الجامعة الأردنيـة
كلية الدراسات العليا

نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير والدكتوراة

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Effect of viral respiratory infections by Influenza A virus, Parainfluenza viruses and Adenoviruses on immuno-inflammatory gene expression/inhibition of human white blood cells

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EFFECT OF VIRAL RESPIRATORY INFECTIONS BY INFLUENZA A VIRUS, PARAINFLUENZA VIRUSES AND ADENOVIRUSES ON IMMUNO-INFLAMMATORY GENE EXPRESSION / INHIBITION OF HUMAN WHITE BLOOD CELLS

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COMMITTEE DECISION

This Thesis/Dissertation (Effect of Viral Respiratory Infections: Influenza Virus, Parainfluenza Virus and Adenovirus on Immunological Gene expression / Inhibition of Human White Blood Cells) was successfully Defended and Approved on 15-7-2010

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DEDICATION

To the man who taught me the paths of life, path by path

My father, may Allah bless him

To the woman who instill in me the essence of passion, step by step

My Mother, may Allah bless her

To the angels who fed me the honey of happiness, drop by drop

My wife and children

To the persons who taught me the letters of knowledge, letter by letter

My teachers

I present to them my humble efforts……..Abdulrhem

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LIST OF ABBREVIATIONS

ACTB	Actin, beta
AdV	Adenovirus
APCs	Antigen presenting cells
AP1	Activating protein-1
ASMC	Airway smooth muscle cells
CCL1	CC chemokines ligant-1
CCR1	Chemokine receptor-1
cDNA	Complementary DNA
C5	Complement-5
CMC	Conserved microbial components
CMV	Cytomegalovirus
COPD	Cronic obstruction pulmonary disease
CSF	Cerebro-spinal fluid
CTL	Cytotoxic T lymphocytes
C_t	Threshold cycle
CXCL-1	Chemokine-ligant-1
dATP	Deoxy-nucleotide adenine tri-phosphate
DCs	Dendretic cells
dNTP	Deoxy-nucleotide tri-phosphate
dsDNA	Double stranded DNA
ELISA	Enzyme-linked immunosorbent assay
Erk	Extracellular-signal protein kinase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDC	Genomic DNA Control
GM-CSF	Granulocyte monocyte colony-stimulating factor
HIV	Human immunodeffeciency virus
HGDC	Human genomic DNA contamination
HKG	House keeping genes
HPIV	Human parainfluenza virus
HPRT1	Hypoxathine phosphoribosyltransferase 1
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule-1
IFNA2	Interferon alpha-2
IFNAR	IFN-α receptor
IL1A	Interleukin-1 alpha
IL1B	Interleukin-1 beta
IMPs	Inhibitors of metalloproteases
IP-10	Interferon induced protein-10
IRF 3	Interferon regulatory factor 3
IRF 7	Interferon regulatory factor 4
ISG	Interferon-stimulated gene
ITR	Inverted terminal repeat
IAV	Influenza A virus
Jak	Janus kinases
KIRs	Killer inhibitory receptors
MCP-1	Monocyt chemoattractant protein-1

M-CSF	Monocyte –CSF
Mda-5	Melanoma differentiation-associated gene 5
mDCs	Myeloid dendritic cells
MHC-I	Major histocompatibility complex-1
MHC-II	Major histocompatibility complex-2
MIF	Microphage migration inhibitory factor
MIG	Monokine induced by interferon-gamma
MIP-1α	Macrophage inflammatoryprotein-1 alpha
MIP-1β	Macrophage inflammatoryprotein-1 beta
M-MLVRT	Moloney Murine Leukemia Virus Reverse Transcriptase
MMPs	Matrix metalloproteases
MxA	Matrix A genes
NF-kB	Nuclear factor-kappa B
NKs	Natural killer cells
NNSV	Nonsegmented negative-sense RNA viruses
OAS	Oligoadenylate synthetases
OD	Optical density
PAMP	Pathogen associated molecular patterns
PBS	Phosphate buffer solution
PBMC	Peripheral blood mononuclear cell
pDCs	Plasmacytoid dendritic cells
PIV	Parainfluenza virus
PKR	Protein kinase R
PPC	Positive PCR control
RANTES	Rregulated upon activation, normal T cell expressed and secreted
Rb	Rretinoblastoma (tumor suppressor protein)
RFA	rheumatoid factors –Absorbent
RPL13A	Ribosomal protein L13a
RTC	Reverse transcription control
RT-PCR	Reverse transcription-polymerase chain reaction
TBE	Tris Boric Acid EDTA
TCR	T cell receptor
Th1	T-helper cell type 1
Th2	T-helper cell type 2
TLRs	toll-like receptors
TMB	Tetra methyl benzidine
TNF	Tumor necrosis factor
SCYE1	Small inducible cytokine subfamily E, member 1
SeV	Sendai virus
ssRNA	Single stranded RNA
SOCS	Suppressors of cytokine signalling
STAT	Signal transducers and activators of transcription
WBCs	White blood cells

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ABSTRACT

Influenza A virus (IAV), parainfluenza virus (PIA) and adenovirus (Adv) are major causes of respiratory infections with high rates of morbidity and mortality worldwide. An understanding of how IAV, PIV and Adv affect host cellular responses is critically important to explore the molecular mechanisms of viral-host interaction. The aims of this study are; to detect changes in the mRNA expression in a panel of inflammatory genes (84 genes) in the leukocyte concentrate of IAV, PIV and Adv infected patients after 48 hrs of infection and to determine the relationship between the inflammatory gene expression and asthma in IAV, PIV and Adv patients. Blood samples from 90 hospitalized patients suffering from respiratory tract infection were tested for the presence of IgM - IAV, PIV and Adv. Only 12 patients were positive. Eight patients were positive for IAV, two patients were positive for each PIV and Adv. Leukocytes from these positive patients were tested for a panel of 84 inflammatory genes using real time-PCR array technology. In IAV, only 22 inflammatory genes showed a significant upregulation ranging between 1.01 and 121.35 folds compared to control cells after recovery in all the eight patients with different frequencies. The upregulation of IL8, IL

10, IL13, C5, CCL7, CCL5 / RANTES, CXCL1 and CCL18 mRNA with high significance ($P > 0.05$) might suggest that the asthma complication during IAV infection is due to the stimulation of immune response by IAV. Thirty one inflammatory genes showed a decrease in their mRNA expression after IAV infection with folds ranging between 250.99 and 1.11 compared to control cells after recovery but with variable frequencies indicating a time dependent response. PIV infection which caused upregulation of the mRNA of 48 genes with mean fold change ranging between 752.42 for IFNA2 and 1.13 for IL10RA. Seven of these upregulated genes (IL8, IL 10, IL13, C5, CCL5 / RANTES, CXCL1 and CCL18) may cause asthma complication. On the other hand, PIV downregulated only 3 genes, MIF, CCL24 and CEBPB with 19.30, 19.25 and 5.56 folds, respectively. This suggests that the host-viral interaction is a cellular response against viral effect. Adv infection significantly stimulates upregulation of 11 mRNA of 11 genes (four of them might cause asthma) and downregulates 44 genes in two infected patients. This suggests that Adv overcomes most inflammatory genes for its favor. This suggests that the relationship between the host (leukocytes) and Adv is a viral influence. In conclusion: This study revealed several alterations of many Leukocyte`s inflammatory gene expressions induced by IAV, PIV and Adv. The common upregulated genes after the IAV, PIV and Adv infections stimulate the activity of different immunological functional pathways represented by cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and asthma pathway Nine of the over expressed genes in all 12 patients infected by the three viruses are expected to be involved in asthma. All 12 patients are mostly infected with related subtypes of influenza A viruses, PIV and Adv. These results may help in further analysis of viral-host interaction.

1. INTRODUCTION

Viral respiratory tract infections are the most common infectious illnesses, though they are usually self-limiting and confined to the respiratory tract. The rapid recognition of viruses and their effective elimination with minimal local and systemic inflammation is a good indication to the efficiency of the innate immune response within the airways and lungs. A failure of this response appears to occur in those with asthma and chronic obstructive pulmonary disease, where viral infection is an important trigger for acute exacerbations (Schaller *et al*., 2006).

The innate immune response to viruses requires their early detection through pathogen recognition receptors and the recruitment of the efficient antiviral response that is centered on the release of type 1 interferon (See and Wark, 2008).

The immune system consists of white blood cells, leukocytes, which form a complex network along with soluble mediators, cytokines, by which the cells communicate with each another. In addition to secreted soluble mediators, the cells have also direct interactions with each other via their cell surface receptors. The immune system can be divided into two parts, innate and adaptive. Innate immunity, which consists of complement system, phagocytes, e.g. granulocytes and macrophages and natural killer cells, are activated within the first hours after viral infection (Janeway *et al*., 2001).

The activation of innate immunity does not require specific viral antigen recognition but the recognition of conserved microbial structures or molecules (e.g. dsRNA) those which are abundant in microbes but missing in the host. These structures are recognized by Toll-like receptors (TLR). On the contrary, adaptive immunity includes specific antigen recognition by B and T cells. B cells can bind antigens directly but T cell activation requires presentation of the antigen by

macrophages or dendritic cells with the association of MHC molecules (Schaller *et al*., 2006).

Effective communication between the cells of the immune system is essential for the proper functions of a whole complex network. Cells secrete cytokines that mediate signals between cells. These signals lead to alterations in the gene expression by the target cell. Tens of different cytokines can be divided into several groups based on their structure, function, or the structure of the receptors to which they bind (Green, 2000).

Respiratory tract infections occur currently with high frequency and are caused by various agents including fungi, bacteria and viruses. Viruses such as influenza viruses (IV), parainfluenza viruses (PIV), human respiratory syncytial viruses (RSV), human metapneumovirus (MPV), human enteroviruses, human rhinoviruses (RV), human coronaviruses (CoV) and adenoviruses (AdV) are responsible for these infections (Sen, 2001). Diagnosis of these viral infections may be achieved by viral isolation in culture, direct antigen detection by immunofluorescence, indirect ELISA test and molecular diagnosis (Mitchell,*et al***,** 2009).

Viral infections are often associated with significant morbidity and mortality causing various diseases that range from mild to severe respiratory tract infection. Therefore, rapid and accurate diagnosis is essential for therapy (Barenfanger,*et al*., 2000).

The influenza virus (IAV) and the parainfluenza viruses (PIV) are both single stranded RNA viruses. The adenovirus (AdV) is a double stranded DNA virus. These viruses have been chosen for this study to investigate their effect on immune gene expression in white blood cells including gene up-regulation and down-regulation after infection. Up-regulated and down-regulated genes provide insights into functional responses of both host and pathogen (Penelope, *et al*., 2004). Detection of these pathogens and

determination of their effects on gene expression in white blood cells is very important for initiating antiviral therapy, avoiding unnecessary antimicrobial therapy, preventing nosocomial spread, decreasing the durationof hospital stays, and reducing management costs (Henrickson, 2005).

Influenza virus causes 5–15% of viral respiratory infections whereas parainfluenza virus and adenovirus cause 5% of cases (Heikkinen and Jarvinen, 2003). Most previous studies have emphasized on the epidemiological aspects of these infections and only few studies have examined the effect of viral infections on human gene expression using advanced techniques. Expression microarray analysis is a powerful method to determine global profiles of gene in cells and tissues under a variety of complex biological conditions (Clewley, 2004).

While much of the original analysis in this field focused on the host response to pathogen infection in tissue culture models, recent methodological improvements in experimental design and data analysis permit the adaptation of this method to more complex biological systems. These new model systems now include a more extensive use of tissue biopsies and complex cell populations from human subjects, as well as the analysis of naturally occurring and experimental infections (Penelope *et al*., 2004).

The typical viruses known to cause exacerbations are rhinoviruses, which cause most common colds, followed by respiratory syncytial virus (RSV), influenza, coronaviruses and parainfluenza viruses (PIVs). In addition, Hogg, (2001), has proposed that latent adenovirus infection might also contribute to the pathogenesis of chronic obstructive pulmonary disease (COPD). Most of recent medical and scientific debates have concentrated on: how some viral respiratory tract infections develop into immune sensitivity, if this correlation occurs what are the molecular changes that take place at the

gene level, which genes will be up-regulated or down-regulated that may lead to immune sensitivity, which virus will be the most effective to stimulate such genes, what is the interpretation for these complications and if there is a possibility to treat these cases at the molecular gene level. Recently, Simpson*et al*., (2006) described different inflammatory (neutrophilic, eosinophilic and granulocytic) subtypes of asthma based on the predominant granulocytic cell in induced sputum. Whether and infection induces disease depends on viral type, host (genetic susceptibility, age, immune responses) and environmental (allergen exposure, season) factors (Schaller,*et al*., 2006; Haller, *et al*., 2006). Many genes are implicated in susceptibility to asthma including those involved in inflammatory responses, IgE regulation, cytokine and chemokine production (Umetsu*et al*., 2002).

1.1. The importance of the study

The current study aims to detect changes in human blood cell gene expression profiles following the natural infection of upper respiratory tract with IV, PIV and AdV and record alteration with the gene expression signatures. Based upon this analysis, this study try to identify specific human blood cell's gene expression signatures that are characteristic for these viruses. We will also detect the genes that are affected by these viral respiratory infections and cause asthma. Identification of host gene products and gene expression patterns that may be used in the diagnosis and treatment of specific virus infections. Respiratory viruses characteristically differ from bacteria in that viruses have the ability to evade the protection offered by inflammatory mechanisms. In addition, the pathogenesis of respiratory viruses and their effect on gene expression / inhibition of white blood cells is not fully understood. This may be due to different viruses adopting different manners of infection and altering different genes either as up-regulation or down-regulation of different genes especially inflammatory genes. Therefore, this study

will concentrate on the effect of IV, PIV and AdV on inflammatory gene profiles of human blood cell using molecular methods, including real time quantitative polymerase chain reaction (RT-qPCR) and PCR-Array technique (Han*et al*., 2008).

The *in vivo* studies of respiratory virus infections on human cells using molecular assays (mostly RT-qPCR and Microarrays) are highly sensitive and specific (Connolly, 2004). Several genes whose expression profiles are altered in response to virus infection have been identified by microarray analyses (Samuel, 2001). In this study RT-PCR-Array technique was chosen for a number of reasons. First, little is known about the blood cells genomic response to infections with these viruses. Second, only microarrays and RT-PCR-Arrays could definitely determine whether the leukocyte-viral infections will be accompanied by a particular pattern of change in gene expression. Third, it would be necessary to have data on blood cell's gene regulation (Myski*et al*., 2009; Campeau *et al*., 2009; Sundararaj *et al*., 2009; Montgomery and Daum 2009; Baqai *et al*., 2009; Silver *et al*., 2008; Aubin *et al*., 2007).

1.2. The aims of the study

The main aim of the present study is to analyze the gene-expression profiles in the peripheral WBCs after infection with respiratory viruses: IV, PIV and AdV. In addition, the gene-expression profiles of patients infected by these viruses are compared with the gene-expression profiles after recovery as a control. The other aims of this study can be summarized as follows:

1- Determine the inflammatory gene expression profile for each virus under study.

2- Determine the differences and similarities of the inflammatory gene expression profile between viruses.

3- Determine which virus is responsible for the development of conventional respiratory tract infections into asthma complications.

2.8. The reasons for this study

From the previous studies it is clear that most studies investigate the viral effect on the cellular gene expression profile*in vitro* cell lines. This*in vitro* model of study not gives precise results compared with *in vivo* studies since results of gene expression in all*in vitro* studies gave completely different results from*in vivo* model studies (David, 2008; Penelope *et al*., 2004). The reason for these variations in results of gene expression in the two models is the inability to provide the suitable conditions for virus and host cells in side *in vitro* studies. For this reason this study investigates the effect of*in vivo* viral infection on blood cell population (leukocytes) to provide all suitable conditions for virus and host cell and get precise results. Also most previous studies concentrate on the effect of only one virus on cell line culture on the gene expression of the host and not investigate the comparative effect of two or more viruses. Therefore this study perform a comparative research to illustrates the effect of three different viruses on the gene expression of human leukocytes and get a complete image about the differences and similarities that occur in the gene expression profile after the infection of each virus. From these differences and similarities, much important information can be obtained such as; determine the specific effect for each virus on leukocytes gene expression, determine the gene expression profile of leukocyte after the infection of each virus and determine the nature of leukocyte response to each virus. Many previous studies not take in the consideration the effect of nature of the viral genome on the cellular gene expression, whereas this study investigated the effect of two different viral genome (double stranded DNA represented by Adv and single stranded RNA represented by IAV and PIV) on the gene expression of human leukocytes.

2. LITERATURE REVIEW

2.1. Respiratory viral infections and immune system

The human respiratory viral invasion is faced by immune system which acts against foreign pathogens as well as removes malignant cells from the body. The immune system consists of two parts, innate and adaptive immunity. The soluble and cellular components of the innate immune system form the first line of defense against invading pathogens. The soluble components of the innate immunity are complement, chemokines and cytokines (Khatri *et al*., 2010).

The adaptive immunity has efficient antigen presentation by antigen presenting cells (APC) and recognition of foreign antigens by lymphocytes. This leads to rapid expansion of B and T lymphocytes with specificity for the inducing antigen. The function of B cells is the production of antimicrobial antibodies. The T cell-mediated cellular immunity and protects body from intracellular pathogens (Janeway*et al*., 2001). The immune response to respiratory viral infection can cause an intense inflammatory reaction associated with asthma. Respiratory viral infection stimulates the immune response associated with chronic obstructive pulmonary disease (COPD). Specific aspects of these immune responses are determined by the biology of the virus, the genetic variability of the host, and the cytokine-chemokine phenotype of the involved tissue (Schaller*et al*., 2006).

A number of studies have shown that many respiratory viruses can induce the secretion of pro-inflammatory cytokines (IL1, IL6, IL11, IL8 and TNF) in epithelial cells derived from bronchial and nasal tissues, in the absence of substantial virus replication (Mosser *et al*., 2005; Edwards *et al*., 2007). These results have led to the hypothesis said that the symptoms associated with virus-induced common colds and other respiratory complications are the result of the increased levels of pro-inflammatory cytokines, rather

than direct effects of virus replication. The same may be true for other viruses associated with upper respiratory infections, such as influenza virus, respiratory syncytial virus, and adenovirus, which can also induce pro-inflammatory cytokine secretion in epithelial cells (Bonville *et al*., 1999). All of these viruses bring about cold and flu symptoms indirectly by stimulating secretion of pro-inflammatory cytokines (Szretter *et al*., 2007).

2.2. The immunity of T- lymphocytes

The bone marrow produces lymphoid precursors which migrate to the thymus in order to differentiate into T lymphocytes. The most important transmembrane structure of T cells is the T cell receptor (TCR). T cells can be divided into two subtypes on the basis of TCR expression. TCRαβ cells recognize antigens associated with self-MHC molecules, whereas TCRγδ cells poorly recognize antigens that are not MHC associated (Janeway *et al*., 2001). The cells that recognize antigen in association with MHC-II molecule become CD4+ T cells and those recognizing antigen with MHC-I molecule become CD8+ T cells. Newly differentiated CD4+ and CD8+ T cells leave thymus as T cells (Laky and Fowlkes, 2005). Recently it was illustrated that this differentiation of CD8+ T cells is stimulated by influenza virus infection (Song *et al*., 2010). Each CD4+ T cell has the potential to further differentiate into either Th1-cell or Th2-cell type CD4+ subset. A number of factors, in addition to antigen stimulus influence this differentiation process. The most important factor is the cytokine environment present during differentiation. IL12, IL18, IL23, IL27 and IFNγ significantly drive the differentiation towards Th1-cell direction whereas IL4 is the most important cytokine in Th2-cell differentiation (Agnello *et al*., 2003).

2.3. Cellular component of the innate immunity

The immune system contains a group of cells that are present in the blood and tissues. The development of blood cells occurs in the bone marrow where hematopoietic stem cells first develop to myeloid progenitors and lymphoid progenitors, or erythroblasts. Red blood cells (erythrocytes) differentiate from erythroblasts. White blood cells (leukocytes) on the other hand, develop from two lineages; myeloid progenitors and lymphoid progenitors. Lymphoid progenitors give rise to B, T lymphocytes, NK cells, and plasmacytoid dendritic cells. Myeloid progenitors produce monocytes, which further differentiate into macrophages and myeloid dendritic cells. Granulocytes develop also from myeloid progenitors into three different cell types, neutrophils, basophiles, and eosinophiles, which all have cytoplasmic granules that give them their characteristic phenotype (Janeway*et al*., 2002). The cells of the immune system can also be defined based on their functions in the immune response. Innate immunity comprises phagocytic cells like macrophages, DCs, and neutrophils as well as NK cells. These cells have a rapid response and do not require specific antigen recognition because they recognize conserved microbial components (CMC). These components abundant in pathogens but absent in the host, are recognized by specific receptors such as toll-like receptors (TLRs), scavenger receptors, and mannose-binding lectins (Takeda and Akira. 2004). These receptors are a group of type I transmembrane proteins that recognize pathogen associated molecular patterns (PAMP) on invading bacteria or virus. There are currently 13 types of TLRs described in human. TLR2, TLR4, TLR5, and TLR9 are the receptors associated mostly with recognition of bacterial components like lipopeptides, lipopolysaccharide (LPS) and flagellins. In contrast, TLR3, TLR7, and TLR8 recognize structures like ssRNA and dsRNA that are usually present in virus infections (Adam*et al*., 2005). TLR signaling leads to activation of many

transcription factors (TFs) such as necrosis factor-kB proteins (NF-kB) that regulate inflammatory response, Interferon regulatory factor (IRF-3). IRF-7 and activating protein-1 (AP1). These TFs regulate expression of various target genes (Kokkinopoulos *et al*., 2005).

The rapid responses of innate immunity do not result in the development of immune memory whereas the activation of adaptive immunity creates an immune memory represented by production of memory B cells and T cell (Yokoyama*et al*., 2004).

2.3.1. The innate immunity of natural killer cells

NKs are cells of the innate immune system whose principal role is to kill virus infected or malignant tumor cells (Moretta *et al*., 2002). Natural killer (NK) cells are large granular lymphocytes that represent about one tenth of all circulating leukocytes (Cooper *et al*., 2001).

NKs monitor the level of MHC-1 proteins, which are expressed on the surface of most vertebrate cells. Presence of high levels of MHC-1 inhibits the killing activity of NKs, so that the NKs selectively kill cells expressing low levels of MHC-1, including both virally-infected cells and some cancer cells. Many viruses have developed mechanisms to inhibit the expression of MHC-1 molecules on the surface of the cells they infect, in order to avoid detection by cytotoxic T lymphocytes (Semino*et al*., 1998). NK cells recognize virus-infected or tumor cells as non-self and eliminate them. On the other hand, NK cells express also stimulatory receptors that activate NK cell cytotoxic response upon ligand binding (Diefenbach*et al*., 2003). NK cell cytotoxic activity against virus-infected cells has been demonstrated in many viral models like in herpes viruses (herpes simplex). orthomyxoviruses (Influenza) and picornaviruses (Coxsackie virus) (French and Yokoyama, 2003).

2.3.2 The innate immunity of dendritic cells

Dendritic cells are able to capture and process the antigen and present it to T cells. DCs are regarded as the typical antigen presenting cells (APC). DCs are produced in the bone marrow and differentiate into myeloid or lymphoid progenitor cells. Myeloid progenitor differentiates into myeloid DCs. Lymphoid precursors differentiate to lymphoid (plasmacytoid) DCs (pDCs). But this differentiation is inhibited by the infection of IAV (Saikat, and Thomas, 2010). Myeloid DCs (mDCs) are responsible for efficient antigen uptake and presentation to T cells, whereas lymphoid DCs produce significant amounts of type I interferon. The migratory features of mDCs and pDCs differ too. The mDCs are responsive to several chemokines and thus are rapidly recruited at the site of infection. The pDCs are responsive only to CXCL12 chemokine (Colonna*et al*., 2004). The ingestion and recognition of antigen stimulates DC to mature. This maturation is accompanied by the upregulation of chemokine receptors and induction of adhesion molecules that allow DCs to migrate to secondary lymphoid tissues and present antigens to T cells (Sozzani *et al*., 2000).

2.3.3. The innate immunity of Cytokines

Cytokines are signaling molecules that are used in cellular communication. They are proteins (glycoproteins). They are produced widely throughout the body by cells of diverse embryological origin (Khatri*et al*., 2010).

Cytokines play a key role in the development and functioning of both the innate and adaptive immune response. They are often secreted by immune cells that have encountered a pathogen such as viruses, thereby activating and recruiting immune cells to increase the system's response to the pathogen (David*et al*., 2008). Each cytokine has a cell-surface receptor that activates subsequent cascades of intracellular signaling via

second messengers often tyrosine kinases (phosphorylation or dephosphorylation of signaling protein mechanism) to alter the cell behavior and then alter specific genes expression that lead to alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcriptional factors resulting in the production of other cytokines (Tian *et al*., 2005).

The effect of a particular cytokine on a given cell depends on the cytokine type, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface and downstream signals activated by receptor binding; these last two factors can vary by cell type. Cytokines are characterized by considerable "redundancy", in that many cytokines appear to share similar functions (David *et al*., 2008). Production of cytokines is usually very low and their production is regulated by various activating stimuli at the level of transcription or translation. Cytokine production is transient and they act by binding to their specific cell surface receptors on target cells. Most cytokine effects result from an altered gene expression pattern in the target cells (Vilcek, 2003). It is common for different cell types to secrete the same cytokine or for a single cytokine to act on several different cell types (pleiotropy). Cytokines can also act synergistically (two or more cytokines acting together) or antagonistically (cytokines causing opposing activities) (Khatri *et al*., 2010).

The common human cytokines include: Tumor necrosis factor (TNF) superfamily, member 5 (TNFSF5). IFNA2, IL1A, IL1B, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL1F10, IL5, IL8, IL9, IL10, IL13, IL17C, IL22, lymphotoxin A (LTA), lymphotoxin B(LTB), Microphage migration inhibitory factor (MIF), small inducible cytokine subfamily E, member 1(SCYE1) (Green, 2000). Cytokines, having so diverse range of effects, can be classified based on their functions into seven families: Interleukins (ILs); Interferons

(IFNs). Chemokines, Growth factors (GFs), Tumor necrosis factors (TNFs), Colony stimulating factors (CSFs) and transforming growth factor (TGF) (Janeway and Medzhitov, 2002).

2.3.3.1. Interleukins

Interleukins were first demonstrated to be expressed by white blood cells (leukocytes). It has since been found that interleukins are produced by a wide variety of body cells. The function of the immune system depends in a large part on interleukins. The majority of interleukins are synthesized by helper CD4+ T lymphocytes as well as monocytes, macrophages and endothelial cells. They promote the development and differentiation of T, B, and hematopoietic cells (Watford *et al*., 2003).There are 35 types of interleukins from IL1 to IL35 and few are classified into interlukine-1 superfamily which includes IL1, IL18 and IL33. Interlukine-6 family includes; IL6, IL 11, IL 27, IL30 and IL 31. Interlukine-10 family includes; IL10, IL19, IL 20, IL22, IL 24 and IL26. Interferon type 3 family includes; IL 28 and LI 29. Common Gamma- chain family includes; IL2/15, IL3, IL4, IL7, IL 9, IL13 and IL 21. Interlukine-12 family includes IL12 and IL 23. Other interleukins, include; IL5, IL8, IL14, IL16, IL17 /IL25 and IL32 (Noosheen and Sehy 2007).

Interleukin 1(IL1) superfamily activates T cells. IL-2, a type of cytokine immune system signaling molecule, is a leukocytotrophic hormone that is effective in the body's natural response to microbial infection and in discriminating between foreign antigen (non-self) and self molecule (Thornton *et al*., 2004).

IL4 is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. Upon activation by IL4, Th2 cells subsequently produce additional IL4. Its receptor is the interleukin-4 receptor. It has many biological roles, including the stimulation of

activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cell into Th2 cells (Olver *et al*., 2007).

IL4, IL5, and IL6 stimulate proliferation and differentiation of B cells. IL-6 is secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation (Febbraio and Pedersen, 2005). IL3, IL7 and granulocyte monocyte colony-stimulating factor (GM-CSF) stimulate hematopoiesis (Inoue *et al*., 2000). IL7 is a hematopoietic growth factor secreted by the stromal cells of the red marrow and thymus. IL7 stimulates the differentiation of multipotent (pluripotent) hematopoietic stem cells into lymphoid progenitor cells (as opposed to myeloid progenitor cells where differentiation is stimulated by IL13) and stimulates proliferation of all cells in the lymphoid lineage (B and T lymphocytes and NK cells) (Terry and Crystal, 2002). IL8 is secreted and is an important mediator of the immune reaction in the innate immune response (Utgaard *et al*., 1998). IL10, also known as human cytokine synthesis inhibitory factor (CSIF) is an anti-inflammatory cytokine. This cytokine has pleiotropic effects in immunoregulation and inflammation (Grimbaldeston, 2007).

IL12 family, cytokines that are produced by hematopoietic and endothelial cells, the main producers of these cytokines are monocytes, macrophages and DC. The gene expression of IL12 subunits is regulated independently (Goriely *et al*., 2001). In addition, IL12 especially in combination with IL18 induces IFN-γ production in DC (Frucht *et al*., 2001). IL35 is an IL12 family cytokine that is produced by regulatory, but not effectors T cells and plays a role in immune suppression (Noosheen and Sehy 2007). IL13 is a cytokine secreted by many cell types, but especially T helper type 2 (Th2) cells that is an important mediator of allergic inflammation and disease (Wills-Karp *et al*., 2003). IL15 is a cytokine with structural similarity to IL12 that is secreted by mononuclear phagocytes (and some other cells) following infection by viruses. This cytokine induces cell

proliferation of NKs and induces effectorsof NK (Lodolce *et al*., 2003). IL18 is a member of the IL1 cytokine superfamily and it was originally identified as an IFN-γ inducing factor in the presence of IL12. The principal source of IL18 is macrophages but IL18 is produced by a wide range of cell types such as DC, keratinocytes, Langerhans cells and intestine epithelial cells (Lakshmanan and Porter, 2007; Puren *et al*., 1999). IL21 is the most recently identified member of gamma-c-cytokine family. IL21 regulates NK cell functions in many ways. Activated NK cell functions are inhibited by IL21 as IL21 reduces IL15 which induces proliferation of NK cells (Kasaian *et al*., 2002). IL27 belongs to the IL12 family that is produced by antigen-presenting cells and plays an important function in regulating the activity of B and T lymphocytes (Larousserie *et al*., 2006). IL28 plays a role in immune defense against microbes especially viruses and its gene is highly upregulated in cells infected with viruses. It is highly similar (in amino acid sequence) to IL29 (Kempuraj, 2004; Sheppard, 2003). IL32 (belong to other interleukins) is a cytokine that can induce cells of the immune system (such as monocytes and macrophages) to secrete tumor necrosis factor-alpha (TNF-α) in addition to chemokines such as MIP-2/CXCL2 (Kim *et al*., 2005). IL33 is a cytokine belonging to the IL1 superfamily. IL33 induces helper T cells, mast cells, eosinophiles and basophiles to produce IL5 and IL13 (Puren *et al*., 1999).

2.3.3.2. Interferons

Interferons (IFNs) are natural cell-signaling proteins produced by the cells of the immune system of most vertebrates in response to challenges such as viruses, parasites and tumor cells. Interferons are produced by a wide variety of cells in response to the presence of double-stranded RNA, a key indicator of viral infection. The double-stranded RNA binds with TLR, this binding stimulates signal transduction pathway which leads to

activate IRF3 which inters the nucleus and stimulates the expression of interferons (Kotenko *et al*., 2003). Interferons assist the immune response by inhibiting viral replication within host cells, activating natural killer cells and macrophages, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection. Old classification divided the Interferons in two groups; Type I interferon are interferon α, β, δ, κ, ω, τ, and two recently characterized lambda-interferon (Kotenko*et al*., 2003). Recent classification divide the interferon into three major classes that have been described for humans according to the type of receptor through which they signal: Interferon type-1: All type I IFNs bind to a specific cell surface receptor complex known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. The type I interferon present in humans are IFN-α, IFN-β and IFN-ω (Liu, 2005). Interferon type II: Type II interferon group contains only one member in humans; interferonγ which binds to interferon γ receptor (IFNGR) (Kanda *et al*., 2009; Vilcek, 2003; Liu, 2005). In human tissues, IFNs are expressed in a low basal level even in the absence of specific inducer. Generally, microbial infections (viruses, bacteria) and other microbial components induce IFN production. Interferons are produced by many cell types but macrophages and dendritic cells are considered as the main producers of interferon (Muller*et al*., 1994). IFN receptors are composed of two chains (IFN-α/βR1, IFN-α/βR2, IFN-γR1 and IFN-γR2) dimerized by binding with their ligands. Binding of IFNα/β induces Jak1 and Tyk2 activation, which leads to tyrosine phosphorylation of intracellular domains of each receptor chain and STAT proteins. Dimerized STAT proteins translocate into nucleus and bind to regulatory elements in the promoters of IFN-activated genes (Caraglia*et al*., 2005). IFNα/β promotes innate and adaptive immune responses by activating the expression of several cytokines and their receptor genes and having a number of immunomodulatory effects (Matikainen*et al*., 2001). In addition, IFNα induces

maturation and activation of DCs. IFNα-induced IL15 stimulates the proliferation of memory T cells (Dalod *et al*., 2002). Type II interferon, IFNγ, is produced mainly by NK and T cells (especially Th1 cells). Other APC-derived cytokines TNFα, IL-15, IL-18 as well as type I interferon (in synergy with IL-12) stimulate IFNγ production from NK and T cells (Nakahira *et al*., 2002). IFN-γ promotes innate immune responses by activating phagocytosis and NK cell effectors functions (Schroder *et al*., 2004). The recently classified type III interferon group consists of three IFNλ (lambda) molecules called IFN-λ1, IFN-λ2 and IFN-λ3 (also called IL29, IL28α and IL28β respectively). These IFNs signal through the receptor complex IL10R2 (Bartlett, 2005).

2.3.3.3 Chemokines

Chemokines are small peptides that are potent activators and chemoattractants for leukocyte they selectively recruit monocytes, neutrophils, and lymphocytes (Rot and Adrean, 2004). Members of the chemokine family are categorized into four groups: CC chemokines, CXC chemokines, C chemokines and CX3C chemokines depending on the space between the first two cysteine residues. The first group CC chemokines (or ß-chemokines) have two adjacent cysteines near their amino terminus. There have been at least 28 distinct members of this subgroup reported for mammals, called CC ligand chemokines (Laing, 2004). CC chemokines (CCL2, CCL28 and CCL5) induce the migration of monocytes, NK cells and dendretic cells (Fernandez and Lolis, 2002). CC chemokine ligand-1 (CCL1) is a potent attractant for Th2 lymphocytes. These findings suggest that CC L1 may play a role in lymphocyte recruitment in bronchial asthma (Montes *et al*., 2006). One of the most promising markers of allergic inflammation is CCL11, which has a selective influence on the migration of eosinophiles. A study has tested weather influenza vaccination has any influence on serum expression of this

chemokine. The results in patients with bronchial asthma, influenza vaccinations assure efficient protective antibody level and modulate the serum level of CCL11 (Jahnz-Rozyk *et al*., 2004) CC chemokines such as CCL11, CCL5, and CCL3 are central mediators in the pathogenesis of asthma. They are mainly associated with the recruitment and the activation of specific inflammatory cells, such as eosinophiles, lymphocytes, and neutrophils. The CC L16 exerts chemotactic activity on human monocytes and lymphocytes. An adenovirus encoding CCL16/ AdCCL16 was used to determine whether this chemokine might also block pre-existing tumors. AdCCL16, when injected in established nodules significantly delayed tumor growth (Guiducci *et al*., 2004). Microarray technology offers a new opportunity to gain insight into global gene and protein expression profiles in asthma. To identify novel factors produced in the asthmatic airway, a study analyzed sputum samples by using a membrane-based human chemokine ligand 18 (CCL18) microarray technologies in patients with bronchial asthma (Kim *et al*., 2009). The second group CXC chemokines the two N-terminal cysteines of CXC chemokines (or α-chemokines) are separated by one amino acid, represented in this name with an "X". There have been 17 different CXC chemokines described in mammals; CXC chemokines specifically induce the migration of neutrophils (Huo *et al* 2001; Stokke *et al*, 1995). The third group of chemokines is known as the C chemokines (or γ chemokines). and is unlike all other chemokines in that it has two cysteines; one N-terminal cysteine and one cysteine at C- terminal. Two chemokines have been described for this subgroup and are called XCL1 (lymphotactin-α) and XCL2 (lymphotactin-ß). These chemokines attract T cell precursors to the thymus (Fernandez and Lolis, 2002).

The fourth group member has three amino acids between the two cysteines and is termed CX_3C chemokine (or delta-chemokines). The only CX_3C chemokine is called fractalkine (or CX_3CL1). It is a polypeptide structure which differs from the typical

structure of other chemokines. Soluble CX3CL1 potently chemoattracts T cells and monocytes, and act as an adhesion molecule between epithelial cells and immune cells (Matloubian *et al* 2000). Viral infection results in a significant upregulation of CCL2 gene (8.9-fold), CXCL1 gene (30.5-fold), CXCL2 gene (26-fold) and IL-6 gene (63.3-fold), IL-8 gene (8.1-fold) (Crompton *et al*., 1988). The upregulation of CCL2, CXCL1, CXCL2 and IL-8 gene expression was significantly correlated to the upregulation of IL-6 gene expression. Interestingly, the viral infection-induced upregulation of CCL2, IL-6 and IL-8 gene expression was positively correlated to the onset of acute inflammatory pain (Papandopoulos *et al*., 2001; Wang *et al*., 2009).

The most important chemokine genes that are associated with inflammatation are: complement-5 (C5), CCL1 (I-309), CCL2 (mcp-1), CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8 (mcp-2), CCL11 (eotaxin), CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4) CCL17, CCL18, CCL19, CCL20 (MIP-3a), CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2 / eotaxin-2), CCL25 (TECK) , CCL26, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6 (GCP-2), CXCL9, CXCL10 (IP-10), CXCL11, CXCL12 , CXCL13 and CXCL14 (Green, 2000; Kaplanisky and Bongrand, 2001).

One study showed that complement-5 (C5), induces the activation and suppression of inhibitory proteins that are crucial for cytokine production and neutrophile recruitment in lung pathology (Shushakova *et al*., 2002; Qian *et al*., 2008). Chemokines induce chemotaxis through the activation of G-protein-coupledreceptors, which belong to a family of 7-transmembraneG-protein-coupled receptor, the size of which has grown considerablyin recent years and now includes 18 members (Albert*set al*., 2002). Chemokine receptorexpression on different cell types and their binding and responseto

specific chemokines are highly variable (Lee *et al*., 2008). Chemokine receptors are implicated in viral inflammation. The most important chemokine receptor genes associated with inflammation include: CCLR13, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, XCR1 (Murdoch and Finn, 2000).

2.3.3.4 Tumor necrosis factors

Tumor necrosis factors (TNF-α, TNF-β) are produced from cells of the innate immune system such as macrophages in response to infection. This proinflammatory cytokine binds to cell surface receptors and activate NF-kB, which is normally stored in an inactive form in the cytoplasm of almost all human cells. Once activated, NF-kB inters into the nucleus and turns on the transcription of more than 60 known genes that participate in activation of proinflammatory mediators and apoptosis of infected cells (Mehta *et al*., 2003; Alberts *et al*., 2002).

2.3.3.5. Growth factors

Growth factors include Erythropoietin (EPO), Thrombopoietin TPO, Epidermal growth factor (EGF), Fibroblast growth factor (FGF), and Platelet-derived growth factor (PDGF), which activate growth of immune cells (Vilcek, 2003).

