RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS IN PATIENTS WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME

AUTHOR: William G. Hodge (7913090HO)

DEPARTMENT OF EPIDEMIOLOGY AND BIOSTATISTICS

McGILL UNIVERSITY, MONTREAL

Submitted: September 2002

A thesis submitted to the Faculty of Graduate Studies and Research in Partial Fulfilment of the Requirements of the Degree of Doctor of Philosophy in Epidemiology and Biostatistics

Your file Votre référence
ISBN: 0-612-88490-2
Our file Notre référence
ISBN: 0-612-88490-2

CONTRIBUTION OF CO-AUTHORS AND ACKNOWLEDGEMENTS

Jean-François Boivin: Primary Supervisor. He regularly attended thesis meetings throughout my training and was the principal source of advice for the design and implementation of the study. He proofread the manuscripts. He is a co-author of all manuscripts to be submitted. He was the primary mentor of my epidemiology training.

Stan Shapiro: Co-supervisor. He regularly attended thesis meetings throughout my training and was the principal source of advice for the analysis of the study. He is a co-author of all manuscripts to be submitted. He was the primary mentor of my biostatistical training.

Richard Lalonde: Co-supervisor. He regularly attended thesis meetings throughout my training and was the principal source of advice for the content expertise aspect of my study. He is a co-author of all manuscripts to be submitted. He helped develop the study base in Montreal. He was the primary mentor of my infectious disease training and has been a mentor since my second year of medical school, in 1989.

Bruce Jackson: My main academic mentor in ophthalmology from medical school through residency and as a junior staff. He has always put academic issues first.

Kirtida Shah: She was hired as a post-residency research fellow for 2 years, from 1998 to 1999. She was the primary abstractor of data from medical records. She is a co-author of all manuscripts to be submitted.

Brad Murphy: He was a resident at the University of Ottawa Department of Ophthalmology from 1996 to 2001. He was involved in abstracting data from medical records and co-ordinating the laboratory-based analysis of stored blood. He is a co-author of all manuscripts to be submitted.

Michel Dionne: He was hired as a summer medical student in 1997. He was one of the abstractors of data from medical records. He is a co-author of manuscripts I and III.

Ashish Goela: He was hired as a summer medical student in 1998. He was one of the abstractors of data from medical records. He is a co-author of manuscripts I and III.

Francisco Diaz-Mitoma: He is head of the Regional Virology Laboratory of Eastern Ontario. He was instrumental in the decisions and mechanics of the laboratory testing done for manuscript II. He is a co-author of manuscript II.

Jean Deschênes: He helped with some administrative hurdles for the Montreal arm of the study.

Rita Frost: She was the hired technician assigned to perform the laboratory analysis for manuscript II, and her expertise and experience are appreciated.

University of Ottawa: Through its Young Investigator Award, financial support for the bulk of this study, especially the laboratory-based part, was obtained.

Medical Research Council of Canada: Through its summer medical research grants, several of the above-mentioned medical students were hired.

Coherent Medical Group, Palo Alto, California: Through a generous and flexible industry grant, funds to hire Kirtida Shah were obtained.

Kim Cavanagh: Through her knowledge of computer word processing, she magically merged and marginated the manuscript into a presentable final version.

Gloria Baker: A professional editor who smoothed out all the edges.

Brigitte Lavigne: My wife. I always keep the most important acknowledgment for last.

TABLE OF CONTENTS

ABSTRACT

BACKGROUND: Cytomegalovirus (CMV) retinitis is the most common and most devastating ocular opportunistic infection among patients with the acquired immunodeficiency syndrome (AIDS). The lifetime cumulative incidence of this infection in patients with AIDS ranges from 25% to 40%. With the advent of highly active antiretroviral treatment (HAART) in 1996, the incidence of CMV retinitis is declining in industrialized nations. However, at least 20% of patients do not respond to HAART. Furthermore, it is possible that more resistance to HAART may develop in the not too distant future, with an explosive increase in the incidence of CMV retinitis. In developing and mixed-economy countries, the incidence is actually increasing. No other ocular opportunistic infection among patients with AIDS has a lifetime cumulative incidence over 1–2%. Without treatment, blindness occurs in 100% of patients within 6 months. When treatment is undertaken, it is expensive, not always effective and exhausting for the patient.

Over two dozen descriptive studies, prospective cohort studies and clinical trials have been conducted to examine the treatment of CMV retinitis. Surprisingly, very few studies have investigated the risk factors for this disease. Identifying these risk factors will have many benefits, including helping establish screening regimens, examination frequency regimens and targeted prophylaxis with oral ganciclovir or other anti-CMV agents.

Risk factors for CMV infection of other organ systems in other immunosuppressive settings have been identified but rarely studied as potential determinants of CMV retinitis in patients with AIDS. These risk factors include corticosteroid therapy, therapy with

other immunosuppressive medications, previous organ function, HLA type, previous blood transfusion and co-infections. Results from these studies provide a strong foundation for investigating potential risk factors for CMV retinitis. Furthermore, serum CMV viral load may also be a useful predictor.

GENERAL OBJECTIVE: To determine risk factors for CMV retinitis in patients with AIDS.

HYPOTHESIS: Many potential risk factors for CMV retinitis may be uncovered by this study. However, the principal hypothesis of the study is that treatment with steroids or other immunosuppressive drugs is a significant risk factor for CMV retinitis.

RESEARCH PLAN:

DESIGN: A case–control study involving cases and controls from the period 1990–1999.

SUBJECTS AND SAMPLING: The sampling was from a primary study base. The vast majority of patients with AIDS and CD4 counts less than 50 cells/μL from western Quebec and eastern Ontario are seen at one of two ophthalmic centers: the Eye Institute of the University of Ottawa or the Department of Ophthalmology of McGill University, in Montreal. All visits have been indexed for easy retrieval. A total of 279 patients were included in the study, 120 cases and 159 controls. The sample size calculation was based on the expectation of an odds ratio (OR) of 3.0 for the primary predictor, corticosteroid therapy.

VARIABLES: The outcome variable was the presence or absence of CMV retinitis (binary). The most important predictor variables that we studied were corticosteroid use and use of other immunosuppressive drugs. These were analyzed in an iatrogenic model

that included other treatments. Another model was developed to study clinical risk factors, and a third model was developed to study laboratory-based risk factors. A fourth model was developed for confounders and was used within the three main exposure models.

STATISTICAL ANALYSIS: Multivariate logistic regression was used to model each of the three exposure categories, controlling for confounding. A systematic model-building strategy was developed, from assumption testing to model building to model checking.

RESULTS: Among the clinical risk factors, flashing lights and floaters (OR 11.42, 95% confidence interval [CI] 3.43–38.01), retinal microinfarction (cotton-wool spots) (OR 2.90, 95% CI 1.01–8.29), number of previous opportunistic infections (OR 1.81, 95% CI 1.24–2.64), nonocular CMV infections (OR 82.99, 95% CI 6.86–1004.58) and homosexual acquisition of human immunodeficiency virus (OR 2.83, 95% CI 1.13–7.12) were significant predictors of CMV retinitis. From the laboratory model, a high CMV viral load was a significant predictor of CMV retinitis (OR 33.03, 95% CI 2.32–469.39), as was a low hemoglobin concentration (OR 0.96, 95% CI 0.94–0.98). Among the HLA types, HLA-Bw4 (OR 11.68, 95% CI 1.29–105.82) and HLA-DRBI15 (OR 9.34, 95% CI 1.14–76.41) were significant predictors of CMV retinitis, whereas HLA-Cw7 was protective against CMV retinitis (OR 0.09, 95% CI 0.01–0.67). From the iatrogenic model, steroid use was predictive of CMV retinitis (OR 6.41, 95% CI 2.35–17.51).

CONCLUSIONS: Based on this study, the use of steroids systemically elevated the risk of CMV retinitis. Other clinical and laboratory variables were found to elevate the risk of (or protect against) this disease. These findings may be useful to clinicians and health

policy experts in developing rational guidelines for screening, examination frequency and targeted prophylaxis for patients with AIDS.

RÉSUMÉ

CONTEXTE : La rétinite à cytomégalovirus (CMV) est l'infection opportuniste la plus fréquente et la plus dévastatrice observée chez les patients atteints du SIDA. L'incidence cumulative de cette infection au cours de la vie d'un patient sidéen a été de 25 à 40 %. Avec l'avènement du traitement antirétroviral hautement actif (HAART), l'incidence de la rétinite à CMV s'est résorbée chez les nations développées. Toutefois, au moins 20 % des patients ne réagissent pas au traitement HAART. De plus, il est certainement possible que la résistance au traitement HAART puisse s'accentuer dans un avenir rapproché en raison d'une nouvelle hausse de l'incidence de la rétinite à CMV. Dans les pays développés et à économie mixte, l'incidence est présentement à la hausse. Au cours de la vie des patients sidéens, aucune autre infection oculaire opportuniste ne présente d'incidence cumulative supérieure à 1-2 %. La cécité survient en moins de six mois chez 100 % des patients qui ne reçoivent aucun traitement. Lorsque le traitement est amorcé, il est coûteux et épuisant pour le patient et il n'est pas toujours efficace.

Plus de deux douzaines d'études descriptives, d'études prospectives par cohortes et d'essais cliniques ont été entreprises pour analyser le traitement de la rétinite à CMV. Curieusement, très peu de recherches ont été entreprises pour étudier les facteurs de risque tout aussi importants de cette maladie. La détermination de ces facteurs de risque procurera beaucoup d'avantages, dont celui d'aider à établir des régimes de sélection et de fréquence d'examen des patients ainsi que des mesures prophylactiques ciblées à l'aide de ganciclovir oral ou d'autres agents anti-CMV.

Les facteurs de risque d'infection à CMV d'autres systèmes d'organes ont été

déterminés dans d'autres traitements immunosuppresseurs, mais rarement étudiés comme facteurs déterminants potentiels de la rétinite à CMV chez les patients sidéens. Ces facteurs de risque incluent la corticothérapie, les autres médicaments immunosuppresseurs, les fonctions antérieures d'organes, le groupage HLA, les transfusions sanguines antérieures et les co-infections. Les résultats de ces recherches constituent une base solide pour l'étude des facteurs de risque potentiels de la rétinite à CMV. De plus, la charge virale en CMV du sérum peut se révéler un prédicteur utile.

OBJECTIF GÉNÉRAL : La détermination des facteurs de risque de la rétinite à CMV chez les patients atteints du SIDA.

HYPOTHÈSE : Plusieurs facteurs de risque possibles de la rétinite à CMV peuvent être découverts grâce à ce plan d'étude. Toutefois, la principale hypothèse de travail de cette étude suppose que le traitement à l'aide de stéroïdes ou d'autres médicaments immunosuppresseurs représente un facteur de risque significatif de la rétinite à CMV.

PLAN DE RECHERCHE :

PLAN D'ÉTUDE : Une étude cas-témoins utilisant des sujets et des témoins de la période de 1990 à 1999.

SUJETS ET ÉCHANTILLONNAGE : L'échantillonnage s'est effectué à partir d'une base d'étude principale. La grande majorité des patients atteints du SIDA (dont la numération des lymphocytes T-CD4 est inférieure à 50 cellules/FL) qui proviennent de l'ouest du Québec et de l'est de l'Ontario sont rencontrés à l'un des deux centres ophtalmiques suivants : à l'Institut de l'œil de l'Université d'Ottawa, ou au département

d'ophtalmologie de l'Université McGill de Montréal. Toutes les consultations ont été indexées pour en faciliter le repérage. Un total de 279 patients ont fait partie de cette étude dont le nombre de sujets a été calculé à partir d'une valeur probable d'un ratio d'incidence approché de 3/1 pour le prédicteur primaire d'une corticothérapie.

VARIABLES : La variable *résultat* a été la présence ou l'absence de la rétinite à CMV (binaire). Les variables *predictive* les plus importantes que nous avons étudiées sont l'utilisation de corticostéroïdes et d'autres médicaments immunosuppresseurs. Ces variables ont été analysées à l'aide d'un modèle iatrogène qui incluait d'autres modalités de traitement. Un autre modèle a été développé pour étudier les facteurs de risques cliniques d'infection. Un troisième modèle a été élaboré pour étudier les facteurs de risque en laboratoire. Un quatrième modèle a également été développé pour les variables confusionnelles et utilisé à l'intérieur des principaux modèles d'exposition décrits plus haut.

ANALYSE STATISTIQUE : La régression logistique multivariée a été utilisée pour modéliser chacune des trois catégories d'exposition en fonction du contrôle des variables confusionnelles. Une stratégie systématique d'élaboration de modèle a été développée à partir de la vérification des hypothèses ainsi que de l'élaboration et de la vérification de modèles.

RÉSULTATS : Parmi les facteurs de risque cliniques, la scintillement et les corps flottants (risque relatif approché [RRA] : 11,42; intervalle de confiance [IC] 95 % : 3,43 à 38,01), la microinfarction rétinienne (RRA : 2,90; IC 95 % : 1,01 à 8,29), un certain nombre d'infections opportunistes antérieures (RRA : 1,81; IC 95 % : 1,24 à 2,64), les

infections à CMV non oculaires (RRA : 82,99; IC 95 % : 6,86 à 1004,58) et l'acquisition homosexuelle du VIH (RRA : 2,83; IC 95 % : 1,13 à 7,12) se sont avérés des prédicteurs significatifs de la rétinite à CMV. À partir de ce modèle de laboratoire, une charge virale élevée de CMV a été un prédicteur significatif de la rétinite à CMV (RRA : 33,03; IC 95 % : 2,32 à 469,39) tout comme une faible concentration en hémoglobine (RRA : 0,96; IC 95 % : 0,94 à 0,98). Parmi les groupages HLA, le HLA BW4 a été un prédicteur significatif de la rétinite à CMV (RRA : 11,68; IC 95 % : 1,29 à 105,82) tout comme le HLA DRBI15 (RRA : 9,34; IC 95 % : 1,14 à 76,41). Finalement, le HLA CW7 a été protecteur contre la rétinite à CMV (RRA : 0,09; IC 95 % : 0,01 à 0,67). D'après le modèle iatrogénique, l'utilisation de stéroïdes s'est révélée prédictive de la rétinite à CMV (RRA : 6,41; IC 95 % : 2,35 à 17,51).

CONCLUSIONS : Selon cette étude, l'utilisation de stéroïdes a systématiquement élevé le risque de développer la rétinite à CMV. De plus, on a identifié d'autres variables cliniques et de laboratoire qui accroissent (ou protègent) le risque de développer cette maladie. Ces variables peuvent s'avérer utiles pour tous les cliniciens et les experts en politiques de santé qui travaillent au développement de directives rationnelles pour la sélection, la fréquence d'examen et la prophylaxie ciblée de ces patients.

INTRODUCTION

Cytomegalovirus (CMV) retinitis is one of the most devastating complications of the acquired immunodeficiency syndrome (AIDS). Several authors have investigated treatment, but far fewer investigators have examined the risk factors for this disease. Well-designed case–control studies would contribute to understanding these risk factors and may provide several clinical benefits. The benefits of understanding the risk factors for CMV retinitis include the possibility of formulating more sophisticated guidelines for screening, determining risk factors to permit earlier detection, and providing a more logical algorithm for prophylactic therapy. One example of potential targeted prophylaxis is oral ganciclovir therapy; however, since this agent is currently very expensive and has only moderate bioavailability, it is seldom used as a prophylactic medication.[1,2] Valganciclovir, antiretroviral therapy or experimental immune-based therapy may prove to play a role in prophylaxis as well.[3] However, the exact method of optimal targeted prophylaxis would have to be developed in separate studies. The purpose of this manuscript-based thesis was to study the risk factors for CMV retinitis in the most comprehensive way to date. To that end, we report three case–control studies, each with the objective of studying a different category of risk factors for this disease. The first paper deals with clinical risk factors, the second with laboratory-based risk factors and the third with iatrogenic risk factors.

A comprehensive review of the basic science and clinical epidemiologic aspects of CMV retinitis in patients with AIDS is first presented. Next, the methodology of the study is presented in detail, far greater detail than can be presented in any one of the

manuscripts. This is especially so when a rationale is given for the dynamics of the exposure variables, which is critical for a good case–control study but which would have to be presented in too much detail for any journal manuscript. However, of necessity, there is some overlap between the comprehensive review and detailed methodology at the beginning of the thesis, and the introduction and methodology in each manuscript. The three manuscripts then follow. Brief bridging material is presented between manuscripts I and II and between manuscripts II and III. References are provided at the end of the comprehensive review/detailed methodology section as well as at the end of each manuscript. Finally, a conclusion and summary is provided.

COMPREHENSIVE REVIEW

DESCRIPTIVE EPIDEMIOLOGY OF AIDS

AIDS was first recognized in North America in 1981. Since then it has become a massive worldwide epidemic, with essentially every country reporting cases. In 1984, the human immunodeficiency virus (HIV) was identified as the principal causative agent, and serologic testing could be performed to identify the carrier state even before full-blown AIDS developed. The World Health Organization estimated that over 40 million people were infected worldwide as of early 2000.[4] Early in the epidemic, Western industrialized countries seemed to have the highest incidence of the disease, but this was at least partly due to better and more open surveillance and reporting mechanisms.[5]

In the 1980s and 1990s, AIDS emerged as the leading cause of nontraumatic death among young males in North America and one of the five leading causes among women.[6] Furthermore, these deaths have a disproportionately large impact on society because of the young age of those affected and the loss of parents of young children.

As the 1990s progressed, the descriptive epidemiology of the disease changed throughout the world. In industrialized nations, an understanding of the biologic, psychologic and social risk factors related to transmission along with the advent of new therapy has decreased the mortality from this disease.[7] Although this is obviously a positive outcome, the pool of patients with the virus has increased as the life expectancy of patients infected with HIV rises. This has led many to speculate that a new, explosive epidemic of opportunistic infection is possible, especially if resistance to new antiviral medications emerges.[7] Furthermore, there are still over 40,000 new infections diagnosed

per year in the USA, and over 50% are in people under 25 years of age, a number that is unacceptably high.[8]

In industrialized countries, the segments of the population infected have changed.[9] In the early days of the epidemic, HIV was mainly a disease of homosexual men. HIV infection has now assumed a greater role among minority populations, street drug users and heterosexuals. For example, in the USA in 1998, the point prevalence of AIDS per 100,000 population was 66.4 for blacks, 28.1 for Hispanics and 8.2 for whites.

In contrast, developing and mixed-economy countries saw an explosive increase in rates of infection and death in the 1990s.[10] In east Asia, over 1.5 million people became infected during a 3-year period in the 1990's.[11] Between 1997 and 2000, the infection rate more than doubled in 27 of the world's less developed countries, and the rate increased even more in several eastern European countries, including Russia.[12] In many of these countries, HIV infection is now one of the leading causes, if not the leading cause, of death, especially among people under 50 years of age.[13] Today, of the over 40 people million infected, approximately 75% are from the poor areas of Africa and Asia.[14]

Another massive social problem created by this illness in developing countries is the high rate of mother-to-child vertical transmission. Perinatal transmission of the virus is widespread because of a lack of availability of chemotherapy designed to reduce the likelihood of infection. Already, over one million children worldwide have died from HIV infection,[15] and an estimated 10 million are now orphaned because of parental deaths.[16] This huge prevalence of sick, orphaned third-world children is one of the most serious consequences in the history of this disease.

The economic costs of the infection are so staggering that they are difficult to calculate. The annual economic burden of treatment and prevention alone is about US$14 billion, but this does not even begin to include the indirect costs of depletion of both skilled and unskilled laborers from these economies. The economic instability is so massive that there are predictions that political instability may result, with average life expectancy decreasing by over 15 years in some of these countries.[17]

CLINICAL DEFINITION OF AIDS

Early in the AIDS epidemic, the disease was principally defined by the presence of opportunistic infections in patients who were serologically positive for HIV. However, in 1993, the US Centers for Disease Control (CDC) expanded its definition to include patients who had not yet manifested an AIDS-defining opportunistic infection but who had a CD4 lymphocyte count less than 200 cells/μL.[18] During this year, the CDC also expanded its list of opportunistic infections to include those listed in Table I.

TABLE 1: OPPORTUNISTIC INFECTIONS INCLUDED IN THE 1993 CENTERS FOR DISEASE CONTROL DEFINITION OF AIDS
Candidiasis of bronchi, trachea or lungs
Candidiasis of esophagus
Invasive cervical cancer
Coccidioidomycosis, extrapulmonary
Cryptococcosis, extrapulmonary
CMV disease, excluding liver, spleen or lymph nodes
CMV retinitis
HIV-related encephalopathy
Herpes simplex skin ulcers or more than 1 month's duration
Herpes simplex bronchitis or pneumonitis
Herpes simplex esophagitis
Histoplasmosis, extrapulmonary
Isosporiasis, intestinal, of more than 1 month's duration
Kaposi's sarcoma
Burkitt's lymphoma
Immunoblastic lymphoma
Primary intracerebral lymphoma
Mycobacterium avium–intracellulare complex infection, extrapulmonary
Mycobacterium kansasii infection, extrapulmonary
Mycobacterium tuberculosis infection, any site
Infection with other *Mycobacterium* species, extrapulmonary
Pneumocystis carinii pneumonia
Recurrent pneumonia, any type
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis, intracerebral
HIV wasting syndrome

BIOLOGY OF HIV INFECTION

Although the macrophage is probably the first cell type infected, the interaction of HIV with the T-helper subclass of immunologic cells (CD4 cells) plays the central role in HIV infection. HIV binds to the CD4 cell via its surface glycoprotein gp120.[19] Once the virus is in the CD4 cell, a virus-enabled reverse transcriptase transcribes the viral genomic RNA into proviral DNA, which then integrates into the host cell DNA,[20] although some HIV DNA remains unintegrated in the cell cytoplasm. In an activated cell, the HIV DNA is then transcribed into both progeny genomic and messenger RNA, which is then spliced and translated into viral proteins after several post-translational modifications. Some infected CD4 lymphocytes may revert back to a state of inactivation; such lymphocytes may harbor latent proviral DNA (the latent phase) which can again lead to active infection when the cell is activated. The mature virion is then formed from virion RNA along with structural proteins and cell envelope proteins, some of which bud from the membrane surface.[21] The regulatory nature of this viral cycle is highly complex.[22] Soon after infection with HIV, the virus appears in large quantities in plasma. During this acute phase, HIV triggers various immune responses which then can partially control the rate of viral replication, leading to a decline but rarely a disappearance of the plasma viremia. After an initial burst of plasma viremia, most infected persons then have a lower constant viremia which is sustained at a stable level over a long period of time and is termed the chronic active phase. The level of viremia attained in the chronic active phase is called the viral set point. This level varies from

individual to individual. This level of plasma viremia is a strong predictor of the rate of immunologic decline and progression to symptomatic disease. HIV replication occurs continuously leading to a level of CD4 cell destruction which is not fully balanced by CD4 cell production, gradually leading to a decline in the CD4 cell count and increasing degrees of immunosuppression. At some point, the level of immunosuppression is sufficient to allow the emergence of opportunistic infection. This usually occurs at CD4 levels below 200 cells/uL and especially below 50 cells/uL for CMV retinitis. Regulatory proteins that interact with HIV DNA are produced by the virus itself and may interact with both host cell proteins and other regulatory proteins to determine which phase of activation the virus enters.

Ultimately, HIV kills CD4 cells, which is the major pathophysiologic mechanism of HIV infection and clinical AIDS. Several mechanisms contribute to cell death. Virus budding may form holes in the CD4 cell membrane.[23] Much of the HIV DNA and RNA accumulate in the CD4 cell cytoplasm, destroying mitochondrial and cytoplasmic machinery.[24] It has also been shown that some HIV envelope proteins may enter the CD4 cell and contribute to cell death.[25] HIV likely also infects progenitor cells in the bone marrow, thus contributing to loss of the CD4 lymphocyte pool. Some CD4 cells may also bind to other CD4 cells after HIV infection, creating multinucleated giant cell-like structures that are cytotoxic.[26] There may also be an autoimmune role for cell death, as B lymphocyte upregulation may be important in autoimmune cell death. Finally, programmed cell death, or apoptosis, may also play an important role in CD4 depletion. For example, there is evidence that anti-gp120 antibodies may cross-link other CD4 cells

and set off intracellular signals important in programmed cell destruction.[27]

Besides CD4 cell death, HIV infection also causes CD4 cell dysfunction. It is not clear whether CD4 cell dysfunction is merely in the pathway on the road to cell death. It is known that HIV-infected CD4 cells do not induce B lymphocytes to secrete immunoglobulin nearly as efficiently as uninfected CD4 cells.[28] Infected cells also respond less well to soluble antigens. In addition, the amount of soluble CD4 cytokines, such as interleukin-2 (IL-2), is reduced in infected cells, with a resultant decrease in CD4 clonal activation.[29] Besides a decrease in production of IL-2, another postulated mechanism of CD4 cell dysfunction is the inability of HLA type II proteins to bind properly with CD4 cells because of HIV–gp120 molecular interactions, which leads to down-regulation of the immune response.[30]

In the early stage of viremia, HIV is widely disseminated throughout the body, but a partial immune response forms.[31] However, the virus still replicates in some CD4 cells and peripheral lymph nodes, creating a chronic phase of infection that may last for several years. Although patients are clinically stable and free of opportunistic infections, immune function as measured by CD4 cell count slowly deteriorates. The persistence of CD4 cells in the germinal centers of lymph nodes is an important factor in the pathogenesis and progression of HIV infection. Regional immunologic activation in these areas, including the local production of cytokines such as IL-2, help recruit more CD4 cells to the areas that are vulnerable to infection by HIV. This is thought to be one factor responsible for the slow deterioration in immune function, occurring over many years, in these patients. Pathologic studies reveal that disruption of nodal architecture occurs with

time, and this may be one of the reasons why the lymphatic system cannot keep the virus in a latent or chronic stage.[32] Cloyd et al.[33] theorized that the determining factor in how HIV causes AIDS is via up-regulation of L-selectin after CD4 infection. According to their homing theory, L-selectin causes CD4 cells to slowly leave the plasma and enter lymph node germinal centers. Other important events in HIV pathogenesis include HIV mutations that produce more virulent strains, a decrease in neutralizing antibodies and a decrease in CD8 cells.[33]

Several factors have been shown to up-regulate the HIV replicative cycle both in lymph nodes and in CD4 cells. These include genes from other viruses, including CMV and herpes simplex virus (HSV), as well as a host of bacteria.[34] The main mechanism is likely via promoting the up-regulation of DNA-binding proteins. Local cytokines and B lymphocytes are also an important factor. However, down-regulation of HIV infection of CD4 cells also occurs. Tomaras et al.[35] found that CD8 cells mediate a suppressive activity of CD4 HIV infection, likely sometime after HIV infects the cells. Brodie et al.[36] tracked CD8 cells via gene marker techniques and determined that the plasma level of these cells correlate with the level of CMV-infected CD4 cells.

HIV is also able to infect macrophages and monocytes. These cells do not die off at anywhere near the same rate as CD4 cells, and the biology of infection is different.[37] HIV remains sequestered in the cell cytoplasm within vesicles and usually does not incorporate its DNA into the macrophage/monocyte DNA. However proviral DNA can integrate into macrophage DNA allowing the virus to complete its life cycle. As a result, macrophages and monocytes can harbor the virus for long periods and may be a reservoir

for viral infection in the blood over the long term. Also, it may be via these cells that HIV is brought into central nervous system tissue, including the eye.[38]

NONOPHTHALMIC CLINICAL FEATURES OF AIDS

Once the CD4 count falls below 200 cells/μL, clinical deterioration becomes more rapid. For most patients with AIDS, the most life threatening infection is *Pneumocystis carinii* pneumonia (PCP). The most common symptoms are shortness of breath, nonproductive cough and fever.[39] Chest x-rays typically demonstrate interstitial infiltrates; occasionally, other diagnostic tests, such as pulmonary function tests, are necessary to demonstrate pulmonary abnormality. Trimethoprim–sulfamethoxasole has been shown to be effective for prophylaxis of PCP.[40] Other opportunistic infections that can occur are listed in Table I, including those caused by CMV. Neoplasms such as Kaposi's sarcoma and central nervous system lymphoma also occur at much greater incidence in patients with AIDS than in the general population. Kaposi's sarcoma is much more aggressive in patients with AIDS than in HIV-negative people and can involve visceral structures.

AIDS AND THE EYE

Many ocular infections and malignant disorders have been reported among patients with AIDS, including CMV retinitis,[41–45] *Herpes zoster* ophthalmicus,[46–48] *Toxoplasma* retinitis,[49–53], *Pneumocystis* choroidopathy,[54–57] *Herpes simplex* keratitis,[58,59] ocular tuberculosis,[60] ocular syphilis,[61–65] fungal retinitis,[66] Kaposi's sarcoma of the lid and

conjunctiva[67] and ocular lymphoma.[68] Since all these conditions have fairly distinct clinical features, confusion with CMV retinitis is unlikely.

Herpes zoster ophthalmicus is the second most common eye infection in patients with AIDS, after CMV retinitis.[69] As is typical for zoster eye disease, many structures can be involved. The lid involved typically has a dermatomal inflammation with vesicles and, later, scarring. Entropion or ectropion as well as ptosis may result. Typical dendritic keratitis may develop, the dendrites being smaller and more superficial than those due to HSV. Iritis and glaucoma can also occur. Optic neuropathy is rare but possible, as are ischemic ocular misalignments. The two ophthalmic manifestations of zoster that are unique to patients with AIDS are painful, diffuse keratitis and the progressive outer retinal necrosis syndrome. The keratitis demonstrates a diffuse erosion-type syndrome that can be diagnosed by culturing the virus from the cornea. The most important ocular *Herpes zoster* infection with respect to this study is the progressive outer retinitis necrosis syndrome, which at least superficially resembles CMV retinitis. However, in zoster outer retinal necrosis, the infection is in the outer retina, not the inner retina. Furthermore, it is much more rapidly progressive, has less hemorrhage and is much less responsive to treatment than CMV retinitis.[69]

Toxoplasmosis is the third most common ocular infection in patients with AIDS, with a lifetime incidence of approximately 2%.[70] The classic lesion is that of a healed scar with a new area of activation adjacent to it. This area of activation appears as a creamy white infiltrate that does not respect the blood vessels, with overlying vitritis. However, in patients with AIDS, more atypical lesions are also possible, including multifocal

lesions, bilateral lesions and lesions without previous scarring. The necrosis from Toxoplasmosis in patients with AIDS may also be diffuse. It is felt that this atypical presentation of Toxoplasmosis retinitis is due to a higher probability of having an acquired form of the disease rather than the congenital form. The lesions are easily distinguished from CMV retinitis by the lack of vascular involvement, the vitritis, the adjacent scarring and the lack of hemorrhage.

Fungal retinitis is uncommon in patients with AIDS, with a lifetime incidence of less than 1%.[70] Its presentation is typical, with one or two areas of necrotic infiltrates on the retina and an overlying lesion of the "multiple pearls" type. The accompanying vitritis is less severe than in immunocompetent patients. Although the lesions may be confused with an atypical Toxoplasmosis lesion, it would be extremely unlikely to confuse them with CMV retinitis, for the same reasons described in the preceding paragraph.

PCP is the most important opportunistic infection in patients with AIDS. *P. carinii* can infect the eye as well, but at nowhere near the same frequency with which it can infect the lung. In fact, except for a brief period in the 1990's, eye infection with this organism is exceptionally rare. During the early to mid 1990's, aerosolized pentamidine was commonly administered to patients with AIDS to prevent PCP. However, because the rest of the body apart from the lung was not protected, *Pneumocystis* choroidopathy was diagnosed more commonly than in any other period.[71,72] This disease appears as several small yellow-orange circles or plaques at the level of the choroid. The lesions are always observed posterior to the equator of the eye, likely owing to the nature of the choroidal blood supply there. Sometimes the lesions grow and coalesce. Anecdotal

observations indicate that *Pneumocystis* choroidopathy and CMV retinitis may occur in the same patient at a rate higher than would be expected by chance, giving fuel to the theory that a risk factor for CMV retinitis is co-infection with other organisms.[73] However, the two are very distinct entities, with *Pneumocystis* choroidopathy not following blood vessels, being circular in nature, having a distinct color and having no hemorrhage.

Other retinal infections or neoplasms that may occur in patients with AIDS, including syphilis, tuberculosis, lymphoma and bacterial retinitis, are extremely rare and present with a very different morphology from that of CMV retinitis.

BIOLOGY OF CYTOMEGALOVIRUS INFECTION

CMV, unlike HIV, is a DNA virus of the herpesvirus family. It is a species-specific virus. The double-stranded DNA core is surrounded by a capsid composed of 162 capsomeres, all six-sided in cross-section. The capsid is surrounded by an outer envelope. The DNA of CMV is the largest of the herpesvirus family. The genome contains both long and short unique sequences and has a coding capacity for over 230 proteins.[74] Different CMV strains are defined by the terminal sequences of the genome. There is some similarity in DNA sequence to human DNA, especially the HLA class I genes. CMV DNA most readily infects human fibroblasts, but most cell types are probably permissive.[75]

Synthesis of protein from CMV DNA occurs in a series of interdependent steps. The immediate early proteins are synthesized first, which allows transcription of messenger

RNA for the second set of proteins, the early proteins. Early proteins allow for DNA replication by helping in the coding of DNA polymerase. The vast majority of the immediate early and early proteins are enzymatic in nature, whereas the late proteins are mostly structural.

The transmission of CMV infection is not perfectly understood, but transmission via body fluids, including serum, saliva, urine, blood transfusion or organ transplantation is the most likely mechanism.[76] Casual transmission, as from hand-to-mouth contact, does not seem likely in most cases, as studies of health care workers caring for congenitally infected children showed no increase in the infection rate over that in the general population.[77]

CMV infection may occur from primary exogenous infection, from congenital infection or from reactivation of latent virus. After primary infection, CMV is disseminated by the bloodstream to many organs.[78] A primary infection may be asymptomatic or may cause a CMV mononucleosis syndrome in persons who are otherwise healthy. A primary infection is potentially very serious for the unborn child of a pregnant woman and for various categories of immunosuppressed patients, particularly recipients of transplanted organs and persons with advanced HIV infection and a low CD4 lymphocyte count. CMV activity is likely most prominent in granulocytes during primary infection. As the virus is transported to reticuloendothelial cells, an intense level of viremia may occur.[79] When there is clinically apparent CMV disease in visceral organs, CMV viremia is almost always present, at levels 20 to 30 times that after primary infection.[80] However, the same level of viremia is not seen in CMV retinitis, perhaps

because the viral load needed for retinal infection is much lower. In fact, CMV retinitis may occur despite negative serologic testing for CMV. A high level of viremia is also present in organ transplant recipients with CMV infection. Free CMV DNA can also be detected in the plasma of patients with visceral CMV infections. Even when one visceral organ is infected, CMV can be found in many other organs, including the kidney, spleen and brain. In immunocompetent patients, despite multiple organ seeding of the virus, usually the infection is clinically mild or even not apparent; the virus may not be detected in secretions. However, in immunosuppressed people, there is a prolonged period of virus replication, and virus can be found in most secretions.[81]

After primary infection in immunocompetent patients, CMV enters a latent state. The location of latency is likely granulocytes or reticuloendothelial cells.[82] However, more than one type of cell may be involved, as several species-specific strains can infect a multitude of host cells. During latency, dampening of the host immune response occurs as surface glycoproteins that become apparent during the active stage of infection are not epitopic during latency. Natural killer cells and macrophages are likely the main cells involved in early regulation of the immune response. T lymphocytes are the most important defense against CMV. Infected cells alter their cytolytic activity and decrease their production of HLA class I antigens[83] but increase their production of HLA-DR.[84] Serum IL-6 and IL-8 levels are elevated in patients infected with CMV, and production of tumor necrosis factor alpha is up-regulated, which likely is one other explanation for the retinal necrosis seen in CMV infection. CMV infection also enhances the production of transforming growth factor beta, which down-regulates the immune response to viral

antigens.[85] Production of antibody against CMV is probably not as important as cell-mediated immunity but likely plays some role. Most of the antibody detected is of the IgA class near epithelial surfaces and IgG or IgM in serum. The antibodies are usually against viral surface proteins or glycoproteins such as protein p150 or the envelope glycoprotein complex gc11.[86] Because of its protean effects on the immune system, CMV is in itself felt to be immunosuppressive.

