
 if(fit$conv == T){
   bhat<-fit$bh
   sd<-fit$sd
   A = chol(sd)
   bh = bhat + t(A)%*%rnorm(4, 0, 1)

   #generate q1,q2
   sc1<-log(c2/(c2-c1))
   sc2<-log(1/(1-c2))
   q1<-1+rgamma(1,shape=1,scale=sc1)
   q2<-1+rgamma(1,shape=1,scale=sc2)
 } else {
   c1 = cj[1]
   c2 = cj[2]
 }
# cat('bhat=', bhat,'\n')

 qj<-c(q1,q2)
 cj<-c(c1,c2)
 #cat('c1 = ', c1, 'c2 = ', c2, 'beta = ', bh, '\n')
 return(list(q=qj,c=cj,b=bh))
}

#Replication and Estimation
```

```
getmle<-function(dat, X, n,cj, qj, bh, r0,r1){
 #replication from 1 to r0(burn-in)
 for (i in 1:r0){
  g<-gibbs(qj,cj, bh, dat, X)
  cj<-g$c
  qj<-g$q
  bh = g$b
 }

 #replications from r0 to r1(total length of Markov Chain is r0+r1)
 qg = matrix(NaN, r1, 2)
 cg = qg
 bg = matrix(NaN, r1, 4)
 for (i in 1:r1){
  g<-gibbs(qj,cj, bh, dat, X)
  qj = g$q
  cj = g$c
  bh = g$b
  qg[i, ]=g$q
  cg[i, ]=g$c
  bg[i, ]=g$b
 }

 #Estimate the mean and credible intervals
 c.m<-apply(cg,2,mean)
 q.m<-apply(qg,2,mean)
 bh.m<-apply(bg,2,mean)

 ptl<-c(0.025,0.975)
 c.q<-apply(cg,2,quantile,ptl)
 q.q<-apply(qg,2,quantile,ptl)
 bh.q<-apply(bg,2,quantile,ptl)
 est<-c(c.m,c.q,q.m,q.q,bh.m,bh.q)
 h<-list(est=est,c=cg,b=bg,q=qg)
 #cat('c = ', c.m, '\n')
 #cat('beta = ', bh.m, '\n')

 return(h)
}
```

(B) Main codes for Simulation

```
source("lib.R")
# simulation parameters
set.seed(1)
```

```
n = 4000
b0e<-0.1
b1e<-1
b2e<-1.5
b3e<-3.5
b0 = c(log(b0e),log(b1e),log(b2e),log(b3e))
c10 = 0.2
c20 = 0.5
r0 = 200
r1 = 200

#run r2 replications of gibbs sampling
r2<- 40
nc = 24
out<-matrix(NaN, nrow = r2, ncol=nc)
j = 1
while (j  <= r2){
 #generate initial values for parameters
  qj = c(2, 2)
  d = datgen(n, b0, c10, c20)
  dat = d$dat
  #print(mean(dat[, 1]))
  X   = d$X
  # new code to find a good initial value for c1 and c2
  plgk = -n*3
  for (w2 in c(0.2, 0.4, 0.6, 0.8)) {
    for (w1 in c(0.1, 0.3, 0.5, 0.7)) {
      if (w1 < w2) {
        fit = logitfit(w1,w2,dat)
        if (fit$lglk > plgk) {
          plgk = fit$lglk
          c1 = w1
          c2 = w2
          bh = fit$bh
        }
      }
    }
  }
  cj = c(c1, c2)
  #bh = logitfit(c1,c2,dat)$bh
  h = try(getmle(dat, X, n, cj, qj, bh, r0, r1))
  if(class(h) == "try-error") next
  #h<-getmle(dat, X, n, cj, qj, bh, r0, r1)
  est<-h$est
  #print(est)
  out[j,]=est
```