2.3.3.6. Transforming growth factor

Transforming growth factors (TGF)-β family including inhibins/activins and bone morphogenetic proteins which activate immunomodulation, morphogenesis and development of immune cells (Mehta *et al*., 2003).

2.3.3.7. Colony stimulating factors

Colony stimulating factors (CSF) include granulocyte-CSF (G-CSF), Monocyte–CSF (M-CSF) and IL 3. They are responsible for growth and differentiation of hematopoietic cells (Vilcek, 2003). Pleiotropic actions can be explained by having the ability to activate multiple signaling pathways wherein different signaling pathways differentially contribute to different functions (Ozaki and Warren, 2002). In addition to functional grouping of cytokines, they can be classified based on the structure of their receptors (Dy *et al.*, 1999). The biological functions of cytokines are achieved by binding of cytokines with high affinity to their specific cell-surface receptors. Receptor binding triggers signal transduction cascades that regulate cell activation, differentiation, proliferation, and apoptosis (Yamaoka *et al*., 2004).

2.4. The effect of Influenza A virus on inflammatory genes expression

Cytokine responses to viruses are central to IAV pathogenesis in mammals. To better understand differences in host responses to IAV infections, it is important to compare the levels of gene expression in infected cells and control cells using gene array technology (Kempuraj, 2004). Burioni *et al*., (2010) showed that the IAV-human infection cause viral effect on cell and the viral-host relationship is explained as crucial effect on host. IAV infection significantly increases the tumor necrosis factors which stimulates important immune pathway such as NF-kB pathway stimulates the production of inflammatory cytokines and chemokines (Zhu *et al*., 2010; NG *et al*., 2010).

The infection of human lung and trachea with IAV stimulates higher expression of genes related to respiratory immune system and innate response (Reemers *et al*., 2010). The upregulation of many genes causes asthma complication with IAV infection and play a key role in the cytokine-cytokine receptor interaction pathway (Huang *et al*., 2009).

Human infection with IAV stimulates inflammatory genes that stimulate transcriptional factors used to stimulate more inflammatory genes such as IFNs, TNF and interleukins gene which lead to bronchiolitis and ends with asthma (Huang*et al*., 2009). For example the infection with IAV stimulates the expression of interleukin-8 cytokine which is the most highly activated pro-inflammatory gene. The expression of this interleukin indicates its involvement in the IAV inflammatory response and stimulates the innate immune system response (Utgaard *et al*., 1998). Early IAV infection activates the production of anti-inflammatory cytokines such as the interleukin-13 which inhibits the production of pro-inflammatory cytokines and chemokines (Humbert*et al*., 1997).

Many cytokines induced by IAV infection that function as potent chemoattractants produced by innate and adaptive cells such as NK cells, macrophages and T-lymphocyte and then act against viral responses (Guan*et al*., 2002).

The infection of A549 cells with IAV stimulates the upregulation of cytokines and chemokines that recruit many immune cells to the sites of infection. Chemokine ligant- 5 activates the recruitment of many immune cells and Chemokine Ligant-1 was found to stimulate monocyte chemotactic activity (Geiler*et al*., 2010). The infection of mice pulmonary cells with H1N1 A/Puerto Rico/8/34 lead to produce the multi-immune functional cytokine (IL1A) (Aleksandr*et al*., 2005).

The infection with IAV stimulates the upregulation of IFN which is the most important gene for viral response. It acts as; a natural cell-signaling protein produced by the cells of the immune system in viral response and gives a viral protective role (Kotenko*et al*., 2003; Huang *et al*., 2010). The infection of vertebrates with IAV stimulates initial expression of many IFNs with high significance (Dillon, and Runstadler, 2010). For example the infection of mouse with IAV activates the expression of tens of interferon induced genes (Novoselova *et al*., 2010). In general viral infections stimulates hundreds

of IFN stimulating genes (ISGs) that provide antiviral state to infected and para-infected cells (Dalod *et al*., 2002). IAV infection induces the production of many chemokines (such as CXCL1) and cytokines such as interleukin-6 and interleukine-8 (Wang *et al*., 2009).

The response of human leukocytes includes different cytokines, chemokines and other inflammatory genes. The infections of respiratory cells lead to increase the production of proinflammatory genes represented by IL1, IL6, and IFNswhich initiate the cascades of antiviral responses in infected cells (Geiler *et al*., 2010).

IAV infection increases the level of multi-functional cytokine (interleukin 1- beta) which is involved in many cellular activities, including cell proliferation, differentiation and apoptosis (Stuart and Nancy, 2001; Adams *et al*., 2010).

IAV /H1N1 infection to human increase the level of mRNA of many pro inflammatory genes that activate different immune pathway as a response to IAV /H1N1 infection within the first day of infection (Zhang *et al*., 2006). Most IAV infected patients produce high level of cytokines that perform immune functions and other cellular activities such as the differentiation of T-cells during IAV infections (Zhu *et al*., 2010; Olver *et al*., 2007).

The infection of human lung cells with IAV-H1N1 stimulates high production of interferon induced protein (also called chemokine ligand-10) which is potent chemo-attractant to many immune cells (Wu *et al*., 2010; Geiler *et al*., 2010). The *in vitro* monocytes infection with IAV lead to stimulates the expression of chemokine ligant-2 which recruits immune cells to infected site (Bubfeld *et al*., 1998).

Many inflammatory cytokines are produced after the infection of IAV. These cytokines activate the migration of adhesion molecules (allow binding of immune cells to epithelial

cells in lung air ways) and immune cells to infected sites and also facilitates the recognition of IAV after infection (Matloubian*et al*., 2000).

The IAV infection to human epithelial cell causes high expression of inflammatory cytokines especially cytokines that play a key role in stimulation of initial inflammatory response during infection (Zhu*et al*., 2010)

Recent study showed that IAV infect most vertebrates and stimulates the production of IFN at initial stage of infection which in turn stimulates the expression of Mx-protein which plays a key role in innate immune response to viral infection (Dillon, and Runstadler, 2010). Viral infection causes recruitment and activation of leukocytes which are important for elimination of viruses from infected areas but in the same time the IAV infection inhibit the differentiation on monocytes into dendretic cells because IAV interferes with DC differentiation and evades virus-specific immune response (Boliar and Chambers, 2010). Research findings suggest that dendritic cells make use of distinct cellular signal transduction pathways to sense viruses and activate their type I IFN genes (Diebold *et al*., 2003). Mononuclear blood cells have an important role in immunity as they produce different cytokines in response to viral infections such as IFN-α, IFN-γ, interferon regulatory factor-1(IRF-1). MxA genes and interferon induced protein-10 (IP-10) (Peiris, *et al*, 2004). Influenza A virus capability of inducing the expression of a variety of chemokine genes in infected human macrophages or leukocytes causes activation of cell signaling pathways leading to an augmentation of inflammation (Bubfeld, *et al*., 1998; Sprenger, *et al*., 1996).

Infection of influenza virus induce host cell to produce large number of interleukins such as IL-1 receptor, L12, IL-23, IL10, IL-5, eotaxin, IFN-β, and IL-8. In result asthma

is triggered by this infection (Terry*et al*., 2002; Koenraad, *et al*., 2006; Yamamoto *et al*., 2003; Kawai and Akira, 2006; Akira*et al*., 2006).

In compared study between *in vivo* and *in vitro* infections of human influenza virus A showed significant production of IL-6, TNF-α, INF-α, INF-γ and IL-10 in response to community acquired influenza A illness*in vivo*. The response was as the release of IL-6 only (Kaiser, *et al*., 2001). Another study determined increasing in the levels of IL-1β, IL-2, IL-6, IL-8, IFN-α, TGF-β, and TNF-α in nasal fluid, plasma, and serum obtained serially from 19 volunteers experimentally infected with influenza A/Texas/36/91 (H1N1). None of IL-1 β, IL-2, or TGF-β levels increased significantly (Hayden*et al*., 1998).

The cytokines IFN-□/□, TNF-□, IL-1 and IL-6, and mononuclear cell attractants chemokines: MIP-1□ MIP-1□, CCL4, monocytic chemoattractant protein (MCP-1) (CCL2). MCP-3 (CCL7). IP-10 (CXCL10) and CCL5 have been found in influenza virus-infected cultures of human monocytes (Bubfeld*et al*., 1998). In contrast, IL-8 (CXCL8) production is suppressed in human monocytes infected with influenza A/Puerto Rico/8/34 (A/PR/8/34) (Hofmann *et al*., 1997). Exposure of human peripheral blood monocytes cell (PBMC) to influenza virus resulted in an immediate up-regulation of NK activity of PBMC via IL-15 induction. Results clearly indicated that IL-15 was specifically responsible for the NKs activity (Fawas*et al*., 1999).

2.5. Effect of parainfluenza viruses on inflammatory genes expression

Human infection with PIV stimulates the host cell to initiate early antiviral response represented by increase the expression of interferon with high level at the initial period of infection. Interferon type 1 is released byinfected cells to prevent the spreading of virus from one infected site to another (Andrejeva *et al*., 2002).

PIV infection to human respiratory tract stimulates high expression of anti-inflammatory interleukins that play an important immune-regulatory role by inhibit the antigen presentation, interleukins secretion, immune blastogenesis and proliferation (Sieg *et al*., 1996).

The expression of many human chemokines is increased after infection with PIV especially those chemokines that play important role in initial recruitment of immune cells and adhesion molecules to infected foci (Jacob*et al*., 2008). Also the PIV infection stimulates the function of many chemokines that recruit lymphocytes and dendritic cells to the site of infection (Liao *et al*., 1999). PIV induces expression of tumor necrosis factors in a high rate after infection. This cytokine stimulates a major pro-inflammatory cytokine that inhibit the viral infection through activating the NF-kB translocation to the nucleus which stimulates the transcription many inflammatory genes that activate proinflammatory and apoptosis activity in the infected cells (Mehtæt *al*., 2003; Bienhof *et al*., 2002; Lin, *et al*., 2007).

The infection of human epithelial tissue in the air ways with PIV lead to the production of anti-inflammatory factors that act as antagonist agent during the PIV infection. This antagonist activity results in the reduction of T cell activation and immune response (Salkind *et al*., 1991). Human PIV infection stimulates various gene expressions that activate immune system processes against viral infection through the enhancement of expression of many interleukin after PIV infection (Weiss*et al*., 2004).

The infection of *in vitro* A549 cells with PIV stimulates the expression of MHC1 and this increasing is companioned with the expression of interleukin type 1-A during the initial stage of PIV infection. On the other hand the PIV infection also activates the macrophages during the 24 h of infection which in turn stimulates the expression of many pro-inflammatory cytokine genes that mediate the inflammatory response, and is involved in a variety of immune cellular activities, including cell proliferation, differentiation, and apoptosis (Garofalo *et al*., 1996).

HPIV are major agents for respiratory illness, HPIVs are associated with immune-modulatory phenomenon. In addition, viral infection induces potent inflammatory responses that are largely the main cause for pathogenesis. This immunomodulatory phenomenon interaction is very clear between HPIV type 3 and dendritic cells (DCs). DCs are often the first immune cells to come in contact with viruses after infection, and can become primary targets of the invading virus. Hence, viral interference with DCs functions may have evolved as a viral adaptive mechanism to disrupt host immune responses (Horga *et al*., 2005).

HPIV3 and HPIV2, inhibit IFN-signaling to overcome the IFN response by the C-protein for HPIV3 and the V-protein for the HPIV2, both of which are multifunctional accessory proteins expressed from the P gene. Anotherwork suggests that slowing the cell cycle might be one way theV protein favors viral replication (Tuffaha*et al*., 2000). HPIV3 suppresses IFN stimulated tyrosine phosphorylation of STATs at an early phase of infection without degrading any of the signaling components in most cell lines (Gotoh *et al*., 2009).

Interferon system is designed to block the spread of virus infection in the body, sometimes at the expense of accelerating the death of the infected cells. Thus, in nature interferons and viruses maintain an equilibrium that allows regulated viral replication

(Sen, 2001). IFNs can upregulate the expression of many cellular genes, such as (PKR), OAS and Mx proteins which inhibit virus replication. All members of the *Paramyxovirinae* so far examined block IFN signaling (Diepen *et al*., 2010), thereby inhibiting the expression of IFN-stimulated genes.

It is unlikely that blocking IFN signaling alone is sufficient to allow these viruses to fully circumvent the IFN response because any IFN released by infected cells would still induce an antiviral state in surrounding uninfected cells, thereby making it difficult for the virus to spread from the initial foci of infection (Andrejeva *et al*., 2002; Conzelmann, 2005). Consequently, as well as blocking IFN signaling, most paramyxoviruses also limit the production of IFN by infected cells (He *et al*., 2002). HPIV1 infection inhibits the IFN response by inhibiting IRF3 activation and IFN production (Van *et al*., 2006). One of the initial responses of an organism to infection by pathogenic viruses is the synthesis of antiviral cytokines such as the type I interferons (IFN-α/β), interleukins, and other proinflammatory cytokines and chemokines (Carlos *et al*., 2005).

Interferons are also generating an intracellular environment that restricts virus replication and signals the presence of a viral pathogen to the adaptive arm of the immune response (Grandvaux *et al*., 2002; Chatziandreou *et al*., 2002). IFN antagonists of non segmented negative-strand RNA viruses (NNSVs) have been identified. These antagonists follow quite distinct strategies. It becomes evident that members of all NNSV families encode IFN antagonists that actively interfere with pathways activating IRF-3 and IRF-7 (Schlender *et al*., 2005). Human parainfluenza virus 2 (HPIV2) infections cause degradation of STAT2, and not of STAT1 (Young *et al*., 2000; Parisien *et al*., 2001; sun *et al*., 2004). Another studies showed that many paramyxoviruses have evolved specific proteins that inhibit the IFN-induced antiviral responses through direct

inhibition of cellular STAT proteins (Rodriguez*et al*., 2003; Nishio *et al*., 2005; Malur, 2005). As well as blocking IFN signaling, the paramyxovirus V proteins also limit the production of IFN-β by binding to the cellular RNA helicase enzyme (Childs*et al*., 2007). It is known that IFN dependent interference with viral infection relies on a failure of viral mRNA translation or of viral nucleic acid replication (Goodbourn*et al*., 2000; Arimilli*et al*., 2006; Poole *et al*., 2002).

A study report that the highly conserved domain of the V proteins of a wide variety of paramyxoviruses inhibit melanoma differentiation-associated gene 5 (mda-5) product of infected cell (mda5 is an IFN-inducible gene in host cell) (Andrejeva*et al*., 2004). In another research line one study investigate the effect of paramyxoviruses at the molecular level of proteosome mechanisms. It was known that cellular expression of V proteins from simian virus 5 (SV5) and HPIV2 induce polyubiquitylation of STAT1 and STAT2 targets (Ulane and Horvath, 2002; Alberts *et al*., 2002).

Numerous studies have highlighted the importanceof these proteins in virus transcription and inhibition of interferon signaling,including the observation on the role of HPIV-3 C protein in the transcription of viralgenome (Malur *et al*., 2005; Malur *et al*., 2004; Young *et al*., 2000).

IL10 is known to play an important immunoregulatory role in infection with many viruses by decreasing antigen presentation, IL2 secretion, T cell blastogenesis and preventing T cell proliferation (De Waal*et al*., 1991). The HPIV3 infection increased monocyte survival, accelerated dendritic cell (DC) apoptosis (Plotnicky*et al*., 2000; Horga, *et al*., 2005; Nouen *et al*., 2009). Interleukin6 (IL6) along with TNFα and IL1β, is a major proinflammatory cytokine that plays important roles in clearing virus infection through inflammatory responses. Many viruses have developed strategies to block IL6

expression. Wild-type PIV5 infection induces little expression of cytokines such as IL6 or TNFα, whereas infection by a mutant PIV5 induced high levels of IL6 expression (Lin *et al*., 2007).

2.6. Effect of human adenoviruses on inflammatory genes expression

The infection of human respiratory cells with Adv cause considerable effect on the expression of most immune and inflammatory genes after infection. These sever effects of Adv infection on immune cells suggests that the relationship between the stimulated cell and Adv is called as a virulence effect relationship (Burioni *et al*., 2010).

Generally most previous studies revealed that Adv infection causes inhibition of immune response during the initial period of infection. This effect of Adv on infected cells belongs to its virulence which controls the expression of most cellular gene such as; cellular transcription genes, cell cycle genes, cell growth genes cell structural genes (Granberg *et al*., 2006).

The inflammatory genes represented by many interleukins and their receptors are affected by Adv infection. The reduction of these cytokines occurs due to the ability of Adv to develop different anti-immune mechanisms against the host cell (Huang *et al*., 2009). Adv infection also decreases the production of different chemokines in the immune cells and disarms the cellular immune activities. These viral effects lead to inhibit important inflammatory pathway then subject the host cell to adenoviral replication and translation (Zhao *et al*., 2007).

The Adv infection also reduces the expression of chemokine receptors which activate the membrane-bound G protein-coupled receptors to stimulate immune-inflammatory response and activate the migration and activation of lymphocytes to the infected air ways in lung (Thomas *et al*., 2007; Back *et al*., 2005).

In vitro Adv infection to respiratory cells induces the activation of many inflammatory genes expression that leads to recruit and activate large number of immune cells to the site of infection then overcome the viral infection (Scibetta*et al*., 2005). The Adv infection causes high impact on many and different cytokines such as interferon type 1, interleukin 1, 6, and 8 that act synergistically against Adv infection at the early stages of infection (Higginbotham*et al*., 2002).

Interferon type 1 expression increased with high level during the Adv infection to human cells. The expressed interferon then enhance many immune pathway that end up with activation of huge number of inflammatory genes and interferon stimulating genes that counteract the Adv infection (Hutnick *et al*., 2010). Many chemokines are activated during Adv infection. Some of these chemokines stimulate immune cellular responses, immune cells recruitment, and migration of adhesion proteins to the infected tissues (Ronald, 2003; Constantin *et al*., 2000; Zlotnik *et al*., 2006; Bernt *et al*., 2005).

Adv infection to human respiratory cells activates the expression of tumor necrosis factor cytokine which involved in many inflammatory processes that act to overcome the Adv infection. For example this cytokine stimulates the NF-kB which activates many inflammatory cytokines against Adv infection (Hutnick*et al*., 2010; Sallusto *et al*., 2000). The infection of HeLa cell lines with Adv activates various chemokines that recruit different immune cells and control the trafficking of immune cells migration from blood circulation to Adv infected sites (Borgland*et al*., 2000).

The expression of many chemokine receptors is also stimulated by Adv infection. These receptors are coupled with transmembrane G-protein and stimulates different immune pathway against Adv infection. Some of these pathways activate the migration of dendretic cells and lymphocytes to the infected sites (Naoki*et al*., 2003). On the other hand Adv infection also activates the production of different proteins that perform

different immune function such as the inhibition of viral proteins, production of enzymes that degraded the viral transcripts and repress viral translational proteins (Bone*et al.*, 2005).

Immunity to viral pathogens depends on coordinated control of host cell genes, so adenoviruses have developed strategies to redirect the normal genetic program. As one of the most informative examples, the adenoviral early region 1A (E1A) gene encodes an oncoprotein that activates the host cell cycle and enables DNA synthesis that is needed for viral replication (Endter and Dobner, 2004).

The adenovirus-5 E1A was found to reverse-transform many human tumor cells. Tumor suppression apparently results from the ability of E1A to re-programme transcription in tumor cells. These discoveries have provided a tool with which to study the regulation of fundamental cellular processes (Frisch and Mymryk, 2002; Gallimore and Turnell, 2001).

Adenovirus infection might stimulate immune response associated with chronic obstructive pulmonary disease (COPD) and this may lead to immune sensitivity. The host's immune response plays a critical mechanistic role through the production of certain cytokines and chemokines against AdV (Schaller,*et al*., 2006). After adenovirus infection most of cellular genes are found to be down-regulated indicating that adenovirus employs additional strategies to redirect the cellular synthesis machinery to virus production during the late stages of infection (Zhao, *et al*., 2007; Granberg *et al*., 2006).

The infection of an adenovirus induced significant apoptosis of human pancreatic cancer cells (Miura *et al*., 2005). So far, little is known about the exact nature of the target genes. Therefore, cDNA microarray technique was used to study the changes in the cellular

gene expression profile in primary human cells after infection with adenovirus type 2 (Granberg, *et al*., 2006).

Studies have revealed that human adenovirus-encoded E1A protein promotes cell proliferation through the targeted interaction with cellular proteins that act as key negative regulators of cell growth. The interferon (IFN) negatively regulates cell growth in part through the Rb/E2F pathway by dephosphorylation of Rb and change the active Rb/E2F complex into repressor complex which inhibit the cell cycle progression (Xin *et al*., 2001). E1A reprograms the cell for DNA synthesis and induces the intrinsic cellular apoptosis program (Burgert *et al*., 2002).

Anti-adenoviral immunity involves innate and adaptive mechanisms. The innate immune response includes the release of inflammatory mediators such as chemokines, which regulate the activation, differentiation, and migration of leukocytes, amplify immunity and promote the conversion of innate to adaptive immune responses (Zhang H, *et al*., 2003).

Several inflammatory mediators and chemokine/receptor pairs known to be induced by AdV vectors including IFN-γ, IL-4 and IL8 (Cheng *et al*., 2009; Wu *et al*., 2006). Adenovirus, particularly its E1A protein, has been investigated in the pathogenesis of chronic obstructive pulmonary disease (COPD). High levels of E1A DNA were found in the lungs of COPD patients, where its expression increased with disease severity (Hayashi and Hogg, 2007).

A study demonstrates a correlation between AdE1A-induced sensitization and stabilization of p53 protein (human tumor suppressor protein its functions are cell cycle control, apoptosis and maintenance of genetic stability) (Barkic*et al*., 2009; Zhang *et al*., 2006). Many tumor cells are resistant to TNFα induced apoptosis. AdE1A sensitizes the

resistant cells to TNFα (Albert *et al*., 2002). TNF is a key inflammatory cytokine with antiviral properties. Human adenoviruses encode several intracellular proteins that mediate the effects of TNF that induce apoptosis (Wold, 2004).

2.7. Microarray technique

The principle of microarray assisted mRNA profiling has first been described in 1987 using a collection of cDNA fragments spotted on filter paper (Kulesh*et al*., 1987). Since then the technology has maturated considerably and presently a range of high quality commercial microarray platforms is available either based on long DNA oligonucelotides (single probe per transcript) or short DNA oligonucleotides (multiple probes per transcript) (Kwan *et al*., 2008).

Microarrays of DNA probes have at least three roles in clinical virology. These are: firstly, in diagnosis, to recognize the causative agent of viral illness; secondly, for molecular typing for (i) patient management, (ii) epidemiological reasons (e.g. investigating routes of viral transmission), (iii) purposes related to vaccine use; and thirdly, in research, to investigate the interactions between the virus and the host cell especially the effect of viral infections on gene expression profile (Clewley, 2004; Venkatasubbarao, 2004). Applications included the identification and comparison of mRNA or DNA from across tissues or organisms by hybridization against thousands of oligomeric DNA probes immobilized on planar surfaces, such as glass slides or PCR-array trays. This provided researchers with important view of the gene content present or absent within a given microbial genome (Schena, 2004). Development of microarrays facilitated the screening of viral pathogens from across broad viral families. A randomized primer was used to amplify any viral RNA that was present in the sample

using reverse transcriptase-PCR followed by hybridization on a microarray (Uttamchandani, 2009).

The affymetrix respiratory pathogen-microarray was used to detect the respiratory viruses influenza A and adenovirus (Lin, 2006). The array's large screening capacity meant that both the forward and reverse strands of the DNA targets could be sequenced to provide added sequencing accuracy (Lodes, 2007). Systematic studies of gene expression patterns using cDNA microarrays provide a powerful approach to molecular dissection of cells and tissues by comparing expression levels of tens of thousands of genes at a time (Tanaka, 2004; Bone, *et al*., 2005). Real-time-reverse transcription PCR-array (RT-PCR-array) (SABioscience Company) is the most sensitive and reliable method for gene expression analysis. Its wide dynamic range makes real-time RT-PCR the preferred choice for the simultaneous quantification of both rare and abundant genes in the same sample (Campeau *et al*., 2009; Myskiw *et al*., 2009).

The reverse transcriptase PCR-array takes advantage of real-time PCR performance and combines it with the ability of microarrays to detect the expression of many genes simultaneously and with high accuracy due to the presence of internal control genes that are used for normalization with the sample genes. RT-PCR-arrays are designed to analyze a panel of genes related to a disease state or biological pathway (Chitture*t al*., 2009).

For all these scientific reasons this study select the RT-PCR-Array technique (SABioscience Company) (Campeau*et al*., 2009; Myskiw *et al*., 2009) to investigate the

effect of infections of three well known respiratory pathogenic viruses (IV, PIV and Ad) on the inflammatory and immune genes in infected human WBCs.

3. MATERIALS AND METHODS

3.1 Instruments and equipments.

Autoclave (Selecta, Spain)

Centrifuge (Biofuge, Heraeus, Germany)

Digital balance (Denver, UK)

Electrophoresis chambers (Clever Scientific, UK)

Electrophoresis power supply (Power Pac Basic TM, BIORAD, USA).

ELIZA reader (Tipo, UK)

Gel documentation system (UVP, USA)

PCR tubes (Bio basic inc, Canada).

Pipettes (Witopet, Germany)

Spectrophotometer (Smart Spec TM plus, BIORAD, USA).

Thermal cycler (iCycler, Bio-Rad, USA)

Ultra-viol transilluminator (UVP, USA)

Vortex mixer (HARRIS, UK)

3.2 Reagents.

Absolute ethanol (Reidel-de Haen, Germany).

Blood RNA Kit (Invitrogen, USA).

Chloroform (Reidel-de Haen, Germany).

Deoxy nucleotides triphosphate (dNTPs) (Bio basic inc, Canada).

DNA ladder (Promega, USA).

EDTA (Invitrogen. USA).

Ethidium bromide (Promega, USA).

Forward primer (Promega, USA).

Free nuclease water (Promega, USA).

Isopropanol alcohol (Invitrogen, USA).

IgM-ELISA Kit (IBL-Hamburg Corporation, Germany).

LE-agarose powder (Promega, USA).

Loading dye (Promega, USA).

$MgCl_2$ (Promega, USA).

M-MLV RT enzyme (Promega, USA)

NaCl (Promega, USA).

PCR buffer 10x (Bio basic inc, Canada).

Random primers (Promega, USA).

Reward primer (Promega, USA).

Ribonuclease inhibitor (Invitrogen, USA).

RT-PCR-Array Kits (SABioscience, USA)

Sodium acetate (Reidel-de Haen, Germany)

Taq DAN polyemerase (Bio basic inc, Canada).

TE-saturated phenol (Promega, USA).

Tris-Borate-EDTA 10X (Bio Basic Inc, Canada).

Trisol reagent (Invitrogen, USA).

TE buffer (Promega, USA).

3.3. Methods

3.3.1. Patients

Through the period from May 17 to July 8/ 2009, 90 patients were hospitalized at Ibn AL-Haytham Hospital in Amman/Jordan for respiratory tract infection symptoms, a group of 12 patients were included in this study and subjected to analysis. The institutional review board (IRB) was obtained to collect blood samples from all patients in Ibn AL-Haytham Hospital in Amman/ Jordan.

A specialist physician has reviewed all data related to patients including: disease symptoms, onset of infection, age, sex and risk factors. Blood samples were collected for viral detection and molecular investigations.

3.3.2. Patients questionnaire

Patients were interviewed according to Table 1.

Table 1: Patients questionnaire

Patient's number	
Date of sampling	
Address/Region	
Age	
Sex	
Weight	
Disease onset	
Signs and Symptoms	
Other notes	

3.3.3. Blood samples

3.3.3.1. Collection and transport of specimens.

Blood samples (5ml) were obtained from hospitalized patients with symptoms of respiratory infections (patients with bacterial infection were excluded. Specimens were placed on ice and transported to the molecular laboratory/ Faculty of Medicine / University of Jordan. Blood samples were divided into two parts:

First part (2 ml) is placed in a heparinized tube for viral-type detection using indirect IgM-ELISA test (Kim, 2003).

The second part of blood sample (3 ml) is placed in an EDTA tube to extract RNA from WBCs within 24 h after collection for studying the immune gene expression of leukocytes.

Blood samples were collected from patients in the acutephase of the disease (0–2 days) when the patientshad high-grade fever (>38.0 °C). Second samples were taken after recovery (After 1 month as the control samples).

Total RNA is purifiedwith blood RNA kits (Invitrogen, USA) according to the manufacturer'sprotocols.

3.4. Detection of respiratory tract viruses

Infection by any of the three viruses (IVA, PIV and AdV) was diagnosed bythe indirect IgM-ELISA test (IBL-Hamburg Corporation, Germany).

The principle of the assay is the same with the three viruses which include the quantitative immunoenzymic determination of IgM-class antibodies against these viruses based on the ELISA (Enzyme –linked immunosorbant assay) technique (Wu *et al*., 2010).

3.4.1. Adenovirus assay identification

Diagnosis of AdV is performed using the indirect-IgM-ELISA test (Mitchell,*et al*, 2009). Before test procedure the wash buffer is diluted by adding 60 ml of wash buffer to 600 ml of distilled water (1:10). The mixture is warmed up at 37ºC to dissolve crystals (Mix vigorously) and stored at 2-8 ºC.

The samples are diluted with diluent buffer (1:101)(e.g. 5 µL + 500 µL) and treated with rheumatoid factors –Absorbent (RFA) in order to avoid interferences of specific IgG and rheumatoid factors. Patient sera should be treated with RF absorbent.

The RF-Absorbent (20 µl) is added to 400 µL of 1:101 diluted samples. The mixture is mixed well and is incubated ≥1 min (< 15 min) at 18-25°C.

The blood samples are collected in heparinzed tubes and centrifuged at 4000 xg for 2-3 min. The well tray is unveiled; leaving the first 4 wells for blank, negative control, cut-off control and positive control.

From each standard and diluted patient 100 µl were pipetted into the respective wells of the microtiter plate and use the following standards: standard A-D: 1; 10; 40; 150 U/ml: Standard A (Negative Control), standard B (Cut-Off Control), standard C (Weakly Positive Control), standard D (Positive control containing IgM antibodies against adenovirus), PBS and stabilizers.

The plate is covered with adhesive foil and incubated 60 min at 18-25°C. The adhesive foil is removed and the incubation solution is discarded. The plate is washed 3 times with 300 µl of diluted wash buffer. The excess solution removed by tapping the inverted plate on a paper towel.

The enzyme conjugate (100 µl) was pipetted into each well. The tray is covered with new adhesive foil and incubated for 30 min at 18-25°C. The adhesive foil is removed and the incubation solution is discarded.

The plate is washed 3 times with 300 µl of diluted wash buffer. The excess solution is removed by tapping the inverted plate on a paper towel. TMB substrate solution (100 µl) pipette into each well. The plate is incubated for 20 min at 18-25°C in the dark (without adhesive foil).

The substrate reaction is stopped by adding 100 µl of TMB-stop solution into each well. The contents are briefly mixed by gently shaking the plate. Color changes from blue to yellow.

The optical density is measured with a spectrophotometer at 450 nm within 60 min after pipetting of the Stop Solution.

3.4.2. Calculation of results

The concentration of the samples can be read from the standard curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher pre-dilution have to be multiplied with the dilution factor. The typical calibration curve is used to illustrate the optical density for each standard.

Standard	U/mL Mean	OD
A	1	0.022
B	10	0.535
C	40	1.046
D	150	1.926

3.4.3. Interpretation of results

The results are accepted according to the following values

U/mL	Interpretation
< 8	negative
8 - 12	equivocal
> 12	positive

3.5. Real time- PCR array (RT-PCR Array)

After diagnosis of infection by these viruses the second step in this study was to study the effect of viral infections on 84 inflammatory genes (SABioscience) (Campeau *et al*., 2009; Myskiw *et al*., 2009) to determine the inflammatory gene expression profile of patient's white blood cells. The inflammatory genes that will be examined in this study include the following classes:

3.5.1. Cytokine genes:

CD40LG (TNF-SF5). IFNA2, IL10, IL13, IL17C, IL1A, IL1B, IL1F10, IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, IL22, IL5, IL9, LTA, LTB, MIF, SCYE1, SPP1, TNF.

3.5.2. Cytokine receptors genes:

IFNA2, IL10RA, IL10RB, IL13, IL13RA1, IL5RA, IL9, IL9R.

3.5.3. Chemokine genes:

C5, CCL1 (1-309), CCL11 (eotaxin)., CCL13 (mcp-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19, CCL2 (mcp-1), CCL20 (MIP-3a). CCL21 (MIP-2), CCL23 (MPIF-1), CCL24 (MPIF-2 / eotaxin-2), CCL25 (TECK) , CCL26, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (mcp-3), CCL8

(mcp-2), CXCL1, CXCL10 (IP-10), CXCL11 (I-TAC / IP-9), CXCL12 (SDF1), CXCL13, CXCL14, CXCL2, CXCL3, CXCL5 (ENA-78 / LIX), CXCL6 (GCP-2), CXCL9, IL8.

3.5.4. Chemokine receptors genes:

CCL13 (mcp-4), CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CX3CR1, IL8RA, XCR1 (CCXCR1).

3.5.5. Other genes involved in inflammatory response:

ABCF1, BCL6, C3, C4A, CEBPB, CRP, CARD18, IL1R1, IL1RN, IL8RB, LTB4R, TOLLIP (Campeau *et al*., 2009; Myskiw *et al*., 2009).

3.6. Extraction of RNA from patient's leukocytes

The RNA of the 12 infected patients (acute samples) with the three viruses were extracted within 24 hours of sampling and stored under -87C°. After one month (following recovery) a second sample was obtained from the same patients (control samples) and their RNA was extracted within 24 hours and stored at -87C°. Thus each patient has two extracted RNAs; acute samples RNAs and control samples RNAs.

The blood sample (3ml) is placed in EDTA tube and centrifuged at 2500 rpm for 17 min. The Buffy coat (which contains the leukocytes) is pulled from the upper layer (300 µl) and put into another tube. Trizol reagent (900 µl) of Phenol-guanidin-isothiocyanate (Invitrogen, USA) is added at a ratio (1:3) to maintain the integrity of total RNA during blood cells rupturing. The tube is inverted several times (10-15 times) (Diepen *et al* 2010; Riny, 2005).

The homogenized sample is incubated for 5 min at 15– 30 ºC to permit the dissociation of nucleoprotein complexes. The chloroform (250 µl) is added. The tube is vigorously shacked by hand for 15 sec then incubated at 15 – 30 ºC for 5-7 min.

The tube is centrifuged at 12,000 rpm for 15 min. the mixture is separated into three layers, lower-red phenol chloroform phase, an inter-phase and a colorless upper aqueous phase (RNA remains exclusively in the aqueous phase).

The aqueous phase is transferred into a clean tube and isopropanol alcohol (500µl) is added. The tube is inverted 50 times and incubated at 15 – 30 ºC for 10 min then it is centrifuged at 12,000 rpm for 10 min, where gel-like pellet (transparent) is observed.

The supernatant is removed and 500 µl of 70% ethanol is added to remove any impurities. The tube is then inverted 7-10 times and then centrifuged at 7,000 rpm for 5 min. Finally the RNA pellet is dried for 5 min then re-suspended in 50100 µl RNase-DNnse-free water (Invitrogen, USA).

The extracted RNA is ready at this stage for production of cDNA. Before conversion of mRNA to cDNA, the isolated mRNA was tested for its quantity and quality

3.6.1. RNA quantity:

Spectrophotometer analysis is carried out to determine the amount of RNA by measuring the optical density (O.D) at λ_{260} as in the following procedure:

The RNA sample is diluted (1:25) by mixing (4 µl RNA sample + 96 µl autoclaved distilled water. The spectrophotometer at λ_{260} is blanked (zero) using 1000 µl autoclaved distilled water.

The O.D of diluted RNA samples is checked at λ= 260 (An O.D of 1 corresponds to approximately 40 µg/ml). The concentration of RNA is calculated (µg/ml) according to the following equation:

RNA concentration = 40 (O.D factor) x 25 (dilution factor) x O.D at λ_{260}. Adequate concentration must be between 0.01 – 1.0 µg/ml.

RNA quality is checked using two methods:

The $O.D_{260/280}$ ratio (Ratio of RNA to protein) is checked and RNA is considered pure if the O.D ratio is between1.7 – 2.0. Lower ratio (O.D< 1.7) indicates presence of protein contamination.

3.6.4. Gel electrophoresis

The electrophoresis tank and comb are soaked with 5% H_2O_2 for 30 min, and then 0.8 % agarose gel is prepared. The RNA sample (10µl) is added to 5µl of 6X loading dye into the gel. The gel is run at 75 volt for 45 min, and then photographed using the gel documentation system (UVP, USA). Good quality RNA appears as a sharp clear two bands (28S and 18S) of ribosomal RNA. Presence of smears means that there is protein contamination. Partially degraded RNA appears as thick band with smearing background.

3.7. Test the RNA efficiency to produce the cDNA strand

Before performing the RT PCR-Array, the RNA samples are tested for the efficiency of the reverse transcription to produce the cDNA from RNA samples.

3.7.1. First strand synthesis of cDNA

The RNA sample is placed (using 2 to 8 µg of RNA, based on the OD value of each sample) in a sterile PCR- tube; the random primer is added (1.0µg) (Promega, USA). The volume is completed to 10µl by RNase free water. The tube is heated to 70°C for 10 minutes to melt secondary structure within the template. The tube is cooled immediately on ice to prevent secondary structure from reforming.

3.7.2. Preparation of reverse transcriptase mixture (RT mixture)

To prepare the cDNA first strand synthesis, the following components are added to the annealed primer/template in the order shown.

Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) 5X Reaction (SABioscience) Buffer of enzyme 5µl

dATP, 10mM 1.25µl

dCTP, 10mM 1.25µl

dGTP, 10mM 1.25µl

dTTP, 10mM 1.25µl

Ribonuclease Inhibitor 25 units 0.7µl

M-MLV RT 200 units 1 µl

Nuclease-Free Water, complete to final volume 15µl

Add the master mix (RT mixture) to the RNA sample. Mix gently and incubate for 60 minutes at 37°C for synthesis of first strand cDNA, store at -20°C until next step (Roth and Goft, 1985).

3.7.3. PCR reaction

Add the following components (Promega, USA) to a PCR-tube:

10x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl) 5µl

$MgCl_2$ (50 mM) 1.5 µl

dNTPs Mix (10mM) 1.0 µl

Forward primer (10µM) 1.0 µl

Reward primer (10µM) 1.0 µl

Taq-DNA polymerase (5 U/ µl) 0.4

cDNA first strand synthesis 2 µl

RNase free water complete to 50 µl.

The components are mixed and the PCR-tube is placed in the thermo-cycler (Bio-Rad, USA) and run the following program;

Cycles	Repeat	Duration	Temperature
1	1	10 minutes	95 °C
2	40	30 seconds	95 °C
		30 seconds	58 °C
		60 seconds	72 °C

Repeat the cycle 2 for 40 times

3.7.4. Gel electrophoresis

Agarose gel 2% (10X Tris Boric Acid EDTA) is prepared. An adequate volume of electrophoresis 1X TBE buffer is prepared by dilution of one volume of 10X TBE buffer (Promega, USA) in nine volumes of distilled water. Then 2 g of agarose powder were added (Promega,USA) into 100 mL of 1X TBE buffer and then the mix was melted in a microwave with swirling to ensure even mixing and clear appearance. Then 5µl of 2.5mg/ml ethidium bromide was added to the mix and poured into themini gel casting platform and allowed to harden.