CMV infection can cause abortive or passive infection in cells of immunocompetent patients and lytic infection in cells of immunocompromised patients.[87] In the human retina during CMV retinitis, the lytic cycle is felt to predominate. The envelope of the virus is acquired from the host cell endoplasmic reticulum and cell membrane. The glycoproteins from these structures along with the viral capsid proteins are very immunogenic, which probably explains the hemorrhagic necrosis so visible with CMV ocular infection.[88]

The infection of retinal tissue is felt to begin via vascular endothelial cells, with spread to perivascular glia, Müller cells and the retina pigment epithelium. Rao et al.[89] studied autopsy eyes of patients with CMV retinitis using immunohistochemistry, CMV DNA probes and electron microscopic examination of retinas at different stages of infection. They found that CMV in the retinal glial cells and adjacent vascular tissue was almost always devoid of endothelia. They hypothesized that after infecting vascular endothelial cells, the virus destroys this tissue before invading adjacent retinal tissue. Scholz et al[90] studied this phenomenon in vitro in order to determine which cell-to-cell anatomic functions are damaged to produce endothelial cell permeability and then

destruction. Using cell monolayers, they found that the cadherin–catenin–actin complex is damaged by CMV infection and is likely the first important anatomic alteration allowing CMV to gain access to retinal tissue.

Once infected by CMV, cells demonstrate a unique histopathologic response consisting of the presence of giant ("cytomegalic" cells) with both intranuclear and cytoplasmic inclusions.[91] The intranuclear inclusions are typically surrounded by a clear halo. The cytoplasmic inclusions are granular in nature.

CYTOMEGALOVIRUS RETINITIS BACKGROUND

CMV retinitis is the most common and most devastating ocular opportunistic infection among patients with AIDS. It is a necrotizing, blinding form of retinitis that tends to occur late in the course of the disease. Other AIDS-related ocular problems pale in magnitude and severity compared to CMV retinitis. For many, if not most, patients, the threat of blindness during their remaining lifetime is a source of great anxiety. Undoubtedly, this is one of the most important AIDS-related illnesses of any organ system that has manifested itself since the onset of the AIDS epidemic.

Descriptive Epidemiology

The reported lifetime cumulative incidence of AIDS-related CMV retinitis ranges from 25– 40%.[92] Two large population-based incidence studies, one Canadian and the other American, demonstrated an increasing incidence of this disorder in the late 1980's and the 1990's.[93,94] No other ocular opportunistic infection among patients with AIDS has

a lifetime cumulative incidence over 1–2%.[95] Because CMV retinitis is a relatively late manifestation of AIDS, it is the index disease defining AIDS in only about 2% of patients. Based on data from the pre-HAART era, the estimated mean time from development of AIDS to manifestation of CMV retinitis is 9–18 months.[96–98] Although CMV retinitis can occur among other immunocompromised people, especially organ transplant recipients,[99] it is much less common among these groups than among patients with AIDS. CMV retinitis may also develop in patients immunosuppressed only by steroid therapy, but this, too, is rare.[100] Indeed, it is only with the advent of the AIDS epidemic that CMV retinitis has emerged as an enormous clinical and public health problem.

With the recent development of HAART,[101] the incidence of CMV retinitis is declining. Casado et al.[102] studied a cohort of 172 HIV-infected patients with a CD4 count less than 100 cells/μL at the time of protease inhibitor introduction. The cumulative incidence of CMV retinitis was 5% at 1 year and 6% at 2 years. Doan et al.[103] found that the annual risk of CMV retinitis in 1995 (without protease inhibitors) was 6.1%, compared to 1.2% in 1997 (with protease inhibitors); the annual risk of relapse after anti-CMV therapy decreased from 36% to 17%. Nevertheless, we should be cautiously optimistic regarding this new treatment. At least 20% of patients respond poorly or not at all to HAART,[104,105] and the risk of CMV retinitis has not changed for them. Furthermore resistance to HAART therapy can develop, with a gradual decrease in CD4 count and increase in HIV viral loads in many patients despite an initial response to therapy. Finally, in most parts of the world, HAART therapy is not available.[14] Hence,

CMV retinitis remains one of the most important irreversibly blinding diseases in ophthalmology.

Clinical Characteristics[41]

The clinical symptoms of early CMV retinitis include flashing lights and floaters (the presence of dark mobile spots in the visual field). These symptoms may even signify pre-clinical infection. Some patients also describe blind spots in their visual field, which can be detected on visual field examination. Even small lesions far away from the posterior pole of the eye can cause these symptoms, presumably due to changes at the vitreoretinal interface caused by the infectious retinitis. Furthermore, most patients, regardless of the location of their retinitis, report a decrease in the quality of their vision even if it is measured as normal. Of course, lesions in the posterior pole of the eye, anywhere near the fovea or optic nerve, produce a decrease in central vision that is measurable.

Examination reveals exudative hemorrhagic retinopathy (fulminant/edematous variant) that usually follows the retinal blood vessels and begins in the posterior pole of the eye, often near the macula. Since the areas of retinal involvement are dense, details of the choroid cannot be seen below the retinal lesions. With progression, patients notice significant loss of both peripheral and central vision. In advanced cases there are massive areas of hemorrhagic necrosis of the retina. Satellite lesions may be present, and progression occurs with very little overlying vitritis or anterior chamber inflammation. A retinal detachment that is very difficult to treat may develop. These rhegmatogenous (resulting from a tear) retinal detachments result from fluid's gaining access to the

subretinal space through multiple necrotic holes in the retina. The 1-year risk of retinal detachment in patients with CMV retinitis may be as high as 50%.[106] The risk of these rhegmatogenous retinal detachments seems related to the duration of disease, extent of necrotic retina and perhaps location near the vitreous base;[107] most would therefore be potentially preventable with early diagnosis and treatment. Some authors have speculated that detachment rates might be increased by treatment because of the greater chance of vitreoretinal cicatrization;[108] however, harder data seem to refute this.[109] Patients who have had a retinal detachment in one eye are at higher risk for detachment in the fellow eye.[110] Most of these detachments are complicated to treat, with the need for vitrectomy, scleral buckle and intraocular silicone oil. There is good anatomic success with this procedure,[111] with macular reattachment rates up to 90%. However, visual results typically are not as good. The silicone oil causes a severe hyperopic shift and can be toxic to the retina. In addition, the retinitis may progress and typically has already involved an extensive area of the retina. There is also a high incidence of optic neuropathy in these patients, which may be secondary to the retinitis, the oil or poor perfusion pressure during surgery.[112] Finally, cataracts are a typical complication of intraocular inflammation and are exacerbated by silicone oil. Serous retinal detachments can also occur in the fulminant forms of CMV retinitis.

The vitritis and anterior chamber inflammation of CMV retinitis are so mild that obscuration of the retinal or iris detail is extremely rare. However, there is clearly at least some anterior chamber inflammation in that stellate keratic precipitates can usually be seen. Variants of this classic type of CMV retinitis have been reported, including a

granular-appearing type (indolent/granular variant).[113] This rarer form is characterized by grainy opacification of the retina through which the underlying detail of the choroid can be seen. There is much less hemorrhage and much less vascular sheathing. In some cases a clear atrophic central area is seen whose shape tends to be more circular or oval. This type often does not follow blood vessels, and the most opaque aspect of the lesion is at its border. Interestingly, these two presentations may not be distinct at all. Treated fulminant lesions often have a granular nature that resembles the granular type of CMV lesions. Given that some fulminant lesions have shown at least partial response to nonclassic anti-CMV drugs such as acyclovir,[114] it is possible that the granular form is actually a fulminant form that has been partially treated through systemic anti-HIV or anti-HSV drug therapy. Supporting this theory is the fact that the granular lesions tend to progress more slowly than the fulminant lesions.[115] Very rarely, other presentations such as retinal vasculitis[116] or early macular lesions dominate the clinical picture. With the predominant vasculitis presentation, one or several areas of typically fulminant CMV retinitis are usually seen in other locations in the retina. This clinical variant is a type of frosted branch angiitis (named after its appearance similar to a tree branch in winter), a syndrome that can occur in patients without AIDS or CMV infection. Occasionally the optic nerve is the principal site of CMV retinal infection.[117] The clinical presentation is very variable, with some patients demonstrating optic disc swelling adjacent to a clearly defined area of CMV retinitis, and others having optic nerve swelling as the only sign of retinitis. Furthermore, some patients have only mild findings, including normal visual acuity, no afferent pupillary defect and no visual field defect, whereas others have severe visual

function deficits. These findings have led some investigators to believe that in the former case only inflammation is present in the nerve, while in the latter, a frank CMV infection is present.[117]

Principally for study purposes, some authors have divided the retina into three zones.[113] Zone I encompasses the area closest to the optic nerve and macula, extending 1500 μ from the center of the optic nerve medially and 3000 μ from the center of the fovea temporally. Zone II extends from the end of zone I to the area connecting the vortex veins. Zone III encompasses the rest of the retina. Some authors think the fulminant variety may be more common in zone I and the granular variety more common in zone III.[106]

The point prevalence among patients who already have bilateral CMV retinitis at the time of first diagnosis of CMV retinitis is 20–40%.[118] Depending on treatment modality, this value will increase to 50–75% during the patient's lifetime.

Occasionally, other ocular tissues besides the retina can be affected by CMV. These include the conjunctiva[119] most commonly and, rarely, the iris[120] or cornea. Some authors believe that CMV infection of the lacrimal gland may be the reason for the dry eye.[121] As mentioned, these other syndromes are clinically minor compared to CMV retinitis and are very rare.

Pathologic Findings

The unique aspects of CMV infection are the presence of both intracytoplasmic and intranuclear inclusions as well as the large size ("cytomegalic") of the cells. These cells

are usually four to five times larger than noninfected cells, and the nucleus of the cell is typically moved to the periphery. Another unique feature is that the intranuclear inclusions are surrounded by a halo. CMV retinitis produces full-thickness retinal necrosis.[122] Electron microscopy confirms the presence of CMV particles in all retinal layers and occasionally even in retinal pigment epithelial cells. However, it is rare to find CMV antigens in the underlying choroid and even rarer to find them in other parts of the eye. As would be expected from the clinical picture, retinal vessels and their endothelium may also be infected. In fact, the endothelial cells themselves can necrose and break through the circulation to travel to other parts of the retina, which may be how hematogenous spread occurs.[123] Mononuclear cells such as lymphocytes predominate, although neutrophils may be present as well.[123] In fact, the perivascular infiltrate seen clinically in CMV retinitis is mostly neutrophils. The choroid under the area of CMV retinitis does not have viral antigen but may have a scanty inflammatory cell infiltrate, hinting that at least some of the inflammatory cell recruitment may come from the choroid.[124] The decrease in helper T-type lymphocytes in the blood of patients with advanced AIDS is paralleled in the retina. Despite extensive retinitis, the vitreous is usually relatively free of inflammatory cells. Finally, subretinal fluid may be seen correlating with the serous retinal detachments seen clinically. Furthermore, retinal holes eventually develop in the most necrotic retinal areas, predisposing patients to the relentless retinal detachments seen clinically.

Laboratory Diagnosis

In recent years, the use of laboratory-based serologic assessments of CMV infection has gained wide attention, with the purpose of predicting which patients will manifest CMV retinitis. Culturing the virus is possible and depends on detection of the typical cytopathic effect of CMV in tissue culture.[82] However, it can take as long as 2 to 3 weeks for the result to be obtained. Rapid culture techniques have been used; they depend on detection of CMV early or immediate early proteins in tissue culture systems by using fluorescent monoclonal antibodies. In some techniques, a monoclonal antibody is added after centrifugation in order to increase sensitivity. Rapid culture techniques take approximately 24 hours to produce a result. However, they may be less sensitive than conventional culture as they rely on specific CMV proteins that may not be identical across all CMV types.

A positive result of a serologic test confirms that a person has been infected by CMV but, of course, does not necessarily confirm destructive CMV retinitis or any other active end organ CMV disease. Development of IgG antibodies to CMV in a previously seronegative patient indicates recent infection. Elevated IgM levels may suggest recent infection, but false-negative results can occur in the face of severe immunosuppression, and false-positive results may occur in the presence of rheumatoid factor.

Another antibody detection technique, the complement fixation test has been used to test for CMV infection, but it has poor sensitivity.[125] The indirect immunofluorescence test can give false-positive results. The anticomplement immunofluorescence test is more sensitive than the complement fixation test and more specific than the immuno-

fluorescent antibody test.[126] Other tests that have been used to detect CMV include virus neutralization tests, enzyme-linked immunosorbent assays (ELISAs) and radioimmunoassays. The presence of CMV pp65 protein in circulating leukocytes is usually detected by using a fluorescent-labeled monoclonal anti-CMV antibody in a direct fluorescent assay. Occasionally, antibody levels in the eye have been compared to levels in the blood in order to diagnose CMV retinitis more accurately.[127]

Amplification of viral DNA via polymerase chain reaction (PCR) has been studied in several papers, which are reviewed below in the section on understanding risk factors.

Natural History and Treatment

Without treatment, complete blindness (inability to perceive light) occurs in 100% of patients with CMV retinitis within 6 months.[115] The progression is due to movement of the border of the retinitis, with adjacent satellite lesions also contributing. There are no well-documented cases of spontaneous resolution without appropriate anti-CMV or anti-HIV therapy. Classic CMV retinitis usually begins in zone I or II of the retina (in or near the posterior pole of the eye). The lesions typically spread at a rate of 25–50 μ (about one-fourth to one-half the width of a retinal blood vessel) per day.[115] Early detection and intervention are likely important, as there is usually time from detection to intervention to halt the process. Furthermore, the most destructive posterior movement that destroys central vision via the macula may take the longest amount of time. Before the advent of specific therapy for CMV retinitis, this slow progression was nevertheless relentless, as autopsy studies demonstrated CMV retinitis at the fovea in a high proportion of cases.[124]

Several treatment modalities exist for CMV retinitis. One of the present treatment standards is intravenous ganciclovir. This medication is given to the patient first in induction dosages of 5 mg/kg twice a day for 2 weeks. Next, maintenance therapy is undertaken at a dosage of 5 mg/kg per day for the rest of the patient's life. Intravenous ganciclovir has several side effects, including myelosuppression, a devastating side effect for an already immunosuppressed patient. Alternatively, intravenous foscarnet can be used, but side effects from this drug are even more common than from ganciclovir. Both drugs are virostatic, and CMV retinitis will recur if either medication is stopped. This has been confirmed with electron micrographic studies, which have demonstrated viral particles at the border of treated lesions.[128] Furthermore, even with continuous maintenance therapy, the median time to recurrence of CMV retinitis is approximately 60 days.[106] A randomized clinical trial comparing these two drugs showed that the median time to retinitis recurrence was similar in the two study groups.[106] To combat the recurrence of CMV retinitis as well as the emergence of resistant strains, some authors recommend higher than usual doses, switching from one drug to the other or continued combination therapy.[129]

Other forms of treatment include weekly intraocular ganciclovir or foscarnet injections.[130] These injections have the advantage of providing a higher dose of medication at the site of active retinitis but the disadvantage of not treating potential CMV infections elsewhere in the body and creating ocular side effects such as endophthalmitis and early retinal detachment. Other treatment modalities include a depot intraocular sustained-release device,[131] a variant of the weekly injection route.

A randomized clinical trial demonstrated that with local administration of ganciclovir into the eye, retinitis progression took a median of 226 days,[132] far superior to that reported for intravenous ganciclovir. The fact that maintenance ganciclovir levels in the vitreous after intravenous infusion are often subtherapeutic supports the benefit of local therapy.[133] However local therapy does not protect the other eye or any non-ocular CMV infection. The use of intraocular cidofovir and other intraocular medications, especially antisense nucleotides, has also been reported. Fomivirsen (ISIS 2922) is an antisense oligonucleotide that inhibits replication of human CMV by binding to complementary sequences on messenger RNA transcribed from the major immediate early transcriptional unit of the virus. Perry and Balfour[134] reported a phase I clinical trial in which 18 patients with unilateral CMV retinitis were treated with 165 μg of this drug once a week for 3 weeks, followed by once every 2 weeks. The median time to progression was 71 days, compared with 14 days in patients not receiving the drug. The most common adverse reaction was decreased intraocular pressure and intraocular inflammation, which was in general mild and not nearly as clinically significant as it usually is with cidofovir.

The Spanish Cidofovir Study Group reported on the use of intravenous cidofovir in 51 patients receiving HAART in whom CMV retinitis developed.[135] After a median of nine doses, 49 patients showed no retinitis activity. However, 22 patients needed to stop treatment prematurely because of side effects, including death, nephrotoxicity and iritis. The Studies of Ocular Complications of AIDS Research Group found intravenous cidofovir to be efficacious at low or high doses in a randomized clinical trial.[136] This group was able to decrease the incidence of side effects by using probenecid, aggressive

hydration and intermittent dosing and by monitoring proteinuria. A study from San Francisco with similar results was published in 1998.[137]

Cidofovir and ganciclovir have synergistic effects in vitro. As a result, some authors have speculated that together these two agents may prove to represent a beneficial treatment for CMV disease. Jacobson et al.[138] reported a small phase I study in which seven patients with CMV retinitis were treated with intravenous cidofovir combined with oral ganciclovir. During a median of 5.5 months of follow-up, one patient showed progression of CMV retinitis. However, three patients had cidofovir complications, including iritis and hypotony.

There have been reports of slowing or regressing of CMV retinitis without specific anti-CMV treatment, even in the pre-HAART era. Most of these patients were taking zidovudine (AZT) orally.[139] Since this drug has no anti-CMV activity in vitro, the presumed mechanism is via enhancement of the patient's immune function. However, this drug seems ineffective in the vast majority of patients in changing the natural history of this disease. Fay et al.[140] confirmed the occasional benefit of AZT but also found that the anti-HSV drug acyclovir showed similar benefit in an occasional patient. Although acyclovir has weak anti-CMV activity, it is generally felt to be ineffective against CMV in vivo.

Prophylaxis

Prophylactic therapy has the intuitive and obvious appeal of decreasing the incidence of serious CMV-related diseases and improving quality of life. However, a potentially

even greater effect was demonstrated in the Aquitaine cohort in France.[141] In studying the factors predictive of the occurrence of CMV disease, Saillour et al.[141] found that after other factors were controlled for, CMV disease was one of the strongest predictors of death in the cohort; perhaps because of its own immunosuppressive effect. Prophylactic therapy with oral ganciclovir (1000 mg three times daily) shows promise for patients at high risk[1,2] but has drawbacks, including cost and moderate bioavailability. Hence, not all patients are candidates for therapy with this medication, and without a complete understanding of the risk factors for this disease, targeting patients at high risk is impossible at this point. Other anti-CMV agents are also being studied, such as valganciclovir, whose bioavailability is far superior to that of ganciclovir.

BENEFITS OF UNDERSTANDING RISK FACTORS

Although present therapies and potential prophylactic treatments are far from ideal, there is no doubt that understanding the risk factors for CMV retinitis will be beneficial. Some patients at high risk, once identified, may benefit from targeted prophylaxis. In addition, an understanding of the risk factors may lead to earlier diagnosis. The retinitis would be discovered at an earlier stage; hence, smaller amounts of the retina would be involved. This could result in less severe visual complications from this illness, in terms of both peripheral retinal involvement and central vision. It is very frustrating for a clinician to see a patient for the first time with large areas of the retina or even the macula already involved by CMV retinitis. Once this latter point is reached, central vision cannot be restored.

PREVIOUS STUDIES ON UNDERSTANDING RISK FACTORS

Dozens of descriptive studies, prospective cohort studies and clinical trials have been carried out to investigate the treatment of CMV retinitis. Surprisingly, far fewer studies have been performed to examine the equally important question of risk factors for this devastating disease. In fact, apart from the DNA quantification techniques that have gained widespread attention in the past few years, there is a paucity of studies looking at risk factors for CMV retinitis.

Clinical Risk Factors

Two studies have shown that a low CD4 lymphocyte count (helper T cells) is a strong risk factor for CMV retinitis.[142,143] Few, if any, other clinical risk factors have been discovered. A cross-sectional study also showed an association between CD4 count and CMV retinitis as well as an association with homosexual acquisition of HIV and CMV retinitis.[144] In a larger study, the Multicenter AIDS Group used a prospective cohort design to study patients with a diagnosis of AIDS or AIDS-related complex and CD4 counts less than 250 cells/μL who were receiving AZT.[145] AZT therapy had to have been started between April 1987 and April 1988. Patients were followed for 2 years or until death. CMV retinitis was identified by an ophthalmologist. Gastrointestinal disease was determined by endoscopic biopsy. Other CMV end-organ disease was also confirmed histologically. CMV viremia or viruria was not considered as evidence of CMV disease. Only incident cases were studied and compared with control subjects without CMV disease. In all, 1002 patients were followed for a median of 600 days. CMV disease developed in 109 patients (10.9%); the vast majority (85.3%) had CMV retinitis. In a

Cox proportional hazards model, baseline CD4 count less than 100 cells/μL (hazard ratio [HR] 2.32), an enrolment diagnosis of AIDS (HR 1.95) and homosexuality (HR 3.42) were risk factors for CMV disease.

In the Aquitaine cohort study,[141] 3525 patients were followed over an 8-year period, and patients with CD4 counts less than 200 cells/μL were eligible for the study. Using a Cox proportional hazards model, these investigators also found an increased risk of CMV disease among patients with homosexual acquisition of HIV.

End-organ damage has been postulated as a risk factor given that it has been studied as a risk factor for CMV disease in other organ systems. In other words, patients with abnormal retinas to begin with may be at increased risk for CMV retinitis. The most common noninfectious retinal abnormality in patients with AIDS is retinal microvascular infarction, manifesting as cotton-wool spots. These lesions, present in a high proportion of patients, are commonly seen before the development of CMV retinitis,[146] but they have never been established as a risk factor in a study using appropriate methodology.

The hypothesis in studies examining previous organ function as a risk factor for CMV infection is that an organ or organ system that has been previously damaged, regardless of the mechanism, may be more vulnerable to infections in general and perhaps to CMV in particular. In a prospective cohort study, Horak et al.[147] studied pretransplantation lung function using pulmonary function tests as a predictor of CMV pneumonia after bone marrow transplantation. Forced expiratory volume in 1 second was an important predictor of subsequent CMV pneumonia. Gorensek et al.[148] found that type of liver disease before liver transplantation was one of the predictors of CMV infection

after transplantation. Paya et al.[149] confirmed these findings and reported that acute fulminant hepatitis was the most important diagnosis involved. Among primary leukemic diagnoses for which bone marrow transplantation may be necessary, acute myeloblastic leukemia seems to be an independent risk factor for CMV infection in bone marrow transplant recipients.[150]

In patients with systemic CMV infections, there is evidence that co-infection with other viruses or bacteria may predispose to CMV infection. Schiffer et al.[151] found a strong correlation between CMV disease and co-infection with bacteria. In the Aquitaine cohort, infection with *Toxoplasma* was a predictor of CMV disease.[141] In a large Multicenter retrospective cohort study performed in 17 European countries, patients with PCP, toxoplasmosis or extraocular CMV infection were at increased risk for CMV retinitis.[152] HLA type was also studied in these systemic infections and may contribute to CMV infection additively with other bacteria or viruses. Other instances where HLA type may be an important element in the causation of infection include Reiter's syndrome. HLA-B27-positive patients with this disorder manifest a syndrome of conjunctivitis, arthritis and urethritis or colitis after infection with a number of possible agents, including *Chlamydia trachomatis*, *Campylobacter* species, *Yersinia* species, *Salmonella* species or *Shigella* species. Kraat et al.[153] found HLA-DR7 to be an important risk factor for CMV infection after renal transplantation. Boland et al.[154] studied this same problem and found HLA-B7 to be a risk factor for CMV infection when the data from all solid-organ transplants were pooled.

Laboratory-Based Risk Factors

Qualitative Methods

There is hope that determining the existence and the amount of CMV viremia by the PCR technique as well as other molecular methods may prove to be a very useful tool in both diagnosis and management of CMV retinitis. Preliminary studies have been completed in this area,[155–158] and others are ongoing. In a cross-sectional study, Pannuti et al.[159] studied the frequency of CMV viremia using cell culture isolation and a CMV antigenemia assay, which involved peroxidase staining of neutrophils with monoclonal antibodies to the pp65 matrix protein of CMV. Eight of 24 patients with AIDS who had untreated CMV retinitis had a positive assay, compared with 0 of 24 patients with AIDS who did not have CMV retinitis matched for sex, age, Karnovsky score and CD4 count. In a cross-sectional study in Minnesota, Jackson et al.[160] found that almost 100% of homosexual men who were HIV positive were also CMV positive by latex agglutination, as were about 60% of hemophiliacs from the same population. However, these authors concluded that the presence of CMV infection could not be used as a reliable indicator of stage of HIV infection or of predictability of CMV disease.

Wetherill et al.[155] conducted a prospective case–control study and found that all 11 patients with CMV disease had a positive antigenemia assay on peripheral blood leukocytes, compared with 4 of 11 patients without CMV disease. In a prospective cohort study in Paris, CMV viremia as determined by rapid culture methods was highly predictive of CMV retinitis (relative risk [RR] 22.03) in the 192 patients followed for 1 year or more.[161]

Dodt et al.[162] studied 200 HIV-positive patients with CD4 cell counts less than 100 cells/μL who were monitored by CMV PCR, the antigenemia test, blood cultures and CMV IgG and IgM tests every 2 months for 1 year. CMV disease developed in 38 patients. The median time between detection and onset of disease was 46 days with PCR, 34 days with the antigenemia test and 1 day with blood cultures. Multivariate analysis showed that the qualitative CMV PCR predicted disease better than the other tests.

Gorensek et al.[148] reported on 33 consecutive liver transplant recipients studied prospectively for evidence of CMV infection at the Cleveland Clinic Foundation between 1984 and 1987. Sixteen (48%) episodes of CMV infection were identified, nine primary and seven recurrent. Unfortunately, gamma globulin prophylaxis was routinely administered to several patients, thereby rendering the results rather inhomogeneous. The authors evaluated 12 potential risk factors for CMV infection, including pretransplantation serologic status of donor and recipient, recipient's age, gender, race, liver disease, number and type of blood products transfused, type and intensity of immunosuppression, and occurrence of rejection. Patients had to survive for at least 1 week. The mean length of follow-up was 2 months (range 7–148 days). Immunosuppression consisted of tapering doses of corticosteroids and cyclosporine or, if patients could not tolerate cyclosporine, azathioprine. Rejection was determined by liver biopsy. Several definitions of CMV infection were created, including primary and recurrent symptomatic and asymptomatic infection. However, any serologic change or positive culture was considered as evidence of infection. The etiologic period was defined as follows. Corticosteroids were quantitated in the first week and first month

after transplantation. Neither pretransplantation serologic status date nor time of blood product transfusion was defined. Appropriate multivariate analysis was used, including time to CMV infection (Cox proportional hazards model) and subgroup analysis for type of CMV infection (symptomatic, asymptomatic etc.). Positive donor CMV serostatus was the only significant risk factor identified.

In a cross-sectional study, Hamprecht et al.[163] reported on the detection of human CMV DNA from peripheral blood mononuclear cells, granulocytes and plasma. They studied 220 blood samples from 75 immunosuppressed patients with clinically suspected primary or recurrent CMV infection. For granulocyte CMV detection, granulocyte separation techniques were used to amplify CMV DNA. Plasma PCR techniques had the highest predictive value, followed by mononuclear CMV amplification and granulocyte amplification.

Gellrich et al.[164] performed a retrospective cohort study looking at the usefulness of testing for CMV viruria in defining risk for CMV retinitis among 130 patients with CD4 counts lower than 50 cells/μL enrolled between January 1990 and April 1995. CMV viruria was determined by shell vial cell culture. Nineteen of 54 patients with CMV viruria had CMV retinitis, compared with 4 of 76 patients without CMV viruria. CMV was detected in urine within 3 months of the development of CMV retinitis in 83% of patients; appropriate index dating for controls was not defined. The authors concluded that CD4 counts less than 50 cells/μL and CMV viruria together constitute a viable ophthalmic screening regimen.

Cope et al.[165] prospectively followed 162 liver transplant recipients between

November 1992 and April 1996. Using a qualitative PCR assay, they examined 1433 surveillance blood samples for human CMV infection. The patients were receiving a triple immunosuppressive regimen (azathioprine, methylprednisolone and cyclosporine). No antiviral prophylaxis was given to treat CMV infection. Human CMV disease was diagnosed by PCR, culture, antibody titre or pathology. The International Standard for CMV disease in liver transplantation was followed. Fifty-one patients had CMV viremia, and 20 had CMV disease. Patients with a sustained increase in viral load had a greater chance of manifesting CMV disease, but this could be studied in only four patients. In multivariate analysis, viral load was associated with an odds ratio (OR) of 2.70 (95% confidence interval [CI] 1.41–5.17) for the development of CMV disease. The etiologic period studied was 2 months; the index time for controls was not defined. Another risk factor found to be significant was steroid administration (OR 1.61 [95% CI 1.04–2.51]). The etiologic period for steroid use was not provided. Donor-positive, recipient-negative serostatus were also found to be significant. There was a 50% probability of disease when the viral load reached $10^{5.1}$ genomes/mL of blood and a 90% probability at $10^{5.5}$ genomes/mL. When steroid use was factored in, the risk curves were shifted down to reflect increasing risk of CMV disease at lower viral loads. Thus, at a methylprednisolone dosage of 12 g, the 50% probability level shifted to $10^{4.1}$ genomes/mL. These findings suggest that prednisone therapy should be reduced whenever possible during CMV infection. The authors did not attempt to define a critical viral load above which CMV prophylaxis would be strongly suggested.

Quantitative PCR Detection

Bowen et al.[166] studied HIV-positive patients with a CD4 count less than 50 cells/μL and previous evidence of CMV infection as defined by IgG seropositivity. Patients were recruited in 1995. DNA was extracted from 200 μL of whole blood collected in a citrated tube and analyzed using qualitative PCR. All samples were analyzed twice, and those with a positive result were analyzed three times. Patients who were CMV positive on PCR had monthly eye examinations and repeat PCR. Patients who were initially CMV negative were followed every 3 months with eye examinations and repeat PCR. Patients were followed for one year. Those who did not manifest the event of interest were nevertheless included in the analysis by means of survival analysis. The final date of follow-up was May 31, 1996. To take into account covariates, the authors used the Cox proportional hazards model to calculate the HR. Among the 27 patients with positive results of PCR at study entry, CMV disease developed in 16 (59.2%), compared with 3 (4.3%) of the 70 patients with negative PCR results giving a HR of 20.15 of manifesting CMV disease. Other covariates studied were patients' age and CD4 count; these were not significant predictors of the development of CMV disease. Quantitative analysis of patients who were initially CMV positive on PCR showed that CMV viral loads at study entry were higher among patients who went on to manifest CMV disease. In patients initially CMV positive, each 0.25 $\log_{10}$ increase in viral load over time was significantly associated with a 37% increase in the likelihood of the development of CMV disease (HR 1.37, 95% CI 1.15–1.63). This effect was unchanged after adjustment for age and CD4 count. All three CMV-negative patients in whom CMV retinitis developed

eventually did have positive PCR results. Overall, the positive predictive value of PCR was 0.60 after 12 months of follow-up (median 5 months).

Rasmussen et al.[158] carried out a cross-sectional study to determine whether quantities of CMV DNA from white blood cells were different in HIV-positive and HIV-negative patients. They also wanted to determine whether quantities of CMV DNA were different in HIV-infected patients with and without CMV retinitis. The long-term goal was to look at CMV copy number in white blood cells as a surrogate of CMV end-organ disease. For PCR, immediate early primers were used for a 162-base-pair product. A detector probe was used with ^{32}phosphorus. Cases were enrolled from the Stanford University School of Medicine and controls from among laboratory personnel. There were 15 HIV-negative people in the study and 105 HIV-positive people, 25 of whom had retinitis. The median number of CMV copies isolated was 37 from HIV-negative patients, 25 from HIV-positive patients without retinitis and 89 from HIV-positive patients with retinitis. A total of 35% of those without CMV retinitis had more than 40 copies, compared with 56% of those with CMV retinitis. No other clinical variables were studied except to ensure a comparable CD4 count, and all analysis were univariate. The higher number of virus particles may reflect more cells infected or a higher number of viral particles per cell. The authors reported that systemic infection may not occur in all patients with CMV retinitis, as 12 patients with CMV retinitis were CMV negative using PCR.

In a subsequent prospective cohort study, the same authors looked at quantification of CMV burden by viral PCR in leukocytes, plasma and urine.[167] They enrolled consecutive patients with CD4 counts less than 100 cells/μL who were undergoing ophthalmic

screening for CMV retinitis in San Francisco. Patients were excluded if they were pregnant, had a prior history of CMV disease or were treated with an anti-CMV drug. Eyes were examined every 2 months. The mean time from enrolment to development of CMV retinitis was 10 months. The authors studied 550 samples from 75 patients; each sample was examined in duplicate. DNA was quantified by the optical density method. Fewer patients with retinitis than controls were being treated with AZT. Anti-HIV drug use was defined as that taken on study entry rather than medication taken in the past. Other potential confounders documented at study entry included demographic characteristics, duration of AIDS, CD4 count and hematologic parameters. Sensitivity, specificity, and positive and negative predictive value were used to evaluate quantitative CMV DNA amplification, CMV isolation and CD4 counts as predictors of retinitis. The use of leukocyte DNA to detect CMV burden was more sensitive than the use of plasma, but plasma was more specific. The positive predictive value was highest among plasma samples. The highest positive predictive value occurred at copy levels greater than 32 in plasma and greater than 320 in leukocytes. A single value above threshold was significant, but it was even more significant if copy number was sustained in two or more consecutive samples. Mere isolation of CMV from blood was also a significant predictor of CMV disease. The authors pointed out that the threshold copy number is variable among laboratories because of differences in amplification and detection methods.

In a prospective cohort study, Shinkai et al.[168] used urine and blood leukocyte cultures and qualitative plasma PCR as well as quantitative competitive PCR to identify patients with AIDS at risk for CMV disease. Ninety-four patients were followed for a mean of 12

months. Urine and blood specimens were obtained every 3 months. PCR was performed on plasma. Any positive result during follow-up was considered positive for the purpose of their analysis. Quantitative PCR on plasma samples proved to be the most sensitive and specific test, with a mean of 1510 CMV copies/μL among patients in whom CMV disease developed, compared with 161 copies/μL among those in whom CMV disease did not develop.

Wattanamano et al.[169] performed a prospective cohort study involving 18 patients with CD4 counts less than 100 cells/μL and no CMV retinitis on baseline screening examinations to investigate the sensitivity and specificity of three different methods of CMV detection: pp65 antigenemia leukocyte assay, the Digene Hybrid Capture CMV DNA assay and the Roche Amplicor Qualitative PCR test. The Digene assay, with cut-offs at 1400 genome copies/mL, proved to have the highest sensitivity and specificity.