```
 filename=paste("out",c10*100,c20*100,b0e*10,b1e*10,b2e*10,b3e*10,sep="")
 write(t(out[1:j,]),file=filename,ncol=nc,sep="\n")
 j = j + 1
}
```

(C) Codes for Summarizing the Results of Gibbs Sampling

```
b0e<-0.1
b1e<-1
c10 = 0.2
c20 = 0.4
nc = 24

for (b2e in c(1.5)){
 for (b3e in c(3.5)){
  b0 = c(log(b0e),log(b1e),log(b2e),log(b3e))

  #read-in output data
  filename<-paste("out",c10*100,c20*100,b0e*10,b1e*10,b2e*10,b3e*10,sep="")
  out<-try(matrix(scan(filename,quiet=T),ncol=nc,byrow=T),silent=T)
  if (class(out)=="type-error")next

  #calculate mean estimation for c and beta from replications
 B=length(out[,1])
  #cat("length=",B,"\n")
  xm<-apply(out[1:B,c(1,2,7,8,13,14,15,16)],2,mean)

  #calculate coverage probability for c and beta
  c1l<-out[,3]
  c1h<-out[,4]
  c2l<-out[,5]
  c2h<-out[,6]
  c1cp<-mean(ifelse(c1l<=c10&c10<=c1h,1,0))*100
  c2cp<-mean(ifelse(c2l<=c20&c20<=c2h,1,0))*100

  bh3l<-out[,23]
  bh3h<-out[,24]
  bh30<-b0[4]
  bh3cp<-mean(ifelse(bh3l<=bh30&bh30<=bh3h,1,0))*100

  bh0l<-out[,17]
  bh0h<-out[,18]
  bh00<-b0[1]
  bh0cp<-mean(ifelse(bh0l<=bh00&bh00<=bh0h,1,0))*100
```

```
 bh1l<-out[,19]
  bh1h<-out[,20]
  bh10<-b0[2]
  bh1cp<-mean(ifelse(bh1l<=bh10&bh10<=bh1h,1,0))*100

  bh2l<-out[,21]
  bh2h<-out[,22]
  bh20<-b0[3]
  bh2cp<-mean(ifelse(bh2l<=bh20&bh20<=bh2h,1,0))*100

  #calculate rej for beta3
  bh3rej<-mean(ifelse(bh3l>0|bh3h<0,1,0))*100

  #print-out results
  cat(B,c10,c20,b0e,b1e,b2e,b3e,"\t")
  #cat("c1=",xm[1],"bias=",xm[1]-c10,'cp=',c1cp,"\n")
  #cat("c2=",xm[2],"bias=",xm[2]-c20,'cp=',c2cp,"\n")
  #cat("q1=",xm[3],"q2=",xm[4],"\n")
  #cat("beta3=",xm[8],"bias=",xm[8]-bh30,'cp=',bh3cp,"rej=",bh3rej,"\n")

  cat(xm[1],xm[1]-c10,c1cp,"\t")#c1h
  cat(xm[2],xm[2]-c20,c2cp,"\t")#c2h
  cat(xm[3],xm[4],"\t")#q1h &q2h
 cat(xm[8],xm[8]-bh30,bh3cp,bh3rej,"\t")#beta3h
  cat(xm[5],xm[5]-bh00,bh0cp,"\t")#beta0h
  cat(xm[6],xm[6]-bh10,bh1cp,"\t")#beta1h
  cat(xm[7],xm[7]-bh20,bh2cp,"\n")#beta2h
 }
}
```

Appendix B

R Coding for Standard Test in Simulation Studies