3.7.5. Loading the samples

The gel casting platform containing the set gel was placed in the electrophoresis tank supplied with electrophoresis buffer. The PCR product (10µl) is mixed with 5µl of loading dye (Promega, USA) and loaded into the well. The run has 3µl of DNA ladder mixed with 2µl of loading dye to determine the size of the bands of the PCR product. The gel is supplied with 120 volt for 20 minutes. The DNA was visualized by placing on a UV light source and was photographed directly.

3.8. Real time PCR-Array:

The real time PCR-array takes advantage of real-time PCR performance and combines it with the ability of microarrays to detect the expression of manybiological genes lines in human cells (Campeau*et al*., 2009; Myskiw *et al*., 2009; Adams *et al*., 2010).

3.8.1. Genomic DNA elimination mixture:

The isolated RNA will be treated with DNase:

For each RNA sample, the following contents are mixed in a sterile PCR tube:

Total RNA	25.0 ng to 5.0 µg
GE (5X gDNA Elimination Buffer)	2.0µl
RNase-free H_2O to a final volume of …	10.0µl .

The same amount of total RNA in this reaction is used for every sample starting with 0.5 or 1.0 µg of total RNA for 96-well plate formats. The contents are mixed gently with a pipette followed by brief centrifugation. The mixture is incubated at 42°C for 5 min and chilled on ice immediately for at least one minute.

3.8.2. cDNA synthesis

The cDNA is prepared from RNA samples (one sample cDNA from patient after recovery of infection as a control sample and second sample cDNA from patient in infection period as an acute sample). The reverse transcriptase (RT) mixture is prepared as follows:

RT mixture	1 reaction	2 reaction	4 reaction
BC3 (5X RT Buffer 3) contains dNTPs	4 µl	8 µl	16 µl
Primer (P2 - Oligo-dt primer & external Control Mix)	1 µl	2 µl	4 µl
RE3 (Rev.T Enzyme Mix 3)	2 µl	4 µl	8 µl
RNase-free H_2O	3 µl	6 µl	12 µl
Final Volume	10 µl	20 µl	40 µl

The RT mixture (10µl) is added to each 10µl genomic DNA elimination mixture and the contents are mixed well but gently with a pipette, incubated at 42°C for exactly 15 min and then the reaction is stopped immediately by heating at 95°C for 5 minutes.

The ddH_2O (91µl) is added to each 20µl of cDNA synthesis reaction then the contents are mixed well. The finished first strand cDNA synthesis reaction is placed on ice until the next step or stored overnight at -20 °C (Hideshima *et al*., 2009).

3.8.3. Addition of cDNA to RT-qPCR Master Mix

The cDNA is added to RT-qPCR Master Mix (Master mixes contain SYBER Green and reference dye). Then the master mix is used to prepare the experimental mixture as follows:

The following components are mixed in a 5-ml tube	.
2X SABiosciences RT-qPCR Master Mix	1350 µl
Contains; syber green, Taq-DAN polymerase	
Forward and reverse primers and dNTPs	
Diluted First Strand cDNA Synthesis reaction	102 µl
ddH_2O	1248 µl
Total Volume	2700 µl

3.8.4. Loading the 96-Well PCR-array

The mixture is aliquot across the PCR-arrays, each PCR-array profiles the expression of 84 pathway-specific geneplus 12 wells for controls. The experimental mixture (cDNA and RT-qPCR Master Mix) is added (25µl) to each well of the PCR-array.

3.8.5. Real-Time PCR detection

Seal the PCR-array carefully but tightly with the optical thin-wall 8-cap strips. Be sure that no bubbles appear in any of the wells of the PCR-array. To remove bubbles, the plate is tapped gently on the bench top or the plate is centrifuged briefly

The plate is placed on ice while setting up the PCR cycling as mentioned below.

One plate is placed in real-time thermal cycler.PCR-arrays containing experimental mixture may be stored at -20 °C wrapped in aluminum foil for up to one week until ready to run. The appropriate program is entered and run using the real-time instrument software.

3.8.6. Perform thermal cycling

The real-time amplification data is collected using software instrument to get two profiles (profileone as control sample and profile two as acute sample). The program is a two-step cycling used for Bio-Rad-iCycler.

Cycles	Repeat	Duration	Temperature
1	1	10 minutes [1]	95 °C
2	40	15 seconds	95 °C
		1 minute [2]	60 °C

1- The 10-minute step at 95 °C is required to activate the Hot-Start DNA polymerase.

2 - SYBR-Green fluorescence is detected and recorded from every well during the annealing step of each cycle.

3.8.7. Recommended Quality Control (Dissociation Melting Curve):

Melting curve program is run immediately after the above cycling program. It generates a first derivative dissociation curve for each well in the entire plate using instrument software. No more than one peak should appear in each reaction at temperatures greater than 80 °C (Figure 1, Appendix-1). The following program was run:

Cycles	Repeat	Duration	Temperature
1	1	1 minutes	95 °C
2	1	1 minute	55 °C
3	80	10 second	55 °C

Increase set point temperature after cycle 2 by 0.5°C.

Visually, the plate is inspected after the run for any signs of evaporation from any well. If evaporation is observed, the well is marked so that it may qualify the data analysis appropriately.

3.8.8. Calculation of the threshold cycle (Ct) for each well

Using the instrument's software the threshold cycle (Ct) is calculated for each well. The baseline and threshold values were determined. The baseline is determined at the two cycles before the cycle that gave the earliest visible amplification, usually around (Figure 2, Appendix-1). The threshold value was determined within the lower one-third part of the linear phase of the amplification plot (Figure 3, Appendix-1).

The same thresholds are used across all PCR-array runs in the same analysis. If the RNA sample quality has been adequately controlled, the cycling program has been executed properly, and the thresholds have been defined correctly. Then the value of threshold cycle of positive PCR control (C_t^{PPC}) should be 20 ± 2 across all of PCR-arrays or samples. The resulting threshold cycle (Ct) values for all wells were analyzed using a Blank Excel Spread-sheet SABioscience Data Analysis Template Excel File (Campeau *et al*., 2009; Myskiw *et al*., 2009).

3.8.8.1. Data Analysis: $\Delta\Delta C_t$ Method

According to the PCR-array Data Analysis Web Portal change all Ct values reported as greater than 35 are considered as negative calls. The threshold cycle values of the control wells are examined as follows:

a. Genomic DNA Control (GDC):

The C_t^{GDC} is calculated. If the value is greater than 35, then the level of genomic DNA contamination is too low to affect gene expression profiling results and no action is needed. If the value is less than 35, then genomic DNA contamination is evident.

b. Reverse Transcription Control (RTC):

To determine any impurities in RNA sample that affect the reverse transcription of the RT- First Strand built-in external RNA control.

The ΔC_t is calculated as follows:

$\Delta C_t = AVG\ C_t^{RTC} - AVG\ C_t^{PPC}$

c. Positive PCR Control (PPC):

To determine any impurities in RNA sample that affect the PCR amplification of the positive control also affect the PCR amplification.The average C_t^{PPC} value should be 20 ± 2 on each PCR-array and should not vary by more than two cycles between PCR-arrays being compared. Larger differences in average C_t^{PPC} values between samples indicate the presence of different amounts of PCR amplification inhibitors in each sample and that all of the RNA samples require further purification.

An average value of C_t^{PPC} that is consistently greater than 22 for all samples may indicate a problem with the cycling conditions or may simply be indicative of the relative sensitivity of instrument.

The ΔC_t for each gene is normalizedfor the house keeping genes (Beta-2-microglobulin, Hypoxanthine phosphoribosyltransferase 1, Ribosomal protein L13a, Glyceraldehyde-3-phosphate dehydrogenase, and Actin, beta) in each plate as follows:

$\Delta C_t = C_t^{GOI} - C_t^{AVG\ HKG}$

The $\Delta\Delta C_t$ is calculated for each gene across two PCR-arrays (or groups) as follows: $\Delta\Delta C_t = \Delta C_t$ (group 2) - ΔC_t (group 1). Where group 1 is the control (control sample) and group 2 is the experimental sample (acute sample).

The fold-change is calculated for each gene from group 1 to group 2 as $2 \wedge (-\Delta\Delta C_t)$.

If the fold-change is greater than 1, then the result may be reported as a fold upregulation. If the fold-change is less than 1, then the negative inverse of the result may be reported as a fold down-regulation. The fold-change ratios may also be reported as is.

3.8.8.2. Detailed mathematical explanation of ΔΔCt data analysis

Due to the inverse proportional relationship between the threshold cycle (G) and the original gene expression level, and the doubling of the amount of product with every cycle, the original expression level (L) for each gene of interest is expressed as:

$L = 2^{-Ct}$

The expression level of each gene of interest (GOI) was normalized to a housekeeping gene (HKG). as follow:

$2^{\wedge\text{-Ct (GOI)- Ct (HKG)}} = 2^{\wedge\text{-}\Delta Ct}$

To determine fold change in gene expression, the normalized expression of the GOI in the experimental sample is divided by the normalized expression of the same GOI in the control sample:

$2^{\wedge\text{-}\Delta Ct\text{ (acute)}} - 2^{\wedge\text{-}\Delta Ct\text{ (Con.)}} = 2^{-\Delta\Delta Ct}$

3. 9. Statistical analysis

Since each gene has 24 values (12 values for acute samples and 12 values for control samples) the difference between the two values for each gene is analyzed using the non-parametric Wilcoxon signed ranks-test to calculate the T-value (similar to t-test value) and P-value for each gene (Joshua *et al.*, 2006). The *P*-value is an important parameter for significance level that helps to establish the reliable range for ΔΔCt estimation. *P*-values are important to claim differential expression between the two Ct values (acute samples and control samples) for each gene. The *P*-values are derived from testing the null hypothesis that ΔΔCt are equal to 0.0. Therefore, a small *P*-value indicates that the ΔΔCt is significantly different from 0.0 which demonstrates a significant effect. If the $P \geq 0.05$ for each gene in acute samples versus control samples, the gene expression is significantly different among the two samples (Table 1, Appendex.1)

4. Results

4.1. Patients and serological screening

Sera from all of the 90 patients were tested by ELISA-IgM and 18 patients gave positive results with respiratory infection, 14 patients gave a positive result for IVA virus (15.55 %). two patients gave a positive result for PIV (2.22 %) and two patients gave a positive result for Ad (2.22 %). From the 18 patients, only 12 patients (35- 40 years old) were chosen to analyze the effect of IAV, PIV and Adv on the inflammatory gene expression profile in leukocytes using RT-PCR-Array analysis including 8 patients with IAV, two patients with PIV and two patients with AdV. Specialist physician reviewed all of the data relating to patients as shown in Table 2.

Table 2: The age, sex, weight, symptoms and ELISA test results of all 18 infected patients (14 infected with IVA, 2 infected with PIV and 2 infected with Adv).

Patients number	Age (years)	Sex	Weight (Kg)	Signs and symptoms onset (days)	Signs and symptoms	ELISA-IgM result
1	37	F	70	2	Runny nose, fever, asthma	IAV
2	35	F	72	2	Runny nose, fever sneezing, cough	IAV
3	36	F	94	2	Runny nose, fever, headache	IAV
4	40	F	78	2	Runny nose, fever, chills	IAV
5	35	F	58	2	Runny nose, Fever, arthritis	IAV
6	40	F	67	2	Runny nose, Fever, asthma, headache, arthritis	IAV
7	36	F	87	2	Fever, headache, arthritis, cough	IAV
8	39	F	65	2	Runny nose, fever, arthritis, sore throat cough	IAV
9	40	M	72	2	Runny nose, sneezing, cough	IAV
10	35	F	71	2	Runny nose, fever, sore throat, headache	IAV
11	36	M	61	2	Runny nose, fever,	IAV

					teary eyes, congested throat	
12	35	M	71	2	Runny nose, fever,	IAV
13	37	F	54	2	Runny nose, fever, sneezing, cough	IAV
14	14	F	39	2	Runny nose, cough, arthritis.	IAV
15	40	F	55	2	Runny nose, fever, arthritis	PIV
16	34	F	75	2	Runny nose, fever, headache, arthritis, otitis, cough.	PIV
17	35	F	60	2	Runny nose Fever,sneezing,cough	Adv
18	40	F	61	2	Fever, asthma, headache	Adv

4.2. RNA preparation

From the 18 patients with positive ELISA test, only 12 patients (35- 40 years old) were choosed for analysis. The RNA concentrations of the 12 patients (acute samples and control samples) ranged between 121.3 and 1218.2 µg/ml for acute samples, and between 130.5 and 607.8 µg/ml for control samples. The RNA quality (purity) of the 12 patients (acute samples and control samples) ranged from 1.7082 to 1.8570 for acute samples and from 1.7049 to 2.0328 for control samples (Table 3).

Table 3: The RNA quantity (concentration of acute and control RNA samples as measured by OD-spectrophotometer) and RNA quality (the purity of acute and control RNA samples at OD_{260} compared with protein at OD_{280} as measured by spectrophotometer).

	During infection (Acute samples)		After recovery (Control samples)	
Sample Number	RNA concentration OD/concentration µg/ml	OD_{260}/OD_{280}	RNA concentration OD/concentration µg/ml	OD_{260}/OD_{280}
1	174.2512	1.7082	195.0	1.7329
2	895.8364	1.7631	590.318	2.0328
3	1451.5575	1.8326	324.0552	1.8806
4	306.8629	1.7736	588.5579	1.8317
5	281.8319	1.7670	532.0560	1.7278
6	1218.2847	1.8223	548.7266	1.7049
7	121.3187	1.7020	247.1611	1.867
8	173.6380	1.7202	181.5276	1.8571
9	1195.0665	1.8332	130.5861	1.9273
10	367.9903	1.7660	499.3196	1.7298
11	242.6133	1.7728	320.9909	1.7071
12	1032.8713	1.8570	607.8282	1.9804

The volumes (µL) of RNA samples (acute and control) are calculated from the RNA concentrations that were calculated by the spectrophotometer. Each volume of each sample must contains µg/µL and then used in the RT-PCR-Array as illustrated in Table 4.

Table 4: The concentrations of 12 acute and 12 control RNA samples that are calculated by spectrophotometer (µg/ml) and then converted into RNAvolume (contains 1 µg/ml) for each sample and then used in the RT-PCR Array.

	During infection (Acute samples)		After recovery (Control samples)	
Sample Number	RNA concentration µg/ml	RNA volume (µL) used in the RT-PCR Array	RNA concentration µg/ml	RNA volume (µL) used in the RT-PCR Array
1	174.2512	5.7	195.0	5.1
2	895.8364	1.1	590.318	1.7
3	1451.5575	0.7	324.0552	3.0
4	306.8629	3.2	588.5579	1.7
5	281.8319	3.5	532.0560	1.9
6	1218.2847	0.8	548.7266	1.8
7	121.3187	8.0	247.1611	4.0
8	173.6380	5.7	181.5276	5.5
9	1195.0665	0.8	130.5861	7.6
10	367.9903	2.7	499.3196	2.0
11	242.6133	4.1	320.9909	3.1
12	1032.8713	1.0	607.8282	1.6

The result of gel electrophoresis test showed good quality RNA samples represented by two sharp and clear bands (28S and 18S) rRNA (Figure1).

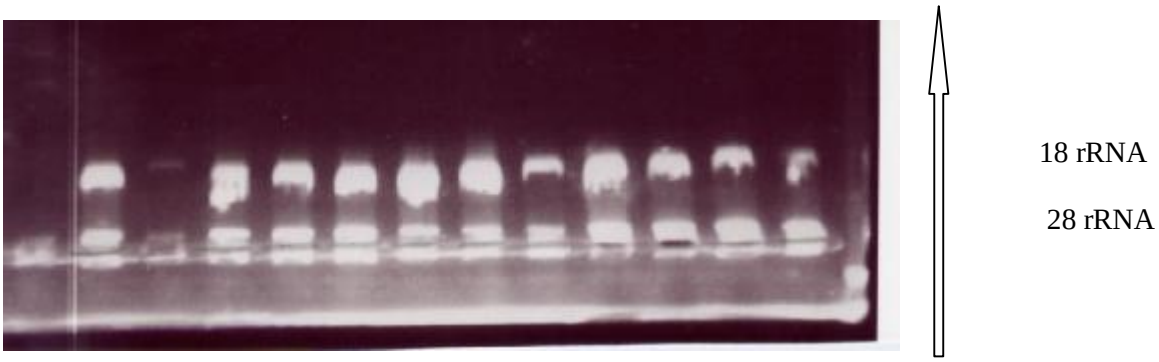

Figure 1: Gel electrophoresis for RNA (acute and control samples) showing the two rRNA bands (28S and 18S). These bands give indication that the quality of RNA samples is adequate and can be used to produce cDNA and perform RT-PCR-Array.

4.2.1. Test the RNA efficiency to produce the cDNA strand

The RNA samples were tested for the efficiency of the reverse transcription to produce the cDNA. The results showed a clear 500 bp DNA band for each samples (Figure 2). The RNA samples are of high quality and can be used to produce the cDNA strand to be used for the RT-PCR Array technique.

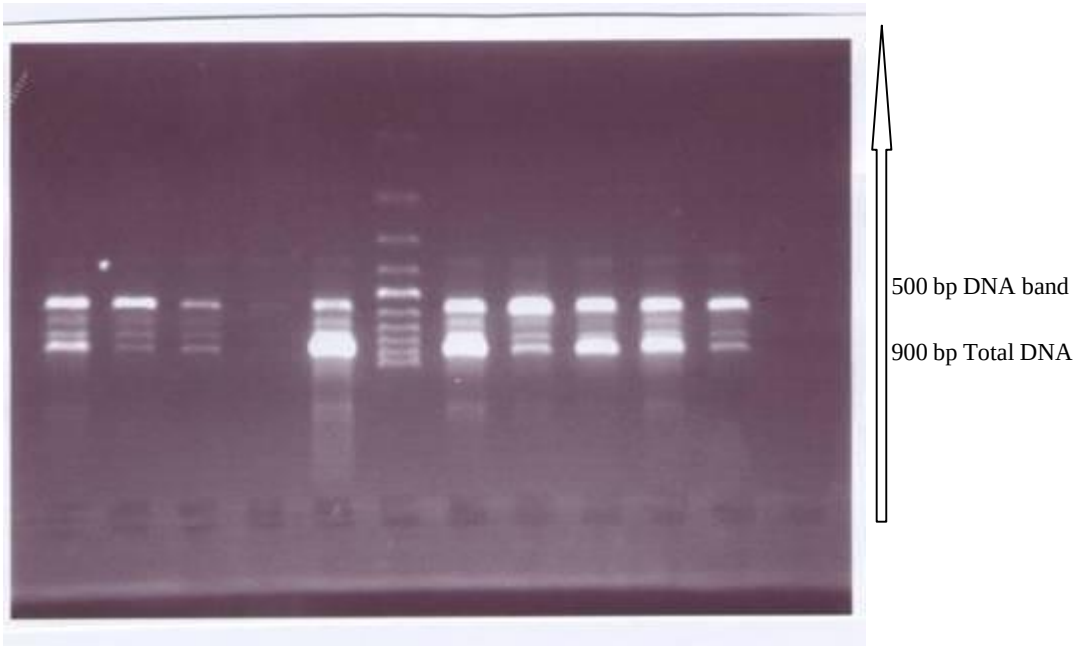

Figure 2: Gel electrophoresis test for the quality of RNA acute samples to produce the cDNA with 500 bp DNA band. The production of 500 bp DNA band indicates that the RNA samples (for acute and control) are efficient to produce cDNA from RNA samples with presence of reverse transcriptase enzyme. The 900 bp DNA bands represent the total DNA that used and these bands appear because the procedure not used DNase.

4.3. Real time PCR –Array:

4.3.1. Ct values:

This study chose the immuno-inflammatory genes line provided by SABioscience, USA (Campeau *et al*., 2009; Myskiw *et al*., 2009) to analyze the immuno-inflammatory gene expression profile that results due to the infection of the three viral infections (IAV, PIV and Adv) to human leukocytes. The immuno-inflammatory genes line consists of 84 genes and 12 controls arranged in the 96 wells of the RT-PCR-Array (Appendix A: Table 1).

The Ct values of the 84 immuno-inflammatory genes and 12 controls for the 12 patients (12 acute samples and 12 control samples) are obtained after RT-PCR-Array run (Bio-Rad software, USA) and illustrated as 8 samples infected by IAV (Appendix A: Table 2), two samples infected by PIV and two samples infected by AdV (Appendix A: Table 3).

The Ct values represent the threshold value for each gene after amplification by RT-PCR-Array, from these Ct values the fold change for each gene is calculated using SABioscince Excel-Analysis of RT-PCR-Array.

4.4. Leukocytes gene expression analysis after IAV infection:

In patient 1, the infection with IAV up regulated 26 (30.95%) genes by more than 1 fold change and 23(27.38%) genes were down regulated (Appendix A: Table 4, Appendix B: Figure 4).

The fold changes of the 26 upregulated genes is variable and ranged between 1.13 fold change for B-cell CLL/lymphoma 6, Chemokine (C-C motif) ligand 7, Interleukin 1 family, member 6 (epsilon). Small inducible cytokine subfamily E, member 1 (endothelial

monocyte-activating) and 1009 fold change with Interleukin 10 (IL10) (Appendix B: Figure 5). The fold change of the downregulated 23 genes ranged between -1.5 in Interleukin 5 (colony-stimulating factor, eosinophile) and -1.16 in Chemokine (C-C motif) ligand 26 (Appendix B: Figure 6). The gene expression profile of IAV infection in patient 1 is depicted in (Appendix A: Table 5).

In patient 2, 62 (73.80%) genes were up regulated and 15 (17.85%) genes were down regulated after 48 h of IAV infection (Appendix A: Table 6, Appendix B: Figure 7).

The up regulated genes in patient 2 ranged between 1.148 in the interleukin 1 family, member 8 (eta). Chemokine (C-C motif) receptor 9 (CCR9) genes and 27.85 fold change with interleukin 8 gene (Appendix B: Figure 8).

On the other hand the down regulated 15 genes in patient 2 ranged between -2.80 as in IL 8 receptor, alpha and -1.14 in macrophage migration inhibitory factor (glycosylation-inhibiting factor) (Appendix B: Figure 9). The gene expression profile of IAV in patient 2 is depicted in (Appendix A: Table 7).

The infection of patient 3 with IAV showed alteration of gene expression profile represented with 49 (58.33%) up regulated genes (>1 fold change) and 29 (34.52%) down regulated genes (< 1 fold change) (Appendix A: Table 8, Appendix B: Figure 10).

The up regulated 49 genes in this sample gave fold changes ranged between 1.10 in Interleukin 13 receptor, alpha 1 and 4.11 in the Toll interacting protein (Appendix B: Figure 11). The down regulated 29 genes in patient 3 ranged between -19.15 in Chemokine (C-C motif) ligand 11 and -1.04 in ABCF1 (Appendix B: Figure 12). The gene expression profile of IAV infection in patient 3 is depicted in (Appendix A: Table 9).

In the fourth patient 70 (83.33%) genes were up regulated (> 1 fold change) and 11(13.09%) genes were down regulated (< 1 fold change) (Appendix A: Table 5,

Appendix B: Figure 10). The up regulated 70 genes in patient 4 ranged between 1.10 fold change in CD40 ligand and 46.52 fold change with Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (CXCL1) (Appendix B: Figure 14).

The down regulated 11 genes ranged between -1992 fold change in Chemokine (C-C motif) receptor 1 and -1.11 fold change in CCAAT/enhancer binding protein (C/EBP). beta (Appendix B: Figure15). The gene expression profile of IAV in patient 4 is depicted in (Appendix A: Table 11). With patient 5 the IAV infection caused up regulation of 16 (19.04%) genes and down regulation to 66 (78.57%) genes (Appendix A: Table 12, Appendix B: Figure 16). The up regulated 16 genes in patient 5 gave fold change ranged between 1.14 fold with Chemokine (C-C motif) receptor 2 and 90.51 fold with Chemokine (C-C motif) ligand 11 (CCL11) (Appendix B: Figure 17).

The down regulated 66 genes in patient 5 ranged between – 5. 278 fold as in Chemokine (C-X-C motif) ligand 2 (CXCL2). Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating) (SCYE1) and -1.14 fold as in Chemokine (C-C motif) ligand 7 and (Appendix B: Figure 18).

The gene expression profile of IAV in patient 5 is depicted in (Appendix A: Table 13).

The infection of patient 6 with IAV caused up regulation to 27 (32.14%) genes and down regulation of 48 (57.14%) genes (Appendix A: Table 14, Appendix B: Figure 19).

The up regulated 27 genes in patient 6 are ranged between 1.16 fold as in Chemokine (C-C motif) receptor 1and 79.89 fold as in Chemokine (C-X-C motif) ligand 9 (CXCL9) (Appendix B: Figure 20).

The down regulated 48 genes in patient 6 are ranged between 3.94 fold as in Chemokine (C-C motif) ligand 26 CCL26) and 1.13 as in Complement component 4A (Rodgers blood group) (Appendix B: Figure 21).

The gene expression profile of IAV infection in patient 6 is depicted in (Appendix A: Table 15).

IAV infection to patient 7 up regulates 83 (98.80%) genes which represent the largest number of immune-inflammatory up regulated genes within all samples that infected with IAV (Appendix A: Table 16, Appendix B: Figure 22). On the other hand there are no down regulated genes in this sample.

The fold changes of the 83 up regulated genes in patient 7 are ranged between 1.23 fold as in Interleukin 10 receptor, alpha and 68.59 fold as in Chemokine (C-X-C motif) ligand 10 (CXCL10) (Appendix B: Figure 23).

The gene expression profile of IAV in patient 7 is depicted in (Appendix A: Table 17).

The infection of IAV in patient 8 up regulates 38 (45.23%) genes and down regulates 24 (28.57%) genes (Appendix A: Table 18, Appendix B: Figure 24).

The up regulated 38 genes in patient 8 gave fold changes that ranged between 1.10 fold as Chemokine (C motif) receptor 1 and 37.79 fold change as in Interleukin 8 (IL8) (Appendix B: Figure 25). The fold change in the 24 down regulated genes ranged between 1.37 in Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) and 1.11 in Chemokine (C-C motif) ligand 26 (Appendix B: Figure 26).

The gene expression profile of IAV infection in patient 8 is depicted in (Appendix A: Table 19).

4.4.1. The common upregulated genes in IAV infected patients

The infection with IAV caused common upregulation (upregulated in two or more patients) for 22 inflammatory genes in the 8 infected patients but with different frequencies (frequency) ranged between 100% frequency (upregulated in all the infected samples) and 62.5% frequency (upregulated in 5 infected samples). The most prominent and common upregulated genes are 19 genes with fold change between 121.35 and 4.5

(Appendix A, table 28).The remaining genes (IL9, ABCF1 and BCL6) are upregulated with 1.58, 1.45 and 1.01 fold change respectively.

4.4.2. The common downregulated genes in IAV infected patients

The common 31-downregulated genes in all samples infected by IAV also occurred with different frequencies ranged between 87.5% and 25% frequency and with different fold change. The most prominent downregulated genes are 13 with fold change between -250.14 and -2.06 (Appendix A, table 29). The remaining 18 downregulated genes with fold change between -1.9 and -1.11 include IL1F9, LTB3, IL8RA, IL22, CXCL5, SPP1, CCR9, LTA1, IL1F5, CXCL9, IL10RA, IL1R1, MIF, IL9R, CXCL3, CEBPB, IL1F and IL1F6.

The common up and downregulated genes are gathered in one heat map through the clustering of all 84 genes in all 8 infected patients to give a complete image for the inflammatory gene expression profile in the IAV infected leukocyte (Figure 3).

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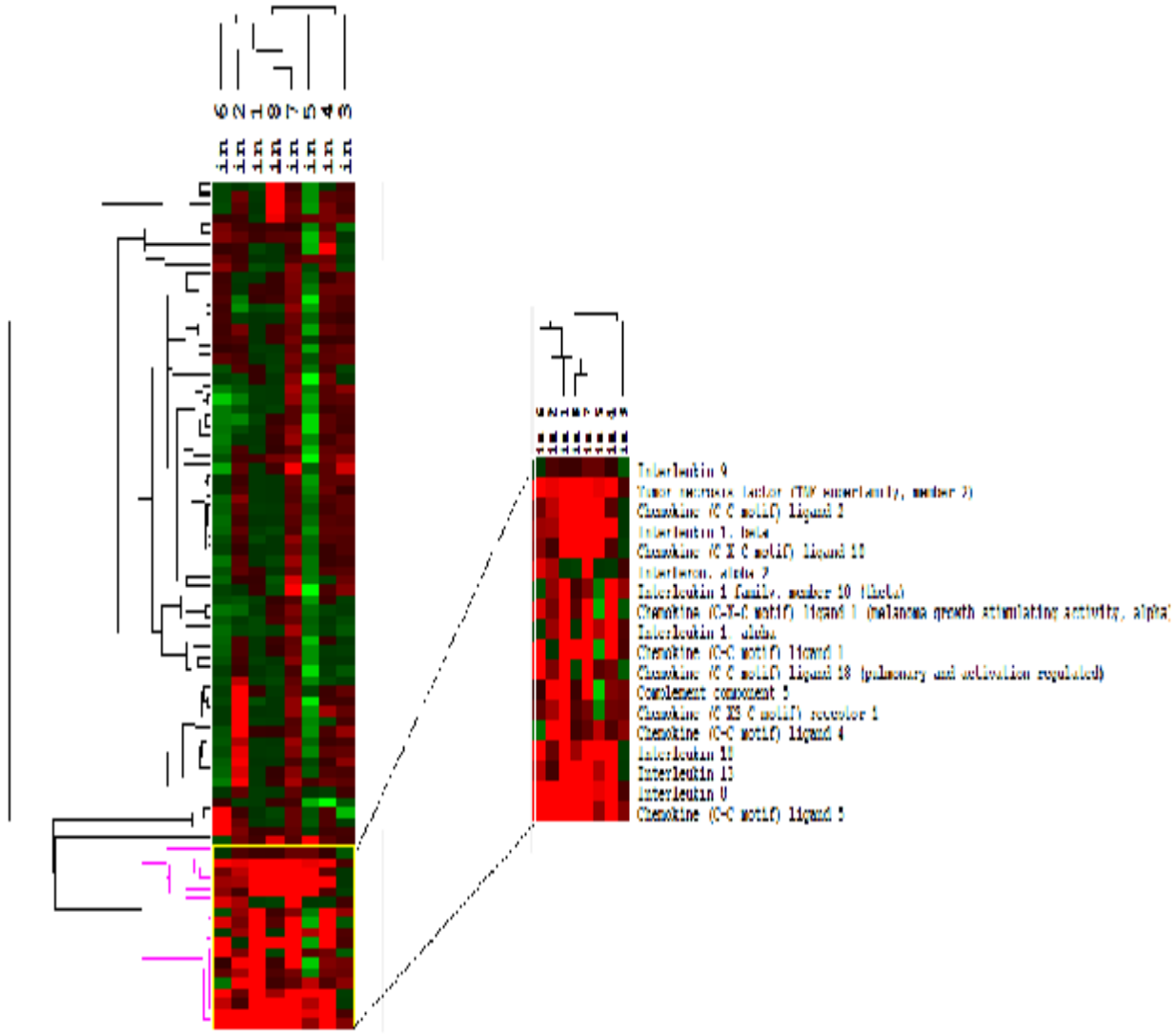

Figure 3: Cluster of 84 immune genes expression in 8 samples of the human leukocytes after IAV infections. The dendogram depicts the 48 inflammatory-gene profile of leukocytes. Each panel represents one patient with 84 genes. The up regulated genes are with red color and the down regulated genes are with green color.

4.5. Leukocyte gene expression analysis after PIV infection:

Parainfluenza virus infected two samples and caused up regulation of 48 (57.14%) genes in patient 1 and down regulation of 20 (23.8%) genes (Appendix A: Table 20, Appendix B: Figure 27). The fold changes of up regulated genes ranged from 1.11 fold as in secreted phosphoprotein 1 and 101.13 fold in Interleukin 1, alpha (IL1A) (Appendix B: Figure 28). The fold change of the 20 downredulated genes ranged between -1.91 in

Chemokine (C-X-C motif) ligand 14 and -1.11 in Chemokine (C-C motif) receptor 6 (Appendix B: Figure 29). The gene expression profile of PIV in patient 1 is depicted in (Appendix A: Table 21). In patient 2 the PIV infection caused up regulation to 75(89.28%) genes and down regulated 7 (8.33%) genes (Appendix A: Table 22, Appendix B: Figure 30).

The up regulated 75 genes ranged between 1.13 fold as in Lymphotoxin beta (TNF superfamily, member 3) and 1428.22 fold as in Interferon, alpha 2 (IFNA2) which represents the most highly up regulated gene in this study (Appendix B: Figure 31).

The down regulated 7 genes in patient 2 after 48 h of PIV infection are ranged between – 37.27 fold as in each of Interleukin 13 receptor, alpha 1 (IL13RA1). Macrophage migration inhibitory factor (glycosylation-inhibiting factor) (MIF) and - 2.67 fold in Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating) (SCYE1) (Appendix B: Appendix B: Figure 32). The gene expression profile of PIV in patient 2 is depicted in (Appendix A: Table 23).

4.5.1. The common up regulated genes in PIV infected patients.

The most prominent and common upregulated genes in the two patients infected with PIV is 25 genes with different fold changes ranged between 752.42 in IFNA2 and 2.22 in CCR7 (Appendix A, table 30). The remaining 23 common upregulated genes with fold change between 1.84 and 1.13 fold include; IL1RA, CCL16, CCL15, IL17C, IL9, CXCL3, C4A, CCL8, IL9R, CCL21, B4R, CXCL9, SPP1, IL8RB, ABCF1, C3, IL1F6, IL1F9, IL5, IL13, CCL23 and IL10RA.

4.5.2. The common downregulated genes in PIV infected patients

The common downregulated genes in the 2 patients infected by PIV are represented by only 3 genes with fold changes ranged between 19.30 for MIF and 5.56 for CEPBP gene (Appendix A, Table 31).

The complete gene expression profile of 84 inflammatory genes in the 2 PIV infected patients gathered in a cluster (Figure 4).

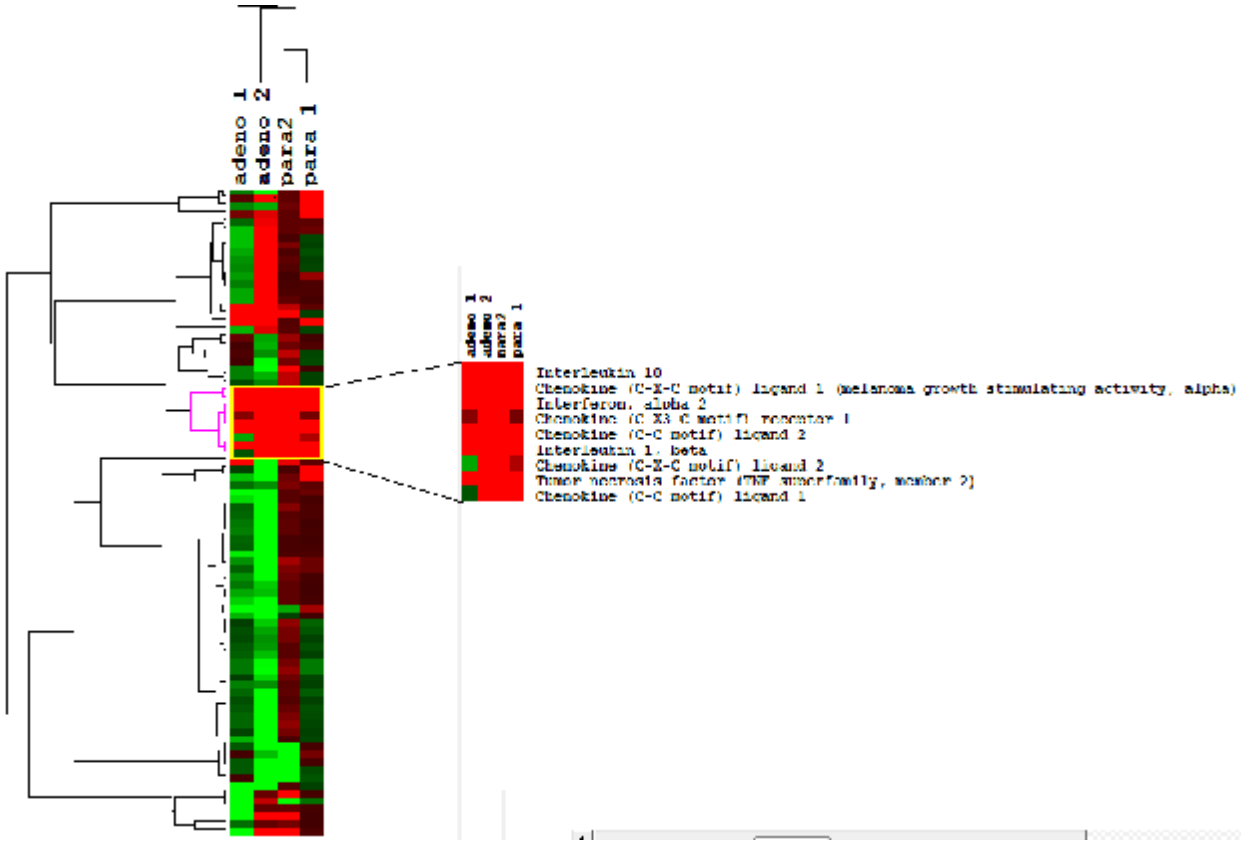

Figure 4: Cluster of 84 inflammatory gene expressions in 2 patients of the human leukocytes after PIV infection and 2 samples infected by Adv. Each panel represents one sample with up regulated genes (Red color) and down regulated genes (Green color)

4.6. Leukocytes gene expression analysis after Adv infection:

Adenovirus infects two samples, in patient 1 the infection up regulated 15 (17.85%) genes and down regulated 61(72.61%) genes (Appendix A: Table 24, Appendix B: Figure 33). The up regulated 15 genes in patient 1 ranged between 1.23 fold as in Chemokine (C motif) receptor 1 and 9.18 fold in Chemokine (C-C motif) receptor 4 (CCR4) (Appendix B: Figure 34). The down regulated 61 genes in patient 1 after 48 h of Adv infection ranged between – 5404 as in Interleukin 8 receptor, beta (IL8RB) and – 1.14 in both Chemokine (C-C motif) receptor 17 (CCR17) and CCL7 (Appendix B: Figure 35). The gene expression profile of Adv in patient 1 is depicted in (Appendix A: Table 25).

In patient 2 the Adv infection up regulated 32 (38.09%) and down regulated 52 (61.90%) after 48 h of infection (Appendix A: Table 26, Appendix B: Figure 33). The up regulated 32 genes in patient 2 after 48 h of Adv infection ranged between 1.53 as in Interleukin 8 receptor, beta and 49.18 as in Interleukin 8 (IL8) (Appendix B: Figure 34).

The down regulated 52 genes in patient 2 after 48 h of Adv infection ranged between– 4039.60 fold in Interleukin 1 family, member 6 (epsilon) (IL1F6) and -2.11in Chemokine (C-C motif) ligand 7 (Appendix B: Figure 35). The gene expression profile of Adv infection in patient 2 is depicted in (Appendix A: Table 27).

4.6.1. The common upregulated genes in Adv infected patients

The common upregulated genes in the 2 AdV infected patients are represented by 11 genes with fold changes ranged between 27.51 in IL8 and 2.6 in the CCL5 (Appendix A, Table 32).

4.6.2 The common downregulated genes in AdV infected patients

The common downregulated genes in the 2 AdV infected patients were 44 genes with different fold changes ranged between -2021.5 with IL1F6 and -1.6 with CCL7. The most prominent and common downregulated genes are 17 genes with fold change between -2021.5 and -4.15 fold (Appendix A, table 33). The remaining 27 common down regulated genes with fold change between -3.8 and -1.6 include; CCL3, IL9R, CCL23, CCL21, ABCF1, CCR6, IL10RA, SPP1, IL17 C, CCR2, CRP, CCL25, C3, IL5RA, CCR8, IL1A, CCL24, CCL11, CXCL14, CCR3, LTB4, CCR1, CCL15, CCR9, CCL4, CCL17 and CCL7. The common up and downregulated genes gathered in on clustering Figure to give complete image for the gene expression profile of the AdV infection (Figure 4).