Some authors have examined CMV levels in the eye itself using PCR. Verbraak et al.[170] measured paired serum and aqueous humor samples from patients with necrotizing retinitis over a 5-year period. They attempted to correlate CMV levels with final clinical diagnosis. A similar technique was used by Doornenbal et al.[171] and Danise et al.[172] Knox et al.[173] performed PCR-based assays of vitreous samples as a diagnostic test in patients with known CMV or herpetic disease and compared the results to those of noninfectious controls in order to improve the sensitivity and specificity of the assay. Although this method may be valuable in established retinitis when there is a question of diagnosis, it is impractical as a predictive tool for CMV retinitis.

A recent study has tempered somewhat the enthusiasm about diagnostic assays to

help predict CMV retinitis. Verbraak et al.[174] compared the CMV strains in ocular samples and peripheral blood leukocytes of 13 patients with AIDS who had CMV retinitis. The DNA sequence of the immediate early gene was determined and amplified with PCR. Of the 10 patients with CMV isolated from both compartments, 7 showed different DNA sequences, implying different strains of virus. Hence, any measurement of serum CMV DNA might be considered invalid as a predictor of ocular CMV disease.

In the era of protease inhibitor therapy, some authors have intentionally studied the incidence of CMV retinitis as well as the use of PCR assays in this clinical situation. As part of the Spanish CMV–AIDS Study Group, Casado et al.[102] studied a cohort of 172 patients with AIDS with a CD4 count less than 100 cells/μL. They found that a positive CMV PCR assay at initiation of therapy was the most important predictor of CMV retinitis (HR 4.41). A positive result was more important than the quantitative value in predicting the occurrence of CMV retinitis.

Other Methods

CD8 lymphocyte counts have also been studied as a risk factor for CMV retinitis. Although CD8 counts have been associated with emergence of CMV retinitis, investigators were not able to determine whether this was independent of CD4 counts. In a cross-sectional study, Oka et al.[175] studied CD8 counts in 14 patients with CMV retinitis compared to 24 controls with PCP. They found a slightly lower mean CD8 count among cases than among controls. In a small cross-sectional study, Butler and Griedman[176] identified other hematologic parameters that may predict retinitis, but no

other study has been performed in an attempt to replicate their findings.

Immunogenetic predisposition to CMV retinitis was studied in a prospective cohort study.[177] Despite a small sample (21 patients), this study showed a significant association between HLA-DR7, HLA-B44 and HLA-B51 and the appearance of CMV retinitis. It will be important to reproduce this finding in a larger study and determine whether there are other immunogenetic markers for CMV retinitis. In a Canadian study of female sex workers in Kenya, MacDonald et al.[178] showed that the incidence of infection with HIV (not CMV) was lower in workers who were HLA-A2 and HLA-DRB1*01 positive than in those positive for other HLA types.

Iatrogenic Risk Factors

Iatrogenic risk factors for CMV infection of other organ systems in other immunosuppressive settings have been determined but have been studied only superficially, if at all, as potential determinants of CMV retinitis. Clues from these studies may help us better understand CMV retinitis. One such risk factor is corticosteroid therapy. Nagler et al.[150] conducted a case–control study to examine risk factors for CMV pneumonia (diagnosed via bronchiolar lavage) in bone marrow transplant recipients. CMV pneumonia developed in 18 of 197 patients (the exact time frame was not specified). In 3 of the 18, the disease occurred before engraftment. Acute myeloid leukemia was not diagnosed in any of these 3 patients (vs. 6 of 15 controls), all of whom were less than 18 years old. None manifested acute graft-versus-host disease

(vs. 9 of 15 controls). None of the patients without CMV pneumonia were treated with steroids, compared with 15 of those with CMV pneumonia, although the etiologic period was not specified. In summary, a strong association was found between steroid use and development of CMV pneumonia.

Manez et al.[179] performed a historical cohort study on the risks of CMV intestinal disease after intestinal transplantation and also found steroids to be an important risk factor. From May 1990 to March 1993, 20 adults and 18 children received 40 intestinal autografts at their institution. Immunosuppressive therapy included FK506, steroids and, in selected cases, low-dose azathioprine. Steroid therapy was begun intraoperatively. Surveillance endoscopy was performed one or two times per week postoperatively. Thirteen patients (34%) received prophylaxis with ganciclovir and/or acyclovir. Culture for CMV was performed whenever CMV disease was clinically suspected. Several risk factors were studied: age, type of intestinal transplant, donor and recipient serostatus, use of ganciclovir prophylaxis, use of seronegative blood products, number of units of blood products transfused, treatment with muromonab-CD3 (Orthoclone OKT 3), average blood levels of FK506 per day, average daily dosage of maintenance steroids adjusted to weight, azathioprine dosage and total amount of steroid boluses adjusted to weight. Univariate and multivariate analyses were performed using the Cox proportional hazards model. CMV disease developed in 15 patients, a median of 54 days after transplantation. This has been roughly assumed to be the etiologic period for this study, but the range was 21–274 days. CMV enteritis accounted for 81% of the infections. Multivariate analysis showed that significant risk factors for the first episode of CMV disease were donor

seropositive/recipient seronegative status (HR 3.86, 95% CI 1.21–12.36), amount of steroids given as boluses (HR 2.90, 95% CI 1.46–50.53) and average daily blood level of FK506 (HR 2.15, 95% CI 1.02–4.52).

Nelson et al.[180] performed a case–control study on the risk of CMV disease of any organ system in patients with AIDS and also found corticosteroids to be an important determinant. Morris et al.[181] studied the risk of CMV disease in 165 renal allograft recipients treated with cyclosporine for at least 6 months between March 1985 and December 1986. CMV disease was diagnosed in 54 patients. The etiologic period of prednisone use was at least 4 of the previous 6 months. CMV disease developed in 36 of the 94 patients treated with prednisone, compared with 18 of the 71 who did not receive prednisone. Gorensek et al.[182] studied the risk factors for CMV infection in heart transplant recipients enrolled from August 1994 to August 1996. The etiologic period was 120 days. The only significant predictor of CMV infection was greater than average steroid use.

Analogously, immunosuppressive (nonsteroidal cytotoxic) therapy has been studied as a possible risk factor for systemic CMV disease. In a multicenter case–control study of CMV infection after liver transplantation, Schiffer et al.[151] found use and duration of immunosuppressive therapy to be the most important nonserologic determinant of CMV infection. More specifically, Kraat et al.[153] found triple immunosuppressive therapy to be an important risk factor for CMV infection after renal transplantation. The type and intensity of immunosuppression were found to be an important risk factor for CMV infection after liver transplantation.[148] In a cohort study, Kirklin et al.[183] analyzed the

Cardiac Transplant Research Database to look at risk factors for CMV infection after heart transplantation. Patients were enrolled from Jan. 1, 1990, to June 30, 1992. Among the 1553 patients in the cohort, CMV disease developed in 230. Higher than average dosages of cytotoxic induction therapy agents was the most important risk factor found.

Some authors have observed an increase in the frequency of CMV infections in patients who have received multiple blood transfusions.[149] The pathogenesis of this association is unclear, although it does seem to be more complicated than simple seroconversion. Possible mechanisms include transfer of an increased viral load by the transfusions ("CMV viremia"), transfer of new, slightly modified strains of CMV that cannot be determined with present serologic testing, or activation of preexisting CMV by a factor(s) in the transfusion. This last theory was championed by Sloand et al.[184] in the discussion of their historical cohort study looking at CMV infection of any organ in patients with AIDS who had received blood transfusions. Paya et al.[149] reported similar results, and, in fact, these authors found transfusion to be the most important risk factor for CMV infection after liver transplantation. However, Gorensek et al.[148] reported conflicting findings.

In patients seen at the University of Ottawa Eye Institute and the Department of Ophthalmology of McGill University, Montreal, Canada, anecdotal clinical observations strongly support the previously reported association between low CD4 count and CMV retinitis. Moreover, we have observed that patients with AIDS who receive immunosuppressive chemotherapy or steroids (typically for Kaposi's sarcoma or PCP) frequently manifest CMV retinitis or have a worsening of preexisting retinitis.

Furthermore, most patients with CMV retinitis are cachectic. While other covariates may be operating in these patients, nutrition should also be studied as a risk factor.

In summary, there are several important gaps in our knowledge with respect to risk factors for CMV retinitis. Very few studies and even fewer comprehensive studies have been performed to examine the risk factors for this important disease. In fact, most of the studies investigating the etiology of CMV retinitis have dealt with CMV disease of nonophthalmic organs in non-AIDS immunosuppressive settings. Several of the hypotheses regarding risk factors in the current work have come from these studies.

DETAILED METHODOLOGY

Overview

A case–control study was conducted among patients in whom CMV retinitis had been diagnosed between 1990 and 1999 seen at the University of Ottawa Eye Institute and the Department of Ophthalmology of McGill University. Based on theoretical and practical grounds, the analysis of risk factors was divided into three sections: 1) clinical risk factors, 2) laboratory-based risk factors and 3) iatrogenic risk factors. Cases were patients with AIDS and CMV retinitis who had CD4 counts below 50 cells/μL at the time of diagnosis of retinitis. Controls were patients with AIDS without CMV retinitis who had a first CD4 count below 50 cells/μL, chosen from the same period as the cases. The inclusion criteria were: 1) HIV seropositivity, 2) a diagnosis of AIDS meeting the 1993 CDC definition of the disease[5] (HIV positive with at least one opportunistic infection, or HIV positive and an absolute CD4 count less than 200 cells/μL), 3) a CD4 count less

than 50 cells/μL at the time of enrolment in the study and 4) age 18 years or more.

Patients who met the eligibility criteria but were not followed in the study base for at least 3 months before being eligible to be a case or control were excluded.

Sampling and Study Base

All eligible cases who received a diagnosis of CMV retinitis in the two study centers in 1990–1999 were enrolled. Controls were sampled randomly from the study base at both institutions. The characteristics of the study base at the two institutions were slightly different. In eastern Ontario, most patients with advanced HIV disease are referred to the Immunodeficiency Service of the General Campus of the Ottawa Hospital. Most of these patients are then sent to the Uveitis Service of the University of Ottawa Eye Institute for eye examinations whenever symptoms occur or occasionally for CMV surveillance eye examinations. In western Quebec, one of the main referral sources for the Uveitis Service of the Royal Victoria Hospital is the Montreal Chest Hospital. Hence, the study base for the western Quebec patients consisted of patients with AIDS from the Montreal Chest Hospital who were sent to the Ophthalmology Department of the Royal Victoria Hospital for ocular evaluation. The geographic area of this primary study base for both hospitals together extends westward from Drummondville and Trois-Rivières, Que., through the greater metropolitan Montreal area and continues on to western Quebec and eastern Ontario, including the Pontiac region and the greater metropolitan Ottawa area. The area excludes the Kingston metropolitan area. The southern boundary is the USA border, and the northern boundary is the Laurentian region in Quebec and the Pembroke region in

Ontario. In eastern Ontario, almost all patients with AIDS and CD4 counts less than 50 cells/μL are seen at the University of Ottawa Eye Institute by one ophthalmologist (W.G.H.). In western Quebec, there was only one uveitis specialist during the study period, and so most patients with AIDS in this area would be referred to him, at the Royal Victoria Hospital, Montreal. However, it is possible that some patients would be seen by retina specialists at hospitals affiliated with the Université de Montréal, and therefore there would not be 100% referral in this area.

It is important to point out that the control group (patients with AIDS without CMV retinitis) was obtained from the same institutions over the same period as the cases.

Index Date

Cases were patients with AIDS and a diagnosis of CMV retinitis. The date of diagnosis of retinitis represented the index date, and risk factors for CMV retinitis were then determined relative to this index date. We also needed to determine an index date for the controls. The most appropriate index date for this group would have been the date corresponding to the same duration of HIV infection as for the cases. However, this could not be determined accurately in our study, since the exact time of HIV infection is typically difficult to ascertain because initial infection with HIV is asymptomatic or may be associated with a "flu"-like illness unrecognized by patient or physician. We therefore decided to restrict our study population — cases and controls — to subjects with CD4 counts below 50 cells/μL. Our assumption was that cases and controls would then be approximately matched for duration of HIV infection. Specifically, the date of the first

CD4 count recorded below 50 cells/μL represented the index date for controls.

Detailed Case Definition

A case of CMV retinitis was diagnosed by clinical examination based on the finding of slowly progressive hemorrhagic necrotic retinitis that followed the retinal blood vessels and in which the vitreous and anterior chambers were free of inflammatory cells. Although very occasionally this diagnosis can be confused at any one particular visit with *Toxoplasma* retinitis or zoster retinitis, one or two reexaminations every 3–4 days virtually always distinguishes between these diagnoses because of the lack of inflammatory cells in the vitreous (compared to toxoplasmosis) and the relatively slow progression (compared to zoster retinitis) of CMV retinitis. Therefore, the probability of misclassifying the diagnosis of a case is extremely small and would have a negligible effect on the measure of association.

EXPOSURES STUDIED

General Perspectives

Broadly speaking, there are three types of risk factors for CMV retinitis that have been studied or can be conceptually considered as possible risk factors for this condition. Examples of *clinical risk factors* include the patient's symptoms, the presence of retinal microinfarction, or cotton-wool spots, on clinical examination or previous opportunistic infections. By definition, clinical risk factors can be determined by history or physical examination at the index date. Examples of *laboratory-based risk factors* include

hematologic parameters, HLA type and CMV PCR. Examples of *iatrogenic risk factors* include the use of steroid medication or immunosuppressive drugs and the amount of blood transfused. The conceptual separation of these risk factors can be defended on several grounds. First, it is common for patients who present for care to fit into one of these risk factor strata. Some patients present with CMV retinitis as their index illness or one of their early illnesses in the course of their HIV infection. These patients would be most easily considered in the clinical risk factor stratum. Their eye findings and symptoms are likely to be the most important clinical variables on which to base etiologic decisions regarding screening frequency or prophylactic medications. Other patients present relatively late in the course of their illness. For these patients, the iatrogenic risk factors would be most important, as, typically, they would have received multiple treatments and medications. Laboratory-based risk factors are a separate entity from the other two types of risk factor even on theoretical grounds. One can study HLA type, for example, independently of the other risk factors. On a practical level, hundreds of risk factors were recorded for a final sample of 279 patients. Using one model for this number of exposure variables would be very imprecise. Even more important, reducing this model would force several variables, likely to be important clinically and practically, out of the model.

EXPOSURE DYNAMICS, INCLUDING PATTERN, DURATION AND RISK PERIOD

Definitions

1) Pattern: The pattern of exposure defines the frequency of the exposure that we considered significant for the study. Examples include a single episode, multiple episodes, a single exposure to a drug, a full course of a drug or a single laboratory test.

2) Duration: The duration of exposure was classified as short (less than 1 month) or long (ongoing for 1 month or more) for the purpose of the study.

3) Risk Period: The risk period is the time window we considered important for the study. We attempted to define the risk periods as precisely as possible from previous clinical work or the biology of the exposure. Short risk periods were considered to be 3 months, intermediate risk periods, 6 months, and long risk periods, 12 months or more.

Clinical Variables

1) Patient Symptoms (floaters/flashing lights/scotomas, pain/irritation): These easily documented symptoms and signs may provide clues to the early onset of CMV retinitis or its subclinical antecedent.[113] Since any positive finding may be significant, we used a binary distribution (yes/no or 0, 1 in the analysis) for the purpose of this study. These variables need to be of long duration, as a moment of transient irritation or one transient

floater would likely not signify ensuing disease. The risk period would need to be relatively recent, as these symptoms are likely to develop shortly before the clinical disease manifests. A risk period of 3 months was chosen.

Summary: Pattern: Single episode. Duration: Long. Risk period: 3 months.

2) Cotton-Wool Spots: Cotton-wool spots are microinfarctions of the retina and are transient.[146] Some authors feel that breakdown of the blood–ocular barrier reflects the beginning of the cascade for CMV retinal infection.[146] The exposure dynamics of cotton-wool spots are not known, and, to our knowledge, there are no previous studies examining them. Nevertheless, the most important exposure dynamic of this risk factor is likely the risk period: given the transient nature of retinal microinfarctions, the development of a cotton-wool spot long before the onset of CMV retinitis is most probably unimportant. The risk period should therefore be relatively short, and we chose 3 months. We also chose any cotton-wool spot as important. The duration of these insults is short.

Summary: Pattern: Single episode. Duration: Short. Risk period: 3 months.

3) Overall Infection Pattern: Some studies have demonstrated an increased rate of CMV retinitis in patients with concurrent or previous infections with bacteria or other viruses.[141,151,152] It is not clear whether this is a proxy for another risk factor or whether the co-infection is itself causal. Basic science of HIV and CMV infection supports this possibility, as upregulation of HIV replication occurs in the presence of CMV, HSV and

several bacteria.[34] If the basic science studies are correct, the upregulation would occur over a short period (as in vitro studies demonstrate almost instantaneous upregulation), and any infection would be important. Hence, a single episode of infection over a short period is likely to be etiologically relevant. Furthermore, the risk periods were not clearly defined in previous clinical studies.[141,152]

The disadvantage of using clinically based infections is that any nonclinical infection or silent infection, which may be etiologically relevant, would be missed. Nevertheless, it would not be possible to detect subclinical infections in a case–control study, and, in any case, their usefulness as exposure variables is questionable. The variables we studied were the crude binary variable "infection vs. no infection" and the continuous variable "total number of opportunistic infections."

Summary: Pattern: Any single documentation of infection in the risk period. Duration: Assumed short. Risk period: 3 months.

4) Specific Infections: A list of specific infections and one miscellaneous category were also recorded. The assumption was that it is conceivable that overall infection or number of infections may not be important, but a specific infection (such as HSV infection[140]) may be. The other assumptions in this category were the same as in "Overall Infection Pattern."

Summary: Pattern: Any single documentation of infection in the risk period. Duration: Assumed short. Risk period: 3 months.

5) Risk Factors for Acquisition of HIV Infection (homosexual transmission, heterosexual transmission, intravenous drug use): Superficially, this variable seems not to have biologic plausibility as a risk factor for CMV retinitis. However, clinical studies support mode of acquisition as an important risk factor for CMV disease.[141,144] Specifically, homosexual acquisition seems to be an important variable in previous studies. HIV mutations resulting in different strains[31] may be more or less resistant to anti-HIV medication. Hence, we hypothesized that mode of acquisition is likely a marker for a pool of strains of HIV or CMV that may be more pathogenic with respect to CMV retinitis. There may be some nondifferential misclassification of this variable, as one must rely exclusively on the patient's history for the information.

Summary: Pattern: One transmission event. Duration: Not applicable. Risk period: Not applicable.

Laboratory-Based Risk Factors

1) HLA Type: HLA type is a genetic allele and therefore constitutes a lifelong continuous exposure. We determined the HLA genotype using the lymphocyte microcytotoxicity technique.[185] This provides a simple, reproducible and sensitive assay for HLA-A, -B, -C and most -DR antigens on lymphocytes. The basis of the procedure is cytolysis mediated by specific antibody in the presence of complement. Since the majority of reagents currently in use have been operationally defined, the procedure is sensitive to minor alterations in the protocol. The protocol used is described in Appendix I.

2) CMV Infection: CMV infection (a binary variable) and quantitative CMV load (a continuous variable) were measured once in the risk period. This is important as a practical matter. It resembled the clinical situation of a periodic test's being used to estimate the risk of disease. The duration of exposure was assumed to be relatively constant throughout the risk period, although studies aimed at this question exclusively might demonstrate a more complicated risk function. The risk period is based on the biology of infection and previous clinical studies. The biology of infection would warrant an intermediate risk period, as it is clear that clinical infection does not occur immediately after biologic infection, likely at least in part owing to the latent aspect of infection described in the introduction.[82] Furthermore, previous clinical studies have demonstrated risk periods between 3 months and 1 year, with most at about 6 months.[148,158,162–170]

Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 6 months.

The protocol for CMV PCR[186] can be found in Appendix II.

3) HIV Copy Number: The HIV copy number is a continuous variable. The assumptions regarding single episode testing and constant duration are similar to those for CMV infection. HIV biology was described in detail in the introduction. Based on its latent stage[22] as well as chronic active stage, we chose an intermediate risk period.

Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 6

months.

The method used for HIV detection and quantification was the nucleic acid sequence-based amplification (NASBA) procedure,[187] summarized in Appendix III.

4) Hematologic Parameters (hemoglobin, hematocrit, leukocytes, platelets): To our knowledge, there are no detailed studies on the etiologic dynamics of hematologic parameters. Butler and Griedman[176] used a short etiologic period (2 months) to study hematologic parameters as risk factors for CMV retinitis. The biology of these variables as potential risk factors would also support a short etiologic period, as fluctuations can be great with a change in the clinical course. Furthermore, on a pragmatic level, since the results of these tests are variable and depend on many factors, a test in the far past would likely have little clinical relevance.
Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 3 months.

5) CD8 Count: CD8 counts have been studied as risk factors for CMV disease,[175] but these studies have been cross-sectional; hence, they do not help us with predicting the risk period. The biology of CD8 cell interaction with HIV (which could potentially alter the risk of CMV retinitis) suggests a relatively short risk period.[33,36]
Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 3 months.

6) Nutrition Variables (body mass index, serum albumin, total protein): Nutrition variables have not been studied to date. These risk factors have been generated from our observations. We have chosen a short risk period, as distant nutrition parameters are likely unrelated to outcome.

Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 3 months.

Iatrogenic Risk Factors

1) Steroid Use (binary use of steroid, binary use of prednisone, and their total amounts): Corticosteroids are well-known immunosuppressants.[188] They inhibit both the early (redness, edema, pain) and the late (leukocyte recruitment) components of the inflammatory response. One of their main mechanisms of action is to reduce the ability of neutrophils to adhere to capillary and capillary-like tissue in vitro and in vivo. Corticosteroids also modulate the activity of cytokines and other chemical mediators of inflammation. Corticosteroids seem to have a linear effect clinically. Small amounts equivalent to approximately 5 mg per day of prednisone are necessary to sustain life. Intermediate dosages, such as 20 mg per day, have a moderate therapeutic effect and a moderate side effect profile. Larger dosages, such as 80 mg per day, have a profound anti-inflammatory and immunosuppressive effect and a grave side effect profile. Higher dosages than this are used only in life-threatening situations. This linear trend also applies to eye inflammation, in which it is a well-known practice to reduce corticosteroid dosages in a linear way in order to maintain anti-inflammatory activity.[188] Only

occasional side effects of corticosteroids are idiosyncratic (eg aseptic necrosis of the femur). Steroids do not increase risk to patients immediately (days to weeks) after use; there is a therapeutic delay. Furthermore, since the dose is usually tapered, there is a delay to normalcy as well. Finally, in clinical studies examining this variable in similar clinical settings, such as CMV infection in organ transplant recipients, the risk period was 4 to 6 months.[180–182]

Summary: Pattern: Binary use and continuous use were studied (binary and continuous variables, including total dose). Duration: Long. Risk period: 6 months.

2) Chemotherapeutic Agents: Along the same reasoning as for steroids, we also studied other immunosuppressive chemotherapeutic agents. These agents invariably suppress the leukocyte count and were studied as potential iatrogenic risk factors. Less is known about the exposure dynamics of these agents at a clinical level compared to steroids. However, two groups have looked at these agents in this context, both in a binary exposure but with very different risk periods. Kirklin et al.[183] studied these agents as risk factors for CMV infection in cardiac transplant recipients with an etiologic period of 24 months, while Kraat et al.[153] studied them as risk factors for CMV infection in renal transplant recipients with a risk period of 1–3 months. The alkylating agents demonstrate immunosuppression for up to 8 weeks after cessation of therapy, with a rebound phenomenon for up to 2 more weeks.[189] The folic acid analogues demonstrate similar dynamics. The vinca alkaloids rebound quicker, at about 4 weeks. Hence, the basic science of these drugs support the use of a relatively short risk period.

Summary: Pattern: At least one full course of the drug. Duration: Long. Risk period: 3 months.

3) Radiotherapy: Some side effects of radiotherapy, including skin and mucous membrane necrosis, are very acute and typically occur in epithelial cells.[190] However, there are also late effects, such as fistula formation, nonhealing ulceration and radiation retinopathy, which tend to occur in slowly metabolizing tissues. The spectrum of radiation-induced secondary infections is therefore intermediate to long term. Furthermore, there is an exponential dose–response effect of radiotherapy for most of its biologic effects, including complications.

Summary: Pattern: Any full course of radiotherapy. Duration: Long. Risk period: 12 months.

4) Blood Transfusions: The explanation for the potential etiologic role of blood transfusions is not clear. It may be that blood transfusion is a surrogate for an infectious etiology. Based on this explanation, the choice of an exposure dynamic similar to that of the clinical infections would be wise. However, other factors may be at play, and in at least one clinical study[184] 6 months was used as the risk period when studying any CMV disease in patients with AIDS.

Summary: Pattern: Any single transfusion in the risk period and total number of transfusions in the risk period. Duration: Short. Risk period: 6 months.

Confounders

The confounders chosen were variables associated with the clinical variables under study that also independently increase or decrease the risk of retinitis. They included CD4 count, anti-HIV medications, anti-CMV medications, anti-HSV medications and hospital center.

1) CD4 Count: All patients had CD4 counts less than 50 cells/μL at enrolment in the study. However, based on our understanding of markers of disease severity, CD4 count before enrolment is the most important confounder in this study. It is associated with several exposures and is an extremely important predictor of CMV retinitis. The risk period is known to be relatively short.[142–144] This has been supported by studies of CD4 counts and function that seem to indicate significant improvement in immune function in the short term in patients receiving HAART.[101,102]

Summary: Pattern: Single episode (testing). Duration: Not applicable. Risk period: 3 months.

2) Anti-HIV Medications: This is a very large category. A detailed summary of the principal anti-HIV medications[191] is provided in Appendix IV. These medications are potentially associated with many exposure variables, and patients receiving them would be at reduced risk for CMV retinitis. The exact etiologic period is unknown, but given that patients typically receive these medications for the long term, if not life, a long etiologic period was assumed.

Summary: Pattern: At least one full course of drug. Duration: Continuous. Risk period: We measured treatments received in the 2 years before the index date.

3) Anti-CMV and Anti-HSV Medications: Anti-CMV medications used to treat nonocular CMV infections were controlled for, as they would be associated with predictors of CMV retinitis and would protect against CMV retinitis itself. The anti-HSV medication acyclovir has some anti-CMV activity[114] and therefore should be controlled for in a similar way as anti-CMV medications. The etiologic period for these agents is not clear but was assumed to be similar to that for anti-HIV medications.
Summary: Pattern: At least one full course of drug. Duration: Continuous. Risk period: 2 years.

4) Hospital Center: Because there are incalculable potential differences between the two hospital centers that may confound the exposure–disease association, hospital center was also controlled for.

SAMPLE SIZE AND POWER

The sample size in a case–control study with noncontinuous predictor and outcome variables can be determined by specifying alpha, beta, P1 (the proportion of cases expected to have been exposed to the risk factor) and P2 (the proportion of controls expected to have been exposed to the risk factor).[192] We used the following values:

alpha = 0.05 (two tailed)

beta = 0.20 (power = 80%)

The power of the study was based on corticosteroids as the principal risk factor for CMV retinitis.

P2: The proportion of controls expected to have the risk factor is low, somewhere between 5–15%[179,180]. Let us use 10% for our sample size estimation.

P1: To calculate P1, we need to specify the OR we would like to detect. Often, other studies can provide guidelines as to the OR that can be expected. This is difficult to estimate a priori, but two groups that looked at corticosteroids as a risk factor for CMV disease found ORs of 12.4 and 5.9 respectively.[179,180] In the former study, multivariate analysis was used appropriately, but the study concerned intestinal transplantation. The latter study looked at CMV disease in patients with AIDS, but the analysis was very elementary. The OR of 5.9 is the more conservative estimate, but even this value is quite high and is probably unrepresentative of the OR that we would expect to find in our study. Let us use an OR of 3.0 to be both conservative and realistic.

$P1 = OR \times P2/[(1 - P2) + (OR \times P2)] = 3.0 \times 0.10/(0.90 + 0.30) = 0.25$

According to Table 13.B of Hulley and Cummings,[192] **N = 99 cases and 99 controls.**

In the event that 99 cases could not be found who met our inclusion/exclusion criteria and/or did not have sufficient information about variables available, we had a contingency plan. To maintain the same power, we used a control to case ratio of 4:1 (in general, the amount of benefit of using a ratio greater than 4:1 is minimal). To determine the number of cases needed, we used the following formula:[192]

$[(c + 1)/2c]n$

where c is the number of controls per case and n is the number of cases.

$[(4 + 1)/(2 \times 4)]n = 5/8n$

$5/8 \times 99 =$ **62 cases and 248 controls.**

This provided us with a range of cases and controls needed.

DATA MANAGEMENT AND QUALITY CONTROL

Data were recorded on a standardized form that was created to ensure proper recording of the outcome, its date and all exposure variables, including their dates and especially the time relative to the index date. After the data for 10 subjects were recorded, the form was revised as necessary. Two researchers recorded data for every patient. In cases of disagreement, the principal investigator acted as the adjudicator. The data forms were inspected for completeness, errors and inconsistencies, and additions or deletions were performed as required. The data were then entered into a database using Microsoft Excel software. Range checks were performed on all individual variables. Logic checks were also incorporated so that errors could be detected immediately and before processing. To ensure data integrity and accuracy, a double data entry protocol was adopted for all variables. This required that data entry be performed a second time using identical error verification parameters. Error lists were generated after every 20 patients and corrected.

Data security and confidentiality were ensured by storing all data in a locked office. All staff were briefed on confidentiality procedures. Once data editing was complete, all data sets identified patients by number only to ensure confidentiality. Data were backed

up daily.

STATISTICAL ANALYSIS

Univariate statistics were obtained to check for implausible values as a final data editing step. Bivariate analyses were then conducted for all exposure variables using the unpaired *t*-test for continuous variables (or the Wilcoxon rank-sum test if non-normal) and the chi-square test for discrete variables. Logistic regression was used for multivariate analyses. A p value of 0.10 or less in the bivariate analyses was used as a cut-off for inclusion in the multivariate analyses. Next, the assumption of linearity of the logit was checked in a bivariate analysis for all exposure and confounding variables individually. This was done by graphing the ORs against each of the midpoints of multiple equal-interval variables. For example, CD4 count was divided into four approximately equal intervals: 0–12, 13–24, 25–36, and 37–49 cells/μL. Then the OR pertaining to case status corresponding to each interval was graphed against the midpoint of each interval (6, 18.5, 30.5 and 43.0 cells/μL respectively). From this plot, linearity of the logit was assessed. Next, a correlation matrix was obtained for all exposure variables to assess multicollinearity. Any correlation coefficient greater than 0.8 was recorded, and if a multicollinearity problem arose in the multivariate model, an algorithm for omitting one variable was used. This algorithm consisted of first keeping the variable that was a more biologically plausible exposure, then keeping the variable with fewer missing data, and finally keeping the variable with less measurement error as determined a priori.

For confounding, we determined a priori that the model would include CD4 count,

hospital site (city), at least one anti-HIV medication variable, at least one anti-CMV medication variable and at least one anti-HSV medication variable. Although these five categories of confounding variables were chosen a priori, it was not clear for anti-HIV medication, anti-CMV medication and anti-HSV medication which specific representation of the variable to use (e.g., for anti-HIV medication, specific anti-HIV medication, total dose of anti-HIV medication etc.). Incorporating all possible representations of each variable would not be realistic because of precision limitations. Therefore, logistic regression was used in the same way as described in this section previously to identify at least one variable for each of the preselected drug treatment categories. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. These confounding variables were then introduced into the main exposure models.

Statistical techniques were not used to reduce the number of confounders in the model but were used to reduce exposure variables for the final model. Model reduction for the exposure variables was performed with the likelihood ratio test. If data were missing, cases and controls were deleted in the multivariate analysis. However, when the model was reduced or expanded, these data were then used again if the variable with the missing data was eliminated from the model. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. The performance of the final model against chance and its fit were then

determined.

The study protocol was submitted to and met the requirements of the Research Ethics Boards of both the Ottawa Hospital and the Royal Victoria Hospital/Montreal Chest Hospital.

APPENDIX I: PROTOCOL FOR HLA TYPING USING THE LYMPHOCYTE MICROCYTOTOXICITY TECHNIQUE[185]

A patient's lymphocyte suspension was prepared using a count of 1 - 3 × 10^6/mL in RPMI-1640. The following sequence was then carried out: The concentration of cell suspension was adjusted to 1 - 3 × 10^6 cells/mL and thoroughly mixed. The typing trays were thawed immediately before use, and 1 μL of the thoroughly mixed cell suspension was added to each well dropping on top of oil, care being taken not to touch serum with the needle tip in order to avoid carryover. It was sometimes necessary to mix cells and serum samples thoroughly with a wire. The solutions were then incubated at room temperature (21–23°C) for 30 minutes. Next, 5 μL of pretested rabbit complement was added to each well, and the solutions were incubated at room temperature (21–23°C) for 60 minutes. The solutions were then stained by adding 2 μL of eosin-Y directly to the well or by soft-drop technique, and 4–8 μL of formalin was added directly into the well by soft-drop technique.

DNA was prepared, and polymerase chain reaction (PCR) primer tubes were labeled. A tube was prepared for each patient, for DNA dilution and for control. A total of 96 μL of PCR solution was pipetted into each patient tube, and 2.5 μL of Taq polymerase was added into the PCR solution. For the control, 6 μL of PCR solution, 0.15 μL of Taq and 3.9 μL of autoclaved ddH20 were used. In the biohazard hood, the required DNA was added to finish making the dilution to give a final concentration of 40–60 ng/μL for DR-SSP and 80 ng/μL for DQ-SSP. Then 62 μL of the appropriate DNA dilution was added

into the PCR solution/Taq mix. The PCR solution/Taq/DNA was mixed well by vortexing, and 5 μL was placed into each of the primer tubes. Next, 10 μL of PCR solution/Taq/ddH20 was mixed into the control tube, and the tubes were given a quick spin in the eppendorf centrifuge. The sample tray was placed into the heating block of the Cetus 9600 Thermalcycler. After amplification was finished, 10 μL of PCR product was loaded directly onto a 2% agarose gel and was run at 145 V for 25–30 minutes.

For the HLA-DR and -DQ subtypes, a restriction fragment length polymorphism technique was used. The genes that code for the DR1 to DR18, DR52, DR53 and DQ proteins have been sequenced on chromosome 6. Within the gene sequences there are many common bases among the different groups, but there are also small base differences. These differences are what specify the DR and DQ subtypes. Primers have been developed that amplify only those areas in the sequences that give each type its specificity. These primers are used as a screening tool to classify a patient's DR and DQ type into a broad category. Once this is achieved, subgroups can be determined using other primers and restriction fragment length polymorphisms.

APPENDIX II: PROTOCOL FOR CYTOMEGALOVIRUS POLYMERASE CHAIN REACTION TECHNIQUE[186]

Cytomegalovirus (CMV) PCR was performed as follows. The probe/primer set was intended for the amplification and detection of a 435-base-pair region of the major immediate early gene of CMV. The materials used were: 1 × 200 μL CMV Primer MIE-5, 25 pmol/μL unbiotinylated primer, 1 × 200 μL CMV Primer MIE-4B, 25 pmol/μL primer biotinylated on the 5′ end, 1 × 100 μL CMV RNA Probe, 50X RNA probe complementary to the biotinylated strand of the amplification product in buffered solution, 1 × 200 μL CMV Positive Assay Control and PCR product generated from primers MIE-5 and MIE-4B. The reagents used were GeneAmp® PCR Core Reagents, Ampliwax™ PCR Gem 100 and Digene *SHARP SIGNAL* System Assay for PCR Products as well as a positive and negative PCR control. A DNA Thermal Cycler (Perkin-Elmer, catalogue no. N801-0150) was used, and reagents were stored at −20°C.

