DETECTION OF HHV-8 IN AUTOPSY SAMPLES FROM AIDS PATIENTS

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Human herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is the most recently identified human herpesvirus. A key question regarding HHV-8 is the location of infected cells within HHV-8 seropositive individuals. Outside of tumor tissues, HHV-8 viral proteins have been detected in saliva, circulating B cells, and semen of some, but not all HHV-8 seropositive individuals. HHV-8 is the causative agent of Kaposi's sarcoma (KS) and is associated with two other distinct proliferative disorders: primary effusion lymphoma and some forms of multicentric Castleman's disease. To better understand viral infection including the cellular targets of infection, we have begun a systematic screening of autopsy tissues from HHV-8 seropositive men who died with AIDS. Using immunohistochemistry (IHC), my goals were to determine reservoirs of HHV-8 infection and latency in organ tissues, determine the type of viral infection (lytic and/or latent) of each tissue type, and attempt to identify the infected cell type. In this report, using IHC, we document the presence of HHV-8 infected cells in several organs including kidney, lung, liver, and gastrointestinal tract samples from the Multicenter AIDS Cohort Study (MACS). Both lytic and latent infections have been detected and the infected cells appear to consist of both immune and non-immune cells. These results demonstrate the ability of HHV-8 to establish infections in various organs which may affect the pathogenesis of the virus in infected individuals. Kaposi's sarcoma is currently

a major public health concern, as it is the most common malignancy found in individuals with AIDS and iatrogenic KS is a key concern in the field of solid-organ transplantation. This study will attempt to identify reservoirs of HHV-8 infection within the body in order to better understand the biology of HHV-8 in infected individuals, and the role HHV-8 plays in disease pathogenesis.

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goes to the 1st year class of 2005. We had a lot of great times and fun memories. I wish them nothing but the best, and I hope as time passes our careers cross paths often.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

1.0 INTRODUCTION

1.1 HHV-8

1.1.1 Discovery and associated cancers

Human herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is the most recently identified human herpesvirus. HHV-8 was first identified in AIDS-KS tissue in 1994 [1], and is required for the development of all forms of Kaposi's sarcoma (KS) [2]. Besides KS, HHV-8 is also etiologically linked to several rare types of lymphomas often found in AIDS patients, namely primary effusion lymphoma (PEL) and some forms of multicentric Castleman's disease (MCD) [3].

1.1.1.1 Kaposi's sarcoma

KS was first described as an "idiopathic multiple pigmented sarcoma of the skin" by Moritz Kaposi in 1872 [123]. KS is a multicentric angioproliferative disorder characterized by proliferation of spindle-shaped cells, neo-angiogenesis, inflammation, and edema [4-7]. There are four epidemiological variants of KS that develop in distinct populations of subjects: classic KS, endemic KS, AIDS-associated KS, and iatrogenic KS [8]. KS will be discussed more thoroughly in section 1.2 of this thesis.

1.1.1.2 Primary effusion lymphoma

First identified as a subset of body-cavity-based lymphomas, primary effusion lymphoma (PEL) has been shown to contain HHV-8 DNA sequences [9]. The consistent presence of HHV-8 DNA in PEL implies an important role for HHV-8 in the pathogenesis of this rare lymphoma which accounts for only 0.13% of all AIDS malignancies in AIDS patients in the USA [10]. HHV-8 DNA is always associated with PEL, and the majority of the cases are also EBV positive [11].

1.1.1.3 Multicentric Castleman's Disease

HHV-8 is also linked to another B cell lymphoproliferative disorder called Multicentric Castleman's Disease (MCD), which can be described by generalized lymphadenopathy with polyclonal hyperimmunoglobulinemia and high levels of serum IL-6 [12, 13]. Unlike KS and PEL, HHV-8 DNA sequences have only been detected in a subset of MCD patients, who are usually co-infected with HIV [13].

1.1.2 HHV-8 prevalence and transmission

The seroprevalence rates of HHV-8 in the general population vary depending on geographic location as shown by Figure 1.

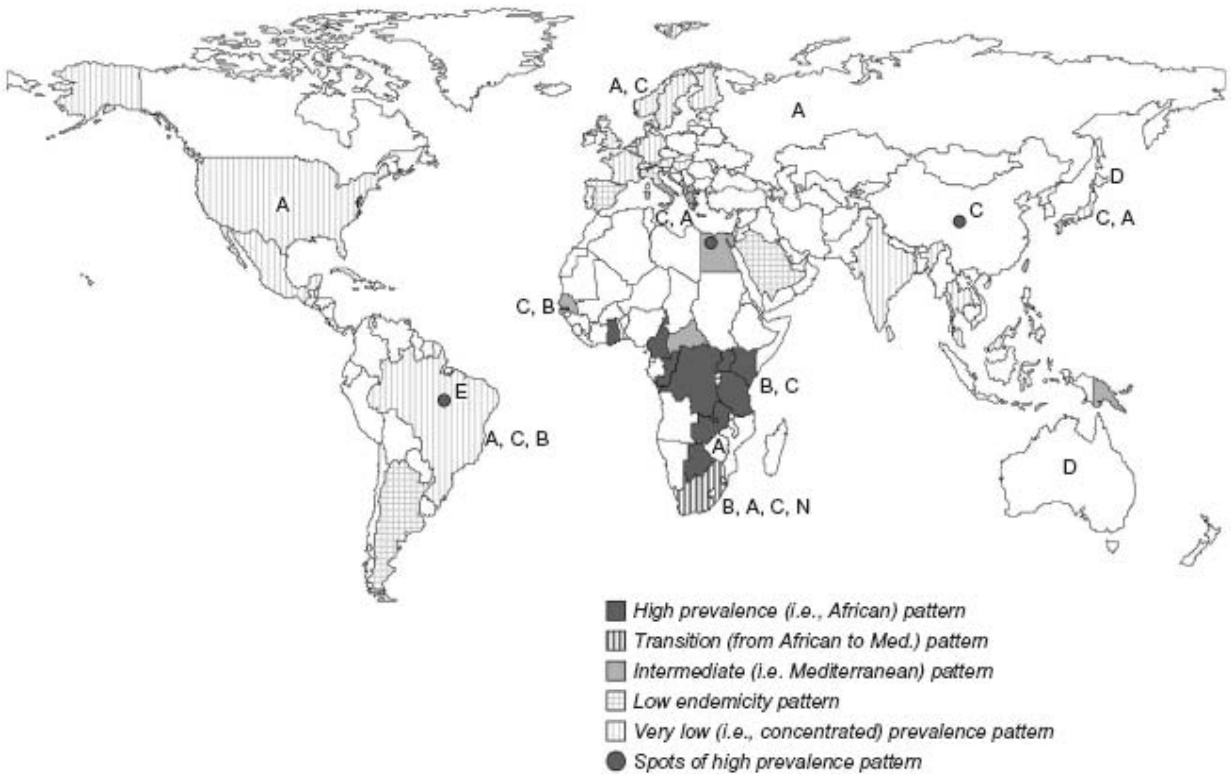


Figure 1. Epidemiological patterns of HHV-8 infection. Countries were allocated to specific patterns on the basis of HHV-8 prevalence data. For Mediterranean countries such as Greece, which lacks seroprevalence data, or Egypt, where data are available only for the city of Alexandria, allocation was based on patterns of KS occurrence. The letters in the figure refer to HHV-8 genotype. (Figure reprinted with permission from Dukers, N.H. and G. Rezza, *Human herpesvirus 8 epidemiology: what we do and do not know*. Aids, 2003. **17**(12): p. 1717-30.)

HHV-8 seroprevalence is higher among HIV-seronegative homosexual subjects compared with the HIV-seronegative heterosexual population, ranging from 20% to 38% [14-16], and is even higher in HIV-seropositive homosexual populations, ranging from 30% to as high as 48% [17-19]. In homosexual men who are seropositive for both HHV-8 and HIV, the presence of HHV-8 DNA in peripheral blood mononuclear cells precedes and predicts the development of KS [20, 21]. Currently virus has been detected most reproducibly in saliva [22, 23], and occasionally in genital secretions [22, 24-26]. These data show an important role for saliva in the transmission of HHV-8.

Other modes of transmission have also been debated as some large cohorts of intravenous drug users and highly exposed hemophiliacs did not show increased seroprevalence over the general population [27, 28]. Other studies, however have detected a relationship between seropositivity and the frequency or duration of injection drug use [16, 29]. In the higher-prevalence societies of the Mediterranean Basin and Africa, a different epidemiologic pattern occurs. Infection is equally prevalent in adult men and women, and infection begins in childhood. It is currently thought that saliva, possibly originating from the mothers' premastication of food for child, and close interpersonal contacts, such as contact of non-intact skin or mucous membranes with blood containing secretions are the methods of transmission in endemic regions [30].

1.1.2.1 Sexual practices and HHV-8 infection

Heterosexual transmission is not likely to be a major route and seems to require frequent sexual exposure to occur [31]. Prevalence seems to be elevated mainly in heterosexuals who have a high number of sexual partners or a sexually transmitted disease [32, 33]. Among homosexual men, there is agreement that the transmission of HHV-8 is primarily sexual in nature [14]. However, the specific mechanism of transmission is still a topic of debate. Some studies on the relationship between KS and sexual practices have reported an association between oral-anal sex and AIDS-KS [34, 35]. Cross-sectional studies have demonstrated an association between the presence of HHV-8 antibodies and anal-genital sex, oral-anal sex, and deep kissing with an HIV positive partner [22, 36, 37]. Currently virus has been detected most reproducibly in saliva [22, 23], and occasionally in genital secretions [22, 24-26]. Anal-genital sex is a common practice

among homosexual men, as is oral-anal sex. Therefore, if HHV-8 is present in saliva it may be transmissible by either of these two routes.

1.1.2.2 HHV-8 epidemic and the AIDS epidemic

KS was endemic in parts of Africa long before the beginning of the AIDS epidemic [38]. In these areas, and among populations of homosexual men in industrialized countries, widespread immunosuppression caused by HIV opened the door for HHV-8 to cause numerous cases of KS. Some studies conducted among homosexual men in North America and Europe suggested that there existed concurrent epidemics of HHV-8 and HIV infections and that the two infections potentially share modes of transmission [15, 36]. However, other studies suggested that the prevalence of HHV-8 infection was already high among homosexual men when the HIV epidemic began. Although some initial increases in the prevalence of HHV-8 infection were reported among homosexual men at the start of the HIV epidemic, the prevalence has since remained nearly constant [39-41]. It has been suggested that this discrepancy in prevalence trends over time between HIV and HHV-8 indicate that there must be different or additional transmission routes for HHV-8 when compared to HIV [42].

1.1.3 The genetic structure of HHV-8

HHV-8 is a member of the lymphotropic γ-herpesviruses in the subfamily rhadinovirus [43]. The HHV-8 genome is a double–stranded linear DNA of 165 to 170 kilobasepairs (kbp) [44]. The genome contains a central unique region of approximately 137 kbp, which includes all of the

viral open reading frames, flanked by a series of highly GC-rich direct terminal repeats which make up the difference [44].

1.1.3.1 Phases of infection

As with other herpesviruses, HHV-8 can establish either a latent or a lytic infection within infected cells. During latency, the viral genome is maintained as a circular episome whose replication is coupled to cellular replication. The few viral gene products that are expressed are important for maintaining the viral genome and for ensuring the survival of an infected population of cells. No infectious virus is produced during latency, yet there is a clonal expansion of the infected population because viral replication is coupled to cellular replication. The cells that support latent replication are at risk for tumor development because viral proteins expressed during latency disrupt important regulatory pathways within the cell. HHV-8 latent genes; latency associated nuclear antigen-1 (LANA-1), latency associated nuclear antigen-2 (LANA-2), viral-cyclin, viral-FLICE inhibitory protein (v-FLIP), and kaposin have a widespread distribution in advanced AIDS-KS lesions [45]. v-FLIP is associated with anti-apoptotic activity [46], and activation of NF-kB by v-FLIP is associated with prolonged survival of HHV-8 infected primary effusion lymphoma cell lines [47]. LANA-1 can antagonize p53-mediated apoptosis [48], and v-cyclin can inactivate tumor suppressor Rb and cyclin dependent kinase pathway inhibitor p27, thus promoting cell cycle progression [49]. LANA-1 and v-FLIP can also induce IL-6 secretion in HHV-8 infected cells [50, 51]. The kaposin gene also encodes several microRNAs (miRNA), which may regulate gene expression by binding to complementary messenger RNAs [52].

Latently infected cells have the potential to shift to lytic replication. Lytic replication is accompanied by expression of numerous viral proteins needed for making mature virions. Cells supporting lytic replication are generally not at risk for malignant transformation because the host cell is usually destroyed when the replication cascade is complete, and hundreds of viral particles can be released from a single cell [53, 54]. Lytic replication is critical for transmission to uninfected cells, including dissemination to new hosts. The production of infectious virus is often associated with a viral syndrome that results from multiple factors, including the altered function of newly infected cells, the lysis of infected cells, and the expression of viral gene products such as v-IL-6, and a viral-G protein-coupled receptor (v-GPCR), that have systemic and paracrine effects [55-57].

1.1.3.2 Relevant gene expression

For the purposes of this thesis, we will focus on three major proteins (one latent phase, two lytic phase) expressed by HHV-8. The latent phase protein of interest is the latency associated nuclear antigen type 1 (LANA-1). The two lytic phase proteins we are observing are K8.1 and viral-interleukin-6 (v-IL-6).

1.1.3.2.1 LANA-1

The latency associated nuclear antigen type 1 (LANA-1) protein is a latent nuclear antigen of HHV-8 considered important in the establishment and maintenance of the HHV-8 latent viral

episome in the nucleus [58, 59]. LANA-1 is a 222-234 kDa protein that is expressed in the majority of nuclei in KS spindle cells [60, 61]; however, LANA-1 protein expression is variable and can depend upon the clinical stage of the KS tumor [60, 62].

1.1.3.2.2 K8.1

The K8.1 glycoprotein is a structural component of the KSHV particle and is thought to play a role in virus entry by binding to heparan sulfate moieties on cell surfaces. However, it has been shown that K8.1 is dispensable for HHV-8 entry [63]. K8.1 has a molecular weight of 35-40 kDa and is unique to HHV-8 [64]. Two transcripts are produced, K8.1A and K8.1B, of which K8.1B is shorter. K8.1A is very antigenic, and will be the form focused on in this study

1.1.3.2.3 v-IL-6

The role of IL-6 in KS and MCD had been suspected prior to the discovery of HHV-8 [44]. IL-6 enhanced the proliferation of KS cells in culture and elevated levels of IL-6 corresponded with disease development in MCD [65, 66]. The HHV-8 homologue of IL-6, v-IL-6, shares roughly 25% amino acid identity with the human cytokine, and has been shown to share similar biological properties with IL-6 [67].

1.2 Kaposi's sarcoma

The most important disease state with which HHV-8 has been associated is Kaposi's sarcoma (KS). KS is an angioproliferative lesion that can occur in many tissues, but is most commonly localized to the skin [68]. Previously a rare disease, it is now a global and clinical health problem because of its association with the HIV pandemic and other immunosuppressed states. There are four epidemiological variants of KS that develop in distinct populations of subjects: classic KS, endemic KS, AIDS-associated KS, and iatrogenic KS [69, 70]. Classic KS is an indolent tumor that affects the elderly, primarily males, in Mediterranean countries such as Italy, Israel, and Turkey [71]. Classic KS legions are characteristically found in the lower extremities and typically are non-aggressive and non-lethal [70]. HHV-8 was prevalent in Africa prior to the HIV epidemic which was the cause of the large prevalence of KS seen on the continent before HIV changed the scope of KS presentations [72]. Endemic KS presents in four clinical forms with one form similar to classic KS, but found in younger adults; the other three forms are more aggressive, similar to AIDS-associated KS [70]. In the context of AIDS, KS is the most common malignancy and is an AIDS defining illness [73]. AIDS-associated KS is a more aggressive tumor than classic KS and can disseminate into the viscera with a greater likelihood of death [74]. AIDS-associated KS often presents multifocally and more frequently on the upper body and head regions [70]. In the context of immunosuppression, as with organ transplants, both primary infection and reactivation of HHV-8 becomes a potential concern. It has been shown that post-transplant immunosuppression can cause iatrogenic KS to appear [75]. Post-transplant KS can cause significant clinical manifestations such as rejection of the transplant

and/or death of the patient. In a study of 356 post-transplant patients with KS, 40% had visceral involvement, and 17% of those with visceral KS died from the cancer [75].

1.2.1 Prevalence

KS has a much higher prevalence in areas endemic for HHV-8 such as Africa and Mediterranean countries [76-78]. Outside of these endemic areas, KS is most frequently seen in immunocompromised hosts, especially in patients with HIV who have a high rate of infection with HHV-8 [79, 80]. Only a small fraction of healthy adults infected with HHV-8 will ever develop symptomatic disease [81, 82]. This suggests that HHV-8 infection is necessary but not sufficient for the development of KS. The disease is more likely to develop when there is dysfunction of the host immune system resulting from infection with HIV or from treatments to prevent organ transplant rejection. Not all individuals who develop KS are overtly immunosuppressed, however. Classic KS occurs primarily in elderly Mediterranean or Ashkenazi Jewish men who live in areas where seroprevalence is high [83]. In addition, men, women, and children in certain regions of Africa can develop an endemic form of KS without co-infection with HIV, but the incidence of KS in these regions increases when patients are also infected with HIV [78].

1.2.2 At risk populations

The incidence of KS after organ transplantation is 500-1000 times greater than in the general population [84]. Approximately 0.5 to 5% of organ transplant recipients develop KS, depending

on their geographical origin and the type of organ received [75]. HIV-infected individuals have a risk of KS that is thousands of times higher than that of the general population. In a study of 143 HIV-infected homosexual men, who were followed for a median of 30 months, more than 50% of those who initially had HHV-8 sequences detectable by polymerase chain reaction in the peripheral blood mononuclear cells had progressed to KS within 3-5 years [20]. Studies of HHV-8 co-infected individuals with known dates of HIV seroconversion found a progression rate of nearly 30% after HIV seroconversion [85]. The hazard of KS from dual infection appears to increase by more than 27% for each year of infection with HIV before HHV-8 seroconversion [86]. These data suggest that KS among HIV-infected individuals is more likely to occur when an individual becomes more immunocompromised. Also supporting this concept is the association between the progression to KS and low CD4 cell counts [86, 87]. Other indicators of the development of KS among HIV/HHV-8 co-infected individuals, such as high HHV-8 antibody titres and high HHV-8 viral loads, have also been identified [87, 88].

KS also seems to occur more often with HIV-1 infection, as compared to those infected with HIV-2, even when the two HIV types are accompanied by the same level of HHV-8 prevalence. These findings suggest that other co-factors specifically related to HIV-1, as opposed to HIV-2, are involved in the development of KS [89].

There is very little information on the risk of KS development among immunocompetent HHV-8 positive individuals. This lack of information is due mostly to the low incidence of KS in the general population. However, a study conducted among individuals over fifty years of age on three islands of the Mediterranean (Sardinia, Sicily, and Malta) showed yearly rates ranging from

1:3108 to 1:3574 among HHV-8 positive men and from 1:4,866 to 1:10,970 among HHV-8 positive women. The male to female ratio ranged between 1.4 and 3.5, suggestive of a role for gender-related factors in the development of KS [90]. How gender differences contribute to the risk of the development of KS remains undetermined.

Other possible roles of other host-related factors also need to be additionally investigated. These factors include malnutrition, especially in highly endemic areas of KS in Africa, or genetic factors that could either increase the risk of KS (HLA DR5) or decrease this risk (HLA DR3) [91, 92].

1.2.3 Gene expression

The viral transcripts that are detected in most cells in KS lesions are primarily associated with latency. These include LANA-1, v-cyclin, v-FLIP, and kaposin [46, 61, 93, 94]. LANA-1 is essential for latent replication of HHV-8 and for proper segregation of HHV-8 in daughter cells [58]. LANA-1 also affects important cellular regulatory proteins, including the tumor suppressor genes p53 and retinoblastoma gene product Rb [95, 96]. Due to the effects that LANA-1 has on p53 and Rb, latently infected cells are more likely to replicate both cellular and viral DNA and are less likely to undergo apoptosis, thereby increasing the population of latently infected cells. The v-cyclin expressed during latency stimulates cell division [97], and v-FLIP blocks apoptosis, enhancing survival of virally infected cells [47, 98]. As stated above, most cells in KS lesions are latently infected, however it has been reported that a few cells do continue to undergo lytic replication [99]. Some of the proteins expressed during lytic replication are currently

hypothesized to contribute to the tumorigenic phenotype. v-GPCR, which is expressed early in the lytic cascade induces the expression of vascular endothelial growth factor (VEGF), a cytokine that is important in the angiogenesis that is characteristic in KS lesions [57].

1.2.4 HIV and HHV-8 crosstalk

Recent studies have shown that HIV replication stimulates HHV-8 production in PEL cell lines and peripheral blood mononuclear cells from KS patients, possibly due to the activating functions of HIV-transactivation protein (Tat) [100]. ORF50, the major transactivator of HHV-8 lytic cycle can in turn induce increased levels of HIV replication by interacting synergistically with HIV-1 Tat leading to increased cell susceptibility to HIV infection and transient permissiveness to HIV replication [101].

1.2.5 Previous reports of HHV-8 anatomical mapping

There have been numerous reports of different serological and PCR based assays for the detection of HHV-8 all with varying levels of sensitivity [102]. Even with these methods, a comprehensive anatomical mapping of HHV-8 had not been attempted, until recently. A group from the University of Texas Medical Branch-Galveston attempted to anatomically map the reservoirs of infection of the eight human herpesviruses using fresh tissue specimens from forty major anatomical sites, from eight autopsies, using a recently developed real-time PCR method for detection of all eight human herpesviruses [103]. HHV-8 was detected in only two lymph nodes from two cases, a 43-year old HIV-positive male and a 73-year old HIV-negative male.

There were two major drawbacks to this study that our study was hoping to improve on. The first was that there was no attempt to determine seroprevalence of the subjects used in the study. With a small n=8, there is a legitimate concern that none of the subjects were HHV-8 seropositive. With our study, seroprevalence was verified and all five of our subjects were seropositive for HHV-8. The second drawback, with any PCR based assay, is the inability to identify individual infected cells. With IHC, individual infected cells can be visualized and a better knowledge of cell morphology and locations within tissues can be gained. Therefore, the combined lack of mapping attempts, as well as the shortcomings of the above mentioned study, led us to the current study using IHC to anatomically map HHV-8 infection.

2.0 THESIS AIMS

Human herpesvirus-8 (HHV-8), also known as Kaposi's sarcoma-associated herpesvirus, is the most recently identified human herpesvirus. A key question regarding HHV-8 is the location of infected cells within HHV-8 seropositive individuals. Until recently, commercial reagents for the detection of this virus have not been available. A small number of studies have been done trying to identify HHV-8 reservoirs within the body using PCR, but they have been ineffective due to various shortcomings as described in section 1.2.5. The rationale for using immunohistochemistry (IHC) to detect viral antigen in tissues of the autopsy cases was two-fold. First, IHC has been known to be sensitive and antibodies are available for the detection of HHV-8 lytic and latent antigens. Secondly, with IHC, individual infected cells are detected, which allows us to histologically attempt to determine infected cell types. Therefore, the overall goal of this study was to use IHC to gain a better understanding of HHV-8 reservoirs within HIV+, HHV-8+ individuals.

SPECIFIC AIMS

Specific Aim #1: Determine if the presence of HHV-8 infection will correspond to the known biology of the virus.

Hypothesis: We expect to see the virus in areas of high immune surveillance and lymphoid tissue concentration such as organs of the mucosa-associated lymphoid tissue as well as, gut-associated lymphoid tissue.

Specific Aim #2: Determine what, if any, effect the presence of Kaposi's sarcoma will have on the pattern and location of viral expression.

Hypothesis: KS status of the individual will have an effect on the prevalence and/or location of viral expression in the KS+ autopsy cases.

3.0 MATERIALS AND METHODS

3.1 Sample selection criteria

Samples were selected from Pittsburgh Multicenter AIDS Cohort Study (MACS) participants using the following criteria. First, autopsy tissue had to be available from the patient. Second, the patient had to be seropositive for HHV-8. Using these criteria, a list of potential tissue donors was created and we randomly chose three patients without KS and two patients with KS. For each patient we received between twenty-three and thirty-four different tissue types.

3.2 Immunohistochemistry

Paraffin sections were cut at 5 μm and mounted on Surgipath microslides snowcoat X-tra. The serial sections were deparaffinized in a 6-minute xylene wash and rehydrated in a series of ethanol grades to water starting with 2-five minute washes in 100% ethanol, followed by 1-5 minute wash in 95% ethanol, 1-5 minute wash in 70% ethanol, and 2-5 minute washes in water. Antigen retrieval was performed using Target Retrieval Solution (Dako Cytomation Cat. #S1699) in a Black and Decker rice steamer for 40 (LANA-1) or 20 (v-IL-6, K8.1) minutes. Blocking of endogenous peroxidases was done in a 15 minute wash of 450 ml of methanol and 5 ml of 30% hydrogen peroxide. Non-specific proteins were blocked with either normal rabbit

serum (LANA-1) or normal goat serum (v-IL-6, K8.1) for 20 minutes at room temperature in a humidified chamber. Anti-ORF 73 (anti-LANA-1) rat monoclonal, anti-ORF K8.1 mouse monoclonal, and anti-HHV-8 v-IL-6 rabbit polyclonal were used as primary antibodies at a 1:500 dilution (ABI Cat. #13-210-100, 13-213-101, & 13-214-050 respectively). Primary incubation occurred overnight at 4°C in a humidified chamber. Biotinylated rabbit anti-rat IgG, goat anti-mouse IgG, and goat anti-rabbit IgG were used as secondary antibodies (Vector Labs Cat. #BA-400, BA 9200, & BA-1000 respectively). Secondary incubation occurred for 60 minutes at room temperature in a humidified chamber. Vector ABC Elite (Cat. #PK-7200) and Vector NovaRED substrate (Cat. #SK4800) were used to develop the staining. Hematoxylin (Dako Cytomation Cat. #S3301) was used as the counter stain. Slide mounting was done using Fisher Scientific permount (Cat. #SP15-100).

3.3 Imaging

Imaging was done using a Fisher Scientific USB 1.1 Digital/Video Micromaster II Brightfield microscope (Cat. # 12-563-313). IHC stained paraffin slides were placed on the microscope stage and images were digitally captured using the Westover Scientific Micron 1 imaging software program. Image resizing and insets were done using Microsoft Photo Editor Version 3.0.2.3.

4.0 RESULTS

4.1 Section I : Description of autopsy cases and tissues received

4.1.1 Description of autopsy cases

The demographic information for each patient is shown in table 1.

Patient #1

Patient #1 was a 40 year old homosexual male who became seropositive for HIV in 1983 and enrolled in the Pitt Men's Study in 1985. He had a history of intravenous drug abuse and multiple sexual partners which put him at risk for developing AIDS. No one entity was identified as the cause of death in this patient however, multiple organ failure due to cytomegalovirus (CMV) and HIV infection played a major role. There were signs of nephritis which most likely resulted in renal failure, and pneumonia, coupled with bilateral pleural effusions, resulted in respiratory compromise. His disease course was characterized by disseminated herpes zoster, CMV retinitis which resulted in blindness, multifocal leukoencephalopathy and decreasing CD4 counts. Over the course of the disease he was treated with AZT, foscarnet and bactrim and he died in April, 1995.