4.7. The common upregulated genes in all samples infected by the three viruses.

The infection of the leukocytes of the 12 patients by the three viruses caused upregulation for 10 common genes with frequency ranged between 83.33% and 100% (Table 5).

Table 5: The frequency of 10 common up regulated genes in all (12) patients infected with the three viruses (IAV, PIV and Adv) after 48h of infection.

No.	Unigene	Gene name	Frequency/12 patients			Fold change	Total Frequency(%)
			IAV	PIV	Adv		
1	Hs.193717	IL 10	8	2	2	16.14	100
2	Hs.241570	TNF	8	2	2	18.22	100
3	Hs.303649	CCL 2	8	2	2	39.73	100
4	Hs.126256	IL1B	8	2	2	60.94	100
5	Hs.789	CXCL1	8	2	2	26.04	100
6	Hs.78913	C-X3-CR 1	8	2	2	21.16	100
7	Hs.624	IL8	8	2	2	53.02	100
8	Hs.514821	CCL 5	8	2	2	13.51	100
9	Hs.211575	IFNA 2	6	2	2	262.60	83.33
10	Hs.1722	IL1A	6	2	2	34.08	83.33

4.8. The common downregulated genes in samples infected by the three viruses.

The infection of the leukocytes of the 12 patients by the three viruses caused 7 common downregulated genes with different frequencies ranged between 75% and 50% (Table 6).

Table 6: The frequency of 7 common downregulated genes in all (12) patients infected with the three viruses (IAV, PIV and Adv) after 48h of infection.

No.	Unigene	Gene name	Frequency/12 patients			Fold change	Total Frequency (%)
			IAV	PIV	Adv		
	Hs.301921	CCR1	6	---	2	-250.1	66.66
	Hs.407995	MIF	5	2	---	-19.30	58.33
	Hs.247838	CCL24	4	2	----	-10.68	50
	Hs.376208	LTB	4	---	2	-6.86	50
	Hs.517106	CEBPB	7	2	---	-5.56	75
	Hs.414629	CCL13	4	---	2	-5.35	50
	Hs.591680	SCYE1	4	---	2	-4.68	50

The type of viral infections, the number, percentage, and the lower and upper fold changes in all common up and downregulated genes in the 12 patients infected by the three viruses IAV, PIV and AdV were summarized in table 7 and clustered in figure 5 in order to give complete image for the common genes that affected by these respiratory viruses.

Table 7: Type of viral infection and the total number, percentage, lower and upper fold change of up and downregulated genes in all patients infected by the 3 viruses.

Patient Number	Viral infection	Number And % Of upregulated genes	Number Of down regulated genes	Fold change of upregulated gene (gene name)		Fold change of downregulated gene (gene name)	
				lower fold	upper fold	lower fold	upper fold
1	IAV	26(30.95)	23(27.38)	1.13 (BCL6, (CCL7,SCYE1)	1009 (IL10)	-1.16 (CCL26)	-1.5 (IL5)
2	IAV	62(73.80)	15(17.85)	1.14 (IL1F8,CCR9)	27.85 (IL8)	-1.14 (MIF)	-2.80 (IL8R)
3	IAV	49(58.33)	29(34.52)	1.10 (IL13R)	4.11 (TOLLP)	-1.11 (CCL8)	-19.15 (CCL11)
4	IAV	70(83.33)	11(13.09)	1.10 (CD40LG)	46.52 (CXCL1)	-1.11 (CEBPB)	-1992 (CCR1)
5	IAV	16(19.04)	66(78.57)	1.14 (CCR2)	90.51 (CCL11)	-1.14 (CCL7)	-5.27 (CXCL2)
6	IAV	27(32.14)	48(57.14)	1.16 (CCR1)	79.89 (CXCL9)	-1.13 C4A	-3.94 (CCL26)
7	IAV	83(98.80)	----------	1.23 (IL10RA)	68.59 (CXCL10)	--------	-------
8	IAV	38(45.23)	24(28.57)	1.10 (CCR1)	37.79 (IL8)	-1.11 (CCL26)	-1.37 CCL18
1	PIV	44(52.38)	20(23.80)	1.11 (SPP1)	101.13 (IL1A)	-1.11 CCR6	-1.91 (CXCL14)
2	PIV	75(89.28)	7(8.33)	1.13 TNF	1428.22 (IFNA2)	-2.67 (SCYE1)	-37.27 (IL13R1 MIF)
1	Adv	15(17.85)	61(72.61)	1.23 (CCR1)	9.18 (CCR4)	-1.14 (CCR17, CCR7)	-5404 (9IL8RB)
2	Adv	32(38.09)	52(61.90)	1.53 (IL8RB)	49.18 (IL8)	-2.11 (CCL7)	-4039 (IL1F6)

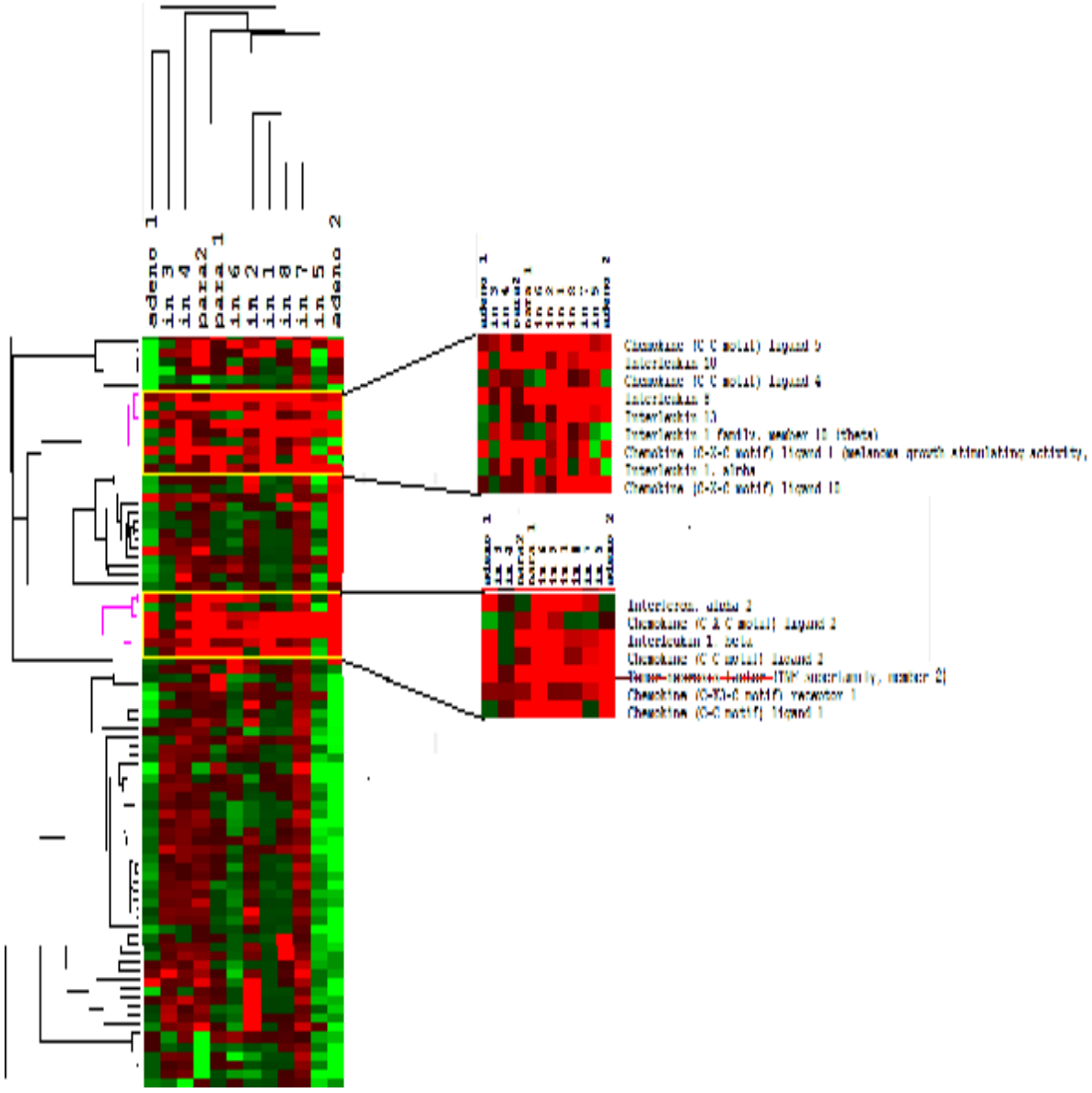

Figure 5: The common up and downregulated genes in all 12 patients infected with the three viruses (IAV, PIV and Adv). The 12 patients are represented by 12 panels, each panel represents I patient with 84 inflammatory genes that affected by the three viruses. Each gene is represented by on rectangular (Red, upregulation and green, downregulated). The upper dendogram represents the clad possibilities of the viruses based on the unity or diversity in their effect on the gene expression. The two branches to the right represent the most common 16 upregulated genes (Red color) in all infected 12 patients based on the fold change as a first respect and on the gene frequency as a second respect.

4. 9. Gene ontology

The infection with the IAV, PIV and Adv caused considerable changes in the gene expression of human WBCs. The most prominent upregulated genes are 19, 25, and 11 for IAV, PIV and Adv respectively. All upregulated genes share common inflammatory functions and play a key role in different inflammatory and cellular pathways that can be determined using gene ontology (GO) with KEGG tool (Grosu *et al*., 2002; Huang *et al*., 2009) for each group of upregulated genes during the infection of each virus or for common upregulated genes in all infected human leukocytes.

4.9.1. Gene ontology for upregulated genes after IAV infection

The infection with IAV stimulates the expression of 22 inflammatory genes. The most prominent upregulated genes are 19 genes which share different important immunological functional pathways represented by cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and Asthma pathway (Table 8).

Table 8: Gene ontology for upregulated genes after IAV, PIV and Adv infections. Each virus upregulated specific number of genes which stimulate specific inflammatory pathways.

Gene pathway	Upregulated genes by IAV infection		Upregulated genes by PIV infection		Upregulated genes by Adv infection	
	Gene No. and (%)	Gene name	Gene No. and (%)	Gene name	Gene No. And (%)	Gene name
Cytokine-cytokine receptor interaction pathway	17/19 (89.5)	CCL1,CCL11, CCL18,CCL2, CCL4, CCL5, CCL7, CXCL1, CXCL10, CX3CR1, IFNA2, IL1A, IL1B, IL8, IL10, IL13, TNF	22/25 (91.7)	CCL1,CCL11, CCL13, CCL18,CCL2, CCL5, CCR2, CCR4, CCR7, CXCL1, CXCL10, CXCL13, CX3CR1, IFNA2, IL1A, IL1B, IL8, IL10, IL10RB, IL22, LTB, TNF	11/11 (100)	CCL2,CCL5, CCR5, CCR7, CXCL1, CX3CR1, IFNA2, IL1B, IL8, IL10, TNF
NOD-like receptor signaling pathway	8/19 (42.1)	CCL2, CCL5, CCL11,CCl7, CXCL1, IL1b, IL8, TNF	8/25 (42.1)	CCL2, CCL5, CCL11,CCLl3, CXCL1, IL1b, IL8, TNF	6/11 (54.5)	CCL2, CCL5, CXCL1, IL1B, IL8, TNF
Toll-like receptor signaling pathway.	7/19 (36.8)	CCL4,CCL5, CXCL10, IFNA2, IL1B, IL8, TNF	6/25 (25)	CCL5,CXCL10 , IFNA2, IL1B, IL8, TNF	5/11 (45.5)	CCL5, IFNA2, IL1B, IL8, TNF
Asthma pathway	4/19 (21.1)	CCL11, IL10, IL13, TNF	3/25 (21.5)	CCL11, IL10, TNF	2/11 (18.2)	IL10, TNF

4.9.1.1. Gene pathway analysis for IAV infection

The IAV infection upregulated 19 inflammatory genes, from which 17 (89%) genes involved in cytokine-cytokine receptor interaction pathways (Figure 6), 8 (42.1%) genes involved in the NOD-like receptor signaling pathway (Figure 7), 7 (36.8%) inflammatory genes which participate in the Toll-like receptor signaling pathway (Figure 8), 4 inflammatory genes involved in asthma pathway (Figure 9).

4.9.2. Gene ontology for upregulated genes after PIVinfection

The infection of PIV activates 48 inflammatory genes. The most prominent upregulated genes are 25 genes which play important role in the immunological functional pathways represented by cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and asthma pathway (Table 8).

4.9.2.1. Gene pathway analysis for PIV infection

The prominent upregulated genes after the PIV infection to human leukocytes were 25 genes. Four groups of these 25 genes stimulate four inflammatory pathways including; 22 (91.7%) genes, activate the cytokine-cytokine receptor interaction pathway (figure 6), 8 (42.1%) genes involved in the NOD-like receptor signaling pathway (Figure 7), 6 (25%) genes involved in Toll-like receptor signaling pathway (Figure 8), and 3(21.5%) genes involved in asthma pathway (Figure 9).

4.9.3. Gene ontology for upregulated genes after Adv infection

The infection with Adv upregulated 11 genes which stimulate four different immunological functional pathways represented by cytokine-cytokine receptor interaction pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and asthma pathway (Table 8).

4.9.3.1. Gene pathway analysis for Adv infection

Adv infection upregulated 11 inflammatory genes in human leukocytes. Four groups from these 11 genes participate in important inflammatory pathways represented by; 11(100%) genes activate the cytokine-cytokine receptor interaction pathway (Figure 6), 6 (54.5 %) genes involved in the NOD-like receptor signaling pathway (Figure 7), 5 (45.5%) genes involved in Toll-like receptor signaling pathway (Figure 8), and 2 (18.2%) genes involved in asthma pathway (Figure 9).

4.9.4. Gene ontology for 10 common upregulated genes after infection of all samples with 3 viruses.

The common upregulated 10 genes after the IAV, PIV and Adv infections stimulate the activity of different immunological functional pathways represented by cytokine-cytokine receptor interaction, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and asthma pathway (Table 9).

Table 9: Gene ontology for 10 common upregulated genes after infection of all samples with 3 viruses. Four groups from these 10 upregulated genes stimulate four inflammatory pathways.

Gene pathway	Gene number	Gene percentage	Gene name
Cytokine-cytokine receptor interaction pathway	10	100	CCL2,CCL5, CXCL1, CX3CR1, IFNA2, IL1A, IL1B, IL8, IL10, TNF
NOD-like receptor signaling pathway	6	60	CCL2, CCL5, CXCL1, IL1B, IL8, TNF
Toll-like receptor signaling pathway.	5	50	CCL5, IFNA2, IL1B, IL8, TNF
Asthma pathway	2	20	IL10, TNF

4.9.4.1. Gene pathway analysis for common upregulated genes after IAV, PIV and Adv infections

The infection of all patients with the 3 viruses stimulates the upregulation of 10 common inflammatory genes. All these genes (10 with 100%) activate the cytokine-cytokine receptor interaction pathway (Figure 6). From 10 genes, 6 (60 %) genes involved in the NOD-like receptor signaling pathway (Figure 7), 5 (50%) genes involved in Toll-like receptor signaling pathway (Figure 8), and 2 (20%) genes involved in asthma pathway (Figure 9).

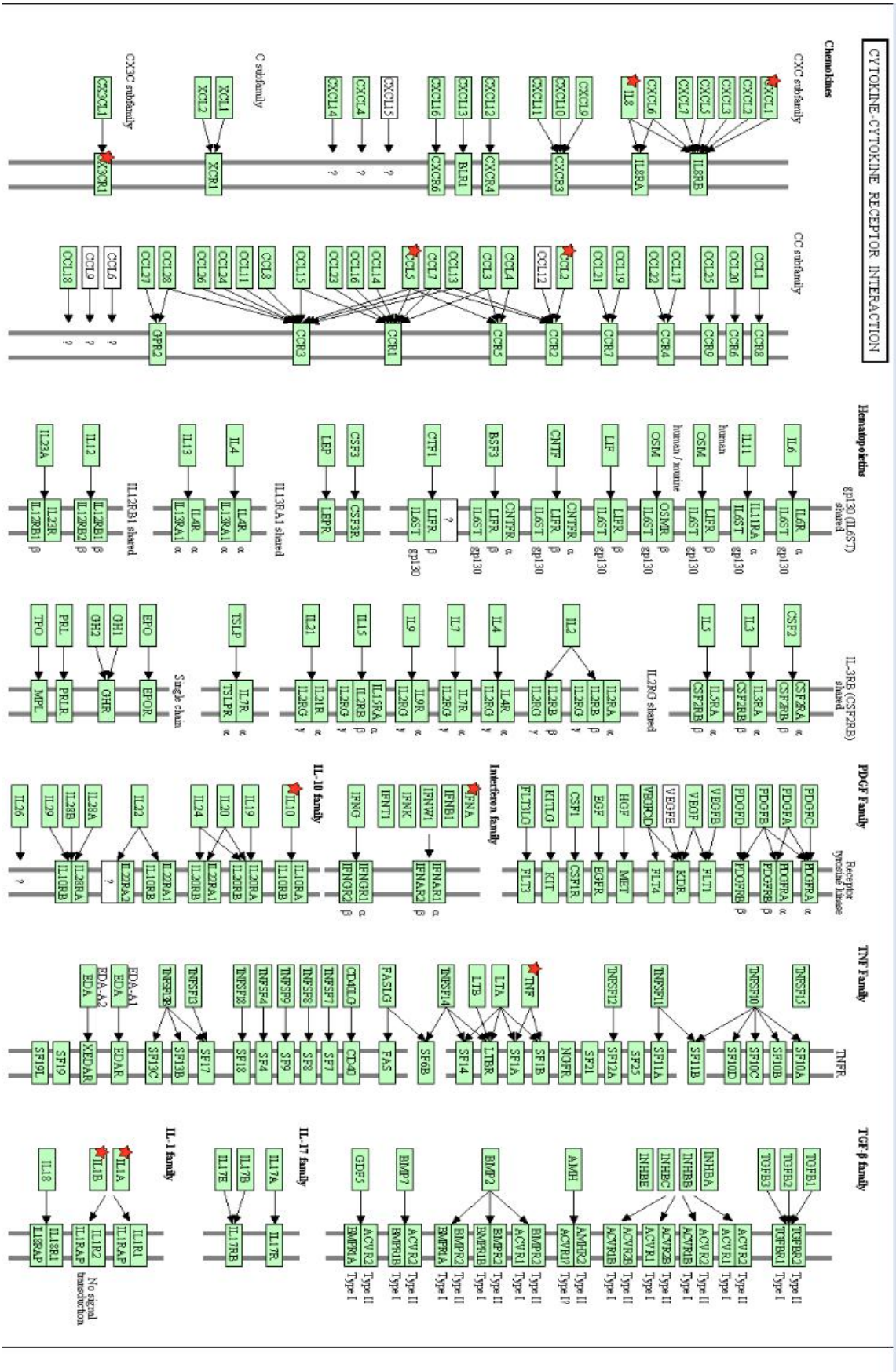

Figure 6: Cytokine-cytokine receptor interaction pathway.

Figure 6 depicted cytokine-cytokine receptor interaction pathway. The 17 upregulated genes during IAV infection that produce cytokines which play crucial intercellular regulators and mobilizers of cells engaged in innate as well as adaptive inflammatory host defenses. These cytokines (red stars) induce responses through binding to specific receptors on the cell surface of target cells. These bindings then stimulate various signaling pathways end up with activation of many transcriptional factors that stimulate large number of inflammatory genes which act against the invading pathogens.

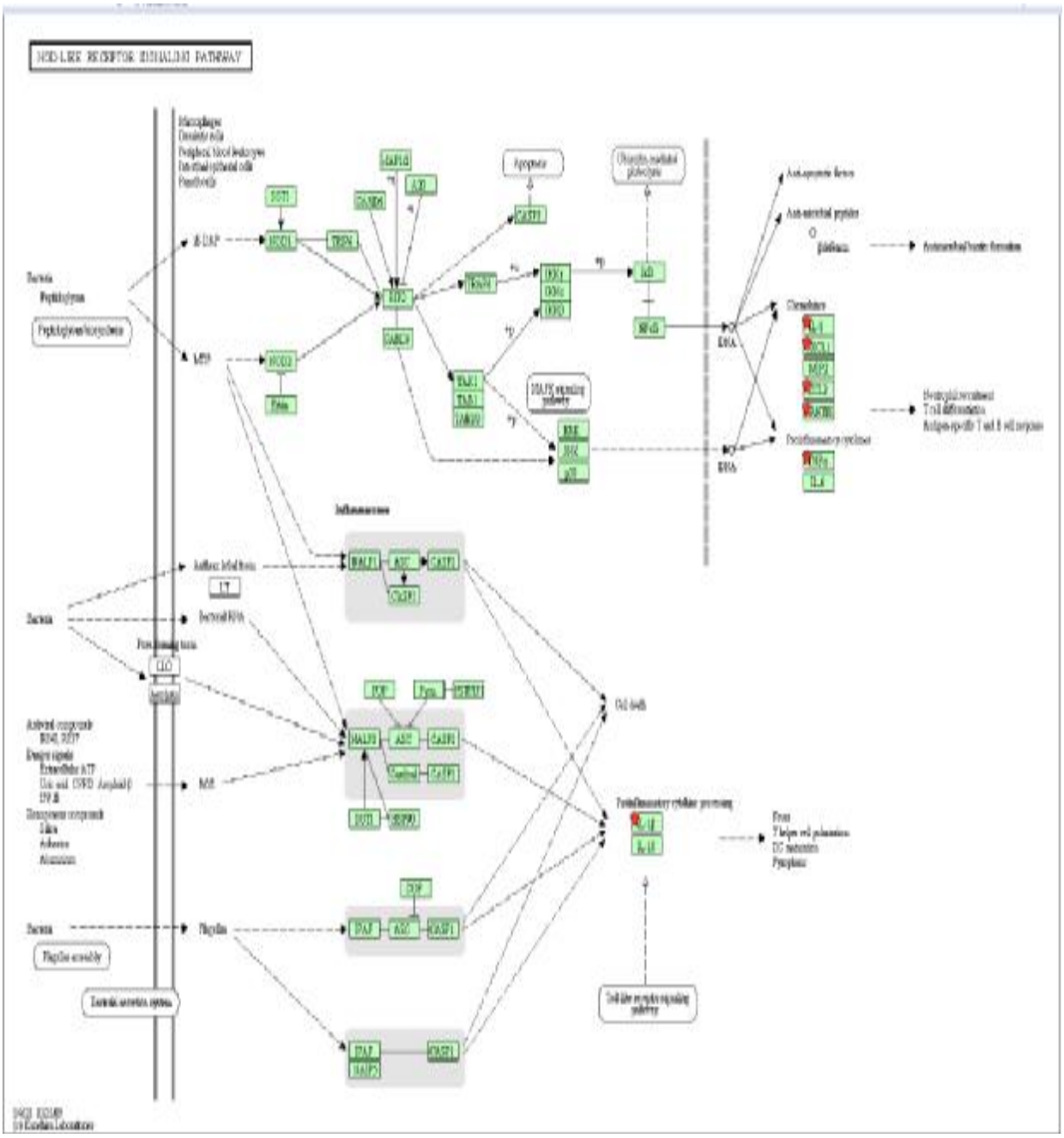

Figure 7: NOD-like receptor signaling pathway
The infection of IAV stimulates upregulation of 8 inflammatory genes (red stars) which activate specific families of pattern recognition receptors that are responsible for

detecting various pathogens and generating innate immune responses. The intracellular NOD-like receptor driving the activation of NF-kB and MAPK, cytokine production and apoptosis. On the other hand, they induce caspase-1 activation through the assembly of multiprotein complexes called inflammasomes. The inflammasomes are critical for generating mature proinflammatory cytokines in concert with Toll-like receptor signaling pathway.

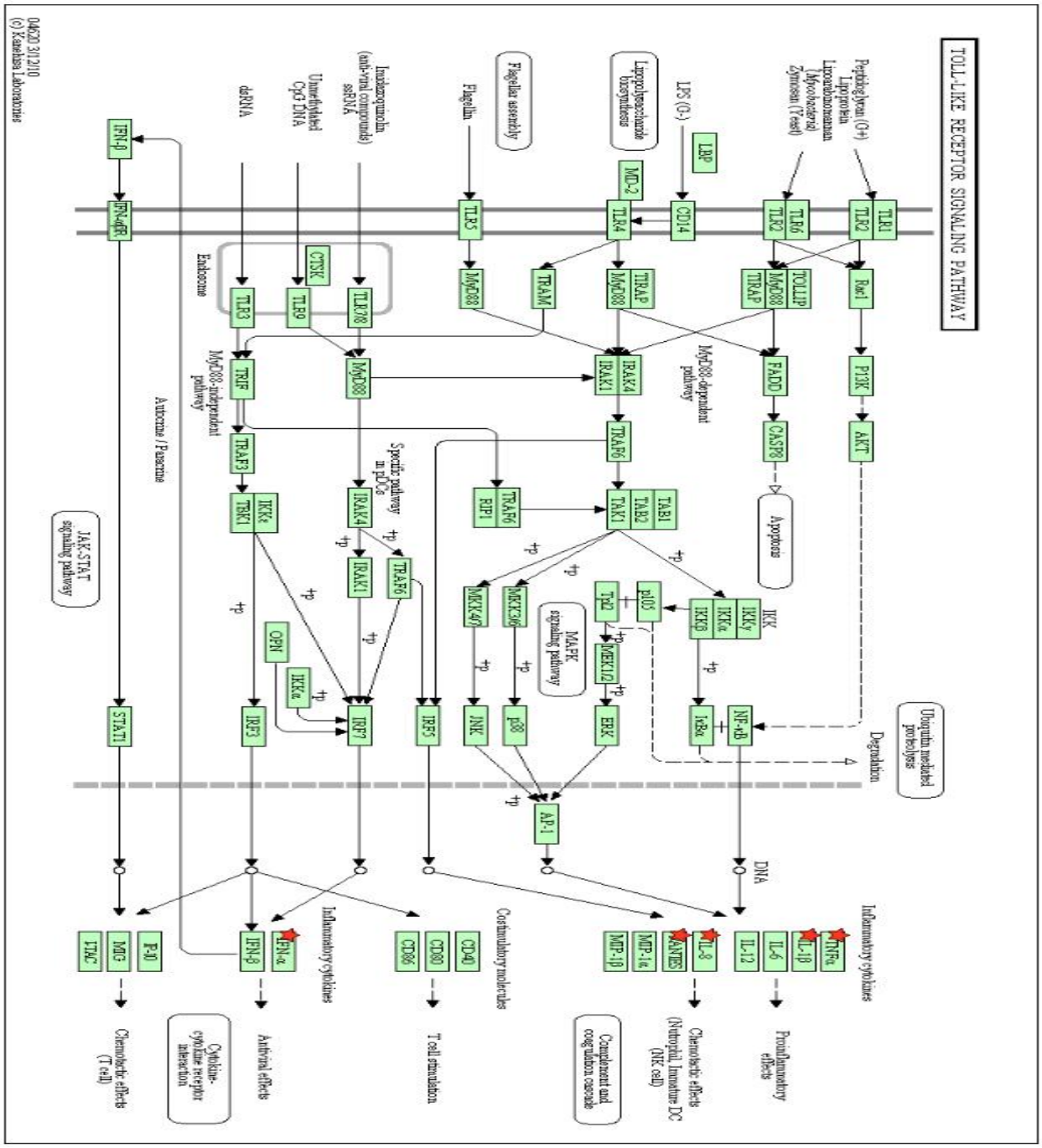

Figure 8: Toll-like receptor signaling pathway.
The IAV infection upregulates 7 inflammatory genes (red stars) involved in the activation of Toll-like receptors (TLRs). TLRs are specific families of pattern recognition receptors that are responsible for detecting microbial pathogens and generating innate immune

responses. Pathogen recognition by TLRs provokes rapid activation of innate immunity by inducing production of proinflammatory cytokines and upregulation of costimulatory molecules. TLR signaling pathways are separated into two groups: a MyD88-dependent pathway (Myeloid differentiation primary response gene-88) that leads to the production of proinflammatory cytokines with quick activation of NF-kB and MAPK, and a MyD88-independent pathway associated with the induction of IFN-beta and IFN-inducible genes, and maturation of dendritic cells with slow activation of NF-kB and MAPK.

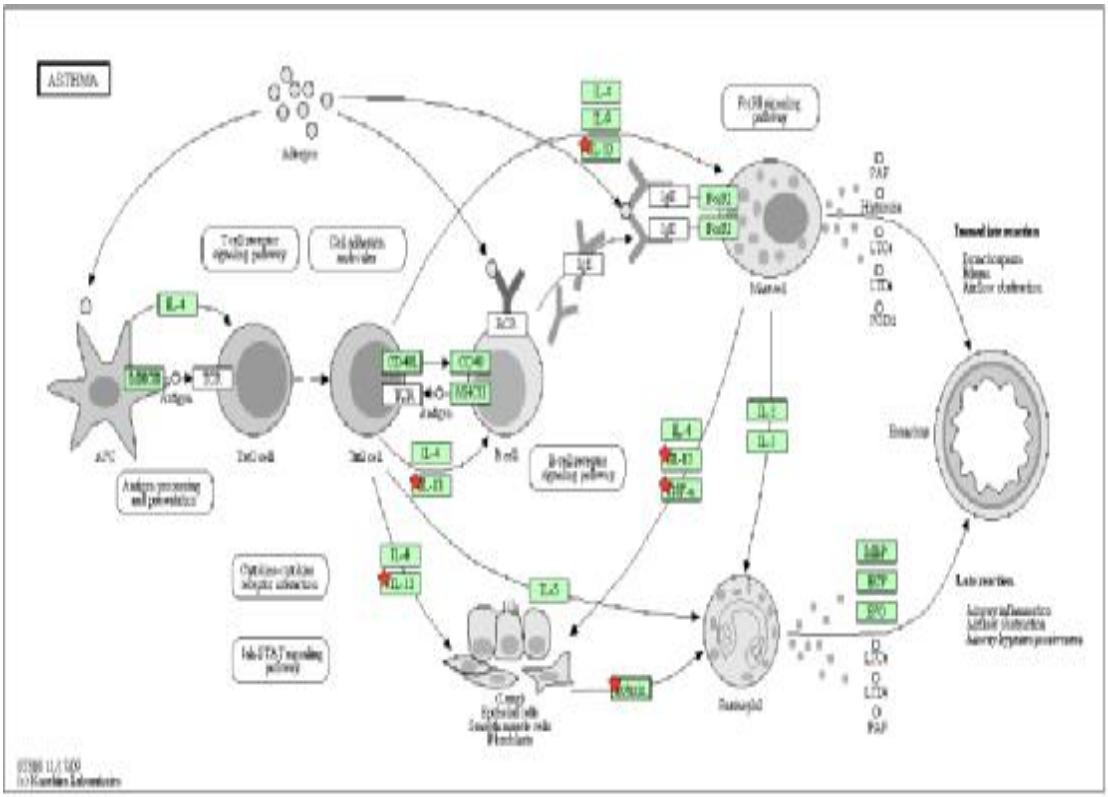

Figure 9: Asthma pathway

Viral infection encounter antigen presenting cells (APC) that line the airway. Upon recognition of the antigen and activation by APC, naive T cells differentiate into Th2 cells, a process that is promoted by interleukin 4 (IL-4). Activated Th2 cells stimulate B cells to produce IgE antibodies in response to IL-4 and IL-13 (red stars). IgE binds the high affinity IgE receptor at the surface of mast cells, the proliferation and differentiation of which is promoted by IL-9. The crosslinking of mast-cell-bound IgE by pathogen leads to the release of biologically active mediators (histamine, leukotrienes) by means of degranulation and, so, to the immediate symptoms of allergy. Activated mast cells and Th2 cells also induce the production of IL-5. IL-5 travels to the bone marrow and regulates the differentiation and egress of eosinophiles from the bone marrow into the blood. Moreover activated mast cells and Th2 cells in the lung generate the cytokines interleukin IL-4, IL-13 and tumor necrosis factor (TNF)-alpha. These cytokines stimulate the generation of eotaxin (CCL11) by lung epithelial cells, fibroblasts and smooth muscle cells. Eotaxin then stimulates the selective recruitment of eosinophiles from the airway microvessels into the lung tissue. The activation of eosinophiles leads to release of toxic granules and oxygen free radicals that lead to tissue damage and promote the development of chronic inflammation.

5. Discussion

Respiratory viral infections frequently cause worldwide epidemics. Each year, viral epidemics inflict an enormous number of human diseases, which results in tremendous economic cost both due to admissions to hospitals and loss of productivity (Huang*et al*., 2010). Host innate immunity is the first line of protection against infection by virus and is essential in local control of invading microbes. The innate immune system is composed of macrophages, natural killer cells and dendritic cells which play crucial roles in the initiation and subsequent production of the adaptive immune response, as well as in promoting localized inflammation and producing cytokines that recruit additional leukocytes to the site of infection. In this study three respiratory viruses (IAV, PIA and Adv) were investigated to study their effect on 84 inflammatory genes of human leukocytes using RT-PCR-Array kit (SABioscience Company) (Campeau*et al*., 2009; Myskiw *et al*., 2009).

5. 1. Effect of IAV, PIV and Adv infections on leukocytes gene expression

This study examines the interaction of IAV, PIV and Adv with human leukocytes gene expression after 48h of IAV infection. The infection with these 3 viruses induces a strong pro-inflammatory cytokine and a leukocyte chemokine response in human leukocytes, as shown by RT-PCR-Array. Recent study showed that the infection of human lung and trachea with IAV stimulates higher expression of genes related to respiratory immune system and innate response (Reemers*et al*., 2010). This result is in agreement with this study which revealed that the leukocytes-infection by IAV, PIV and Adv causes considerable changes in leukocytes-gene expression profile.

5. 1. 1. The common upregulated genes after IAV, PIV and Adv infections

The response of human leukocytes to IAV, PIV, and Adv infections includes the induction of different cytokines, chemokines and other inflammatory genes. The upregulation of most inducible genes in this study were proinflammatory genes which stimulated at initial infection. These results are in agreement with a recent study showed that the infection of respiratory cells lead to increase the production of proinflammatory genes and apoptosis genes (Geiler *et al*., 2010). The early cellular response in this study (24- 48 h), suggests that the expression of these genes may initiate the cascades of an antiviral responses that result in upregulation of different inflammatory genes. Some of these upregulated genes cause asthma complication with IAV infection and play a key role in the cytokine-cytokine receptor interaction pathway (Figure 6) (Huang*et al*., 2009).

The infection with IAV, PIV and Ad viruses caused common upregulation of 10 genes with high frequencies (8.33% to 100%) and with fold change ranged between 13.51 fold for CCL5 and 262.6 fold for IFNA2 (Table 5 and Figure 3). The most upregulated gene is IFNA2 (262.6 fold, $P < 0.003$). Interferon alpha 2 upregulated with high frequency (83.33%) in all infected patients. IFNA2 acts as; a natural cell-signaling protein produced by the cells of the immune system in response to the presence of double-stranded RNA, a key indicator of viral infection (Kotenko*et al*., 2003). For IAV infection recent study showed that infection with IAV stimulates the upregulation of IFN which give a protective role against viral infections (Huang*et al*., 2010). IFN production also increased significantly in most vertebrates after the infection of IAV (Dillon, and Runstadler, 2010).

Another recent study revealed that the infection of mouse with IAV cause upregulation of 46-interferon induced genes in addition to other cytokines (Novoselova*et al*., 2010). In the present study the mRNA transcription of IFNA2 upregulated in 37.5% (25.95 fold;*P* < 0.003) of the patients after 48 h of IAV infection. Similar results were recorded previously where IFNA2 is highly upregulated in viral infections and stimulates gene expression in NK cells and antigen processing and induces hundreds of IFN stimulating genes (ISGs) which activate antiviral state in the infected cells (Matikainen*et al*., 2001; Dalod *et al*., 2002; Sato *et al*., 2000).

The infection of PIV also stimulates the upregulation of IENA2 gene which degrades the mRNA in host cell and blocks the spreading of PIV infection in the body, sometimes at the expense of accelerating the death of the infected cells. When IFN is released by infected cells it induces an antiviral state in surrounding uninfected cells, thereby making it difficult for PIVto spread from the initial foci of infection (Andrejeva*et al*., 2002).

For the effect of Adv infection on IFNA2, the upregulation of interferon, alpha2-mRNA with high significance (*P* < 0.003) induces several antiviral proteins, including protein kinase R (PKR) and oligo-adenylate synthetases (OAS). The activation of PKR leads to general inhibition of protein synthesis and replication of the invader virus. OAS activation leads to degradation of host cell mRNAs and viral RNA (Bone*et al*., 2005). Recent studies showed that Adv infection to human cells induces the expression of IFN type 1 with high significance (Kahl*et al*., 2010; Hutnick *et al*., 2010).

Interleukin 1-B cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. It activates IL-1R type 2 which in turn stimulates IL-2 release, B-cell

maturation and proliferation, and fibroblast growth factor activity (Stuart and Nancy, 2001). The high significant mRNA expression (60.94 fold;$P < 0.002$) of IL1B gene in all infected patient with high frequency (100%) in this study suggests its immune role against the IAV, PIV and Adv by activation of many immune response activities which may lead to asthma symptoms. For the effect of IAV on IL1B level, previous study showed that IL1B and IL6 cytokine levels were upregulated during the IAV infection (Adams *et al*., 2010).

On the other hand PIV infection stimulates the IL1B cytokine production by activated macrophages as a proinflammatory protein. This cytokine is released during inflammation of the central nervous system (Asadullah*et al*., 2003). In the Adv infected patient the IL1B mRNA-transcription is upregulated in leukocytes to counteract Adv by cooperative action with IL-6, IL-8, and IFN-α as recorded by a previous study (Higginbotham*et al*., 2002).

Interleukin 8 cytokine is upregulated in all samples that infected with the 3 viruses with 100% frequency and high significance (53.02 fold;$P < 0.002$). The activation of IL8 was found to cause bronchiolitis and ends with asthma (Huang*et al*., 2009). Another study showed that IL8 is upregulated after IAV and then stimulates the innate immune system response and may cause asthma complication (Utgaard*et al*., 1998).

PIV infection upregulates the IL8 gene which in turn activates immune system processes to overcome the PIV infection and participates in the NFkB activation pathway that activates many transcriptional factors in nucleus and thereby stimulates various inflammatory gene expressions (Weiss*et al*., 2004). Another study also showed expression of IL8 after PIV infection and may activate immune system processes to overcome the viral infection (Young*et al*., 2006).

During the Adv infection a previous study used array analysis found that Adv infection *in vitro* also induced over expression of IL-8 genes (Scibetta *et al*., 2005).

CCL 2 is upregulated with high significance (39.73 fold; $P < 0.002$) in all patients (100% frequency) that infected with the three viruses. The CCL2 has direct and indirect effect on immune responses toward viral infections through its functions as a chemotactic factor for monocytes and basophiles. It activates CCR2 which in turn acts as a receptor for the monocyte chemoattractant proteins (MCP-1, MCP2, MCP-3 and MCP-4) (Joshua *et al*., 2006). CCL2 transcription is also upregulated in human monocyte culture after IAV infection (Bubfeld *et al*., 1998).