Quality control was performed by testing primers by PCR amplification of 0, 10^2 and 10^4 copies of target DNA according to the amplification protocol. Efficient amplification was verified by visual analysis of the amplification products on an ethidium bromide-stained agarose gel and by analysis with the *SHARP SIGNAL* System Assay for PCR Products. Probes were tested in the *SHARP SIGNAL* System with a known amount of PCR amplification product. Positive Assay Controls were tested with the appropriate probe in the *SHARP SIGNAL* System and had to meet a minimum threshold A_{405} value to be acceptable. The PCR test procedure

involved a final PCR volume per tube of 100 μL. Each tube had the following reagents: Taq polymerase (0.5 μL), dATP (10mM), dTTP (10mM), dCTP (10mM), dGTP (10mM), Primer A (25μM), Primer B (25μM), $MgCl_2$ (25mM), 10X PCR Buffer II (2.5 μL) and sterile dH_2O (6.5 μL). The tubes were placed in the Thermal Cycler and were run at 80°C for 5 minutes and at 4°C for the hold cycle.

APPENDIX III: METHOD OF DETECTION AND QUANTIFICATION OF HIV[187]

HIV-1 was quantitated by the nucleic acid sequence-based amplification (NASBA) procedure. First, nucleic acid was isolated in the following steps: The buffer was washed, and lysis tubes were thawed at 37°C until no crystals were present. The tubes were then vortexed and quick spun. Next, 10 to 200 μL of plasma was added to the lysis buffer and vortexed. The lysis tubes were allowed to sit at room temperature while the Q-calibrations were prepared. The Q-sphere was reconstituted with 220 μL of elution buffer and vortexed lightly. The lysis tubes were spun, and 20 μL of calibrator was added to each lysis buffer tube and vortexed. Next, 50 μL of vortexed silica was added to each tube and was revortexed and spun. The buffer was then removed with a vacuum changing tip, and 1000 μL of wash buffer was added to each tube and was vortexed and spun. Next, the wash buffer was removed, and these steps were repeated for a total of five washes. After the final wash with acetone, the solution was spun at 10,000*g* for exactly 10 seconds to pellet silica. All the acetone was removed. The silica was dried in a heat block at 56°C for 12 minutes. The tubes were dried thoroughly, and 50 μL of elution buffer was added and vortexed. The tubes were incubated in a heat block at 56°C for 10 minutes and vortexed after 5 minutes. The silica/lysis tubes were spun for 3 minutes at 10,000*g*. Then 5 μL of supernatant was removed. The next major step was nucleic acid amplification. The enzyme and primer diluent were thawed, and 45 μL of enzyme diluent

was added to the enzymes and shaken at room temperature. The primer diluent was then prepared, and 120 µL of primer diluent was added to the primer sphere and vortexed. Next, 10 µL of primer mix was added to each reaction tube containing a 5-µL aliquot, and the tubes were placed in a 65°C heat block for 5 minutes and then a 41°C heat block for 5 minutes. Finally, 5 µL of enzymes was added to each tube, and the tubes were incubated for 90 minutes. The next major step was nucleic acid hybridization. Four ruthenium-labeled probes were prepared (WT, Qa, Qb, Qc). Forty-one culture tubes were also prepared. A total of 20 µL of WT mix was added to each of the first 11 tubes and used as a negative control. Qa, Qb and Qc tubes were prepared accordingly. Next, 10 eppendorf tubes were prepared, and the volume of detection diluent specified was added. Then 5 µL of amplified sample was added to each diluent tube and vortexed, and 5 µL of diluted sample was added to each of the four culture tubes containing the above-mentioned mixes. Finally, 5 µL of detection diluent was added to the assay negative tube. All the tubes were incubated in a 41°C water bath for 30 minutes and shaken every 10 minutes. The final step was nucleic acid detection. The reference solution was vortexed, and 300 µL was placed in a separate tube. After hybridization, 300 µL of assay buffer was added to each tube using an eppendorf repeater and 5-mL tip. The carousel was then loaded, with the reference tube first, the assay negative second, then WT, Qa, Qb, Qc tubes for each sample. The copy number was calculated by means of a computer algorithm.

APPENDIX IV: SUMMARY OF ANTI-HIV DRUGS[191]

A. Nucleoside inhibitors of the viral enzyme reverse transcriptase

1. Didanosine (ddI)

1.1 Mechanism of Action: Phosphorylation of ddI by cellular enzymes gives the active metabolite, dideoxyadenosine triphosphate (ddATP). The latter inhibits HIV reverse transcriptase through competition with endogenous deoxyadenosine triphosphate (dATP) for binding to the active site of the enzyme. In addition, ddATP is a substrate for reverse transcriptase, and the resulting nucleoside, dideoxyadenosine (ddA), is incorporated into the growing DNA chain. This prevents further chain extension and aborts proviral DNA synthesis.

1.2 Possible Serious Adverse Effects: Pancreatitis is the major toxic clinical effect. Peripheral neuropathy occurs in patients treated with didanosine; the frequency appears to be dose related. Liver failure and retinal depigmentation can also occur.

1.3 Oral Dosage:

Adults:

Patient Weight	Tablets	Buffered Powder
≥ 60 kg	200 mg bid	250 mg bid
< 60 kg	125 mg bid	167 mg bid

2. Lamivudine (3TC)

2.1 Mechanism of Action: Lamivudine is phosphorylated intracellularly to its active metabolite, lamivudine triphosphate (L-TP), which inhibits HIV reverse transcriptase via viral DNA chain termination. In addition, L-TP inhibits both RNA- and DNA-dependent DNA polymerase activities of reverse transcriptase.

2.2 Possible Serious Adverse Effects: Pancreatitis, especially in children.

2.3 Oral Dosage:

Adults and Adolescents (≥ 12 years): 150 mg bid, with zidovudine 200 mg tid. For adults with body weights below 50 kg, the recommended oral dosage of lamivudine is 2 mg/kg bid administered in combination with zidovudine.

Children: 4 mg/kg bid (up to a maximum of 150 mg bid) administered with zidovudine 180 mg/m^2 every 6 hours.

3. Stavudine (d4T)

3.1 Mechanism of Action: Stavudine is converted to the active metabolite, the triphosphate, by cellular kinases. It is a potent competitive inhibitor (competing with the natural substrate thymidine triphosphate) of HIV reverse transcriptase. In addition, stavudine triphosphate is used by HIV reverse transcriptase in vitro for incorporation into the nascent DNA chain and functions as a DNA chain terminator

in vitro.

3.2 Possible Serious Adverse Effects: Peripheral neuropathy is the major toxic clinical effect.

3.3 Oral Dosage:

Adults: 15 or 30 mg bid if patient weighs less than 60 kg, and 20 or 40 mg bid if patient weighs 60 kg or more.

4. Zalcitabine (ddC)

4.1 Mechanism of Action: Zalcitabine is converted to its active metabolite, dideoxycytidine 5′-triphosphate (ddCTP), by cellular enzymes. The latter serves as an alternative substrate to deoxycytidine triphosphate (dCTP) for HIV reverse transcriptase. The incorporation of ddCTP into a growing DNA chain leads to premature chain termination, inhibiting the in vitro replication of HIV-1 by inhibition of viral DNA synthesis. ddCTP serves as a competitive inhibitor of the natural substrate, dCTP, for the active site of the viral reverse transcriptase and thus further inhibits viral DNA synthesis.

4.2 Possible Serious Adverse Effects: Peripheral neuropathy is the major toxic effect. Pancreatitis, hepatic toxicity, oral ulcers, esophageal ulcers, cardiomyopathy with congestive heart failure and anaphylactoid reactions can also occur.

4.3 Oral Dosage: 0.75 mg tid

5. Zidovudine (AZT)

5.1 Mechanism of Action: Zidovudine is converted to the monophosphate and further converted to the diphosphate by cellular thymidine kinase and cellular thymidylate kinase respectively. The diphosphate is then converted to the active metabolite, the triphosphate, by other cellular enzymes. Zidovudine triphosphate interferes with HIV reverse transcriptase and thus inhibits viral replication. In vitro, zidovudine triphosphate has been shown to be incorporated into growing chains of DNA by viral reverse transcriptase. When incorporation by the viral enzyme occurs, the DNA chain is terminated.

5.2 Possible Serious Adverse Effects:

- Bone marrow suppression: Anemia and granulocytopenia are the most significant adverse events observed. There have also been reports of pancytopenia, which was reversible in most instances after therapy with the drug was stopped.
- Myopathy
- Lactic acidosis/severe hepatomegaly with steatosis
- Other: Pancreatitis, sensitization reactions, vasculitis and seizures have been rare.
- Changes in skin pigmentation have been associated with the use of zidovudine.

5.3 Oral Dosage (capsules and syrup):

Adults:

- Asymptomatic HIV infection: 100 mg every 4 hours while awake for a total daily dosage of 500 mg.
- Symptomatic HIV disease: 100 mg every 4 hours around the clock for a total daily dosage of 600 mg.
- In combination with zalcitabine: 200 mg of zidovudine + 0.75 mg of zalcitabine administered concomitantly tid. Children (3 months to 12 years): 180 mg/m^2 every 6 hours.

B. Protease inhibitors

Mechanism of Action: Proteolytic cleavage of the polypeptide precursor to the viral capsid proteins and reverse transcriptase of HIV type 1 by the enzyme HIV-1 protease is crucial to the production of mature infectious virions. Protease inhibitors inhibit the enzyme HIV-1 protease, resulting in the production of virions that are noninfectious and immature in appearance. HIV type 2 and simian immunodeficiency virus are also inhibited, although higher drug concentrations are required for inhibition of these strains.

Oral Dosage and Common Adverse Effects:

Drug	Dosage	Common Adverse Effects
Saquinavir mesylate	600 mg tid	Diarrhea and abdominal discomfort; asymptomatic elevations in levels of transaminases and creatine phosphokinase
Ritonavir	600 mg bid	Nausea, vomiting, diarrhea, altered taste, and circumoral and peripheral paresthesia
Indinavir sulfate	800 mg tid	Insomnia, dry throat and dry skin; asymptomatic elevation in levels of bilirubin; nephrolithiasis
Nelfinavir	750 mg tid	Diarrhea, asthenia, headache and hypertension

REFERENCES

1. Spector SA, McKinley GF, Lalezari JP, et al. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. *N Engl J Med* 1996;334:1491-1497.
2. Anderson RD, Griffy KG, Jung D, et al. Ganciclovir absolute bioavailability and steady-state pharmacokinetics after oral administration of two 3000 mg/d dosing regimens in HIV and CMV seropositive patients. *Clin Ther* 1995;17:425-432.
3. Masur H, Whitcup SM, Cartwright C, et al. Advances in the management of AIDS-related cytomegalovirus retinitis. *Ann Intern Med* 1996;125(2):126-136.
4. Fauci AS. The AIDS epidemic: considerations for the 21st century. *N Engl J Med* 1999;341(14):1046-1050.
5. Centers for Disease Control and Prevention. *HIV/AIDS Surveillance Report* 1993;5(3):1-20.
6. Selik RM, Chu SY, Buehler JW. HIV infection as leading cause of death among young adults in U.S. cities and states. *JAMA* 1993;269:2991-2994.
7. Mann JM, Tarantola DJM. HIV 1998: the global picture. *Sci Am* 1998;279:62-63.
8. Rosenberg PS. Scope of the AIDS epidemic in the United States. *Science* 1995;270:1372-1375.
9. Centers for Disease Control and Prevention. *HIV/AIDS Surveillance Report* 1998;10(2):1-43.
10. Gottlieb MS. AIDS — past and future. *N Engl J Med* 2001;344(23):1788-1791.
11. Pisani E. AIDS into the 21st century: some critical considerations. *Reprod Health Matters* 2000;8(15):63-76.

12. Piot P. "Bridging the gap." Conference Proceedings of the Opening ceremony from *12th World AIDS Conference*. Geneva, 1998.

13. DeCock KM. AIDS — the leading cause of adult death in the west African city of Abidjan, Ivory Coast. *Science* 1990;249:793-796.

14. *Confronting AIDS: Public Priorities in a Global Epidemic*. World Bank policy research report. New York: Oxford University Press; 1997. p. 1-353.

15. American Academy of Pediatrics Committee on Pediatric AIDS. Surveillance of pediatric HIV infection. *Pediatrics* 1998;101(2):315-319.

16. American Academy of Pediatrics Committee on Pediatrics AIDS. Planning for children whose parents are dying of HIV/AIDS. *Pediatrics* 1999;103(2):509-511.

17. Department of Economic and Social Affairs of the United Nations Secretariat. *The Demographic Impact of HIV/AIDS*. New York: The Department; 1999. p. 1-44.

18. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992;41(RR-17):1-19.

19. Klatzmann D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* 1984;312:767-768.

20. Picker LJ, Maino VC. The CD4(+) T cell response to HIV-1. *Curr Opin Immunol* 2000;12(4):381-386.

21. Dewhurst SL, da Cruz RL, Whetter L. Pathogenesis and treatment of HIV-1 infection: recent developments (Y2K update). *Front Biosci* 2000;5:D30-D49.

22. Luciw PA, Potter SJ, Steimer K, et al. Molecular cloning of AIDS-associated

retrovirus. *Nature* 1984;312:760-763.

23. Leonard R, Zagury D, Desportes I, et al. Cytopathic effect of human immunodeficiency virus in T4 cells is linked to the last stage of virus infection. *Proc Natl Acad Sci U S A* 1988;85:3570-3574.

24. Koga Y, Lindstrom E, Fenyo EM, et al. High levels of heterodisperse RNAs accumulate in T cells infected with human immunodeficiency virus and in normal thymocytes. *Proc Natl Acad Sci U S A* 1988;85:4521-4525.

25. Hoxie JA, Alpers JD, Rackowski JL, et al. Alterations in T4 (CD4) proteins and mRNA synthesis in cells infected with HIV. *Science* 1986;234:1123-1127.

26. Sodroski J, Goh WC, Rosen C, et al. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature* 1986;332:470-474.

27. Losa GA, Graber R. Spontaneous apoptosis, oxidative status and immunophenotype markers in blood lymphocytes of AIDS patients. *Anal Cell Pathol* 2000;21(1):11-20.

28. Gupta S, Safai B. Deficient autologous mixed lymphocyte reaction in Kaposi's sarcoma associated with deficiency of Leu-3+ responder T cells. *J Clin Invest* 1983;71:296-300.

29. Kutza J, Hayes MP, Clouse KA. Interleukin-2 inhibits HIV-1 replication in human macrophages by modulating expression of CD4 and CC-chemokine receptor-5. *AIDS* 1999;13(4):527-528.

30. Clayton LK, Sieh M, Pious DA, et al. Identification of human CD4 residues affecting class II MHC versus HIV-1 gp120 binding. *Nature* 1989;339:548-551.

31. Parren PW, Moore JP, Burton DR, et al. The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity. *AIDS* 1999;13:S137-S162.

32. Wood GS. The immunohistology of lymph nodes in HIV infection: a review. *Prog AIDS Pathol* 1990;2:25-32.

33. Cloyd MW, Chen JJ, Wang I. How does HIV cause AIDS? The homing theory. *Mol Med Today* 2000;6(3):108-111.

34. Gendelman HE, Phelps W, Feigenbaum L, et al. Trans-activation of the human immunodeficiency virus long terminal repeat sequence by DNA viruses. *Proc Natl Acad Sci U S A* 1986;83:9759-9763.

35. Tomaras GD, Lacey SF, McDanal CB, et al. CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. *Proc Natl Acad Sci U S A* 2000;97(7):3503-3508.

36. Brodie SJ, Patterson BK, Lewinsohn DA, et al. HIV-specific cytotoxic T lymphocytes traffic to lymph nodes and localize at sites of HIV replication and cell death. *J Clin Invest* 2000;105(10):1407-1417.

37. Crowe S, Mills J, McGrath MS. Quantitative immunocytofluorographic analysis of CD4 surface antigen expression and HIV infection of human peripheral blood monocyte/macrophages. *AIDS Res Hum Retroviruses* 1987;3:135-145.

38. Ho DD, Rota TR, Schooley RT, et al. Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. *N Engl J Med* 1985;313:1493-1497.

39. Haverkos HW. Assessment of therapy for *Pneumocystis carinii* pneumonia: PCP Therapy Project Group. *Am J Med* 1984;76:501-508.

40. Centers for Disease Control. Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for adults and adolescents infected with human immunodeficiency virus. *MMWR Recomm Rep* 1992;41(RR-4):1-11.

41. Holland GN, Gottlieb MS, Yee RD, et al. Ocular disorders associated with a new severe acquired cellular immunodeficiency syndrome. *Am J Ophthalmol* 1982;93:393-402.

42. Kestelyn P, Lepage P, Perre PVD. Perivasculitis of the retinal vessels as an important sign in children with AIDS-related complex. *Am J Ophthalmol* 1985;100:614-615.

43. Kestelyn P, Van de Perre P, Rouvroy D, et al. A prospective study of the ophthalmologic findings in the acquired immune deficiency syndrome in Africa. *Am J Ophthalmol* 1985;100:230-238.

44. Newman NM, Mandel MR, Gullett J, et al. Clinical and histologic findings in opportunistic infections. *Arch Ophthalmol* 1983;101:396-401.

45. Newsome DA, Green WR, Miller ED, et al. Microvascular aspects of acquired immune deficiency syndrome retinopathy. *Am J Ophthalmol* 1984;98:590-601.

46. Forster DJ, Dugel PU, Frangieh GT, et al. Rapidly progressive outer retinal necrosis in the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1990;110:341-348.

47. Margolis TP, Lowder CY, Holland GN, et al. Varicella–zoster virus retinitis in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol*

1991;112:119-131.

48. Sellitti TP, Hwang AJ, Schiffman J, et al. Association of herpes zoster ophthalmicus with acquired immunodeficiency syndrome and acute retinal necrosis. *Am J Ophthalmol* 1993;116:297-301.

49. Berger BB, Egwuagu CE, Freeman WR, et al. Miliary toxoplasmic retinitis in acquired immunodeficiency syndrome. *Arch Ophthalmol* 1993;111:373-376.

50. Cochereau-Massin I, Le Hoang P, Lautier-Fraw M, et al. Ocular toxoplasmosis in human immunodeficiency virus-infected patients. *Am J Ophthalmol* 1992;114:130-135.

51. Holland GN, Engstrom RE, Glastow BJ, et al. Ocular toxoplasmosis in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1988;106:653-667

52. Parke DW, Font RL. Diffuse toxoplasmic retinochoroiditis in a patient with AIDS. *Arch Ophthalmol* 1986;104:571-575.

53. Wiess A, Margo CE, Ledford DK, et al. Toxoplasmic retinochoroiditis as an initial manifestation of the acquired immune deficiency syndrome. *Am J Ophthalmol* 1987;103:248-249.

54. Rao NA, Zimmerman PL, Boyer D, et al. A clinical histopathologic and electron microscopic study of *Pneumocystis carinii* choroiditis. *Am J Ophthalmol* 1989;107:218-228.

55. Foster RE, Lowder CY, Meisler DM, et al. Presumed *Pneumocystis carinii* choroiditis: unifocal presentation, regression with intravenous pentamidine, and

choroiditis recurrence. *Ophthalmology* 1991;98:1360-1365.

56. Holland GN, McArthur LJ, Roos RY. Choroidal pneumocystosis. *Arch Ophthalmol* 1991;109:1454-1459.

57. Koser MW, Jampol LM, MacDonell K. Treatment of *Pneumocystis carinii* choroidopathy. *Arch Ophthalmol* 1990;108:1214-1219.

58. Rosenwasser GOD, Greene WH. Simultaneous herpes simplex type I and II keratitis in acquired immunodeficiency syndrome. *Am J Ophthalmol* 1992;113:102-103.

59. Shuler JD, Engstrom RE Jr, Holland GN. External ocular disease and anterior segment disorders associated with AIDS. *Int Ophthalmol Clin* 1989;29:98-104.

60. Blodi BA, Johnson MW, McLeish WM, et al. Presumed choroidal tuberculosis in a human immunodeficiency virus affected host. *Am J Ophthalmol* 1989;108:606-607.

61. Centers for Disease Control. Primary and secondary syphilis — United States, 1981–1990. *MMWR Morb Mortal Wkly Rep* 1991;40:314-315, 321-323.

62. Beauvais DA, Michelson JB, Seybold ME, et al. Retroviruses and their play-pals. *Surv Ophthalmol* 1989;34:59-64.

63. Becerra LI, Ksiazek SM, Savino PJ, et al. Syphilitic uveitis. *Am J Ophthalmol* 1989;96:1727-1730.

64. Bouisse V, Cochereau-Massin I, Jobin D, et al. Uvéite syphilitique et infection par le virus de l'immunodéficience humaine. *J Fr Ophtalmol* 1991;14:605-609.

65. Hungerbuhler U, Kulstrunk M, Osusky R. Uveitis luetica bein einen AIDS-patienten. *Schweiz Med Wochenschr* 1988;118:1761-1766.

66. Jabs DA, Green WR, Fox R, et al. Ocular manifestations of acquired immune

deficiency syndrome. *Ophthalmology* 1989;96:1092-1099.

67. Shuler JD, Holland GN, Miles SA, et al. Kaposi sarcoma of the conjunctiva and eyelids associated with the acquired immunodeficiency syndrome. *Arch Ophthalmol* 1989;197:858-862.

68. Centers for Disease Control. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. *MMWR CDC Surveill Summ* 1987;36(suppl 1S):1S-5S.

69. Margolis TP, Milner MS, Shama A, et al. Herpes zoster ophthalmicus in patients with human immunodeficiency virus infection. *Am J Ophthalmol* 1998;125(3):285-291.

70. Hodge WG, Seiff SR, Margolis TP. Ocular opportunistic infection incidences among patients who are HIV positive compared to patients who are HIV negative. *Ophthalmology* 1998;105(5):895-900.

71. Dugel PU, Rao NA, Forster DJ, et al. *Pneumocystis carinii* choroiditis after long-term aerolized pentamidine therapy. *Am J Ophthalmol* 1990;110:113-117.

72. Freeman WR, Gross JG, Labelle J, et al. *Pneumocystis carinii* choroidopathy: a new clinical entity. *Arch Ophthalmol* 1989;107:863-867.

73. Wasserman L, Haghighi P. Otic and ophthalmic pneumocystosis in AIDS. Report of a case and review of the literature. *Arch Pathol Lab Med* 1992;116(5):500-503.

74. Landini MP, Michelson S. Human cytomegalovirus proteins. *Prog Med Virol* 1988;35:152-185.

75. Knowles WA. In-vitro cultivation of human cytomegalovirus in thyroid epithelial

cells. *Arch Virol* 1990;50:119-124.

76. Kinney JS, Onorato IM, Steward JA, et al. Cytomegaloviral infection and disease. *J Infect Dis* 1985;151:772-774.

77. Dworsky ME, Welch K, Cassady G. Occupational risk for primary cytomegalovirus infection among pediatric health-care workers. *N Engl J Med* 1983;309:950-953.

78. Cox F, Meyer D, Hughes WT. Cytomegalovirus in tears from patients with normal eyes and with acute cytomegalovirus chorioretinitis. *Am J Ophthalmol* 1975;80:817-824.

79. Collins TM, Quirk MR, Jordan MC. Biphasic viremia and viral gene expression in leukocytes during acute cytomegalovirus infection of mice. *J Virol* 1994;68:6305-6311.

80. Saltzman RL, Quirk MR, Jordan MC. High levels of circulating cytomegalovirus DNA reflect visceral organ disease in viremic immuno-suppressed patients other than marrow recipients. *J Clin Invest* 1992;90:1832-1838.

81. Dworsky M, Yow M, Stagno S, et al. Cytomegalovirus infection of breast milk and transmission in infancy. *Pediatrics* 1983;72:295-299.

82. Ho M, editor. Cytomegalovirus. In: *Biology of Infection*. 2nd ed. New York: Plenum; 1991. p. 1-66.

83. Grundy JE, Ayles HM, McKeating JA, et al. Enhancement of class 1 HLA antigen expression by cytomegalovirus: role in amplification of virus infection. *J Med Virol* 1988;25:483-495.

84. Scholz M, Hamann A, Blaheta RA, et al. Cytomegalovirus and interferon related

effects on human endothelial cells: cytomegalovirus infection reduces upregulation of HLA class II antigen expression after treatment with interferon-gamma. *Hum Immunol* 1992;35:230-238.

85. Michelson S, Alcami J, Kim SF, et al. Human cytomegalovirus infection induces transcription and secretion of transforming growth factor beta 1. *J Virol* 1994;68:5730-5737.

86. Rasmussen L, Matkin C, Spaete R, et al. Antibody response to human cytomegalovirus glycoproteins gB and gH after natural infection in humans. *J Infect Dis* 1991;164:835-842.

87. Lafemina RL, Hayward GS. Differences in cell-type specific blocks to immediate early gene expression and DNA replication of human, simian and murine cytomegalovirus. *J Gen Virol* 1988;69:355-374.

88. Marshall GS, Stout GG, Knights ME, et al. Ontogeny of glycoprotein gB-specific antibody and neutralizing activity during natural cytomegalovirus infection. *J Med Virol* 1994;43:77-83.

89. Rao NA, Zhang J, Ishimoto S. Role of retinal vascular endothelial cells in development of CMV retinitis. *Trans Am Ophthalmol Soc* 1998;96:111-126.

90. Scholz M, Blaheta RA, Vogel J, et al. Cytomegalovirus-induced transendothelial cell migration: a closer look at intercellular communication mechanisms. *Intervirology* 1999;42(5-6):350-356.

91. Cowdry A. The problem of intranuclear inclusions in virus disease. *AMA Arch Pathol* 1934;18:525-542.

92. Holland GN, Pepose JS, Simons KB, et al. Ocular disease in the acquired immune deficiency syndrome: clinicopathological correlations. In: Secchi AG, Fregona IA, editors. *Modern Trends in Immunology and Immunopathology of the Eye*. Milan: Saunders; 1989. p. 1233-1247.

93. Montaner JS, Le T, Hogg R, et al. The changing spectrum of AIDS index diseases in Canada. *AIDS* 1994;8(5):693-696.

94. Munoz A, Schrager LK, Bacellar H, et al. Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study 1985–1991. *Am J Epidemiol* 1993;137(4):423-438.

95. Holland G, Tufail A, Jordan MC. Cytomegalovirus diseases. In: Pepose J, Holland G, Wilhelmus KR, editors. *Ocular Infection & Immunity*. New York: Mosby; 1996. p. 1088-1129.

96. Holland GN, Sison RF, Jatulis DE, et al. Survival of patients with the acquired immune deficiency syndrome after development of cytomegalovirus retinopathy. *Ophthalmology* 1990;97:204-211.

97. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1992;5:1069-1074.

98. Mansour AM, Uchida T, Rodenko G, et al. Ocular–systemic interrelationships in acquired immunodeficiency syndrome. *Int J STD AIDS* 1991;2(1):25-29.

99. Egbert PR, Pollard RB, Gallagher JG, et al. Cytomegalovirus retinitis in immunosuppressed hosts: II. Ocular manifestations. *Ann Intern Med* 1980;93:664-

670.

100. Berger BB, Weinberg RS, Tessler HH, et al. Bilateral cytomegalovirus panuveitis after high-dose corticosteroid therapy. *Am J Ophthalmol* 1979;88:1020-1025.

101. McDonald CK, Kuritzkes DR. Human immunodeficiency virus type 1 protease inhibitors. *Arch Intern Med* 1997;157:951-959.

102. Casado JL, Arrizabalaga J, Montes M, et al. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV-AIDS Study Group. *AIDS* 1999;13(12):1497-1502.

103. Doan S, Cochereau I, Gwenisik N, et al. Cytomegalovirus retinitis in HIV-infected patients with and without highly active antiretroviral therapy. *Am J Ophthalmol* 1999;128(2):250-251.

104. Lewis JS, Terriff CM, Coulston DR, et al. Protease inhibitors: a therapeutic breakthrough for the treatment of patients with HIV. *Clin Ther* 1997;19:187-214.

105. Le Moing V, Chene G, Carrieri MP, et al. Predictors of virologic rebound in HIV-1 infected patients initiating a protease inhibitor-containing regimen. *AIDS* 2002;16:21-29.

106. Studies of Ocular Complications of AIDS (SOCA) Research Group in collaboration with the AIDS Clinical Trials Group (ACTG). Foscarnet–ganciclovir cytomegalovirus retinitis trial: IV. Visual outcomes. *Ophthalmology* 1994;7:1250-1261.

107. Freeman WR, Henderly DE, Wan WL, et al. Prevalence, pathophysiology, and treatment of rhegmatogenous retinal detachment in treated cytomegalovirus

retinitis. *Am J Ophthalmol* 1987;103:527-536.

108. Holland GN, Sidikaro Y, Kreiger AE, et al. Treatment of cytomegalovirus retinopathy with ganciclovir. *Ophthalmology* 1987;94:815-823.

109. Jabs DA, Enger C, Haller J, et al. Retinal detachments in patients with cytomegalovirus retinitis. *Arch Ophthalmol* 1991;109:794-799.

110. Freeman WR, Friedberg DN, Berry C, et al. Risk factors for development of rhegmatogenous retinal detachment in patients with cytomegalovirus retinitis. *Am J Ophthalmol* 1993;116:713-720.

111. Lim JI, Enger C, Haller JA, et al. Improved visual results after surgical repair of cytomegalovirus-related retinal detachments. *Ophthalmology* 1994;101:264-269.

112. Freeman WR, Quiceno JI, Crapotta JA, et al. Surgical repair of rhegmatogenous retinal detachment in immunosuppressed patients with cytomegalovirus retinitis. *Ophthalmology* 1992;99:466-474.

113. Holland GN, Buhles WC, Mastre B, et al. A controlled retrospective study on ganciclovir treatment for cytomegalovirus retinopathy: use of a standardized system for the assessment of disease outcome. *Arch Ophthalmol* 1989;107:1759-1766.

114. Holland GN. AIDS: retinal and choroidal infections. In: Ryan SJ, Lewis H, editors. *Medical and Surgical Retina: Advances, Controversies and Management*. St. Louis: Mosby; 1994. p. 325-339.

115. Holland GN, Shuler JD. Progression rates of cytomegalovirus retinopathy in ganciclovir-treated and untreated patients. *Arch Ophthalmol* 1992;110:1435-1442.

116. Geier SA, Nasemann J, Klauss V, et al. Frosted branch angiitis associated with

cytomegalovirus retinitis. *Am J Ophthalmol* 1992;114:514-516.

117. Roarty JD, Fisher EJ, Nussbaum JJ. Long-term visual morbidity of cytomegalovirus retinitis in patients with acquired immune deficiency syndrome. *Ophthalmology* 1993;100:1685-1688.

118. Gross JG, Bozzette SA, Mathews WC, et al. Longitudinal study of cytomegalovirus retinitis in acquired immune deficiency syndrome. *Ophthalmology* 1990;97:681-686.

119. Garau J, Kabins S, DeNosaquo S, et al. Spontaneous cytomegalovirus mononucleosis with conjunctivitis. *Arch Intern Med* 1977;137:1631-1632.

120. Hart WM, Reed CA, Freedman HL, et al. Cytomegalovirus in juvenile iridocyclitis. *Am J Ophthalmol* 1978;86:329-331.

121. England AC, Miller SA, Maki DG. Ocular findings of acute cytomegalovirus infection in an immunologically competent adult. *N Engl J Med* 1982;307:94-95.

122. De Venecia G, Zu Rhein GM, Pratt MV, et al. Cytomegalic inclusion retinitis in an adult. *Arch Ophthalmol* 1971;86:44-57.

123. Palestine AG, Rodrigues MM, Macher AM, et al. Ophthalmic involvement in acquired immunodeficiency syndrome. *Ophthalmology* 1984;91:1092-1099.

124. Pepose JS, Holland GN, Nestor MS, et al. Acquired immune deficiency syndrome: pathogenic mechanisms of ocular disease. *Ophthalmology* 1985;92:472-484.

125. Stagno S, Reynolds DW, Huang ES, et al. Congenital cytomegalovirus infection. *N Engl J Med* 1977;296:1254-1258.

126. Rao N, Waruszewski DT, Armstrong JA, et al. Evaluation of anti-complement

immunofluorescence test in cytomegalovirus infection. *J Clin Microbiol* 1977;6:633-638.

127. David JL, Feuer W, Culbertson WW, et al. Interpretation of intraocular and serum antibody levels in necrotizing retinitis. *Retina* 1995;15:233-240.

128. Pepose JS, Newman C, Bach MC, et al. Pathologic features of cytomegalovirus retinopathy after treatment with the antiviral agent ganciclovir. *Ophthalmology* 1987;94:414-424.

129. Jacobson MA, Drew WL, Feinberg J, et al. Foscarnet therapy for ganciclovir-resistant cytomegalovirus retinitis in patients with AIDS. *J Infect Dis* 1991;163:1348-1351.

130. Ussery FM, Gibson SR, Conklin RH, et al. Intravitreal ganciclovir in the treatment of AIDS associated cytomegalovirus retinitis. *Ophthalmology* 1988;95:640-648.

131. Martin DF, Parks DJ, Mellow SD, et al. Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant: a randomized controlled clinical trial. *Arch Ophthalmol* 1994;112:1531-1539.

132. Musch DC, Martin DF, Gordon JF, et al. Treatment of cytomegalovirus retinitis with a sustained release ganciclovir implant. The Ganciclovir Implant Study Group. *N Engl J Med* 1997;337(2):83-90.

133. Kuppermann BD, Quiceno JI, Flores-Aguilar M, et al. Intravitreal ganciclovir concentration after intravenous administration in AIDS patients with cytomegalovirus retinitis: implications for therapy. *J Infect Dis* 1993;168:1506-1509.

134. Perry CM, Balfour JA. Fomivirsen. *Drugs* 1999;57(3):375-381.

135. Berenguer J, Mallolas J. Intravenous cidofovir for compassionate use in AIDS patients with cytomegalovirus retinitis. Spanish Cidofovir Study Group. *Clin Infect Dis* 2000;30(1):182-184.

136. Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group. Parenteral cidofovir for cytomegalovirus retinitis in patients with AIDS: the HPMPC peripheral cytomegalovirus retinitis trial. A randomized, controlled trial. *Ann Intern Med* 1997;126(4):264-274.

137. Lalezari JP, Holland GN, Kramer F, et al. Randomized, controlled study of the safety and efficacy of intravenous cidofovir for the treatment of relapsing cytomegalovirus retinitis in patients with AIDS. *J Acquir Immune Defic Syndr* 1988;17(4):339-344.

138. Jacobson MA, Wilson S, Stanley H, et al. Phase I study of combination therapy with intravenous cidofovir and oral ganciclovir for cytomegalovirus retinitis in patients with AIDS. *Clin Infec Dis* 1999;28(3):528-533.

139. D'Amico DJ, Skolnik PR, Kosloff BR, et al. Resolution of cytomegalovirus retinitis with zidovudine therapy: a case report. *Arch Ophthalmol* 1988;106:1168-1169.

140. Fay MT, Freeman WR, Wiley CA, et al. Atypical retinitis in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1988;105:483-490.

141. Saillour F, Bernard N, Dequae-Merchadou L, et al. Predictive factors of occurrence of cytomegalovirus disease and impact on survival in the Aquitaine Cohort in France, 1985 to 1994. Groupe d'Épidémiologie Clinique du SIDA en Aquitaine. *J*

Acquir Immune Defic Syndr Hum Retrovirol 1998;17:171-178.

142. Kuppermann BD, Petty JG, Richman DD, et al. Correlation between CD4+ counts and prevalence of cytomegalovirus retinitis and human immunodeficiency virus-related noninfectious retinal vasculopathy in patients with acquired immunodeficiency syndrome. *Am J Ophthalmol* 1993;115:575-582.

143. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1992;5:1069-1074.