```
#092812
#traditional analysis in simulation studies

#generate one dataset
datgen<-function(n,b,c1,c2){
  n2<-n/2
  x0<-rep(1,n) #intercept
  x1<-c(rep(0,n2),rep(1,n2))#create values for treatment

  t<-runif(n,0,1)#create values for time interval
  x2<-ifelse (c1<=t&t<=c2,1,0)#create values for time indicator

  x3=x1*x2 #create values for interaction
  X<-cbind(x0,x1,x2,x3)
  xbeta<-X%*%b

  p<-exp(xbeta)/(1+exp(xbeta))
    #calculate the probablity for the occurence of outcome event
  # y<-matrix(rbinom(n,size=1,prob=p),nrow=n)
  y = rbinom(n,size=1,prob=p)
    #create values for outcome

  dat<-data.frame(cbind(y,X,t))
  names(dat)=list("y","x0","x1","x2","x3","t")
  return(list(dat = dat, X = X))
}

# simulation parameters set-up
n = 2000
b0e<-0.1
b1e<-1
b2e<-1.0
b3e<-1.5
b0<-c(log(b0e),log(b1e),log(b2e),log(b3e))
c10<-0.2
c20<-0.4

c1<-0.6
c2<-0.7

r<-500
```

```
nc = 12
out<-matrix(NaN, nrow = r, ncol=nc)
j = 1
while (j  <= r){
  d = datgen(n, b0, c10, c20)
  dat = d$dat
  y = dat[, 1]
  x1 = dat[, 3]
  t<-dat[,6]
  x2 <-ifelse(c1<=t&t<=c2,1,0)
  x3 = x1 * x2
  bh<-matrix(NaN,nrow=1,ncol=4)

  fit<-glm(y~x1+x2+x3, family=binomial("logit"))
  bh<-fit$coef
  conf<-confint(fit)
  #cat('bh=',bh,'\n','confint=',conf,'\n','\n')
  est<-c(bh,conf)
  #print(est)

  out[j,]=est
  j=j+1
}

#print(out)

bh.m<-apply(out[1:r,c(1,2,3,4)],2,mean)

#caculate cp for beta3
bh3l<-out[,11]
bh3h<-out[,12]
bh30<-b0[4]
bh3cp<-mean(ifelse(bh3l<=bh30&bh30<=bh3h,1,0))*100

#calculate rej for beta3
bh3rej<-mean(ifelse(bh3l>0|bh3h<0,1,0))*100

#print bias, cp, and power for beta3
#cat(bh.m[4]-bh30,bh3cp,bh3rej,'\n')
summary<-c(bh.m[4]-bh30,bh3cp,bh3rej)

filename=paste("out")
write(summary, file=filename, ncol=3)
```

Appendix C

R Coding for Permutation Test in Simulation Studies

```
#generate one dataset
datgen<-function(n,b,c1,c2){
  n2<-n/2
  x0<-rep(1,n) #intercept
  x1<-c(rep(0,n2),rep(1,n2))#create values for treatment

  t<-runif(n,0,1)#create values for time interval
  x2<-ifelse (c1<=t&t<=c2,1,0)#create values for time indicator

  x3=x1*x2 #create values for interaction
  X<-cbind(x0,x1,x2,x3)
  xbeta<-X%*%b

  p<-exp(xbeta)/(1+exp(xbeta))
  # calculate the probablity for the occurence of outcome event
  # y<-matrix(rbinom(n,size=1,prob=p),nrow=n)
  y = rbinom(n,size=1,prob=p)
  #create values for outcome

  dat<-data.frame(cbind(y,X,t))
  names(dat)=list("y","x0","x1","x2","x3","t")
  return(dat)
}

#find out risk windows with >=5 patients
testn = function(c1, c2, X, t) {
 threshold = 5
 n = length(t)
 X[,3]<-ifelse (c1<=t&t<=c2,1,0)
 trtn = sum(X[,2]*X[,3])
 ctln<-sum((1-X[,2])*X[,3])
 if( trtn >= threshold & ctln >= threshold )  rpt = F else rpt = T
 return(rpt)
}