For PIV another studies showed that the expression of CCL2 chemokine was increased after infection with PIV and other respiratory viruses (Jacob *et al*., 2008). The infection with Adv also stimulates the upregulation of CCL2 mRNA-expression significantly and induces leukocyte motility. It stimulates the migration primarily of monocytes and T cells to infected sites (Zlotnik *et al*., 2006). Another study showed that systemic injection of adenovirus vectors into mice increases plasmalevels of proinflammatory CCL2 which is a potent inducer of dendritic cell maturation (Bernt *et al*., 2005).

In this study the interleukin-1A is upregulated with high significance (34.08 fold; $P < 0.003$) in 83.33% frequency. Interleukin-1A is a member of the IL-1 family which seems to participate in the pulmonary immune response against pathogens (Rosseau *et al*., 2007). IL1A pulmonary concentration was also upregulated and elevated in IAV (H1N1 A/Puerto Rico/8/34) after mice infection (Aleksandr *et al*., 2005). Another study showed that the IL1A is significantly upregulated and increased in the broncheoalveolar fluid (BALF) of IAV asthmatic patients (Montes *et al*., 2006).

The cytokine IL1 A is upregulated and increased in A549 cells after PIV infection. High level of IL1A increases the activity of class I MHC which enhances the immune response against PIV (Garofalo *et al*., 1996). Adv infection also activates the expression of IL1 which acts against the Adv infection at initial stage of infection (Higginbotham *et al*., 2002).

CXCL1 chemokine upregulated in this study in all patients (100% frequency) with high significance (26.04 fold;$P < 0.002$). The function of CXCL1 chemokine gene (the melanoma growth stimulatory activity/growth-regulated protein) is to regulate the cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane G protein-coupled receptors. It also plays fundamental roles in the development, homeostasis, and function of the immune system (Huang *et al*., 2009). Previous studies reported that CXCL1 acts through G-protein to activate IL-6 gene expression since the viral infection induce upregulation of IL-6 and IL-8 gene expression which are positively correlated to the onset of acute inflammatory pain and lead to asthma complication (Wang *et al*., 2009; Papdopoulos *et al*., 2001). In another study, the CXCL1 showed high levels of expression (30.5-fold) during IAV infection (Crompton *et al*., 1988). The results of these studies are consistent with the findings of these studies especially in the highly upregulation of CXCL1 after IAV infection and the presence of asthma complication signs and symptoms in some of the patients.

Another study showed that the infection with PIV and other respiratory viruses increase the expression of these CXCL1 after 24 h of infection with PIV (Jacob *et al*., 2008). In previous study the Adv infection increases the mRNA transcript of the Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha). This increase may enhance its ability to specifically recruit polymorphonuclear leucocytes (PMNLs) into inflamed tissues (Constantin *et al*., 2000).

CX3CR1 is upregulated with high significance (21.16 fold; $P < 0.006$) in all patients (100% frequency). CX3CR1 (chemokine fractalkine) gene has two important functions, one acts as a mediator for both adhesive and migratory leukocytes to activate endothelial cells, where it is primarily expressed and second acts as a co-receptor for viral envelope protein (Huang *et al*., 2009). IAV infection stimulates the mRNA expression of this gene which may interact with IAV invasion and mediates different immune processes and facilitate the recognition and binding with the IAV after invading the host cell (Matloubian *et al*., 2000). The PIV infection also upregulates CX_3CR1 significantly, this chemokine acts as a receptor for CX3CL1 (fractalkine). The binding of this receptor with its ligant induce leukocyte chemotaxis and displaying a range of CX3C biological activities (Tripp *et al*., 2001). A previous study also revealed that Adv infection caused high increase in CX3CR1 gene expression which plays a role in adaptive immunity and participates in the response to immune activities and viral inflammation (Ronald, 2003).

TNF is upregulated in this study with high significance (18.22 fold; $P < 0.002$) in all infected patients (100% frequency). This gene encodes a multifunctional proinflammatory cytokines through binding to its receptors TNFRA and TNFRB leading to the translocation of transcriptional factor NF-kB to the nucleus which turns on the transcription of more than 60 known genes that participate in activation of proinflammatory activities and apoptosis of infected cells (Mehta *et al*., 2003). It was recorded previously that TNF mRNA was significantly increased by infection of influenza virus and Sendai virus (Zhu *et al*., 2010; Veckman *et al*., 2006). Recent study also showed that IAV infection to human cells cause upregulation of TNF and IL6 at the first 24 h of infection (NG *et al*., 2010).

PIV infection stimulates TNF as a major pro-inflammatory cytokine that plays important roles in clearing virus infection through inflammatory responses (Lin *et al*., 2007). Another study showed that PIV induces expression of TNF in high rate after PIV infection (Bienhof *et al*., 2002).

Recent study performed on Adv infection showed that the TNF mRNA-transcription is significantly upregulated at initial period of Adv infection and stimulates further inflammatory pathways (Hutnick *et al*., 2010). TNF also creates signal transduction pathways and lead to the activation of transcription factors, such as NF-kB that induce secretion of inflammatory cytokines like IL-6, TNFα, MIP1α, MIP1β and IL-1β (Sallusto *et al*., 2000).

Interleukin-10 cytokine upregulated with 16.14 fold ($P < 0.002$) in all infected patients (100% frequency). The upregulation of this potent anti-inflammatory cytokine may be induced by the invading virus to make use of its anti-inflammatory function and to provide a safe environment for viral replication. IL10 represses the expression of TNFα, IL6 and IL1 by activating macrophages. Recent study reported that IL10 appears to play a detrimental role during host response to acute influenza virus infection (Keer *et al*., 2008). Another study showed a significant increase in IL10 mRNA expression in virus infection accompanied with acute asthma, but not observed in virus infection with out asthma (Grissell *et al*., 2005).

Previous studies showed that PIV infection also stimulates high expression of Interleukine-10 and activates signal transducer activity to stimulate growth factor binding and cytokine binding (Sieg *et al*., 1996; De Waal *et al*., 1991).

Another published work revealed a wide involvement of interleukin 10 to prevent viral infections and produce regulatory cells that are involved in protection against allergic diseases (Mege *et al*., 2006).

CCL 5 is upregulated in all patients (100% frequency) infected with the IAV, PIV and Ad viruses with high significant (13.51 fold; $P < 0.05$). Previous studies showed that CCL5 is upregulated and accumulated in respiratory secretion after 24 h of IAV infection (Brydon *et al*., 2003; Cully *et al*., 2006). Recent study also showed that the infection of A549 cells with IAV stimulates the upregulation of CCL5 and other cytokines (Geiler *et al*., 2010). For PIV infection another study showed that the expression of CCL5 chemokine was increased after infection with PIV (Jacob *et al*., 2008).

Recent study showed that Adv infection stimulates the expression of chemokine (C-C motif) receptor 5 gene which play a crucial role to accelerate recruitment of memory $CD8^+$ T cells to the lung airways during virus infection (Jacob *et al*., 2008). Another previous study showed that Adv infection activates chemokine (C-C motif) ligand 5 mRNA-expression significantly in HeLa and A549 cell lines (Borgland *et al*., 2000).

5.1.1.2. Gene ontology

The function of gene ontology (GO) is to classify functional categories based on co-occurrence with sets of genes in a gene list and rapidly unraveling new biological processes associated with cellular functions and pathways. Gene ontology mainly provides typical batch annotation and gene analysis to highlight the most relevant GO associated with a given gene list. Also GO provides investigators with much more power to analyze their genes using many different biological aspects in a single space (Huang *et al*., 2009)

Gene ontology uses a novel algorithm to measure relationships among the annotation terms based on the degrees of their co-association genes to group the similar, redundant, and heterogeneous annotation contents from the same or different resources into annotation groups. This reduces the burden of associating similar redundant terms and makes the biological interpretation more focused in a group level. The tool also provides

a look at the internal relationships of the clustered terms by comparing it to the typical linear, redundant term report, over which similar annotation terms may be distributed among hundreds or thousands of other terms (Dennis *et al*., 2003).

Currently the GO covering various categories, protein-protein interactions, protein functional domains, disease associations, bio-pathways, sequence general features, homologies, gene functional summaries, and gene tissue expressions. In addition, to take full advantage of GO this method provides the most possible and well-known pathways and can display genes from a user's list on pathway maps to facilitate biological interpretation in a network context (Huang *et al*., 2009)

5.1.1.2.1. Gene ontology for upregulated genes after the infection with IAV, PIV and Adv.

The analysis of upregulated genes after the infection with IAV, PIV and Adv provides many scientific facts about the nature of cellular response against viral infections. In this study the analysis of upregulated genes using GO give important information about the molecular genetic pathway response of human leukocytes toward these three viruses. This cellular response (represented by upregulated genes) is the main informative source to enrich the scientific knowledge for gene therapy, viral vaccine, pathogenesis and immune-inflammation mechanisms (Ashburner *et al*., 2000).

The infection of the human leukocytes with IAV, PIV and Adv lead to common upregulation of 10 inflammatory genes. These genes stimulate the activity of four different immunological functional pathways represented by cytokine-cytokine receptor interaction pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and asthma pathway (Table 10) (Huange *et al*., 2009). Thus the same four inflammatory pathways are activated by IAV, PIV and Adv but with different number of upregulated genes for each virus (Table 8).

The cytokine-cytokine receptor interaction pathway is activated by the IAV, PIV and Ad viruses (Figure 6). The first step in this pathway is the stimulation of many CXC-chemokines family including; CXCL1, IL8, and CXCL10. All these CXC-chemokines in turn activate different inflammatory receptors (IL8RB, IL8RA, CXCR3 and CX3CR1). IL8RA and IL8RB are powerful chemotactic factors that stimulate the migration of many immune cells. CXCR3 function is to mediate the proliferation of human mesangial cells. The function of CX3CR1 is to mediate both adhesive and migratory function. On the other hand the IAV, PIV and Adv infections also activate the CC-chemokines family member represented by CCL1, CCL2, CCL4, CCL5, CCL7, CCL11 and CCL18. These chemokines in turn activate different inflammatory receptors (CCR1, CCR2, CCR3, CCR5 and CCR8). CCR1 activates stem cell proliferation, CCR2, CCR3 and CCR5 function is transduction of a signal by increasing the intracellular calcium ions level to control granulocytes proliferation. CCR8 function is to regulate monocytes chemotaxis and thymic cell line apoptosis (Denni*set al*., 2003).

The infection with the IAV, PIV and Ad viruses also activate the hematopoietins (IL13) cytokine which activates IL4RA1 and IL13RA1. Both of these receptors promote Th2-proliferation. The IFNA is also activated by the three viruses within the cytokine-cytokine receptor interaction pathway. The IFNA activation stimulates IFNAR1 and IFNAR2; both of these receptors trigger tyrosine phosphorylation of a number of proteins including JAKs, TYKs and STAT proteins and IFNRs themselves.

The IAV, PIV and Adv infections also stimulate the IL10 (IL10-family) in this pathway. IL10 in turn activates IL10RA and IL10RB; both of them stimulate a high level of IL10 expression in monocytes, B-cells, large granular lymphocytes and T-cells.

The TNF (TNF-family) also activated during the infection with IAV, PIV and Adv. This activation of TNF stimulates two TNF-receptors; TNF-supper family 1A (SF1A) and TNF-supper family 1B (SF1B), both of them regulate TNFA function by antagonizing its biological activities. At the late stage of cytokine-cytokine receptor interaction pathway, the infection with IAV, PIV and Adv stimulate the expression of TGF-B family (IL-1 family) including IL1A and IL1B, both of them activate the IL1R1 and IL1RAP. These receptors then stimulate the activity of NF-kB signaling that activates various inflammatory cytokines such as IL1AP and TOLLIP (Huange*et al*., 2009).

The NOD-like receptor signaling pathway (nucleotide-binding oligomerization domain containing-1) enhances caspase-9 mediated apoptosis and induces NF-kB. The infection with IAV, PIV and Adv stimulate the production of inflammosomes caspase-1which cleaves IL1B cytokine. Interleukin 1B cytokine then involved in a variety of inflammatory processes important for defense against pathogens. This activity leads to form fever, T- helper cell polarization, DC maturation and pyroptosis.

On the other hand the IAV, PIV and Adv activate many anti-apoptotic factors, antimicrobial peptides represented by IL8, CXCL1, MIP2, CCL2, CCL5 (RANTES) and pro-inflammatory cytokine (TNF A). The first function of these cytokines is the stimulation of expression of different molecules (mostly chemical compound) like beta-defensin which leads to antimicrobial barrier formation. The second function of these cytokines is the activation of neutrophile recruitment, T-cell differentiation and antigen-specific T and B-cell response (Huange*et al*., 2009).

The Toll-like receptor pathway is also stimulated by the IAV, PIV and Adv infections. This pathway involved in antiviral, antibacterial and anticancer activities. It belongs to interferon family (IFN beta-1 fibroblast). The function of Toll-like receptor pathway is mediated by JAK-STAT pathway. Once IAV, PIV and Adv infect the host, many JAK-STAT pathways are activated and lead to stimulate different transcriptional factors (TFs) represented by; NF-kB, IKB- alpha (inhibitor kappa-B- alpha). ERK, P38 (mitogen-activated protein kinases, IRF3, IRF5 and IRF7. These TFs then activate various processes including; first, the activation of inflammatory cytokines such as TNF-alpha, IL1B, IL8, CCL5 (RANTES), and MIP-1B. All these inflammatory cytokines then activate proinflammatory effects against viruses and activate chemotactic effects for neutrophile, immature DC, NK cells and complement compound cascade.

Second, they activate IFN-A which causes antiviral effects through cytokine- cytokine receptor interaction pathway, third, they activate IP-10 which stimulates chemotactic effects for T-cells (Huange *et al*., 2009; Dennis *et al*., 2003).

The asthma pathway occurs either due to microbial infections or by allergens. The viral infections with IAV, PIV and Adv stimulate APCs to bind to viral antigen with the presence of MHC-2. The APC then bind to T-cell receptor on Th0-cell (naïve T-cell) which become active and differentiated to Th2-cell. Th2-cell then produces IL13 which is first activates B-cell to bind to viral antigen and produce specific antibodies against it. Second, IL13 stimulates the epithelial cells in the lung to produce eotaxin (CCL11) which in turn activates the migration of eosinophile to bronchus and cause air way inflammation, air flow obstruction and air way hyper-responsiveness as a late reaction.

At the same time Th2 cell also produces IL10, which activates mast cells to produce histamine. Histamine level then increased in bronchus and causes bronchospasm, edema

and air flow obstruction as intermediate reaction. The activated mast cell also produces TNF-alpha which stimulates epithelial cells again to express eotaxin (CCL11) which activates eosinophile migration to bronchus and causes a late reaction as mentioned above (Huange *et al*., 2009).

5.1. 2. The common downregulated genes after IAV, PIV and Adv infections

The most common down regulated genes in all 12 samples after IAV, PIV and Adv infections are 7 inflammatory genes with different frequencies ranged between 50% and 75% frequency. The downregulation of these genes means that the three viruses have considerable effects on infected leukocytes and this result comes in agreement with another recent study showed that the IAV-human infection cause vital effect on cell and the viral-host relationship is explained as crucial effect on Host (Burioni *et al*., 2010).

IAV and Adv stimulate significantly the downregulation of CCR1 gene (Table 6) compared with the control cells. CCL4 (MIP-1β) and CCL5/ RANTES signaling is mediated via CCR1 and CCR5. Another study showed that IAV activates the expression of CCR1 but its function was insufficient to induce chemokine response of human monocytes during influenza A infection (Salentin *et al*., 2003). In another study, airway smooth muscle cells showed a significant level of CCR1 mRNA and protein, both *in vitro* and *in vivo* (Joubert *et al*., 2008). In this study the CCR1 mRNA transcription level showed a highly significant downregulation (-250.14; $P < 0.005$) in 75% of the infected patients.

Both IAV and PIV infections downregulate the MIF gene with high significance (-10.40 fold; $P < 0.034$) in patients infected with the IAV and PIV viruses. This gene share many functions represented by activation of immune system, response to

stimulation and mediating cellular biological processes that act against these two viruses (Huang *et al*., 2009).

The IAV, PIV and Adv viruses downregulate the expression of CCL24 (-10.68 fold change; $P < 0.05$) and 66.66% frequency. The downregulation of CCL24 gene leads to reduce the immune system stimulation and mediating cellular biological processes during viral infection (Huang*et al*., 2009).

IAV and Adv infections reduce the expression of lymphotoxin beta (LTB) (-6.86 fold; $P < 0.021$) after 48 h of infection, thus its function in the development of secondary lymphoid organs may be considerably reduced (Spahn*et al*., 2005).

IAV infection reduce the production of CEBPB gene with -5.56 fold change ($P<0.05$). CEBPB gene is an important transcriptional activator in the regulation of genes involved in immune and inflammatory responses. Thus, the down regulation of this gene may be induced by the IAV to facilitate viral replication by inhibiting the specific role of IL6 signaling pathway, therefore this gene is also called NF-IL6 gene.

In another study, the mRNA expression of CEBPB gene was downregulated in a combination with the effect of Ras-protein that is activated under immune-inflammatory responses against microbial infection (Sebastian and Johanson, 2009).

PIV infection also downregulates CEBPB gene which leads to reduces its functions represented by activation of immune system, response to stimulation and mediating cellular biological processes (Huang*et al*., 2009).

IAV and Adv infection reduce the expression of CCL13 which play important role in inflammatory response and involved in immunoregulatory and inflammatory processes (Huang *et al*., 2009). In the present study both viruses (IAV and Adv) downregulated the production of CCL13 significantly ($P < 0.003$) with moderate fold change (-5.35 fold) and 50% frequency.

The infection with IAV and Adv stimulate the reduction of the small inducible cytokine subfamily-E1 (SCYE1) gene. This gene seems to have both an inflammatory cytokine activity and tRNA-binding domain. It is involved in the stimulation of inflammatory responses against microbes. The mRNA of this gene is downregulated to a low level (-4.68; $P < 0.009$) and with 50% frequency. Another study reported that the mRNA of SCYE1 is slightly induced in the respiratory viral infection (Reghunathan *et al*., 2005).

5. 1. 3. The cluster of gene expression profile after IAV infection.

The extent of similarities and differences between the expression profiles of the 8 IAV patients were investigated using the software 'Cluster' and displayed as heat maps using 'Tree View' (Eisen *et al* 1998). This analysis has the advantage of demonstrating all possible relationships between the examined genes at once. The cluster of leukocytes gene expression profile after IAV infection reveals significant alterations in the expression of inflammatory genes (Figure 3). Most of samples showed upregulated genes and this general similarity in the gene expression profile suggests three points; first, the viral-cellular interaction is a cellular response to the viral infection because the leukocytes stimulate the upregulation of most inflammatory genes. Second, all samples are mostly infected with related subtypes of influenza A viruses because the viral subtypes stimulate the downregulation of the same genes. Third, clearly, the most important component of the heat map is the bottom part, which shows a clear up regulation mostly in all cases. In the other heat map parts the changes were relatively insignificant and played a minor role in revealed dendogram that segregate the 8 patients into two groups, one containing 1, 2, 5, 6, 7, and 8, and the other including 3 and 4. Thus the samples 1, 2, 5, 6, 7 and 8 are related since they originated from one cluster, whereas 3 and 4 are more similar and clustered from one origin (Figure 3).

5. 1.4. The cluster of leukocytes gene expression after PIV infection.

The gene expression profiles of PIV and Adv are clustered together (Figure 4). The main feature of gene expression in the two patients that were infected by PIV is the upregulation of most genes. The panel of patient 1(para1) show three different regions; the top region which mostly occupies one third of all panel (84 gene) has showed an alternative up and down regulated genes. The second region at the middle of the panel, represents the most important region and includes 9 highly upregulated genes (IL10, IL1B, CXCL1, CXCL2, CCL1, CCL2, CX3CR1, IFNA2 and TNF). These genes are upregulated in the two patients infected by PIV (Red color). This suggests that the two subtypes of PIV may belong to one origin since they are clustered from one origin. The third region at the lower part of the panel represents the most downregulated genes which also showed alternative up and down regulated genes. Panel 2 (para 2) represents the second patient that is infected by PIV. Panel 2 generally showed more up regulated genes and shared the panel 1 with many upregulated genes especially in the middle region.

5.1.5. The cluster of Leukocytes gene expression after Adv infection.

The two infected samples are represented in the two panels in the cluster as; adeno1 and adeno2 (Figure 4). The cluster shows that the effect of Adv on the leukocyte gene expression is divided into three parts. At the top of the cluster, the two samples showed different gene expression; in sample one (adeno1) mostly showed downregulated genes whereas in contrast sample two (adeno2) showed mostly upregulated genes. The middle part of the cluster represents the most important area since it includes the common 9 upregulated genes in the two patients infected by Adv. These genes include; IL10, CXCL1, IFNA2, CXCR1, CCL2, IL1B, CXCL2, TNF and CCL1. The third lower part in the cluster showed common downregulated genes in both samples. This lower part with

the similarity in the downregulated genes in the two panels (adeno1 and adeno2) is different from all other patients and this indicates the unique effect of Adv on the leukocyte gene expression. This viral influence was also shown by another recent study (Burioni *et al*., 2010).

6. Conclusions

In conclusion, the results of this study revealed that:

The generation of RT-PCR-Array allows the comparison of different viruses with respect to their impact on cellular gene expression.

Gene ontology showed that IAV, PIV and Ad viruses stimulate four different inflammatory pathways with specific genes for each virus, thus the cellular response mechanisms to these different viruses are similar.

Clear differences were found in human WBC gene expression induced by IAV, PIV and Adv. These differences may be due to the viral virulence and the nature of the viral genome (DNA or RNA).

Human WBC gene expression profiles, induced by IAV, PIV and Adv demonstrate different alterations of inflammatory genes which are involved in the complex interaction between these viruses and human WBCs.

Ad viruses have different effects on the human leukocytes gene expression represented as a viral influence. On the other hand the response of human WBCs to IAV and PIV infections represented as a cellular response.

The infection with IAV, PIV and Adv stimulate asthma pathway which leads to asthma complication.

7. Recommendations

A complete analysis of changes in cellular functions including the production of gene products and protein- protein interactions within the stimulated cells will be required to explore the underlying mechanism of the effects of IAV, PIV and Adv infection during the course of infection.

Further studies using reverse genetics are required to identify viral genes that are critical for the differential up-regulation or down-regulation of host genes.

The information obtained from this study will enhance our view of IAV, PIV and Adv infections and its influence on host cell biology and may eventually lead to new therapeutic targets.

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9. Appendices

Appendix A – Tables

Statistical analysis for each inflammatory gene after the infection with the three viruses. This analysis includes the*t*-test value, *P*- value, Wilcoxon *t*-value (also called Z-value), Wilcoxon *P*-value, and Pearson *P*-value.

Gene position	Gene symbol	Paired t-value with P-value	Wilcoxon T-valuewith P-value	Pearson r-value With P-value
A01	ABCF1	-4.241, (P < 0.001)	-2.668, (P< 0.008)	0.7, (P < 0.12)
A02	BCL6	-2.735, (P < 0.019)	-2.276, (P< 0.023)	0.527, (P< 0.078)
A03	C3	-4.091, (P < 0.002)	-2.667, (P< 0.008)	0.676, (P< 0.016)
A04	C4A	-4.430, (P< 0.001)	-2.864, (P< 0.004)	0.702, (P<0.011)
A05	C5	-2.6, (P< 0.025)	-2.197, (P< 0.028)	0.422, (P< 0.172)
A06	CCL1	-8.360, (P< 0.001)	-3.061, (P< 0.002)	0.509, (P< 0.095)
A07	CCL11	-2.319, (P< 0.041)	-2.864, (P< 0.004)	0.304, (P< 0.336)
A08	CCL13	-3.468, (P< 0.005)	-2.510, (P< 0.012)	0.6, (P< 0.047)
A09	CCL15	-4.479, (P< 0.001)	-2.903, (P< 0.004)	0.642. (P< 0.024)
A10	CCL16	-3.385, (P< 0.006)	-2.394, (P< 0.017)	0.503, (P< 0.095)
A11	CCL17	-4.468, (P< 0.001)	-2.866, (P< 0.004)	0.750, (P< 0.005)
A12	CCL18	-4.160, (P< 0.002)	-2.511, (P< 0.012)	0.430, (P< 0.164)
B01	CCL19	-3.058, (P< 0.011)	-2.433, (P< 0.015)	0.682, (P< 0.015)
B02	CCL2	-6.809, (P< 0.000)	-3.061, (P< 0.002)	0.435, (P< 0.157)
B03	CCL20	-6.156, (P< 0.001)	-2.981, (P< 0.003)	0.787, (P< 0.002)
B04	CCL21	-4.013, (P< 0.002)	-2.578, (P< 0.010)	0.712, (P< 0.009)
B05	CCL23	-3.886, (P< 0.003)	-2.668, (P< 0.008)	0.653, (P< 0.021)
B06	CCL24	-1.749, (P< 0.108)	-1.609, (P< 0.108)	0.343, (P< 0.274)
B07	CCL25	-3.993, (P< 0.002)	-2.748, (P< 0.006)	0.693, (P< 0.012)
B08	CCL26	-1.796, (P< 0.100)	-1.726, (P< 0.085)	0.326, (P< 0.302)
B09	CCL3	-3.802, (P< 0.003)	-2.535, (P< 0.011)	0.666, (P< 0.018)
B10	CCL4	-4.628, (P< 0.001)	-2.903, (P< 0.004)	0.688, (P< 0.013)
B11	CCL5	-8.651, (P< 0.000)	-3.059, (P< 0.002)	0.722, (P< 0.008)
B12	CCL7	-4.994, (P< 0.001)	-2.824, (P< 0.005)	0.718, (P< 0.008)
C01	CCL8	-6.687, (P< 0.000)	-3.062, (P< 0.002)	0.836, (P< 0.001)
C02	CCR1	-2.047, (P< 0.065)	-2.826, (P< 0.005)	0.350, (P< 0.260)
C03	CCR2	-4.490, (P< 0.001)	-2.669, (P< 0.008)	0.656, (P< 0.020)
C04	CCR3	-4.482, (P< 0.001)	-2.825, (P< 0.005)	0.736, (P< 0.006)
C05	CCR4	-4.162, (P< 0.002)	-2.756, (P< 0.006)	0.632, (P< 0.028)
C06	CCR5	-5.805, (P< 0.000)	-2.936, (P< 0.003)	0.660, (P< 0.020)
C07	CCR6	-3.874, (P< 0.003)	-2.758, (P< 0.006)	0.536, (P< o.072)
C08	CCR7	-6.250, (P< 0.000)	-2.981, (P< 0.003)	0.692, (P< 0.013)
C09	CCR8	-3.993, (P< 0.002)	-2.746, (P< 0.006)	0.658, (P< 0.020)
C10	CCR9	-4.275, (P< 0.001)	-2.824, (P< 0.005)	0.719, (P< 0.008)
C11	CEBPB	-1.854, (P< 0.091)	-1.569, (P< 0.117)	0.554, (P< 0.062)
C12	CRP	-4.203, (P< 0.001)	-2.829, (P< 0.009)	0.672, (P< 0.017)
D01	CX3CR1	-0.428, (P< 0.677)	-2.119, (P< 0.034)	-0.405, (P< 0.191)
D02	CXCL1	-9.080, (P< 0.000)	-3.061, (P< 0.002)	0.673, (P< 0.017)
D03	CXCL10	-6.096, (P< 0.002)	-3.061, (P< 0.002)	0.442, (P< 0.151)

D04	CXCL11	-5.784,(P< 0.000)	-2.983, (P< 0.003)	0.765,(P<0.004)
D05	CXCL12	-5.384,(P< 0.000)	-2.981, (P< 0.003	0.758, (P< 0.004)
D06	CXCL13	-2.397, (P< 0.035)	-2.046, (P< 0.041)	0.353, (P< 0.260)
D07	CXCL14	-4.340, (P< 0.001)	-2.828, (P< 0.005)	0.710, (P< 0.010)
D08	CXCL2	-4.995, (P< 0.000)	-3.059, (P< 0.002)	0.749, (P< 0.005)
D09	CXCL3	-6.403, (P< 0.000)	-2.983, (P< 0.003)	0.847, (P< 0.001)
D10	CXCL5	-4.128, (P< 0.002)	-2.746, (P< 0.006)	0.752, (P< 0.005)
D11	CXCL6	-3.338, (P< 0.007)	-2.433, (P< 0.015)	0.628, (P< 0.029)
D12	CXCL9	-4.549, (P< 0.001)	-2.934, (P< 0.003)	0.681, (P< 0.015)
E01	CARD18	-1.129,(P< 0.283)	-1.334, (P< 0.182)	0.308, (P< 0.330)
E02	IFNA2	-4.753, (P< 0.001)	-2.944, (P< 0.003)	0.219, (P< 0.495)
E03	IL10	-4.028, (P< 0.002)	-2.433, (P< 0.015)	-0.063, (P< 0.846)
E04	IL10RA	-3.085, (P< 0.010)	-2.353, (P< 0.019)	0.428, (P< 0.165)
E05	IL10RB	-3.188, (P< 0.009)	-2.136, (P< 0.033)	0.594, (P< 0.042)
E06	IL13	-7.261, (P< 0.000)	-3.061, (P< 0.002)	0.641, (P< 0.025)
E07	IL13RA1	-3.545, (P< 0.005)	-2.550, (P< 0.011)	0.696, (P< 0.012)
E08	IL17C	-3.581, (P< 0.004)	-2.491, (P< 0.013)	0.560, (P< 0.058)
E09	IL1A	-4.714, (P< 0.001)	-2.943, (P< 0.003)	0.441, (P< 0.152)
E10	IL1B	-11. 518, (P< 0.00)	-3.061, (P< 0.002)	0.581, (P< 0.048)
E11	IL1F10	-4.807, (P< 0.001)	-2. 748, (P< 0.006)	0.691, (P< 0.013)
E12	IL1F5	-3.852, (P< 0.003)	-2.492, (P< 0.013)	0.630, (P< 0.028)
F01	IL1F6	-0.236, (P< 0.816)	-2.118, (P< 0.034)	0.257, (P< 0.421)
F02	IL1F7	-5.627, (P< 0.000)	-2.845, (P< 0.004)	0.801, (P< 0.002)
F03	IL1F8	-3.436, (P< 0.006)	-2.595, (P< 0.009)	0.724, (P< 0.008)
F04	IL1F9	-3.062, (P< 0.011)	-2.315, (P< 0.021)	0.650, (P< 0.022)
F05	IL1R1	-5.470, (P< 0.000)	-2.825, (P< 0.005)	0.809, (P< 0.001)
F06	IL1RN	-6.381, (P< 0.000)	-3.061, (P< 0.002)	0.862, (P< 0.000)
F07	IL22	-4.843, (P< 0.001)	-2.981, (P< 0.003)	0.710, (P< 0.010)
F08	IL5	-4.966, (P< 0.000)	-2.981, (P< 0.003)	0.732, (P< 0.007)
F09	IL5RA	-3.425, (P< 0.006)	-2.447, (P< 0.014)	0.591, (P< 0.043)
F10	IL8	-9.494, (P< 0.000)	-3.061, (P< 0.002)	0.670, (P< 0.017)
F11	IL8RA	-3.677, (P< 0.004)	-2.589, (P< 0.010)	0.641, (P< 0.025)
F12	IL8RB	-2.196, (P< 0.050)	-3.059, (P< 0.002)	0.212, (P< 0.508)
G01	IL9	-1.326, (P< 0.212)	-2.119, (P< 0.034)	0.245, (P< 0.443)
G02	IL9R	-3.489, (P< 0.005)	-2.472, (P< 0.013)	0.719, (P< 0.008)
G03	LTA	-5.747, (P< 0.000)	-2.937, (P< 0.003)	0.800, (P< 0.002)
G04	LTB	-2.923, (P< 0.014)	-2.312, (P< 0.021)	0.449, (P< 0.143)
G05	LTB4R	-4.099, (P< 0.002)	-2.825, (P< 0.005)	0.678, (P< 0.015)
G06	MIF	-2.622, (P< 0.024)	-2.120, (P< 0.034)	0.498, (P< 0.100)
G07	SCYE1	-2.825, (P< 0.017)	-2.275, (P< 0.023)	0.600, (P< 0.039)
G08	SPP1	-3.738, (P< 0.003)	-2.629, (P< 0.009)	0.691, (P< 0.013)
G09	TNF	-12.626, (P< 0.00)	-3.061, (P< 0.002)	0.691, (P< 0.013)
G10	CD40LG	-3.360, (P< 0.006)	-2.446, (P< 0.014)	0.627, (P< 0.029)
G11	TOLLIP	-4.323, (P< 0.001)	-2.845, (P< 0.004)	0.647, (P< 0.023)
G12	XCR1	-4.341, (P< 0.001)	-2.758, (P< 0.006)	0.625, (P< 0.030)

Table 1: The gene position, gene symbol and gene name of the 84 inflammatory genes as arranged in the RT-PCR-Array kit (SABioscience, USA)

Gene position	Gene symbol	Gene name
A01	ABCF1	ATP-binding cassette, sub-family F (GCN20). member 1
A02	BCL6	B-cell CLL/lymphoma 6
A03	C3	Complement component 3
A04	C4A	Complement component 4A (Rodgers blood group)
A05	C5	Complement component 5
A06	CCL1	Chemokine (C-C motif) ligand 1
A07	CCL11	Chemokine (C-C motif) ligand 11
A08	CCL13	Chemokine (C-C motif) ligand 13
A09	CCL15	Chemokine (C-C motif) ligand 15
A10	CCL16	Chemokine (C-C motif) ligand 16
A11	CCL17	Chemokine (C-C motif) ligand 17
A12	CCL18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
B01	CCL19	Chemokine (C-C motif) ligand 19
B02	CCL2	Chemokine (C-C motif) ligand 2
B03	CCL20	Chemokine (C-C motif) ligand 20
B04	CCL21	Chemokine (C-C motif) ligand 21
B05	CCL23	Chemokine (C-C motif) ligand 23
B06	CCL24	Chemokine (C-C motif) ligand 24
B07	CCL25	Chemokine (C-C motif) ligand 25
B08	CCL26	Chemokine (C-C motif) ligand 26
B09	CCL3	Chemokine (C-C motif) ligand 3
B10	CCL4	Chemokine (C-C motif) ligand 4
B11	CCL5	Chemokine (C-C motif) ligand 5
B12	CCL7	Chemokine (C-C motif) ligand 7
C01	CCL8	Chemokine (C-C motif) ligand 8
C02	CCR1	Chemokine (C-C motif) receptor 1
C03	CCR2	Chemokine (C-C motif) receptor 2
C04	CCR3	Chemokine (C-C motif) receptor 3
C05	CCR4	Chemokine (C-C motif) receptor 4
C06	CCR5	Chemokine (C-C motif) receptor 5
C07	CCR6	Chemokine (C-C motif) receptor 6
C08	CCR7	Chemokine (C-C motif) receptor 7
C09	CCR8	Chemokine (C-C motif) receptor 8
C10	CCR9	Chemokine (C-C motif) receptor 9
C11	CEBPB	CCAAT/enhancer binding protein (C/EBP). beta
C12	CRP	C-reactive protein, pentraxin-related
D01	CX3CR1	Chemokine (C-X3-C motif) receptor 1
D02	CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
D03	CXCL10	Chemokine (C-X-C motif) ligand 10
D04	CXCL11	Chemokine (C-X-C motif) ligand 11
D05	CXCL12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)

D06	CXCL13	Chemokine (C-X-C motif) ligand 13
D07	CXCL14	Chemokine (C-X-C motif) ligand 14
D08	CXCL2	Chemokine (C-X-C motif) ligand 2
D09	CXCL3	Chemokine (C-X-C motif) ligand 3
D10	CXCL5	Chemokine (C-X-C motif) ligand 5
D11	CXCL6	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
D12	CXCL9	Chemokine (C-X-C motif) ligand 9
E01	CARD18	Caspase recruitment domain family, member 18
E02	IFNA2	Interferon, alpha 2
E03	IL10	Interleukin 10
E04	IL10RA	Interleukin 10 receptor, alpha
E05	IL10RB	Interleukin 10 receptor, beta
E06	IL13	Interleukin 13
E07	IL13RA1	Interleukin 13 receptor, alpha 1
E08	IL17C	Interleukin 17C
E09	IL1A	Interleukin 1, alpha
E10	IL1B	Interleukin 1, beta
E11	IL1F10	Interleukin 1 family, member 10 (theta)
E12	IL1F5	Interleukin 1 family, member 5 (delta)
F01	IL1F6	Interleukin 1 family, member 6 (epsilon)
F02	IL1F7	Interleukin 1 family, member 7 (zeta)
F03	IL1F8	Interleukin 1 family, member 8 (eta)
F04	IL1F9	Interleukin 1 family, member 9
F05	IL1R1	Interleukin 1 receptor, type I
F06	IL1RN	Interleukin 1 receptor antagonist
F07	IL22	Interleukin 22
F08	IL5	Interleukin 5 (colony-stimulating factor, eosinophil)
F09	IL5RA	Interleukin 5 receptor, alpha
F10	IL8	Interleukin 8
F11	IL8RA	Interleukin 8 receptor, alpha
F12	IL8RB	Interleukin 8 receptor, beta
G01	IL9	Interleukin 9
G02	IL9R	Interleukin 9 receptor
G03	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
G04	LTB	Lymphotoxin beta (TNF superfamily, member 3)
G05	LTB4R	Leukotriene B4 receptor
G06	MIF	Macrophage migration inhibitory factor
G07	SCYE1	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)
G08	SPP1	Secreted phosphoprotein 1
G09	TNF	Tumor necrosis factor (TNF superfamily, member 2)
G10	CD40LG	CD40 ligand
G11	TOLLIP	Toll interacting protein
G12	XCR1	Chemokine (C motif) receptor 1
H01	B2M	Beta-2-microglobulin
H02	HPRT1	Hypoxanthine phosphoribosyltransferase 1
H03	RPL13A	Ribosomal protein L13a

H04	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
H05	ACTB	Actin, beta
H06	HGDC	Human Genomic DNA Contamination
H07	RTC	Reverse Transcription Control
H08	RTC	Reverse Transcription Control
H09	RTC	Reverse Transcription Control
H10	PPC	Positive PCR Control
H11	PPC	Positive PCR Control
H12	PPC	Positive PCR Control

Table 2: The threshold values (Ct values) of the 84 immuno-inflammatory genes after the infection of 8 patients with IAV.