144. Drew WL, Mintz L, Miner RC, et al. Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981;143(2):188-192.

145. Gallant JE, Moore RD, RichmanDD, et al. Incidence and natural history of CMV disease in patients with advanced human immunodeficiency virus disease treated with zidovudine (AZT). *J Infect Dis* 1992;166(6):1223-1227.

146. Holland GN, Pepose JS, Pettit TH, et al. Acquired immune deficiency syndrome: ocular manifestations. *Ophthalmology* 1983;90:859-873.

147. Horak DA, Schmidt GM, Zaia JA. Pretransplant pulmonary function predicts cytomegalovirus-associated interstitial pneumonia following bone marrow transplantation. *Chest* 1992;102(5):1484-1490.

148. Gorensek MJ, Carey WD, Vogt D, et al. A multivariate analysis of risk factors for cytomegalovirus infection in liver-transplant recipients. *Gastroenterology* 1990;98(5 Pt 1):1326-1332.

149. Paya CV, Wiesner RH, Hermans PE, et al. Risk factors for cytomegalovirus and

severe bacterial infections following liver transplantation: a prospective multivariate time-dependent analysis. *J Hepatol* 1993;19(2):325-329.

150. Nagler A, Elishoov H, Kapelushnik Y, et al. Cytomegalovirus pneumonia prior to engraftment following T-cell depleted bone marrow transplantation. *Med Oncol* 1994;11(3-4):127-132.

151. Schiffer V, Mentha G, Giostra E, et al. [Cytomegalovirus in liver transplantation: incidence and groups at risk.] *Schweiz Med Wochenschr* 1994;124(15):631-636.

152. Burke M, Yust I, Katlama C, et al. Cytomegalovirus retinitis in patients with AIDS in Europe. AIDS in Europe Study Group. *Eur J Clin Microbiol Infect Dis* 1997;16(12):876-882.

153. Kraat YJ, Christiaans MH, Nieman FH. Risk factors for cytomegalovirus infection and disease in renal transplant recipients: HLA-DR7 and triple therapy. *Transpl Int* 1994;7(5):362-367.

154. Boland GJ, Hene RJ, Ververs C, et al. Factors influencing the occurrence of active cytomegalovirus (CMV) infections after organ transplantation. *Clin Exp Immunol* 1993;94(2):306-312.

155. Wetherill PE, Landry ML, Alcabes P. Use of a quantitative CMV antigenemia test in evaluating HIV+ patients with and without CMV disease. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;12(1):33-37.

156. Hansen KK, Ricksten A, Hofmann B, et al. Detection of cytomegalovirus DNA in serum correlates with clinical cytomegalovirus retinitis in AIDS. *J Infect Dis* 1994;170(5):1271-1274.

157. MacGregor RR, Pakola SJ, Graziani AL, et al. Evidence of active CMV infection in clinically stable HIV-infected individuals with CD4+ lymphocyte counts below 100/microliters of blood: features and relation to risk of subsequent CMV retinitis. *J Acquir Immune Defic Syndr Hum Retrovir* 1995;10(3):324-330.

158. Rasmussen L, Morris S, Zipeto D, et al. Quantitation of human CMV DNA from peripheral blood cells of human immunodeficiency virus-infected patients could predict CMV retinitis. *J Infect Dis* 1995;171(1):177-182.

159. Pannuti CS, Kallas EG, Muccioli C, et al. Cytomegalovirus antigenemia in acquired immunodeficiency syndrome patients with untreated cytomegalovirus retinitis. *Am J Ophthalmol* 1996;122(6):847-852.

160. Jackson JB, Erice A, Englund JA, et al. Prevalence of cytomegalovirus antibody in hemophiliacs and homosexuals infected with human immunodeficiency virus type 1. *Transfusion* 1988;28(2):187-189.

161. Gerard L, Leport C, Flandre P et al. CMV viremia and the CD4 lymphocyte count as predictors of CMV disease in patients infected with HIV. *Clin Infect Dis* 1997;24(5):841-842.

162. Dodt KK, Jacobsen PH, Hofmann B, et al. Development of cytomegalovirus (CMV) disease may be predicted in HIV-infected patients by CMV polymerase chain reaction and the antigenemia test. *AIDS* 1997;11(3):21-28.

163. Hamprecht K, Steinmassl M, Einsele H, et al. Discordant detection of human cytomegalovirus DNA from peripheral blood mononuclear cells, granulocytes and plasma: correlation to viremia and HCMV infection. *J Clin Virol* 1998;11(2):125-

136.

164. Gellrich MM, Baumert E, Rump JA, et al. Clinical utility of CMV urine cultures for ophthalmic care in patients with HIV. *Br J Ophthalmol* 1996;80(9):818-822.

165. Cope AV, Sabin C, Burroughs A, et al. Interrelationships among quantity of human cytomegalovirus DNA in blood, donor–recipient serostatus and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. *J Infect Dis* 1997;176:1484-1490.

166. Bowen EF, Sabin CA, Wilson P, et al. CMV viremia detected by polymerase chain reaction identifies a group of HIV positive patients at high risk of CMV disease. *AIDS* 1997;11(7):889-893.

167. Rasmussen L, Ziputo D, Wolitz RA, et al. Risk for retinitis in patients with AIDS can be assessed by quantitation of threshold levels of CMV DNA burden in blood. *J Infect Dis* 1997;176(5):1146-1155.

168. Shinkai M, Bozzette SA, Powderley W, et al. Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for identification of AIDS patients at risk for developing human cytomegalovirus disease. *J Infect Dis* 1997;175:302-308.

169. Wattanamano P, Clayton JL, Kopicko JJ, et al. Comparison of three assays for cytomegalovirus detection in AIDS patients at risk for retinitis. *J Clin Microbiol* 2000;38(2):727-732.

170. Verbraak FD, Galema M, van den Horn GH, et al. Serological and polymerase chain reaction-based analysis of aqueous humour samples in patients with AIDS and necrotizing retinitis. *AIDS* 1996;10(10):1091-1099.

171. Doornenbal P, Sheerpbaarsma G, Quint WG, et al. Diagnostic assays in cytomegalovirus retinitis: detection of herpes virus by simultaneous application of the polymerase chain reaction and local antibody analysis on ocular fluid. *Br J Ophthalmol* 1996;80(3):235-240.

172. Danise A, Cinque P, Vergani S, et al. Use of polymerase reaction assays of aqueous humor in the differential diagnosis of retinitis in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1997;24(6):1100-1106.

173. Knox CM, Chandler D, Short GA, et al. Polymerase chain reaction-based assays of vitreous samples for the diagnosis of viral retinitis. Use in diagnostic dilemmas. *Ophthalmology* 1998;105(1):37-44.

174. Verbraak FD, Bruinenberg M, van den Horn GJ, et al. Cytomegalovirus (CMV) strain differences between the eye and blood in AIDS patients with CMV retinitis. *AIDS* 1998;12(7):713-718.

175. Oka S, Nagata Y, Fujino Y, et al. CD8+ T lymphocyte counts as an adjunctive predictor of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome. *Intern Med* 1997;36(7):461-465.

176. Butler GA, Griedman AH. Screening indices for cytomegalovirus retinitis in patients with human immunodeficiency virus. *Mt Sinai J Med* 1992;59(1):61-65.

177. Schrier RD, Freeman WR, Wiley CA, et al. Immune predispositions for cytomegalovirus retinitis in AIDS. The HNRC Group. *J Clin Invest* 1995;95(4):1741-1746.

178. MacDonald KS, Fowke KR, Kimani J, et al. Influence of HLA supertypes on

susceptibility and resistance to human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;181(5):1581-1589.

179. Manez R, Kusne S, Green M, et al. Incidence and risk factors associated with the development of cytomegalovirus disease after intestinal transplantation. *Transplantation* 1995;59(7):1010-1014.

180. Nelson MR, Erskine D, Hawkins DA, et al. Treatment with corticosteroids — a risk factor for the development of clinical cytomegalovirus disease in AIDS. *AIDS* 1993;7(3):375-378.

181. Morris DJ, Martin S, Dyer PA, et al. HLA mismatching and cytomegalovirus infection as risk factors for transplant failure in cyclosporin-treated renal allograft recipients. *J Med Virol* 1993;41(4):324-327.

182. Gorensek MJ, Stewart RW, Keyes TK, et al. A multivariate analysis of the risk of cytomegalovirus infection in heart transplant recipients. *J Infect Dis* 1988;157(3):515-522.

183. Kirklin JK, Naftel DC, Levine TB, et al. Cytomegalovirus after heart transplantation. Risk factors for infection and death: a multiinstitutional study. The Cardiac Transplant Research Database Group. *J Heart Lung Transplant* 1994;13(3):394-404.

184. Sloand E, Kumar P, Klein HG, et al. Transfusion of blood components to persons infected with HIV type 1: relationship to opportunistic infection. *Transfusion* 1994;34(1):48-53.

185. Allannic H, Fauchet R, Lorcy Y, et al. HLA and Graves' disease: an association

with HLA-DRw3. *J Clin Endocrinol Metab* 1980;51(4):863-867.

186. Demmler GJ, Buffone GJ, Schimbor CM, et al. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. *J Infect Dis* 1988;158(6):1177-1184.

187. Kievits T, van Gemen B, van Strijp D, et al. NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. *J Virol Methods* 1991;35:273-286.

188. Schimmer BP, Parker KP. Adrenocorticosteroids. In: Goodman LS, Gilman AG, editors. *The Pharmacologic Basis of Therapeutics*. 9'th ed, New York: MCGraw Hill; 1996. p. 1459-1485.

189. Chabner BA, Allegra CJ, Curt GA et al.Antineoplastic Agents. In: Goodman LS, Gilman AG, editors. *The Pharmacologic Basis of Therapeutics*. 9'th ed. New York: McGraw Hill; 1996. p. 1233-1288.

190. Sausville EA, Longo DL. Principles of cancer therapy. In: Harrison TR, editor. *Principles of Internal Medicine*. 15'th ed, New York: McGraw-Hill; 2001. p. 530-547.

191. Rachlis AR, Zarowny DP. Guidelines for antiretroviral therapy for HIV infection. *CMAJ* 1998;158:496-505.

192. Browner WS, Black D, Newman TB. Estimating sample size and power. In: Hulley SB, Cummings SR, editors. *Designing Clinical Research*. San Francisco: Williams & Wilkins; 1988. p. 139-151.

MANUSCRIPT I: CLINICAL RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

CLINICAL RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

RUNNING TITLE: Clinical Risks for CMV-R

AUTHORS: William G. Hodge MD, MPH, FRCSC[1,2]

Jean-François Boivin MD, ScD, FRCPC[2]

Stan Shapiro PhD[2]

Richard Lalonde MD, FRCPC[3]

Kirtida Shah MD[1]

Brad Murphy MD[1]

Michel Dionne MD[1]

Ashish Goela MD[1]

1- University of Ottawa Eye Institute, Ottawa, Ontario, Canada

2- Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada

3- Division of Infectious Diseases, Royal Victoria Hospital of McGill University Health Sciences Center, Montreal, Quebec, Canada

Corresponding Author:

Dr. William G. Hodge

University of Ottawa Eye Institute

501 Smyth Road

Ottawa ON Canada

K1H 8L6

ABSTRACT

BACKGROUND: Over two dozen descriptive studies, prospective cohort studies and clinical trials have been conducted to examine the treatment of cytomegalovirus (CMV) retinitis. Surprisingly, very few studies have investigated the equally important question of the risk factors for this disease. Identifying these risk factors will have many benefits, including helping establish screening regimens, examination frequency regimens, and targeted prophylaxis with oral ganciclovir or other anti-CMV agents.

PURPOSE: To determine the clinical risk factors for CMV retinitis in patients with the acquired immunodeficiency syndrome (AIDS).

METHODS: We conducted a case–control study involving 120 patients in whom CMV retinitis had been diagnosed in 1990–1999 and 159 patients without CMV retinitis from the same period. The sampling was from a primary study base in western Quebec and eastern Ontario of patients with AIDS and CD4 counts less than 50 cells/μL at the time of diagnosis of retinitis in the cases or the corresponding date for the controls. Clinical risk factors studied included visual symptoms, retinal microinfarctions (cotton-wool spots), previous opportunistic infections and risk factors for HIV acquisition. Confounders controlled for included CD4 count, hospital center and a series of variables to control for confounding by drug treatment. Statistical analysis was performed by multivariate logistic regression. A systematic model-building strategy was developed, from assumption testing to model building to model checking.

RESULTS: The following clinical risk factors were significant predictors of CMV

retinitis: flashing lights/floaters (odds ratio [OR] 11.42, 95% confidence interval [CI] 3.43–38.01), cotton-wool spots (OR 2.90, 95% CI 1.01–8.29), number of previous opportunistic infections (OR 1.81, 95% CI 1.24–2.64), previous nonocular CMV infection (OR 82.99, 95% CI 6.86–1004.58), previous *Mycobacterium* infection (OR 3.41, 95% CI 0.99–11.85) and homosexuality (OR 2.83, 95% CI 1.13–7.12).

CONCLUSIONS: Based on this study, clinical variables have been identified that elevate the risk of CMV retinitis. These findings may be useful to clinicians and health policy experts in developing rational guidelines for screening, examination frequency and targeted prophylaxis for CMV retinitis in patients with AIDS.

KEY WORDS: CMV Retinitis, Clinical Risk Factors

INTRODUCTION

Cytomegalovirus (CMV) retinitis is one of the most devastating complications of the acquired immunodeficiency syndrome (AIDS). It is the most common ocular opportunistic infection among patients with AIDS. It is a necrotizing, blinding form of retinitis that tends to occur late in the course of AIDS. The reported lifetime cumulative incidence of AIDS-related CMV retinitis ranges from 25% to 40%.[1] Two large population-based studies demonstrated an increasing incidence of this disorder in the late 1980's and the 1990's.[2,3] No other ocular opportunistic infection among patients with AIDS has a lifetime cumulative incidence over 1-2%.[4] Based on data from the era before highly active antiretroviral therapy (HAART), the estimated mean time from development of AIDS to manifestation of CMV retinitis is 9–18 months.[5]

With the advent of HAART,[6] the incidence of CMV retinitis is declining. Casado et al.[7] studied a cohort of 172 HIV-infected patients with a CD4 count less than 100 cells/μL at the time of protease inhibitor introduction. The cumulative incidence of CMV retinitis was 5% at 1 year and 6% at 2 years. Doan et al.[8] found that the annual risk of CMV retinitis in 1995 (without protease inhibitors) was 6.1%, compared to 1.2% in 1997 (with protease inhibitors); the annual risk of relapse after anti-CMV therapy decreased from 36% to 17%. Nevertheless, we should be cautiously optimistic regarding this new treatment. At least 20% of patients respond poorly or not at all to HAART,[9,10] and the risk of CMV retinitis has likely not changed for them. Furthermore, since emerging resistance to a component of HAART is always possible, the ophthalmic and public health community should be prepared for new CMV outbreaks at all times. Finally, in

developing nations where HAART is not yet available, AIDS and its opportunistic infections are more devastating today than they ever have been. Hence, CMV retinitis remains one of the most important irreversibly blinding diseases in all of ophthalmology.

Several authors have investigated treatment,[11–13] but far fewer investigators have examined risk factors for CMV retinitis. Well designed case–control studies would be able to contribute to understanding these risk factors and may provide several clinical benefits. The benefits of understanding these risk factors include the possibility of creating more sophisticated guidelines for screening, determining risk factors to permit earlier detection, and providing a more logical algorithm for prophylactic therapy. One example of potential targeted prophylaxis is oral ganciclovir therapy; however, since this agent is currently very expensive and has only moderate bioavailability, it is seldom used as a prophylactic medication.[11,12] Valganciclovir, antiretroviral therapy or experimental immunologic therapy may prove to play a role in prophylaxis as well.[13] However, the exact method of optimal targeted prophylaxis would have to be developed in separate studies.

Two early studies established that a low CD4 lymphocyte (helper T cell) count is a strong risk factor for CMV retinitis.[5,14] A cross-sectional study confirmed the CD4 finding and also showed an association with homosexuality as a risk factor for CMV retinitis.[15] A larger study from the Multicenter AIDS Group in the United States demonstrated that baseline CD4 count less than 100 cells/μL, an enrolment diagnosis of AIDS (versus AIDS-related complex or simply HIV seropositivity) and homosexuality were risk factors for CMV disease.[16] In the Aquitaine cohort study in France, 3525

patients were followed over an 8-year period, and patients with CD4 counts less than 200 cells/μL were eligible for the study.[17] This study, too, showed an increased risk of CMV disease among homosexual patients.

Preexisting end-organ disease has also been postulated as a risk factor. In other words, patients with preexisting retinal abnormalities may be at increased risk for CMV retinitis later. The most common noninfectious retinal abnormality in patients with AIDS is retinal microvascular infarction, manifesting as "cotton-wool spots." Cotton-wool spots are commonly seen in patients before the development of CMV retinitis,[18] but they have never been established as a risk factor in a study using an appropriate methodology.

In systemic CMV infections in patients with AIDS, there is evidence that previous infection or co-infection with other viruses or bacteria may predispose to the reactivation of CMV infection. Schiffer et al.[19] found a strong correlation between CMV disease and co-infection with bacteria. In the Aquitaine cohort, toxoplasmosis was a predictor of CMV disease.[17] In a large multicenter retrospective cohort study performed in 17 European countries, patients with *Pneumocystis carinii* pneumonia, toxoplasmosis or extraocular CMV infection were at increased risk for CMV retinitis.[20]

The goal of this study was to determine the clinical risk factors (i.e., factors determined by history or physical examination) for CMV retinitis in a large group of patients from two Canadian centers.

METHODS

A case–control study was conducted among patients with AIDS seen at the

University of Ottawa Eye Institute and the Department of Ophthalmology of McGill University in Montreal, Canada. Cases were patients with AIDS and a new diagnosis of CMV retinitis who had a CD4 count below 50 cells/μL at the time of diagnosis of the retinitis. Cases were eligible for inclusion in the study between 1990 and 1999, but none were found in 1999. Controls were patients with AIDS but without CMV retinitis and a first CD4 count below 50 cells/μL chosen from the same period as the cases. Cases and controls were not matched by year of inclusion in the study. The inclusion criteria were as follows: 1) HIV seropositivity, confirmed by Western blot analysis, 2) a diagnosis of AIDS meeting the 1993 Centers for Disease Control definition of this disease[4] (HIV positive with at least one opportunistic infection, or HIV positive and absolute CD4 count less than 200 cells/μL), 3) a CD4 count less than 50 cells/μL at the time of inclusion in the study and 4) age 18 years or more at the index date (see definition below).

Patients who met the eligibility criteria but did not return to the participating clinics for at least 3 months after enrolment were excluded.

Sampling and Study Base

All eligible cases who received a diagnosis of CMV retinitis in the two study centers in 1990–1999 were enrolled. Controls were sampled randomly from the study base at both institutions. The characteristics of the study base at the two institutions were slightly different. In eastern Ontario, most patients with advanced HIV disease are referred to the Immunodeficiency Service of the General Campus of the Ottawa Hospital. Most of these

patients are then sent to the Uveitis Service of the University of Ottawa Eye Institute, for eye examinations whenever symptoms occur or occasionally for CMV surveillance eye examinations. In western Quebec, one of the main sources of referral to the Uveitis Service of the Royal Victoria Hospital, Montreal, is the Montreal Chest Hospital. Hence, the study base for western Quebec consisted of patients with AIDS seen at the Montreal Chest Hospital who were sent to the Ophthalmology Department of the Royal Victoria Hospital for ocular evaluation.

Index Date

The date of diagnosis of retinitis represented the index date for cases; risk factors for CMV retinitis were determined relative to this index date. We also needed to identify an index date for controls. The most appropriate index date would have been the date corresponding to the same duration of HIV infection as for the cases. However, this could not be determined accurately, since the exact time of HIV infection is typically difficult to ascertain because initial infection with HIV is asymptomatic or may be associated with a "flu"-like illness unrecognized by patient or physician. We therefore decided to restrict our study population — cases and controls — to subjects with CD4 counts below 50 cells/μL. Our assumption was that the duration of HIV infection would then be similar, on average, for cases and controls. Specifically, the date of the first CD4 count recorded below 50 cells/μL represented the index date for controls.

Exposure Variables

1) Visual Symptomatology (floaters/flashing lights, ocular irritation): These variables are associated with early CMV retinitis or its subclinical antecedent. Since they are likely to be present as the retinitis becomes clinically manifest or shortly before,[21] we considered any positive finding within 3 months before the index date to be significant.

2) Retinal Microinfarctions (cotton-wool spots): Since retinal microinfarctions are transient, a cotton-wool spot observed long before the index date is most probably unimportant.[18] The risk period should therefore be relatively short. We retained cotton-wool spots observed within 3 months before the index date.

3) Overall Infection Pattern: A single opportunistic infection shortly before the onset of CMV retinitis is likely to be etiologically relevant.[22] Furthermore, previous clinical studies have not clearly defined the risk period.[17,20] The variables we studied were history of infection (dichotomous variable) and the total number of opportunistic infections (continuous variable). The risk period was chosen to be 3 months before the index date.

4) Specific Infections: Specific opportunistic infections and one miscellaneous category were also recorded. Our assumption was that it is conceivable that a history of any infection or number of infections may not be important, but a specific infection may be.[23]

5) Risk Factors for Acquisition of HIV Infection (homosexual transmission, heterosexual

transmission, intravenous drug use, blood transfusion): It is hypothesized that the mode of acquisition of HIV infection is associated with different strains of HIV varying in pathogenic potential with respect to the eventual occurrence of CMV retinitis.[15,16] Because any one patient may have acquired HIV from more than one source, this category was divided into four dichotomous variables.

Confounders

The confounders chosen were variables associated with the clinical variables under study that also independently increase or decrease the risk of retinitis. They included CD4 count, anti-HIV medications, anti-CMV and anti-HSV (herpes simplex virus) medications, and hospital center.

1) CD4 Count: All patients had CD4 counts less than 50 cells/μL at enrolment into the study. Based on our understanding of markers of disease severity, CD4 count before enrolment is the most important confounder in this study. It is associated with opportunistic infections and may be associated with patient symptomatology. Furthermore, it is an extremely important predictor of CMV retinitis. The risk period for increased risk of CMV retinitis in patients with a low CD4 count is known to be short.[5] This has been supported by studies of CD4 counts and function that seem to indicate significant improvement in immune function in the short term in patients receiving HAART.[7] We therefore used the CD4 level measured in the 3 months before the index date.

2) Anti-HIV Medications: These medications are potentially associated with many exposure variables, as patients with more symptoms, more previous infections and a more pathogenic strain of HIV would be more likely to be taking more anti-HIV medications and higher cumulative dosages of these medications. Furthermore, patients receiving anti-HIV medication would be at reduced risk for CMV retinitis. We measured treatments received in the 2 years before the index date.

3) Anti-CMV and Anti-HSV Medications: Anti-CMV medications used to treat nonocular CMV infections were controlled for, as these medications would be associated with predictors of CMV retinitis and would protect against CMV retinitis itself. Furthermore, the anti-HSV medication acyclovir has some anti-CMV activity[24] and therefore should be controlled for in a similar way as anti-CMV medications. The etiologic period chosen was 2 years.

4) Hospital Center: Because there are unmeasurable potential differences between the two hospital centers that may confound the exposure–disease association, hospital center was also controlled for.

All data were abstracted from the subjects' medical records.

Statistical Analysis

Univariate statistics were obtained to check for implausible values as a final data

editing step. Bivariate analyses were then conducted for all clinical exposure variables using the unpaired *t*-test for continuous variables (or the Wilcoxon rank-sum test if nonnormal) and the chi-square test for discrete variables. Logistic regression was used for multivariate analyses. A *p* value of 0.10 or less in the bivariate analyses was used as a cut-off for inclusion in the multivariate analyses. Next, the assumption of linearity of the logit was checked in a bivariate analysis for all exposure and confounding variables individually. This was done by graphing the odds ratios (ORs) against each of the midpoints of multiple equal-interval variables. For example, CD4 count was divided into four approximately equal intervals: 0–12, 13–24, 25–36, and 37–49 cells/μL. Then the OR pertaining to case status corresponding to each interval was graphed against the midpoint of each interval (6, 18.5, 30.5 and 43.0 cells/μL respectively). From this plot, linearity of the logit was assessed. Next, a correlation matrix was obtained for all exposure variables to assess multicollinearity. Any correlation coefficient greater than 0.8 was recorded, and if a multicollinearity problem arose in the multivariate model, an algorithm for omitting one variable was used. This algorithm consisted of first keeping the variable that was a more biologically plausible exposure, then keeping the variable with fewer missing data, and finally keeping the variable with less measurement error as determined a priori.

For confounding, we determined a priori that the model would include CD4 count, hospital site (city), at least one anti-HIV medication variable, at least one anti-CMV medication variable and at least one anti-HSV medication variable. Although these five categories of confounding variables were chosen a priori, it was not clear for anti-HIV

medication, anti-CMV medication and anti-HSV medication which specific representation of the variable to use (e.g., for anti-HIV medication, specific anti-HIV medication, total dose of anti-HIV medication etc.). Incorporating all possible representations of each variable would not be realistic because of precision limitations. Therefore, logistic regression was used in the same way as described above to identify at least one variable for each of the preselected drug treatment categories. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. These confounding variables were then introduced into the main clinical model.

Statistical techniques were not used to reduce the number of confounders in the model but were used to reduce exposure variables for the final model. Model reduction for the exposure variables was performed with the likelihood ratio test. If data were missing, cases and controls were deleted in the multivariate analysis. However, when the model was reduced or expanded, these data were then used again if the variable with the missing data was eliminated from the model. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. The performance of the final model against chance and its fit were then determined.

The study protocol was approved by the Research Ethics Boards of both the Ottawa Hospital and the Royal Victoria Hospital/Montreal Chest Hospital.

RESULTS

Demographic and Descriptive Results

There were 279 patients in the study, 148 from Montreal and 131 from Ottawa. Of the 279, 120 were cases (88 from Montreal) and 159 were controls (99 from Ottawa). Table I summarizes the demographic and descriptive results for the two groups. The average age at the index date was 38.6 years for cases and 39.6 years for controls. Complete data were available for age, race, gender, medication use, previous infections, CD4 count and hospital site. For other variables, the completeness of the medical records was as follows: retinal microinfarctions 277 (99.3%), visual symptomatology 276 (98.9%) and mode of HIV acquisition 243 (87.1%).

TABLE I: DEMOGRAPHIC AND DESCRIPTIVE RESULTS		
	No. (and %) of Subjects	
Variable	**Cases (N = 120)**	**Controls (N = 159)**
Hospital site		
Ottawa	32 (26.7)	99 (62.3)
Montreal	88 (73.3)	60 (37.7)
Gender		
Male	109 (90.8)	136 (85.5)
Female	11 (9.2)	23 (14.5)
Caucasian race	107 (89.2)	131 (82.4)
Index date, year		
1990	6 (5.0)	5 (3.1)
1991	5 (4.2)	15 (9.4)
1992	15 (12.5)	13 (8.2)
1993	21 (17.5)	19 (11.9)
1994	23 (19.2)	33 (20.8)
1995	25 (20.8)	46 (28.9)
1996	20 (16.7)	23 (14.5)
1997	4 (3.3)	4 (2.5)
1998	1 (0.8)	1 (0.6)

Clinical Variable Model

Table II presents the bivariate dichotomous data on the clinical variables. Based on the criteria described in the methods section, the following variables were retained for the multivariate model: presence of floaters/flashing lights by history, presence of cotton-wool spots, history of opportunistic infection, homosexuality, heterosexual risk factors, intravenous drug use and blood transfusion.

TABLE II: BIVARIATE DICHOTOMOUS DATA FOR CLINICAL VARIABLES

Variable	No. (and %) of Cases Exposed	No. (and %) of Controls Exposed	Odds Ratio (OR)	95% Confidence Interval (CI)	*p* Value
Caucasian race	107 (89.2)	131 (82.4)	0.57	0.28–1.14	0.11
Cotton-wool spots	31 (26.3)	12 (7.6)	4.36	2.15–8.84	0.00001
Floaters/flashing lights	31 (26.5)	7 (4.4)	7.83	3.37–18.13	0.00001
Pain/irritation	1 (0.9)	6 (3.8)	0.22	0.03–1.42	0.13
Any opportunistic infection	94 (78.3)	94 (59.1)	2.50	1.46–4.26	0.0007
Homosexuality	83 (87.4)	83 (56.1)	5.42	2.75–10.66	0.00001
Heterosexual risk factors	12 (12.6)	54 (36.5)	0.25	0.13–0.50	0.00001
Intravenous drug use	5 (5.3)	21 (14.2)	0.34	0.13–0.90	0.03
Blood transfusion	2 (2.1)	12 (8.1)	0.24	0.02–1.00	0.05

Table III summarizes the results of specific infections and their association with risk of CMV retinitis. *Mycobacterium avium–cellulare* complex infection, nonocular CMV infections, HSV infections, systemic candidiasis and other infections were retained in the final multivariate model.

TABLE III: INFECTIONS AMONG CASES AND CONTROLS

Infection	No. (and %) of Cases Exposed	No. (and %) of Controls Exposed	OR	95% CI	*p* Value
Pneumocystis pneumonia	30 (25.0)	39 (24.5)	1.03	0.59–1.77	0.93
Mycobacterium avium–cellulare complex infection	15 (12.5)	8 (5.0)	2.70	1.13–6.44	0.02
Nonocular cytomegalovirus (CMV) infection	22 (18.3)	3 (1.9)	11.67	3.61–37.51	0.00001
Herpes simplex virus (HSV) infection	24 (20.0)	18 (11.3)	1.96	1.02–3.77	0.04
Gram-positive sepsis	5 (4.2)	7 (4.4)	0.94	0.31–2.90	0.92
Escherichia coli sepsis	2 (1.7)	5 (3.1)	0.52	0.054–2.38	0.44
Systemic candidiasis	39 (32.5)	35 (22.0)	1.71	1.00–2.91	0.05
Herpes zoster	3 (2.5)	8 (5.0)	0.48	0.14–1.72	0.28
Central nervous system toxoplasmosis	3 (2.5)	8 (5.0)	0.48	0.14–1.72	0.28
Other infections	24 (20.0)	11 (6.9)	3.36	1.59–7.09	0.001

The bivariate analysis for continuous variables is presented in Table IV. Total number of infections was retained in the final clinical model.

TABLE IV: BIVARIATE CONTINUOUS VARIABLES FROM CLINICAL DATA			
	Mean ± Standard Deviation (SD)		
Variable	**Cases**	**Controls**	***p* Value**
Age, years	38.62 ± 8.09	39.58 ± 8.21	0.33
Total number of opportunistic infections	1.43 ± 1.18	0.89 ± 0.93	0.0001

Confounding by Medication

It was decided a priori that at least one drug or drug amount from each of the anti-HIV, anti-CMV and anti-HSV medication categories would be used in the final multivariate model. CD4 and hospital site were also included. The specific variables to be included were determined by logistic regression.

Table V presents the bivariate descriptive statistics and ORs for the dichotomous treatment variables. Variables with a p value less than 0.10 were retained for the subsequent logistic regression.

TABLE V: BIVARIATE DISCRETE STATISTICS FOR THE DICHOTOMOUS TREATMENT VARIABLES

Medication	No. (and %) of Cases Exposed	No. (and %) of Controls Exposed	OR	95% CI	p Value
Anti-HSV	53 (44.2)	35 (22.0)	2.80	1.67–4.71	0.0001
Anti-CMV	17 (14.2)	9 (5.7)	2.75	1.20–6.29	0.02
Ganciclovir	12 (10.0)	4 (2.5)	4.31	1.42–12.99	0.008
Foscarnet	7 (5.8)	0 (0.0)	–	–	0.002
CMV test drug	3 (2.5)	6 (3.8)	0.65	0.18–2.44	0.55
Anti-CMV combination therapy	6 (5.0)	0 (0.0)	–	–	0.004
Anti-human immunodeficiency virus (HIV)	93 (77.5)	106 (66.7)	1.72	1.01–2.95	0.05
Zidovudine (AZT)	80 (66.7)	92 (57.9)	1.46	0.89–2.38	0.13
Didanosine (ddI)	47 (39.2)	60 (37.7)	1.06	0.65–1.73	0.81
Zalcitabine (ddC)	30 (25.0)	37 (23.3)	1.10	0.63–1.91	0.74
Lamivudine (3TC)	27 (22.5)	19 (12.0)	2.14	1.31–4.04	0.02
Stavudine (d4T)	5 (4.2)	1 (0.6)	6.87	1.04–12.32	0.04
Saquinavir	6 (5.0)	0 (0.0)	–	–	0.004
Ritonavir	6 (5.0)	0 (0.0)	–	–	0.004
Indinavir	3 (2.5)	0 (0.0)	–	–	0.05

The total number of anti-HIV medications and total duration of anti-HIV drug use among cases and controls are shown in Table VI. In this bivariate analysis, cases took more drugs on average than controls, and the duration of medication use was longer

among cases than among controls.

TABLE VI: BIVARIATE STATISTICS FOR NUMBER OF ANTI-HIV MEDICATIONS AND DURATION OF DRUG USE			
Mean ± SD			
Variable	**Cases**	**Controls**	***p* Value**
Total number of anti-HIV medications	1.68 ± 1.41	1.32 ± 1.20	0.06
Duration of anti-HIV medication use, months	12.82 ± 9.50	10.27 ± 9.50	0.02

Whenever possible, the quantity of drug was also recorded for the risk period given in the methods section. With some drugs, such as the protease inhibitors and foscarnet, too few observations were available, and therefore the data are not presented for these drugs. The results for those with reasonable precision are summarized in Table VII. Acyclovir was used in significantly greater amounts among cases than among controls.

TABLE VII: BIVARIATE STATISTICS FOR TOTAL AMOUNT OF MEDICATION USED			
Mean ± SD, g			
Medication	**Cases**	**Controls**	***p* Value**
Acyclovir	69.12 ± 126.20	37.28 ± 110.54	0.003
Ganciclovir	3.67 ± 18.17	0.67 ± 4.89	0.19
Zidovudine (AZT)	141.04 ± 149.50	125.71 ± 143.15	0.30
Didanosine (ddI)	45.86 ± 90.07	35.13 ± 64.68	0.61
Zalcitabine (ddC)	0.14 ± 0.38	0.11 ± 0.27	0.90
Lamivudine (3TC)	26.24 ± 60.86	12.54 ± 52.38	0.17

The final confounding model was then built (Table VIII). CD4 count, hospital center and treatment variables with a p value less than 0.10 were all initially forced into the model.

TABLE VIII: FINAL CONFOUNDING MODEL				
Variable	**OR**	**Standard Error (SE)**	**95% CI**	***p* Value**
Any ganciclovir use	3.11	1.95	0.91–10.61	0.07
Any acyclovir use	2.05	0.65	1.10–3.83	0.02
Number of anti-HIV drugs	1.43	0.18	1.13–1.82	0.03
CD4 count	0.96	0.01	0.94–0.98	0.0001
Hospital center	5.19	1.62	2.82–9.58	0.0001

The clinical variables retained from the univariate analysis and the confounders were used to build the final model. The model was then reduced as described in the methods section. The final model is presented in Table IX.