Patient #2

Patient #2 was a 47 year old male who was diagnosed as HIV positive in March 1987, when he enrolled in the Pitt Men's Study. At his first visit in July 1987 his CD4 count was 511. His CD4 counts thereafter were always less than 200 and his most recent clinic visit in March 1993 revealed a CD4 count of 30. He had no significant family or social history. He had a history of diarrhea, sinusitis, *Candida* infection, peripheral neuropathy, leukopenia, anemia and wasting. Upon autopsy, basal cell carcinoma of the anal canal and a poorly differentiated malignant neoplasm of uncertain origin in the oropharynx were discovered. The cause of death was pneumonia, and gram negative septicemia, and the patient died in May, 1993.

Patient #3

Patient #3 was a 49 year old male, who was diagnosed with HIV in approximately 1985. His only risk factor for HIV infection was homosexuality. He had no history of intravenous drug use and was a nondrinker and a nonsmoker. He was treated with AZT and prophylactic bactrim but stopped these medications due to side effects. He had a history of CMV retinitis, pneumonia due to *Pneumocystis carinii* and CMV. Despite anti-viral therapy with foscarnet, anti-pneumocystis therapy with intravenous pentamidine and anti-fungal therapy with intravenous fluconazole, the patient had a downhill course and expired. The cause of death was disseminated Cryptococcus, *Pneumocystis carinii* pneumonia (PCP) and CMV infection, and the patient died in July, 1995.

Patient #4

Patient #4 was a 45 year old homosexual male, who became seropositive for HIV in 1989. He was diagnosed with AIDS in 1993 and Kaposi's sarcoma in 1994. The patient reported a 17 pack/year history of smoking, quitting in 1989, and social alcohol use prior to 1989. Postmortem examination revealed a large volume of melanotic stool distal to multiple jejunal ulcers. The lungs were congested and both lower lobes had extensive discrete nodules that microscopically were consistent with a large cell lymphoproliferative disorder. The neoplastic lymphocytes were within angiolymphatic spaces, consistent with a diagnosis of intravascular lymphomatosis. These abnormal lymphocytes were also seen in sections of liver, small and large bowel, and anus. Kaposi's sarcoma lesions were only seen grossly. Cause of death was bilateral pneumonia, lymphoproliferative disorder (LPD) involving lungs and colon, gastrointestinal hemorrhage and disseminated Kaposi's sarcoma. The patient died in January, 1994.

Patient #5

Patient #5 was a 40 year old homosexual male who tested positive for HIV in April 1984 when he joined the Pitt Men's Study. He was relatively asymptomatic until October 1990 when he developed PCP and oral Candidiasis. In February 1992 he developed Kaposi's sarcoma of the left chest wall which was treated by radiation therapy. In August 1992, he was diagnosed with *Mycobacterium avium* complex disease. Patient #3 was a smoker for 10 years but quit smoking in 1990. He had no history of drug or alcohol abuse. He had a history of Hepatitis B in 1977 or

1978. Postmortem examination showed residual tumor at the site previously treated for Kaposi's sarcoma on the left chest wall. In addition, multiple lesions were seen in the rectum extending from the mucosa to the serosa. There were also Kaposi's sarcoma lesions in the lymph node, trachea and liver. Cause of death was systemic *Mycobacterium avium intracellulare* complex infection complicated by multiple Kaposi's sarcoma in the skin, rectum and liver and the patient died in June, 1993.

Table 1. This table shows an overview of the demographic information of each patient we received tissue samples from.

Case #	Age	KS Status	Date of Death	Cause of Death	HIV
1	40	-	04/1995	CMV induced organ failure	Seroconvert 1983
2	47	-	05/1993	Pneumonia and gram – septicemia	Diagnosed 1987
3	49	-	07/1995	Disseminated *Cryptococcus*, PCP, and CMV	Diagnosed 1985
4	45	+	01/1994	Pneumonia, LPD, GI hemorrhage, KS	Seroconvert 1989
5	40	+	06/1993	Systemic *Mycobacterium avium intracellulare*	Diagnosed 1984

4.1.2 Tissue received by case

In this study we received tissue from five autopsy cases of men who were HHV-8 seropositive and died with AIDS. Of the five cases, two of these men were diagnosed with KS. The tissue samples that we received varied by case. Tables 2 and 3 show what tissue types were received for each case.

Table 2. This table shows the autopsy tissue samples received from the three patients without KS.

Tissue Type	Patient #1	Patient #2	Patient #3
Abdominal skin	x		
Adrenals	x	x	x
Anorrectum			x
Anus	x	x	x
Bladder	x	x	x
Bone marrow	x	x	x
Colon	x	x	x
Esophagus	x	x	x
Ganglion	x		
Heart		x	x
Ileum	x	x	
Jejunum	x	x	x
Kidney	x	x	x
Liver	x	x	x
Lung	x	x	x
Lymph Node	x	x	x
Muscle		x	x
Nerve			x
Pancreas	x	x	
Penile lesion	x		
Rectum		x	
Right atrium with thrombus	x		
Skin			x
Spleen	x	x	x
Stomach	x	x	x
Supravocal cord lesion		x	
Sural nerve	x		
Testes	x	x	x
Thyroid/parathyroid	x	x	x
Tongue	x		
Vagus nerve	x		
Vocal cord inferior lesion		x	

Table 3. This table shows the autopsy tissue samples received from the two patients with KS.

Tissue Type	Patient #4	Patient #5
Adrenals	x	x
Anal Skin	x	x
Appendix	x	
Bile duct	x	
Bladder		x
Bone marrow	x	x
Colon	x	x
Distal small bowel	x	
Duodenal lesion	x	
Esophagus		x
Foot lesion	x	
Gall bladder	x	
Ganglion	x	
Gastroesophageal Junction	x	
Heart	x	x
Ileum	x	x
Jejunum	x	x
Kidney	x	x
Liver	x	x
Lung	x	x
Lymph Node	x	
Muscle	x	x
Nerve		x
Pancreas	x	x
Prostate		x
Rectal lesion		x
Rectum	x	
Skin		x
Spleen	x	x
Stomach	x	x
Stomach lesions	x	
Testes	x	
Thyroid/parathyroid	x	x
Tongue		
Trachea		x
Vagus nerve	x	

4.1.3 Description of patient staining results

Patient #1 (KS-) (Table 5)

In patient 1, K8.1 staining was very prominent in the abdominal lymph node and this stain was the only staining seen in immune system tissue of patient 1. In the major organs, K8.1 staining was seen sporadically in the kidney and spleen, and very prominently in the lung. No v-IL-6 staining was observed in any of the major organs. In the upper GI, K8.1 staining was observed in all tissues except the stomach, and v-IL-6 staining was seen in all upper GI tissues of patient 1. In the lower GI, K8.1 staining was very prevalent, as it was observed in all lower GI tissues of patient 1. v-IL-6 staining was also prominent in the lower GI, as it was absent only from anus tissue. No staining was observed in any of the nervous tissue. Both K8.1 and v-IL-6 staining was quite heavy in testes tissue from patient one. In the miscellaneous tissue, K8.1 staining was seen sporadically in a penile lesion and thyroid tissue, and focally in the adrenal and right atrium tissue, whereas v-IL-6 was seen sporadically in the adrenal tissue. No LANA staining was done on this patient due to time constraints.

Patient #2 (KS-) (Table 6)

In patient 2, K8.1 staining of the immune system tissue was very prominent. There was also some sporadic LANA staining in the bone marrow tissue we received. This was the only LANA staining observed in patient 2. K8.1 staining was seen in the lung, heart, and kidney tissue of patient 2. In the lower and upper GI, K8.1 staining was very prevalent, as it was seen each tissue sample tested from these systems. The only observed v-IL-6 staining within patient 2 was seen in the testes tissue. K8.1 staining was also observed in two vocal cord lesions in patient 2.

Patient #3 (KS-) (Table 7)

In patient 3, lytic staining of the bone marrow tissue was very prominent, whereas in the lymph node, only LANA staining was observed. K8.1 staining of the major organs was seen in the lung, spleen, and kidney of patient 2, with v-IL-6 being observed only in the kidney. LANA staining was seen more prominently in patient 3 and was observed in the liver and kidney of patient 3. In the upper and lower GI, lytic antigen staining was highly prevalent, especially K8.1 in the lower GI, where all three tissues were of the highest staining level. LANA staining was also observed in the lower GI in the colon and jejunum. Similar to patient 1, no staining was observed in the nervous system tissue we received from patient 3. In the testes tissue we received, only v-IL-6 staining was observed.

Patient #4 (KS+) (Table 8)

Patient 4 was found to be a special case and will be discussed further in the results section. K8.1 staining was seen to be very prevalent in the lymph nodes, as well as the upper and lower GI tissues of patient 4. It was also observed in some of the organs such as the kidney and lung tissue. v-IL-6 was seen less prominently than K8.1, but was observed in the lung, testes, and some of the lower and upper GI tissues. LANA staining was also quite prevalent in patient 4, and was seen in the lung and kidney tissue, as well as in some of the upper and lower GI tissue.

Patient #5 (KS+) (Table 9)

In patient 5, LANA staining was observed in the bone marrow, kidney, esophagus, and three of the tissues of the lower GI. K8.1 staining was less prevalent in patient 5 and was seen prominently in the lower GI, but seldom elsewhere. v-IL-6 staining was very prevalent in the

major organs and was seen in lung, liver, and kidney tissue. Elsewhere it was only observed in the prostate and ileum.

4.2 Section II: Immunohistochemistry Data

IHC

Using IHC our goal was to determine reservoirs of HHV-8 infection and latency, determine the type of viral infection (lytic and/or latent) of each tissue type, and attempt to identify the infected cell types. In this report, using IHC, we document the presence of HHV-8 infected cells in several organs including kidney, lung, liver, and gastrointestinal tract samples from the Multicenter AIDS Cohort Study (MACS). Both lytic and latent infections have been detected and the infected cells appear to consist of both immune and non-immune cells.

4.2.1 Control Staining

Our control slides, which were done in parallel with the staining of the tissue slides, consisted of a 2:1 mixture of BCBL/BJAB cells. BCBL-1 cells are a body cavity based lymphoma cell line that is infected with HHV-8. BJAB cells are a Burkitt Lymphoma cell line that is HHV-8 uninfected. Serial slides were done in duplicate and stained with either the primary antibody or a normal serum control. Staining with the anti-LANA-1 antibody shows a nuclear stain, whereas staining with anti-v-IL-6 and anti-K8.1 antibodies show a cytoplasmic stain, with a visible nucleus. Figures 2-7 are examples of each of these control stains.

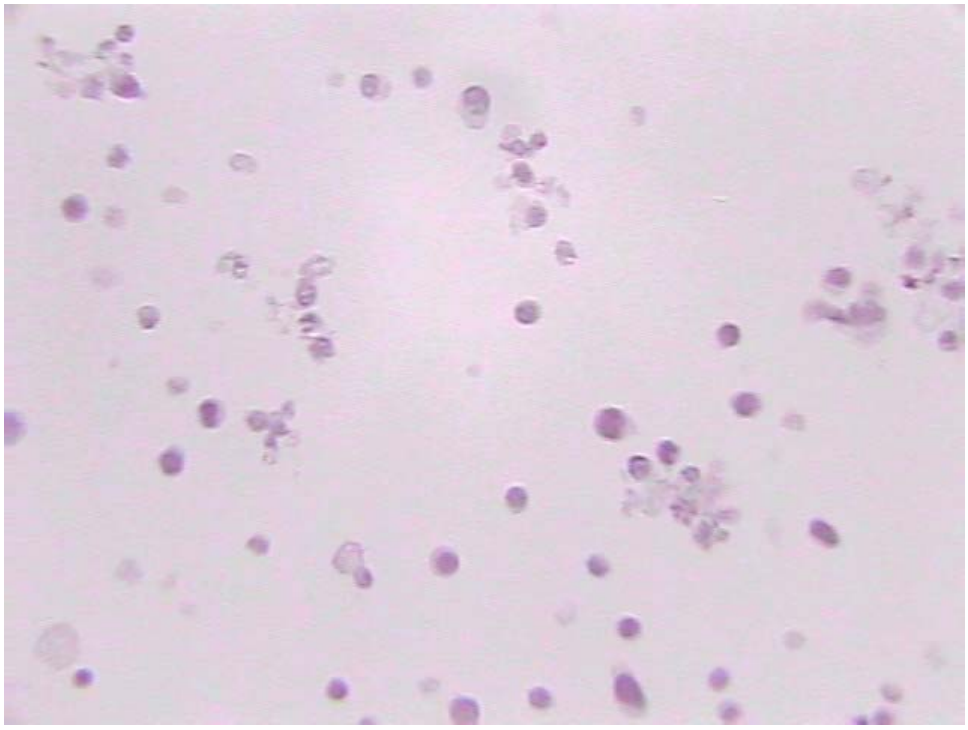


Figure 2. BCBL/BJAB Normal Rat Serum 40x

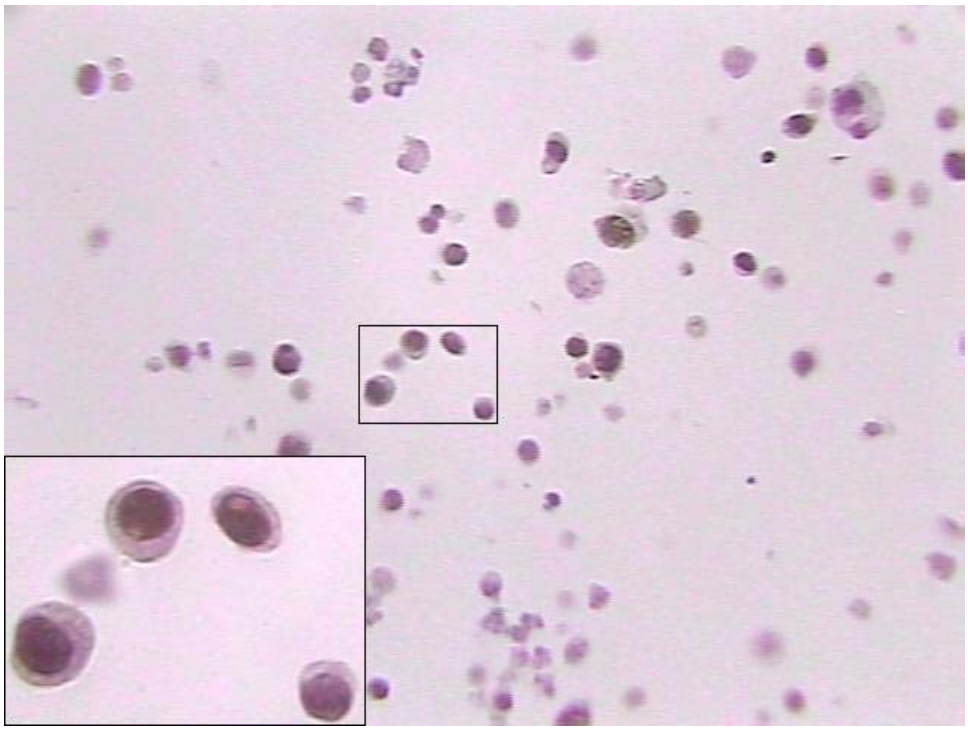


Figure 3. BCBL/BJAB LANA-1 40x (Inset 100x)

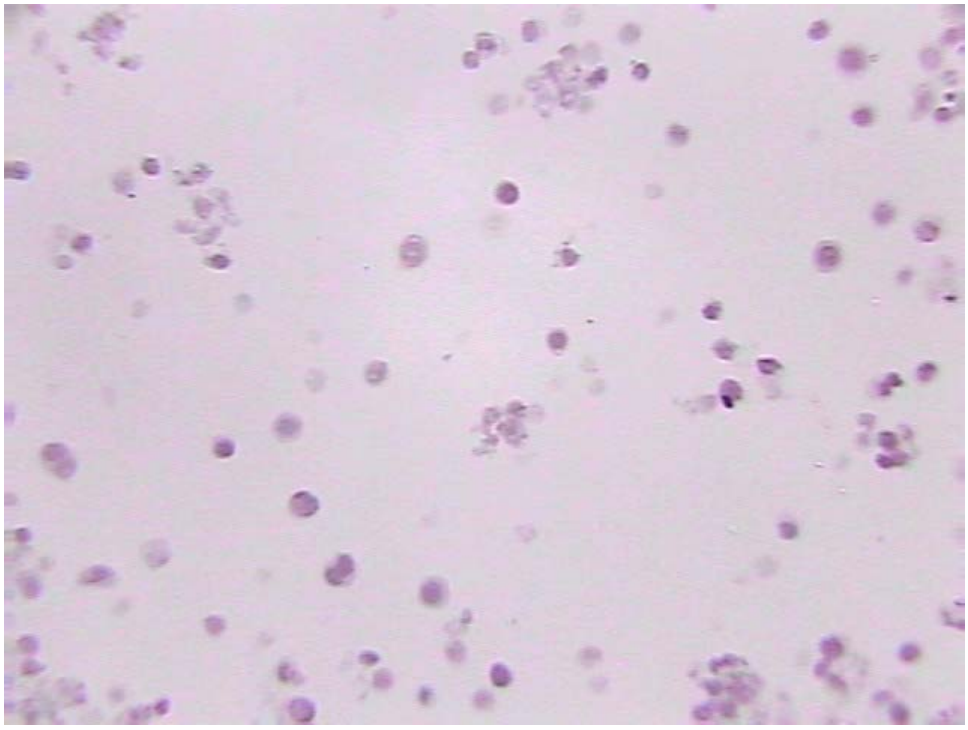


Figure 4. BCBL/BJAB Normal Rabbit Serum 40x

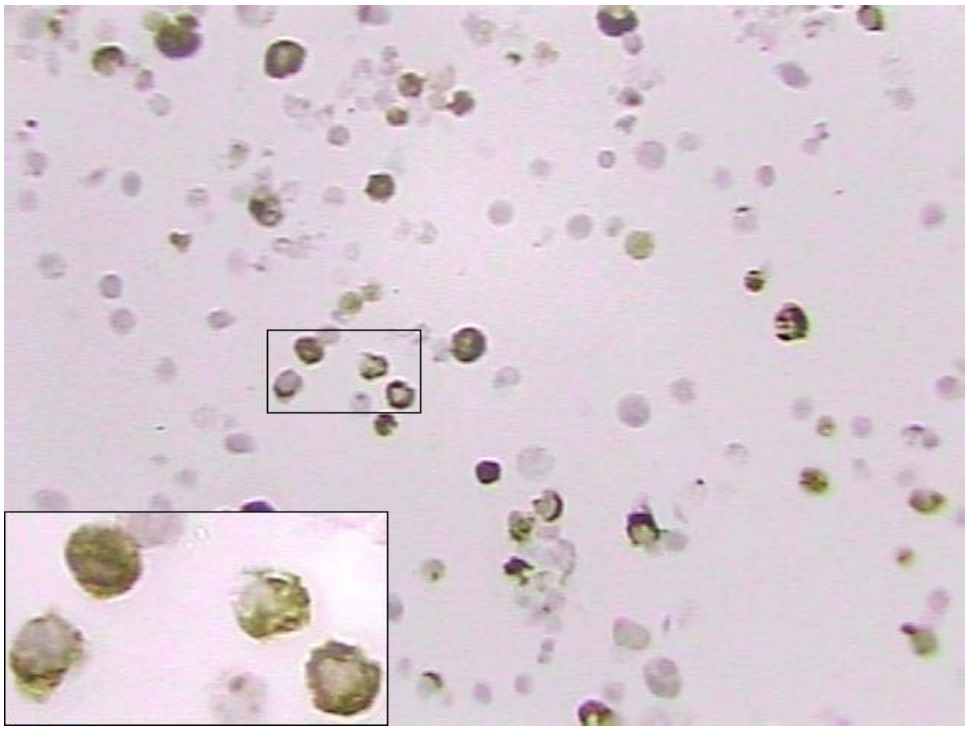


Figure 5. BCBL/BJAB v-IL-6 40x (Inset 100x)

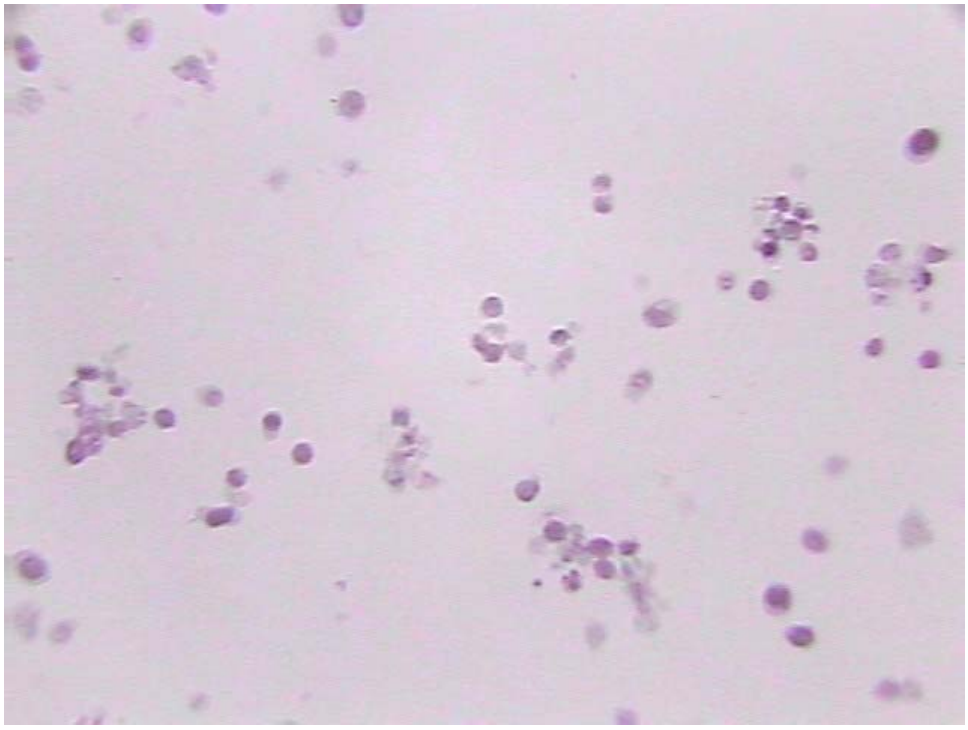


Figure 6. BCBL/BJAB Normal Mouse Serum 40x

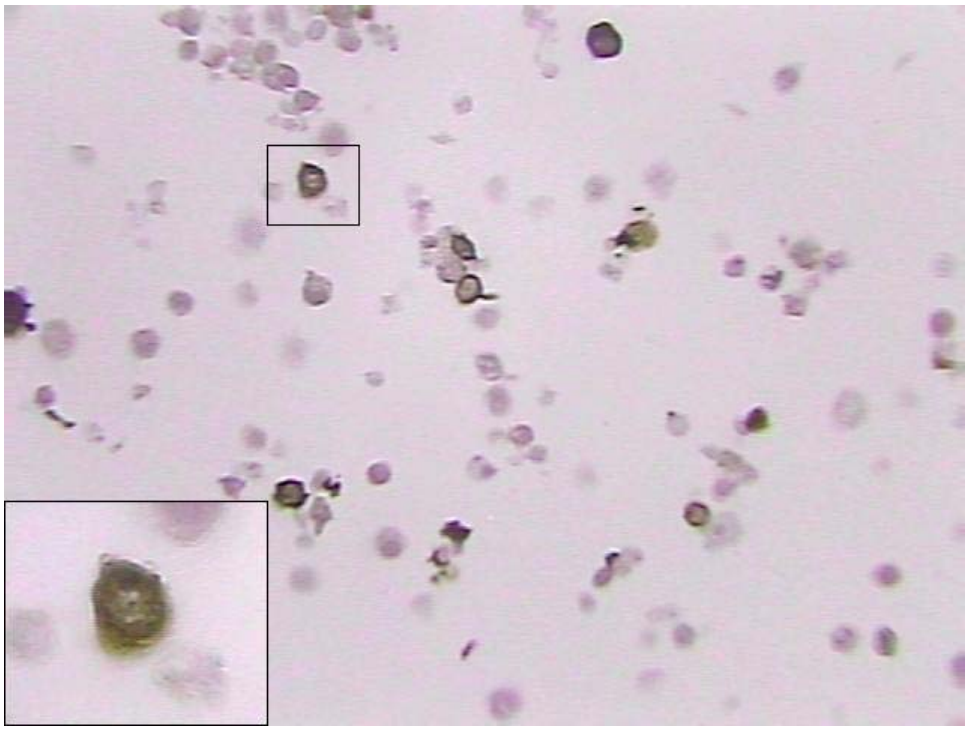


Figure 7. BCBL/BJAB K8.1 40x (Inset 100x)

4.2.2 Semi-quantitation

In order to be able to compare the IHC staining between samples, we developed a semi-quantitative scale for the positive samples with five levels of staining. Level 0, represents a negative staining and is characterized by absolutely no staining observed on the entire slide. Level 1, termed sporadic, represents staining of <25 cells scattered throughout the entire slide with no apparent foci. Level 2, termed focal, represents staining of small clusters of cells dispersed throughout the slide. Level 3, termed diffuse 1 represents staining similar to sporadic in the sense that there were no apparent foci; however, diffuse 1 overall has more positive staining than sporadic. Level 4, termed diffuse 2, was the most positive category, with many positive cells densely spread throughout the sample. Figures 8-15 show examples of each staining category.