X2test = function(c1,c2,dat){
  t<-dat[,6]
  x2<-ifelse(c1<=t&t<=c2,1,0)

  dat = dat[x2>0, ] #inside the risk window
  y = dat[, 1]
```

```
  x1 = dat[, 3]

  n1 = sum(y)+1
  n = length(y)+2
  n0 = n-n1
  m1 = sum(x1)+1
  m0 = n - m1
  d = sum(y*x1)+0.5
  c = n1 - d
  b = m1 - d
  a = n0 - b
  lor = log(a*d/(b*c))
  lsd = sqrt(1/a+1/b+1/c+1/d)
  z = lor/lsd
  p = 2*pnorm(-abs(z), 0, 1)

  return(p)
}


#find out p-value
findp<-function(density, nc, dat){
  r<-(1/density+1)*(1/density)/2
  #out<-matrix(NaN, nrow = r, ncol=nc)
  out = rep(NaN, r)
  X   = dat[, 2:5]
  t   = dat[, 6]

  j=1
  hi<-(1-density)
  c1_seq<-c(seq(0,hi, by=density))
  for (c1 in c1_seq){
    c2_st<-c1+density
    c2_seq<-c(seq(c2_st,1.0,by=density))
    for (c2 in c2_seq){
      if (testn(c1, c2, X, t)) next;
      est<-X2test(c1,c2,dat)
      out[j]=est
      j=j+1
    }
  }
  pmin = min(out, na.rm = T)
  return(pmin)
}
```

```
#parameter set-up
n = 2000
b0e<-0.1
b1e<-1
b2e<-1.0
c10<-0.2
c20<-0.4
b3e<-1.5
b0<-c(log(b0e),log(b1e),log(b2e),log(b3e))
density = 0.05
nc = 10

#pB<-read.table("1015.txt",head=F)
#find out permutation p-value distribution
B = 10000
dat = datgen(n, b0, c10, c20)
y = dat[, 1]
pB = rep(NaN, B)
for (b in 1:B) {
  print(b)
  yb = sample(y)
  datb = dat
  datb[, 1] = yb
  outb<-findp(density, nc, datb)
  pB[b] = outb
}
print(pB)

#find out actual p-value of 500 replications
R = 500
p0 = rep(0, R)
p = p0
for (i in 1:R){
  dat = datgen(n, b0, c10, c20)
  density<-0.05
  nc<-10
  p0[i]<-findp(density, nc, dat)

  p[i]<-mean(pB<p0[i])
  rej<-mean(ifelse(p[1:i]<0.05,1,0))*100

  cat('p-value=',p[i], 'pwr = ', rej, '\n')

}

p.m<-mean(p)
```

```
rej<-mean(ifelse(p<0.05,1,0))*100

output<-c(p.m,rej)
cat('p-value=',p.m,' ','rej=',rej,'\n')

filename=paste("permutation",b2e*10,b3e*10, '.txt', sep="")
write(output,file=filename,ncol=1)
```

Appendix D

R Coding for CVT data analysis

(A) Library Codes

```
#generate regression coefficients
logitfit <-function(c1,c2,dat){
 y = dat[, 1]
 x1 = dat[, 3]
 t<-dat[,9]
 x2 <-ifelse(c1<=t&t<=c2,1,0)
 x3 = x1 * x2
 x4<-dat[,6]
 x5<-dat[,7]
 x6<-dat[,8]

 fit<-glm(y~x1+x2+x3+x4+x5+x6, family=binomial("logit"))
 sd = vcov(fit)
 # new: add loglike function as output
 out = list(bh = fit$coef, sd = sd, conv = fit$converged, lglk = logLik(fit))
 return(out)
}

#calculate the joint probability (log likelihood) of y
lj<-function(c1,c2,bh,X,t,y){
 X[,3]<-ifelse (c1<=t&t<=c2,1,0)
 X[,4] = X[,2]*X[,3]
 xbeta = X%*%bh
 exb = exp(xbeta)
 li = y*xbeta - log(1+exb)
 lj = sum(li)
 return(lj)
}