TABLE IX: FINAL CLINICAL MODEL				
Variable	**OR**	**SE**	**95% CI**	***p* Value**
Floaters/flashing lights	11.42	7.01	3.43–38.01	0.0001
Cotton-wool spots	2.90	1.55	1.01–8.29	0.05
Total number of opportunistic infections	1.81	0.35	1.24–2.64	0.002
Nonocular CMV infections	82.99	105.58	6.86–1004.58	0.001
Mycobacterium infection	3.41	2.17	0.99–11.85	0.05
Systemic candidiasis	2.12	0.89	0.93–4.83	0.07
Other infections	3.98	2.23	1.32–11.96	0.01

TABLE IX: FINAL CLINICAL MODEL				
Homosexual acquisition of HIV	2.83	1.33	1.13–7.12	0.03
Any ganciclovir use	0.09	0.13	0.006–1.39	0.09
Any acyclovir use	1.26	0.54	0.55–2.90	0.59
Number of anti-HIV drugs	1.66	0.29	1.17–2.34	0.004
CD4 count	0.94	0.01	0.92–0.97	0.0001
Hospital center	3.99	1.83	1.62–9.82	0.003

CONCLUSION

The purpose of this study was to determine whether risk factors for CMV retinitis could be uncovered that could eventually be used in the monitoring, surveillance, treatment and prophylaxis of patients at risk for this disorder. We used a case–control design, which allowed us to study many risk factors simultaneously. We identified potential risk factors from a review of previous work in the field of CMV retinitis as well as from studies of risk factors for CMV infection of other organ systems, typically among organ transplant recipients. We believe that this is the most comprehensive study undertaken to date to study the clinical risk factors for this blinding disease, which is one of the most important diseases of the AIDS epidemic.

Among the clinical risk factors studied, both cotton-wool spots and floaters/flashing lights were found to be important predictors of CMV retinitis. Cotton-wool spots are areas of microinfarction and are therefore, by definition, areas where the retinal microcirculation is damaged. Hence, this certainly could be an avenue whereby CMV can gain access to the retina. Using 8-μm pores in a cell transit analyzer, Tufail et al.[25] compared neutrophil rigidity in HIV-positive patients with CMV retinitis and in HIV-positive patients without CMV retinitis and HIV-negative controls. They found significantly increased rigidity in patients with CMV retinitis and hypothesized that this neutrophil rigidity phenomenon may be responsible for HIV-related cotton-wool spots. Glasgow[26] infused microspheres impregnated with fluorescent dye to compare the integrity of the retinal microvasculature in autopsy eyes from patients with AIDS with that in control eyes from subjects without AIDS. Vascular breaches, including

microaneurysms, were found much more commonly in the AIDS group than in the control group. Using a trypsin digest preparation, Glasgow and Weisberger[27] showed that there was more vessel attenuation and more microaneurysms in patients with AIDS than in controls; the former also had an abnormal endothelial cell:pericyte ratio. Rao et al.[28] studied autopsy eyes of patients with CMV retinitis. They observed CMV in the retinal glial cells and found that adjacent vascular tissue was almost always devoid of endothelium. They hypothesized that after vascular endothelial cell infection, the virus destroys this tissue before invading adjacent retinal tissue. Scholz et al.[29] studied this phenomenon in vitro and found that the cadherin–catenin–actin complex is damaged by CMV infection and is likely the first important anatomic alteration allowing CMV to gain access to retinal tissue.

Hence, cotton-wool spots may represent the manifestation of neutrophil rigidity leading to capillary occlusion and damage, which permits access of CMV into retinal tissue.

Flashing lights and floaters are common, nonspecific symptoms of various retinal disorders. We could not determine whether this symptom is due to early frank CMV retinal infection or more proximal events in the chain of pathogenesis of CMV retinitis. More proximal events could be the invasion of CMV through the retinal vessel walls or even earlier events, such as early vessel wall damage even before CMV breaches the vessel wall.

Basic science studies have shown that upregulation of HIV may occur in conjunction with that of other viruses or bacteria.[22] However, the exact relationship is complex. Our

study uncovered several interesting findings with respect to other infections as risk factors for CMV retinitis. First, the total number of opportunistic infections within the 3 months before the index date was higher among cases than controls (OR 1.81, 95% CI 1.24–2.64). When we studied specific infections, we found nonocular CMV infections to be a very strong predictor of CMV retinitis (OR 82.99, 95% CI 6.86–1004.58). This has face validity, as a CMV infection in another organ system would be expected to be predictive of CMV infection in the eye. *Mycobacterium* infections were also predictive of CMV retinitis. To our knowledge, no previous study in the literature has shown this association. However, in the Multicenter AIDS Cohort Study, a similar result was found but not published (Doug Jabs, personal communication 1999). *M. avium–cellulare* complex infection may upregulate CMV replication, or, alternatively, *M. avium–cellulare* complex and CMV may share an as yet undiscovered common genetic or acquired pathogenic mechanism.

Homosexuality was found to be a significant risk for CMV retinitis in our study. Other investigators have also reported this association.[5,15] Neither heterosexual risk factors nor intravenous drug use increased the risk for CMV retinitis in the multivariate model. One possible explanation for this is the strain of CMV or HIV transmitted. More homogeneous "pools" of viral strains likely exist among population subgroups. The data for patients in our study base in the 1990's suggest that the CMV or HIV strains circulating in the homosexual population were more virulent with respect to CMV retinitis than those in other populations.

In any epidemiologic study, alternate explanations for the results may be due to

chance, bias or confounding. With a sample size of 279, we feel the study had adequate statistical power for most of the analyses that we conducted. In addition, it is one of the largest studies of clinical risk factors for CMV retinitis ever undertaken. The tightness of our confidence intervals was generally adequate. The major biases of concern in this case–control study were selection bias and misclassification bias. We should point out that we chose a case–control design for two main reasons. First, it allowed us to examine multiple potential exposure variables, which was one of the goals of the study. Second, with the advent of HAART, in 1996, the incidence of CMV retinitis began declining to the point where it would not have been realistic to recruit a cohort of unaffected patients that would provide a study with adequate power. We attempted to minimize selection bias by adhering strictly to the study base principle. In fact, for the Montreal patients, our initial hope was to use the patients at the ophthalmology clinic of the Royal Victoria Hospital as our study base. However, while cases were well recorded in that clinic, controls were not adequately retrievable from this study base. We therefore changed our study base to patients referred from the Montreal Chest Hospital to the Royal Victoria Hospital to ensure that cases and controls would arise from the same population base, thereby respecting the study base principle. As for misclassification bias, we purposely chose at least 3 months' follow-up for all patients to ensure that any misclassification would be nondifferential. Differential misclassification could have occurred if, for example, cases were followed more closely and more frequently before the index date than controls; however, the minimum length of follow-up required in our study decreased the chances that this would occur. Nondifferential misclassification is always possible

when medical records are used as the main source of information, especially for a variable such as mode of HIV acquisition. However, this would have biased our results toward the null, so that the statistically significant estimates reported here may underestimate the actual ORs. Finally, it is possible that for some exposure variables, the temporal association between the exposure and CMV retinitis can be questioned. This is most possible for patient symptomatology, unlikely for retinal microinfarctions, and not possible for previous opportunistic infections or HIV risk factors.

We took great efforts to minimize the effect of confounding. CD4 count was controlled for a priori as it is both the most important indicator of disease severity and an approximate indicator of time since HIV infection. Hospital center was also controlled for a priori because of the obvious potential for confounding from one study base to another. Regarding confounding by medication, we created a model and decided a priori that it would contain at least one variable for anti-HIV medication, one variable for anti-CMV medication and at least one variable for anti-HSV medication (since anti-HSV medications may also have some anti-CMV effects). This model was then used in the main exposure model to control for confounding. While it is impossible to ever be absolutely certain that confounding has been completely eliminated in any epidemiologic study, we feel confident that we minimized its effect.

In conclusion, this study is one of the most comprehensive studies to date examining the clinical risk factors for CMV retinitis. It is hoped that the results will help guide both internists and ophthalmologists in the surveillance, diagnosis and management of this disease.

ACKNOWLEDGEMENTS: Supported by the University of Ottawa Young Investigator Award and the Medical Research Council of Canada. The authors wish to acknowledge Dr. Jean Deschênes for helping with administrative issues for patient recruitment in Montreal.

REFERENCES

1. Holland GN, Pepose JS, Simons KB, et al. Ocular disease in the acquired immune deficiency syndrome: clinicopathological correlations. In: Secchi AG, Fregona IA, editors. *Modern Trends in Immunology and Immunopathology of the Eye*. Milan: Saunders; 1989. p. 132-155.
2. Montaner JS, Le T, Hogg R, et al. The changing spectrum of AIDS index diseases in Canada. *AIDS* 1994;8(5):693-696.
3. Munoz A, Schrager LK, Bacellar H, et al. Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am J Epidemiol* 1993;137(4):423-438.
4. Holland G, Tufail A, Jordan MC. Cytomegalovirus diseases. In: Pepose JS, Holland GN, Wilhelmus KR, editors. *Ocular Infection & Immunity*. New York: Mosby; 1996. p. 1088-1129.
5. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1992;5:1069-1074.
6. McDonald CK, Kuritzkes DR. Human immunodeficiency virus type 1 protease inhibitors. *Arch Intern Med* 1997;157:951-959.
7. Casado JL, Arrizabalaga J, Montes M, et al. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV-AIDS Study Group. *AIDS* 1999;13(12):1497-1502.

8. Doan S, Cochereau I, Guvenisik N, et al. Cytomegalovirus retinitis in HIV-infected patients with and without highly active antiretroviral therapy. *Am J Ophthalmol* 1999;128(2):250-251.

9. Lewis JS, Terriff CM, Coulston DR, et al. Protease inhibitors: a therapeutic breakthrough for the treatment of patients with HIV. *Clin Ther* 1997;19:187-214.

10. Le Moing V, Chene G, Carrieri MP, et al. Predictors of virologic rebound in HIV-1 infected patients initiating a protease inhibitor-containing regimen. *AIDS* 2002;16:21-29.

11. Spector SA, McKinley GF, Lalezari JP, et al. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. *N Engl J Med* 1996;334:1491-1497.

12. Anderson RD, Griffy KG, Jung D, et al. Ganciclovir absolute bioavailability and steady-state pharmacokinetics after oral administration of two 3000 mg/d dosing regimens in HIV and CMV seropositive patients. *Clin Ther* 1995;17:425-432.

13. Masur H, Whitcup SM, Cartwright C, et al. Advances in the management of AIDS-related cytomegalovirus retinitis. *Ann Intern Med* 1996;125(2):126-136.

14. Kuppermann BD, Petty JG, Richman DD, et al. Correlation between CD4+ counts and prevalence of cytomegalovirus retinitis and human immunodeficiency virus-related noninfectious retinal vasculopathy in patients with acquired immunodeficiency syndrome. *Am J Ophthalmol* 1993;115:575-582.

15. Drew WL, Mintz L, Miner RC, et al. Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981;143(2):188-192.

16. Gallant JE, Moore RD, Richman DD, et al. Incidence and natural history of CMV disease in patients with advanced human immunodeficiency virus disease treated with zidovudine (AZT). *J Infect Dis* 1992;166(6):1223-1227.

17. Saillour F, Bernard N, Dequae-Merchadou L, et al. Predictive factors of occurrence of cytomegalovirus disease and impact on survival in the Aquitaine cohort in France, 1985 to 1994. Groupe d'Épidémiologie Clinique du SIDA en Aquitaine. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;17:171-178.

18. Holland GN, Pepose JS, Pettit TH, et al. Acquired immune deficiency syndrome: ocular manifestations. *Ophthalmology* 1983;90:859-873.

19. Schiffer V, Mentha G, Giostra E, et al. [Cytomegalovirus in liver transplantation: incidence and groups at risk.] *Schweiz Med Wochenschr* 1994;124(15):631-636.

20. Burke M, Yust I, Katlama C, et al. Cytomegalovirus retinitis in patients with AIDS in Europe. AIDS in Europe Study Group. *Eur J Clin Microbiol Infect Dis* 1997;16(12):876-882.

21. Holland GN, Buhles WC, Mastre B, et al. A controlled retrospective study on ganciclovir treatment for cytomegalovirus retinopathy: use of a standardized system for the assessment of disease outcome. *Arch Ophthalmol* 1989;107:1759-1766.

22. Gendelman HE, Phelps W, Feigenbaum L, et al. Trans-activation of the human immunodeficiency virus long terminal repeat sequence by DNA viruses. *Proc Natl Acad Sci U S A* 1986;83:9759-9763.

23. Fay MT, Freeman WR, Wiley CA, et al. Atypical retinitis in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1988;105:483-490.

24. Rachlis AR, Zarowny DP. Guidelines for antiretroviral therapy for HIV infection. *CMAJ* 1998;158:496-505.

25. Tufail A, Holland GN, Fisher TC, et al. Increased polymorphonuclear leucocyte rigidity in HIV infected individuals. *Br J Ophthalmol* 2000;84(7):727-731.

26. Glasgow BJ. Evidence for breaches of the retinal vasculature in acquired immune deficiency syndrome angiopathy. A fluorescent microsphere study. *Ophthalmology* 1997;104(5):753-760.

27. Glasgow BJ, Weisberger AK. A quantitative and cartographic study of retinal microvasculopathy in acquired immunodeficiency syndrome. *Am J Ophthalmol* 1994;118(1):46-56.

28. Rao NA, Zhang J, Ishimoto S. Role of retinal vascular endothelial cells in development of CMV retinitis. *Trans Am Ophthalmol Soc* 1998;96:111-123.

29. Scholz M, Blaheta RA, Vogel J, et al. Cytomegalovirus induced transendothelial cell migration: a closer look at intercellular communication mechanisms. *Intervirology* 1999;42(5-6):350-356.

LINKING TEXT I

In the previous paper, clinical risk factors for CMV retinitis were examined. We defined clinical risk factors as those that could be determined by history and physical examination. They included visual symptomatology, retinal signs, a history of previous opportunistic infections and the method of acquisition of HIV. Family practitioners, infectious disease specialists and ophthalmologists participate in the day-to-day care of patients with AIDS. A variety of other health care personnel, including nurses, also play a key role. One very practical positive aspect of these clinical risk factors is that almost all of those mentioned in the previous paper may be probed for by any of these personnel. The only exception is cotton-wool spots, for which ophthalmologic training would provide an advantage for retinal examination. Hence, in the day-to-day caregiving of these patients, including trying to uncover predictors of early CMV retinitis, clinical risk factors provide a practical, simple and economic group of predictors for this disease.

The same cannot be said for all the laboratory-based risk factors to be studied in the next paper. Because they are laboratory based, they are more cumbersome to identify, and testing is expensive. On a day-to-day basis for medical caregivers, laboratory tests such as hematologic parameters and nutrition parameters are fairly simple to obtain. However, testing for viral load and HLA type is expensive and sometimes not always uniformly reliable across different laboratories. The use of these laboratory tests may be optimized by ordering them for certain patients at high risk, identified through clinical or iatrogenic risk factors. The laboratory-based risk factors will be summarized in detail in the next paper.

MANUSCRIPT II: LABORATORY-BASED RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

LABORATORY-BASED RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

RUNNING TITLE: Lab Risks for CMV-R

AUTHORS:

William G. Hodge MD, MPH, FRCSC[1,2]

Jean-François Boivin MD, ScD, FRCPC[2]

Stan Shapiro PhD[2]

Richard Lalonde MD, FRCPC[3]

Kirtida Shah MD[1]

Brad Murphy MD[1]

Francisco Diaz-Mitoma[4]

1- University of Ottawa Eye Institute, Ottawa, Ontario, Canada

2- Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada

3- Division of Infectious Diseases, Royal Victoria Hospital of McGill University Health Sciences Centre, Montreal, Quebec, Canada

4- Regional Virology Laboratory of Eastern Ontario, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada

Corresponding Author: Dr. William G. Hodge

University of Ottawa Eye Institute

501 Smyth Road

Ottawa ON Canada K1H 8L6

ABSTRACT

BACKGROUND: Over two dozen descriptive studies, prospective cohort studies and clinical trials have been conducted to examine the treatment of cytomegalovirus (CMV) retinitis. Surprisingly, very few studies have investigated the equally important question of risk factors for this disease. Identifying these risk factors will have many benefits, including helping establish screening regimens, examination frequency regimens, and targeted prophylaxis with oral ganciclovir or other anti-CMV agents.

PURPOSE: To determine the laboratory-based risk factors for CMV retinitis in patients with the acquired immunodeficiency syndrome (AIDS).

METHODS: We conducted a case–control study involving 120 patients in whom CMV retinitis had been diagnosed in 1990–1999 and 159 patients without CMV retinitis from the same period. The sampling was from a primary study base in western Quebec and eastern Ontario of patients with AIDS and CD4 counts less than 50 cells/μL at the time of diagnosis of retinitis in the cases or the corresponding date for the controls. There were two components to the study. The first component (N = 279) examined standard-of-care laboratory tests done during the study period (hematologic parameters and nutrition variables). The second component (N = 57), which was a subset of the first, examined laboratory tests on stored blood samples from the eastern Ontario site only (HLA type, qualitative and quantitative CMV polymerase chain reaction (PCR), and human immunodeficiency virus load). Multivariate logistic regression was used to model the data, controlling for confounding. A systematic model-building strategy was developed, from assumption testing to model building to model checking.

RESULTS: A low hemoglobin level was a statistically significant predictor of CMV retinitis (odds ratio [OR] 0.96, 95% confidence interval [CI] 0.94–0.98). Both qualitative CMV PCR (OR 21.71, 95% CI 1.80–261.67) and quantitative CMV PCR (OR 33.03, 95% CI 2.32–469.39) were strong predictors of CMV retinitis. Among the HLA types, HLA-Bw4 (OR 11.68, 95% CI 1.29–105.82) and HLA-DRBI15 (OR 9.34, 95% CI 1.14–76.41) were significant predictors of CMV retinitis, whereas HLA-Cw7 was protective against CMV retinitis (OR 0.09, 95% CI 0.01–0.67).

CONCLUSIONS: Based on this study, laboratory variables have been identified that elevate (or decrease) the risk of CMV retinitis. These findings may be useful to clinicians and health policy experts in developing rational guidelines for screening, examination frequency and targeted prophylaxis for patients with AIDS.

KEY WORDS: CMV Retinitis, Risk Factors, PCR

INTRODUCTION

Cytomegalovirus (CMV) retinitis is one of the most devastating complications of the acquired immunodeficiency syndrome (AIDS). It is the most common and most devastating ocular opportunistic infection among patients with AIDS. It is a necrotizing, blinding form of retinitis that tends to occur late in the course of AIDS. The reported lifetime cumulative incidence of AIDS-related CMV retinitis ranges from 25% to 40%.[1] Two large population-based studies demonstrated an increasing incidence of this disorder in the late 1980's and the 1990's.[2,3] No other ocular opportunistic infection among patients with AIDS has a lifetime cumulative incidence over 1%–2%.[4] Based on data from the era before highly active antiretroviral therapy (HAART), the estimated mean time from development of AIDS to manifestation of CMV retinitis is 9–18 months.[5]

With the advent of HAART,[6] the incidence of CMV retinitis is declining.[7] Nevertheless, we should be cautiously optimistic regarding this new treatment. At least 20% of patients respond poorly or not at all to HAART,[8,9] and the risk of CMV retinitis has likely not changed for them. Furthermore, since emerging resistance to HAART is always possible, the ophthalmic and public health community should be prepared for new CMV outbreaks at all times. Finally, in developing nations, AIDS and its opportunistic infections are more devastating today than they ever have been. Hence, CMV retinitis remains one of the most important irreversibly blinding diseases in all of ophthalmology.

Well designed case–control studies would be able to contribute to understanding the risk factors for CMV retinitis and may provide several clinical benefits. The benefits of understanding these risk factors include the possibility of formulating more sophisticated

guidelines for screening, determining risk factors to permit earlier detection, and providing a more logical algorithm for prophylactic therapy. One example of potential targeted prophylaxis is treatment with oral ganciclovir therapy; however, since this agent is currently very expensive and has only moderate bioavailability, it is seldom used as a prophylactic medication.[10,11] Valganciclovir, antiretroviral therapy or experimental immunologic therapy may prove to play a role in prophylaxis as well.[12] However, the exact method of optimal targeted prophylaxis would have to be developed in separate studies.

In recent years, the use of laboratory-based assessments of CMV infection has gained wide attention, with the purpose of predicting which patients will manifest CMV retinitis. Viral culture is possible and depends on the typical cytopathic effect of CMV in tissue culture.[13] However, it can take as long as 3 weeks for the result to be obtained. Rapid culture techniques have been used; they depend on fluorescent monoclonal antibody techniques to CMV early or immediate early proteins in tissue culture systems. However, they may be less sensitive than conventional culture, as they rely on specific CMV proteins that may not be identical across all CMV types.

A positive result of a serologic test confirms that a person has been infected by CMV, but does not necessarily confirm destructive CMV retinitis.[14–16] These tests only confirm prior exposure to CMV but because of the high prevalence of seropositivity, they do not provide information on the risk of CMV reactivation and disease, except to identify the rare individual without antibodies who is not at risk.

There is hope that studying the existence and the amount of CMV viremia by the

polymerase chain reaction (PCR) technique as well as other molecular methods may prove to be a very useful tool and may help in the prediction of future CMV retinitis. Preliminary studies have been completed in this area, and others are ongoing.[17–20] Many studies have been undertaken in HIV medicine and others in the field of organ transplantation which may serve as an analogy to the problems of CMV infection in patients positive for HIV.

Cope et al.[21] prospectively followed 162 liver transplant recipients between November 1992 and April 1996. Using a qualitative PCR assay, they examined 1433 surveillance blood samples for human CMV infection. In multivariate analysis, viral load (per 0.25 $\log_{10}$) was associated with an odds ratio (OR) of 2.70 (95% confidence interval [CI] 1.41–5.17) for the development of CMV disease.

Dodt et al.[22] studied 200 HIV-positive patients with CD4 cell counts less than 100 cells/μL who were monitored by CMV PCR, the antigenemia test, blood cultures and CMV IgG and IgM tests every 2 months for 1 year. CMV disease developed in 38 patients. Qualitative CMV PCR predicted disease better than the other tests.

Hamprecht et al.[23] studied the detection of human CMV DNA from peripheral blood mononuclear cells, granulocytes and plasma of HIV patients. Plasma PCR techniques had the highest predictive value for CMV infection, followed by mononuclear CMV amplification and granulocyte amplification.

Bowen et al.[24] studied HIV-positive patients with a CD4 count less than 50 cells/μL and previous evidence of CMV infection (as defined by IgG seropositivity). A positive PCR result was associated with a relative hazard of 20.15 of manifesting future clinical

CMV end-organ disease. Quantitative analysis of patients who were initially CMV positive on PCR showed that CMV viral loads at study entry were higher among patients who went on to manifest CMV end-organ disease.

A study by Rasmussen et al.[25] looked at quantification of CMV burden by viral PCR in leukocytes, plasma and urine. They enrolled 75 consecutive patients with CD4 counts less than 100 cells/μL who were undergoing ophthalmic screening for CMV retinitis in San Francisco. DNA was quantified by the optical density method. The use of leukocyte DNA to detect CMV burden was more sensitive than the use of plasma DNA, but plasma DNA was more specific. The positive predictive value was highest among plasma samples.

In a prospective study, Shinkai et al.[26] used urine and blood leukocyte cultures and qualitative plasma PCR as well as quantitative competitive PCR to identify patients with AIDS at risk for CMV end-organ disease. They followed 94 patients for a mean of 12 months, obtaining urine and blood specimens every 3 months. PCR was performed on plasma. Quantitative PCR on plasma samples proved to be the most sensitive and specific test to predict future disease, with a mean of 1510 CMV copies/μL among patients in whom CMV disease developed, compared with 161 copies/μL among those in whom CMV disease did not develop.

Wattanamano et al.[27] performed a prospective cohort study involving 18 patients with CD4 counts less than 100 cells/μL and no CMV retinitis on baseline screening examinations to investigate the sensitivity and specificity of three different methods of CMV detection: pp65 antigenemia leukocyte assay, the Digene Hybrid Capture CMV

DNA assay and the Roche Amplicor Qualitative PCR test. The Digene Assay, with cut-offs at 1400 genome copies/mL, proved to have the highest sensitivity and specificity.

A recent study has tempered somewhat the enthusiasm about diagnostic assays to help predict CMV retinitis. Verbraak et al.[28] compared the CMV strains in ocular samples and peripheral blood leukocytes of 13 patients with AIDS who had CMV retinitis. The DNA sequence of the immediate early gene was determined and amplified with PCR. Of the 10 patients with CMV isolated from both compartments, 7 showed different DNA sequences, implying different strains of virus. The implication was that one single PCR test may not be easy to reproduce across laboratories.

In the era of protease inhibitor therapy, some authors have intentionally studied the incidence of CMV retinitis as well as the use of PCR assays in this clinical situation. Casado et al.[7] studied a cohort of 172 patients with AIDS with a CD4 count less than 100 cells/μL as part of the Spanish CMV-AIDS Study Group. They found that a positive CMV PCR test at initiation of therapy was the most important predictor of CMV retinitis (relative hazard 4.41). A positive result was more important than the quantitative value in predicting the occurrence of CMV retinitis.

Other laboratory-based parameters, including CD8 lymphocyte counts, have also been studied as risk factors for CMV retinitis. Although CD8 counts have been associated with emergence of CMV retinitis, investigators were not able to determine whether this was independent of CD4 counts. In a cross-sectional study, Oka et al.[29] studied CD8 counts in 14 patients with CMV retinitis compared to 24 controls with *Pneumocystis carinii* pneumonia. They found a slightly lower mean CD8 count among

cases than among controls. In a small cross-sectional study, Butler and Griedman[30] identified other hematologic parameters that may predict retinitis, but no other study has been performed in an attempt to replicate their findings.

Immunogenetic predisposition to CMV retinitis was studied in a prospective cohort study.[31] Despite a small sample (21 cases), this study showed a significant association between HLA-DR7, HLA-B44 and HLA-B51 and the appearance of CMV retinitis. It will be important to reproduce this finding and determine whether there are other immunogenetic markers for CMV retinitis. In a study of female sex workers in Kenya, MacDonald et al.[32] showed that the incidence of infection with HIV (not CMV) was lower in workers who were HLA-A2 and HLA-DRB1*01 positive than in those positive for other HLA types. Other studies have demonstrated an association between HLA type and rate of progression of HIV infection.[33,34]

We performed a case–control study to examine laboratory-based risk factors as predictors of CMV retinitis.

METHODS

A case–control study was conducted among patients with AIDS to determine laboratory-based risk factors for CMV retinitis. There were two distinct components to the study. The first examined standard-of-care laboratory tests (SCLTs) done during the study period. Typically, these tests were done regularly and routinely during the time frame of this study as part of standard clinical practice. The second component of the study examined laboratory tests on stored blood samples (SBS). These samples were

obtained by the Immunodeficiency Service of the Ottawa Hospital from patients who were positive for HIV during the time period of the study, for the purpose of performing laboratory tests in the future as new technology became available. Therefore, these tests were not performed routinely during the study period. For the SCLT component of the study, subjects (N = 279) were from the University of Ottawa Eye Institute and the Department of Ophthalmology of McGill University in Montreal, Canada. Cases were patients with AIDS and a new diagnosis of CMV retinitis who had a CD4 count below 50 cells/μL at the time of diagnosis of the retinitis. Cases were eligible for inclusion in the study between 1990–1999, but none were found in 1999. Controls were patients with AIDS but without CMV retinitis and a first CD4 count below 50 cells/μL chosen from the same period as the cases. Cases and controls were not matched by year of inclusion in the study. The inclusion criteria were as follows: 1) HIV seropositivity confirmed by Western blot analysis, 2) a diagnosis of AIDS meeting the 1993 Centers for Disease Control definition of this disease[4] (HIV positive with at least one opportunistic infection, or HIV positive and a CD4 count less than 200 cells/μL), 3) a CD4 count less than 50 cells/μL at the time of inclusion in the study and 4) age 18 years or more at the index date (see index date definition below).

Patients who met the eligibility criteria but did not return to the participating clinics for at least 3 months after enrolment were excluded.

For the SBS component of the study, the methodology was identical except that the sample size was 57, and all patients were from the University of Ottawa site (of the 131 patients from Ottawa, 74 either did not have any blood stored, or the stored blood was

not drawn during the appropriate risk period; see below). The SBS component of the study represents a subset of the SCLT component.

Sampling and Study Base

For the SCLT component, all eligible cases who received a diagnosis of CMV retinitis in the two study centres between 1990 and 1999 were enrolled. Controls were sampled randomly from the study base at both institutions. For the SBS component, all eligible cases who received a diagnosis of CMV retinitis at the Ottawa site between 1990 and 1999 and had blood stored by the Immunodeficiency Service were enrolled. All eligible controls from the Ottawa site from the same period who had blood stored by the Immunodeficiency Service were enrolled.

The characteristics of the study base at the two institutions were slightly different. In eastern Ontario, most patients with advanced HIV disease are referred to the Immunodeficiency Service of the General Campus of the Ottawa Hospital. Most of these patients are then sent to the Uveitis Service of the University of Ottawa Eye Institute for eye examinations whenever symptoms occur or occasionally for CMV surveillance eye examinations. In western Quebec, one of the main sources of referral to the Uveitis Service of the Royal Victoria Hospital is the Montreal Chest Hospital. Hence, the study base for western Quebec consisted of patients with AIDS from the Montreal Chest Hospital who were sent to the Ophthalmology Department of the Royal Victoria Hospital for ocular evaluation.

Index Date

For both components of the study, the date of diagnosis of retinitis represented the index date for cases, and risk factors for CMV retinitis were then determined relative to this index date. We also needed to determine an index date for controls. The most appropriate index date for this group would have been the date corresponding to the same duration of HIV infection as for the cases. However, this could not be determined accurately, since the exact time of HIV infection is typically difficult to ascertain because initial infection with HIV is asymptomatic or may be associated with a "flu"-like illness unrecognized by patient or physician. We therefore decided to restrict our study population — cases and controls — to subjects with CD4 counts below 50 cells/μL. Our assumption was that the duration of HIV infection would then be similar, on average, for cases and controls. Specifically, the date of the first CD4 count recorded below 50 cells/μL represented the index date for controls.

Exposure Variables

SCLT Component

1) Hematologic Parameters (hemoglobin, hematocrit, leukocytes, platelets — all as continuous variables): To our knowledge, there are no detailed studies on the etiologic dynamics of hematologic parameters. Butler and Griedman[30] used a short etiologic period (2 months) to study hematologic parameters as risk factors for CMV retinitis. The biology of these variables as potential risk factors also supports a short etiologic period, as fluctuations can be large with a change in the clinical course. Furthermore, on a

pragmatic level, since the results of these tests can depend on many factors and have a significant amount of variance, a test performed far in the past would likely have little clinical relevance. Hence, a risk period of 3 months before the index date was chosen.

2) CD8 Count: CD8 counts have been studied as risk factors for CMV disease,[29,30] but since these studies were cross-sectional, they do not help us a great deal in predicting the risk period. The biology of CD8 cells and their interaction with HIV would support a short risk period.[35] Hence, for this variable, we chose a risk period of 3 months.

3) Nutrition Variables (body mass index, serum albumin, total protein): Nutrition variables have not been studied to date. Hypotheses about these risk factors were generated from our clinical observations. We chose a short risk period of 3 months, as distant nutrition parameters are likely unrelated to the risk of retinitis.

SBS Component

1) HLA Type: HLA type is a genetic characteristic and therefore constitutes a lifelong continuous exposure that can be measured at any time relative to the index date. We determined the HLA genotype using the lymphocyte microcytotoxicity technique.[36] This provides a simple, reproducible and sensitive assay for HLA-A, -B, -C and most -DR antigens on lymphocytes. The basis of the procedure is cytolysis mediated by specific antibody in the presence of complement.

2) CMV Infection and CMV Viral Load Quantification: CMV infection (a binary variable) and quantitative CMV load (a continuous variable) were measured once in the risk period. This is important as a practical matter, as it will resemble the clinical situation of a periodic test being used to manage risk of disease. The duration of exposure was assumed to be relatively constant throughout the risk period, although studies aimed at this question exclusively might demonstrate a more complicated function. The risk period of 6 months was chosen based on the biology of infection and previous clinical studies.[13,24] For CMV PCR, amplification and detection of a 435-base-pair region of the major immediate early gene of CMV[37] were used. Quantification of CMV DNA was performed with optical density.

3) HIV Copy Number: The HIV copy number was a continuous variable. The assumptions regarding one-time testing and constant duration of exposure are similar to those for CMV infection. Based on HIV biology,[38] we chose an intermediate risk period. Therefore, the risk period was 6 months before the index date. HIV-1 was quantified by the nucleic acid sequence-based amplification (NASBA) procedure.[39]

Confounders

The confounders chosen were variables associated with the exposure variables under study that also independently increase or decrease the risk of retinitis. They included CD4 count, anti-HIV medications, anti-CMV and anti-HSV (herpes simplex virus) medications, and hospital centre.

1) CD4 Count: All patients had CD4 counts less than 50 cells/μL at enrolment into the study. Based on our understanding of markers of disease severity, CD4 count before enrolment is the most important confounder in this study. It is associated with several exposure variables (e.g., patients with low CD4 counts tend to be more ill than those with higher CD4 counts and would be more likely to have a lower hemoglobin concentration and lower hematocrit; furthermore, CD4 count is inversely associated with HIV viral load). Finally, CD4 count is an extremely important predictor of CMV retinitis. The risk period for increased risk of CMV retinitis in patients with a low CD4 count is known to be short.[5] This has been supported by studies of CD4 counts and function that seem to indicate significant improvement in immune function in the short term in patients receiving HAART.[7] We therefore used the CD4 level measured in the 3 months before the index date. CD4 count was used as a confounder in both components of the study.

2) Anti-HIV Medications: These medications are potentially associated with many exposure variables in the SCLT component of the study, as sicker patients with lower hemoglobin/hematocrit values and poorer nutrition would be more likely to be taking more anti-HIV medications and higher cumulative dosages of these medications. Furthermore, patients receiving anti-HIV medication would be at reduced risk for CMV retinitis. We measured treatments received in the 2 years before the index date. Anti-HIV medication was not used as a confounder in the SBS component of the study.

3) Anti-CMV Medications and Anti-HSV Medications: Anti-CMV medications used to

treat nonocular CMV infections were controlled for in the SCLT component of the study (but not in the SBS component), as these medications would be associated with predictors of CMV retinitis in a similar way as anti-HIV medications and would protect against CMV retinitis itself. Furthermore, the anti-HSV medication acyclovir has some anti-CMV activity[24] and therefore was also controlled for. The etiologic period chosen was two years.

4) Hospital Centre: Because there are unmeasurable potential differences between the hospital centres that may confound the exposure–disease association, hospital centre was also controlled for in the SCLT component of the study.

All data for the SCLT component of the study were abstracted from the subjects' medical records. For the SBS component, laboratory testing was performed on stored blood.

Statistical Analysis

Univariate statistics were obtained to check for implausible values as a final data editing step. Bivariate analyses were then conducted for all clinical exposure variables using the unpaired *t*-test for continuous variables (or the Wilcoxon rank-sum test if nonnormal) and the chi-square test for discrete variables. Logistic regression was used for multivariate analyses; one analysis was conducted for the SCLT part of the study, and one for the SBS component (the analyses were performed identically except where noted

below). A p value of 0.10 or less in the bivariate analyses was used as a cut-off for inclusion in the multivariate analyses. Next, the assumption of linearity of the logit was checked in a bivariate analysis for all exposure and confounding variables individually. This was done by graphing the ORs against each of the midpoints of multiple equal-interval variables. For example, CD4 count was divided into four approximately equal intervals: 0–12, 13–24, 25–36, and 37–49 cells/μL. Then the OR pertaining to case status corresponding to each interval was graphed against the midpoint of each interval (6, 18.5, 30.5 and 43.0 cells/μL respectively). From this plot, linearity of the logit was assessed. Next, a correlation matrix was obtained for all exposure variables to assess multicollinearity. Any correlation coefficient greater than 0.8 was recorded, and if a multicollinearity problem arose in the multivariate model, an algorithm for omitting one variable was used. This algorithm consisted of first keeping the variable that was a more biologically plausible exposure, then keeping the variable with fewer missing data, and finally keeping the variable with less measurement error as determined a priori.