Influenza A virus								
Gene symbol.	Patient 1		Patient 2		Patient 3		Patient 4	
	Acute	Cont.	Acute	Cont.	Acute	Cont.	Acute	Cont.
ABCF1	24.5	24.9	28.6	31.2	22.7	27.2	23.0	24.7
BCL6	23.9	24.2	29.4	31.5	23.1	26.5	23.5	24.6
C3	24.3	24.7	29.0	30.6	22.5	28.1	23.6	24.9
C4A	22.5	22.6	28.2	33.6	21.1	26.6	22.2	24.1
C5	24.9	32.5	28.7	32.7	21.7	27.4	23.1	24.7
CCL1	26.3	33.0	29.9	31.6	23.2	28.3	24.5	29.3
CCL11	25.8	26.3	29.0	31.8	22.8	27.7	24.1	25.2
CCL13	26.9	27.3	30.9	33.5	24.3	29.6	25.6	26.5
CCL15	25.2	25.3	29.1	31.6	23.3	28.3	23.6	25.1
CCL16	25.8	26.3	29.8	31.9	22.9	29.1	24.8	26.1
CCL17	24.9	25.1	30.1	34.2	22.5	28.6	25.0	25.8
CCL18	26.5	34.0	29.7	32.7	24.9	28.6	24.0	25.4
CCL19	24.6	24.8	31.2	31.7	23.1	28.2	23.9	25.0
CCL2	24.8	29.8	28.3	31.9	22.6	27.1	23.3	24.4
CCL20	24.9	24.6	28.3	30.3	22.8	26.9	23.3	27.7
CCL21	24.7	24.7	28.9	31.2	22.8	27.4	23.7	25.0
CCL23	24.7	24.8	28.2	31.1	21.8	27.0	23.0	24.2
CCL24	24.0	23.7	27.7	28.0	22.0	27.0	23.1	24.2
CCL25	23.4	23.0	28.0	30.2	22.0	27.2	23.0	24.0
CCL26	26.7	26.6	31.0	31.4	24.5	29.9	26.3	27.3
CCL3	24.9	24.9	28.0	31.1	21.6	27.0	23.2	24.1
CCL4	23.3	32.0	29.2	33.7	21.2	27.2	23.6	24.5
CCL5	24.1	31.6	29.0	34.1	21.8	27.7	24.3	28.9
CCL7	26.5	26.8	29.1	32.1	23.0	28.0	24.5	25.1
CCL8	25.6	25.7	28.2	30.2	22.8	27.2	23.1	24.4
CCR1	23.9	23.8	28.8	30.2	22.8	26.6	N/A	24.3
CCR2	25.6	25.6	29.1	33.3	23.3	28.7	24.4	25.7
CCR3	24.4	24.5	28.6	30.7	22.5	27.7	23.6	24.6
CCR4	25.5	25.5	28.8	32.6	22.5	27.6	23.7	24.6
CCR5	24.0	24.0	27.6	29.6	21.4	26.8	22.6	23.7

CCR6	24.4	24.4	28.7	31.5	22.4	27.6	23.7	24.8
CCR7	25.1	25.0	30.1	32.1	23.1	28.7	24.1	25.6
CCR8	25.6	25.5	28.5	30.8	22.4	27.7	23.8	25.0
CCR9	24.5	24.4	28.6	30.5	21.8	27.4	23.6	24.6
CEBPB	24.5	24.1	30.0	31.3	23.5	27.3	25.4	25.5
CRP	25.6	25.7	27.8	31.8	21.3	27.3	23.5	23.9
CX3CR1	27.5	34.1	31.6	N/A	25.0	30.6	25.6	27.0
CXCL1	25.7	32.7	28.2	31.1	22.7	26.5	23.7	29.5
CXCL10	25.8	29.9	28.6	30.7	22.9	27.2	23.6	24.5
CXCL11	26.0	25.9	28.3	30.8	22.6	28.0	23.6	24.3
CXCL12	24.7	24.5	29.2	30.2	23.6	27.9	24.4	24.9
CXCL13	25.8	25.8	28.7	31.0	23.6	28.1	24.5	24.9
CXCL14	23.8	23.9	28.3	30.5	21.9	27.4	23.0	24.1
CXCL2	29.1	29.3	32.4	33.6	25.3	29.8	25.9	27.3
CXCL3	28.5	28.0	30.0	32.2	24.8	28.8	24.4	26.0
CXCL5	26.7	26.6	29.0	31.6	21.8	27.2	23.6	24.7
CXCL6	27.4	27.3	28.9	29.9	21.8	27.8	23.8	24.8
CXCL9	26.0	26.0	28.9	31.6	23.1	27.4	24.0	24.9
CARD18	27.3	27.4	29.1	31.4	23.3	28.1	24.7	25.1
IFNA2	26.7	26.6	27.6	31.0	21.7	26.6	22.8	22.9
IL10	23.8	33.9	27.8	30.5	22.5	26.8	23.2	27.6
IL10RA	23.8	23.5	28.1	29.7	22.3	27.2	23.6	23.7
IL10RB	24.9	24.9	29.2	32.7	22.9	27.9	24.3	24.8
IL13	24.7	33.8	28.8	30.9	23.1	27.5	24.0	28.8
IL13RA1	25.3	25.4	30.3	31.7	23.0	27.7	23.9	24.6
IL17C	23.2	23.2	28.5	29.6	22.8	27.7	24.1	25.2
IL1A	24.7	30.9	28	31.3	21.5	26.7	22.9	26.9
IL1B	24.1	29.9	28.3	31.7	21.7	26.2	23.1	27.8
IL1F10	25.2	30.5	28.7	31.9	21.8	27.9	23.8	27.9
IL1F5	25.5	25.5	28.1	32.0	22.0	27.5	23.6	24.7
IL1F6	26.2	26.5	28.1	32.7	22.4	27.9	23.8	24.3
IL1F7	25	24.9	28.9	31.5	22.7	27.6	23.7	23.7
IL1F8	25.7	25.8	28.2	30.1	23.3	27.1	23.7	23.8
IL1F9	26.8	26.6	28.6	30.7	23.2	27.7	23.9	24.1
IL1R1	25.7	25.6	27.9	31.5	22.7	27.1	23.8	23.6
IL1RN	23.8	24.0	28.5	30.9	22.8	26.9	23.2	23.9
IL22	27.2	27.4	29.8	31.6	23.9	29.5	25.1	25.0
IL5	26.1	25.6	27.7	33.5	22	26.9	23.6	24.2
IL5RA	25.3	25.3	29.0	30.0	23.1	28.2	24.6	25.1
IL8	24.1	33.9	27.6	34.1	20.7	25.8	23.1	27.1
IL8RA	24.6	24.3	29.8	30.0	22.4	27.5	24.1	25.2
IL8RB	23.5	23.6	29.0	31.0	23.6	26.3	23.5	24.4
IL9	26.2	26.6	29.4	31.7	25.2	29.0	25.0	25.3
IL9R	26.4	26.3	29.2	33.9	23.7	28.7	24.8	24.5
LTA	24.5	24.5	28.9	31.2	23.7	28	24.5	24.6
LTB	23.8	23.8	30.6	31.3	24.1	28.5	25.6	25.5
LTB4R	24.0	24.1	28.6	30.4	22.6	27.8	23.9	24.3
MIF	23.6	23.2	28.1	29.6	23.1	27.1	23.9	24.1

SCYE1	29.3	29.6	31.1	32.8	23.8	29.7	25.6	26.4
SPP1	27.5	27.6	28.9	31.4	23.0	27.9	24.5	24.9
TNF	23.9	28.9	28.7	32.6	22.8	27.9	24.4	28.9
CD40LG	25.6	25.6	29.9	31.4	22.0	27.5	24.1	24.5
TOLLIP	23.6	23.6	29.4	32.0	22.1	28.7	24.8	25.9
XCR1	26.0	26.0	28.9	30.5	22.5	28.0	25.0	25.6

Table 2 Continued

Influenza A virus								
Gene symbol	Patient 5		Patient 6		Patient 7		Patient 8	
	Acu.	Cont.	Acu.	Cont.	Acut.	Cont.	Acut	Cont.
ABCF1	24.7	26.1	23.4	27.6	22.7	24.9	19.7	24.0
BCL6	24.6	26.5	23.6	27.7	23.3	24.7	20.8	24.7
C3	24.9	26.9	24.6	27.9	22.6	25.2	20.3	24
C4A	24.1	25.8	23.8	26.5	21.7	24.6	19.1	22.6
C5	24.7	26.0	24.0	26.9	22.5	26.8	20.0	23.8
CCL1	25.5	27.1	25.3	31.7	23.6	29.5	21.5	28.0
CCL11	25.2	N/A	25.4	27.7	23.2	25.3	21.3	27.0
CCL13	26.5	28.6	26.5	28.7	24.1	26.6	22.0	25.5
CCL15	25.1	26.8	25.1	27.4	22.8	25.2	21.0	26.9
CCL16	26.1	27.1	26.2	28.4	23.9	26.7	21.3	26.0
CCL17	25.8	27.6	26.3	28.5	23.7	25.9	21.9	25.5
CCL18	25.4	29.5	25.7	31.3	23.3	28.6	21.7	24.8
CCL19	25.0	26.1	24.4	27.9	23.1	25.6	20.4	24.3
CCL2	24.4	31.0	23.9	27.9	22.7	29.4	20.1	27.7
CCL20	24.2	25.7	23.7	26.7	22.6	24.3	19.9	23.4
CCL21	25.0	26.9	24.6	27.3	22.9	25.1	20.5	24.0
CCL23	24.2	25.8	23.6	26.8	22.2	24.5	19.8	23.7
CCL24	24.2	25.4	24.1	25.7	22.4	24.6	19.9	23.6
CCL25	24.0	26.3	24.4	26.7	22.0	24.9	20.1	23.6
CCL26	27.3	28.9	28.5	29.4	25.5	28.4	23.2	26.6
CCL3	24.1	26.3	24.6	26.5	22.5	24.6	20.4	23.9
CCL4	24.5	29.7	25.0	26.7	22.5	24.8	20.4	24.3
CCL5	25.0	29.8	25.7	33.8	23.4	27.9	20.5	28.9
CCL7	25.1	28.2	25.8	31.6	23.2	25.5	21.8	25.6
CCL8	24.4	28.8	23.8	27.6	22.5	24.5	19.6	23.1
CCR1	24.3	25.8	24.1	27.2	22.9	24.7	20.3	24.1
CCR2	25.7	29.5	26.3	27.9	23.3	26.4	21.1	24.8
CCR3	24.6	26.8	25.0	26.8	22.7	24.9	20.0	23.5
CCR4	24.6	27.1	25.1	27.0	22.4	25.2	20.0	23.6
CCR5	23.7	25.4	23.5	26.2	21.6	24.0	19.3	22.8
CCR6	24.8	26.6	24.7	27.1	22.8	24.9	20.6	27.7
CCR7	25.6	29.9	25.6	28.6	23.3	25.5	21.1	26.9
CCR8	25.0	26.4	25.4	27.2	23.2	25.3	21.0	24.6
CCR9	24.6	26.2	24.9	27.8	22.4	24.7	20.6	24.0
CEBPB	25.5	27.8	26.8	28.6	24.1	27.2	22.7	26.0
CRP	23.9	26.7	24.7	26.7	22.6	24.6	20.7	24.2
CX3CR1	27.0	28.9	26.4	30.2	24.6	27.2	21.6	25.8

CXCL1	23.7	25.2	24.3	29.3	22.8	28.3	19.8	24.1
CXCL10	24.5	31.1	24.4	28.7	22.7	30.2	20.1	27.4
CXCL11	24.3	26.8	24.8	26.8	22.6	25.0	20.2	23.6
CXCL12	24.9	26.6	25.3	27.6	23.5	25.6	21.0	24.6
CXCL13	24.9	26.3	25.4	27.3	23.3	25.6	21.0	24.8
CXCL14	24.1	26.7	24.1	27.8	22.0	24.6	19.9	23.2
CXCL2	27.3	28.2	27.7	30.3	24.8	27.6	22.6	26.1
CXCL3	26.0	28.8	26.6	30.9	23.9	26.7	21.7	24.9
CXCL5	24.7	26.5	25.0	26.9	22.8	24.8	20.9	24.0
CXCL6	24.8	26.8	25.0	26.6	22.2	25.3	21.1	24.5
CXCL9	24.9	27.4	25.3	34.5	22.7	25.1	21.1	24.4
CARD18	25.1	28.0	25.0	28.3	23.0	25.6	20.2	24.1
IFNA2	22.9	26.1	23.8	28.8	21.6	26.9	19.2	22.4
IL10	23.7	29.6	24.0	30.5	22.3	27.8	19.8	25.0
IL10RA	23.7	25.5	24.3	26.7	22.6	24.3	20.0	27.0
IL10RB	24.8	27.1	25.6	27.7	22.9	25.9	20.8	24.3
IL13	24.3	29.4	25.1	29.9	22.7	28.9	20.8	28.2
IL13RA1	24.6	26.9	25.1	28.4	22.8	25.3	21.0	24.5
IL17C	25.2	26.4	25.3	27.0	23.1	25.9	20.9	24.9
IL1A	23.8	28.9	24.1	26.7	22.0	27.0	20.0	23.5
IL1B	23.8	31.8	24.6	29.1	21.9	28.7	20.5	29.0
IL1F10	24.8	26.8	25.1	27.3	22.5	25.3	20.6	24.5
IL1F5	24.7	26.6	24.9	27.2	22.4	25.1	21.1	24.5
IL1F6	24.3	27.2	24.6	27.2	22.4	24.9	19.7	23.1
IL1F7	23.7	27.0	24.6	27.4	22.3	24.6	19.9	23.3
IL1F8	23.8	25	24.3	26.9	22.6	24.5	20.1	23.4
IL1F9	24.1	25.9	24.9	27.1	22.6	25.4	20.4	23.9
IL1R1	23.6	25.5	24.4	26.7	22.3	24.6	20.0	23.1
IL1RN	23.9	26.0	24.6	26.8	22.6	24.9	20.6	23.8
IL22	25.0	27.0	26.3	28.2	23.5	27.0	21.8	24.6
IL5	24.2	25.3	24.7	27.1	22.2	25.1	20.4	23.9
IL5RA	25.1	26.9	25.6	27.2	23.1	25.3	21.6	24.8
IL8	23.4	28.9	23.9	29.4	22.0	29.3	20.4	29.2
IL8RA	25.2	26.9	25.4	28.6	23.1	26.0	21.8	24.8
IL8RB	24.4	27.0	24.7	31.5	22.5	24.9	21.0	24.1
IL9	25.3	29.6	25.9	28.7	23.4	25.8	20.9	24.7
IL9R	24.5	26.4	25.5	28.2	22.9	25.0	20.7	24.1
LTA	24.6	26.4	25.3	27.3	22.7	25.1	20.3	23.9
LTB	25.5	27.7	26.4	28.1	24.3	27.0	22.2	26.1
LTB4R	24.3	26.7	24.8	27.3	22.5	25.0	20.1	23.6
MIF	24.1	26.5	24.8	26.9	22.9	25.3	20.5	24.6
SCYE1	26.4	27.3	27.3	29.8	24.0	28.2	22.4	25.4
SPP1	24.9	27.0	25.6	27.3	22.8	25.8	21.6	24.7
TNF	25.2	30.7	25.5	30.8	23.3	28.6	21.5	28.6
CD40LG	24.5	26.9	25.2	28.4	22.7	25.4	21.0	24.7
TOLLIP	25.9	28.4	26.2	27.4	22.6	26.3	21.3	25.2
XCR1	25.6	27.5	25.7	27.3	22.9	25.9	21.4	25.1
B2M	21.3	28.6	22.4	25.6	23.6	21.9	20.3	24.7

HPRT1	25.9	27.4	26.5	29.8	23.6	26.9	21.7	25.0
RPL13A	22.9	24.9	23.4	25.8	21.7	23.3	19.4	23
GAPDH	22.8	25.3	23.9	26.6	21.5	23.9	19.3	22.5
ACTB	22.0	25.2	22.8	25.6	21.8	23.2	19.8	23.1
HGDC	24.4	25.9	25.1	27.1	22.7	25.0	20.8	24.2
RTC	26.6	29.0	27.1	26.8	29.7	28.2	28.4	28.1
RTC	26.8	27.8	27.7	27.1	28.9	28.5	27.9	28.7
RTC	27.5	29.7	28.3	28.6	30.1	28.9	29.5	29.3
PPC	17.6	17.0	17.6	17.8	17.3	18.0	17.2	17.1
PPC	17.7	17.2	17.5	18.0	17.0	17.9	17.2	16.8
PPC	18.0	18.0	18.0	20.2	18.5	18.5	18.2	17.8

Table 3: The threshold values (Ct values) of the 84 immuno-inflammatory genes after the infection of 2 patients with PIV and 2 patients with Adv.

	PIV				Adv			
Gene symbol	Patient 1		Patient 2		Patient 1		Patient 2	
	Acu.	Cont.	Acu.	Cont.	Acu.	Cont.	Acu	Cont.
ABCF1	19.5	23.7	21.7	23.3	22.7	25.2	23.5	21.6
BCL6	19.8	24.7	26.8	23.7	22.4	26.0	23.8	22.6
C3	19.6	23.9	21.9	23.4	22.9	25.7	24.0	22.2
C4A	18.7	23.1	20.9	22.7	21.8	25.4	22.8	21.7
C5	19.7	23.8	21.9	28.6	32.2	25.8	23.6	27.8
CCL1	20.4	29.4	23.0	30.3	23.7	26.8	24.7	28.5
CCL11	20.4	28.2	22.6	24.4	23.7	26.1	24.6	23.5
CCL13	21.2	28.1	23.5	25.3	25.2	27.1	25.8	23.5
CCL15	20.0	24.6	22.3	24.3	23.5	26.2	23.9	23.1
CCL16	21.0	25.8	23.6	25.6	24.1	27.0	24.9	22.0
CCL17	20.6	24.6	22.7	24.6	23.5	26.8	24.4	23.6
CCL18	20.3	24.6	22.7	31.5	31.1	26.7	24.6	29.9
CCL19	20.5	24.6	22.6	23.6	23.7	25.7	24.4	21.8
CCL2	19.5	26.9	21.8	29.9	23.0	29.4	23.1	28.1
CCL20	19.5	23.2	21.7	23.0	22.9	25.0	23.5	27.9
CCL21	19.8	23.9	22.3	23.9	23.1	25.7	24.0	22.0
CCL23	19.4	23.6	21.9	23.1	22.4	25.3	23.6	21.5
CCL24	19.3	23.1	27.7	23.5	22.2	25.2	23.1	21.3
CCL25	19.6	22.7	21.4	23.6	22.2	24.8	23.1	21.3
CCL26	22.4	26.6	29.9	26.3	25.3	28.3	26.7	24.1
CCL3	19.6	23.3	21.6	23.3	22.3	25.3	23.5	21.4
CCL4	19.9	23.5	22.1	24.0	22.4	25.6	23.6	22.6
CCL5	20.6	27.0	22.6	24.2	21.9	26.3	23.8	26.0
CCL7	20.3	24.3	22.4	25.0	23.5	26.8	24.4	23.7
CCL8	19.6	23.8	21.6	23.2	22.8	24.9	25.8	29.9
CCR1	19.8	23.8	21.8	23.3	22.9	25.4	22.7	21.9
CCR2	20.9	25.0	23.0	27.1	23.9	26.4	25.2	23.4
CCR3	19.6	23.6	21.8	24.4	22.6	25.1	23.8	22.7
CCR4	19.7	24.3	21.9	24.8	23.0	29.7	24.1	22.4
CCR5	18.8	22.8	21.0	24.5	21.9	27.9	22.7	28.5
CCR6	19.9	23.8	22.0	23.8	22.8	26.3	24.0	22.6

CCR7	20.1	24.7	22.5	25.1	23.5	29.7	24.4	28.9
CCR8	20.2	24.3	22.6	24.2	23.6	26.2	24.1	22.8
CCR9	19.6	23.3	21.7	23.2	22.7	25.7	23.3	22.4
CEBPB	21.1	24.3	26.9	24.7	30.1	26.6	24.8	26.8
CRP	19.7	23.1	21.6	23.8	22.3	25.8	23.3	22.0
CX3CR1	21.4	26.3	23.9	31.3	25.2	29.8	29.7	33.9
CXCL1	19.9	28.2	22.9	28.7	22.8	28.5	23.2	27.0
CXCL10	19.6	28.8	21.8	23.4	23.0	27.0	22.7	25.5
CXCL11	19.8	23.8	22.1	24.1	23.0	24.9	23.9	28.6
CXCL12	20.7	24.6	22.9	24.3	23.7	26.1	24.3	28.0
CXCL13	20.6	24.8	22.8	29.4	30.7	25.9	24.5	25.6
CXCL14	20.4	23.5	21.6	23.5	22.5	25.1	23.1	21.5
CXCL2	20.9	26.4	23.7	30.8	25.4	27.5	26.7	29.7
CXCL3	20.3	25.1	23.1	24.7	24.8	26.7	25.7	28.8
CXCL5	19.7	23.7	22.1	23.6	22.8	25.7	23.6	22.4
CXCL6	19.7	23.6	21.9	24.1	23.0	25.8	23.9	21.5
CXCL9	19.9	24.5	22.3	23.9	23.6	26.4	24.1	26.4
CARD18	20.4	24.4	22.7	24.1	29.7	25.4	31.3	24
IFNA2	18.8	29.1	21.0	32.5	22.2	28.4	22.7	26.7
IL10	19.7	28.5	21.7	27.8	22.9	29.4	23.2	25.7
IL10RA	19.5	23.6	21.9	23.1	22.1	24.9	22.9	20.9
IL10RB	20.2	25.0	22.9	25.3	23.4	25.7	24.6	21.0
IL13	20.0	24.3	22.4	23.6	23.0	25.5	23.9	27.7
IL13RA1	19.9	24.1	28.0	23.8	23.9	26.9	24.8	22.3
IL17C	20.4	24.7	22.6	24.7	22.8	25.7	24.0	22.0
IL1A	19.2	29.9	21.4	23.0	22.6	25.1	23.2	21.6
IL1B	19.4	27.7	21.4	30.6	22.2	28.8	22.7	27.9
IL1F10	20.0	23.8	22.3	24.3	23.1	25.9	23.8	21.3
IL1F5	20.0	24.0	22.1	24.0	23.2	26.6	23.9	21.5
IL1F6	19.7	23.9	21.8	23.3	23.1	24.8	N/A	23.4
IL1F7	19.8	23.7	21.9	23.1	23.0	24.9	23.5	27.3
IL1F8	19.8	23.7	21.8	23.0	22.9	24.9	23.4	21.5
IL1F9	19.8	24.1	22.4	23.7	23.4	25.0	24.0	21.3
IL1R1	19.6	23.5	21.9	23.4	22.7	24.7	23.4	25.6
IL1RN	19.7	23.8	21.2	23.1	22.6	25.3	21.1	22.1
IL22	20.3	25.6	23.4	24.5	24.3	26.5	25.4	29.1
IL5	19.8	24.1	21.9	23.2	22.8	24.9	23.7	27.8
IL5RA	20.6	24.6	22.9	24.3	23.7	26.9	24.6	22.8
IL8	19.5	27.8	21.7	23.1	22.3	28.1	23.0	29.0
IL8RA	20.3	24.1	22.6	24.5	23.0	26.6	23.8	22.2
IL8RB	19.7	23.9	21.9	23.6	N/A	26.1	22.5	23.5
IL9	21.0	25.8	23.3	24.8	24.6	26.0	34.8	24.9
IL9R	20.5	24.7	22.6	24.2	23.9	25.7	24.4	22.6
LTA	20.2	24.2	22.4	24.0	23.3	25.6	23.8	27.8
LTB	21.0	27.7	24.6	25.8	23.3	26.8	25.3	22.9
LTB4R	20.5	24.6	22.1	23.7	22.9	25.0	24.1	22.9
MIF	20.3	23.9	27.9	23.7	22.2	25.8	23.6	19.7
SCYE1	20.9	26.3	25.3	24.9	25.5	27.0	26.0	23.2

SPP1	20.4	24.6	22.5	24.3	23.6	25.9	24.6	22.8
TNF	20.7	28.9	22.7	29.6	23.1	29.3	24.2	26.8
CD40LG	20.7	24.2	22.4	24.0	24.4	27.2	25.4	22.7
TOLLIP	20.5	24.6	22.6	24.9	22.9	27.1	24.0	23.0
XCR1	20.8	24.7	22.6	25.2	23.8	27.6	24.6	23.8

Table 4: The gene expression analysis for IAV infection in patient1 include; the gene symbol, the average change of C_t values ($\mathbf{AVG\ \Delta C_t}$) between the acut and control samples, the level of gene expression ($\mathbf{2^{-\Delta C_t}}$) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu. 1	Cont.1	Acu. 1	Cont.1	Acu1/Cont.1	Acu 1/Cont.1
ABCF1	1.66	1.94	0.316439	0.260616	1.21	1.21
BCL6	1.06	1.24	0.479632	0.423373	1.13	1.13
C3	1.46	1.74	0.363493	0.299370	1.21	1.21
C4A	-0.34	-0.36	1.265757	1.283426	0.99	-1.01
C5	2.06	9.54	0.239816	0.001343	178.53	178.53
CCL1	3.46	10.04	0.090873	0.000950	95.67	95.67
CCL11	2.96	3.34	0.128514	0.098755	1.30	1.30
CCL13	4.06	4.34	0.059954	0.049378	1.21	1.21
CCL15	2.36	2.34	0.194791	0.197510	0.99	-1.01
CCL16	2.96	3.34	0.128514	0.098755	1.30	1.30
CCL17	2.06	2.14	0.239816	0.226880	1.06	1.06
CCL18	3.66	11.04	0.079110	0.000475	166.57	166.57
CCL19	1.76	1.84	0.295248	0.279322	1.06	1.06
CCL2	1.96	6.84	0.257028	0.008729	29.45	29.45
CCL20	2.06	1.64	0.239816	0.320856	0.75	-1.34
CCL21	1.86	1.74	0.275476	0.299370	0.92	-1.09
CCL23	1.86	1.84	0.275476	0.279322	0.99	-1.01
CCL24	1.16	0.74	0.447513	0.598739	0.75	-1.34
CCL25	0.56	0.04	0.678302	0.972655	0.70	-1.43
CCL26	3.86	3.64	0.068869	0.080214	0.86	-1.16
CCL3	2.06	1.94	0.239816	0.260616	0.92	-1.09
CCL4	0.46	9.04	0.726986	0.001900	382.68	382.68
CCL5	1.26	8.64	0.417544	0.002507	166.57	166.57
CCL7	3.66	3.84	0.079110	0.069830	1.13	1.13
CCL8	2.76	2.74	0.147624	0.149685	0.99	-1.01
CCR1	1.06	0.84	0.479632	0.558644	0.86	-1.16
CCR2	2.76	2.64	0.147624	0.160428	0.92	-1.09
CCR3	1.56	1.54	0.339151	0.343885	0.99	-1.01
CCR4	2.66	2.54	0.158220	0.171943	0.92	-1.09
CCR5	1.16	1.04	0.447513	0.486327	0.92	-1.09
CCR6	1.56	1.44	0.339151	0.368567	0.92	-1.09
CCR7	2.26	2.04	0.208772	0.243164	0.86	-1.16
CCR8	2.76	2.54	0.147624	0.171943	0.86	-1.16
CCR9	1.66	1.44	0.316439	0.368567	0.86	-1.16

CEBPB	1.66	1.14	0.316439	0.453760	0.70	-1.43
CRP	2.76	2.74	0.147624	0.149685	0.99	-1.01
CX3CR1	4.66	11.14	0.039555	0.000443	89.26	89.26
CXCL1	2.86	9.74	0.137738	0.001169	117.78	117.78
CXCL10	2.96	6.94	0.128514	0.008144	15.78	15.78
CXCL11	3.16	2.94	0.111878	0.130308	0.86	-1.16
CXCL12	1.86	1.54	0.275476	0.343885	0.80	-1.25
CXCL13	2.96	2.84	0.128514	0.139661	0.92	-1.09
CXCL14	0.96	0.94	0.514057	0.521233	0.99	-1.01
CXCL2	6.26	6.34	0.013048	0.012344	1.06	1.06
CXCL3	5.66	5.04	0.019777	0.030395	0.65	-1.54
CXCL5	3.86	3.64	0.068869	0.080214	0.86	-1.16
CXCL6	4.56	4.34	0.042394	0.049378	0.86	-1.16
CXCL9	3.16	3.04	0.111878	0.121582	0.92	-1.09
CARD18	4.46	4.44	0.045437	0.046071	0.99	-1.01
IFNA2	3.86	3.64	0.068869	0.080214	0.86	-1.16
IL10	0.96	10.94	0.514057	0.000509	1009.90	1009.90
IL10RA	0.96	0.54	0.514057	0.687771	0.75	-1.34
IL10RB	2.06	1.94	0.239816	0.260616	0.92	-1.09
IL13	1.86	10.84	0.275476	0.000546	504.95	504.95
IL13RA1	2.46	2.44	0.181747	0.184284	0.99	-1.01
IL17C	0.36	0.24	0.779165	0.846745	0.92	-1.09
IL1A	1.86	7.94	0.275476	0.004072	67.65	67.65
IL1B	1.26	6.94	0.417544	0.008144	51.27	51.27
IL1F10	2.36	7.54	0.194791	0.005373	36.25	36.25
IL1F5	2.66	2.54	0.158220	0.171943	0.92	-1.09
IL1F6	3.36	3.54	0.097396	0.085971	1.13	1.13
IL1F7	2.16	1.94	0.223756	0.260616	0.86	-1.16
IL1F8	2.86	2.84	0.137738	0.139661	0.99	-1.01
IL1F9	3.96	3.64	0.064257	0.080214	0.80	-1.25
IL1R1	2.86	2.64	0.137738	0.160428	0.86	-1.16
IL1RN	0.96	1.04	0.514057	0.486327	1.06	1.06
IL22	4.36	4.44	0.048698	0.046071	1.06	1.06
IL5	3.26	2.64	0.104386	0.160428	0.65	-1.54
IL5RA	2.46	2.34	0.181747	0.197510	0.92	-1.09
IL8	1.26	10.94	0.417544	0.000509	820.30	820.30
IL8RA	1.76	1.34	0.295248	0.395021	0.75	-1.34
IL8RB	0.66	0.64	0.632878	0.641713	0.99	-1.01
IL9	3.36	3.64	0.097396	0.080214	1.21	1.21
IL9R	3.56	3.34	0.084788	0.098755	0.86	-1.16
LTA	1.66	1.54	0.316439	0.343885	0.92	-1.09
LTB	0.96	0.84	0.514057	0.558644	0.92	-1.09
LTB4R	1.16	1.14	0.447513	0.453760	0.99	-1.01
MIF	0.76	0.24	0.590496	0.846745	0.70	-1.43
SCYE1	6.46	6.64	0.011359	0.010027	1.13	1.13
SPP1	4.66	4.64	0.039555	0.040107	0.99	-1.01
TNF	1.06	5.94	0.479632	0.016289	29.45	29.45
CD40LG	2.76	2.64	0.147624	0.160428	0.92	-1.09

TOLLIP	0.76	0.64	0.590496	0.641713	0.92	-1.09
XCR1	3.16	3.04	0.111878	0.121582	0.92	-1.09

Table 5: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 1.

No.	Gene name	Up regulated genes	Down regulated genes
1	IL10 Interleukin 10	1009.902	
2	Interleukin 8	820.2956	
3	Interleukin 13	504.9511	
4	Chemokine (C-C motif) ligand 4	382.6814	
5	Complement component 5	178.5272	
6	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	166.5718	
7	Chemokine (C-C motif) ligand 5	166.5718	
8	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	117.784	
9	Chemokine (C-C motif) ligand 1	95.6704	
10	Chemokine (C-X3-C motif) receptor 1	89.2636	
11	Interleukin 1, alpha	67.6492	
12	Interleukin 1, beta	51.2685	
13	Interleukin 1 family, member 10 (theta)	36.2523	
14	Chemokine (C-C motif) ligand 2	29.446	
15	Tumor necrosis factor (TNF superfamily, member 2)	29.446	
16	Chemokine (C-X-C motif) ligand 10	15.7797	
17	Chemokine (C-C motif) ligand 11	1.3013	
18	Chemokine (C-C motif) ligand 16	1.3013	
19	ATP-binding cassette, sub-family F (GCN20). member 1	1.2142	
20	Complement component 3	1.2142	
21	Chemokine (C-C motif) ligand 13	1.2142	
22	Interleukin 9	1.2142	
23	B-cell CLL/lymphoma 6	1.1329	
24	Chemokine (C-C motif) ligand 7	1.1329	
25	Interleukin 1 family, member 6 (epsilon)	1.1329	
26	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	1.1329	
1	Chemokine (C-C motif) ligand 26		-1.1647
2	Chemokine (C-C motif) receptor 1		-1.1647
3	Chemokine (C-C motif) receptor 7		-1.1647
4	Chemokine (C-C motif) receptor 8		-1.1647
5	Chemokine (C-C motif) receptor 9		-1.1647
6	Chemokine (C-X-C motif) ligand 11		-1.1647
7	Chemokine (C-X-C motif) ligand 5		-1.1647
8	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-1.1647
9	Interferon, alpha 2		-1.1647

10	Interleukin 1 family, member 7 (zeta)		-1.1647
11	Interleukin 1 receptor, type I		-1.1647
12	Interleukin 9 receptor		-1.1647
13	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-1.2483
14	Interleukin 1 family, member 9		-1.2483
15	Chemokine (C-C motif) ligand 20		-1.3379
16	Chemokine (C-C motif) ligand 24		-1.3379
17	Interleukin 10 receptor, alpha		-1.3379
18	Interleukin 8 receptor, alpha		-1.3379
19	Chemokine (C-C motif) ligand 25		-1.434
20	CCAAT/enhancer binding protein (C/EBP). beta		-1.434
21	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.434
22	Chemokine (C-X-C motif) ligand 13		-1.5369
23	Interleukin 5 (colony-stimulating factor, eosinophil)		-1.5369

Table 6: The gene expression analysis for IAV infection in patient 2; the gene symbol, the average change of C_t values ($AVG\ \Delta C_t$) between the acut and control samples, the level of gene expression ($2^{-\Delta C_t}$) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu.2	Cont.2	Acu. 2	Cont.2	Acu 2/Cont.2	Acu 2/Cont.2
ABCF1	0.16	1.06	0.895025	0.479632	1.87	1.87
BCL6	0.96	1.36	0.514057	0.389582	1.32	1.32
C3	0.56	0.46	0.678302	0.726986	0.93	-1.07
C4A	-0.24	3.46	1.180993	0.090873	13.00	13.00
C5	0.26	2.56	0.835088	0.169576	4.92	4.92
CCL1	1.46	1.46	0.363493	0.363493	1.00	-1.00
CCL11	0.56	1.66	0.678302	0.316439	2.14	2.14
CCL13	2.46	3.36	0.181747	0.097396	1.87	1.87
CCL15	0.66	1.46	0.632878	0.363493	1.74	1.74
CCL16	1.36	1.76	0.389582	0.295248	1.32	1.32
CCL17	1.66	4.06	0.316439	0.059954	5.28	5.28
CCL18	1.26	2.56	0.417544	0.169576	2.46	2.46
CCL19	2.76	1.56	0.147624	0.339151	0.44	-2.30
CCL2	-0.14	1.76	1.101905	0.295248	3.73	3.73
CCL20	-0.14	0.16	1.101905	0.895025	1.23	1.23
CCL21	0.46	1.06	0.726986	0.479632	1.52	1.52
CCL23	-0.24	0.96	1.180993	0.514057	2.30	2.30
CCL24	-0.74	-2.14	1.670176	4.407620	0.38	-2.64
CCL25	-0.44	0.06	1.356604	0.959264	1.41	1.41
CCL26	2.56	1.26	0.169576	0.417544	0.41	-2.46
CCL3	-0.44	0.96	1.356604	0.514057	2.64	2.64
CCL4	0.76	3.56	0.590496	0.084788	6.96	6.96
CCL5	0.56	3.96	0.678302	0.064257	10.56	10.56
CCL7	0.66	1.96	0.632878	0.257028	2.46	2.46
CCL8	-0.24	0.06	1.180993	0.959264	1.23	1.23
CCR1	0.36	0.06	0.779165	0.959264	0.81	-1.23
CCR2	0.66	3.16	0.632878	0.111878	5.66	5.66
CCR3	0.16	0.56	0.895025	0.678302	1.32	1.32
CCR4	0.36	2.46	0.779165	0.181747	4.29	4.29
CCR5	-0.84	-0.54	1.790050	1.453973	1.23	1.23
CCR6	0.26	1.36	0.835088	0.389582	2.14	2.14
CCR7	1.66	1.96	0.316439	0.257028	1.23	1.23
CCR8	0.06	0.66	0.959264	0.632878	1.52	1.52
CCR9	0.16	0.36	0.895025	0.779165	1.15	1.15
CEBPB	1.56	1.16	0.339151	0.447513	0.76	-1.32
CRP	-0.64	1.66	1.558329	0.316439	4.92	4.92
CX3CR1	3.16	4.86	0.111878	0.034435	3.25	3.25
CXCL1	-0.24	0.96	1.180993	0.514057	2.30	2.30
CXCL10	0.16	0.56	0.895025	0.678302	1.32	1.32
CXCL11	-0.14	0.66	1.101905	0.632878	1.74	1.74
CXCL12	0.76	0.06	0.590496	0.959264	0.62	-1.62

CXCL13	0.26	0.86	0.835088	0.550953	1.52	1.52
CXCL14	-0.14	0.36	1.101905	0.779165	1.41	1.41
CXCL2	3.96	3.46	0.064257	0.090873	0.71	-1.41
CXCL3	1.56	2.06	0.339151	0.239816	1.41	1.41
CXCL5	0.56	1.46	0.678302	0.363493	1.87	1.87
CXCL6	0.46	-0.24	0.726986	1.180993	0.62	-1.62
CXCL9	0.46	1.46	0.726986	0.363493	2.00	2.00
CARD18	0.66	1.26	0.632878	0.417544	1.52	1.52
IFNA2	-0.84	0.86	1.790050	0.550953	3.25	3.25
IL10	-0.64	0.36	1.558329	0.779165	2.00	2.00
IL10RA	-0.34	-0.44	1.265757	1.356604	0.93	-1.07
IL10RB	0.76	2.56	0.590496	0.169576	3.48	3.48
IL13	0.36	0.76	0.779165	0.590496	1.32	1.32
IL13RA1	1.86	1.56	0.275476	0.339151	0.81	-1.23
IL17C	0.06	-0.54	0.959264	1.453973	0.66	-1.52
IL1A	-0.44	1.16	1.356604	0.447513	3.03	3.03
IL1B	-0.14	1.56	1.101905	0.339151	3.25	3.25
IL1F10	0.26	1.76	0.835088	0.295248	2.83	2.83
IL1F5	-0.34	1.86	1.265757	0.275476	4.59	4.59
IL1F6	-0.34	2.56	1.265757	0.169576	7.46	7.46
IL1F7	0.46	1.36	0.726986	0.389582	1.87	1.87
IL1F8	-0.24	-0.04	1.180993	1.028114	1.15	1.15
IL1F9	0.16	0.56	0.895025	0.678302	1.32	1.32
IL1R1	-0.54	1.36	1.453973	0.389582	3.73	3.73
IL1RN	0.06	0.76	0.959264	0.590496	1.62	1.62
IL22	1.36	1.46	0.389582	0.363493	1.07	1.07
IL5	-0.74	3.36	1.670176	0.097396	17.15	17.15
IL5RA	0.56	-0.14	0.678302	1.101905	0.62	-1.62
IL8	-0.84	3.96	1.790050	0.064257	27.86	27.86
IL8RA	1.36	-0.14	0.389582	1.101905	0.35	-2.83
IL8RB	0.56	0.86	0.678302	0.550953	1.23	1.23
IL9	0.96	1.56	0.514057	0.339151	1.52	1.52
IL9R	0.76	3.76	0.590496	0.073812	8.00	8.00
LTA	0.46	1.06	0.726986	0.479632	1.52	1.52
LTB	2.16	1.16	0.223756	0.447513	0.50	-2.00
LTB4R	0.16	0.26	0.895025	0.835088	1.07	1.07
MIF	-0.34	-0.54	1.265757	1.453973	0.87	-1.15
SCYE1	2.66	2.66	0.158220	0.158220	1.00	-1.00
SPP1	0.46	1.26	0.726986	0.417544	1.74	1.74
TNF	0.26	2.46	0.835088	0.181747	4.59	4.59
CD40LG	1.46	1.26	0.363493	0.417544	0.87	-1.15
TOLLIP	0.96	1.86	0.514057	0.275476	1.87	1.87
XCR1	0.46	0.36	0.726986	0.779165	0.93	-1.07