#calculate the (log) conditional probability of c1 & c2
pmh<-function(n,y,X,t,qj,cj,bh){
 c1<-cj[1]
 c2<-cj[2]
 q1<-qj[1]
 q2<-qj[2]
 p1<-lj(c1,c2,bh,X,t,y)
 p2<-log(c1)+(q1-1)*log(c2-c1)-q1*log(c2)+(q2-1)*log(1-c2)
 p<-p1 + p2
 #cat('p1=',p1,'p2=',p2,'\n'
```

```
 return(p)
}

#test whether the risk window has at least 5 subjects in each arm
testn = function(c1, c2, X, t) {
 threshold = 5
 X[,3]<-ifelse (c1<=t&t<=c2,1,0)
 trtn = sum(X[,2]*X[,3])
 ctln<-sum((1-X[,2])*X[,3])
 if( trtn >= threshold & ctln >= threshold & (c2-c1) >= 0.05) rpt = F else rpt = T
 return(rpt)
}

#empirical cdf tranformation used to convert time interval
x.cdf<-function(x){
  p<-rep(0, length(x))
  for (i in 1:length(x)) {
    p[i]<-mean(x<x[i])
  }
  return(p)
}

#one iteration of gibbs sampling
gibbs<-function(qj,cj, bh, dat, X){
 q1<-qj[1]
 q2<-qj[2]
 c1<-cj[1]
 c2<-cj[2]
 t = dat[,9]
 y = dat[, 1]

 #generate c1 and c2
 rpt = T
 pcj = pmh(n,y,X,t,qj,cj,bh)

 while (rpt) {
   D  = min(0.05, (c2-c1)/3)
   D1 = min(0.05 ,c1/2)
   D2 = min(0.05, (1-c2)/2)

   u1 = runif(1, c1-D1, c1+D)
   u2 = runif(1, c2-D, c2+D2)
   uj<-c(u1,u2)
   rpt = testn(u1, u2, X, t)
 }
```

```
 alpha<-exp(pmh(n,y,X,t,qj,uj,bh)- pcj)
 #cat('alpha = ', alpha, '\n')
 U<-runif(1,0,1)
 if(U < alpha) {
    c1 = u1
    c2 = u2
 }

 #generate betas
 fit = logitfit(c1, c2, dat)
 if(fit$conv == T){
   bhat<-fit$bh
   sd<-fit$sd
   A = chol(sd)
   bh = bhat + t(A)%*%rnorm(7, 0, 1)

   #generate q1,q2
   sc1<-log(c2/(c2-c1))
   sc2<-log(1/(1-c2))
   q1<-1+rgamma(1,shape=1,scale=sc1)
   q2<-1+rgamma(1,shape=1,scale=sc2)
 } else {
   c1 = cj[1]
   c2 = cj[2]
 }
# cat('bhat=', bhat,'\n')

 qj<-c(q1,q2)
 cj<-c(c1,c2)
 #cat('c1 = ', c1, 'c2 = ', c2, 'beta = ', bh, '\n')
 return(list(q=qj,c=cj,b=bh))
}

#Replication and Estimation
getmle<-function(dat, X, n,cj, qj, bh, r0,r1){
 #replication from 1 to r0(burn-in)
 for (i in 1:r0){
  g<-gibbs(qj,cj, bh, dat, X)
  cj<-g$c
  qj<-g$q
  bh = g$b
 }

 #replications from r0 to r1(total length of Markov Chain is r0+r1)
 qg = matrix(NaN, r1, 2)
 cg = qg
```

```
bg = matrix(NaN, r1, 7)
for (i in 1:r1){
 g<-gibbs(qj,cj, bh, dat, X)
 qj = g$q
 cj = g$c
 bh = g$b
 qg[i, ]=g$q
 cg[i, ]=g$c
 bg[i, ]=g$b
}