Level 1: Sporadic Example

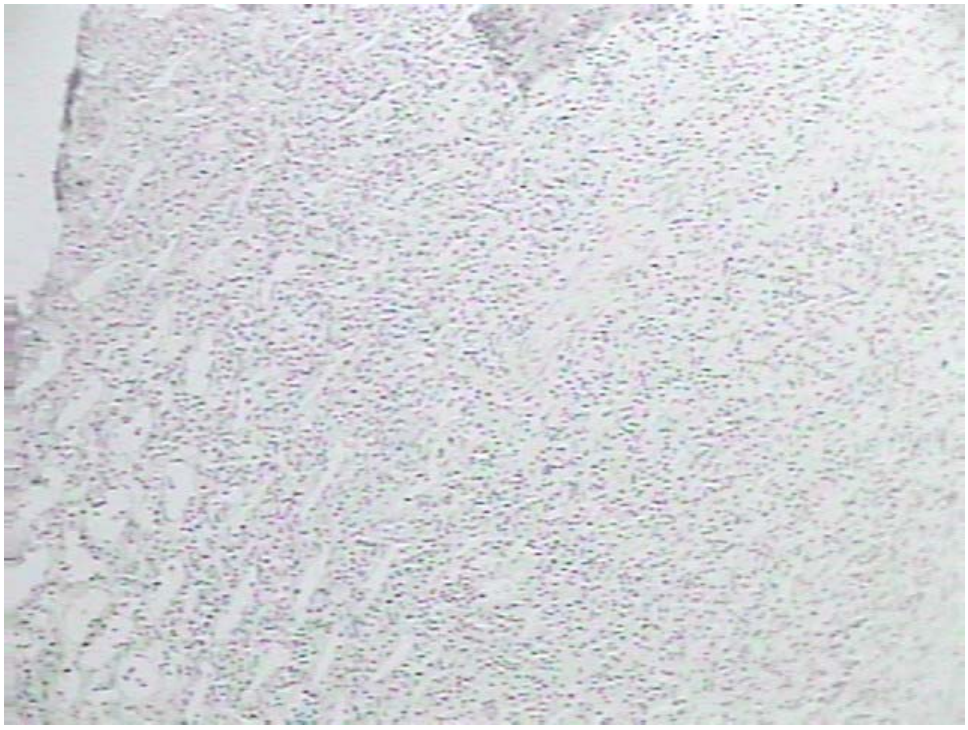


Figure 8. Patient 4 Lung Normal Rabbit Serum 10x

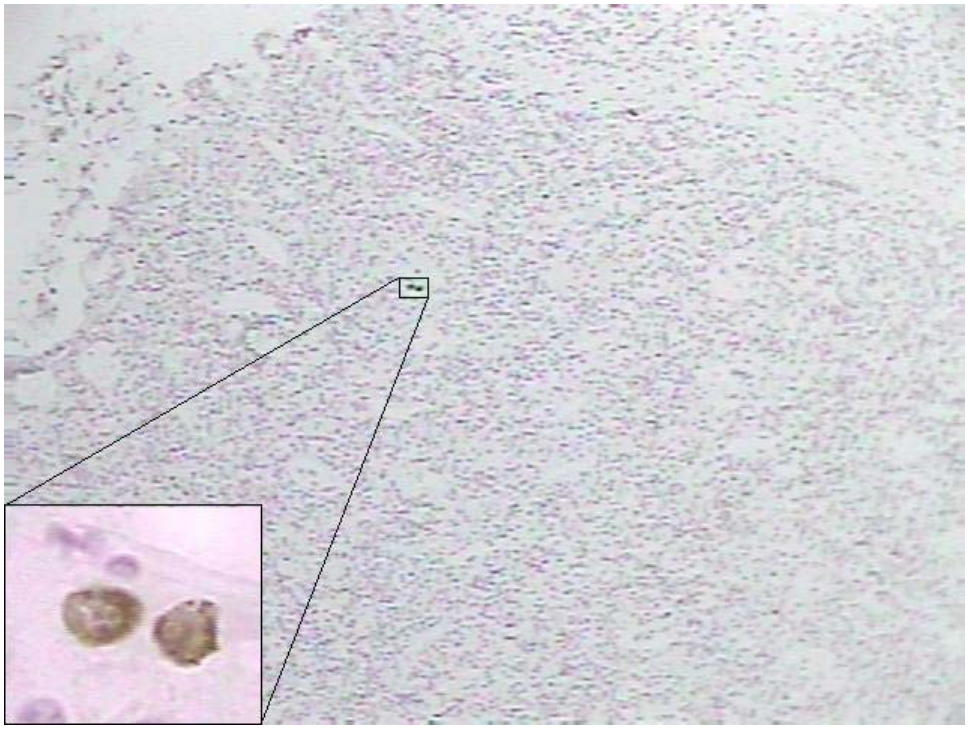


Figure 9. Patient 4 Lung v-IL-6 10x (Inset 100x)

Level 2: Focal Example

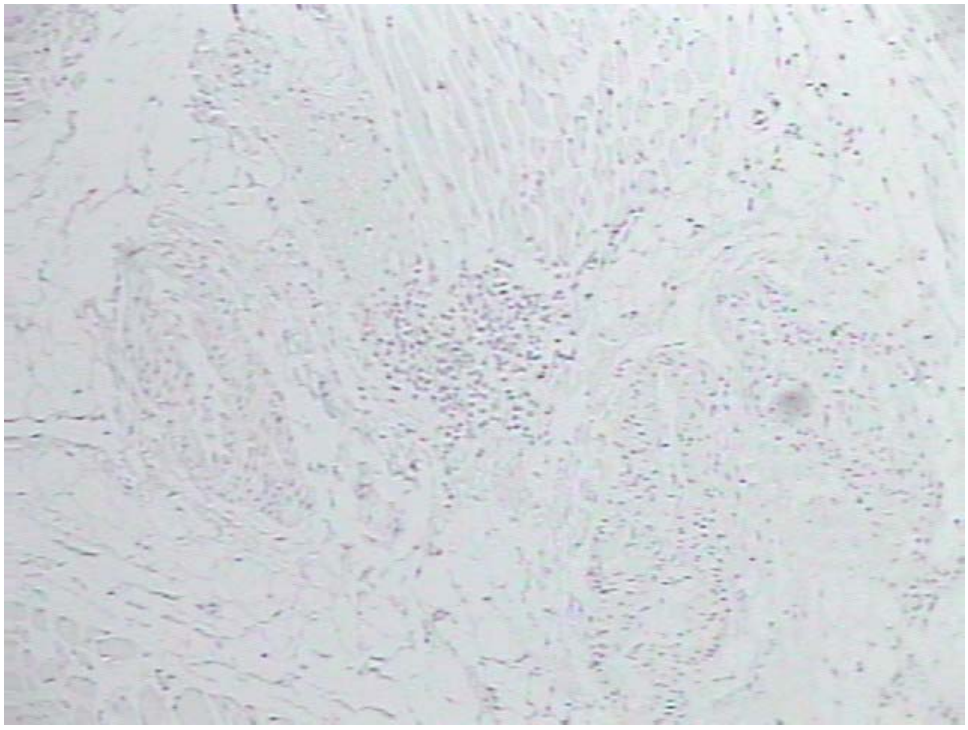


Figure 10. Patient 3 Muscle Normal Mouse Serum 10x

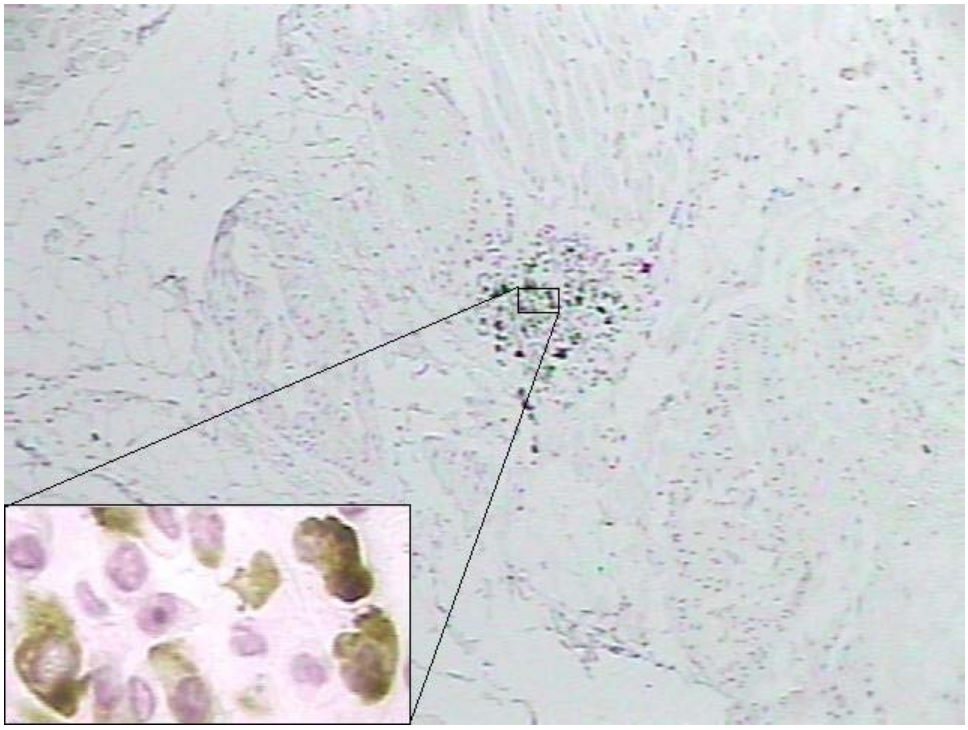


Figure 11. Patient 3 Muscle K8.1 10x (Inset 100x)

Level 3: Diffuse 1 Example

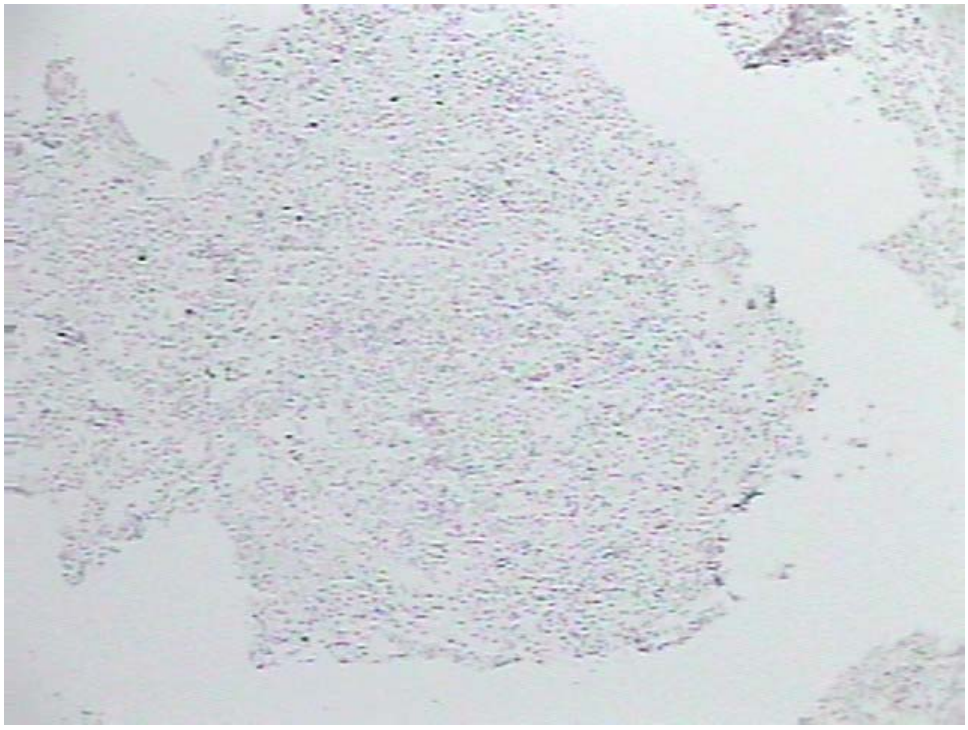


Figure 12. Patient 2 Bone Marrow Normal Mouse Serum 10x

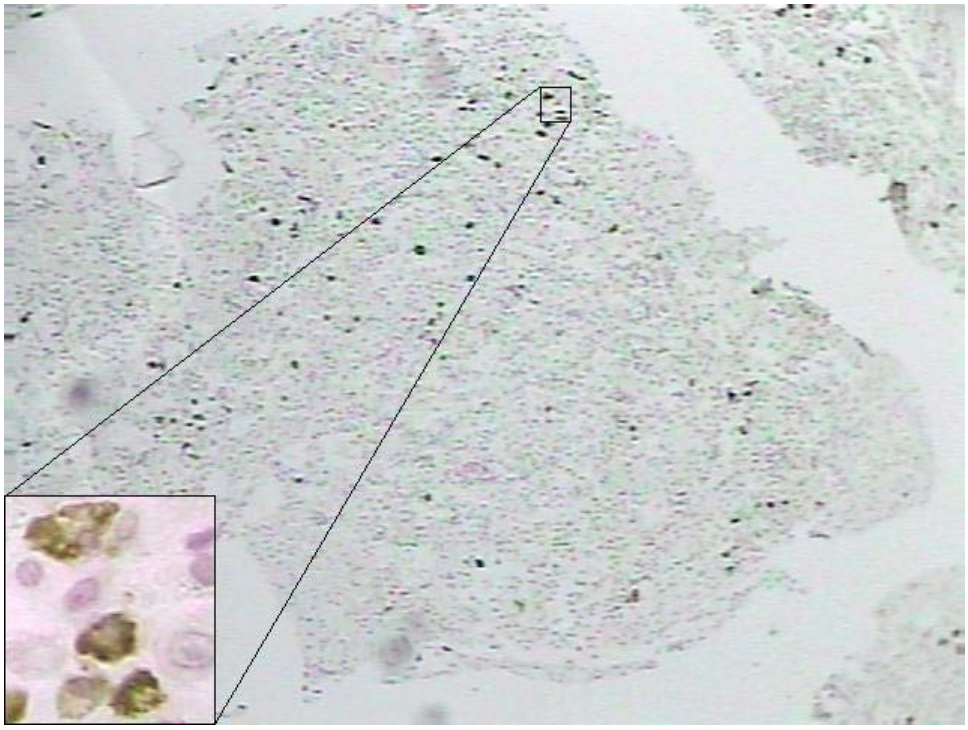


Figure 13. Patient 2 Bone Marrow K8.1 10x (Inset 100x)

Level 4: Diffuse 2 Example

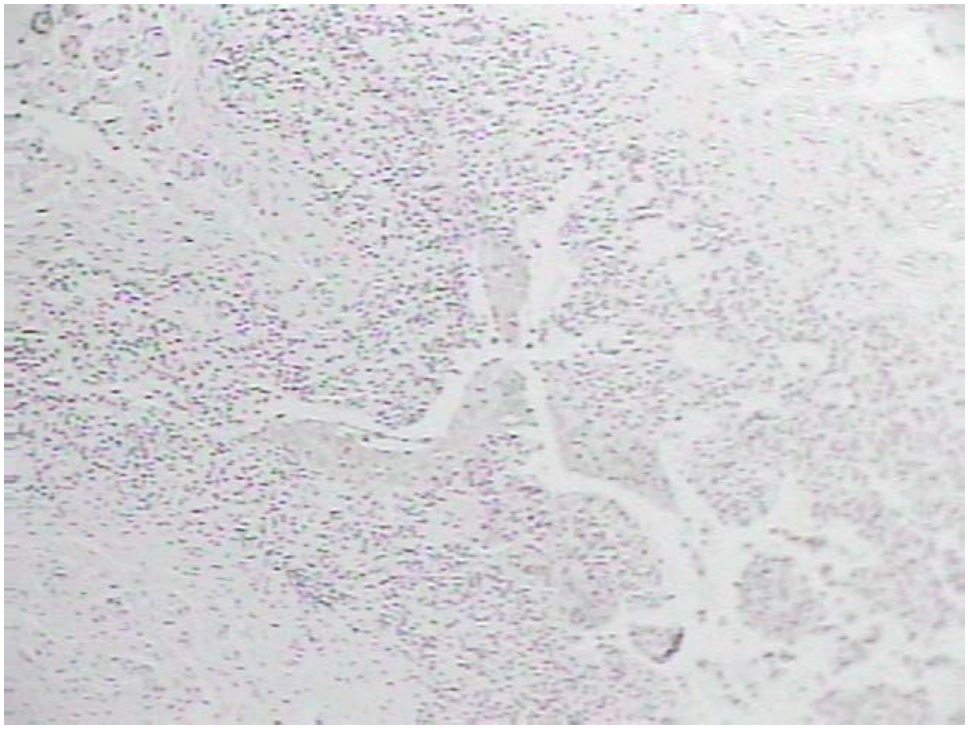


Figure 14. Patient 3 Jejunum Normal Mouse Serum 10x

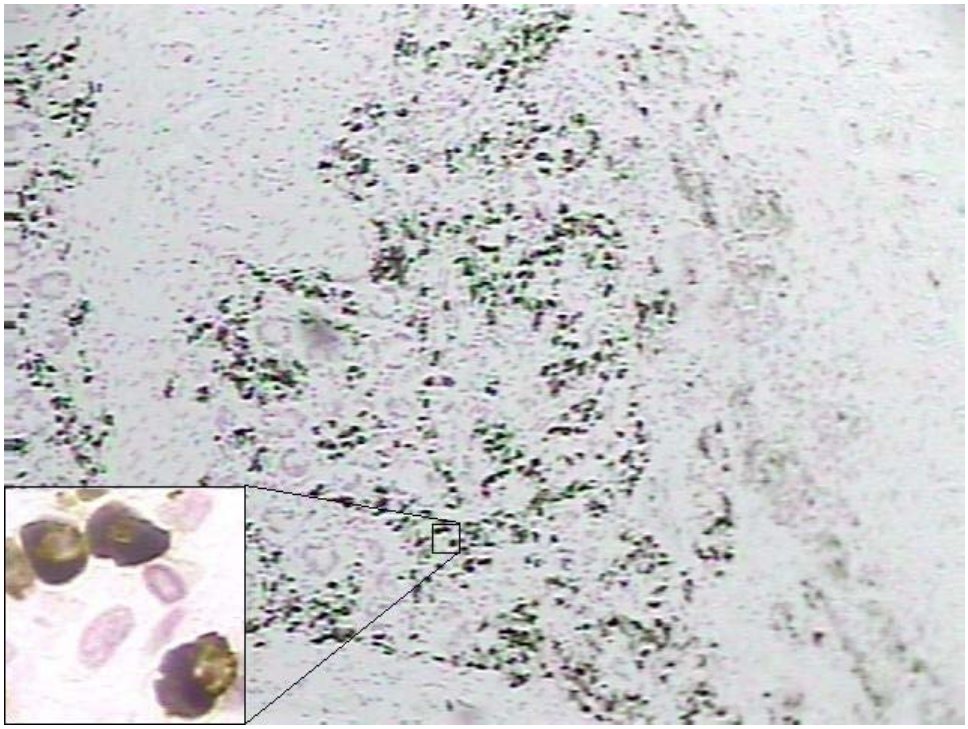


Figure 15. Patient 3 Jejunum K8.1 10x (Inset 100x)

4.2.3 IHC Patient Samples

Samples from each patient were chosen to represent the types of staining seen with different tissue types. Figures 16-55 are tissue samples from each patient chosen as examples.

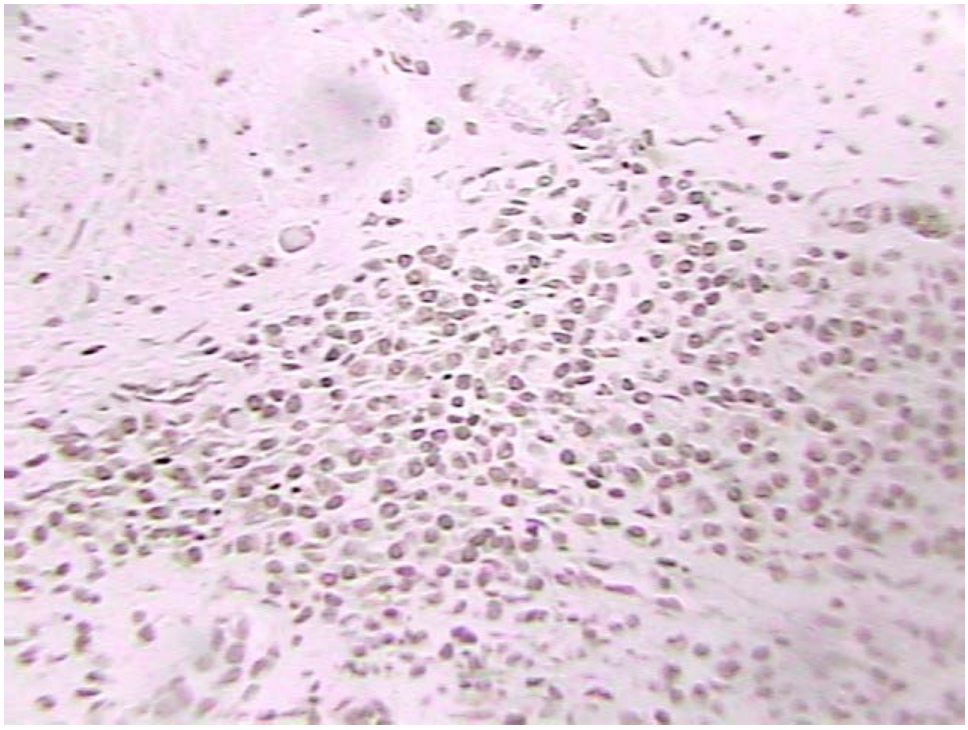


Figure 16. Patient 1 Esophagus Normal Mouse Serum 40x

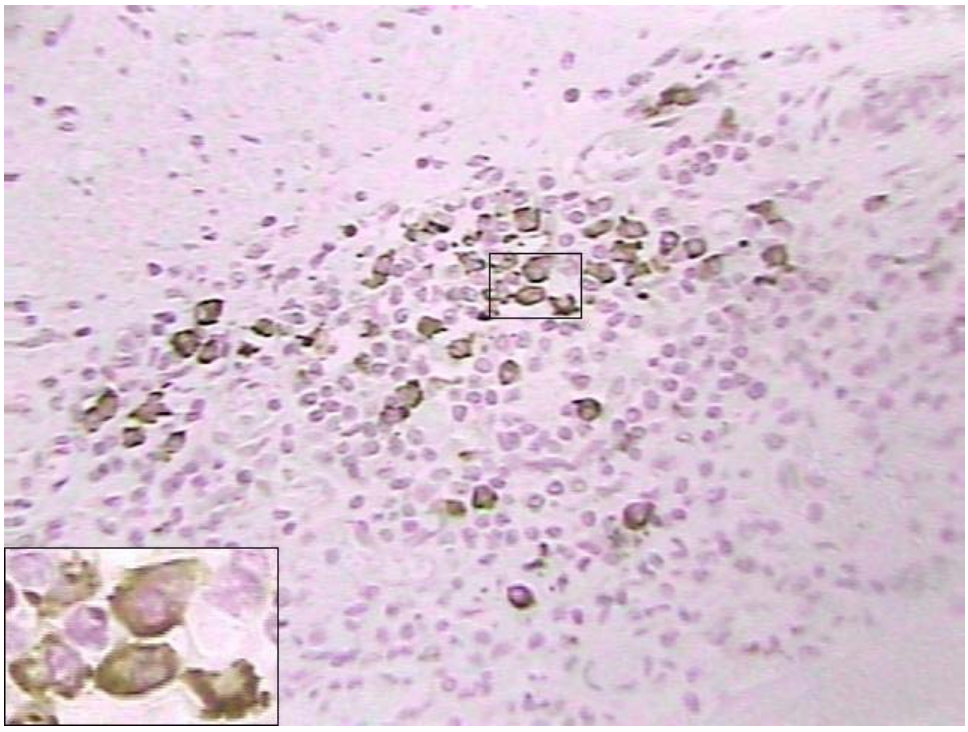


Figure 17. Patient 1 Esophagus K8.1 40x (Inset 100x)

Focal

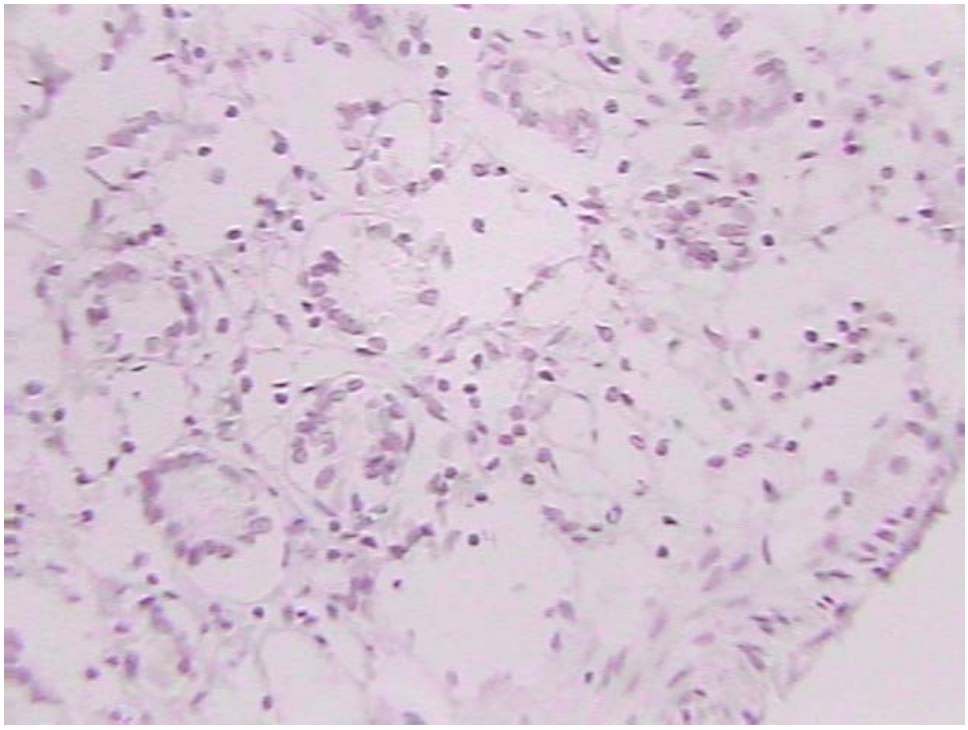


Figure 18. Patient 1 Jejunum Normal Mouse Serum 40x

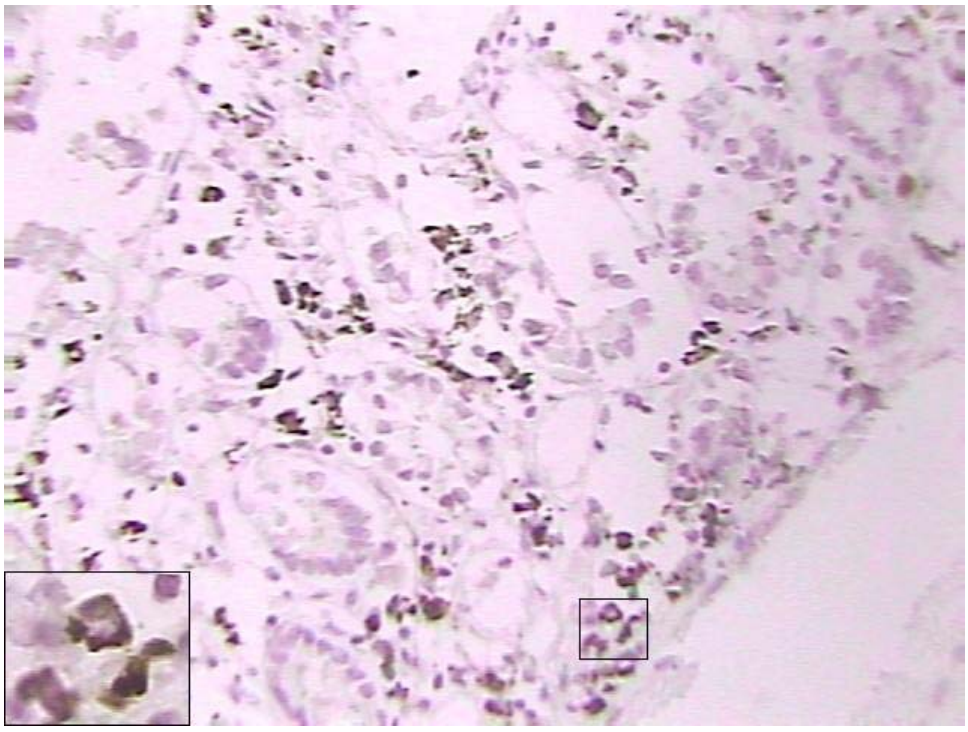


Figure 19. Patient 1 Jejunum K8.1 40x (Inset 100x)