For confounding, we determined a priori that the SCLT model would include CD4 count, hospital site (city), at least one anti-HIV medication variable, at least one anti-CMV medication variable and at least one anti-HSV medication variable. Although these five categories of confounding variables were chosen a priori, it was not clear for anti-HIV medication, anti-CMV medication and anti-HSV medication which specific representation of the variable to use (e.g., for anti-HIV medication, specific anti-HIV medication, total dose of anti-HIV medication etc.). Incorporating all possible representations of each variable would not be realistic because of precision limitations.

Therefore, logistic regression was used in the same way as described above to identify at least one variable for each of the preselected drug treatment categories. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. These confounding variables were then introduced into the main exposure model. For the SBS component of the study, the only confounder used was CD4 count.

Model building was done in a backward manner for the SCLT component of the study and in a forward manner for the SBS component. If data were missing, cases and controls were deleted in the multivariate analysis. However, when the model was reduced or expanded, these data were then used again if the variable with the missing data was eliminated from the model. Statistical techniques were not used to reduce the number of confounders in the model but were used to add or reduce exposure variables for the final models. Model expansion or reduction for the exposure variables was performed with the likelihood ratio test. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. The performance of the final model against chance and its fit were then determined.

The study protocol was submitted to and met the requirements of the Research Ethics Boards of both the Ottawa Hospital and the Royal Victoria Hospital/Montreal Chest Hospital.

RESULTS

Demographic and Descriptive Results

For the SCLT component of the study, there were a total of 279 patients in the study, 148 from Montreal and 131 from Ottawa. Of the 279, 120 were cases (88 from Montreal) and 159 were controls (99 from Ottawa). Table I summarizes the demographic and descriptive results for the cases and controls. The average age at the index date was 38.6 years for the cases and 39.6 years for the controls. Complete data were available for age, race, gender, medication use, CD4 count and hospital site. For other variables, the completeness of the medical records was as follows: hematologic parameters 254 (91.0%), serum albumin 218 (78.1%), CD8 count 203 (72.8%) and total protein 168 (60.2%).

TABLE I: DEMOGRAPHIC AND DESCRIPTIVE RESULTS FROM THE STANDARD-OF-CARE LABORATORY TEST COMPONENT OF THE STUDY

	No. (and %) of Subjects	
Variable	**Cases (N = 120)**	**Controls (N = 159)**
Hospital site		
Ottawa	32 (26.7)	99 (62.3)
Montreal	88 (73.3)	60 (37.7)
Gender		
Male	109 (90.8)	136 (85.5)
Female	11 (9.2)	23 (14.5)
Caucasian race	107 (89.2)	131 (82.4)
Index date, year		

TABLE I: DEMOGRAPHIC AND DESCRIPTIVE RESULTS FROM THE STANDARD-OF-CARE LABORATORY TEST COMPONENT OF THE STUDY		
	No. (and %) of Subjects	
1990	6 (5.0)	5 (3.1)
1991	5 (4.2)	15 (9.4)
1992	15 (12.5)	13 (8.2)
1993	21 (17.5)	19 (11.9)
1994	23 (19.2)	33 (20.8)
1995	25 (20.8)	46 (28.9)
1996	20 (16.7)	23 (14.5)
1997	4 (3.3)	4 (2.5)
1998	1 (0.8)	1 (0.6)

Of the 57 patients in the SBS component of the study, 21 were cases and 36 were controls. At the index date, the average age of the patients was 41.0 years. Most were men (50 [87.7%]), and most were Caucasian (50 [87.7%]). These demographic characteristics were very similar between the cases and controls. The distribution of patients by year of the study was as follows: 1993, 1 (1.8%), 1994, 7 (12.3%), 1995, 22 (38.6%), 1996, 20 (35.1%), 1997, 6 (10.5%) and 1998, 1 (1.8%). Complete data were available for age, race, gender, qualitative CMV PCR, quantitative CMV PCR and CD4 count. For other variables, the completeness of the data was as follows: HIV viral load 55 (96.5%) and HLA type 54 (94.7%).

Hematologic and Nutrition Parameters

The hematologic and nutrition parameters are presented in Table II.

TABLE II: HEMATOLOGIC AND NUTRITION VARIABLES AMONG CASES AND CONTROLS

	Mean ± Standard Deviation		
Variable	**Cases**	**Controls**	***p* Value**
CD8 count, cells/μL	359.44 ± 485.76	424.30 ± 316.96	0.0004
Leukocyte count, cells × 10^9/L	3.19 ± 1.99	3.47 ± 1.77	0.03
Platelet count, × 10^9/L	188.81 ± 79.24	177.39 ± 74.12	0.26
Hemoglobin concentration, g/L	107.01 ± 19.78	119.01 ± 19.77	0.00001
Hematocrit, L/L	0.32 ± 0.05	0.35 ± 0.06	0.00001
Body mass index, kg/m^2	20.21 ± 3.24	21.73 ± 3.57	0.002
Serum albumin level, g/L	37.02 ± 6.08	39.83 ± 5.39	0.0001
Total protein level, g/L	72.98 ± 12.69	73.96 ± 12.10	0.10

HLA Type

Eighty HLA types were processed for each patient. The statistically significant HLA types ($p < 0.10$) are summarized in Table III.

TABLE III: HLA TYPES SIGNIFICANTLY ASSOCIATED WITH CMV RETINITIS AMONG PATIENTS WITH STORED BLOOD SAMPLES (N = 54)

HLA Type	**No. (and %) of Cases Exposed (N = 19)**	**No. (and %) of Controls Exposed (N = 35)**	**Odds Ratio**	***p* Value**
A25	2 (10.5)	0 (0.0)	–	0.05
A31	3 (15.8)	1 (2.9)	6.38	0.08
B7	2 (10.5)	13 (37.1)	0.20	0.04
B18	2 (10.5)	0 (0.0)	–	0.05
B27	4 (21.0)	0 (0.0)	–	0.005

TABLE III: HLA TYPES SIGNIFICANTLY ASSOCIATED WITH CMV RETINITIS AMONG PATIENTS WITH STORED BLOOD SAMPLES (N = 54)				
B40	3 (15.8)	1 (2.9)	6.38	0.08
B52	2 (10.5)	0 (0.0)	–	0.05
Bw4	13 (68.4)	14 (40.0)	3.25	0.05
Cw1	2 (10.5)	0 (0.0)	–	0.05
Cw7	5 (26.3)	21 (60.0)	0.24	0.02
DRBI13	1 (5.3)	12 (34.3)	0.11	0.02
DRBI15	8 (42.1)	7 (20.0)	2.91	0.08
DR51	8 (42.1)	7 (20.0)	2.91	0.08

CMV PCR and HIV Viral Load

Of the 21 cases, 17 (80.9%) had a positive result of qualitative CMV PCR, compared with 18 (50.0%) of the 36 controls (OR 4.25, 95% CI 1.24–14.39). The mean CMV viral load was significantly higher among cases than controls (0.93 vs. 0.25 optical density units) ($p = 0.0005$).

The average HIV viral load was not significantly different between the cases and controls (261,429 copies/μL vs. 224,500 copies/μL) ($p = 0.35$).

Confounding by Medication

It was decided a priori that at least one drug or drug amount from each of the anti-HIV, anti-CMV and anti-HSV medication categories would be used in the final SCLT multivariate model. CD4 and hospital site were also included. The specific variables to be included were determined by logistic regression.

Table IV presents the bivariate descriptive statistics and ORs for the dichotomous treatment variables. Variables with a p value less than 0.10 were retained for the subsequent logistic regression.

TABLE IV: BIVARIATE DISCRETE STATISTICS FOR THE DICHOTOMOUS TREATMENT VARIABLES

Medication	No. (and %) of Cases Exposed	No. (and %) of Controls Exposed	OR	95% CI	p Value
Anti-HSV	53 (44.2)	35 (22.0)	2.80	1.67–4.71	0.0001
Anti-CMV	17 (14.2)	9 (5.7)	2.75	1.20–6.29	0.02
Ganciclovir	12 (10.0)	4 (2.5)	4.31	1.42–12.99	0.008
Foscarnet	7 (5.8)	0 (0.0)	–	–	0.002
CMV test drug	3 (2.5)	6 (3.8)	0.65	0.18–2.44	0.55
Anti-CMV combination therapy	6 (5.0)	0 (0.0)	–	–	0.004
Anti-human immunodeficiency virus (HIV)	93 (77.5)	106 (66.7)	1.72	1.01–2.95	0.05
Zidovudine (AZT)	80 (66.7)	92 (57.9)	1.46	0.89–2.38	0.13
Didanosine (ddI)	47 (39.2)	60 (37.7)	1.06	0.65–1.73	0.81
Zalcitabine (ddC)	30 (25.0)	37 (23.3)	1.10	0.63–1.91	0.74
Lamivudine (3TC)	27 (22.5)	19 (12.0)	2.14	1.31–4.04	0.02
Stavudine (d4T)	5 (4.2)	1 (0.6)	6.87	1.04–12.32	0.04
Saquinavir	6 (5.0)	0 (0.0)	–	–	0.004
Ritonavir	6 (5.0)	0 (0.0)	–	–	0.004
Indinavir	3 (2.5)	0 (0.0)	–	–	0.05

The total number of anti-HIV medications and total duration of anti-HIV drug use among cases and controls are shown in Table V. In this bivariate analysis, cases took more drugs on average than controls, and the duration of medication use was longer among cases than among controls.

TABLE V: BIVARIATE STATISTICS FOR NUMBER OF ANTI-HIV MEDICATIONS AND DURATION OF DRUG USE

Mean ± SD

Variable	Cases	Controls	*p* Value
Total number of anti-HIV medications	1.68 ± 1.41	1.32 ± 1.20	0.06
Duration of anti-HIV medication use, months	12.82 ± 9.50	10.27 ± 9.50	0.02

Whenever possible, the quantity of drug was also recorded for the risk period given in the methods section. With some drugs, such as the protease inhibitors and foscarnet, too few observations were available, and therefore the data are not presented for these drugs. The results for those with reasonable precision are summarized in Table VI. Acyclovir was used in significantly greater amounts among cases than among controls.

TABLE VI: BIVARIATE STATISTICS FOR TOTAL AMOUNT OF MEDICATION USED			
	Mean ± SD, g		
Medication	**Cases**	**Controls**	***p* Value**
Acyclovir	69.12 ± 126.20	37.28 ± 110.54	0.003
Ganciclovir	3.67 ± 18.17	0.67 ± 4.89	0.19
Zidovudine (AZT)	141.04 ± 149.50	125.71 ± 143.15	0.30
Didanosine (ddI)	45.86 ± 90.07	35.13 ± 64.68	0.61
Zalcitabine (ddC)	0.14 ± 0.38	0.11 ± 0.27	0.90
Lamivudine (3TC)	26.24 ± 60.86	12.54 ± 52.38	0.17

The final confounding model was then built (Table VII). CD4 count, hospital center and treatment variables with a *p* value less than 0.10 were all initially forced into the model.

TABLE VII: FINAL CONFOUNDING MODEL				
Variable	**OR**	**Standard Error (SE)**	**95% CI**	***p* Value**
Any ganciclovir use	3.11	1.95	0.91–10.61	0.07
Any acyclovir use	2.05	0.65	1.10–3.83	0.02
Number of anti-HIV drugs	1.43	0.18	1.13–1.82	0.03
CD4 count	0.96	0.01	0.94–0.98	0.0001
Hospital center	5.19	1.62	2.82–9.58	0.0001

The exposure variables retained from the univariate analysis and the confounders were used to build the final model. The model was then reduced as described in the methods section.

Multivariate Analysis

Of the hematologic parameters, only a low hemoglobin level (a continuous variable) was a significant risk factor for CMV retinitis on multivariate analysis (OR 0.96, 95% CI 0.94–0.98). Neither CD8 count nor any of the nutrition parameters were significant in this analysis. Both qualitative CMV PCR (OR 21.71, 95% CI 1.80–261.67) and quantitative CMV PCR (OR 33.03, 95% CI 2.32–469.39) were strong predictors of CMV retinitis. Among the HLA types, HLA-Bw4 was a significant predictor of CMV retinitis (OR 11.68, 95% CI 1.29–105.82), as was HLA-DRBI15 (OR 9.34, 95% CI 1.14–76.41). Finally, HLA-Cw7 was protective against CMV retinitis (OR 0.09, 95% CI 0.01–0.67).

CONCLUSION

The purpose of this study was to determine whether risk factors for CMV retinitis could be uncovered that would help with the monitoring, surveillance, treatment and prophylaxis of patients with this disorder. We used a case–control design, which allowed us to study many risk factors simultaneously. We studied the risk factors systematically based on previous work in the field of CMV retinitis as well as generating etiologic hypotheses from studies of CMV risk factors of other organ systems, typically from organ transplantation. In this paper, we studied laboratory-based risk factors for CMV retinitis.

In the multivariate model of SCLT-based risk factors, only hemoglobin level remained a significant predictor of CMV retinitis: patients with a low hemoglobin level were at higher risk for CMV retinitis. The exact reason for this association is unclear, but it is well known that the life span of erythrocytes may be shortened in the presence of infectious or immunologic disorders. This association remained even after degree of illness was controlled for. It is possible that in the setting of advanced CMV infection, as one would find preceding CMV retinitis, an infectious or immunologic mechanism initiated by CMV may have a deleterious effect on erythrocytes.

CMV infection and amount of serum CMV were also predictive of CMV retinitis in our study. This is biologically plausible, as one would expect a higher viral load to predict CMV retinitis. Our risk period was 6 months; hence, a stored blood sample needed to be available once in the 6 months (our range was 1 to 6 months) preceding the onset of CMV retinitis or, for the controls, from the index date. We were unable to determine the best timing for monitoring viral load or whether sequential viral loads might demonstrate a risk

function even more predictive of CMV retinitis. However, the risk period that we chose is pragmatic on a clinical basis, as obtaining a blood sample for CMV PCR once or twice a year would be practical in a clinical practice and would most likely be economically justifiable as well.

HIV viral load was not a significant predictor of CMV retinitis in this study. However, it should be pointed out that the patient population was restricted to those with CD4 counts less than 50 cells/μL; a more heterogeneous population with respect to CD4 may have produced a different result. Furthermore, the vast majority of patients in this study were not receiving maximal antiretroviral therapy. In today's world of HAART, this result may not be generalizable.

We examined HLA type as a risk factor for CMV retinitis in 54 patients. CMV-infected T lymphocytes are known to decrease their production of HLA class I antigens[40] and increase their production of HLA-DR.[41] Some clinical studies support the importance of HLA type as a risk factor. For example, HLA-DR7 and HLA-B5 have been shown to be risk factors for CMV infection after solid-organ transplantation.[42,43] MacDonald et al[32] studied immunogenetic predisposition to CMV retinitis in a small prospective cohort study and found a significant association between HLA-DR7, -B44 and -B51 and the appearance of CMV retinitis. We found that several HLA types were risk factors for CMV retinitis (HLA-A25, -A31, -B18, -B27, -B40, -B52, -Bw4, -Cw1, -DRBI15 and -DR51) and that some were protective (HLA-B7, -Cw7 and -DRBi13). On multivariate analysis, however, only two HLA types remained significant predictors of CMV retinitis: HLA-Bw4 (OR 11.68, 95% CI 1.29–105.82) and HLA-DRBI15 (OR 9.34, 95% CI 1.14–76.41). Only one

HLA type remained protective against CMV retinitis: HLA-Cw7 (OR 0.09, 95% CI 0.01–0.67). HLA types found to be important in our study are different from those found in other studies. These results need to be interpreted with caution, as both type I and type II errors are possible. Given that we examined 80 HLA types and found different significant associations than other investigators (making any a priori hypotheses invalid), a type I error is certainly possible. With a sample size of 54 for HLA type, a type II error is also a major consideration. However, some HLA types have proven to be important.[42,43] An immunogenetic predisposition to CMV retinitis is one possible reason why the disease develops in some severely immunosuppressed patients with a high viral load but not in others. It is possible that the risk or protection associated with HLA type may even be HIV or CMV strain specific. This association should be studied further in a larger sample of patients.

In any epidemiologic study, alternative explanations for the observed results may be due to chance, bias or confounding. With a sample size of only 57 for the SBS component of the study, statistical power was one of our study's main weaknesses. Although this sample size compares favourably with that in other studies on laboratory-based risk factors for CMV retinitis, it is still a concern, and the findings, especially in the multivariate analysis, should be interpreted carefully.

The major biases of concern in this case–control study were selection bias and misclassification bias. We should first point out that we chose a case–control design for two main reasons. First, it allowed us to examine multiple potential exposure variables, which was one of the goals of the study. Second, with the advent of HAART, in 1996, the

incidence of CMV retinitis began declining to the point where it would not have been realistic to recruit a cohort of unaffected patients that would provide a study with adequate power. We attempted to minimize selection bias by adhering strictly to the study base principle. Nondifferential misclassification is always possible in a laboratory-based risk assessment, as virtually no test has perfect sensitivity or specificity. However, this would have biased our results toward the null, making the association found in this study even more significant.

We took great efforts to minimize the effect of confounding. CD4 count was controlled for a priori for both components of the study as it is both the most important indicator of disease severity and an approximate indicator of time since HIV infection. For the SCLT component of the study, hospital centre was also controlled for a priori because of the obvious potential for confounding from one study base to another. Regarding confounding by medication, we created a model and decided a priori that it would contain at least one variable for anti-HIV medication, one variable for anti-CMV medication and at least one variable for anti-HSV medication (since anti-HSV medications may also have some anti-CMV effects). This model was then used in the main exposure model to control for confounding in the SCLT component of the study. While it is impossible to ever be absolutely certain that confounding has been completely eliminated in any epidemiologic study, we feel confident that we minimized its effect.

In conclusion, we feel that this study is a useful addition to the literature on laboratory-based risk factors for CMV retinitis. It is hoped that the results will help guide both internists and ophthalmologists in the surveillance, diagnosis and management of this blinding disease.

ACKNOWLEDGEMENTS: Supported by the University of Ottawa Young Investigator Award and the Medical Research Council of Canada. The authors wish to acknowledge Rita Frost, of the Regional Virology Laboratory of Eastern Ontario, for her technical expertise.

REFERENCES

1. Holland GN, Pepose JS, Simons KB, et al. Ocular disease in the acquired immune deficiency syndrome: clinicopathological correlations. In: Secchi AG, Fregona IA, editors: *Modern Trends in Immunology and Immunopathology of the Eye*. Milan: Saunders; 1989. p. 132-155.
2. Montaner JS, Le T, Hogg R, Ricketts M, et al. The changing spectrum of AIDS index diseases in Canada. *AIDS* 1994;8(5):693-696.
3. Munoz A, Schrager LK, Bacellar H, et al. Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am J Epidemiol* 1993;137(4):423-438.
4. Holland G, Tufail A, Jordan MC. Cytomegalovirus diseases. In: Pepose JS, Holland GN, Wilhelmus KR, editors. *Ocular Infection & Immunity*. New York: Mosby; 1996. p. 1088-1129.
5. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1992;5:1069-1074.
6. McDonald CK, Kuritzkes DR. Human immunodeficiency virus type 1 protease inhibitors. *Arch Intern Med* 1997;157:951-959.
7. Casado JL, Arrizabalga J, Montes M, et al. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV-AIDS Study Group. *AIDS* 1999;13(12):1497-1502.

8. Lewis JS, Terriff CM, Coulston DR, et al. Protease inhibitors: a therapeutic breakthrough for the treatment of patients with HIV. *Clin Ther* 1997;19:187-214.

9. Le Moing V, Chene G, Carrieri MP, et al. Predictors of virologic rebound in HIV-1 infected patients initiating a protease inhibitor-containing regimen. *AIDS* 2002;16: 21-29.

10. Spector SA, McKinley GF, Lalezari JP, et al. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. *N Engl J Med* 1996;334:1491-1497.

11. Anderson RD, Griffy KG, Jung D, et al. Ganciclovir absolute bioavailability and steady-state pharmacokinetics after oral administration of two 3000 mg/d dosing regimens in HIV and CMV seropositive patients. *Clin Ther* 1995;17:425-432.

12. Masur H, Whitcup SM, Cartwright C, et al. Advances in the management of AIDS-related cytomegalovirus retinitis. *Ann Intern Med* 1996;125(2):126-136.

13. Ho M. Cytomegalovirus. In: *Biology of Infection*. 2nd ed. New York: Plenum; 1991. p. 1-66.

14. Stagno S, Reynolds DW, Huang ES, et al. Congenital cytomegalovirus infection. *N Engl J Med* 1977;296:1254-1258.

15. Rao N, Waruszewski DT, Armstrong JA, et al. Evaluation of anti-complement immunofluorescence test in cytomegalovirus infection. *J Clin Microbiol* 1977;6:633-638.

16. David JL, Feuer W, Culbertson WW, et al. Interpretation of intraocular and serum antibody levels in necrotizing retinitis. *Retina* 1995;15:233-240.

17. Wetherill PE, Landry ML, Alcabes P, et al. Use of a quantitative CMV antigenemia test in evaluating HIV+ patients with and without CMV disease. *J Acquir Immune*

Defic Syndr Hum Retrovirol 1996;12(1):33-37.

18. Hansen KK, Ricksten A, Hofmann B, et al. Detection of cytomegalovirus DNA in serum correlates with clinical cytomegalovirus retinitis in AIDS. *J Infect Dis* 1994;170(5):1271-1274.

19. MacGregor RR, Pakola SJ, Graziani AL, et al. Evidence of active CMV infection in clinically stable HIV-infected individuals with CD4+ lymphocyte counts below 100/microliters of blood: features and relation to risk of subsequent CMV retinitis. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;10(3):324-330.

20. Rasmussen L, Morris S, Zipeto D, et al. Quantitation of human CMV DNA from peripheral blood cells of human immunodeficiency virus-infected patients could predict CMV retinitis. *J Infect Dis* 1995;171(1):177-182.

21. Cope AV, Sabin C, Burroughs A, et al. Interrelationships among quantity of human cytomegalovirus DNA in blood, donor–recipient serostatus and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation. *J Infect Dis* 1997;176:1484-1490.

22. Dodt KK, Jacobsen PH, Hofmann B, et al. Development of cytomegalovirus (CMV) disease may be predicted in HIV-infected patients by CMV polymerase chain reaction and the antigenemia test. *AIDS* 1997;11(3):F21-F28.

23. Hamprecht K, Steinmassl M, Einsele H, et al. Discordant detection of human cytomegalovirus DNA from peripheral blood mononuclear cells, granulocytes and plasma: correlation to viremia and HCMV infection. *J Clin Virol* 1998;11(2):125-136.

24. Bowen EF, Sabin CA, Wilson P, et al. CMV viremia detected by polymerase chain reaction identifies a group of HIV positive patients at high risk of CMV disease. *AIDS* 1997;11(7):889-893.

25. Rasmussen L, Ziputo D, Wolitz RA, et al. Risk for retinitis in patients with AIDS can be assessed by quantitation of threshold levels of CMV DNA burden in blood. *J Infect Dis* 1997;176(5):1146-1155.

26. Shinkai M, Bozzette SA, Powderley W, et al. Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for identification of AIDS patients at risk for developing human cytomegalovirus disease. *J Infect Dis* 1997;175:302-308.

27. Wattanamano P, Clayton JL, Kopicko JJ, et al. Comparison of three assays for cytomegalovirus detection in AIDS patients at risk for retinitis. *J Clin Microbiol* 2000;38(2):727-732.

28. Verbraak FD, Bruinenberg M, van den Horn GJ, et al. Cytomegalovirus (CMV) strain differences between the eye and blood in AIDS patients with CMV retinitis. *AIDS* 1988;12(7):713-718.

29. Oka S, Nagata Y, Fujino Y, et al. CD8+ T lymphocyte counts as an adjunctive predictor of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome. *Intern Med* 1997;36(7):461-465.

30. Butler GA, Griedman AH. Screening indices for cytomegalovirus retinitis in patients with human immunodeficiency virus. *Mt Sinai J Med* 1992;59(1):61-65.

31. Schrier RD, Freeman WR, Wiley CA, et al. Immune predispositions for cytomegalovirus retinitis in AIDS. The HNRC Group. *J Clin Invest* 1995;95(4):1741-1746.

32. MacDonald KS, Fowke KR, Kimani J, et al. Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;181(5):1581-1589.

33. Migueles SA, Sabbaghian MS, Shupert WL, et al. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. *Proc Natl Acad Sci U S A* 2000;97(6):2709-2714.

34. Gao X, Nelson GW, Karachi P, et al. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med* 2001;344(22): 1668-1675.

35. Tomaras GD, Lacey SF, McDanal CB, et al. CD8+ T cell-mediated suppressive activity inhibits HIV-1 after virus entry with kinetics indicating effects on virus gene expression. *Proc Natl Acad Sci U S A* 2000;97(7):3503-3508.

36. Allannic H, Fauchet R, Lorcy Y, et al. HLA and Graves' disease: an association with HLA-DRw3. *J Clin Endocrinol Metab* 1980;51(4):863-867.

37. Compton J. Nucleic acid sequence-based amplification. *Nature* 1991;350:91-92.

38. Luciw PA, Potter SJ, Steimer K, et al. Molecular cloning of AIDS-associated retro-virus. *Nature* 1984;312:760-763.

39. Kievits T, van Gemen B, van Strijp D, et al. NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. *J Virol Methods* 1991;35:273-286.

40. Grundy JE, Ayles HM, McKeating JA, et al. Enhancement of class 1 HLA antigen expression by cytomegalovirus: role in amplification of virus infection. *J Med Virol*

1988;25:483-495.

41. Scholz M, Hamann A, Blaheta RA, et al. Cytomegalovirus and interferon-related effects on human endothelial cells: cytomegalovirus infection reduces upregulation of HLA class II antigen expression after treatment with interferon-gamma. *Hum Immunol* 1992;35:230-238.

42. Kraat YJ, Christiaans MH, Nieman FH, et al. Risk factors for cytomegalovirus infection and disease in renal transplant recipients: HLA-DR7 and triple therapy. *Transpl Int* 1994;7(5):362-367.

43. Boland GJ, Hene RJ, Ververs C, et al. Factors influencing the occurrence of active cytomegalovirus (CMV) infections after organ transplantation. *Clin Exp Immunol* 1993;94(2):306-312.

LINKING TEXT II

Laboratory testing to establish risk levels for CMV retinitis is gaining widespread popularity. The previous paper presented a thorough attempt at studying both common and state-of-the-art laboratory testing as risk factors for CMV retinitis. The risk factors were divided into those identified through standard-of-care tests (those done routinely at the time of the study, such as hematologic parameters, CD8 count and nutrition parameters) and those identified through examination of blood samples that had been stored to permit testing as technology became available (i.e., CMV viral load, HIV viral load and HLA type). Typically, the tests performed on stored blood samples are more expensive than the standard-of-care tests. The results of the laboratory-based risk factor paper can most likely be applied across the entire risk period equally. Furthermore, they may be used together with the results from the other papers. For example, because testing for viral load and HLA type is expensive, ordering these tests routinely for all patients might not be justified. One could use the risk factors found in manuscripts I and III to identify patients at high risk and then order the more expensive laboratory tests only for this subset of patients.

Iatrogenic risk factors for CMV retinitis will be presented in the third paper, which follows. These include corticosteroid use, chemotherapeutic agents administered, radio-therapy and blood transfusion. These variables are extremely easy to determine and can be accurately recorded in medical charts. They are most likely to be important late in the risk period for CMV retinitis (as patients become more immunosuppressed and their CD4 count falls to near 0 cells/μL), since at this time patients will have the greatest need for

these treatments for their accumulating opportunistic infections and malignant disorders. The following paper presents the iatrogenic risk factors in more detail.

MANUSCRIPT III: IATROGENIC RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

IATROGENIC RISK FACTORS FOR CYTOMEGALOVIRUS RETINITIS

RUNNING TITLE: Iatrogenic Risks for CMV-R

AUTHORS:

William G. Hodge MD, MPH, FRCSC[1,2]

Jean-François Boivin MD, ScD, FRCPC[2]

Stan Shapiro PhD[2]

Richard Lalonde MD, FRCPC[3]

Kirtida Shah MD[1]

Brad Murphy MD[1]

Michel Dionne MD[1]

Ashish Goela MD[1]

1- University of Ottawa Eye Institute, Ottawa, Ontario, Canada

2- Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada

3- Division of Infectious Diseases, Royal Victoria Hospital of McGill University Health Sciences Center, Montreal, Quebec, Canada

Corresponding Author: Dr. William G. Hodge

University of Ottawa Eye Institute

501 Smyth Road

Ottawa ON Canada

K1H 8L6

ABSTRACT

BACKGROUND: Over two dozen descriptive studies, prospective cohort studies and clinical trials have been conducted to examine the treatment of cytomegalovirus (CMV) retinitis. Surprisingly, very few studies have investigated the equally important question of risk factors for this disease. Identifying these risk factors will have many benefits, including helping establish screening regimens, examination frequency regimens, and targeted prophylaxis with oral ganciclovir or other anti-CMV agents.

PURPOSE: To determine the iatrogenic risk factors for CMV retinitis in patients with the acquired immunodeficiency syndrome (AIDS).

METHODS: We conducted a case–control study involving 120 patients with CMV retinitis diagnosed in 1990–1999 and 159 patients without CMV retinitis from the same period. The sampling was from a primary study base in western Quebec and eastern Ontario of patients with AIDS and CD4 counts less than 50 cells/μL at the time of diagnosis of retinitis in the cases or the corresponding date for the controls. Iatrogenic risk factors studied included corticosteroid use, treatment with chemotherapy, treatment with radiotherapy and blood transfusions. Confounders controlled for included CD4 count, hospital center and a series of variables to control for confounding by drug treatment. Statistical analysis was performed by multivariate logistic regression. A systematic model building strategy was developed, from assumption testing to model building to model checking.

RESULTS: From the multivariate treatment model, steroid use was the only predictor of CMV retinitis (odds ratio 6.41, 95% confidence interval 2.35–17.51).

CONCLUSIONS: Based on this study, the use of steroids systemically elevated the risk of CMV retinitis. This result may be useful to clinicians and health policy experts in developing rational guidelines for screening, examination frequency and targeted prophylaxis for patients with AIDS.

KEY WORDS: CMV Retinitis, Iatrogenic Risk Factors, Corticosteroids

INTRODUCTION

Cytomegalovirus (CMV) retinitis is one of the most devastating complications of the acquired immunodeficiency syndrome (AIDS). It is the most common and most devastating ocular opportunistic infection among patients with AIDS. It is a necrotizing, blinding form of retinitis that tends to occur late in the course of AIDS. The reported lifetime cumulative incidence of AIDS-related CMV retinitis ranges from 25% to 40%.[1] Two large population-based studies demonstrated an increasing incidence of this disorder in the late 1980's and the 1990's.[2,3] No other ocular opportunistic infection among patients with AIDS has a lifetime cumulative incidence over 1–2%.[4] Based on data from the era before highly active antiretroviral therapy (HAART), the estimated mean time from development of AIDS to manifestation of CMV retinitis is 9–18 months.[5]

With the advent of HAART,[6] the incidence of CMV retinitis is declining. Casado et al.[7] studied a cohort of 172 HIV-infected patients with a CD4 count less than 100 cells/μL at the time of protease inhibitor introduction. The cumulative incidence of CMV retinitis was 5% at 1 year and 6% at 2 years. Doan et al.[8] found that the annual risk of CMV retinitis in 1995 (without protease inhibitors) was 6.1%, compared with 1.2% in 1997 (with protease inhibitors); the annual risk of relapse after anti-CMV therapy decreased from 36% to 17%. Nevertheless, we should be cautiously optimistic regarding this new treatment. At least 20% of patients respond poorly or not at all to HAART,[9,10] and the risk of CMV retinitis has likely not changed for them. Furthermore, since emerging resistance to a component of HAART is always possible, the ophthalmic and public health community should be prepared for new CMV outbreaks at all times. Finally, in developing nations,

AIDS and its opportunistic infections are more devastating today than they ever have been. Hence, CMV retinitis remains one of the most important irreversibly blinding diseases in all of ophthalmology.

Several authors have investigated the treatment of CMV retinitis,[11–13] but far fewer have examined risk factors for this disease. Well designed case–control studies would be able to contribute to understanding these risk factors and may provide several clinical benefits. The benefits of understanding these risk factors include the possibility of creating more sophisticated guidelines for screening, determining risk factors to permit earlier detection, and providing a more logical algorithm for prophylactic therapy. One example of a drug for potential targeted prophylaxis is oral ganciclovir; however, since it is currently very expensive and has only moderate bioavailability, it is seldom used as a prophylactic medication.[11,12] Valganciclovir, antiretroviral therapy or experimental immunologic-based therapy may prove to play a role in prophylaxis as well.[13] However,
the exact method of optimal targeted prophylaxis would have to be developed in separate studies.

Iatrogenic risk factors for CMV infection of nonocular tissue in other immunosuppressive settings have been investigated but have been studied only superficially, if at all, as potential determinants of CMV retinitis. Clues from these studies may help us better understand the risk factors for CMV retinitis. One such risk factor is corticosteroid therapy. Nagler et al.[14] conducted a case–control study to examine risk factors for CMV pneumonia in bone marrow transplant recipients and found a very strong association between steroid use and development of CMV pneumonia. In a historical cohort study on

the risks of CMV intestinal disease after intestinal transplantation, Manez et al.[15] found steroids to be an important risk factor, as did Nelson et al.[16] in a case–control study on the risk of CMV disease in any organ in patients with AIDS. Morris et al.[17] in a study on the risk of CMV disease in renal allograft recipients treated with cyclosporine, found steroids to be an important risk factor. Gorensek et al.[18] studied the risk factors for CMV infection in heart transplant recipients and found that the only significant predictor of CMV infection was greater than average steroid use.

Immunosuppressive (nonsteroidal cytotoxic) therapy has also been studied as a possible risk factor for systemic CMV disease. In a multicenter case–control study of CMV infection after liver transplantation, Schiffer et al.[19] found immunosuppressive medication use and duration to be the most important nonserologic determinants of CMV infection. More specifically, Kraat et al.[20] found triple immunosuppressive therapy to be an important risk factor for CMV infection after renal transplantation. The type and intensity of immunosuppression were found to be important risk factors of CMV infection after liver transplantation.[21] Kirklin et al.[22] analyzed the Cardiac Transplant Research Database to look at risk factors for CMV infection after heart transplantation. Among the 1553 patients in the cohort, CMV disease developed in 230. Higher than average dosages of cytotoxic induction therapy agents was the most important risk factor.

Some authors have observed an increase in CMV infections in patients who have received multiple blood transfusions.[23] The pathogenesis of this association is unclear, although it does seem to be more complicated than simple seroconversion. Possible mechanisms include transfer of an increased viral load by the transfusions ("CMV

viremia"), transfer of new, slightly modified strains of CMV that cannot be determined with present serologic testing, or activation of preexisting CMV by a factor(s) in the transfusion. This last theory was championed by Sloand et al.[23] in the discussion of their historical cohort study looking at CMV infection of any organ in patients with AIDS who had received blood transfusions. Paya et al.[24] published similar results, and, in fact, these authors found transfusions to be the most important risk factor for CMV infection after liver transplantation. However, Gorensek et al.[21] reported conflicting findings.

Thus, there are several important gaps in our knowledge with respect to the iatrogenic risk factors for CMV retinitis. Very few studies have been performed to examine the risk factors for this important disease. In fact, most of the studies investigating the etiology of CMV retinitis have dealt with CMV disease of nonophthalmic organs in non-AIDS immunosuppressive settings. Several of the hypotheses regarding iatrogenic risk factors in the current work have come from these studies.

The goal of this study was to determine the iatrogenic risk factors for CMV retinitis in a large group of patients from two Canadian centers.