#Estimate the mean and credible intervals
c.m<-apply(cg,2,mean)
q.m<-apply(qg,2,mean)
bh.m<-apply(bg,2,mean)

ptl<-c(0.025,0.975)
c.q<-apply(cg,2,quantile,ptl)
q.q<-apply(qg,2,quantile,ptl)
bh.q<-apply(bg,2,quantile,ptl)
est<-c(c.m,c.q,q.m,q.q,bh.m,bh.q)
h<-list(est=est,c=cg,b=bg,q=qg)
#cat('c = ', c.m, '\n')
#cat('beta = ', bh.m, '\n')

 return(h)
}
```

(B) Main Codes

```
source("lib.R")
set.seed(1)
#read in original dataset
cvt<-read.table("cvt092312_multi_preg_dummy.csv",header=T,sep=",")
y<-cvt$outcome
#n<-length(cvt$ID)
x0<-rep(1,4061)
x1<-cvt$treatment
time<-cvt$time_interval
t<-x.cdf(time)
x4<-cvt$age
x5<-cvt$mari_2
x6<-cvt$mari_3
x2<-rep(0,4061)
x3<-x1*x2
X<-cbind(x0,x1,x2,x3,x4,x5,x6)
```

```
dat<-data.frame(cbind(y,X,t))
names(dat)=list("y","x0","x1","x2","x3","x4","x5","x6","t")
d<-list(dat=dat,X=X)