Diffuse 2

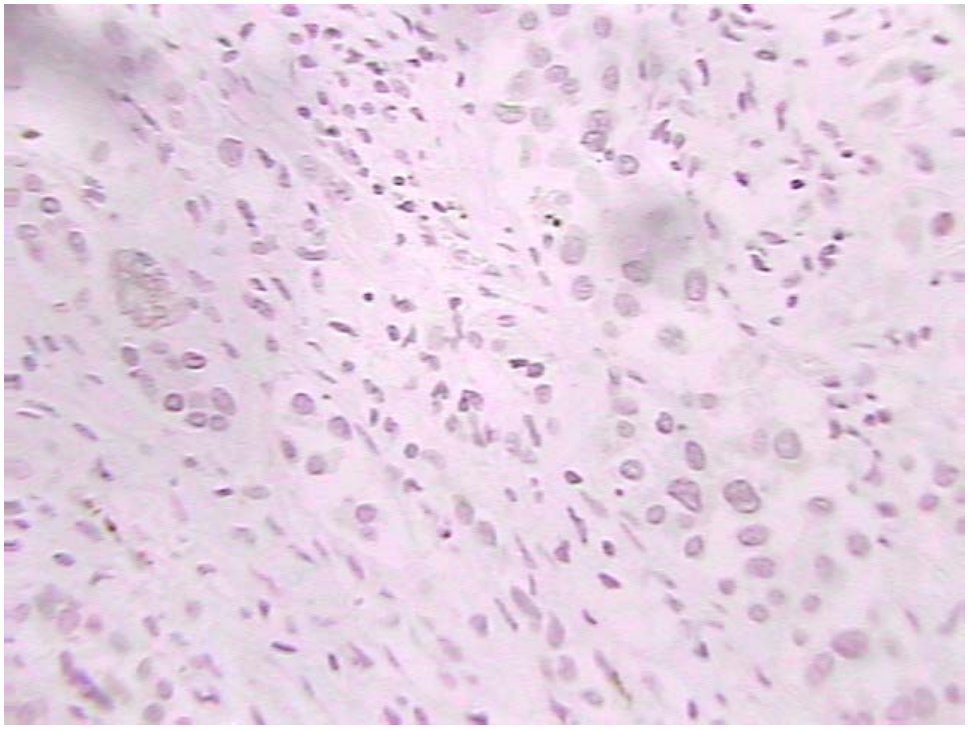


Figure 20. Patient 1 Left Kidney Normal Mouse Serum 40x

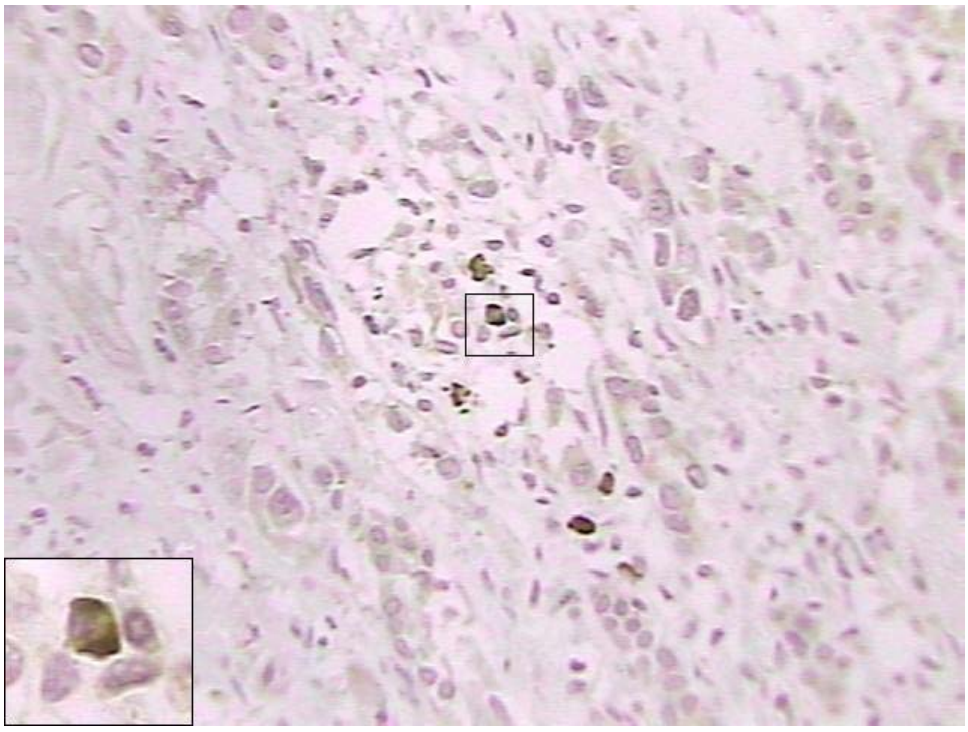


Figure 21. Patient 1 Left Kidney K8.1 40x (Inset 100x)

Sporadic

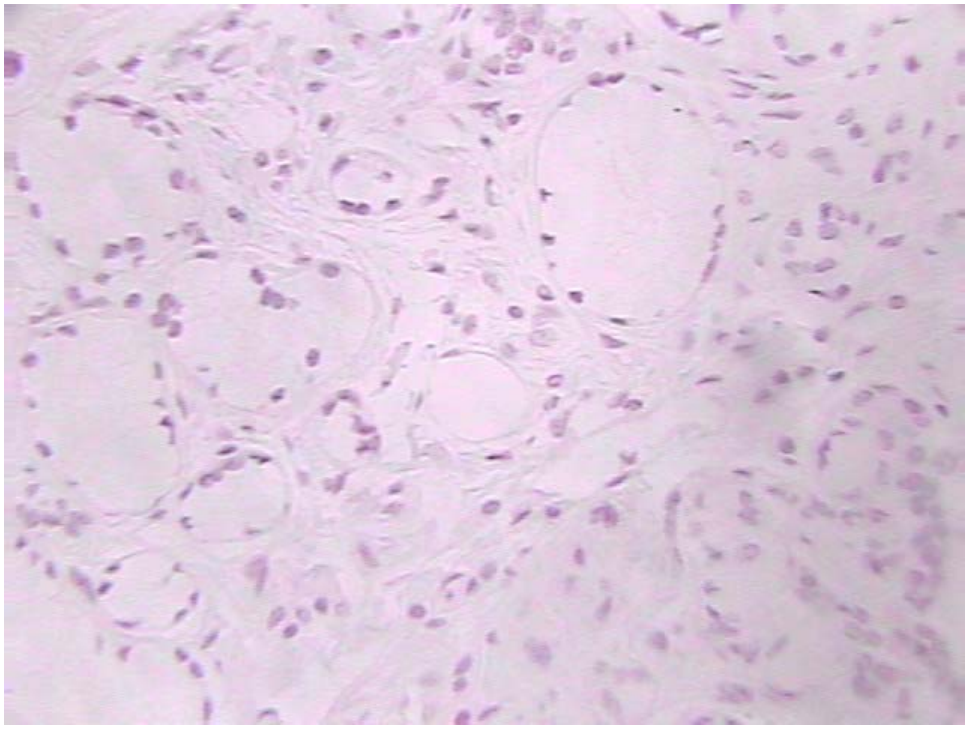


Figure 22. Patient 1 Tongue Normal Mouse Serum 40x

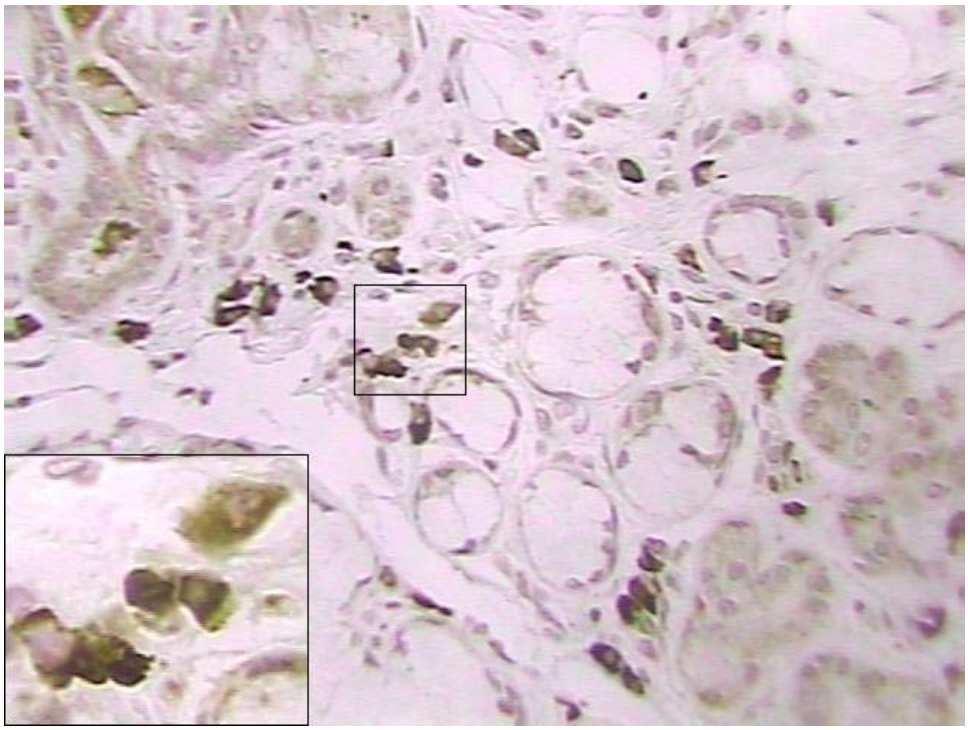


Figure 23. Patient 1 Tongue K8.1 40x (Inset 100x)

Diffuse 2

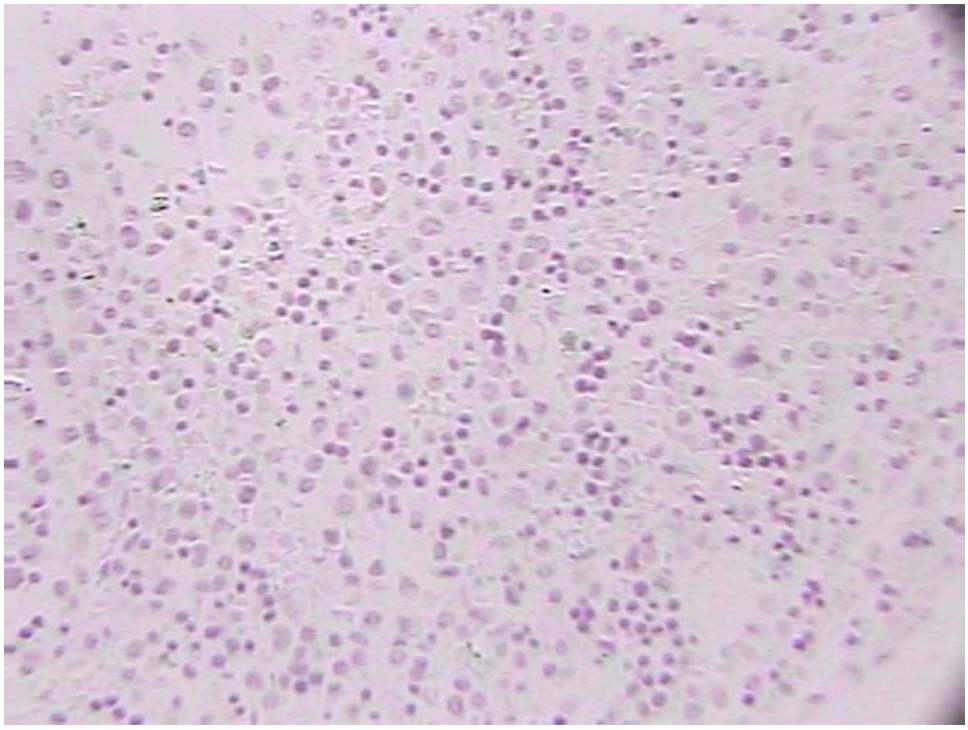


Figure 24. Patient 2 Bone Marrow Normal Mouse Serum 40x

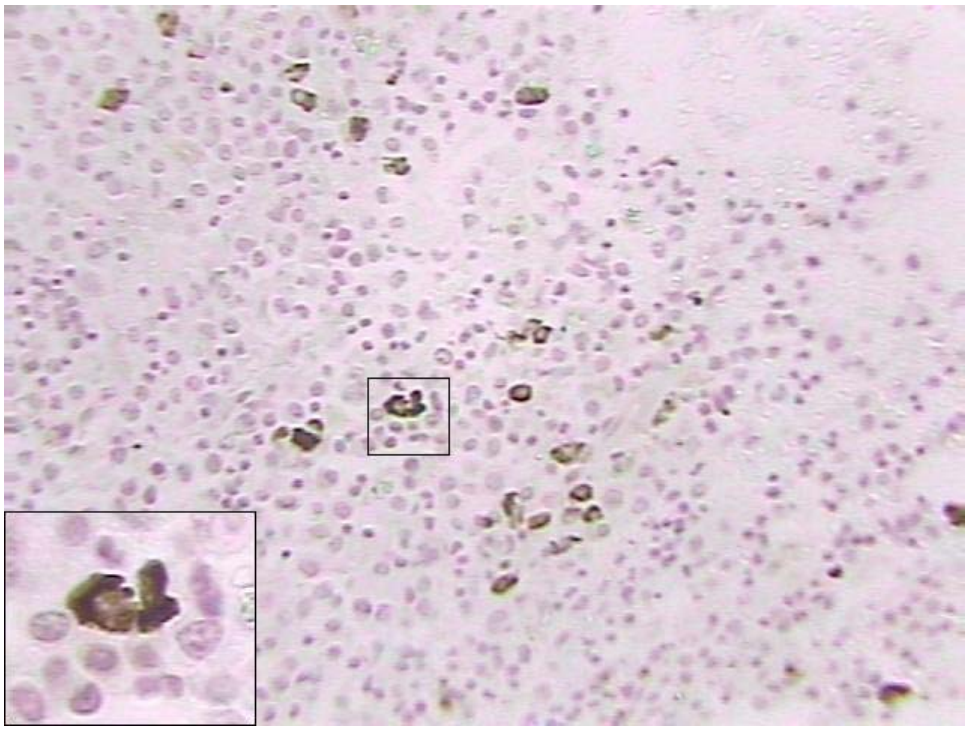


Figure 25. Patient 2 Bone Marrow K8.1 40x (Inset 100x)

Diffuse 2

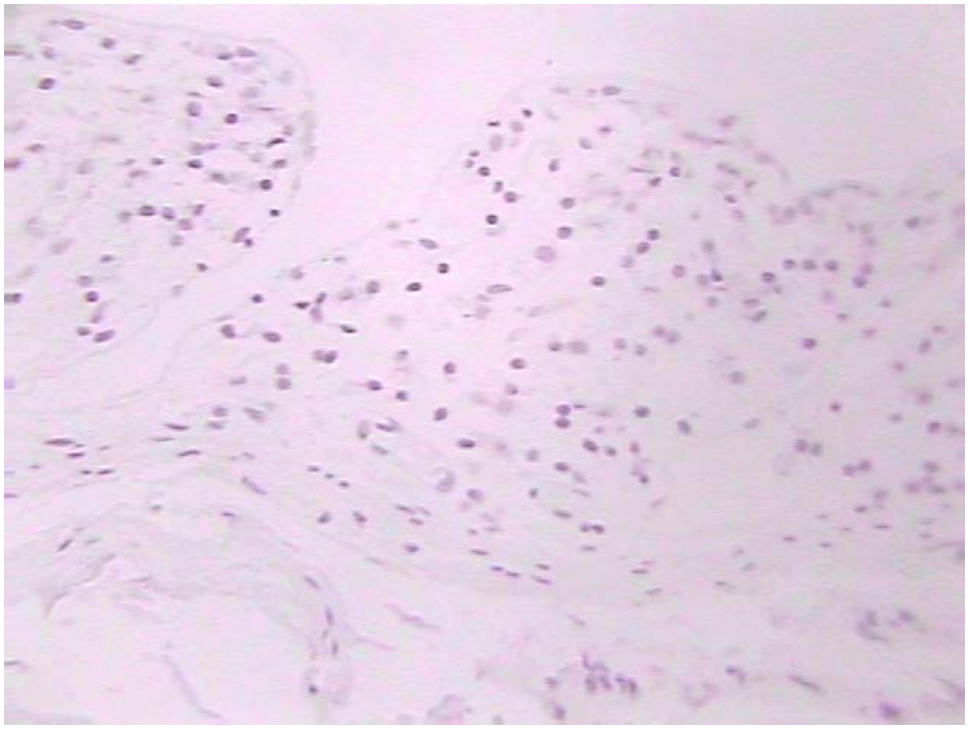


Figure 26. Patient 2 Colon Normal Mouse Serum 40x

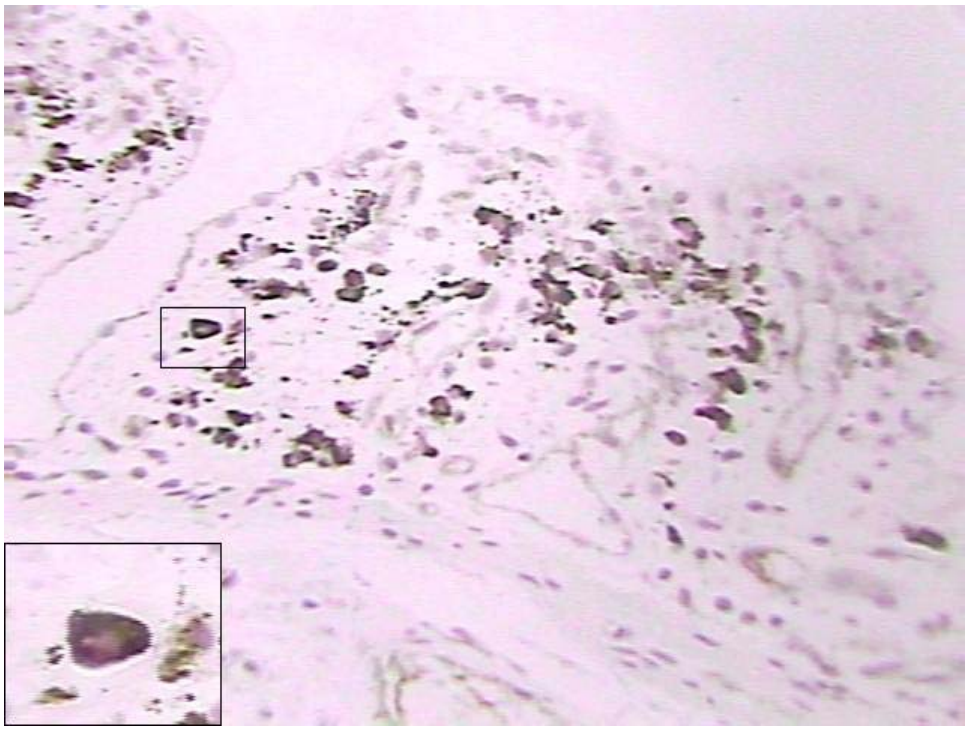


Figure 27. Patient 2 Colon K8.1 40x (Inset 100x)

Diffuse 2

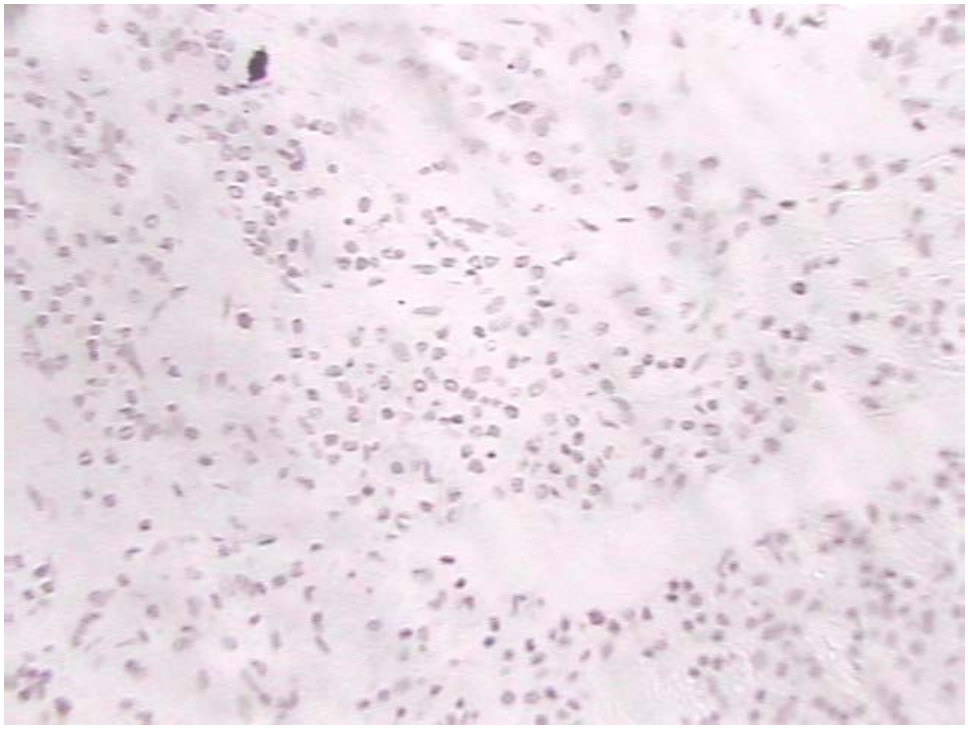


Figure 28. Patient 2 Left Upper Lobe Lung Normal Mouse Serum 40x

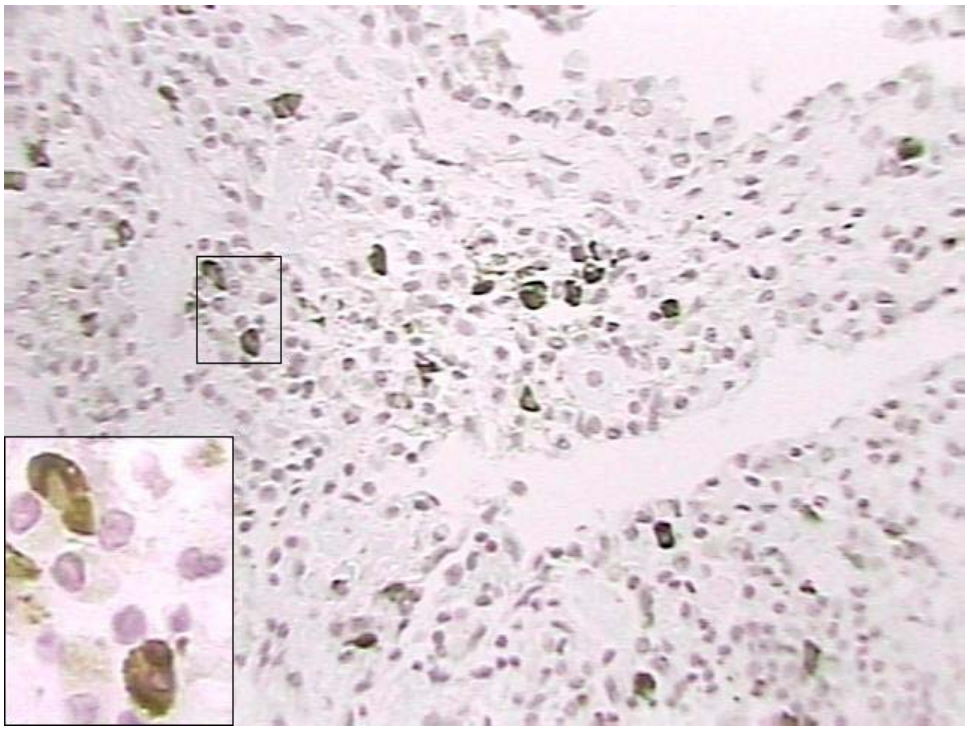


Figure 29. Patient 2 Left Upper Lobe Lung K8.1 40x (Inset 100x)

Diffuse 1

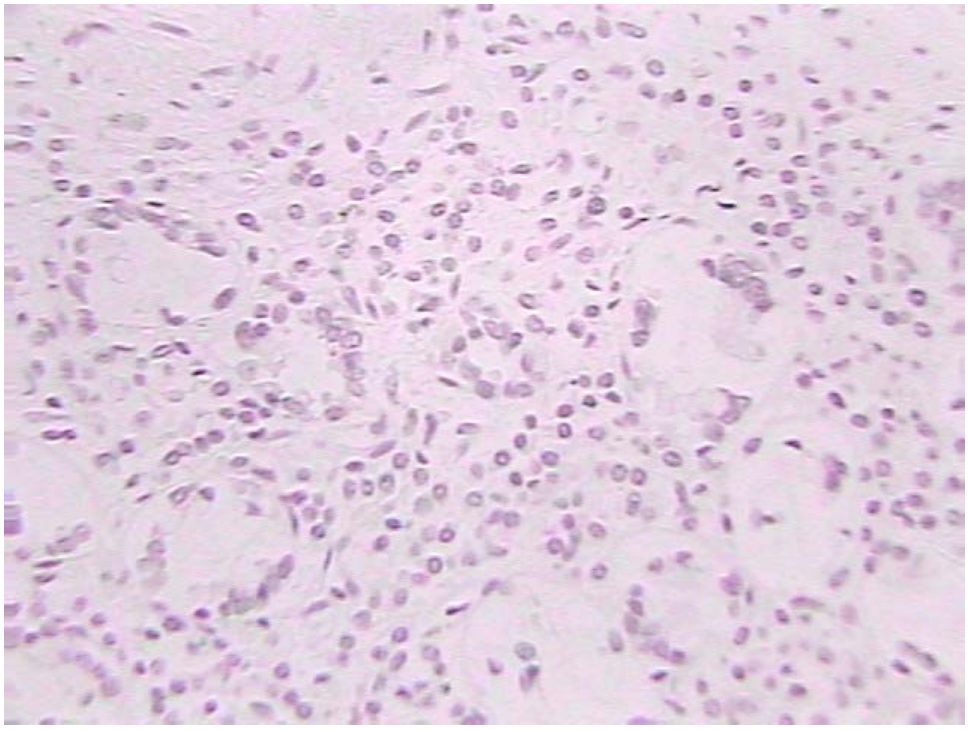


Figure 30. Patient 3 Jejunum Normal Mouse Serum 40x

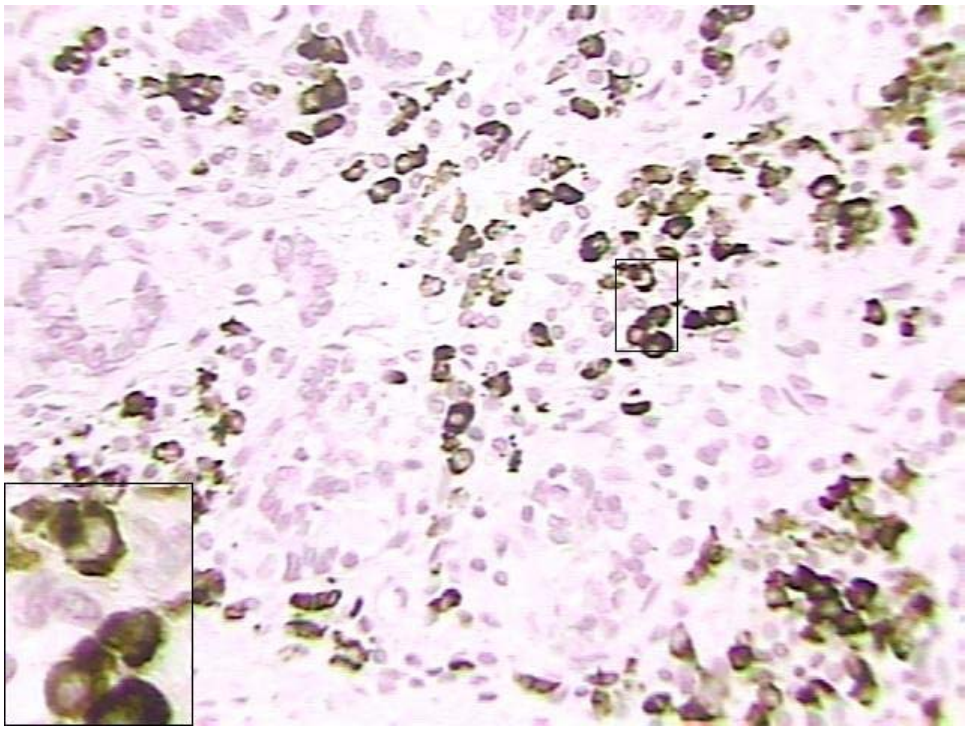


Figure 31. Patient 3 Jejunum K8.1 40x (Inset 100x)