Table 7: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 2

No.	Gene name	Up regulated genes	Down regulated genes
1	Interleukin 8	27.8576	
2	Interleukin 5 (colony-stimulating factor, eosinophil)	17.1484	
3	Complement component 4A (Rodgers blood group)	12.996	
4	Chemokine (C-C motif) ligand 5	10.5561	
5	Interleukin 9 receptor	8.0000	
6	Interleukin 1 family, member 6 (epsilon)	7.4643	
7	Chemokine (C-C motif) ligand 4	6.9644	
8	Chemokine (C-C motif) receptor 2	5.6569	
9	Chemokine (C-C motif) ligand 17	5.278	
10	C- reactive protein	4.9246	
11	Complement component 5	4.9246	
12	Tumor necrosis factor (TNF superfamily, member 2)	4.5948	
13	Interleukin 1 family, member 5 (delta)	4.5948	
14	Chemokine (C-C motif) receptor 4	4.2871	
15	Interleukin 1 receptor, type I	3.7321	
16	Chemokine (C-C motif) ligand 2	3.7321	
17	Interleukin 10 receptor, beta	3.4822	
18	Interleukin 1, beta	3.249	
19	Interferon, alpha 2	3.249	
20	Chemokine (C-X3-C motif) receptor 1	3.249	
21	Interleukin 1, alpha	3.0314	
22	Interleukin 1 family, member 10 (theta)	2.8284	
23	Chemokine (C-C motif) ligand 3	2.639	
24	Chemokine (C-C motif) ligand 7	2.4623	
25	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	2.4623	
26	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	2.2974	
27	Chemokine (C-C motif) ligand 23	2.2974	
28	Chemokine (C-C motif) receptor 6	2.1435	
29	Chemokine (C-C motif) ligand 11	2.1435	
30	Interleukin 10	2.0000	
31	Chemokine (C-X-C motif) ligand 9	2.0000	
32	Toll interacting protein	1.8661	
33	Interleukin 1 family, member 7 (zeta)	1.8661	
34	Chemokine (C-X-C motif) ligand 5	1.8661	
35	Chemokine (C-C motif) ligand 13	1.8661	
36	ATP-binding cassette, sub-family F (GCN20). member 1	1.8661	
37	Secreted phosphoprotein 1	1.7411	
38	Chemokine (C-X-C motif) ligand 11	1.7411	
39	Chemokine (C-C motif) ligand 15	1.7411	
40	Interleukin 1 receptor antagonist	1.6245	
41	Lymphotoxin alpha (TNF superfamily, member 1)	1.5157	

42	Interleukin 9	1.5157	
43	Chemokine (C-X-C motif) ligand 13	1.5157	
44	Chemokine (C-C motif) receptor 8	1.5157	
45	Chemokine (C-C motif) ligand 21	1.5157	
46	Caspase recruitment domain family, member 18	1.5157	
47	Chemokine (C-X-C motif) ligand 3	1.4142	
48	Chemokine (C-X-C motif) ligand 14	1.4142	
49	Chemokine (C-C motif) ligand 25	1.4142	
50	Interleukin 1 family, member 9	1.3195	
51	Interleukin 13	1.3195	
52	Chemokine (C-X-C motif) ligand 10	1.3195	
53	Chemokine (C-C motif) receptor 3	1.3195	
54	Chemokine (C-C motif) ligand 16	1.3195	
55	B-cell CLL/lymphoma 6	1.3195	
56	Interleukin 8 receptor, beta	1.2311	
57	Chemokine (C-C motif) receptor 7	1.2311	
58	Chemokine (C-C motif) receptor 5	1.2311	
59	Chemokine (C-C motif) ligand 8	1.2311	
60	Chemokine (C-C motif) ligand 20	1.2311	
61	Interleukin 1 family, member 8 (eta)	1.1487	
62	Chemokine (C-C motif) receptor 9	1.1487	
1	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.1487
2	CD40 ligand		-1.1487
3	Chemokine (C-C motif) receptor 1		-1.2311
4	Interleukin 13 receptor, alpha 1		-1.2311
5	CCAAT/enhancer binding protein (C/EBP). beta		-1.3195
6	Chemokine (C-X-C motif) ligand 2		-1.4142
7	Interleukin 17C		-1.5157
8	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-1.6245
9	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-1.6245
10	Interleukin 5 receptor, alpha		-1.6245
11	Lymphotoxin beta (TNF superfamily, member 3)		-2.0000
12	Chemokine (C-C motif) ligand 19		-2.2974
13	Chemokine (C-C motif) ligand 26		-2.4623
14	Chemokine (C-C motif) ligand 24		-2.639
15	Interleukin 8 receptor, alpha		-2.8284

Table 8: The gene expression analysis for IAV infection in patient 3; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu. 3	Cont.3	Acu. 3	Cont.3	Acu 3/Cont.3	Acu 3/Cont.3
ABCF1	1.28	1.22	0.411796	0.429283	0.96	-1.04
BCL6	1.68	0.52	0.312083	0.697372	0.45	-2.23
C3	1.08	2.12	0.473029	0.230047	2.06	2.06
C4A	-0.32	0.62	1.248331	0.650671	1.92	1.92
C5	0.28	1.42	0.823591	0.373712	2.20	2.20
CCL1	1.78	2.32	0.291183	0.200267	1.45	1.45
CCL11	1.38	1.72	0.384219	0.303549	1.27	1.27
CCL13	2.88	3.62	0.135842	0.081334	1.67	1.67
CCL15	1.88	2.32	0.271684	0.200267	1.36	1.36
CCL16	1.48	3.12	0.358489	0.115023	3.12	3.12
CCL17	1.08	2.62	0.473029	0.162668	2.91	2.91
CCL18	3.48	2.62	0.089622	0.162668	0.55	-1.82
CCL19	1.68	2.22	0.312083	0.214641	1.45	1.45
CCL2	1.18	1.12	0.441351	0.460094	0.96	-1.04
CCL20	1.38	0.92	0.384219	0.528509	0.73	-1.38
CCL21	1.38	1.42	0.384219	0.373712	1.03	1.03
CCL23	0.38	1.02	0.768438	0.493116	1.56	1.56
CCL24	0.58	1.02	0.668964	0.493116	1.36	1.36
CCL25	0.58	1.22	0.668964	0.429283	1.56	1.56
CCL26	3.08	3.92	0.118257	0.066064	1.79	1.79
CCL3	0.18	1.02	0.882703	0.493116	1.79	1.79
CCL4	-0.22	1.22	1.164734	0.429283	2.71	2.71
CCL5	0.38	1.72	0.768438	0.303549	2.53	2.53
CCL7	1.58	2.02	0.334482	0.246558	1.36	1.36
CCL8	1.38	1.22	0.384219	0.429283	0.90	-1.12
CCR1	1.38	0.62	0.384219	0.650671	0.59	-1.69
CCR2	1.88	2.72	0.271684	0.151774	1.79	1.79
CCR3	1.08	1.72	0.473029	0.303549	1.56	1.56
CCR4	1.08	1.62	0.473029	0.325335	1.45	1.45
CCR5	-0.02	0.82	1.013959	0.566442	1.79	1.79
CCR6	0.98	1.62	0.506980	0.325335	1.56	1.56
CCR7	1.68	2.72	0.312083	0.151774	2.06	2.06
CCR8	0.98	1.72	0.506980	0.303549	1.67	1.67
CCR9	0.38	1.42	0.768438	0.373712	2.06	2.06
CEBPB	2.08	1.32	0.236514	0.400535	0.59	-1.69
CRP	-0.12	1.32	1.086735	0.400535	2.71	2.71
CX3CR1	3.58	4.62	0.083620	0.040667	2.06	2.06
CXCL1	1.28	0.52	0.411796	0.697372	0.59	-1.69
CXCL10	1.48	1.22	0.358489	0.429283	0.84	-1.20
CXCL11	1.18	2.02	0.441351	0.246558	1.79	1.79
CXCL12	2.18	1.92	0.220676	0.264255	0.84	-1.20
CXCL13	2.18	2.12	0.220676	0.230047	0.96	-1.04

CXCL14	0.48	1.42	0.716978	0.373712	1.92	1.92
CXCL2	3.88	3.82	0.067921	0.070805	0.96	-1.04
CXCL3	3.38	2.82	0.096055	0.141610	0.68	-1.47
CXCL5	0.38	1.22	0.768438	0.429283	1.79	1.79
CXCL6	0.38	1.82	0.768438	0.283221	2.71	2.71
CXCL9	1.68	1.42	0.312083	0.373712	0.84	-1.20
CARD18	1.88	2.12	0.271684	0.230047	1.18	1.18
IFNA2	0.28	0.62	0.823591	0.650671	1.27	1.27
IL10	1.08	0.82	0.473029	0.566442	0.84	-1.20
IL10RA	0.88	1.22	0.543367	0.429283	1.27	1.27
IL10RB	1.48	1.92	0.358489	0.264255	1.36	1.36
IL13	1.68	1.52	0.312083	0.348686	0.90	-1.12
IL13RA1	1.58	1.72	0.334482	0.303549	1.10	1.10
IL17C	1.38	1.72	0.384219	0.303549	1.27	1.27
IL1A	0.08	0.72	0.946058	0.607097	1.56	1.56
IL1B	0.28	0.22	0.823591	0.858565	0.96	-1.04
IL1F10	0.38	1.92	0.768438	0.264255	2.91	2.91
IL1F5	0.58	1.52	0.668964	0.348686	1.92	1.92
IL1F6	0.98	1.92	0.506980	0.264255	1.92	1.92
IL1F7	1.28	1.62	0.411796	0.325335	1.27	1.27
IL1F8	1.88	1.12	0.271684	0.460094	0.59	-1.69
IL1F9	1.78	1.72	0.291183	0.303549	0.96	-1.04
IL1R1	1.28	1.12	0.411796	0.460094	0.90	-1.12
IL1RN	1.38	0.92	0.384219	0.528509	0.73	-1.38
IL22	2.48	3.52	0.179244	0.087171	2.06	2.06
IL5	0.58	0.92	0.668964	0.528509	1.27	1.27
IL5RA	1.68	2.22	0.312083	0.214641	1.45	1.45
IL8	-0.72	-0.18	1.647182	1.132884	1.45	1.45
IL8RA	0.98	1.52	0.506980	0.348686	1.45	1.45
IL8RB	2.18	0.32	0.220676	0.801070	0.28	-3.63
IL9	3.78	3.02	0.072796	0.123279	0.59	-1.69
IL9R	2.28	2.72	0.205898	0.151774	1.36	1.36
LTA	2.28	2.02	0.205898	0.246558	0.84	-1.20
LTB	2.68	2.52	0.156041	0.174343	0.90	-1.12
LTB4R	1.18	1.82	0.441351	0.283221	1.56	1.56
MIF	1.68	1.12	0.312083	0.460094	0.68	-1.47
SCYE1	2.38	3.72	0.192109	0.075887	2.53	2.53
SPP1	1.58	1.92	0.334482	0.264255	1.27	1.27
TNF	1.38	1.92	0.384219	0.264255	1.45	1.45
CD40LG	0.58	1.52	0.668964	0.348686	1.92	1.92
TOLLIP	0.68	2.72	0.624165	0.151774	4.11	4.11
XCR1	1.08	2.02	0.473029	0.246558	1.92	1.92

Table 9: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 3.

No.	Gene name	Up regulated genes	Down regulated genes
1	Toll interacting protein	4.1125	
2	Interleukin 1 family, member 10 (theta)	2.9079	
3	Chemokine (C-C motif) ligand 4	2.7132	
4	C-reactive protein, pentraxin-related	2.7132	
5	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	2.7132	
6	Chemokine (C-C motif) ligand 5	2.5315	
7	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	2.5315	
8	Complement component 5	2.2038	
9	Chemokine (C-C motif) receptor 7	2.0562	
10	Chemokine (C-C motif) receptor 9	2.0562	
11	Chemokine (C-X3-C motif) receptor 1	2.0562	
12	Interleukin 22	2.0562	
13	Chemokine (C-X-C motif) ligand 14	1.9185	
14	Interleukin 1 family, member 5 (delta)	1.9185	
15	Interleukin 1 family, member 6 (epsilon)	1.9185	
16	CD40 ligand	1.9185	
17	Chemokine (C motif) receptor 1	1.9185	
18	Chemokine (C-C motif) ligand 26	1.7901	
19	Chemokine (C-C motif) ligand 3	1.7901	
20	Chemokine (C-C motif) receptor 2	1.7901	
21	Chemokine (C-C motif) receptor 5	1.7901	
22	Chemokine (C-X-C motif) ligand 11	1.7901	
23	Chemokine (C-X-C motif) ligand 5	1.7901	
24	Chemokine (C-C motif) receptor 8	1.6702	
25	Chemokine (C-C motif) ligand 23	1.5583	
26	Chemokine (C-C motif) ligand 25	1.5583	
27	Chemokine (C-C motif) receptor 3	1.5583	
28	Chemokine (C-C motif) receptor 6	1.5583	
29	Interleukin 1, alpha	1.5583	
30	Leukotriene B4 receptor	1.5583	
31	Chemokine (C-C motif) ligand 1	1.454	
32	Chemokine (C-C motif) ligand 19	1.454	
33	Chemokine (C-C motif) receptor 4	1.454	
34	Interleukin 5 receptor, alpha	1.454	
35	Interleukin 8	1.454	
36	Interleukin 8 receptor, alpha	1.454	
37	Tumor necrosis factor (TNF superfamily, member 2)	1.454	
38	Chemokine (C-C motif) ligand 24	1.3566	
39	Chemokine (C-C motif) ligand 7	1.3566	
40	Interleukin 10 receptor, beta	1.3566	
41	Interleukin 9 receptor	1.3566	

42	Interferon, alpha 2	1.2658	
43	Interleukin 10 receptor, alpha	1.2658	
44	Interleukin 17C	1.2658	
45	Interleukin 1 family, member 7 (zeta)	1.2658	
46	Interleukin 5 (colony-stimulating factor, eosinophil)	1.2658	
47	Secreted phosphoprotein 1	1.2658	
48	Caspase recruitment domain family, member 18	1.181	
49	Interleukin 13 receptor, alpha 1	1.1019	
1	ATP-binding cassette, sub-family F (GCN20). member 1		-1.04
2	Chemokine (C-C motif) ligand 8		-1.1173
3	Interleukin 13		-1.1173
4	Interleukin 1 receptor, type I		-1.1173
5	Lymphotoxin beta (TNF superfamily, member 3)		-1.1173
6	Chemokine (C-X-C motif) ligand 10		-1.1975
7	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-1.1975
8	Chemokine (C-X-C motif) ligand 9		-1.1975
9	Interleukin 10		-1.1975
10	Lymphotoxin alpha (TNF superfamily, member 1)		-1.1975
11	Chemokine (C-C motif) ligand 20		-1.3755
12	Interleukin 1 receptor antagonist		-1.3755
13	Chemokine (C-X-C motif) ligand 3		-1.4743
14	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.4743
15	Chemokine (C-C motif) receptor 1		-1.6935
16	CCAAT/enhancer binding protein (C/EBP). beta		-1.6935
17	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)		-1.6935
18	Interleukin 1 family, member 8 (eta)		-1.6935
19	Interleukin 9		-1.6935
20	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)		-1.815
21	Interleukin 8 receptor, beta		-3.6301
22	Complement component 3		-8.9383
23	Complement component 4A (Rodgers blood group)		-8.9383
24	B-cell CLL/lymphoma 6		-12.6407
25	Chemokine (C-C motif) ligand 13		-13.5479
26	Chemokine (C-C motif) ligand 15		-14.5203
27	Chemokine (C-C motif) ligand 17		-14.5203
28	Chemokine (C-C motif) ligand 16		-17.8766
29	Chemokine (C-C motif) ligand 11		-19.1597

Table 10: The gene expression analysis for IAV infection in patient 4; the gene symbol, the average change of C_t values ($AVG\ \Delta C_t$) between the acut and control samples, the level of gene expression ($2^{-\Delta C_t}$) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 4	Cont.4	Acu. 4	Cont.4	Acu 4/Cont.4	Acu 4/Cont.4
ABCF1	0.28	1.72	0.823591	0.303549	2.71	2.71
BCL6	0.78	1.62	0.582367	0.325335	1.79	1.79
C3	0.88	1.92	0.543367	0.264255	2.06	2.06
C4A	-0.52	1.12	1.433955	0.460094	3.12	3.12
C5	0.38	1.72	0.768438	0.303549	2.53	2.53
CCL1	1.78	6.32	0.291183	0.012517	23.26	23.26
CCL11	1.38	2.22	0.384219	0.214641	1.79	1.79
CCL13	2.88	3.52	0.135842	0.087171	1.56	1.56
CCL15	0.88	2.12	0.543367	0.230047	2.36	2.36
CCL16	2.08	3.12	0.236514	0.115023	2.06	2.06
CCL17	2.28	2.82	0.205898	0.141610	1.45	1.45
CCL18	1.28	2.42	0.411796	0.186856	2.20	2.20
CCL19	1.18	2.02	0.441351	0.246558	1.79	1.79
CCL2	0.58	1.42	0.668964	0.373712	1.79	1.79
CCL20	0.58	4.72	0.668964	0.037944	17.63	17.63
CCL21	0.98	2.02	0.506980	0.246558	2.06	2.06
CCL23	0.28	1.22	0.823591	0.429283	1.92	1.92
CCL24	0.38	1.22	0.768438	0.429283	1.79	1.79
CCL25	0.28	1.02	0.823591	0.493116	1.67	1.67
CCL26	3.58	4.32	0.083620	0.050067	1.67	1.67
CCL3	0.48	1.12	0.716978	0.460094	1.56	1.56
CCL4	0.88	1.52	0.543367	0.348686	1.56	1.56
CCL5	1.58	5.92	0.334482	0.016516	20.25	20.25
CCL7	1.78	2.12	0.291183	0.230047	1.27	1.27
CCL8	0.38	1.42	0.768438	0.373712	2.06	2.06
CCR1	12.28	1.32	0.000201	0.400535	0.00	-1992.00
CCR2	1.68	2.72	0.312083	0.151774	2.06	2.06
CCR3	0.88	1.62	0.543367	0.325335	1.67	1.67
CCR4	0.98	1.62	0.506980	0.325335	1.56	1.56
CCR5	-0.12	0.72	1.086735	0.607097	1.79	1.79
CCR6	0.98	1.82	0.506980	0.283221	1.79	1.79
CCR7	1.38	2.62	0.384219	0.162668	2.36	2.36
CCR8	1.08	2.02	0.473029	0.246558	1.92	1.92
CCR9	0.88	1.62	0.543367	0.325335	1.67	1.67
CEBPB	2.68	2.52	0.156041	0.174343	0.90	-1.12
CRP	0.78	0.92	0.582367	0.528509	1.10	1.10
CX3CR1	2.88	4.02	0.135842	0.061640	2.20	2.20
CXCL1	0.98	6.52	0.506980	0.010896	46.53	46.53
CXCL10	0.88	1.52	0.543367	0.348686	1.56	1.56
CXCL11	0.88	1.32	0.543367	0.400535	1.36	1.36
CXCL12	1.68	1.92	0.312083	0.264255	1.18	1.18
CXCL13	1.78	1.92	0.291183	0.264255	1.10	1.10

CXCL14	0.28	1.12	0.823591	0.460094	1.79	1.79
CXCL2	3.18	4.32	0.110338	0.050067	2.20	2.20
CXCL3	1.68	3.02	0.312083	0.123279	2.53	2.53
CXCL5	0.88	1.72	0.543367	0.303549	1.79	1.79
CXCL6	1.08	1.82	0.473029	0.283221	1.67	1.67
CXCL9	1.28	1.92	0.411796	0.264255	1.56	1.56
CARD18	1.98	2.12	0.253490	0.230047	1.10	1.10
IFNA2	0.08	-0.08	0.946058	1.057018	0.90	-1.12
IL10	0.48	4.62	0.716978	0.040667	17.63	17.63
IL10RA	0.88	0.72	0.543367	0.607097	0.90	-1.12
IL10RB	1.58	1.82	0.334482	0.283221	1.18	1.18
IL13	1.28	5.82	0.411796	0.017701	23.26	23.26
IL13RA1	1.18	1.62	0.441351	0.325335	1.36	1.36
IL17C	1.38	2.22	0.384219	0.214641	1.79	1.79
IL1A	0.18	3.92	0.882703	0.066064	13.36	13.36
IL1B	0.38	4.82	0.768438	0.035403	21.71	21.71
IL1F10	1.08	4.92	0.473029	0.033032	14.32	14.32
IL1F5	0.88	1.72	0.543367	0.303549	1.79	1.79
IL1F6	1.08	1.32	0.473029	0.400535	1.18	1.18
IL1F7	0.98	0.72	0.506980	0.607097	0.84	-1.20
IL1F8	0.98	0.82	0.506980	0.566442	0.90	-1.12
IL1F9	1.18	1.12	0.441351	0.460094	0.96	-1.04
IL1R1	1.08	0.62	0.473029	0.650671	0.73	-1.38
IL1RN	0.48	0.92	0.716978	0.528509	1.36	1.36
IL22	2.38	2.02	0.192109	0.246558	0.78	-1.28
IL5	0.88	1.22	0.543367	0.429283	1.27	1.27
IL5RA	1.88	2.12	0.271684	0.230047	1.18	1.18
IL8	0.38	4.12	0.768438	0.057512	13.36	13.36
IL8RA	1.38	2.22	0.384219	0.214641	1.79	1.79
IL8RB	0.78	1.42	0.582367	0.373712	1.56	1.56
IL9	2.28	2.32	0.205898	0.200267	1.03	1.03
IL9R	2.08	1.52	0.236514	0.348686	0.68	-1.47
LTA	1.78	1.62	0.291183	0.325335	0.90	-1.12
LTB	2.88	2.52	0.135842	0.174343	0.78	-1.28
LTB4R	1.18	1.32	0.441351	0.400535	1.10	1.10
MIF	1.18	1.12	0.441351	0.460094	0.96	-1.04
SCYE1	2.88	3.42	0.135842	0.093428	1.45	1.45
SPP1	1.78	1.92	0.291183	0.264255	1.10	1.10
TNF	1.68	5.92	0.312083	0.016516	18.90	18.90
CD40LG	1.38	1.52	0.384219	0.348686	1.10	1.10
TOLLIP	2.08	2.92	0.236514	0.132127	1.79	1.79
XCR1	2.28	2.62	0.205898	0.162668	1.27	1.27

Table 11: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 4.

No.	Gene name	Up regulated genes	Down regulated genes
1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	46.5271	
2	Chemokine (C-C motif) ligand 1	23.2636	
3	Interleukin 13	23.2636	
4	Interleukin 1, beta	21.7057	
5	Chemokine (C-C motif) ligand 5	20.2521	
6	Tumor necrosis factor (TNF superfamily, member 2)	18.8959	
7	Chemokine (C-C motif) ligand 20	17.6305	
8	Interleukin 10	17.6305	
9	Interleukin 1 family, member 10 (theta)	14.3204	
10	Interleukin 1, alpha	13.3614	
11	Interleukin 8	13.3614	
12	Complement component 4A (Rodgers blood group)	3.1167	
13	ATP-binding cassette, sub-family F (GCN20). member 1	2.7132	
14	Complement component 5	2.5315	
15	Chemokine (C-X-C motif) ligand 3	2.5315	
16	Chemokine (C-C motif) ligand 15	2.362	
17	Chemokine (C-C motif) receptor 7	2.362	
18	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	2.2038	
19	Chemokine (C-X3-C motif) receptor 1	2.2038	
20	Chemokine (C-X-C motif) ligand 2	2.2038	
21	Complement component 3	2.0562	
22	Chemokine (C-C motif) ligand 16	2.0562	
23	Chemokine (C-C motif) ligand 21	2.0562	
24	Chemokine (C-C motif) ligand 8	2.0562	
25	Chemokine (C-C motif) receptor 2	2.0562	
26	Chemokine (C-C motif) ligand 23	1.9185	
27	Chemokine (C-C motif) receptor 8	1.9185	
28	B-cell CLL/lymphoma 6	1.7901	
29	Chemokine (C-C motif) ligand 11	1.7901	
30	Chemokine (C-C motif) ligand 19	1.7901	
31	Chemokine (C-C motif) ligand 2	1.7901	
32	Chemokine (C-C motif) ligand 24	1.7901	
33	Chemokine (C-C motif) receptor 5	1.7901	
34	Chemokine (C-C motif) receptor 6	1.7901	
35	Chemokine (C-X-C motif) ligand 14	1.7901	
36	Chemokine (C-X-C motif) ligand 5	1.7901	
37	Interleukin 17C	1.7901	
38	Interleukin 1 family, member 5 (delta)	1.7901	
39	Interleukin 8 receptor, alpha	1.7901	
40	Toll interacting protein	1.7901	

41	Chemokine (C-C motif) ligand 25	1.6702	
42	Chemokine (C-C motif) ligand 26	1.6702	
43	Chemokine (C-C motif) receptor 3	1.6702	
44	Chemokine (C-C motif) receptor 9	1.6702	
45	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	1.6702	
46	Chemokine (C-C motif) ligand 13	1.5583	
47	Chemokine (C-C motif) ligand 3	1.5583	
48	Chemokine (C-C motif) ligand 4	1.5583	
49	Chemokine (C-C motif) receptor 4	1.5583	
50	Chemokine (C-X-C motif) ligand 10	1.5583	
51	Chemokine (C-X-C motif) ligand 9	1.5583	
52	Interleukin 8 receptor, beta	1.5583	
53	Chemokine (C-C motif) ligand 17	1.454	
54	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	1.454	
55	Chemokine (C-X-C motif) ligand 11	1.3566	
56	Interleukin 13 receptor, alpha 1	1.3566	
57	Interleukin 1 receptor antagonist	1.3566	
58	Chemokine (C-C motif) ligand 7	1.2658	
59	Interleukin 5 (colony-stimulating factor, eosinophil)	1.2658	
60	Chemokine (C motif) receptor 1	1.2658	
61	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	1.181	
62	Interleukin 10 receptor, beta	1.181	
63	Interleukin 1 family, member 6 (epsilon)	1.181	
64	Interleukin 5 receptor, alpha	1.181	
65	C-reactive protein, pentraxin-related	1.1019	
66	Chemokine (C-X-C motif) ligand 13	1.1019	
67	Caspase recruitment domain family, member 18	1.1019	
68	Leukotriene B4 receptor	1.1019	
69	Secreted phosphoprotein 1	1.1019	
70	CD40 ligand	1.1019	
1	CCAAT/enhancer binding protein (C/EBP). beta		-1.1173
2	Interferon, alpha 2		-1.1173
3	Interleukin 10 receptor, alpha		-1.1173
4	Interleukin 1 family, member 8 (eta)		-1.1173
5	Lymphotoxin alpha (TNF superfamily, member 1)		-1.1173
6	Interleukin 1 family, member 7 (zeta)		-1.1975
7	Interleukin 22		-1.2834
8	Lymphotoxin beta (TNF superfamily, member 3)		-1.2834
9	Interleukin 1 receptor, type I		-1.3755
10	Interleukin 9 receptor		-1.4743
11	Chemokine (C-C motif) receptor 1		-1992

Table 12: The gene expression analysis for IAV infection in patient 5; the gene symbol, the average change of C_t values ($AVG\ \Delta C_t$) between the acut and control samples, the level of gene expression ($2^{-\Delta C_t}$) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 5	Cont.5	Acu. 5	Cont.5	Acu 5/Cont.5	Acu 5/Cont.5
ABCF1	1.72	-0.18	0.303549	1.132884	0.27	-3.73
BCL6	1.62	0.22	0.325335	0.858565	0.38	-2.64
C3	1.92	0.62	0.264255	0.650671	0.41	-2.46
C4A	1.12	-0.48	0.460094	1.394744	0.33	-3.03
C5	1.72	-0.28	0.303549	1.214195	0.25	-4.00
CCL1	2.52	0.82	0.174343	0.566442	0.31	-3.25
CCL11	2.22	8.72	0.214641	0.002371	90.51	90.51
CCL13	3.52	2.32	0.087171	0.200267	0.44	-2.30
CCL15	2.12	0.52	0.230047	0.697372	0.33	-3.03
CCL16	3.12	0.82	0.115023	0.566442	0.20	-4.92
CCL17	2.82	1.32	0.141610	0.400535	0.35	-2.83
CCL18	2.42	3.22	0.186856	0.107321	1.74	1.74
CCL19	2.02	-0.18	0.246558	1.132884	0.22	-4.59
CCL2	1.42	4.72	0.373712	0.037944	9.85	9.85
CCL20	1.22	-0.58	0.429283	1.494849	0.29	-3.48
CCL21	2.02	0.62	0.246558	0.650671	0.38	-2.64
CCL23	1.22	-0.48	0.429283	1.394744	0.31	-3.25
CCL24	1.22	-0.88	0.429283	1.840375	0.23	-4.29
CCL25	1.02	0.02	0.493116	0.986233	0.50	-2.00
CCL26	4.32	2.62	0.050067	0.162668	0.31	-3.25
CCL3	1.12	0.02	0.460094	0.986233	0.47	-2.14
CCL4	1.52	3.42	0.348686	0.093428	3.73	3.73
CCL5	2.02	3.52	0.246558	0.087171	2.83	2.83
CCL7	2.12	1.92	0.230047	0.264255	0.87	-1.15
CCL8	1.42	2.52	0.373712	0.174343	2.14	2.14
CCR1	1.32	-0.48	0.400535	1.394744	0.29	-3.48
CCR2	2.72	3.22	0.151774	0.107321	1.41	1.41
CCR3	1.62	0.52	0.325335	0.697372	0.47	-2.14
CCR4	1.62	0.82	0.325335	0.566442	0.57	-1.74
CCR5	0.72	-0.88	0.607097	1.840375	0.33	-3.03
CCR6	1.82	0.32	0.283221	0.801070	0.35	-2.83
CCR7	2.62	3.62	0.162668	0.081334	2.00	2.00
CCR8	2.02	0.12	0.246558	0.920188	0.27	-3.73
CCR9	1.62	-0.08	0.325335	1.057018	0.31	-3.25
CEBPB	2.52	1.52	0.174343	0.348686	0.50	-2.00
CRP	0.92	0.42	0.528509	0.747425	0.71	-1.41
CX3CR1	4.02	2.62	0.061640	0.162668	0.38	-2.64
CXCL1	0.72	-1.08	0.607097	2.114036	0.29	-3.48
CXCL10	1.52	4.82	0.348686	0.035403	9.85	9.85
CXCL11	1.32	0.52	0.400535	0.697372	0.57	-1.74
CXCL12	1.92	0.32	0.264255	0.801070	0.33	-3.03
CXCL13	1.92	0.02	0.264255	0.986233	0.27	-3.73

CXCL14	1.12	0.42	0.460094	0.747425	0.62	-1.62
CXCL2	4.32	1.92	0.050067	0.264255	0.19	-5.28
CXCL3	3.02	2.52	0.123279	0.174343	0.71	-1.41
CXCL5	1.72	0.22	0.303549	0.858565	0.35	-2.83
CXCL6	1.82	0.52	0.283221	0.697372	0.41	-2.46
CXCL9	1.92	1.12	0.264255	0.460094	0.57	-1.74
CARD18	2.12	1.72	0.230047	0.303549	0.76	-1.32
IFNA2	-0.08	-0.18	1.057018	1.132884	0.93	-1.07
IL10	0.72	3.32	0.607097	0.100134	6.06	6.06
IL10RA	0.72	-0.78	0.607097	1.717131	0.35	-2.83
IL10RB	1.82	0.82	0.283221	0.566442	0.50	-2.00
IL13	1.32	3.12	0.400535	0.115023	3.48	3.48
IL13RA1	1.62	0.62	0.325335	0.650671	0.50	-2.00
IL17C	2.22	0.12	0.214641	0.920188	0.23	-4.29
IL1A	0.82	2.62	0.566442	0.162668	3.48	3.48
IL1B	0.82	5.52	0.566442	0.021793	25.99	25.99
IL1F10	1.82	0.52	0.283221	0.697372	0.41	-2.46
IL1F5	1.72	0.32	0.303549	0.801070	0.38	-2.64
IL1F6	1.32	0.92	0.400535	0.528509	0.76	-1.32
IL1F7	0.72	0.72	0.607097	0.607097	1.00	-1.00
IL1F8	0.82	-1.28	0.566442	2.428390	0.23	-4.29
IL1F9	1.12	-0.38	0.460094	1.301342	0.35	-2.83
IL1R1	0.62	-0.78	0.650671	1.717131	0.38	-2.64
IL1RN	0.92	-0.28	0.528509	1.214195	0.44	-2.30
IL22	2.02	0.72	0.246558	0.607097	0.41	-2.46
IL5	1.22	-0.98	0.429283	1.972465	0.22	-4.59
IL5RA	2.12	0.62	0.230047	0.650671	0.35	-2.83
IL8	0.42	2.62	0.747425	0.162668	4.59	4.59
IL8RA	2.22	0.62	0.214641	0.650671	0.33	-3.03
IL8RB	1.42	0.72	0.373712	0.607097	0.62	-1.62
IL9	2.32	3.32	0.200267	0.100134	2.00	2.00
IL9R	1.52	0.12	0.348686	0.920188	0.38	-2.64
LTA	1.62	0.12	0.325335	0.920188	0.35	-2.83
LTB	2.52	1.42	0.174343	0.373712	0.47	-2.14
LTB4R	1.32	0.42	0.400535	0.747425	0.54	-1.87
MIF	1.12	0.22	0.460094	0.858565	0.54	-1.87
SCYE1	3.42	1.02	0.093428	0.493116	0.19	-5.28
SPP1	1.92	0.72	0.264255	0.607097	0.44	-2.30
TNF	2.22	4.42	0.214641	0.046714	4.59	4.59
CD40LG	1.52	0.62	0.348686	0.650671	0.54	-1.87
TOLLIP	2.92	2.12	0.132127	0.230047	0.57	-1.74
XCR1	2.62	1.22	0.162668	0.429283	0.38	-2.64

Table 13: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 5.

No.	Gene name	Up regulated genes	Down regulated genes
1	Chemokine (C-C motif) ligand 11	90.51	
2	Interleukin 1, beta	25.99	
3	Chemokine (C-C motif) ligand 2	9.85	
4	Chemokine (C-X-C motif) ligand 10	9.85	
5	Interleukin 10	6.06	
6	Interleukin 8	4.59	
7	Tumor necrosis factor (TNF superfamily, member 2)	4.59	
8	Chemokine (C-C motif) ligand 4	3.73	
9	Interleukin 13	3.48	
10	Interleukin 1, alpha	3.48	
11	Chemokine (C-C motif) ligand 5	2.83	
12	Chemokine (C-C motif) ligand 8	2.14	
13	Chemokine (C-C motif) receptor 7	2	
14	Interleukin 9	2	
15	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	1.74	
16	Chemokine (C-C motif) receptor 2	1.41	
1	Chemokine (C-C motif) ligand 7		-1.15
2	Caspase recruitment domain family, member 18		-1.32
3	Interleukin 1 family, member 6 (epsilon)		-1.32
4	C-reactive protein, pentraxin-related		-1.41
5	Chemokine (C-X-C motif) ligand 3		-1.41
6	Chemokine (C-X-C motif) ligand 14		-1.62
7	Interleukin 8 receptor, beta		-1.62
8	Chemokine (C-C motif) receptor 4		-1.74
9	Chemokine (C-X-C motif) ligand 11		-1.74
10	Chemokine (C-X-C motif) ligand 9		-1.74
11	Toll interacting protein		-1.74
12	Leukotriene B4 receptor		-1.87
13	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.87
14	CD40 ligand		-1.87
15	Chemokine (C-C motif) ligand 25		-2
16	CCAAT/enhancer binding protein (C/EBP). beta		-2
17	Interleukin 10 receptor, beta		-2
18	Interleukin 13 receptor, alpha 1		-2
19	Chemokine (C-C motif) ligand 3		-2.14
20	Chemokine (C-C motif) receptor 3		-2.14
21	Lymphotoxin beta (TNF superfamily, member 3)		-2.14
22	Chemokine (C-C motif) ligand 13		-2.3

23	Interleukin 1 receptor antagonist		-2.3
24	Secreted phosphoprotein 1		-2.3
25	Complement component 3		-2.46
26	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-2.46
27	Interleukin 1 family, member 10 (theta)		-2.46
28	Interleukin 22		-2.46
29	B-cell CLL/lymphoma 6		-2.64
30	Chemokine (C-C motif) ligand 21		-2.64
31	Chemokine (C-X3-C motif) receptor 1		-2.64
32	Interleukin 1 family, member 5 (delta)		-2.64
33	Interleukin 1 receptor, type I		-2.64
34	Interleukin 9 receptor		-2.64
35	Chemokine (C motif) receptor 1		-2.64
36	Chemokine (C-C motif) ligand 17		-2.83
37	Chemokine (C-C motif) receptor 6		-2.83
38	Chemokine (C-X-C motif) ligand 5		-2.83
39	Interleukin 10 receptor, alpha		-2.83
40	Interleukin 1 family, member 9		-2.83
41	Interleukin 5 receptor, alpha		-2.83
42	Lymphotoxin alpha (TNF superfamily, member 1)		-2.83
43	Complement component 4A (Rodgers blood group)		-3.03
44	Chemokine (C-C motif) ligand 15		-3.03
45	Chemokine (C-C motif) receptor 5		-3.03
46	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-3.03
47	Interleukin 8 receptor, alpha		-3.03
48	Chemokine (C-C motif) ligand 1		-3.25
49	Chemokine (C-C motif) ligand 23		-3.25
50	Chemokine (C-C motif) ligand 26		-3.25
51	Chemokine (C-C motif) receptor 9		-3.25
52	Chemokine (C-C motif) ligand 20		-3.48
53			
54	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)		-3.48
55	ATP-binding cassette, sub-family F (GCN20). member 1		-3.73
56	Chemokine (C-C motif) receptor 8		-3.73
57	Chemokine (C-X-C motif) ligand 13		-3.73
58	Complement component 5		-4
59	Chemokine (C-C motif) ligand 24		-4.29
60	Interleukin 17C		-4.29
61	Interleukin 1 family, member 8 (eta)		-4.29
62	Chemokine (C-C motif) ligand 19		-4.59
63	Interleukin 5 (colony-stimulating factor, eosinophil)		-4.59
64	Chemokine (C-C motif) ligand 16		-4.92

65	Chemokine (C-X-C motif) ligand 2		-5.28
66	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-5.28

Table 14: The gene expression analysis for IAV infection in patient 6; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 6	Cont.6	Acu. 6	Cont.6	Acu 6/Cont.6	Acu 6/Cont.6
ABCF1	-0.4	0.92	1.319508	0.528509	2.4967	2.4967
BCL6	-0.2	1.02	1.148698	0.493116	2.3295	2.3295
C3	0.8	1.22	0.574349	0.429283	1.3379	1.3379
C4A	0	-0.18	1	1.132884	0.8827	-1.1329
C5	0.2	0.22	0.870551	0.858565	1.014	1.014
CCL1	1.5	5.02	0.353553	0.03082	11.4716	11.4716
CCL11	1.6	1.02	0.329877	0.493116	0.669	-1.4948
CCL13	2.7	2.02	0.153893	0.246558	0.6242	-1.6021
CCL15	1.3	0.72	0.406126	0.607097	0.669	-1.4948
CCL16	2.4	1.72	0.189465	0.303549	0.6242	-1.6021
CCL17	2.5	1.82	0.176777	0.283221	0.6242	-1.6021
CCL18	1.9	4.62	0.267943	0.040667	6.5887	6.5887
CCL19	0.6	1.22	0.659754	0.429283	1.5369	1.5369
CCL2	0.1	1.22	0.933033	0.429283	2.1735	2.1735
CCL20	-0.1	0.02	1.071773	0.986233	1.0867	1.0867
CCL21	0.8	0.62	0.574349	0.650671	0.8827	-1.1329
CCL23	-0.2	0.12	1.148698	0.920188	1.2483	1.2483
CCL24	0.3	-0.98	0.812252	1.972465	0.4118	-2.4284
CCL25	0.6	0.02	0.659754	0.986233	0.669	-1.4948
CCL26	4.7	2.72	0.038473	0.151774	0.2535	-3.9449
CCL3	0.8	-0.18	0.574349	1.132884	0.507	-1.9725
CCL4	1.2	0.02	0.435275	0.986233	0.4414	-2.2658
CCL5	1.9	7.12	0.267943	0.007189	37.2715	37.2715
CCL7	2	4.92	0.25	0.033032	7.5685	7.5685
CCL8	0	0.92	1	0.528509	1.8921	1.8921
CCR1	0.3	0.52	0.812252	0.697372	1.1647	1.1647
CCR2	2.5	1.22	0.176777	0.429283	0.4118	-2.4284
CCR3	1.2	0.12	0.435275	0.920188	0.473	-2.114
CCR4	1.3	0.32	0.406126	0.80107	0.507	-1.9725
CCR5	-0.3	-0.48	1.231144	1.394744	0.8827	-1.1329
CCR6	0.9	0.42	0.535887	0.747425	0.717	-1.3947
CCR7	1.8	1.92	0.287175	0.264255	1.0867	1.0867
CCR8	1.6	0.52	0.329877	0.697372	0.473	-2.114
CCR9	1.1	1.12	0.466516	0.460094	1.014	1.014
CEBPB	3	1.92	0.125	0.264255	0.473	-2.114
CRP	0.9	0.02	0.535887	0.986233	0.5434	-1.8404
CX3CR1	2.6	3.52	0.164938	0.087171	1.8921	1.8921

CXCL1	0.5	2.62	0.707107	0.162668	4.3469	4.3469
CXCL10	0.6	2.02	0.659754	0.246558	2.6759	2.6759
CXCL11	1	0.12	0.5	0.920188	0.5434	-1.8404
CXCL12	1.5	0.92	0.353553	0.528509	0.669	-1.4948
CXCL13	1.6	0.62	0.329877	0.650671	0.507	-1.9725
CXCL14	0.3	1.12	0.812252	0.460094	1.7654	1.7654
CXCL2	3.9	3.62	0.066986	0.081334	0.8236	-1.2142
CXCL3	2.8	4.22	0.143587	0.05366	2.6759	2.6759
CXCL5	1.2	0.22	0.435275	0.858565	0.507	-1.9725
CXCL6	1.2	-0.08	0.435275	1.057018	0.4118	-2.4284
CXCL9	1.5	7.82	0.353553	0.004425	79.8932	79.8932
CARD18	1.2	1.62	0.435275	0.325335	1.3379	1.3379
IFNA2	0	2.12	1	0.230047	4.3469	4.3469
IL10	0.2	3.82	0.870551	0.070805	12.295	12.295
IL10RA	0.5	0.02	0.707107	0.986233	0.717	-1.3947
IL10RB	1.8	1.02	0.287175	0.493116	0.5824	-1.7171
IL13	1.3	3.22	0.406126	0.107321	3.7842	3.7842
IL13RA1	1.3	1.72	0.406126	0.303549	1.3379	1.3379
IL17C	1.5	0.32	0.353553	0.80107	0.4414	-2.2658
IL1A	0.3	0.02	0.812252	0.986233	0.8236	-1.2142
IL1B	0.8	2.42	0.574349	0.186856	3.0738	3.0738
IL1F10	1.3	0.62	0.406126	0.650671	0.6242	-1.6021
IL1F5	1.1	0.52	0.466516	0.697372	0.669	-1.4948
IL1F6	0.8	0.52	0.574349	0.697372	0.8236	-1.2142
IL1F7	0.8	0.72	0.574349	0.607097	0.9461	-1.057
IL1F8	0.5	0.22	0.707107	0.858565	0.8236	-1.2142
IL1F9	1.1	0.42	0.466516	0.747425	0.6242	-1.6021
IL1R1	0.6	0.02	0.659754	0.986233	0.669	-1.4948
IL1RN	0.8	0.12	0.574349	0.920188	0.6242	-1.6021
IL22	2.5	1.52	0.176777	0.348686	0.507	-1.9725
IL5	0.9	0.42	0.535887	0.747425	0.717	-1.3947
IL5RA	1.8	0.52	0.287175	0.697372	0.4118	-2.4284
IL8	0.1	2.72	0.933033	0.151774	6.1475	6.1475
IL8RA	1.6	1.92	0.329877	0.264255	1.2483	1.2483
IL8RB	0.9	4.82	0.535887	0.035403	15.1369	15.1369
IL9	2.1	2.02	0.233258	0.246558	0.9461	-1.057
IL9R	1.7	1.52	0.307786	0.348686	0.8827	-1.1329
LTA	1.5	0.62	0.353553	0.650671	0.5434	-1.8404
LTB	2.6	1.42	0.164938	0.373712	0.4414	-2.2658
LTB4R	1	0.62	0.5	0.650671	0.7684	-1.3013
MIF	1	0.22	0.5	0.858565	0.5824	-1.7171
SCYE1	3.5	3.12	0.088388	0.115023	0.7684	-1.3013
SPP1	1.8	0.62	0.287175	0.650671	0.4414	-2.2658
TNF	1.7	4.12	0.307786	0.057512	5.3517	5.3517
CD40LG	1.4	1.72	0.378929	0.303549	1.2483	1.2483
TOLLIP	2.4	0.72	0.189465	0.607097	0.3121	-3.2043
XCR1	1.9	0.62	0.267943	0.650671	0.4118	-2.4284

Table 15: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 6.