r0 = 500
r1 = 50000

#run r2 replications of gibbs sampling
r2<- 1
nc = 33
out<-matrix(NaN, nrow = r2, ncol=nc)
j = 1
while (j  <= r2){
 #generate initial values for parameters
  qj = c(2, 2)
  X   = d$X
  # new code to find a good initial value for c1 and c2
  plgk = -n*3
  for (w2 in c(0.1,0.2,0.3,0.4,0.5,0.6,0.7,0.8)) {
    for (w1 in c(0.05,0.1,0.2,0.3,0.4,0.5,0.6,0.7)) {
      if (w1 < w2) {
        fit = logitfit(w1,w2,dat)
        if (fit$lglk > plgk) {
          plgk = fit$lglk
          c1 = w1
          c2 = w2
          bh = fit$bh
        }
      }
    }
  }
  cj = c(c1, c2)
  #bh = logitfit(c1,c2,dat)$bh
  h = try(getmle(dat, X, n, cj, qj, bh, r0, r1))
  if(class(h) == "try-error") next
  #h<-getmle(dat, X, n, cj, qj, bh, r0, r1)
  est<-h$est
  #print(est)
  out[j,]=est
  filename=paste("out_multi_age&mari",sep="")
  write(t(out[1:j,]),file=filename,ncol=nc,sep="\n")
  j = j + 1
}
```

Appendix E

Summary Results of Bayesian Inference on β_0, β_1, and β_2 in the Logistic Regression Model in Simulation Studies

(A) Sample size of the simulated data: 1000 ($exp(\beta_0) = 0.1, exp(\beta_1) = 1$)

c_1	c_2	e^{β_2}	e^{β_3}	Marginal $\hat{\beta}_0$		Marginal $\hat{\beta}_1$		Marginal $\hat{\beta}_2$	
				Bias	C.P.	Bias	C.P.	Bias	C.P.
0.1	0.2	1	1.5	-0.07	93	0.07	91	0.26	85
0.1	0.2	1	2.5	-0.03	93	0.02	90	0.17	90
0.1	0.2	1.5	1.5	0.00	95	-0.03	94	-0.01	93
0.1	0.2	1.5	2.5	-0.02	97	-0.01	94	-0.01	94
0.1	0.2	1.5	3.5	-0.01	94	-0.02	94	-0.01	97
0.2	0.3	1	1.5	-0.05	94	0.01	92	0.25	88
0.2	0.3	1	2.5	-0.04	93	-0.03	93	0.17	91
0.2	0.3	1.5	1.5	-0.03	93	-0.03	94	0.00	93
0.2	0.3	1.5	2.5	-0.01	94	-0.05	94	-0.02	96
0.2	0.3	1.5	3.5	-0.02	95	-0.03	95	0.00	95
0.3	0.4	1	1.5	-0.05	93	-0.01	92	0.20	88
0.3	0.4	1	2.5	-0.02	94	-0.06	93	0.13	94
0.3	0.4	1.5	1.5	-0.06	94	-0.04	94	0.02	95
0.3	0.4	1.5	2.5	-0.02	96	-0.05	95	-0.02	96
0.3	0.4	1.5	3.5	-0.03	95	-0.02	95	-0.03	98
0.1	0.3	1	1.5	-0.04	95	0.03	94	0.21	90
0.1	0.3	1	2.5	-0.02	95	0.00	93	0.08	96
0.1	0.3	1.5	1.5	-0.02	93	0.01	94	0.08	92
0.1	0.3	1.5	2.5	0.00	97	-0.02	97	0.00	95
0.1	0.3	1.5	3.5	-0.01	97	0.02	95	-0.03	96
0.2	0.4	1	1.5	-0.04	95	-0.03	95	0.23	90
0.2	0.4	1	2.5	-0.02	94	-0.04	94	0.10	93
0.2	0.4	1.5	1.5	-0.04	95	-0.02	95	0.06	94
0.2	0.4	1.5	2.5	0.00	94	-0.02	97	0.01	96
0.2	0.4	1.5	3.5	-0.03	96	0.02	96	0.00	94
0.2	0.5	1	1.5	-0.02	94	-0.03	94	0.15	90
0.2	0.5	1	2.5	-0.02	95	-0.04	94	0.03	96
0.2	0.5	1.5	1.5	-0.02	94	0.00	95	0.05	95
0.2	0.5	1.5	2.5	-0.02	94	0.00	96	0.00	96
0.2	0.5	1.5	3.5	0.00	95	-0.01	95	-0.02	95

(B) Sample size of the simulated data: 2000

$(\boldsymbol{exp(\beta_0) = 0.1, exp(\beta_1) = 1}$**. Results are based on 500 replications**)

c_1	c_2	e^{β_2}	e^{β_3}	Marginal $\hat{\beta}_0$		Marginal $\hat{\beta}_1$		Marginal $\hat{\beta}_2$	