Diffuse 2

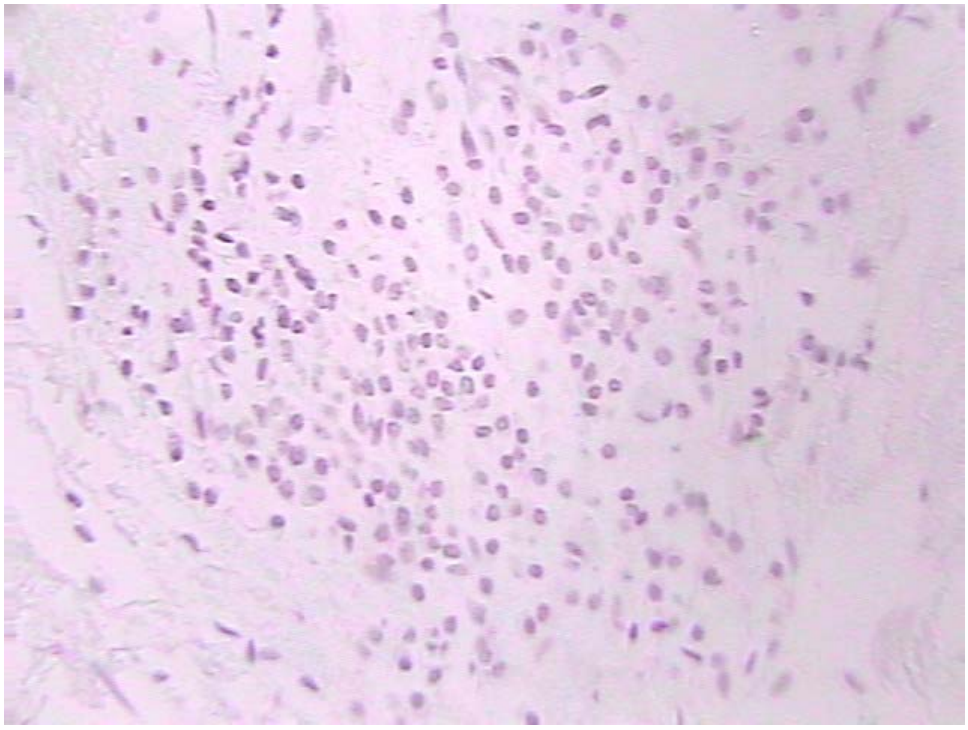


Figure 32. Patient 3 Muscle Normal Mouse Serum 40x

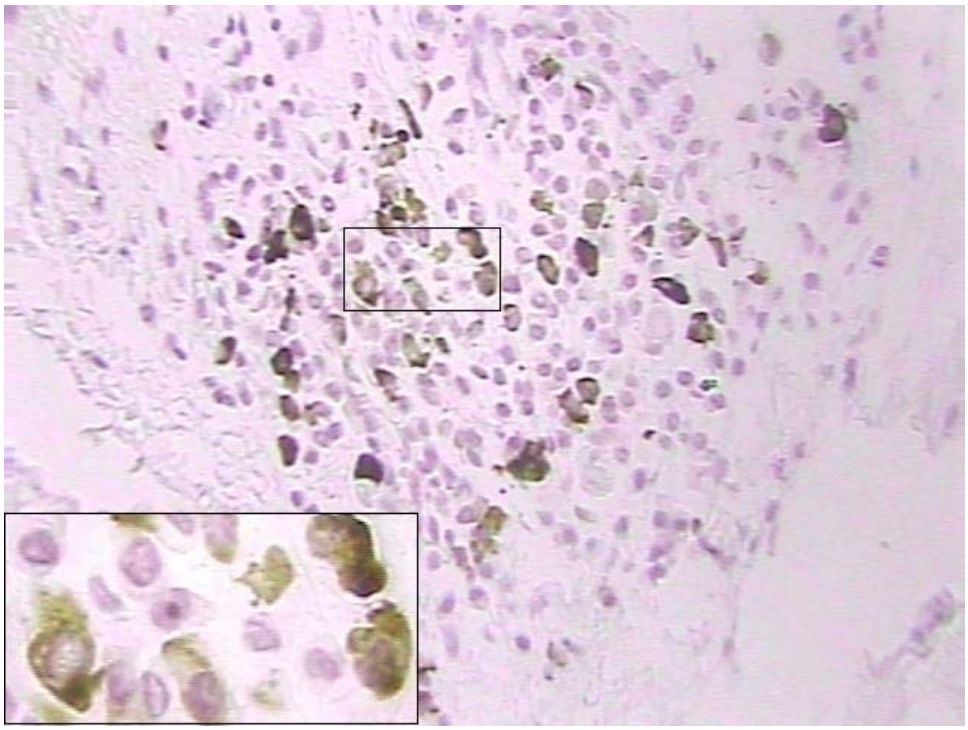


Figure 33. Patient 3 Muscle K8.1 40x (Inset 100x)

Focal

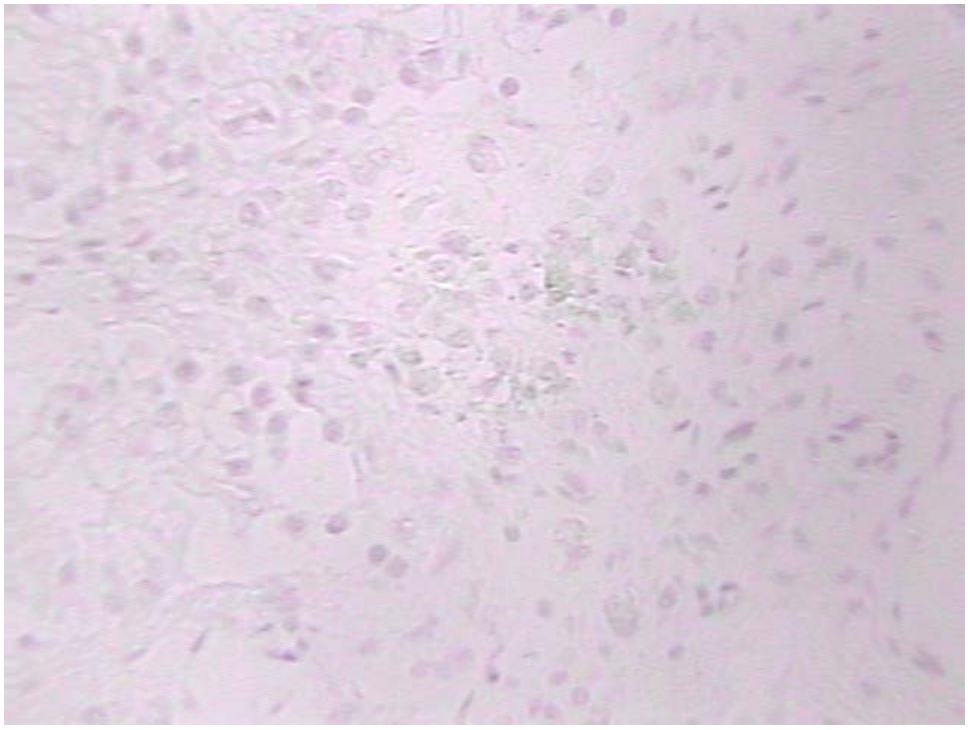


Figure 34. Patient 3 Testes Normal Rabbit Serum 40x

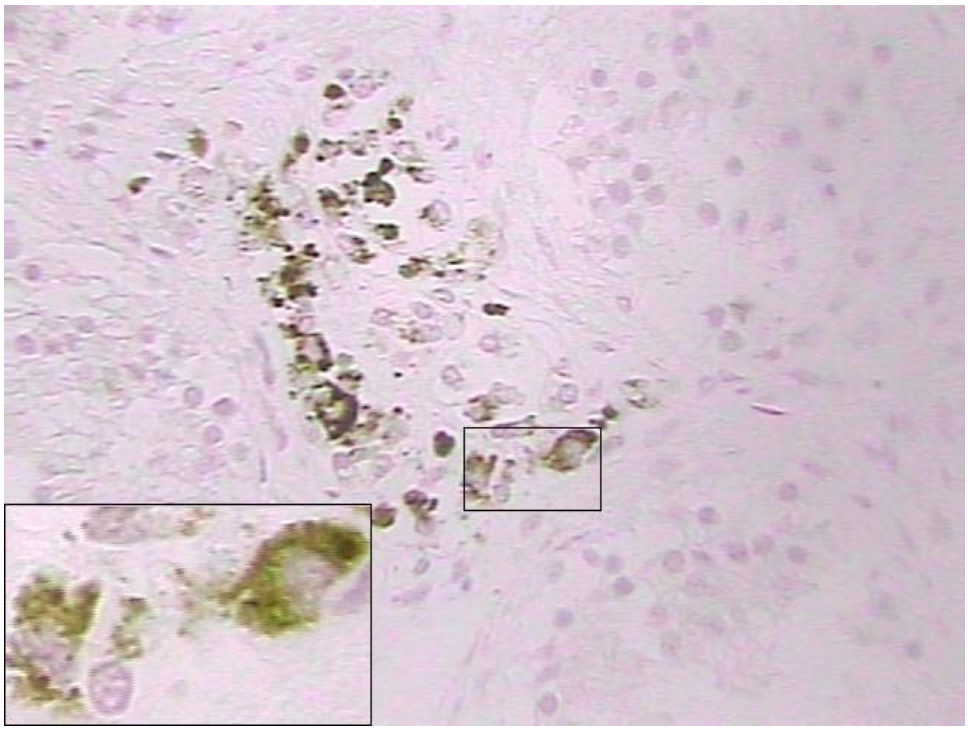


Figure 35. Patient 3 Testes v-IL-6 40x (100x)

Focal

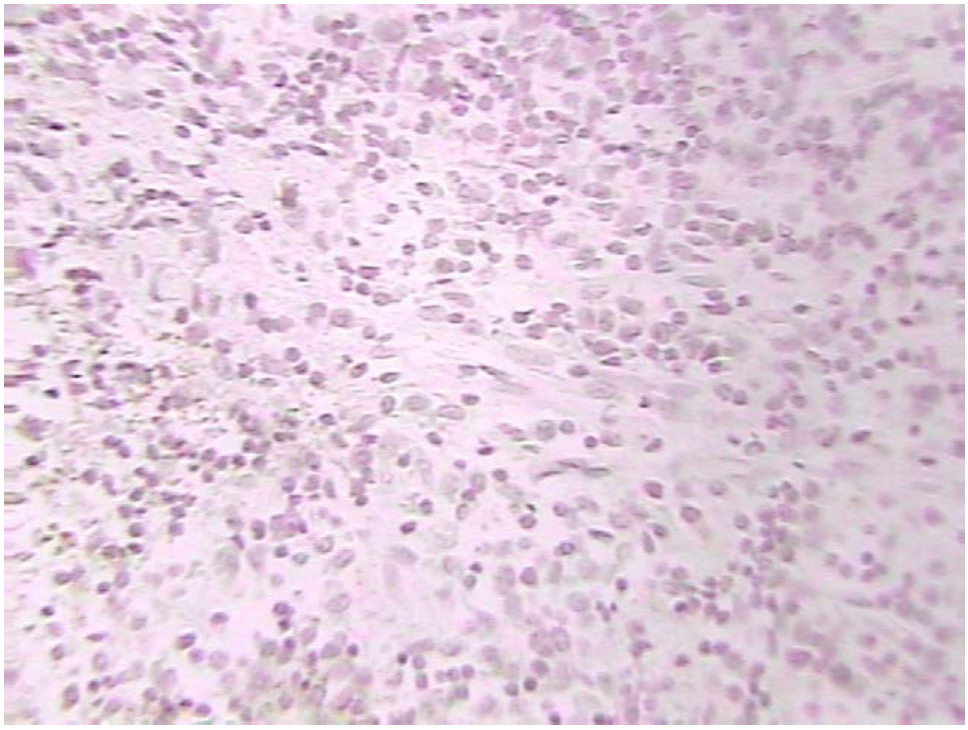


Figure 36. Patient 4 Appendix Normal Rabbit Serum 40x

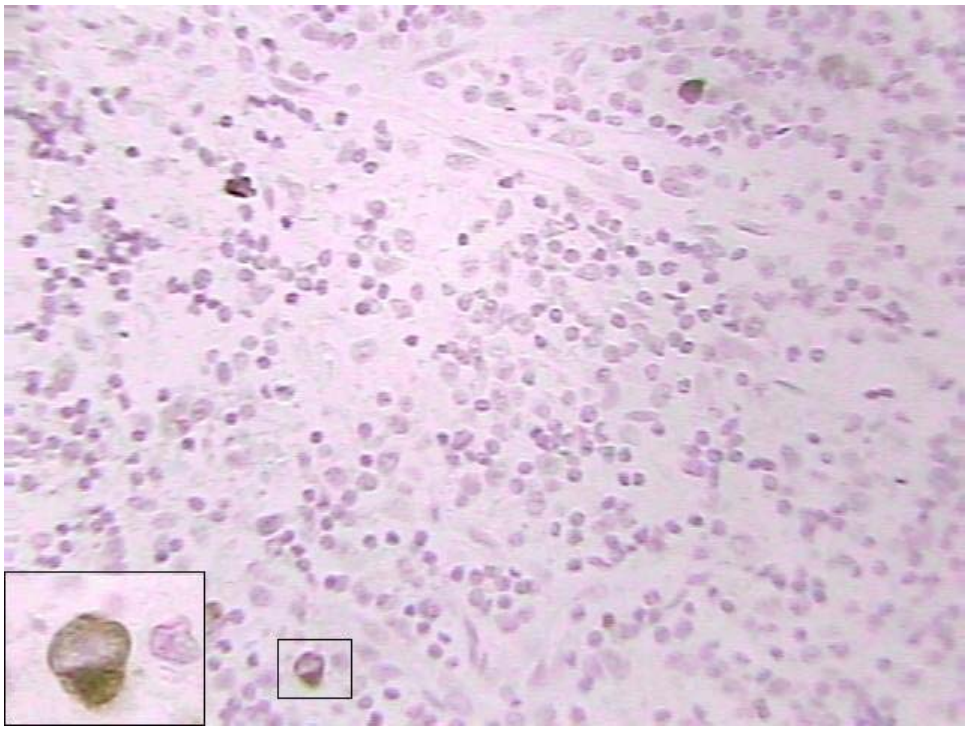


Figure 37. Patient 4 Appendix v-IL-6 40x (Inset 100x)

Sporadic

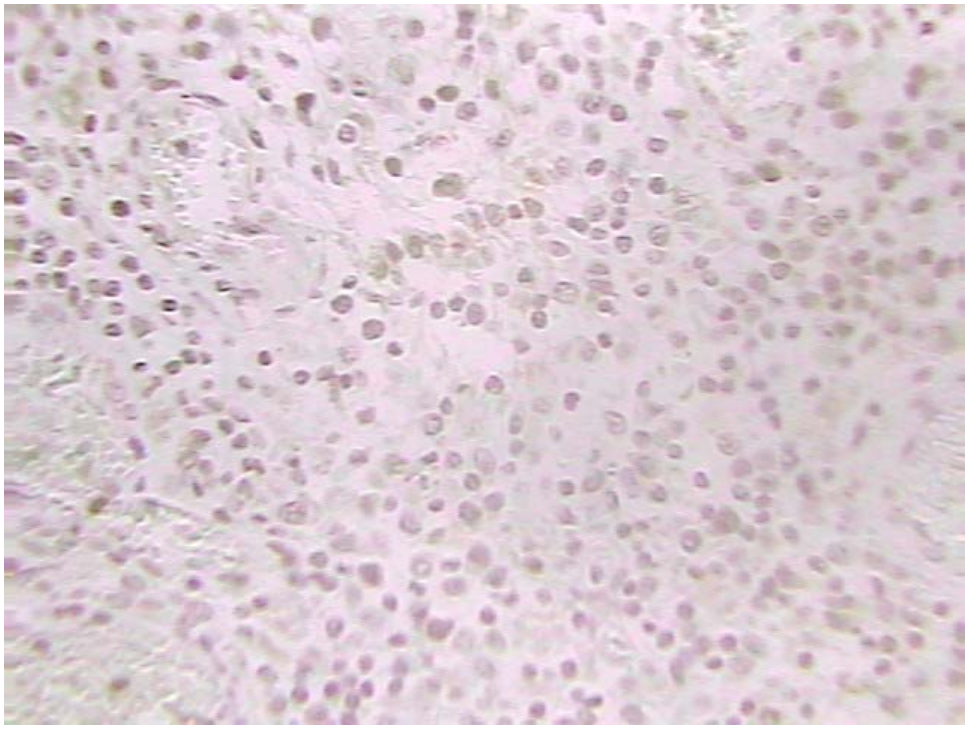


Figure 38. Patient 4 Anal Skin Normal Rat Serum 40x

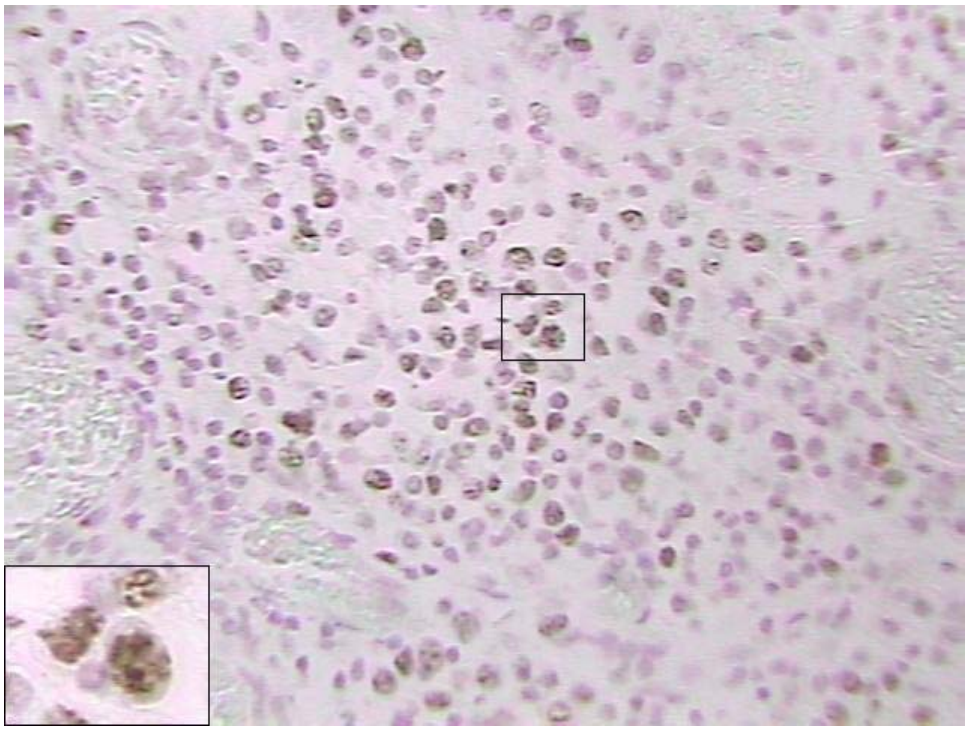


Figure 39. Patient 4 Anal Skin LANA-1 40x (Inset 100x)

Diffuse 2

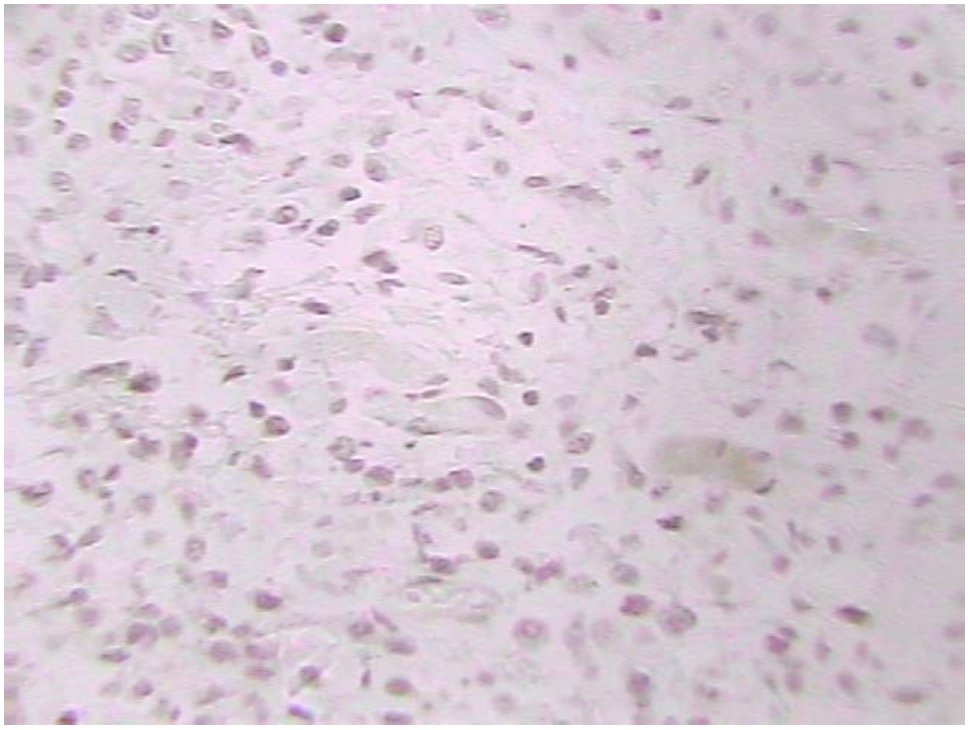


Figure 40. Patient 4 Anal Skin Normal Rabbit Serum 40x

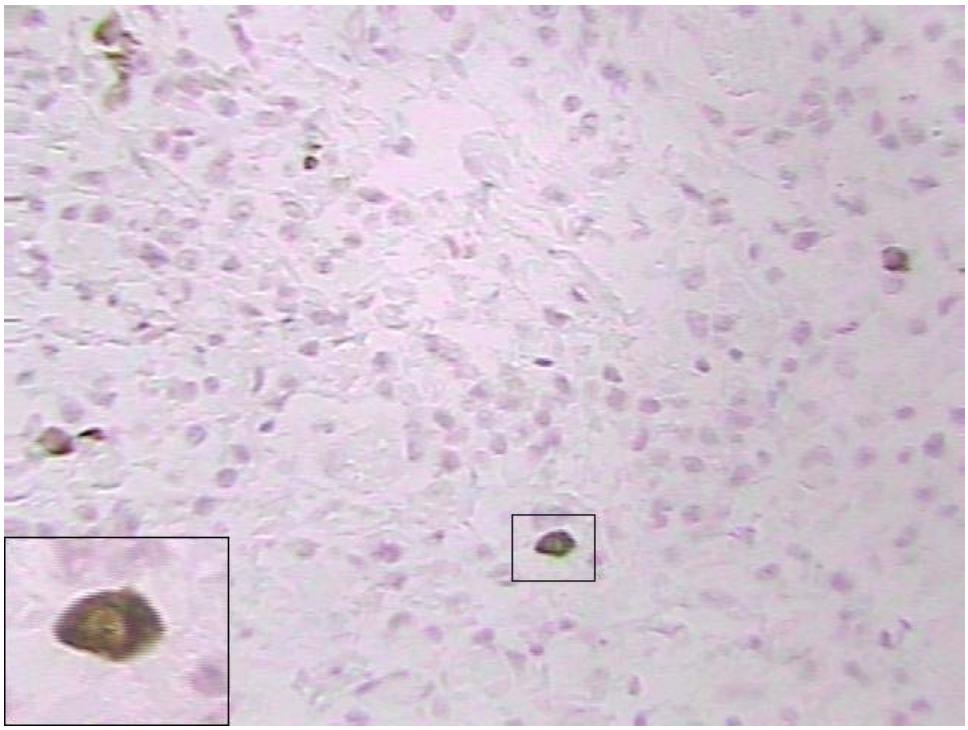


Figure 41. Patient 4 Anal Skin v-IL-6 40x (Inset 100x)

Diffuse 1

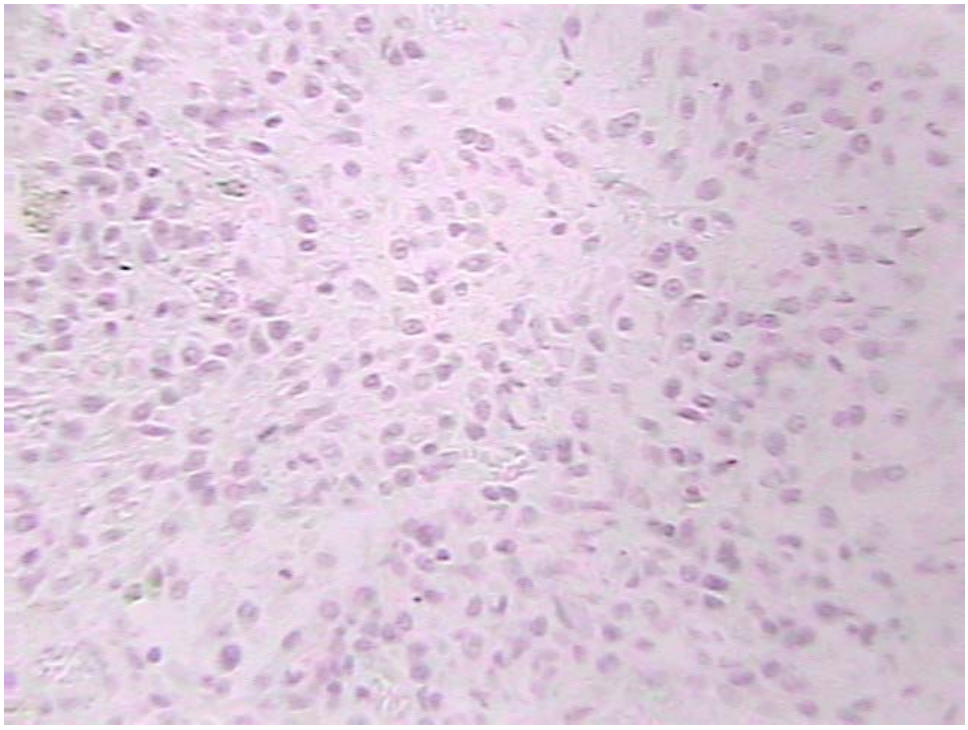


Figure 42. Patient 4 Colon Normal Rabbit Serum 40x

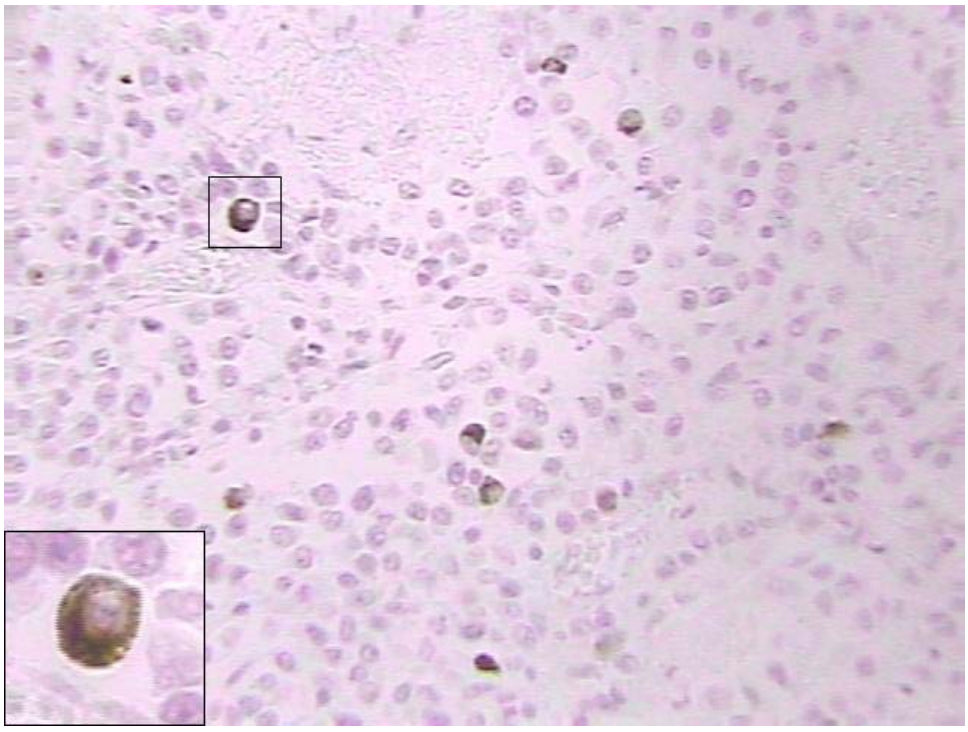


Figure 43. Patient 4 Colon v-IL-6 40x (Inset 100x)