METHODS

A case–control study was conducted among patients with AIDS seen at the University of Ottawa Eye Institute and the Department of Ophthalmology of McGill University in Montreal, Canada. Cases were patients with AIDS and a new diagnosis of CMV retinitis who had a CD4 count below 50 cells/μL at the time of diagnosis of the retinitis. Cases were eligible for inclusion in the study between 1990 and 1999; however, none were found

in 1999. Controls were patients with AIDS but without CMV retinitis who had a first CD4 count below 50 cells/μL, chosen from the same period as the cases. Cases and controls were not matched by year of inclusion in the study. The inclusion criteria were as follows: 1) HIV seropositivity confirmed by Western blot analysis, 2) a diagnosis of AIDS meeting the 1993 Centers for Disease Control definition of this disease[4] (HIV positive with at least one opportunistic infection, or HIV positive and absolute CD4 count less than 200 cells/μL), 3) a CD4 count less than 50 cells/μL at the time of inclusion in the study and 4) age 18 years or more at the index date (see index date definition below).

Patients who met the eligibility criteria but were not followed in the study base for at least 3 months before being eligible to be a case or control were excluded.

Sampling and Study Base

All eligible cases who received a diagnosis of CMV retinitis in the two study centers in 1990–1999 were enrolled. Controls were sampled randomly from the study base at both institutions until the sample size criteria were met (at least 198 patients, based on an assumed odds ratio [OR] of 3.0 for steroid use). The characteristics of the study base at the two institutions were slightly different. In eastern Ontario, most of the patients with advanced HIV disease are referred to the Immunodeficiency Service of the General Campus of the Ottawa Hospital. Most of these patients are then sent to the Uveitis Service of the University of Ottawa Eye Institute for eye examinations whenever symptoms occur or occasionally for CMV surveillance eye examinations. In western Quebec, one of the main sources of referral to the Uveitis Service of the Royal Victoria Hospital is the Montreal

Chest Hospital. Hence, the study base for western Quebec consisted of patients with AIDS from the Montreal Chest Hospital who were sent to the Ophthalmology Department of the Royal Victoria Hospital for ocular evaluation.

Index Date

The date of diagnosis of retinitis represented the index date for the cases. Risk factors for CMV retinitis were then determined relative to this index date. We also needed to determine an index date for the controls. The most appropriate index date for this group would have been the date corresponding to the same duration of HIV infection as for the cases. However, this could not be determined accurately, since the exact time of HIV infection is typically difficult to ascertain because initial infection with HIV may be asymptomatic. We therefore decided to restrict our study population — cases and controls — to subjects with CD4 counts below 50 cells/μL. Our assumption was that the duration of HIV infection would then be similar, on average, for cases and controls. Specifically, the date of the first CD4 count recorded below 50 cells/μL represented the index date for controls.

Exposure Variables

1) Corticosteroid Use (use of steroid, use of prednisone and use of other steroids [all binary variables] and total amounts of these medications): Corticosteroids are a well-known immunosuppressant.[25] Based on previous clinical studies,[16–18] a risk period of 6 months before the index date was used in this study.

2) Chemotherapeutic Agents: Other immunosuppressive chemotherapeutic agents invariably suppress leukocyte counts and were examined as potential iatrogenic risk factors. Based on other studies that have looked at this exposure,[20,22] a risk period of 3 months was chosen.

3) Radiotherapy: Some side effects of radiotherapy, including skin and mucous membrane necrosis, are very acute and typically occur in epithelial cells.[26] However, there are also late effects, such as fistula formation, nonhealing ulceration and radiation retinopathy, which tend to occur in slowly metabolizing tissues. Hence, the risk period chosen was 12 months.

4) Blood Transfusion: The potential etiologic role of blood transfusion is not clear.[23] Explanations range from invoking an infectious etiology to providing a cofactor for CMV infection. In their clinical study, Sloand et al[23] used 6 months as the risk period when studying any CMV disease in patients with AIDS. We also used 6 months as the risk period.

Confounders

The confounders chosen were variables associated with iatrogenic variables that also independently increase or decrease the risk of retinitis. They included CD4 count, anti-HIV medications, anti-CMV medications and anti-HSV (herpes simplex virus) medications, and hospital center.

1) CD4 Count: All patients had CD4 counts less than 50 cells/μL at enrolment in the study. Based on our understanding of markers of disease severity, CD4 count before enrolment is

the most important confounder in this study. It is associated with illnesses that require iatrogenic treatments, such as those that we are studying. Furthermore, it is an extremely important predictor of CMV retinitis. The risk period for increased risk of CMV retinitis in patients with a low CD4 count is known to be short.[5] This has been supported by studies of CD4 counts and function that seem to indicate significant improvement in immune function in the short term in patients receiving HAART.[7] We therefore used the CD4 count measured in the 3 months before the index date.

2) Anti-HIV Medications: These medications are potentially associated with many exposure variables, as patients who need steroid therapy, chemotherapy, radiotherapy and blood transfusions would be more likely to be taking more anti-HIV medications and higher cumulative dosages of these medications. Furthermore, patients receiving anti-HIV medication would be at lower risk for CMV retinitis. We measured treatments received in the two years before the index date.

3) Anti-CMV and Anti-HSV Medications: Anti-CMV medications used to treat nonocular CMV infections were controlled for, as these medications would be associated with predictors of CMV retinitis and would protect against CMV retinitis itself. Furthermore, the anti-HSV medication acyclovir has some anti-CMV activity[27] and therefore should be controlled for in a similar way as anti-CMV medications. The etiologic period chosen was two years.

4) Hospital Centre: Because there are unmeasurable potential differences between the hospital centers that may confound the exposure–disease association, hospital centre was also controlled for.

All data were abstracted from the subjects' medical records.

Statistical Analysis

Univariate statistics were obtained to check for implausible values as a final data editing step. Bivariate analyses were then conducted for all clinical exposure variables using the unpaired *t*-test for continuous variables (or the Wilcoxon rank-sum test if nonnormal) and the chi-square test for discrete variables. Logistic regression was used for multivariate analyses. A p value of 0.10 or less in the bivariate analyses was used as a cut-off for inclusion in the multivariate analyses. Next, the assumption of linearity of the logit was checked in a bivariate analysis for all exposure and confounding variables individually. This was done by graphing the ORs against each of the midpoints of multiple equal-interval variables. For example, CD4 count was divided into four approximately equal intervals: 0–12, 13–24, 25–36, and 37–49 cells/μL. Then the OR pertaining to case status corresponding to each interval was graphed against the midpoint of each interval (6, 18.5, 30.5 and 43.0 cells/μL respectively). From this plot, linearity of the logit was assessed. Next, a correlation matrix was obtained for all exposure variables to assess multicollinearity. Any correlation coefficient greater than 0.8 was recorded, and if a multicollinearity problem arose in the multivariate model, an algorithm for omitting one variable was used. This algorithm consisted of first keeping the variable that was a more biologically plausible

exposure, then keeping the variable with fewer missing data, and finally keeping the variable with less measurement error as determined a priori.

For confounding, we determined a priori that the model would include CD4 count, hospital site (city), at least one anti-HIV medication variable, at least one anti-CMV medication variable and at least one anti-HSV medication variable. Although these five categories of confounding variables were chosen a priori, it was not clear for anti-HIV medication, anti-CMV medication and anti-HSV medication which specific representation of the variable to use (e.g., for anti-HIV medication, specific anti-HIV medication, total dose of anti-HIV medication etc.). Incorporating all possible representations of each variable would not be realistic because of precision limitations. Therefore, logistic regression was used in the same way as described above to identify at least one variable for each of the preselected drug treatment categories. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test was used when choices between parallel models were made. These confounding variables were then introduced into the main exposure model.

Statistical techniques were not used to reduce the number of confounders in the model but were used to reduce exposure variables for the final model. Model reduction for the exposure variables was performed with the likelihood ratio test. If data were missing, cases and controls were deleted in the multivariate analysis. However, when the model was reduced or expanded, these data were then used again if the variable with the missing data was eliminated from the model. Parallel models were used when different representations of the exposure variable were not independent. The Hosmer and Lemeshow goodness-of-fit test

was used when choices between parallel models were made. The performance of the final model against chance and its fit were then determined.

The study protocol was approved by the requirements of the Research Ethics Boards of both the Ottawa Hospital and the Royal Victoria Hospital/Montreal Chest Hospital.

RESULTS

Demographic and Descriptive Results

There were a total of 279 patients in the study, 148 from Montreal and 131 from Ottawa. Of the 279, 120 were cases (88 from Montreal) and 159 were controls (99 from Ottawa). Table I summarizes the demographic and descriptive results for the two groups. The average age at the index date was 38.6 years for the cases and 39.6 years for the controls. Complete data were available for age, race, gender, treatment with radiotherapy, treatment with chemotherapy, medication use, CD4 count and hospital site. For other variables, the completeness of the medical records was as follows: blood transfusion 278 (99.6%) and prednisone use 277 (99.3%).

TABLE I: DEMOGRAPHIC AND DESCRIPTIVE RESULTS		
	No. (and %) of Subjects	
Variable	**Cases (N = 120)**	**Controls (N = 159)**
Hospital site		
Ottawa	32 (26.7)	99 (62.3)
Montreal	88 (73.3)	60 (37.7)
Gender		
Male	109 (90.8)	136 (85.5)
Female	11 (9.2)	23 (14.5)
Caucasian race	107 (89.2)	131 (82.4)
Index date, year		
1990	6 (5.0)	5 (3.1)
1991	5 (4.2)	15 (9.4)
1992	15 (12.5)	13 (8.2)
1993	21 (17.5)	19 (11.9)
1994	23 (19.2)	33 (20.8)
1995	25 (20.8)	46 (28.9)
1996	20 (16.7)	23 (14.5)
1997	4 (3.3)	4 (2.5)
1998	1 (0.8)	1 (0.6)

Iatrogenic variables

The bivariate dichotomous data are summarized in Table II. Based on the criteria summarized in the methods section, all variables except treatment with chemotherapy were

retained for the multivariate model.

TABLE II: BIVARIATE DISCRETE VARIABLES - IATROGENIC DATA					
Variable	**No. (and %) of Cases Exposed**	**No. (and %) of Controls Exposed**	**Odds Ratio (OR)**	**95% Confidence Interval (CI)**	***p* Value**
Steroid use	23 (19.2)	9 (5.7)	3.95	1.78–8.75	0.0005
Prednisone use	13 (11.0)	7 (4.4)	2.69	1.07-6.77	0.04
Treatment with chemotherapy	7 (5.8)	7 (4.4)	1.35	0.48-3.79	0.588
Treatment with radiotherapy	13 (10.8)	4 (2.5)	4.71	1.57-14.08	0.004
Blood transfusion	20 (16.8)	12 (7.6)	2.47	1.17-5.22	0.02

The specific chemotherapeutic agents used in the two groups are shown in Table III.

TABLE III: SPECIFIC CHEMOTHERAPEUTIC AGENTS USED IN THE CASES AND CONTROLS		
Agent	**No. (and %) of Cases Exposed**	**No. (and %) of Controls Exposed**
Doxorubicin	4 (3.3)	2 (1.3)
Bleomycin	4 (3.3)	2 (1.3)
Vincristine	3 (2.5)	3 (1.9)
Vinblastine	2 (1.7)	2 (1.3))
Cyclophosphamide	0 (0.0)	2 (1.3)
Etoposide	0 (0.0)	1 (0.6))
Mitomycin C	0 (0.0)	1 (0.6)
5-Fluorouracil	0 (0.0)	1 (0.6)

Table IV presents the continuous variables from the iatrogenic data. No variable was retained for the final clinical model.

TABLE IV: CONTINUOUS VARIABLES FROM IATROGENIC MODEL

	Mean ± Standard Deviation (SD)		
Variable	**Cases**	**Controls**	***p* Value**
Prednisone dosage, g	125.47 ± 495.86	34.75 ± 233.11	0.35
Radiotherapy dosage, Gy	2.20 ± 15.67	0.15 ± 1.92	0.77
Number of blood transfusions	0.56 ± 1.42	0.95 ± 7.27	0.17

Confounding by Medication

It was decided a priori that at least one drug or drug amount from each of the anti-HIV, anti-CMV and anti-HSV drug categories would be used in the final multivariate model. The specific variables to be included were determined by logistic regression.

Table V summarizes the bivariate descriptive statistics and ORs for the dichotomous treatment variables. Variables with a p value less than 0.10 were retained for the subsequent logistic regression.

TABLE V: BIVARIATE DISCRETE STATISTICS FOR THE DICHOTOMOUS TREATMENT VARIABLES					
Medication	**No. (and %) of Cases Exposed**	**No. (and %) of Controls Exposed**	**OR**	**95% CI**	***p* Value**
Anti-herpes simplex virus	53 (44.2)	35 (22.0)	2.80	1.67–4.71	0.0001
Anti-cytomegalovirus (CMV)	17 (14.2)	9 (5.7)	2.75	1.20–6.29	0.02
Ganciclovir	12 (10.0)	4 (2.5)	4.31	1.42–12.99	0.008
Foscarnet	7 (5.8)	0 (0.0)	–	–	0.002
CMV test drug	3 (2.5)	6 (3.8)	0.65	0.18–2.44	0.55
Anti-CMV combination therapy	6 (5.0)	0 (0.0)	–	–	0.004
Anti-human immunodeficiency virus (HIV)	93 (77.5)	106 (66.7)	1.72	1.01–2.95	0.05
Zidovudine (AZT)	80 (66.7)	92 (57.9)	1.46	0.89–2.38	0.134
Didanosine (ddI)	47 (39.2)	60 (37.7)	1.06	0.65–1.73	0.81
Zalcitabine (ddC)	30 (25.0)	37 (23.2)	1.10	0.63–1.91	0.74
Lamivudine (3TC)	27 (22.5)	19 (12.0)	2.14	1.31–4.04	0.02
Stavudine (d4T)	5 (4.2)	1 (0.6)	6.87	1.04–12.32	0.04
Saquinavir	6 (5.0)	0 (0.0)	–	–	0.004
Ritonavir	6 (5.0)	0 (0.0)	–	–	0.004
Indinavir	3 (2.5)	0 (0.0)	–	–	0.045

The data for total number of anti-HIV medications and total duration of anti-HIV drug use among cases and controls are shown in Table VI. In this bivariate analysis, cases

took more drugs on average than controls, and the duration of medication use was longer among cases than among controls.

TABLE VI: BIVARIATE STATISTICS FOR NUMBER OF ANTI-HIV DRUGS AND DURATION OF DRUG USE			
	Mean ± SD		
	Cases	**Controls**	***p* Value**
Number of anti-HIV medications	1.68 ± 1.41	1.32 ± 1.20	0.06
Duration of anti-HIV medication use, months	12.82 ± 9.50	10.27 ± 9.50	0.02

Whenever possible, the quantity of drug was also recorded for the risk period given in the methods section. With some drugs, such as the protease inhibitors and foscarnet, too few observations were available; therefore, the data are not presented for these drugs. Those with reasonable precision are summarized in Table VII. Acyclovir was used in significantly greater amounts among cases than among controls.

TABLE VII: BIVARIATE STATISTICS FOR TOTAL AMOUNT OF MEDICATION USED			
	Mean ± SD, g		
Drug	**Cases**	**Controls**	***p* Value**
Acyclovir	69.12 ± 126.20	37.28 ± 110.54	0.003
Ganciclovir	3.67 ± 18.17	0.67 ± 4.89	0.19
Zidovudine (AZT)	141.04 ± 149.50	125.71 ± 143.15	0.30
Didanosine(ddI)	45.86 ± 90.07	35.13 ± 64.68	0.61
Zalcitabine(ddC)	0.14 ± 0.38	0.11 ± 0.27	0.90
Lamivudine (3TC)	26.24 ± 60.86	12.54 ± 52.38	0.17

The final confounding model was then built (Table VIII). CD4 count, hospital centre and treatment variables with a *p* value less than 0.10 were all initially forced into the model.

TABLE VIII: FINAL CONFOUNDING MODEL				
Variable	**OR**	**Standard Error (SE)**	**95% CI**	***p* Value**
Any ganciclovir use	3.11	1.95	0.91–10.61	0.07
Any acyclovir use	2.05	0.65	1.10–3.83	0.02
Number of anti-HIV drugs	1.43	0.18	1.13–1.82	0.03
CD4 count	0.96	0.01	0.94–0.98	0.0001
Hospital centre	5.19	1.62	2.82–9.58	0.0001

The iatrogenic variables retained from the univariate analysis and the confounders were now used to build the final model. In the final multivariate model, only steroid use was a

statistically significant predictor of CMV retinitis (OR 6.41, 95% CI 2.35–17.51). This final model also included the confounding variables shown in Table VIII.

CONCLUSION

From the iatrogenic model, several bivariate associations were found to be predictive of CMV retinitis, including steroid use or blood transfusion within 6 months of the diagnosis of CMV retinitis, and administration of radiotherapy with a 12-month risk period. The use of chemotherapeutic agents was not a significant risk factor, nor was therapy with any individual agent. However, the power of the study was not calculated for the assessment of this risk factor, and the numbers were small, especially for individual agents. Only steroid use was found to be a significant predictor of CMV retinitis on multivariate analysis. This finding is in keeping with other clinical studies of CMV risk factors in other immunosuppressive settings.[14–17]

The biology and pharmacology of corticosteroids support their clinical role as immunosuppressants as well as their ability to play a part in the pathogenesis of CMV retinitis.[25] They inhibit both the early (redness, edema, pain) and the late (leukocyte recruitment) components of the inflammatory response. One of their main mechanisms of action is to reduce the ability of neutrophils in vitro and in vivo to adhere to capillary and capillary-like tissue. Corticosteroids also modulate cytokine activity and other chemical mediators of inflammation. Corticosteroids seem to have a linear effect clinically. Small amounts equivalent to approximately 5 mg per day of prednisone are necessary to sustain life. Intermediate dosages, such as 20 mg of prednisone, have a moderate therapeutic effect and a moderate side effect profile. Larger dosages, such as 80 mg per day, have a profound anti-inflammatory and immunosuppressive effect and a grave side effect profile.

This information will be useful for both the internist and the ophthalmologist. Any

HIV-positive person, especially with CD4 counts less than 50 cells/μL, who needs steroid therapy should be followed more carefully by an ophthalmologist. Such patients may be receiving steroids for chemotherapy protocols or may need the drug because of restrictive lung disease from repeated bouts with *Pneumocystis* pneumonia.

In any epidemiologic study, observed results may be due to chance, bias or confounding. We designed this study to look for steroids as the main predictor variable, as this was our primary hypothesis at the outset of the study. The power of the study was calculated based on this assumption. Hence, we feel that the study had adequate statistical power; the fact that our main predictor variable proved to be statistically significant supports this.

The main biases of concern in this case–control study were selection bias and misclassification bias. We should first point out that we chose a case–control design for two main reasons. First, it allowed us to examine multiple potential exposure variables, which was one of the goals of the study. Second, with the advent of HAART, in 1996, the incidence of CMV retinitis began declining to the point where it would not have been realistic to recruit a cohort of unaffected patients that would provide a study with adequate power. We attempted to minimize selection bias by adhering strictly to the study base principle. In fact, for the Montreal patients, our initial hope was to use the patients at the ophthalmology clinic of the Royal Victoria Hospital as our study base. However, although cases were well recorded at the clinic, controls were not adequately retrievable from this study base. We therefore changed our study base to patients referred from the Montreal Chest Hospital to the Royal Victoria Hospital, to ensure that cases and controls would arise from the same population base, thereby respecting the study base principle. As for

misclassification bias, we purposely required at least 3 months' follow-up for all patients in the study base to ensure that any misclassification would be nondifferential. Differential misclassification could have occurred if, for example, cases were followed more closely and more frequently before the index date than controls; however, the minimum length of follow-up required in our study decreased the likelihood that this would occur. Nondifferential misclassification is always possible when medical records are used as the main source of information, but this would have biased our results toward the null, making the association found in this study even more significant. Nevertheless, the small number of patients receiving immunosuppressive cytotoxic drugs and the possible nondifferential misclassification may be two reasons why no association was found between these drugs and the occurrence of CMV retinitis.

We took great efforts to minimize the effect of confounding. It was decided a priori to control for CD4 count, as it is both the most important indicator of disease severity and an approximate indicator of time since HIV infection, important for determining the index date for controls. Hospital centre was also controlled for because of the obvious potential for confounding from one study base to the other. Regarding confounding by medication, we created a model and decided a priori that it would contain at least one variable for anti-HIV medication, one variable for anti-CMV medication and at least one variable for anti-HSV medication (since anti-HSV medications may also have some anti-CMV effects). This model was then used in the main exposure model to control for confounding. While it is impossible to ever be absolutely certain that confounding has been completely eliminated in any epidemiologic study, we feel confident that we minimized its effect.

In conclusion, this study is one of the most comprehensive studies to date examining the iatrogenic risk factors for CMV retinitis. Steroid use represents an important risk factor for this disease in severely immunocompromised patients with AIDS.

ACKNOWLEDGEMENTS: Supported by the University of Ottawa Young Investigator Award and the Medical Research Council of Canada. The authors wish to acknowledge Dr. Jean Deschênes for helping with administrative issues for patient recruitment in Montreal.

REFERENCES

1. Holland GN, Pepose JS, Simons KB, et al. Ocular disease in the acquired immune deficiency syndrome: clinicopathological correlations. In: Secchi AG, Fregona IA, editors. *Modern Trends in Immunology and Immunopathology of the Eye*. Milan: Saunders; 1989. p. 132-155.
2. Montaner JS, Le T, Hogg R, et al. The changing spectrum of AIDS index diseases in Canada. *AIDS* 1994;8(5):693-696.
3. Munoz A, Schrager LK, Bacellar H, et al. Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am J Epidemiol* 1993;137(4):423-438.
4. Holland G, Tufail A, Jordan MC. Cytomegalovirus diseases. In: Pepose JS, Holland GN, Wilhelmus KR, editors. *Ocular Infection & Immunity*. New York: Mosby; 1996. p. 1088-1129.
5. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. *J Acquir Immune Defic Syndr* 1992;5:1069-1074.
6. McDonald CK, Kuritzkes DR. Human immunodeficiency virus type 1 protease inhibitors. *Arch Intern Med* 1997;157:951-959.
7. Casado JL, Arrizabalaga J, Montes M, et al. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV-AIDS Study Group. *AIDS* 1999;13(12):1497-1502.
8. Doan S, Cochereau I, Guvenisik N, et al. Cytomegalovirus retinitis in HIV-infected

patients with and without highly active antiretroviral therapy. *Am J Ophthalmol* 1999;128(2):250-251.

9. Lewis JS, Terriff CM, Coulston DR, et al. Protease inhibitors: a therapeutic breakthrough for the treatment of patients with HIV. *Clin Ther* 1997;19:187-214.

10. Le Moing V, Chene G, Carrieri MP, et al. Predictors of virologic rebound in HIV-1 infected patients initiating a protease inhibitor-containing regimen. *AIDS* 2002;16: 21-29.

11. Spector SA, McKinley GF, Lalezari JP, et al. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. *N Engl J Med* 1996;334:1491-1497.

12. Anderson RD, Griffy KG, Jung D, et al. Ganciclovir absolute bioavailability and steady-state pharmacokinetics after oral administration of two 3000 mg/d dosing regimens in HIV and CMV seropositive patients. *Clin Ther* 1995;17:425-432.

13. Masur H, Whitcup SM, Cartwright C, et al. Advances in the management of AIDS-related cytomegalovirus retinitis. *Ann Intern Med* 1996;125(2):126-136.

14. Nagler A, Elishoov H, Kapelushnik, et al. Cytomegalovirus pneumonia prior to engraftment following T-cell depleted bone marrow transplantation. *Med Oncol* 1994;11(3-4):127-132.

15. Manez R, Kusne S, Green M, et al. Incidence and risk factors associated with the development of cytomegalovirus disease after intestinal transplantation. *Transplantation* 1995;59(7):1010-1014.

16. Nelson MR, Erskine D, Hawkins DA, et al. Treatment with corticosteroids — a risk factor for the development of clinical cytomegalovirus disease in AIDS. *AIDS*

1993;7(3):375-378.

17. Morris DJ, Martin S, Dyer PA, et al. HLA mismatching and cytomegalovirus infection as risk factors for transplant failure in cyclosporin-treated renal allograft recipients. *J Med Virol* 1993;41(4):324-327.

18. Gorensek MJ, Stewart RW, Dyer PA, et al. A multivariate analysis of the risk of cytomegalovirus infection in heart transplant recipients. *J Infect Dis* 1988;157(3): 515-522.

19. Schiffer V, Mentha G, Giostra E, et al. [Cytomegalovirus in liver transplantation: incidence and groups at risk.] *Schweiz Med Wochenschr* 1994;124(15):631-636.

20. Kraat YJ, Christiaans MH, Nieman FH, et al. Risk factors for cytomegalovirus infection and disease in renal transplant recipients: HLA-DR7 and triple therapy. *Transpl Int* 1994;7(5):362-367.

21. Gorensek MJ, Carey WD, Vogt D, et al. A multivariate analysis of risk factors for cytomegalovirus infection in liver-transplant recipients. *Gastroenterology* 1990;98 (5 Pt 1):1326-1332.

22. Kirklin JK, Naftel DC, Levine TB, et al. Cytomegalovirus after heart transplantation. Risk factors for infection and death: a multiinstitutional study. The Cardiac Transplant Research Database Group. *J Heart Lung Transplant* 1994;13(3):394-404.

23. Sloand E, Kumar P, Klein HG, et al. Transfusion of blood components to persons infected with human immunodeficiency virus type 1: relationship to opportunistic infection. *Transfusion* 1994;34(1):48-53.

24. Paya CV, Wiesner RH, Hermans PE, et al. Risk factors for cytomegalovirus and

severe bacterial infections following liver transplantation: a prospective multivariate time-dependent analysis. *J Hepatol* 1993;19(2):325-330.

25. Schimmer BP, Parker KP. Adrenocorticosteroids. In: Goodman LS, Gilman G, editors. *The Pharmacologic Basis of Therapeutics*. 9th ed, New York: McGraw Hill; 1996. p. 1459-1485.

26. Sausville EA, Longo DL. Principles of cancer therapy. In: Harrison TR, editor. *Principles of Internal Medicine*. 15th ed. New York: McGraw-Hill; 2001. p. 530-547.

27. Rachlis AR, Zarowny DP. Guidelines for antiretroviral therapy for HIV infection. *CMAJ* 1998;158:496-505.

FINAL CONCLUSION AND SUMMARY

Since the beginning of the AIDS epidemic, CMV retinitis has been one of the most important complications of this disease. As might be expected, huge resources have been devoted to its treatment, with some success. However, much less effort has been spent trying to understand the predictors or risk factors for CMV retinitis. We performed a case–control study with the goal of trying to uncover some of these risk factors. We used a case–control design for both practical and theoretical reasons. On the practical side, the number of new cases of CMV retinitis started to decrease in the year we began designing the study, largely owing to the emergence of HAART. Hence, following a cohort of patients prospectively became unrealistic, as did even conducting a prospective case–control study. On the theoretical side, a case–control study offers the very real advantage of studying multiple exposures or risk factors. In fact, well over 100 exposure variables were studied in this thesis.

We decided to break the study down into three separate manuscripts: a study of clinical risk factors, a study of laboratory-based risk factors and a study of iatrogenic risk factors. We had several reasons for this. Reducing over 100 exposure variables down to two or three in a multivariate model of 279 patients would eliminate a huge amount of information that would be useful for a clinician interested in the results. Furthermore, a clinician would typically approach the risk factors for CMV retinitis along the lines drawn in these manuscripts. For example, the management of a patient early in the markedly immunosuppressed phase of AIDS might be best approached from clinical risk factors, outlined in manuscript I. The clinician might be most concerned with the following

questions: What is the patient's history of previous opportunistic infection? What are the symptoms? Does s/he have cotton-wool spots on clinical examination? For a severely immunosuppressed patient relatively late in the course of the illness, iatrogenic risk factors might assume a more important role. The clinician might wonder: Has this patient been treated recently with corticosteroids for chronic lung disease from *Pneumocystis carinii* pneumonia or with an immunosuppressive drug for a malignancy? Finally, laboratory-based risk factors that can be assessed cheaply and reproducibly may be important at any time in the course of the patient's illness. Some of the laboratory-based risk factors we studied, such as hematologic parameters and CD8 count, are in this category, while others, such as qualitative and quantitative CMV viral load testing, are still not perfectly reproducible across laboratories given the variety of techniques used.

We hope the results of this study will help change the clinical outcome of CMV retinitis. By identifying risk factors and finding the disease early, we *know* that less retina will be destroyed and treatment can be started earlier. With less destroyed retina, both peripheral and central vision can be saved. Furthermore, we *hope* that understanding risk factors better will help guide screening of these patients in a more rational manner and provide the most rational basis for determining for whom and when to implement prophylactic therapy.

Several risk factors were identified in the three papers. As a final analysis, we created a multivariate logistic regression model that included all the statistically significant risk factors from each component of the study in which the sample size was 279. This model included the confounders as outlined in each study.

The following table summarizes these results.

FINAL MULTIVARIATE MODEL INCORPORATING ALL STATISTICALLY SIGNIFICANT RISK FACTORS (N=279)				
Variable	**Odds Ratio**	**Standard Error**	**95% Confidence Interval**	***p* Value**
Flashing lights/ floaters	8.21	4.92	2.53–26.58	0.0001
Cotton-wool spots	3.70	2.03	1.26–10.86	0.02
Number of opportunistic infections	1.90	0.41	1.25–2.89	0.003
Nonocular cytomegalovirus infections	31.66	39.61	2.72–367.65	0.006
Mycobacterium avium–intracellulare complex infections	1.78	1.11	0.53–6.01	0.35
Other infections	2.75	1.79	0.77–9.86	0.12
Homosexuality	2.41	1.10	0.99–5.88	0.05
Hemoglobin concentration	0.99	0.009	0.97–1.01	0.16
Steroid use	6.74	4.25	1.96–23.17	0.002
CD4 count	0.95	0.01	0.92–0.97	0.0001
Hospital centre	3.15	1.45	1.28–7.74	0.01
Acyclovir use	1.49	0.61	0.07–3.33	0.33
Ganciclovir use	2.12	1.70	0.44–10.26	0.35
Total number of anti-human immunodeficiency virus medications	1.66	0.29	1.18–2.33	0.004

From our results, several conclusions can be drawn. Nonocular CMV infection was a strong predictor of CMV retinitis. This finding has a strong biologic basis. The total number of previous opportunistic infections was also a significant predictor of CMV retinitis,

with the risk increasing as the number of opportunistic infections increased. Flashing lights and floaters were a significant predictor as well, but one needs to be cautious in interpreting this result. In a case–control study, early vascular and perivascular/retinal changes that produce the flashing lights may herald CMV retinitis, or early CMV retinitis itself may cause these symptoms. Cotton-wool spots, or microinfarctions, were predictive of CMV retinitis. Basic science studies examining the pathophysiology of CMV retinitis support this finding. Homosexual acquisition of CMV was a strong risk factor, likely owing to a specific pool of HIV strain. Corticosteroid use was found to be a risk factor for CMV retinitis. The most likely explanation for this finding is that these drugs have potent immunosuppressive effects in an already immunosuppressed population. The power of our study was calculated to examine this risk factor. Other immunosuppressive medications, such as those used for chemotherapy of malignant disorders, were not found to be predictive of CMV retinitis; however, far fewer patients had been exposed to these drugs, making power an important concern.

Our findings in the study of laboratory-based risk factors need to be interpreted with greater caution because our sample was much smaller (57 subjects) than in the other papers. This is especially so for the multivariate analysis. Our bivariate data showed several HLA types to be risk factors for CMV retinitis and others to be protective. However, in the multivariate analysis, only two HLA types (HLA-Bw4 and HLA-DRBI15) were still significant predictors of CMV retinitis, and one HLA type (HLA-Cw7) was protective. Nevertheless, an immunogenetic predisposition, either as a risk or as a protective factor, is certainly plausible and should be studied further. CMV viral load was also

predictive of CMV retinitis, with the highest viral load being most predictive. Our method of determining viral load could not establish a specific cut-off for sensitivity/specificity testing because of technical reasons, but it did seem clear that higher viral load was predictive of CMV retinitis. This finding has strong biologic plausibility and is consistent with several other studies.

The main threat to validity in our study is misclassification bias and residual confounding. However, we feel confident that any misclassification of data from medical records would likely have been nondifferential, further strengthening the relationships found. Residual confounding is always possible in any observational study design, but we went to great lengths to minimize it, including creating a confounding by medication model to run across manuscripts. We minimized selection bias by strictly adhering to the study base principle. The main problem in the study of laboratory-based risk factors was one of precision in the stored blood sample part of the study. With only 57 as our sample size, the results for this part of the study, especially the multivariate results, need to be interpreted with caution.

Finally, this study was designed in 1996 and ended in 2000. Much happened in the field of AIDS medicine over these years. In fact, the development of HAART is one of the most significant advances in the history of this disease. This raises the possibility that our results may not be as generalizable as they would have been before HAART was introduced. The generalizability of the results to third-world countries, where HAART is not available, can also be questioned; however, most of the other anti-HIV medications that were used prevalently in our study also were not available in those countries. It is likely

that our results are very much generalizable to patients in industrialized countries who have not responded to HAART or do not comply with it. Furthermore, if resistance to HAART becomes a significant public health problem in the future, it is likely that the results of this study will be relied on heavily.

With these manuscripts, we hope we have contributed to the understanding of the risk factors for one of the most important diseases in the history of the AIDS epidemic.

June 25, 1998

W.G. Hodge
e Institute
awa General Hospital

ar Dr. Hodge:

RE: OGH-96-037 Risk Factors for Cytomegaloviris (CMV) Retinitis in Patients with AIDS

The Research Ethics Board has reviewed the annual report that you submitted on the above mentioned tocol.

The Board finds the information that you have provided to be acceptable with respect to the ethics of earch with human subjects and has therefore renewed the approval from **May 1998 to May 1999.**

Please note, the Research Ethics Board will only accept the hospital's "Patient/Proxy Consent for dical Research" for research protocols. These forms are available in the REB office.

The new guidelines of the Medical Research Council require a greater involvement of the REB in dies over the course of their execution. The annual renewal letter is one way that the REB monitors the irse of studies through to their completion. You must inform the Board of adverse events encountered ing the study, here or elsewhere, or of significant new information which becomes available after the ewal letter is issued. Both of these may impinge on the ethics of continuing the study. The REB will iew the new information to determine if the protocol would be modified, discontinued, or should continue originally approved.

Please note that annual reports for studies ongoing within your division must be submitted to this mmittee by the principal investigator involved in the study.

Yours sincerely

A. Marchuk, M.D., F.R.C.P.C.
airman
search Ethics Board

M/jt

Institut de Recherche de l'Hôpital Royal Victoria
Research Institute of the Royal Victoria Hospital

February 11, 1998

Dr. Jean Deschênes
Department of Ophthalmology
E4.60

RE: Research Ethics Board Approval
REB Protocol No. 97-472

Dear Dr. Deschênes:

Thank you for submitting the modifications to the consent form of your research study entitled, "*Risk factors for CMV retinitis in patients with AIDS*" as requested by the Medicine-B Subcommittee following ethical review of this study on June 17, 1997.

In accordance with his mandate, Dr. McLean, Acting Chair, has reviewed and accepted the modifications. Therefore, we are pleased to inform you that ethics approval has been granted to the protocol and consent form effective February 11, 1998.

Please keep in mind that a review of all research involving human subjects is required on an annual basis in accord with the initial date of approval. Should any modification to the protocol and/or consent form occur over the next 12 months please advise the REB accordingly.

We trust this is to your satisfaction.

Sincerely,

Lilian Fateen
REB Coordinator