				Bias	C.P.	Bias			
0.1	0.2	1	1.5	0.00	97	0.03	96	-0.04	97
0.1	0.2	1	2.5	-0.01	93	0.04	93	-0.03	95
0.1	0.2	1.5	1.5	0.00	96	0.02	94	-0.16	95
0.1	0.2	1.5	2.5	-0.01	94	0.01	93	-0.05	93
0.1	0.2	1.5	3.5	-0.01	95	0.01	93	-0.03	96
0.2	0.3	1	1.5	0.00	97	0.02	96	-0.03	98
0.2	0.3	1	2.5	0.00	98	-0.01	95	-0.08	97
0.2	0.3	1.5	1.5	0.00	94	-0.01	96	-0.16	96
0.2	0.3	1.5	2.5	0.00	94	-0.02	96	-0.09	93
0.2	0.3	1.5	3.5	0.00	96	0.03	91	-0.11	93
0.3	0.4	1	1.5	-0.01	97	0.01	97	-0.05	97
0.3	0.4	1	2.5	-0.01	96	0.00	94	-0.04	97
0.3	0.4	1.5	1.5	-0.01	95	0.00	96	-0.19	95
0.3	0.4	1.5	2.5	-0.01	94	-0.01	94	-0.05	98
0.3	0.4	1.5	3.5	0.00	94	-0.01	96	-0.06	96
0.1	0.3	1	1.5	0.00	97	0.04	96	-0.01	97
0.1	0.3	1	2.5	-0.01	97	0.02	95	-0.03	96
0.1	0.3	1.5	1.5	0.01	95	0.01	95	-0.04	94
0.1	0.3	1.5	2.5	0.00	96	0.00	94	-0.03	94
0.1	0.3	1.5	3.5	-0.01	95	0.02	93	-0.03	95
0.2	0.4	1	1.5	-0.02	96	0.04	96	-0.01	96
0.2	0.4	1	2.5	-0.01	96	0.01	94	-0.03	95
0.2	0.4	1.5	1.5	0.01	97	0.00	96	-0.06	97
0.2	0.4	1.5	2.5	-0.01	95	-0.01	95	-0.02	98
0.2	0.4	1.5	3.5	-0.01	95	0.00	94	-0.02	95
0.2	0.5	1	1.5	-0.01	94	0.02	95	-0.02	97
0.2	0.5	1	2.5	0.00	95	-0.01	95	-0.02	95
0.2	0.5	1.5	1.5	0.00	94	0.01	94	0.00	96
0.2	0.5	1.5	2.5	0.00	95	0.03	92	-0.03	92
0.2	0.5	1.5	3.5	0.01	94	0.00	94	-0.03	95

(C) Sample size of the simulated data: 4000

$(\boldsymbol{exp(\beta_0) = 0.1, exp(\beta_1) = 1}$. **Results are based on 500 replications**)

c_1	c_2	e^{β_2}	e^{β_3}	Marginal $\hat{\beta}_0$		Marginal $\hat{\beta}_1$		Marginal $\hat{\beta}_2$	
				Bias	C.P.	Bias			
0.1	0.2	1	1.5	0.00	98	0.04	95	-0.05	97
0.1	0.2	1	2.5	0.00	97	0.01	94	-0.05	95
0.1	0.2	1.5	1.5	0.00	97	0.01	95	-0.07	93
0.1	0.2	1.5	2.5	0.00	96	-0.01	95	-0.02	96
0.1	0.2	1.5	3.5	-0.01	97	0.01	97	-0.01	95
0.2	0.3	1	1.5	-0.01	95	0.02	95	-0.03	95
0.2	0.3	1	2.5	0.00	96	0.00	95	-0.04	94
0.2	0.3	1.5	1.5	-0.01	95	0.01	96	-0.08	95
0.2	0.3	1.5	2.5	0.01	94	0.03	89	-0.09	89
0.2	0.3	1.5	3.5	0.00	95	0.02	92	-0.05	91
0.3	0.4	1	1.5	-0.01	96	0.02	95	-0.04	97
0.3	0.4	1	2.5	0.01	94	-0.01	95	-0.04	96
0.3	0.4	1.5	1.5	0.00	95	-0.01	96	-0.11	95
0.3	0.4	1.5	2.5	-0.01	95	0.00	94	-0.02	95
0.3	0.4	1.5	3.5	0.00	98	-0.01	96	-0.02	96
0.1	0.3	1	1.5	-0.01	96	0.05	93	-0.03	97
0.1	0.3	1	2.5	0.00	96	0.00	96	-0.02	94
0.1	0.3	1.5	1.5	0.00	95	0.00	96	-0.01	93
0.1	0.3	1.5	2.5	0.00	96	0.00	96	-0.02	94
0.1	0.3	1.5	3.5	0.00	94	0.01	94	-0.02	93
0.2	0.4	1	1.5	-0.01	95	0.02	96	-0.02	97
0.2	0.4	1	2.5	-0.01	96	0.01	96	-0.01	96
0.2	0.4	1.5	1.5	0.00	95	0.00	95	-0.01	93
0.2	0.4	1.5	2.5	0.00	95	0.00	96	0.00	96
0.2	0.4	1.5	3.5	0.00	95	0.00	95	0.00	96
0.2	0.5	1	1.5	0.00	96	0.03	95	-0.04	96
0.2	0.5	1	2.5	0.00	95	0.00	95	-0.02	94
0.2	0.5	1.5	1.5	0.00	96	0.00	96	0.00	95
0.2	0.5	1.5	2.5	0.00	92	0.02	93	-0.02	90
0.2	0.5	1.5	3.5	0.00	94	0.00	95	-0.01	94