Diffuse 1

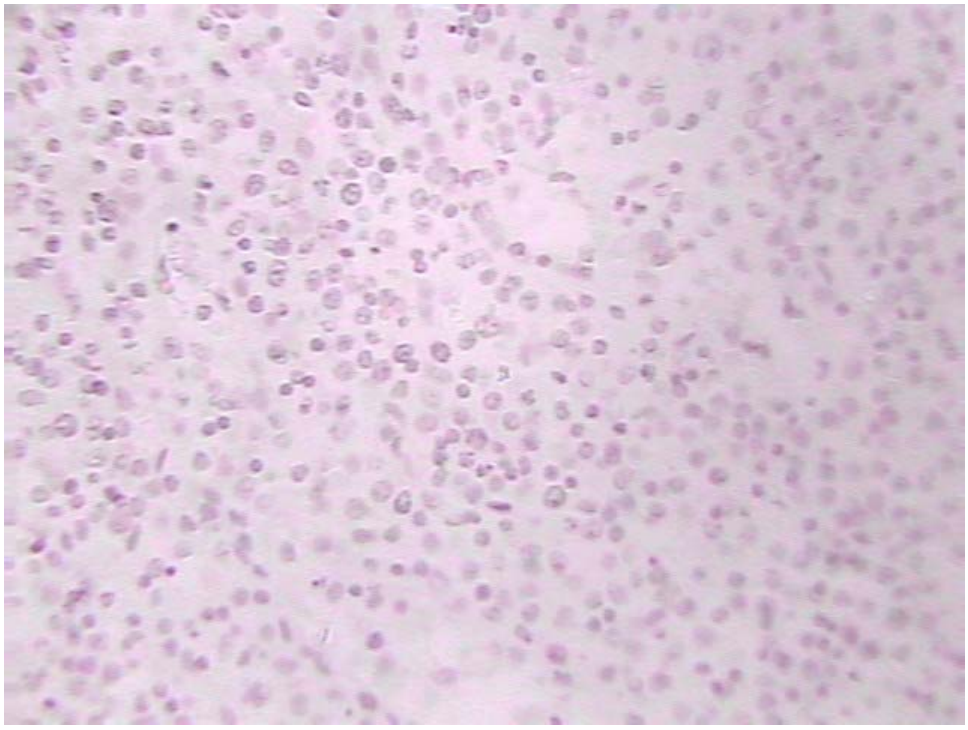


Figure 44. Patient 4 Right Lower Lobe Lung Normal Rabbit Serum 40x

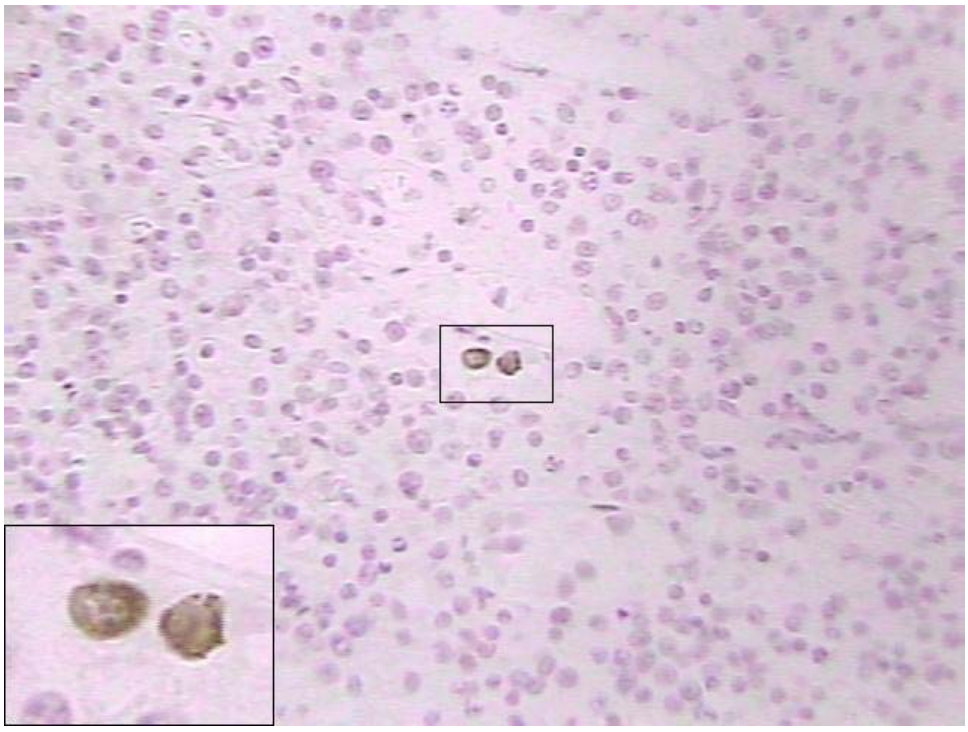


Figure 45. Patient 4 Right Lower Lobe Lung v-IL-6 40x (Inset 100x)

Sporadic

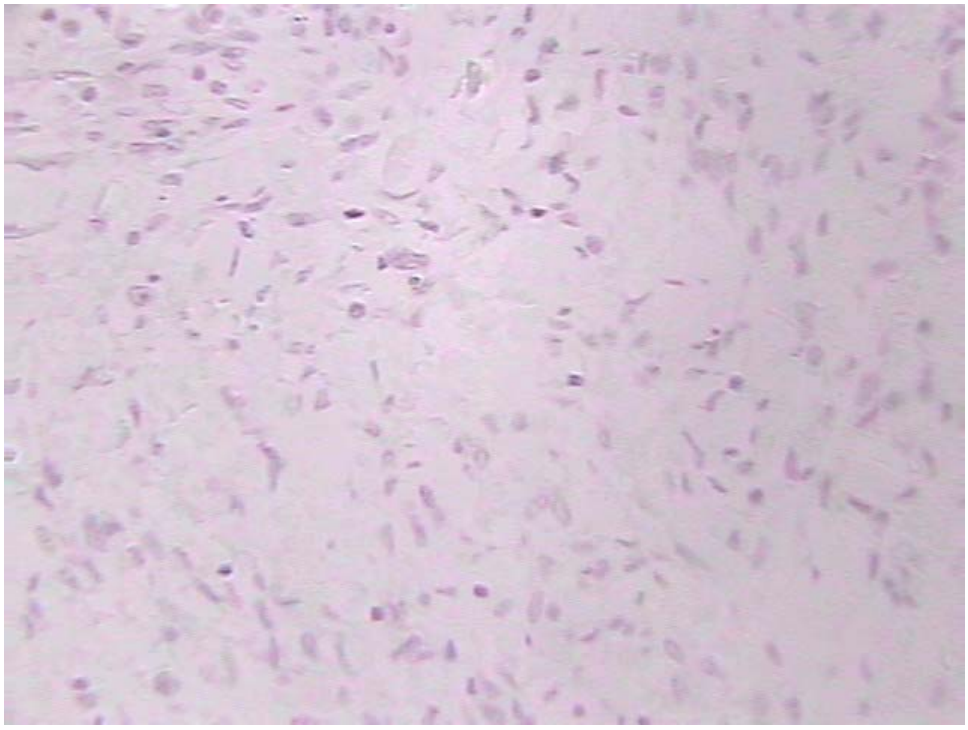


Figure 46. Patient 4 Foot Lesions Normal Rabbit Serum 40x

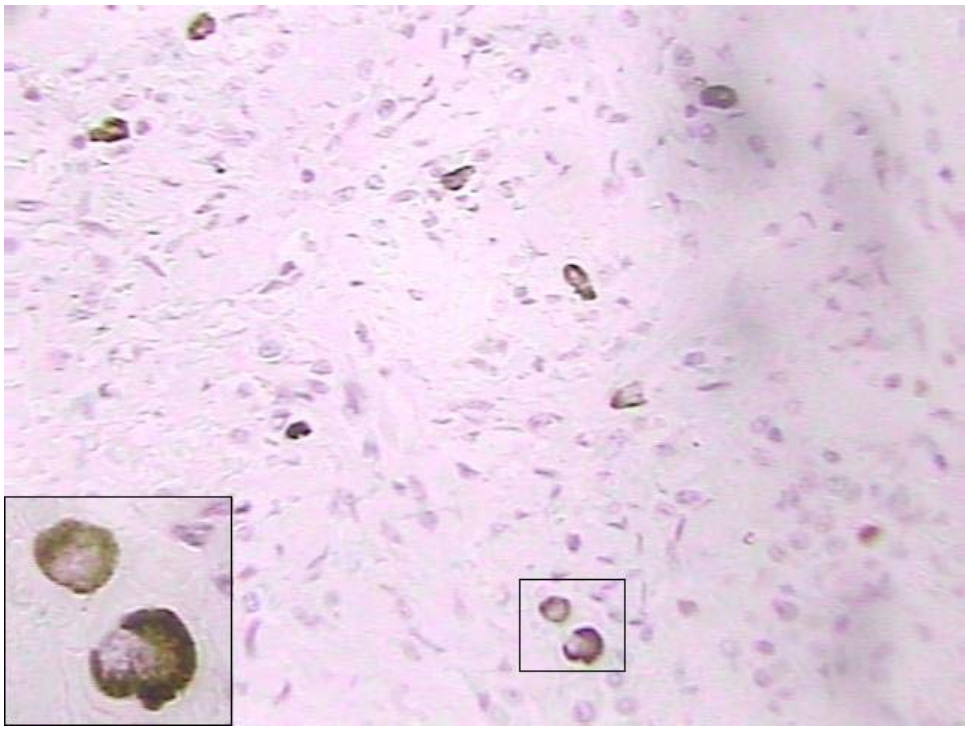


Figure 47. Patient 4 Foot Lesions v-IL-6 40x (Inset 100x)

Diffuse 1

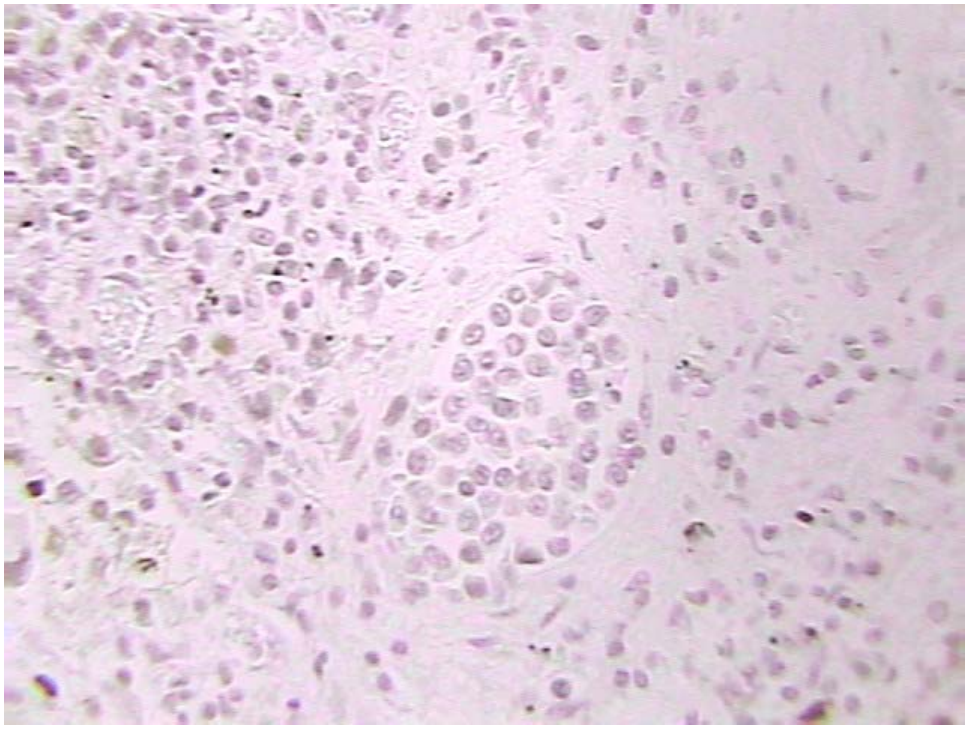


Figure 48. Patient 4 Right Lower Lobe Lung Normal Rat Serum 40x

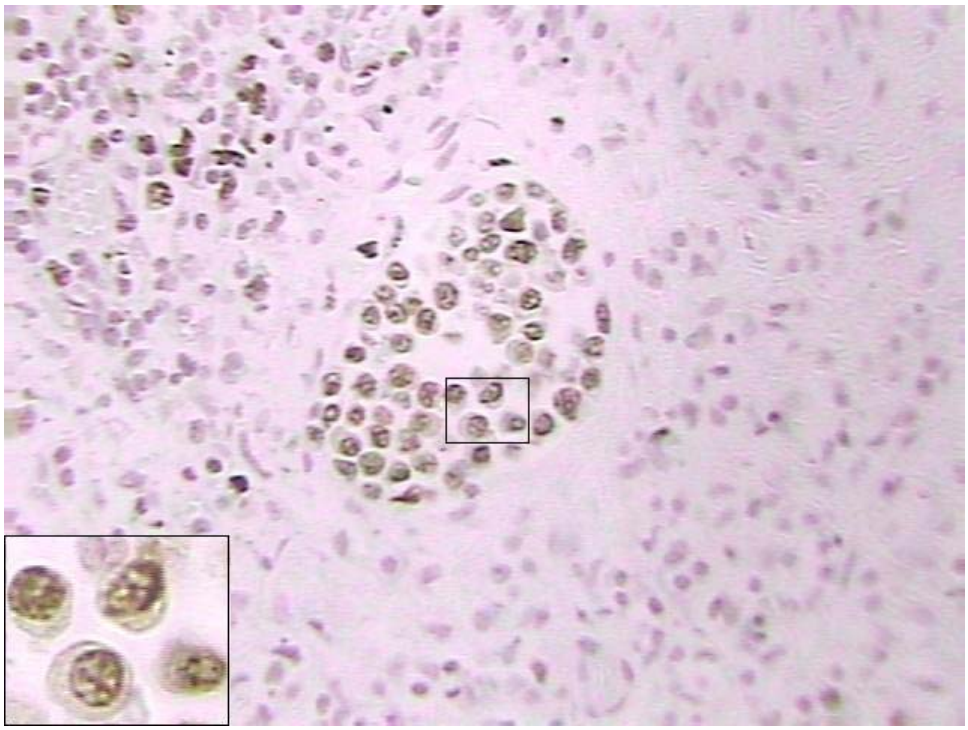


Figure 49. Patient 4 Right Lower Lobe Lung LANA-1 40x (Inset 100x)

Diffuse 2

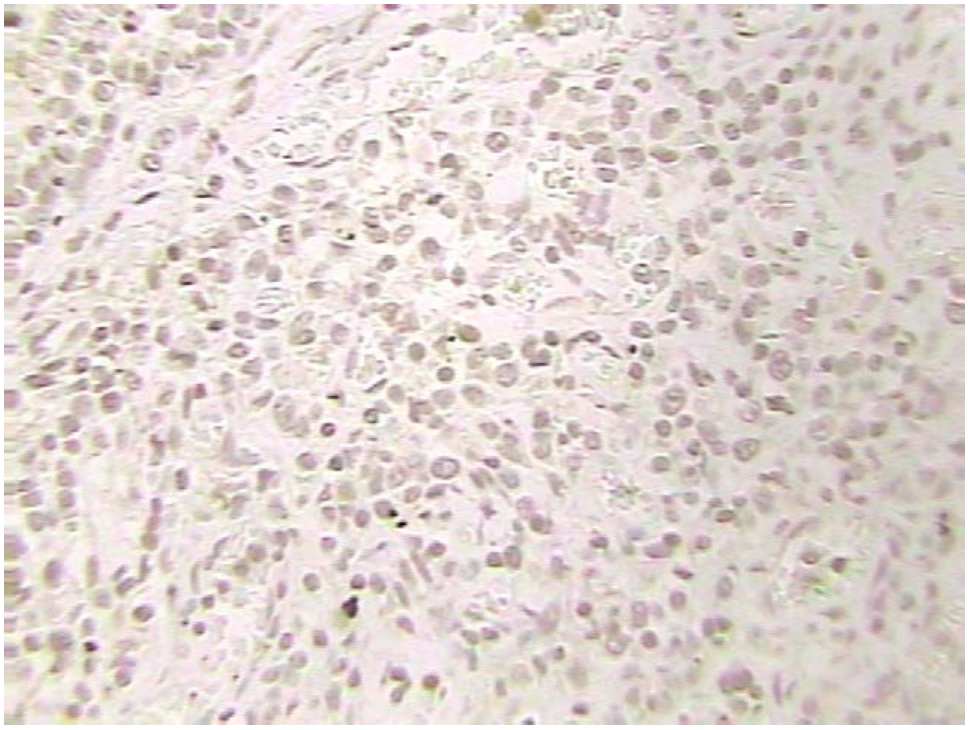


Figure 50. Patient 4 Stomach Lesions Normal Rat Serum 40x

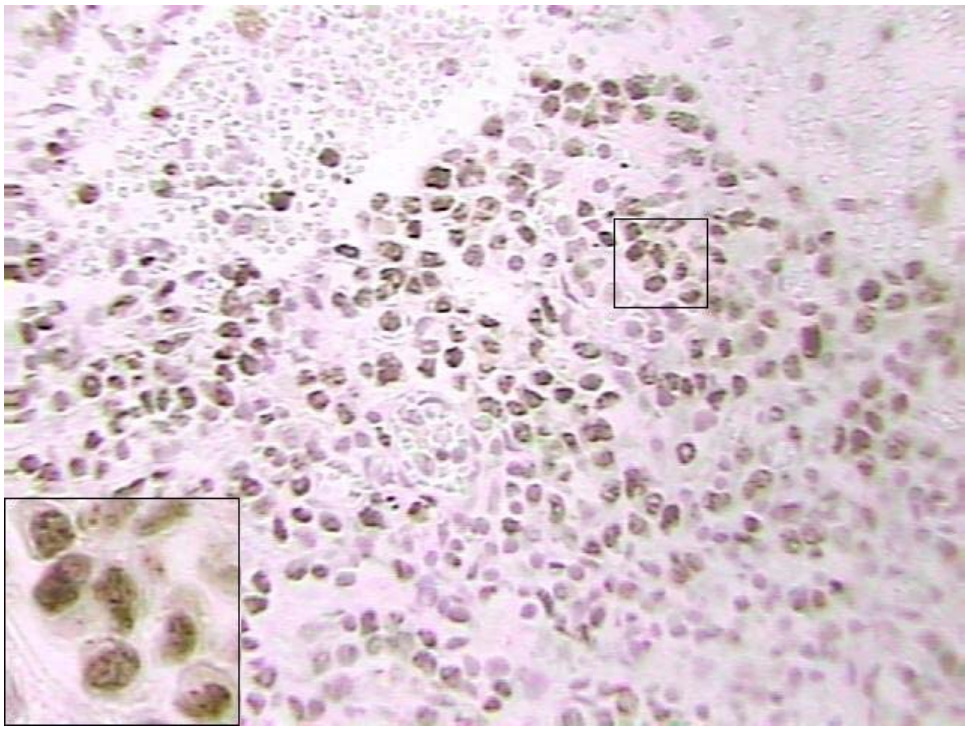


Figure 51. Patient 4 Stomach Lesions LANA-1 40x (Inset 100x)

Diffuse 2

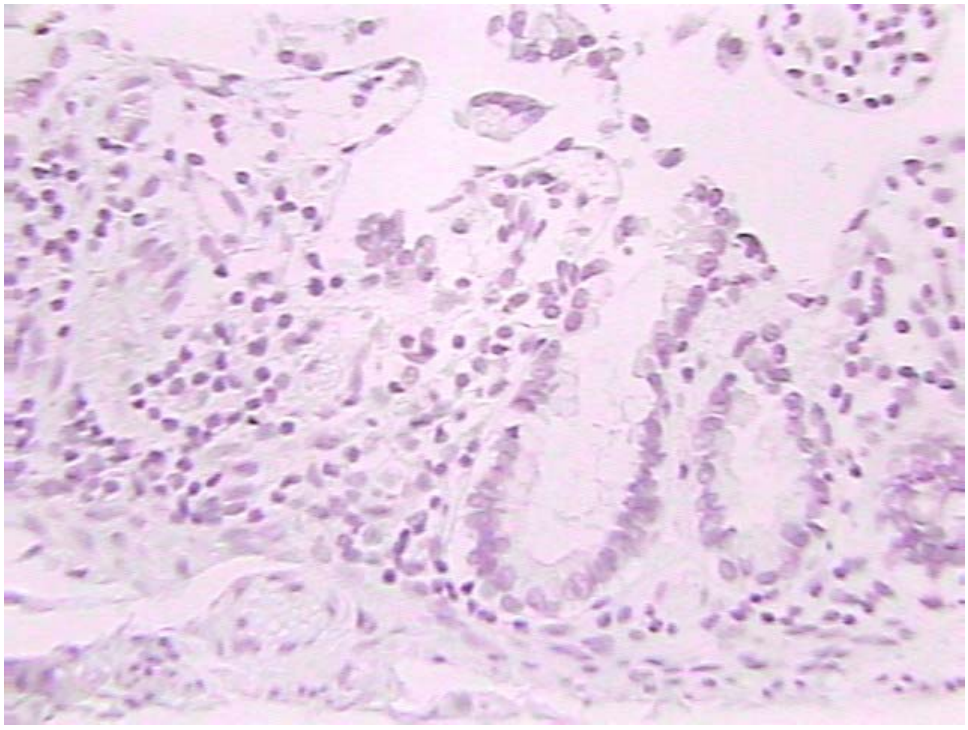


Figure 52. Patient 5 Ileum Normal Rabbit Serum 40x

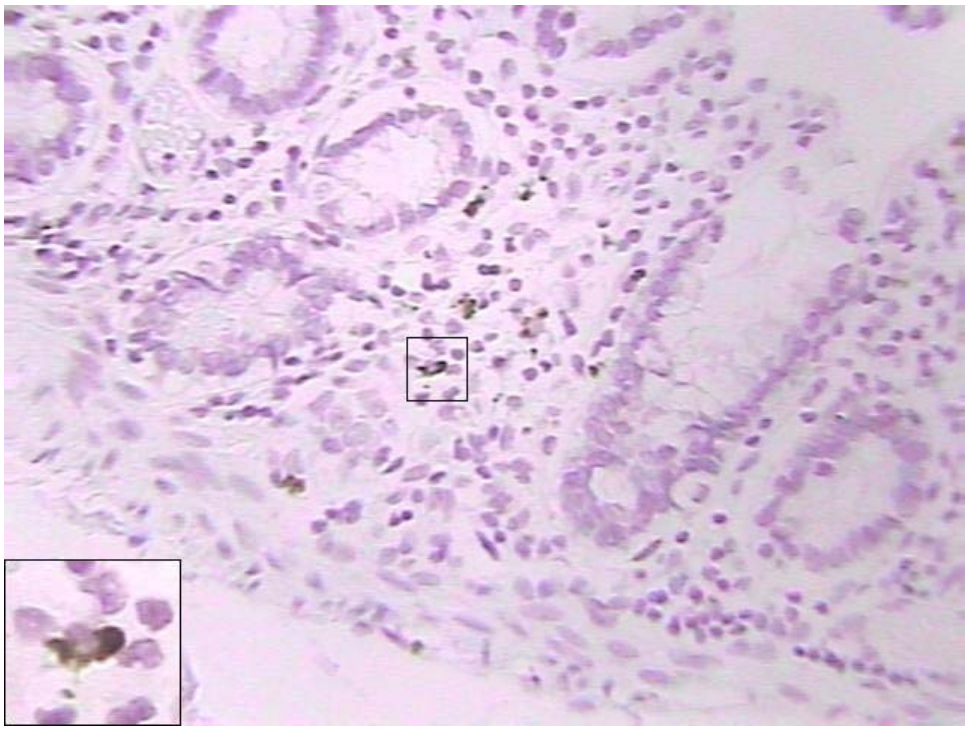


Figure 53. Patient 5 Ileum v-IL-6 40x (Inset 100x)

Diffuse 1

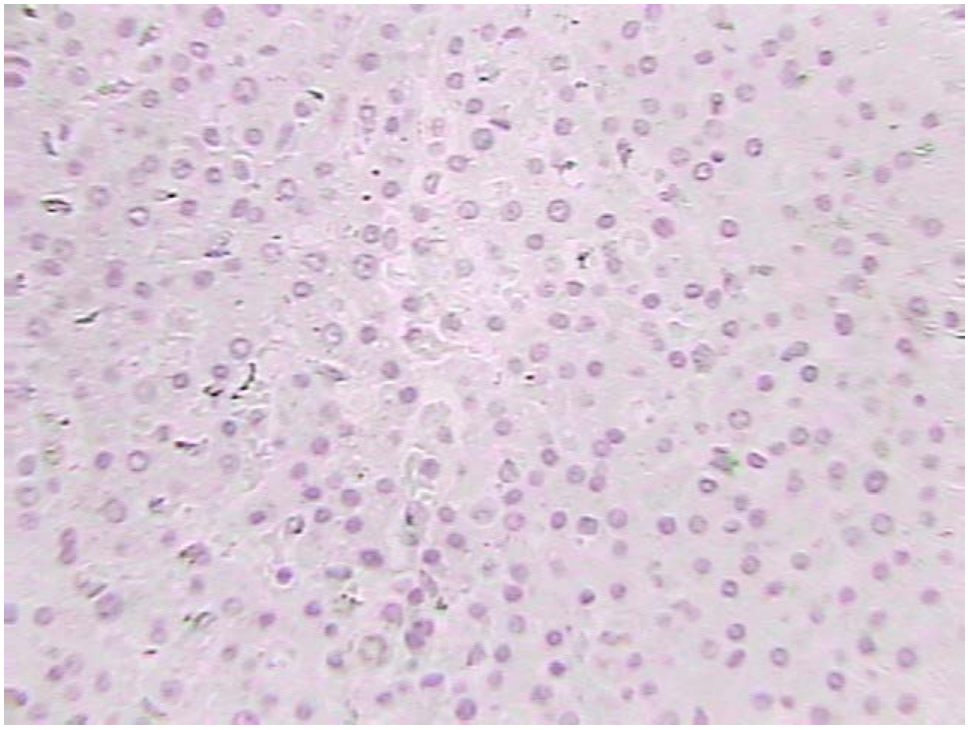


Figure 54. Patient 5 Liver Normal Rabbit Serum 40x

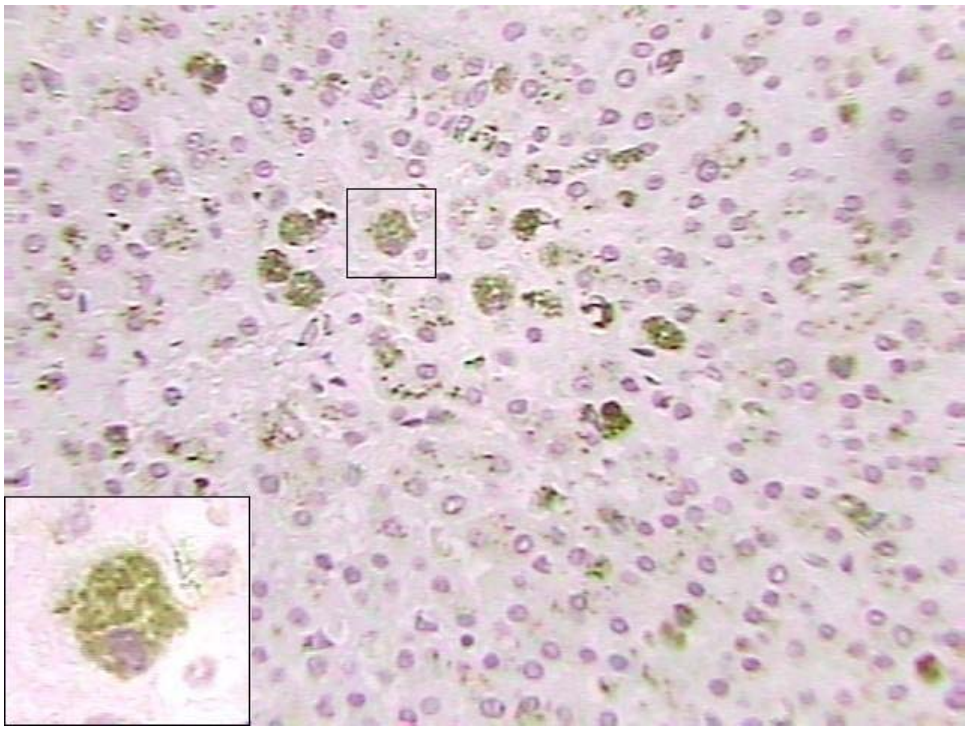


Figure 55. Patient 5 Liver v-IL-6 40x (Inset 100x)

Diffuse 1

4.2.4 IHC results by case

Tables 5-9 show which tissues of each patient tested positive and the level of staining as determined by semi-quantitation discussed earlier in this section. Table 4 is the color legend that will be used in the following tables.

