MSCR 530 – Article Presentation Group C

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**INTRODUCTION**

1. What was the rationale for conducting the study?
   1. There is 30-fold higher rate of anal cancer in HIV positive patients compared to general population.
   2. Men who have sex with men have higher incidence of anal cancer, likely due to increased sexual exposure to anal Human Papilloma Virus (HPV) which subsequently can result in anal cancer.
   3. However, influence of immunodeficiency on anal cancer development is not well understood.
   4. Longer survival associated with combined antiretroviral therapy (cART) may also increase the risk of anal cancer as this risk increases with age.
2. What was the goal of the study?
   1. Study the effect of immunodeficiency and cART on anal cancer development in HIV positive subjects.

**METHODS**

1. What is the study design the authors chose? What population was chosen to conduct this study?

The authors describe a case-control study design aimed to look at the influence of immunodeficiency (and treatment) on anal cancer in patients with HIV. They used the Swiss HIV Cohort Study (SHCS), which has enrolled patients since 1988 to 2011 from 7 large hospitals in Switzerland. This represents 103,000 person-years of follow-up. The design is a retrospective case-control

1. How was the counterfactual experience designed? What other factors would you have considered in creating controls?

The controls were created using a density sampling approach, matching 5 control patients that did not have a diagnosis of anal cancer at that time. Controls were never re-used as controls later on. They did not clarify if the controls ever “became” cases subsequently. The controls were matched on person-years of follow up, the SHCS center, sex, HIV transmission route (e.g. IV needles, MSM), age at enrollment (+/- 9 years), year of enrollment (+/- 2 years). The CD4 (and other immune markers) were collected, but not matched for due to a low number of cases. HPV types were also collected as close to reference dates as possible (only 41 cases and 114 controls had this available data). In addition, their main hypothesis states that ART and immunodeficiency are relevant to anal cancer, but fail to control for these important variables.

1. What did the authors define as the exposure(s)? What was defined as the outcomes? How did they measure the outcomes?

The exposure(s) were not explicitly defined. The covariates that were controlled for are listed, but they looked at several different types of exposures of interest: smoking status, history of ART use, history of AIDS, CD4 nadir, and HPV subtype. The primary outcome was defined as development of anal cancer (68 cases total, 59 eligible cases due to defined time points of the study). They sampled outcomes, and patient clinical variables, every 6 months from enrollment. The authors did not explicitly describe in the methods their statistical approach.

1. The authors used a logistic regression as their major test of association. Was this test the most appropriate in this setting? Why?

The authors are looking at a binary outcome, the development of anal cancer. In this case control setting, an OR is the most straightforward and interpretable approach, and helps the reader to understand the relative increase in risk between groups of developing anal cancer. However, they never specific their methods, including the method the regression is using for generating Beta coefficients. In addition, they separate multiple variables of interest, which may be confounders but are not tested. Their preliminary results also likely suggested the importance of CD4 count, and they use a variety of cut-offs in their results (not consistent). A more appropriate analysis may have been to use a spline of CD4 levels to have a better understanding of when risk changes.

1. How was the subset of patients for HPV serology analysis identified?

Not stated. But 41 of 59 cases and 114 of 295 controls had serology performed on serum samples taken closest in time to reference date. It is possible that this subset used for analysis of HPV serology status was the subset of this study sample who had sufficient amount of banked serum available for analysis in the conduct of this study.

**RESULTS**

1. Create the 2x2 table relating the exposure of E6 oncoprotein of HPV16 status and the disease outcome of anal cancer.

|  |  |  |  |
| --- | --- | --- | --- |
|  | HPV16 E6 Seropositive | HPV16 E6 Seronegative | Totals |
| Anal cancer+ | 9 | 32 | 41 |
| No anal cancer | 0 | 114 | 114 |

1. Calculate the odds ratio from table 1. 3.B. Interpret the infinite odds ratio.

(A) OR = (9\*114)/(0\*32) = 1/0 = ERROR: Cannot divide by Zero!

This OR was reported as infinity in the article.

(B) The odds of disease among those who tested seropostive to HPV 16 E6 oncoproteinwas **close to** infinity times the odds of disease among those who were HPV16 E6 seronegative. This is a very strong association, bounded by infinity. We should be careful of making conclusions off of very small sample sizes; there were only 9 cases of HPV16 E6 oncoprotein seropositive subjects in this case-control study.

**DISCUSSION**

1. The authors identify the small number of anal cancer cases and lack of access to tumor tissue for HPV DNA analysis as limitations and argue that this “should only attenuate the strength of associations.” Do you agree?

The small number of cases is a potential limitation, but given the size of the cohort and length of follow-up, may be the best-case scenario. The authors note that serologic evaluation for HPV may “detect cross-reacting antibodies to other HPV types and/or reflect HPV infection at a nonanal site,” which it seems would overestimate the prevalence of HPV and therefore increase the strength of association in their analysis.

2. Can you identify additional limitations?

The authors argue that there is a difference in CD4 counts between cases and controls (Figure 1 and Table 3), but there is overlap in the confidence intervals for these measures. ART use was broadly categorized as “never” or “ever.” The authors did define “users” as those who had used ART for one month prior to the reference date, but there is still a considerable clinical difference between those who have used ART for a month compared to those who have received medications for years. The study period is lengthy, but the change in guideline recommendations toward the end of this period to initiate ART therapy for all people living with HIV (PLWH) may make the findings less relevant today.

3. What might explain the lack of a protective effect for ART on anal cancer risk?

As noted above, the measure of ART use was somewhat limited, and during the majority of the study period, ART was only prescribed to those with advanced HIV, so the full benefit of ART may not be fully captured. In addition, pre-cancerous lesions may develop during periods of immunosuppression (low CD4 count) and progress despite immune reconstitution after initiation of ART. The authors also note that the population of PLWH has aged after the implementation of ART.

4. Does this study support public health practice of screening for anal cancer in people living with HIV?

Given the finding of an association between low CD4 count and anal cancer, the primary public health intervention should be prevention of HIV and treatment of all PLWH with ART. This study did not evaluate tissue diagnosis of precancerous lesions (AIN) or HPV-associated lesions, and given the limitations of the HPV serology performed, it does not provide support for screening. While the data remain limited even today, there are recommendations from HIVMA to screen those with a history of receptive anal intercourse with cytology.

**CONCLUSION**

1. What were the findings in regard to the effect of immunodeficiency and cART on risk of anal cancer development in HIV positive subjects (the primary objective of the study)?
   1. Low CD4+ cell counts (representative of immunodeficient status) are associated with increased this risk.
   2. No effect of cART on this risk.
2. What were the additional findings of the study?
   1. Smoking increases risk
   2. Exposure of HPV increases the risk (as previously known already).