Table 4. Semi-quantitation legend.

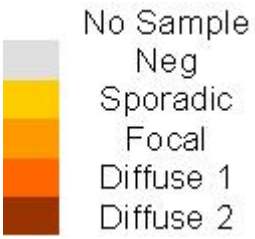


Table 5. Patient 1 IHC staining by tissue type and antigen

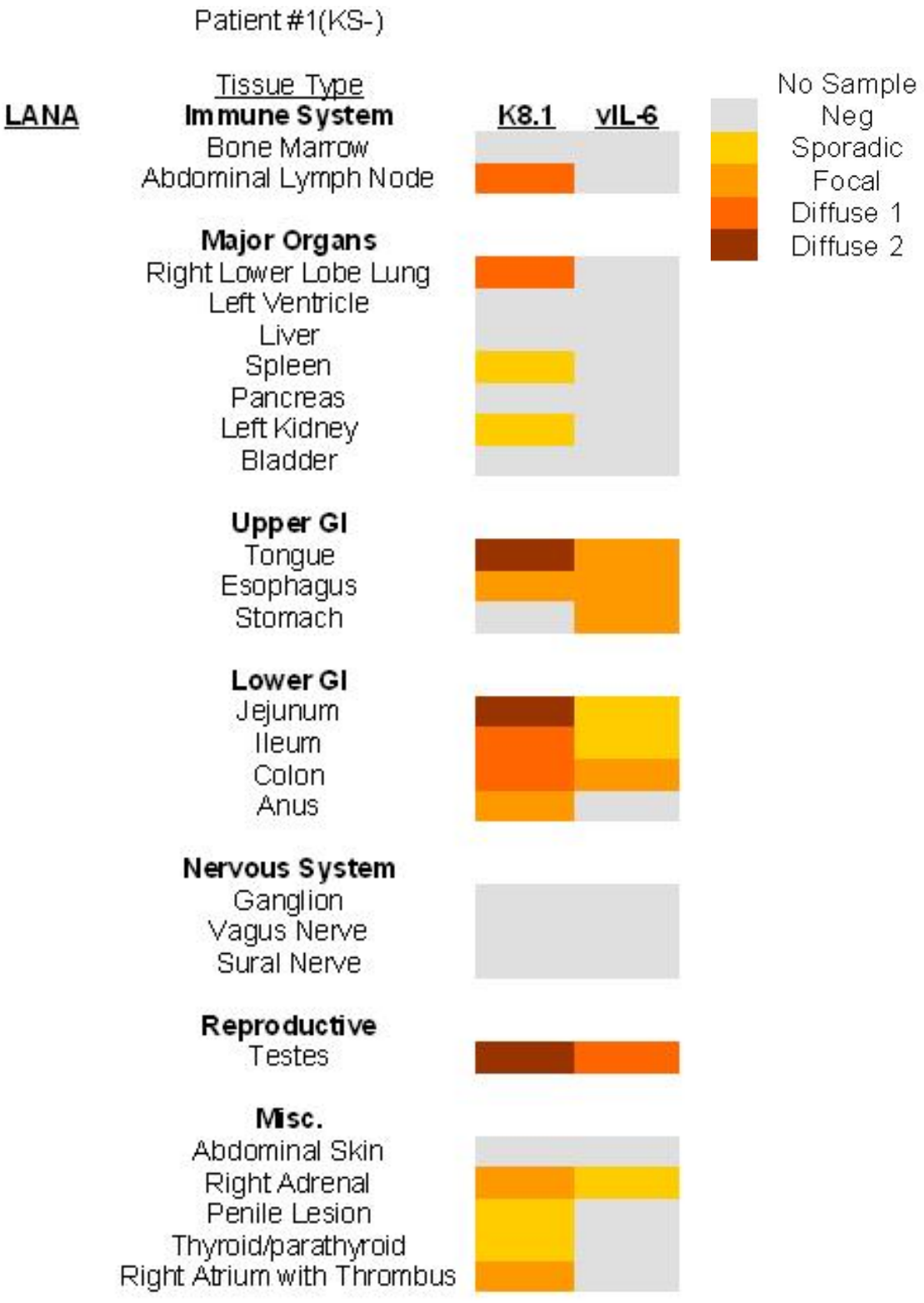


Table 6. Patient 2 IHC staining by tissue type and antigen

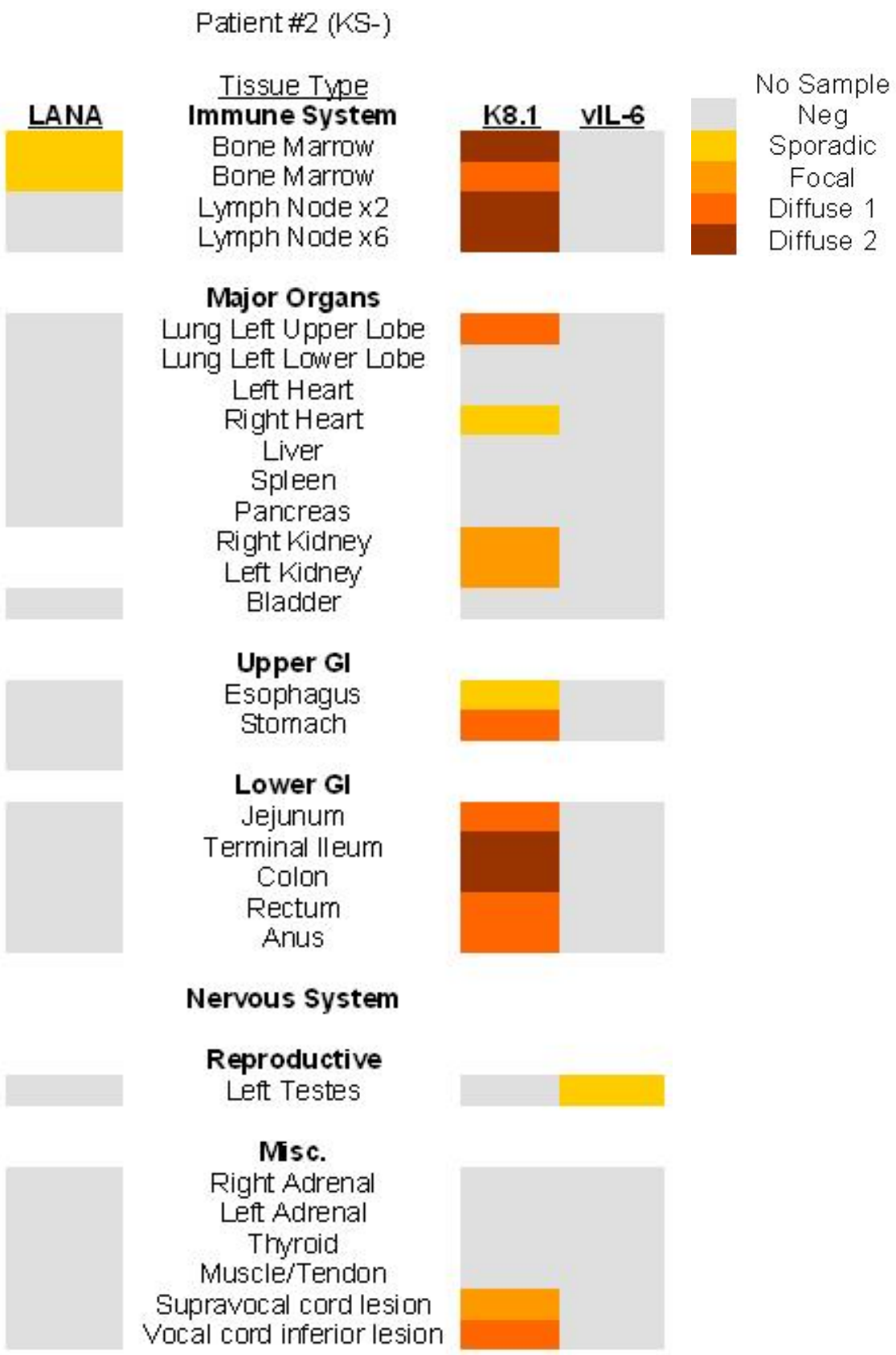


Table 7. Patient 3 IHC staining by tissue type and antigen

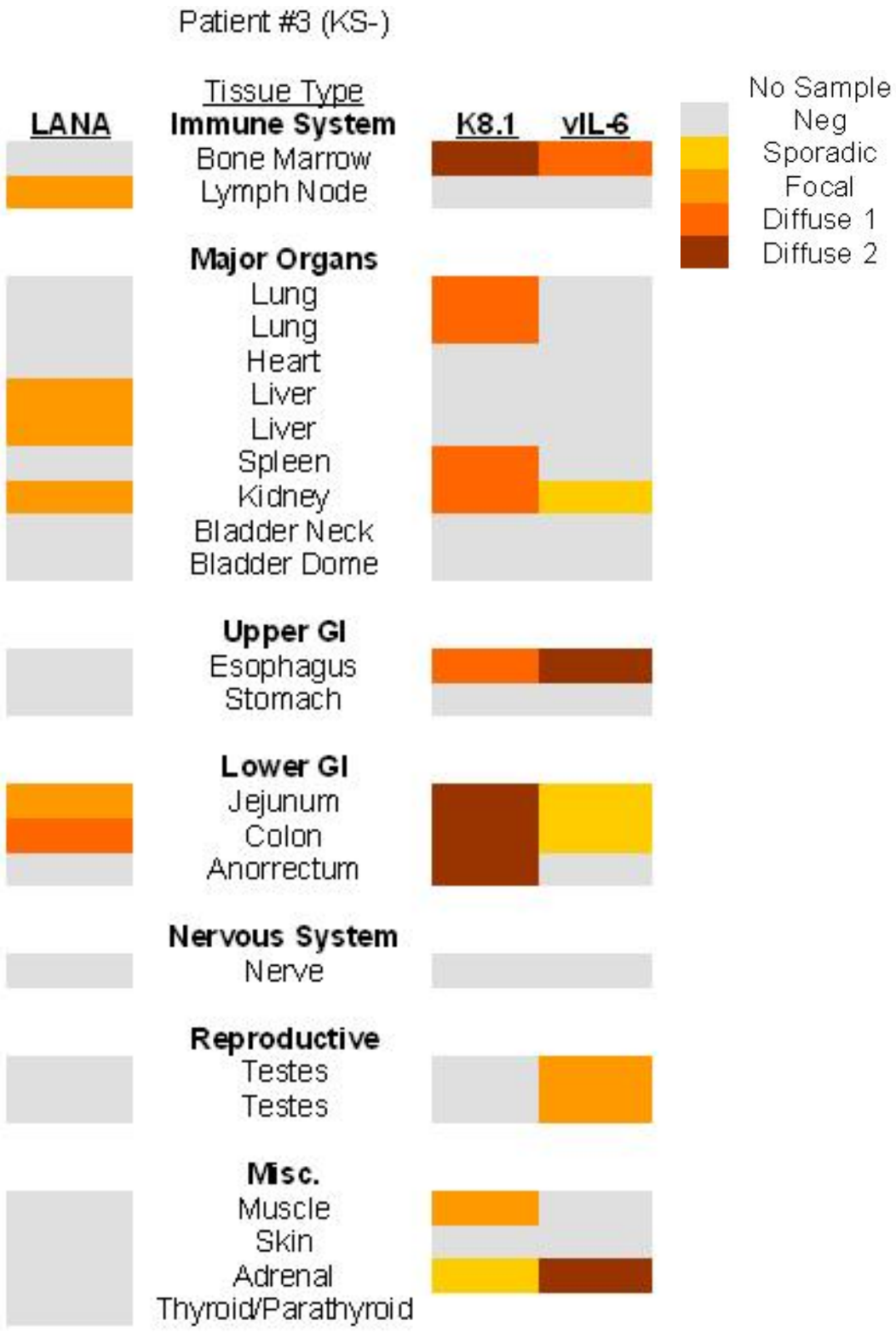


Table 8. Patient 4 IHC staining by tissue type and antigen

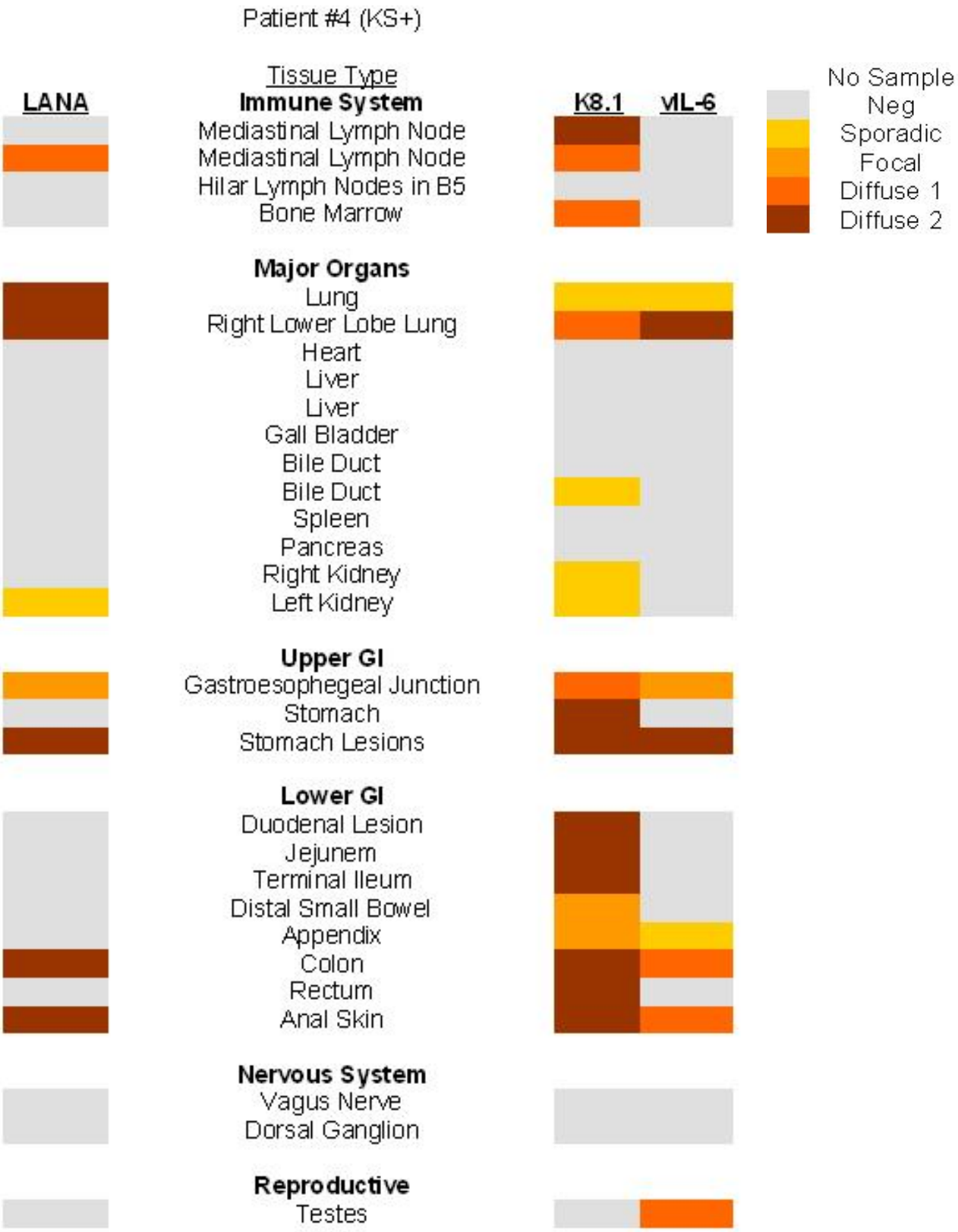
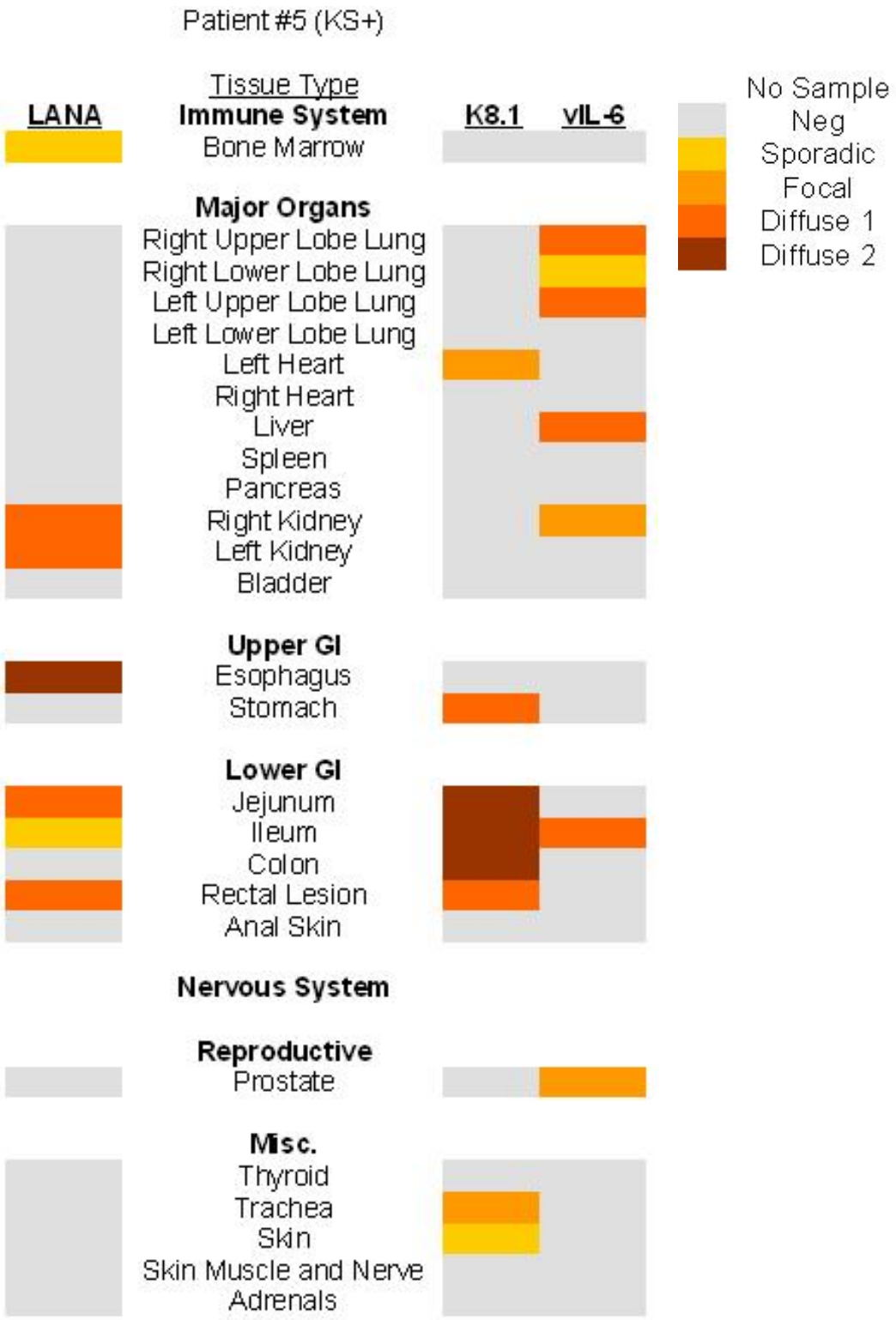


Table 8. (cont.)


Misc.
Right Adrenal
Left Adrenal
Muscle Tissue
Foot Lesions Consistant With Kaposi's
Thyroid

Table 9. Patient 5 IHC staining by tissue type and antigen



4.2.5 IHC results by organ system

Table 10. IHC staining of immune system tissue received from all patients

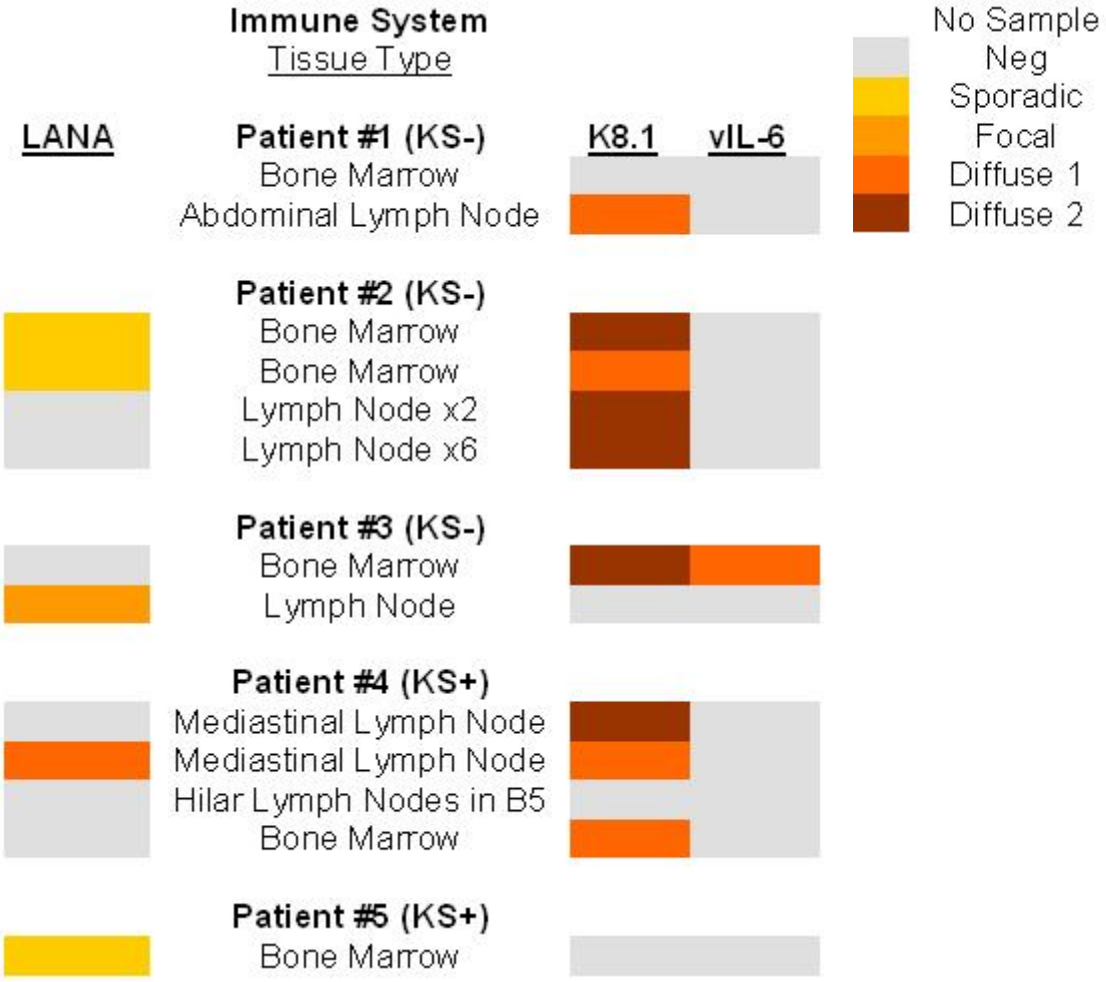


Table 11. IHC staining of upper GI system tissue received from all patients

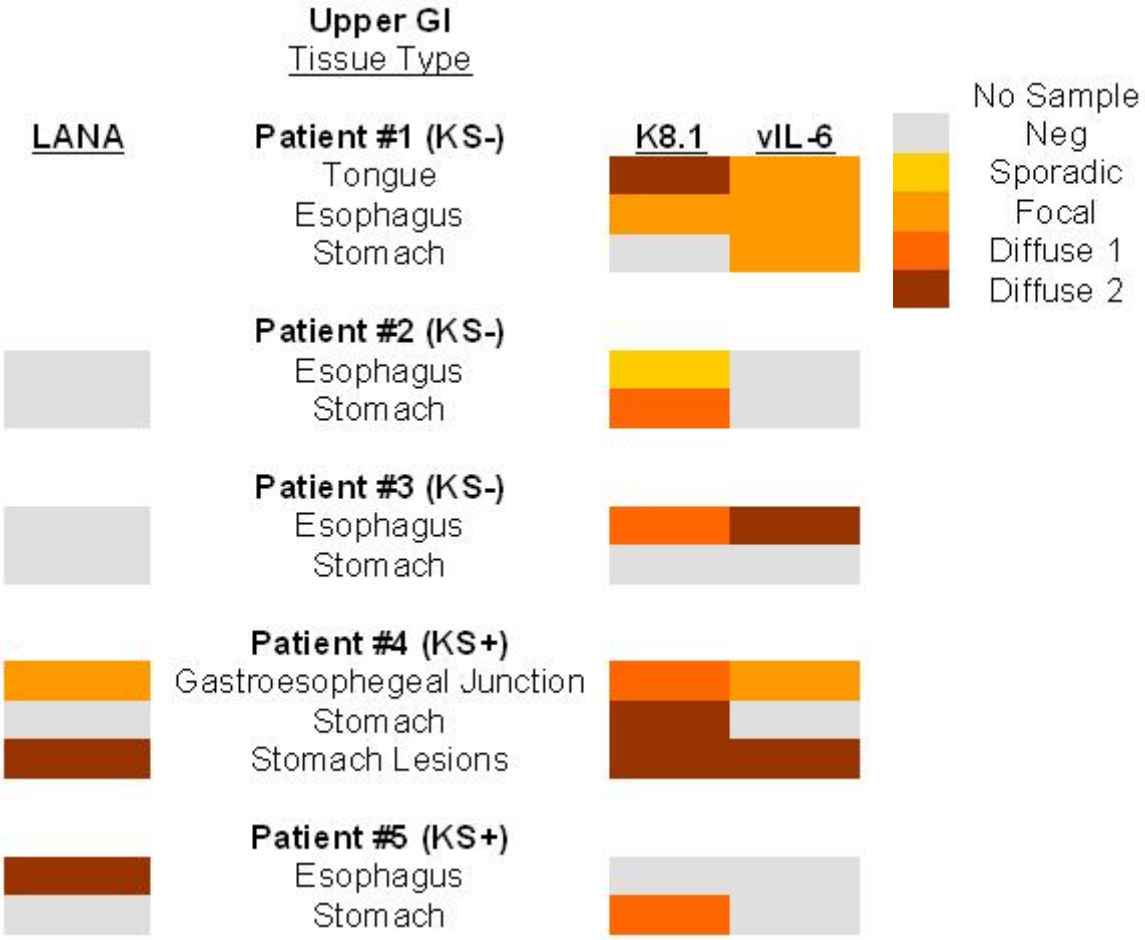


Table 12. IHC staining of lower GI system tissue received from all patients

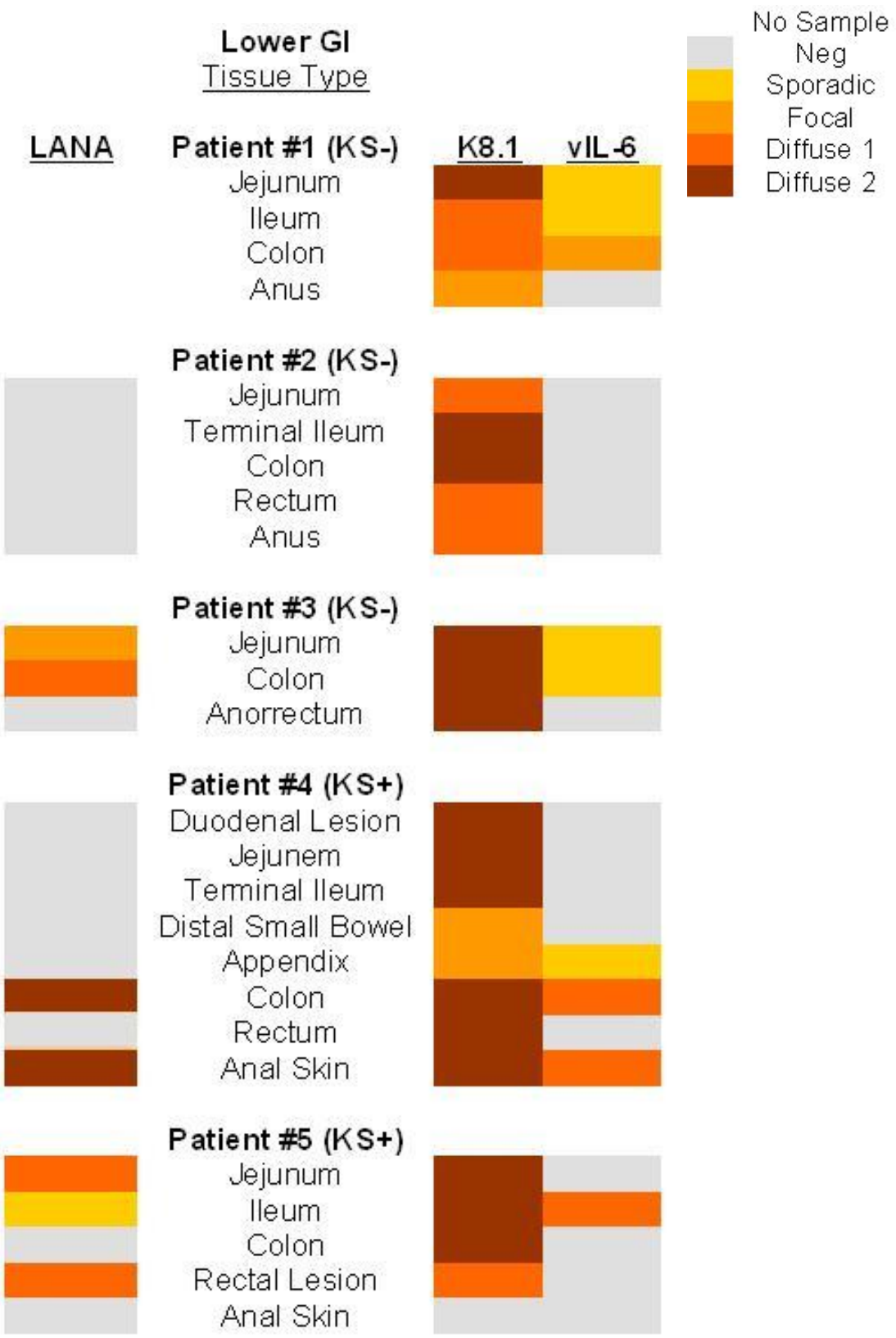


Table 13. IHC staining of the major organ tissue received from all patients

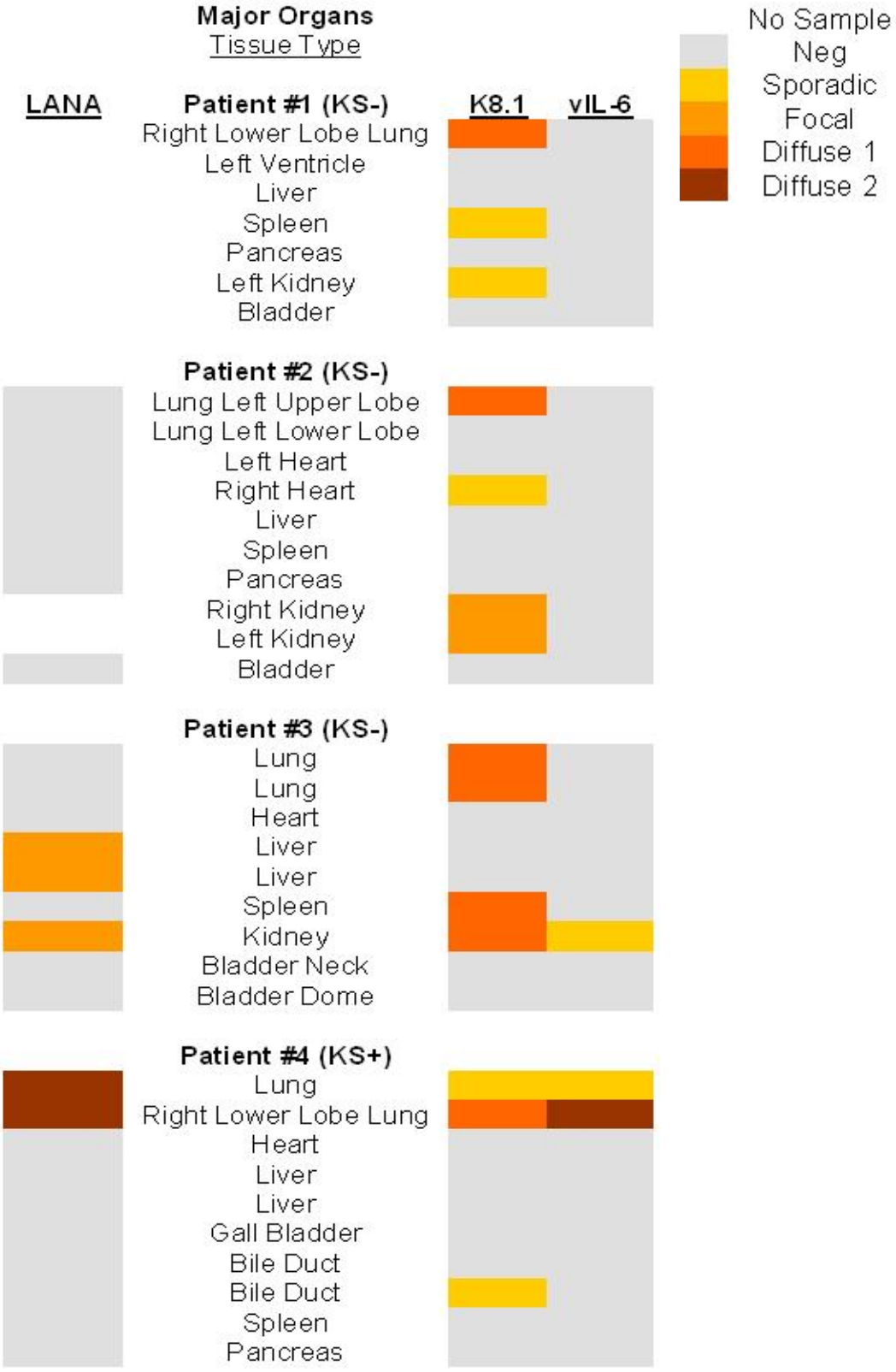


Table 13. (cont.)

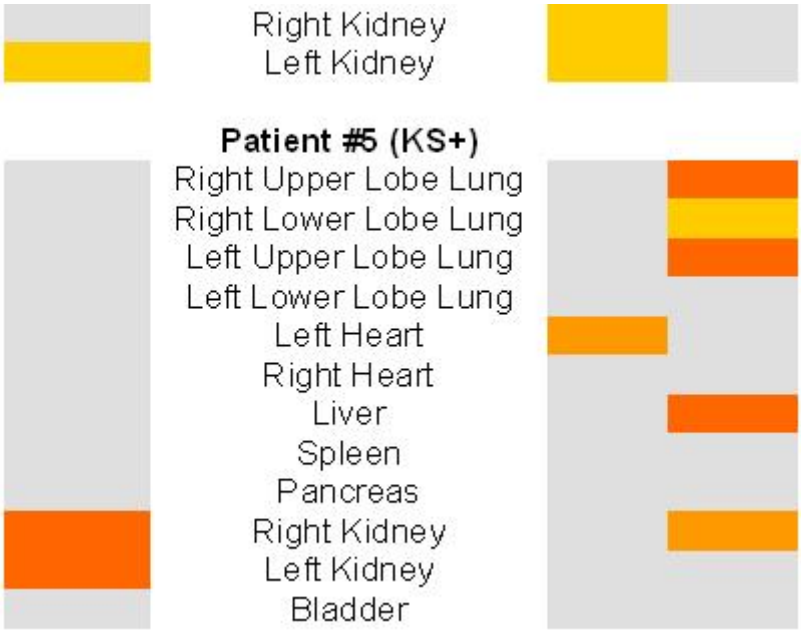


Table 14. IHC staining of the nervous system tissue received from all patients

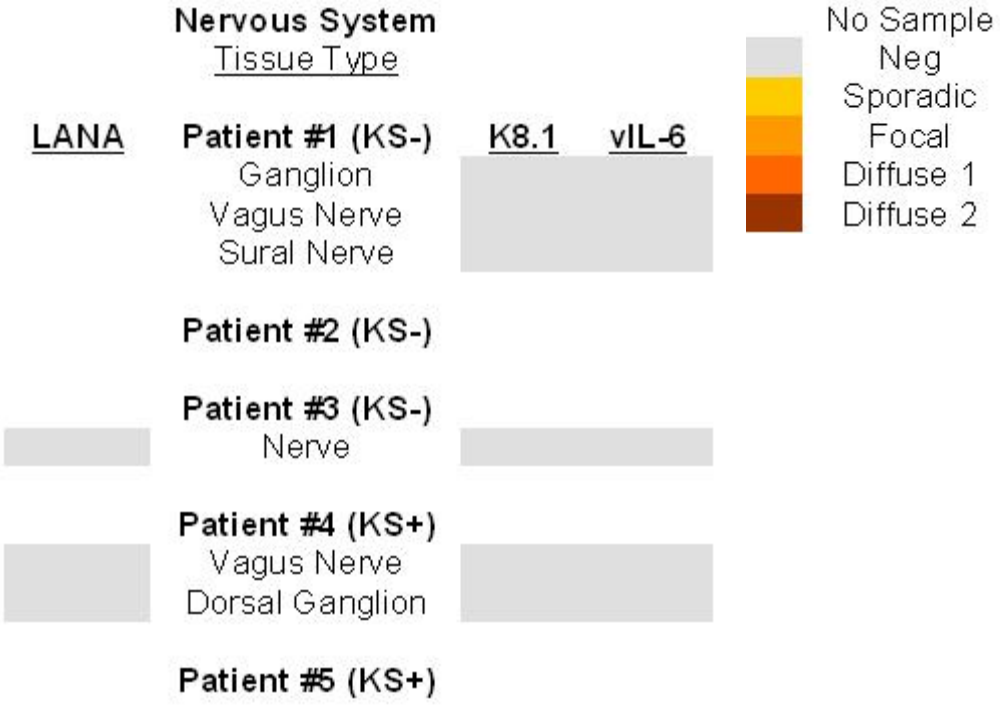


Table 15. IHC staining of the reproductive system tissue received from all patients

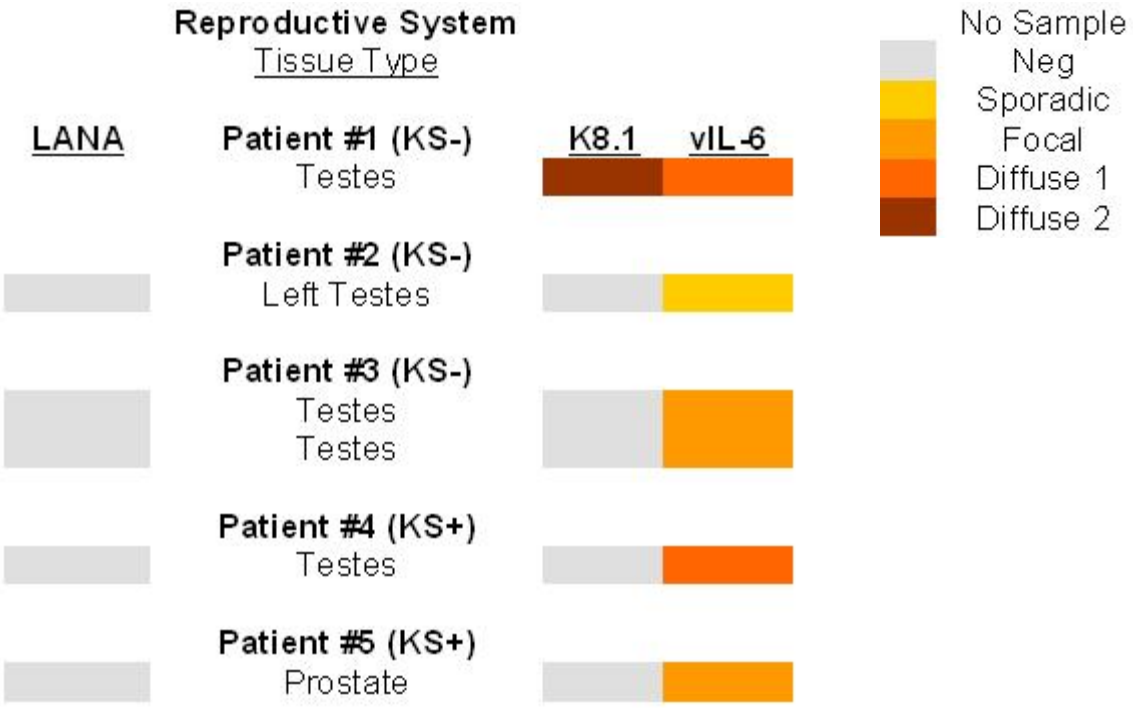
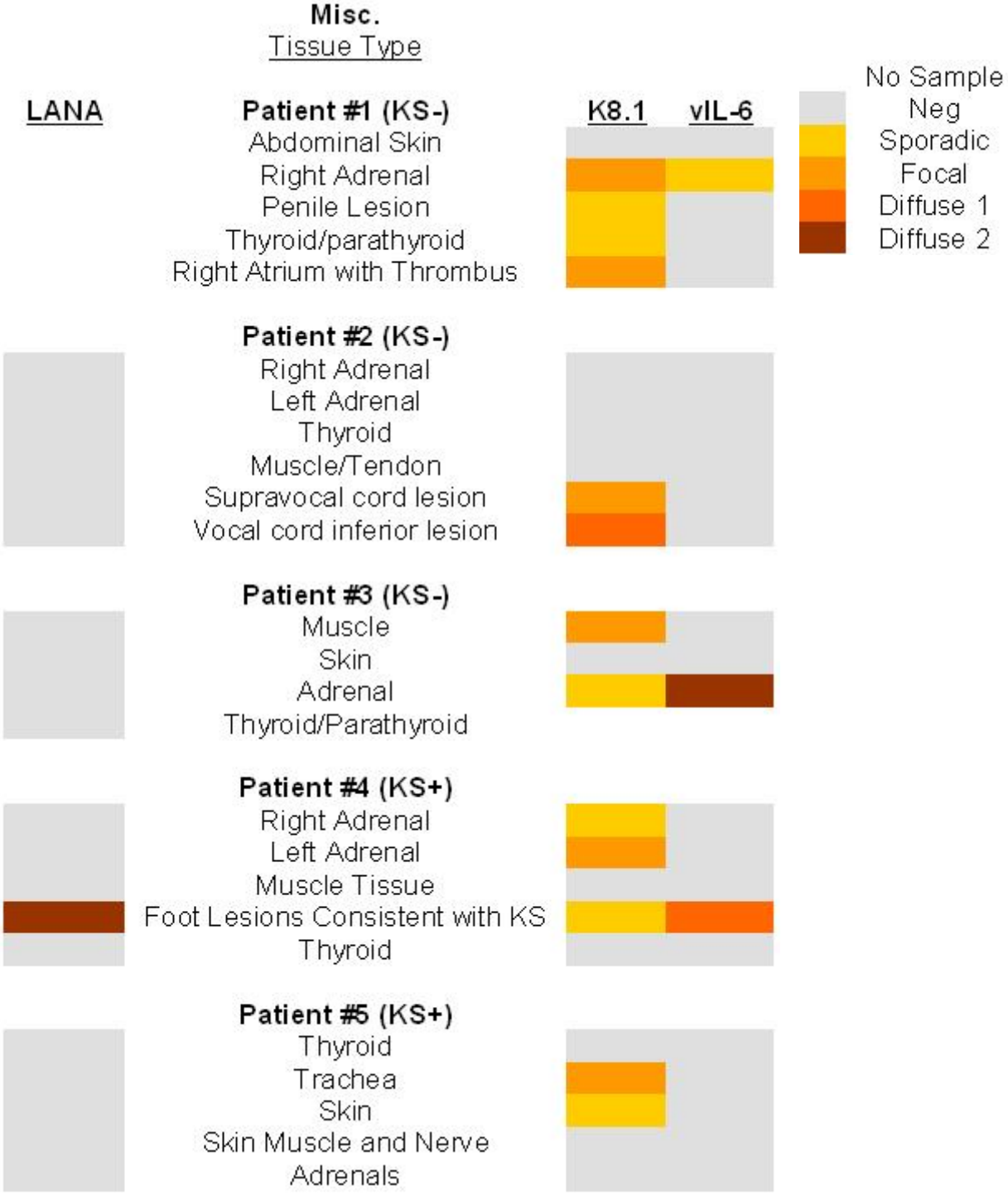


Table 16. IHC staining of the misc. tissue received from all patients



5.0 DISCUSSION

HHV-8 was first isolated from KS tissue in 1994 [1]. Since its discovery, it has been a priority to determine viral tropism *in vivo,* and thus far HHV-8 has been shown to naturally infect CD19+ B-cells [104], endothelium [105], monocytes [106], macrophages [107], dendritic cells [107], prostate glandular epithelium [108], and dorsal root sensory ganglion cells [109]. A comprehensive search for reservoirs of HHV-8 in the human body has not been attempted using IHC, and this study is the first of its kind. Since HHV-8 is the causative agent of KS, PEL, and some forms of MCD, as well as suspected in other diseases, i.e., sarcoidosis [110], multiple myeloma [111], pemphigus vulgaris and pemphigus foliaceus [112], Kikuchi disease [113] and germinotropic lymphoproliferative disorder [114], it is of great importance to identify the reservoirs of HHV-8 within the human body.

Like all other herpesviruses, HHV-8 has two phases of infection; lytic and latent phase. It has been shown *in vitro* however, that expression of certain lytic proteins does not mean full lytic reproduction and progeny virus [115]. In a study done by Krishnan, H.H. *et al.* it was shown that lytic gene expression of HHV-8 is initiated soon after initial infection. However, the initial lytic activation is not successful enough to proceed to a full-fledged lytic replication and progeny virus development. Expression of only a subset of lytic cycle genes (K8.1 included) occurred and subsided rapidly thereafter [115]. While K8.1 staining still indicates viral infection, it may

not necessarily represent a reservoir of latency. Therefore while K8.1, LANA-1, and v-IL-6 staining all represent reservoirs of infection, only LANA-1 staining represents a reservoir of latency.

In this study, the majority of staining was of the two lytic antigens K8.1 and v-IL-6. Lytic antigen staining was the most prevalent in the upper and lower GI sections. There were a number of other tissues that stained positive for HHV-8 lytic antigens including lung, kidney, tongue, esophagus, etc. These tissues are primarily tissues with high levels of immune surveillance and are part of the mucosa-associated lymphoid tissue system. Based on the morphology of the cells staining positive for K8.1, we suspect that they represent immune cells, most likely macrophages. In a separate study from our laboratory, we have demonstrated that macrophages, defined as CD68-positive cells, in the prostates of seropositive men expressed lytic antigens and therefore were infected with HHV-8 [116]. Preliminary data (not shown) from our lab shows that in some of these tissues (tongue, esophagus, colon, bone marrow, jejunum, and lung) the cells that are positive for K8.1 are also positive for CD68, a monocyte/macrophage marker. HHV-8 has also been shown by other labs to infect macrophages with detectable expression of K8.1 [107]. If these are indeed macrophages, there are a few probable explanations. The first explanation is that these are resident, non-inflammatory macrophages, i.e. alveolar macrophages, fixed bone marrow, macrophages, etc. that are getting infected and at the time of staining were expressing lytic proteins. A second possibility is that cells within these tissues are infected with HHV-8 and the CD68+K8.1+ cells are inflammatory macrophages which have responded to HHV-8 infection and subsequently become infected themselves. Another explanation, a combination of the previous explanations, would be that the resident,

non-inflammatory macrophages are becoming infected initially, and are attracting circulating monocytes, which respond to the infection and subsequently become infected. Without more immunostaining and individual cell identification it is difficult to know which of these scenarios is occurring. However, due to location of staining in the body, morphology, and previous studies showing HHV-8 infection of macrophages, I believe that the most likely explanation is an initial infection of resident macrophages which then signal circulating monocytes via chemokines. These monocytes upon becoming inflammatory macrophages, and interaction with infected resident macrophages, become infected as well.

In the first specific aim, my goal was to determine if the presence of HHV-8 infection will correspond to the known biology of the virus. Our hypothesis was that we expect to see the virus in areas of high immune surveillance and lymphoid tissue concentration such as organs of the mucosa-associated lymphoid tissue as well as, gut-associated lymphoid tissue. It is also important to note that the five subjects were all men who had sex with men, as this may play a role in how the virus has been transmitted. As mentioned previously, HHV-8 has been shown to be most consistently detected in saliva and less consistently in semen. Table 11 and 12 (upper GI and lower GI) show a high level of HHV-8 infection. Expression of both lytic antigens was very prevalent among all subjects, while latent antigen expression was more confined to subjects four and five who are both KS positive. Detection of viral infection was found from the tongue and proceeding through the trachea and esophagus leading to the lower GI tract. These findings match well with the know ability for HHV-8 to be detected and transmitted in saliva. In the lower GI, (Table 12) viral expression was very high, especially K8.1 which was detected in every tissue sample, from every patient with the exception of an anal skin sample from patient

five which was negative for v-IL-6 and LANA-1 as well. Not only was K8.1 detected in these tissues, but the majority of the samples (21/25) scored in the top two (diffuse 1 or 2) staining categories. v-IL-6 was detected in nine of the lower GI samples which fits well with the overall trend that v-IL-6 was seen less often than K8.1. Of the thirty-three v-IL-6 positive samples, twenty-three were also positive for K8.1. The major disparity of v-IL-6+, K8.1- samples was seen in the reproductive tissue where, of the five testes samples received, all five were v-IL-6 positive, only one was K8.1 positive, and the lone prostate sample was vIL-6+, K8.1-.

Another point of interest is the complete lack of HHV-8 antigen detected in the nervous system tissue (Table 14). Although HHV-8 has been shown to infect dorsal root sensory ganglion cells, this study was unable to detect any viral antigen in any of the samples.

Among the major organs, detection of HHV-8 was variable (Table 13). Antigens were detected in bone marrow, lung, kidney, spleen, and heart tissue at varying degrees as well as in three of the five adrenal gland tissue samples. From a public health standpoint, this is important to note when considering transplantation and the potential for iatrogenic infection of HHV-8 and post-transplant seroconversion. HHV-8 seroconversion has been documented in heart [117], kidney [118], liver [118, 119], and bone marrow transplant recipients [120]. No documentation of seroconversion in lung transplants was found, nor in the spleen, most probably due to rarity of spleen transplants. With the potential for development of iatrogenic KS, transplant rejection, or even death, much has been made about potentially screening tissue donors for HHV-8 [121], or prophylactically treating with antiviral drugs before solid organ transplantation [122]. Our results from this study show that we did indeed find viral protein staining in tissues of high

immune surveillance and lymphoid tissue concentration, such as the lungs, gut-associated lymphoid tissue, and esophagus. Therefore I believe that our results do correspond with the known biology of HHV-8 as well as provide new information about reservoirs of HHV-8 infection and latency in the body.

In the second specific aim, it was our goal to determine what, if any, effect the presence of Kaposi's sarcoma had on the pattern and location of viral expression. Of the five patients, two were KS positive. In the reproductive and nervous system tissue there was no difference in detection. There was no positive nervous system tissue samples (Table 13) and the reproductive tissue staining was very similar in every patient regardless of KS status (Table 15). Looking at the results from the major organ tissue (Table 13), there doesn't appear to be any great differences in viral antigen staining. In the two patients with KS, lung, kidney, heart, liver and bile duct all were found to be positive for at least one viral antigen. It was similar in the KS negative patients as HHV-8 antigen was detected in lung, kidney, heart, liver, and spleen. The levels of positive staining were also quite similar between the two groups. Lower and upper GI did show differences in LANA-1 expression between the two groups, as LANA-1 was expressed more often in the KS positive patients. However, the lytic antigen expression between KS positive and KS negative patients for the upper and lower GI did not differ. Based on these results, I feel that there was no major difference in prevalence of viral expression between KS+ and KS- subjects.

Of special note in this thesis is patient four. Upon review of the autopsy report, patient four was found to have had a large cell lymphoproliferative disorder known as intravascular

lymphomatosis, located in the bilateral lower lungs, liver, small and large bowel, and anus. Interestingly, LANA-1 staining of these tissues appeared to be associated with the cancer in the lungs, small and large bowel, and anus (no LANA-1 staining was observed in the liver of patient four) (Table 8, Figure 39, 49). However, staining of a different block of liver tissue from the same patient, analyzed under a different study in our laboratory did find substantial staining in the lymphoma cells contained in the liver. This is the first reported instance of HHV-8 being associated with intravascular lymphomatosis.

5.1 Public health significance

The use of IHC to detect reservoirs of HHV-8 infection and latency in the human body has not been attempted, and this is the first study of its kind. This study is significant because we now have a more comprehensive idea of the reservoirs of HHV-8 infection and latency in HIV-seropositive individuals. Hopefully this study will lead to further studies with normal, immunocompetent individuals, and a better screening process for organ and tissue transplant.

6.0 FUTURE DIRECTIONS

In the future, it will be important to identify specific cell types within the tissues that are HHV-8 positive. It has been difficult to identify cell type by merely histological means. Therefore, in the future, it will be necessary to use further immunostaining methods to elucidate the specific cell types.

Another aspect of this project that I feel should be considered is the potential that HIV and HHV-8 are interacting and potentially co-localizing in the gut. Again, this will require more immunostaining for viral antigens of both viruses. Studies have shown that HIV activity in the gut is quite high. It has also been shown that HIV can evade treatment by "hiding" in the gut-associated lymphoid tissue, and there is massive killing of immune cells that occurs in the gut with HIV infection. There is a large amount of lymphoid tissue in the gut and as this study shows, there appears to be a trend of high HHV-8 lytic activity in the gut. Therefore it seems a reasonable assumption that these two viruses may be interacting within the gut by way of their respective transactivator proteins (Tat, and ORF50) to induce viral protein production or viral replication.

Finally, a similar study to this one should be done in healthy, HIV-seronegative individuals, preferably both men and women, as the severe immunocompromised state of these five patients

may have allowed for viral dissemination different from that seen in healthy, HIV-seronegative individuals.

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