No.	Gene name	Up regulated genes	Down regulated genes
1	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	79.8932	
2	Chemokine (C-C motif) ligand 5	37.2715	
3	Interleukin 8 receptor, beta	15.1369	
4	Interleukin 10	12.295	
5	Chemokine (C-C motif) ligand 1	11.4716	
6	Chemokine (C-C motif) ligand 7	7.5685	
7	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	6.5887	
8	Interleukin 8	6.1475	
9	Tumor necrosis factor (TNF superfamily, member 2)	5.3517	
10	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	4.3469	
11	Interferon, alpha 2	4.3469	
12	Interleukin 13	3.7842	
13	Interleukin 1, beta	3.0738	
14	Chemokine (C-X-C motif) ligand 10	2.6759	
15	Chemokine (C-X-C motif) ligand 3	2.6759	
16	ATP-binding cassette, sub-family F (GCN20). member 1	2.4967	
17	B-cell CLL/lymphoma 6	2.3295	
18	Chemokine (C-C motif) ligand 2	2.1735	
19	Chemokine (C-C motif) ligand 8	1.8921	
20	Chemokine (C-X3-C motif) receptor 1	1.8921	
21	Chemokine (C-X-C motif) ligand 14	1.7654	
22	Chemokine (C-C motif) ligand 19	1.5369	
23	Complement component 3	1.3379	
24	Caspase recruitment domain family, member 18	1.3379	
25	Interleukin 13 receptor, alpha 1	1.3379	
26	Chemokine (C-C motif) ligand 23	1.2483	
27	Interleukin 8 receptor, alpha	1.2483	
28	CD40 ligand	1.2483	
29	Chemokine (C-C motif) receptor 1	1.1647	
1	Complement component 4A (Rodgers blood group)		-1.1329
2	Chemokine (C-C motif) ligand 21		-1.1329
3	Chemokine (C-C motif) receptor 5		-1.1329
4	Interleukin 9 receptor		-1.1329
5	Chemokine (C-X-C motif) ligand 2		-1.2142
6	Interleukin 1, alpha		-1.2142
7	Interleukin 1 family, member 6 (epsilon)		-1.2142
8	Leukotriene B4 receptor		-1.3013
9	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-1.3013

10	Chemokine (C-C motif) receptor 6		-1.3947
11	Interleukin 10 receptor, alpha		-1.3947
12	Interleukin 5 (colony-stimulating factor, eosinophil)		-1.3947
13	Chemokine (C-C motif) ligand 11		-1.4948
14	Chemokine (C-C motif) ligand 15		-1.4948
15	Chemokine (C-C motif) ligand 25		-1.4948
16	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-1.4948
17	Interleukin 1 family, member 5 (delta)		-1.4948
18	Interleukin 1 receptor, type I		-1.4948
19	Chemokine (C-C motif) ligand 13		-1.6021
20	Chemokine (C-C motif) ligand 16		-1.6021
21	Chemokine (C-C motif) ligand 17		-1.6021
22	Interleukin 1 family, member 10 (theta)		-1.6021
23	Interleukin 1 family, member 9		-1.6021
24	Interleukin 1 receptor antagonist		-1.6021
25	Interleukin 10 receptor, beta		-1.7171
26	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.7171
27	C-reactive protein, pentraxin-related		-1.8404
28	Chemokine (C-X-C motif) ligand 11		-1.8404
29	Lymphotoxin alpha (TNF superfamily, member 1)		-1.8404
30	Chemokine (C-C motif) ligand 3		-1.9725
31	Chemokine (C-C motif) receptor 4		-1.9725
32	Chemokine (C-X-C motif) ligand 13		-1.9725
33	Chemokine (C-X-C motif) ligand 5		-1.9725
34	Interleukin 22		-1.9725
35	Chemokine (C-C motif) receptor 3		-2.114
36	Chemokine (C-C motif) receptor 8		-2.114
37	CCAAT/enhancer binding protein (C/EBP). beta		-2.114
38	Chemokine (C-C motif) ligand 4		-2.2658
39	Interleukin 17C		-2.2658
40	Lymphotoxin beta (TNF superfamily, member 3)		-2.2658
41	Secreted phosphoprotein 1		-2.2658
42	Chemokine (C-C motif) ligand 24		-2.4284
43	Chemokine (C-C motif) receptor 2		-2.4284
44	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-2.4284
45	Interleukin 5 receptor, alpha		-2.4284
46	Chemokine (C motif) receptor 1		-2.4284
47	Toll interacting protein		-3.2043
48	Chemokine (C-C motif) ligand 26		-3.9449

Table 16: The gene expression analysis for IAV infection in patient 7; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 7	Cont.7	Acu. 7	Cont.7	Acu 7/Cont.7	Acu 7/Cont.7
ABCF1	0.26	1.06	0.835088	0.479632	1.74	1.74
BCL6	0.86	0.86	0.550953	0.550953	1.00	1.00
C3	0.16	1.36	0.895025	0.389582	2.30	2.30
C4A	-0.74	0.76	1.670176	0.590496	2.83	2.83
C5	0.06	2.96	0.959264	0.128514	7.46	7.46
CCL1	1.16	5.66	0.447513	0.019777	22.63	22.63
CCL11	0.76	1.46	0.590496	0.363493	1.62	1.62
CCL13	1.66	2.76	0.316439	0.147624	2.14	2.14
CCL15	0.36	1.36	0.779165	0.389582	2.00	2.00
CCL16	1.46	2.86	0.363493	0.137738	2.64	2.64
CCL17	1.26	2.06	0.417544	0.239816	1.74	1.74
CCL18	0.86	4.76	0.550953	0.036906	14.93	14.93
CCL19	0.66	1.76	0.632878	0.295248	2.14	2.14
CCL2	0.26	5.56	0.835088	0.021197	39.40	39.40
CCL20	0.16	0.46	0.895025	0.726986	1.23	1.23
CCL21	0.46	1.26	0.726986	0.417544	1.74	1.74
CCL23	-0.24	0.66	1.180993	0.632878	1.87	1.87
CCL24	-0.04	0.76	1.028114	0.590496	1.74	1.74
CCL25	-0.44	1.06	1.356604	0.479632	2.83	2.83
CCL26	3.06	4.56	0.119908	0.042394	2.83	2.83
CCL3	0.06	0.76	0.959264	0.590496	1.62	1.62
CCL4	0.06	0.96	0.959264	0.514057	1.87	1.87
CCL5	0.96	4.06	0.514057	0.059954	8.57	8.57
CCL7	0.76	1.66	0.590496	0.316439	1.87	1.87
CCL8	0.06	0.66	0.959264	0.632878	1.52	1.52
CCR1	0.46	0.86	0.726986	0.550953	1.32	1.32
CCR2	0.86	2.56	0.550953	0.169576	3.25	3.25
CCR3	0.26	1.06	0.835088	0.479632	1.74	1.74
CCR4	-0.04	1.36	1.028114	0.389582	2.64	2.64
CCR5	-0.84	0.16	1.790050	0.895025	2.00	2.00
CCR6	0.36	1.06	0.779165	0.479632	1.62	1.62
CCR7	0.86	1.66	0.550953	0.316439	1.74	1.74
CCR8	0.76	1.46	0.590496	0.363493	1.62	1.62
CCR9	-0.04	0.86	1.028114	0.550953	1.87	1.87
CEBPB	1.66	3.36	0.316439	0.097396	3.25	3.25
CRP	0.16	0.76	0.895025	0.590496	1.52	1.52
CX3CR1	2.16	3.36	0.223756	0.097396	2.30	2.30
CXCL1	0.36	4.46	0.779165	0.045437	17.15	17.15
CXCL10	0.26	6.36	0.835088	0.012174	68.59	68.59
CXCL11	0.16	1.16	0.895025	0.447513	2.00	2.00
CXCL12	1.06	1.76	0.479632	0.295248	1.62	1.62
CXCL13	0.86	1.76	0.550953	0.295248	1.87	1.87

CXCL14	-0.44	0.76	1.356604	0.590496	2.30	2.30
CXCL2	2.36	3.76	0.194791	0.073812	2.64	2.64
CXCL3	1.46	2.86	0.363493	0.137738	2.64	2.64
CXCL5	0.36	0.96	0.779165	0.514057	1.52	1.52
CXCL6	-0.24	1.46	1.180993	0.363493	3.25	3.25
CXCL9	0.26	1.26	0.835088	0.417544	2.00	2.00
CARD18	0.56	1.76	0.678302	0.295248	2.30	2.30
IFNA2	-0.84	3.06	1.790050	0.119908	14.93	14.93
IL10	-0.14	3.96	1.101905	0.064257	17.15	17.15
IL10RA	0.16	0.46	0.895025	0.726986	1.23	1.23
IL10RB	0.46	2.06	0.726986	0.239816	3.03	3.03
IL13	0.26	5.06	0.835088	0.029977	27.86	27.86
IL13RA1	0.36	1.46	0.779165	0.363493	2.14	2.14
IL17C	0.66	2.06	0.632878	0.239816	2.64	2.64
IL1A	-0.44	3.16	1.356604	0.111878	12.13	12.13
IL1B	-0.54	4.86	1.453973	0.034435	42.22	42.22
IL1F10	0.06	1.46	0.959264	0.363493	2.64	2.64
IL1F5	-0.04	1.26	1.028114	0.417544	2.46	2.46
IL1F6	-0.04	1.06	1.028114	0.479632	2.14	2.14
IL1F7	-0.14	0.76	1.101905	0.590496	1.87	1.87
IL1F8	0.16	0.66	0.895025	0.632878	1.41	1.41
IL1F9	0.16	1.56	0.895025	0.339151	2.64	2.64
IL1R1	-0.14	0.76	1.101905	0.590496	1.87	1.87
IL1RN	0.16	1.06	0.895025	0.479632	1.87	1.87
IL22	1.06	3.16	0.479632	0.111878	4.29	4.29
IL5	-0.24	1.26	1.180993	0.417544	2.83	2.83
IL5RA	0.66	1.46	0.632878	0.363493	1.74	1.74
IL8	-0.44	5.46	1.356604	0.022718	59.71	59.71
IL8RA	0.66	2.16	0.632878	0.223756	2.83	2.83
IL8RB	0.06	1.06	0.959264	0.479632	2.00	2.00
IL9	0.96	1.96	0.514057	0.257028	2.00	2.00
IL9R	0.46	1.16	0.726986	0.447513	1.62	1.62
LTA	0.26	1.26	0.835088	0.417544	2.00	2.00
LTB	1.86	3.16	0.275476	0.111878	2.46	2.46
LTB4R	0.06	1.16	0.959264	0.447513	2.14	2.14
MIF	0.46	1.46	0.726986	0.363493	2.00	2.00
SCYE1	1.56	4.36	0.339151	0.048698	6.96	6.96
SPP1	1.80	1.80	0.62	0.287175	0.650671	0.44
TNF	1.70	1.70	4.12	0.307786	0.057512	5.35
CD40LG	1.40	1.40	1.72	0.378929	0.303549	1.25
TOLLIP	2.40	2.40	0.72	0.189465	0.607097	0.31
XCR1	1.90	1.90	0.62	0.267943	0.650671	0.41

Table 17: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 7.

No.	Gene name	Up regulated genes	Down regulated genes
1	Chemokine (C-X-C motif) ligand 10	68.5935	Non
2	Interleukin 8	59.7141	
3	Interleukin 1, beta	42.2243	
4	Chemokine (C-C motif) ligand 2	39.3966	
5	Interleukin 13	27.8576	
6	Chemokine (C-C motif) ligand 1	22.6274	
7	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	17.1484	
8	Interleukin 10	17.1484	
9	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	14.9285	
10	Interferon, alpha 2	14.9285	
11	Tumor necrosis factor (TNF superfamily, member 2)	14.9285	
12	Interleukin 1, alpha	12.1257	
13	Chemokine (C-C motif) ligand 5	8.5742	
14	Complement component 5	7.4643	
15	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	6.9644	
16	Toll interacting protein	4.9246	
17	Interleukin 22	4.2871	
18	Chemokine (C-C motif) receptor 2	3.249	
19	CCAAT/enhancer binding protein (C/EBP). beta	3.249	
20	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	3.249	
21	Interleukin 10 receptor, beta	3.0314	
22	Secreted phosphoprotein 1	3.0314	
23	Chemokine (C motif) receptor 1	3.0314	
24	Complement component 4A (Rodgers blood group)	2.8284	
25	Chemokine (C-C motif) ligand 25	2.8284	
26	Chemokine (C-C motif) ligand 26	2.8284	
27	Interleukin 5 (colony-stimulating factor, eosinophil)	2.8284	
28	Interleukin 8 receptor, alpha	2.8284	
29	Chemokine (C-C motif) ligand 16	2.639	
30	Chemokine (C-C motif) receptor 4	2.639	
31	Chemokine (C-X-C motif) ligand 2	2.639	
32	Chemokine (C-X-C motif) ligand 3	2.639	
33	Interleukin 17C	2.639	
34	Interleukin 1 family, member 10 (theta)	2.639	
35	Interleukin 1 family, member 9	2.639	
36	Interleukin 1 family, member 5 (delta)	2.4623	
37	Lymphotoxin beta (TNF superfamily, member 3)	2.4623	
38	CD40 ligand	2.4623	
39	Complement component 3	2.2974	

40	Chemokine (C-X3-C motif) receptor 1	2.2974	
41	Chemokine (C-X-C motif) ligand 14	2.2974	
42	Caspase recruitment domain family, member 18	2.2974	
43	Chemokine (C-C motif) ligand 13	2.1435	
44	Chemokine (C-C motif) ligand 19	2.1435	
45	Interleukin 13 receptor, alpha 1	2.1435	
46	Interleukin 1 family, member 6 (epsilon)	2.1435	
47	Leukotriene B4 receptor	2.1435	
48	Chemokine (C-C motif) ligand 15	2.0000	
49	Chemokine (C-C motif) receptor 5	2.0000	
50	Chemokine (C-X-C motif) ligand 11	2.0000	
51	Chemokine (C-X-C motif) ligand 9	2.0000	
52	Interleukin 8 receptor, beta	2.0000	
53	Interleukin 9	2.0000	
54	Lymphotoxin alpha (TNF superfamily, member 1)	2.0000	
55	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	2.0000	
56	Chemokine (C-C motif) ligand 23	1.8661	
57	Chemokine (C-C motif) ligand 4	1.8661	
58	Chemokine (C-C motif) ligand 7	1.8661	
59	Chemokine (C-C motif) receptor 9	1.8661	
60	Chemokine (C-X-C motif) ligand 13	1.8661	
61	Interleukin 1 family, member 7 (zeta)	1.8661	
62	Interleukin 1 receptor, type I	1.8661	
63	Interleukin 1 receptor antagonist	1.8661	
64	ATP-binding cassette, sub-family F (GCN20). member 1	1.7411	
65	Chemokine (C-C motif) ligand 17	1.7411	
66	Chemokine (C-C motif) ligand 21	1.7411	
67	Chemokine (C-C motif) ligand 24	1.7411	
68	Chemokine (C-C motif) receptor 3	1.7411	
69	Chemokine (C-C motif) receptor 7	1.7411	
70	Interleukin 5 receptor, alpha	1.7411	
71	Chemokine (C-C motif) ligand 11	1.6245	
72	Chemokine (C-C motif) ligand 3	1.6245	
73	Chemokine (C-C motif) receptor 6	1.6245	
74	Chemokine (C-C motif) receptor 8	1.6245	
75	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	1.6245	
76	Interleukin 9 receptor	1.6245	
77	Chemokine (C-C motif) ligand 8	1.5157	
78	C-reactive protein, pentraxin-related	1.5157	
79	Chemokine (C-X-C motif) ligand 5	1.5157	
80	Interleukin 1 family, member 8 (eta)	1.4142	
81	Chemokine (C-C motif) receptor 1	1.3195	
82	Chemokine (C-C motif) ligand 20	1.2311	
83	Interleukin 10 receptor, alpha	1.2311	

Table 18: The gene expression analysis for IAV infection in patient 8; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 8	Cont.8	Acu. 8	Cont.8	Acu 8/Cont.8	Acu 8/Cont.8
ABCF1	-0.40	0.34	1.319508	0.790041	1.67	1.67
BCL6	0.70	1.04	0.615572	0.486327	1.27	1.27
C3	0.20	0.34	0.870551	0.790041	1.10	1.10
C4A	-1.00	-1.06	2.000000	2.084932	0.96	-1.04
C5	-0.10	0.14	1.071773	0.907519	1.18	1.18
CCL1	1.40	4.34	0.378929	0.049378	7.67	7.67
CCL11	1.20	3.34	0.435275	0.098755	4.41	4.41
CCL13	1.90	1.84	0.267943	0.279322	0.96	-1.04
CCL15	0.90	3.24	0.535887	0.105843	5.06	5.06
CCL16	1.20	2.34	0.435275	0.197510	2.20	2.20
CCL17	1.80	1.84	0.287175	0.279322	1.03	1.03
CCL18	1.60	1.14	0.329877	0.453760	0.73	-1.38
CCL19	0.30	0.64	0.812252	0.641713	1.27	1.27
CCL2	0.00	4.04	1.000000	0.060791	16.45	16.45
CCL20	-0.20	-0.26	1.148698	1.197479	0.96	-1.04
CCL21	0.40	0.34	0.757858	0.790041	0.96	-1.04
CCL23	-0.30	0.04	1.231144	0.972655	1.27	1.27
CCL24	-0.20	-0.06	1.148698	1.042466	1.10	1.10
CCL25	0.00	-0.06	1.000000	1.042466	0.96	-1.04
CCL26	3.10	2.94	0.116629	0.130308	0.90	-1.12
CCL3	0.30	0.24	0.812252	0.846745	0.96	-1.04
CCL4	0.30	0.64	0.812252	0.641713	1.27	1.27
CCL5	0.40	5.24	0.757858	0.026461	28.64	28.64
CCL7	1.70	1.94	0.307786	0.260616	1.18	1.18
CCL8	-0.50	-0.56	1.414214	1.474269	0.96	-1.04
CCR1	0.20	0.44	0.870551	0.737135	1.18	1.18
CCR2	1.00	1.14	0.500000	0.453760	1.10	1.10
CCR3	-0.10	-0.16	1.071773	1.117287	0.96	-1.04
CCR4	-0.10	-0.06	1.071773	1.042466	1.03	1.03
CCR5	-0.80	-0.86	1.741101	1.815038	0.96	-1.04
CCR6	0.50	4.04	0.707107	0.060791	11.63	11.63
CCR7	1.00	3.24	0.500000	0.105843	4.72	4.72
CCR8	0.90	0.94	0.535887	0.521233	1.03	1.03
CCR9	0.50	0.34	0.707107	0.790041	0.90	-1.12
CEBPB	2.60	2.34	0.164938	0.197510	0.84	-1.20
CRP	0.60	0.54	0.659754	0.687771	0.96	-1.04
CX3CR1	1.50	2.14	0.353553	0.226880	1.56	1.56
CXCL1	-0.30	0.44	1.231144	0.737135	1.67	1.67
CXCL10	0.00	3.74	1.000000	0.074842	13.36	13.36
CXCL11	0.10	-0.06	0.933033	1.042466	0.90	-1.12
CXCL12	0.90	0.94	0.535887	0.521233	1.03	1.03
CXCL13	0.90	1.14	0.535887	0.453760	1.18	1.18

CXCL14	-0.20	-0.46	1.148698	1.375542	0.84	-1.20
CXCL2	2.50	2.44	0.176777	0.184284	0.96	-1.04
CXCL3	1.60	1.24	0.329877	0.423373	0.78	-1.28
CXCL5	0.80	0.34	0.574349	0.790041	0.73	-1.38
CXCL6	1.00	0.84	0.500000	0.558644	0.90	-1.12
CXCL9	1.00	0.74	0.500000	0.598739	0.84	-1.20
CARD18	0.10	0.44	0.933033	0.737135	1.27	1.27
IFNA2	-0.90	-1.26	1.866066	2.394957	0.78	-1.28
IL10	-0.30	1.34	1.231144	0.395021	3.12	3.12
IL10RA	-0.10	3.34	1.071773	0.098755	10.85	10.85
IL10RB	0.70	0.64	0.615572	0.641713	0.96	-1.04
IL13	0.70	4.54	0.615572	0.042986	14.32	14.32
IL13RA1	0.90	0.84	0.535887	0.558644	0.96	-1.04
IL17C	0.80	1.24	0.574349	0.423373	1.36	1.36
IL1A	-0.10	-0.16	1.071773	1.117287	0.96	-1.04
IL1B	0.40	5.34	0.757858	0.024689	30.70	30.70
IL1F10	0.50	0.84	0.707107	0.558644	1.27	1.27
IL1F5	1.00	0.84	0.500000	0.558644	0.90	-1.12
IL1F6	-0.40	-0.56	1.319508	1.474269	0.90	-1.12
IL1F7	-0.20	-0.36	1.148698	1.283426	0.90	-1.12
IL1F8	0.00	-0.26	1.000000	1.197479	0.84	-1.20
IL1F9	0.30	0.24	0.812252	0.846745	0.96	-1.04
IL1R1	-0.10	-0.56	1.071773	1.474269	0.73	-1.38
IL1RN	0.50	0.14	0.707107	0.907519	0.78	-1.28
IL22	1.70	0.94	0.307786	0.521233	0.59	-1.69
IL5	0.30	0.24	0.812252	0.846745	0.96	-1.04
IL5RA	1.50	1.14	0.353553	0.453760	0.78	-1.28
IL8	0.30	5.54	0.812252	0.021493	37.79	37.79
IL8RA	1.70	1.14	0.307786	0.453760	0.68	-1.47
IL8RB	0.90	0.44	0.535887	0.737135	0.73	-1.38
IL9	0.80	1.04	0.574349	0.486327	1.18	1.18
IL9R	0.60	0.44	0.659754	0.737135	0.90	-1.12
LTA	0.20	0.24	0.870551	0.846745	1.03	1.03
LTB	2.10	2.44	0.233258	0.184284	1.27	1.27
LTB4R	0.00	-0.06	1.000000	1.042466	0.96	-1.04
MIF	0.40	0.94	0.757858	0.521233	1.45	1.45
SCYE1	2.30	1.74	0.203063	0.299370	0.68	-1.47
SPP1	1.50	1.04	0.353553	0.486327	0.73	-1.38
TNF	1.40	4.94	0.378929	0.032577	11.63	11.63
CD40LG	0.90	1.04	0.535887	0.486327	1.10	1.10
TOLLIP	1.20	1.54	0.435275	0.343885	1.27	1.27
XCR1	1.30	1.44	0.406126	0.368567	1.10	1.10

Table 19: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after IAV infection in patient 8.

No.	Gene name	Up regulated genes	Down regulated genes
1	Interleukin 8	37.7918	
2	Interleukin 1, beta	30.6965	
3	Chemokine (C-C motif) ligand 5	28.6408	
4	Chemokine (C-C motif) ligand 2	16.4498	
5	Interleukin 13	14.3204	
6	Chemokine (C-X-C motif) ligand 10	13.3614	
7	Chemokine (C-C motif) receptor 6	11.6318	
8	Tumor necrosis factor (TNF superfamily, member 2)	11.6318	
9	Interleukin 10 receptor, alpha	10.8528	
10	Chemokine (C-C motif) ligand 1	7.6741	
11	Chemokine (C-C motif) ligand 15	5.063	
12	Chemokine (C-C motif) receptor 7	4.724	
13	Chemokine (C-C motif) ligand 11	4.4076	
14	Interleukin 10	3.1167	
15	Chemokine (C-C motif) ligand 16	2.2038	
16	ATP-binding cassette, sub-family F (GCN20). member 1	1.6702	
17	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	1.6702	
18	Chemokine (C-X3-C motif) receptor 1	1.5583	
19	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	1.454	
20	Interleukin 17C	1.3566	
21	B-cell CLL/lymphoma 6	1.2658	
22	Chemokine (C-C motif) ligand 19	1.2658	
23	Chemokine (C-C motif) ligand 23	1.2658	
24	Chemokine (C-C motif) ligand 4	1.2658	
25	Caspase recruitment domain family, member 18	1.2658	
26	Interleukin 1 family, member 10 (theta)	1.2658	
27	Lymphotoxin beta (TNF superfamily, member 3)	1.2658	
28	Toll interacting protein	1.2658	
29	Complement component 5	1.181	
30	Chemokine (C-C motif) ligand 7	1.181	
31	Chemokine (C-C motif) receptor 1	1.181	
32	Chemokine (C-X-C motif) ligand 13	1.181	
33	Interleukin 9	1.181	
34	Complement component 3	1.1019	
35	Chemokine (C-C motif) ligand 24	1.1019	
36	Chemokine (C-C motif) receptor 2	1.1019	
37	CD40 ligand	1.1019	

38	Chemokine (C motif) receptor 1	1.1019	
1	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)		-1.3755
2	Interleukin 22		-1.6935
3	Interleukin 8 receptor, alpha		-1.4743
4	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-1.4743
5	Chemokine (C-X-C motif) ligand 5		-1.3755
6	Interleukin 1 receptor, type I		-1.3755
7	Interleukin 8 receptor, beta		-1.3755
8	Secreted phosphoprotein 1		-1.3755
9	Chemokine (C-X-C motif) ligand 3		-1.2834
10	Interferon, alpha 2		-1.2834
11	Interleukin 1 receptor antagonist		-1.2834
12	Interleukin 5 receptor, alpha		-1.2834
13	CCAAT/enhancer binding protein (C/EBP). beta		-1.1975
14	Chemokine (C-X-C motif) ligand 14		-1.1975
15	Chemokine (C-X-C motif) ligand 9		-1.1975
16	Interleukin 1 family, member 8 (eta)		-1.1975
17	Chemokine (C-C motif) ligand 26		-1.1173
18	Chemokine (C-C motif) receptor 9		-1.1173
19	Chemokine (C-X-C motif) ligand 11		-1.1173
20	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-1.1173
21	Interleukin 1 family, member 5 (delta)		-1.1173
22	Interleukin 1 family, member 6 (epsilon)		-1.1173
23	Interleukin 1 family, member 7 (zeta)		-1.1173
24	Interleukin 9 receptor		-1.1173

Table 20: The gene expression analysis of PIV infection in patient 1; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 1	Cont.1	Acu. 1	Cont.1	Acu 1/Cont.1	Acu 1/Cont.1
ABCF1	0.48	0.64	0.716978	0.641713	1.12	1.12
BCL6	0.78	1.64	0.582367	0.320856	1.82	1.82
C3	0.58	0.84	0.668964	0.558644	1.20	1.20
C4A	-0.32	0.04	1.248331	0.972655	1.28	1.28
C5	0.68	0.74	0.624165	0.598739	1.04	1.04
CCL1	1.38	6.34	0.384219	0.012344	31.12	31.12
CCL11	1.38	5.14	0.384219	0.028360	13.55	13.55
CCL13	2.18	5.04	0.220676	0.030395	7.26	7.26
CCL15	0.98	1.54	0.506980	0.343885	1.47	1.47
CCL16	1.98	2.74	0.253490	0.149685	1.69	1.69
CCL17	1.58	1.54	0.334482	0.343885	0.97	-1.03
CCL18	1.28	1.54	0.411796	0.343885	1.20	1.20
CCL19	1.48	1.54	0.358489	0.343885	1.04	1.04
CCL2	0.48	3.84	0.716978	0.069830	10.27	10.27
CCL20	0.48	0.14	0.716978	0.907519	0.79	-1.27
CCL21	0.78	0.84	0.582367	0.558644	1.04	1.04
CCL23	0.38	0.54	0.768438	0.687771	1.12	1.12
CCL24	0.28	0.04	0.823591	0.972655	0.85	-1.18
CCL25	0.58	-0.36	0.668964	1.283426	0.52	-1.92
CCL26	3.38	3.54	0.096055	0.085971	1.12	1.12
CCL3	0.58	0.24	0.668964	0.846745	0.79	-1.27
CCL4	0.88	0.44	0.543367	0.737135	0.74	-1.36
CCL5	1.58	3.94	0.334482	0.065154	5.13	5.13
CCL7	1.28	1.24	0.411796	0.423373	0.97	-1.03
CCL8	0.58	0.74	0.668964	0.598739	1.12	1.12
CCR1	0.78	0.74	0.582367	0.598739	0.97	-1.03
CCR2	1.88	1.94	0.271684	0.260616	1.04	1.04
CCR3	0.58	0.54	0.668964	0.687771	0.97	-1.03
CCR4	0.68	1.24	0.624165	0.423373	1.47	1.47
CCR5	-0.22	-0.26	1.164734	1.197479	0.97	-1.03
CCR6	0.88	0.74	0.543367	0.598739	0.91	-1.10
CCR7	1.08	1.64	0.473029	0.320856	1.47	1.47
CCR8	1.18	1.24	0.441351	0.423373	1.04	1.04
CCR9	0.58	0.24	0.668964	0.846745	0.79	-1.27
CEBPB	2.08	1.24	0.236514	0.423373	0.56	-1.79
CRP	0.68	0.04	0.624165	0.972655	0.64	-1.56
CX3CR1	2.38	3.24	0.192109	0.105843	1.82	1.82
CXCL1	0.88	5.14	0.543367	0.028360	19.16	19.16
CXCL10	0.58	5.74	0.668964	0.018711	35.75	35.75
CXCL11	0.78	0.74	0.582367	0.598739	0.97	-1.03
CXCL12	1.68	1.54	0.312083	0.343885	0.91	-1.10

CXCL13	1.58	1.74	0.334482	0.299370	1.12	1.12
CXCL14	1.38	0.44	0.384219	0.737135	0.52	-1.92
CXCL2	1.88	3.34	0.271684	0.098755	2.75	2.75
CXCL3	1.28	2.04	0.411796	0.243164	1.69	1.69
CXCL5	0.68	0.64	0.624165	0.641713	0.97	-1.03
CXCL6	0.68	0.54	0.624165	0.687771	0.91	-1.10
CXCL9	0.88	1.44	0.543367	0.368567	1.47	1.47
CARD18	1.38	1.34	0.384219	0.395021	0.97	-1.03
IFNA2	-0.22	6.04	1.164734	0.015198	76.64	76.64
IL10	0.68	5.44	0.624165	0.023035	27.10	27.10
IL10RA	0.48	0.54	0.716978	0.687771	1.04	1.04
IL10RB	1.18	1.94	0.441351	0.260616	1.69	1.69
IL13	0.98	1.24	0.506980	0.423373	1.20	1.20
IL13RA1	0.88	1.04	0.543367	0.486327	1.12	1.12
IL17C	1.38	1.64	0.384219	0.320856	1.20	1.20
IL1A	0.18	6.84	0.882703	0.008729	101.13	101.13
IL1B	0.38	4.64	0.768438	0.040107	19.16	19.16
IL1F10	0.98	0.74	0.506980	0.598739	0.85	-1.18
IL1F5	0.98	0.94	0.506980	0.521233	0.97	-1.03
IL1F6	0.68	0.84	0.624165	0.558644	1.12	1.12
IL1F7	0.78	0.64	0.582367	0.641713	0.91	-1.10
IL1F8	0.78	0.64	0.582367	0.641713	0.91	-1.10
IL1F9	0.78	1.04	0.582367	0.486327	1.20	1.20
IL1R1	0.58	0.44	0.668964	0.737135	0.91	-1.10
IL1RN	0.68	0.74	0.624165	0.598739	1.04	1.04
IL22	1.28	2.54	0.411796	0.171943	2.39	2.39
IL5	0.78	1.04	0.582367	0.486327	1.20	1.20
IL5RA	1.58	1.54	0.334482	0.343885	0.97	-1.03
IL8	0.48	4.74	0.716978	0.037421	19.16	19.16
IL8RA	1.28	1.04	0.411796	0.486327	0.85	-1.18
IL8RB	0.68	0.84	0.624165	0.558644	1.12	1.12
IL9	1.98	2.74	0.253490	0.149685	1.69	1.69
IL9R	1.48	1.64	0.358489	0.320856	1.12	1.12
LTA	1.18	1.14	0.441351	0.453760	0.97	-1.03
LTB	1.98	4.64	0.253490	0.040107	6.32	6.32
LTB4R	1.48	1.54	0.358489	0.343885	1.04	1.04
MIF	1.28	0.84	0.411796	0.558644	0.74	-1.36
SCYE1	1.88	3.24	0.271684	0.105843	2.57	2.57
SPP1	1.38	1.54	0.384219	0.343885	1.12	1.12
TNF	1.68	5.84	0.312083	0.017458	17.88	17.88
CD40LG	1.68	1.14	0.312083	0.453760	0.69	-1.45
TOLLIP	1.48	1.54	0.358489	0.343885	1.04	1.04
XCR1	1.78	1.64	0.291183	0.320856	0.91	-1.10

Table 21: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after PIV infection in patient 1.

No.	Gene name	Up regulated genes	Down regulated genes
1	Interleukin 1, alpha	101.1253	
2	Interferon, alpha 2	76.6386	
3	Chemokine (C-X-C motif) ligand 10	35.7532	
4	Chemokine (C-C motif) ligand 1	31.125	
5	Interleukin 10	27.0958	
6	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	19.1597	
7	Interleukin 1, beta	19.1597	
8	Interleukin 8	19.1597	
9	Tumor necrosis factor (TNF superfamily, member 2)	17.8766	
10	Chemokine (C-C motif) ligand 11	13.5479	
11	Chemokine (C-C motif) ligand 2	10.2674	
12	Chemokine (C-C motif) ligand 13	7.2602	
13	Lymphotoxin beta (TNF superfamily, member 3)	6.3203	
14	Chemokine (C-C motif) ligand 5	5.1337	
15	Chemokine (C-X-C motif) ligand 2	2.7511	
16	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	2.5669	
17	Interleukin 22	2.395	
18	B-cell CLL/lymphoma 6	1.815	
19	Chemokine (C-X3-C motif) receptor 1	1.815	
20	Chemokine (C-C motif) ligand 16	1.6935	
21	Chemokine (C-X-C motif) ligand 3	1.6935	
22	Interleukin 10 receptor, beta	1.6935	
23	Interleukin 9	1.6935	
24	Chemokine (C-C motif) ligand 15	1.4743	
25	Chemokine (C-C motif) receptor 4	1.4743	
26	Chemokine (C-C motif) receptor 7	1.4743	
27	Chemokine (C-X-C motif) ligand 9	1.4743	
28	Complement component 4A (Rodgers blood group)	1.2834	
29	Complement component 3	1.1975	
30	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	1.1975	
31	Interleukin 13	1.1975	
32	Interleukin 17C	1.1975	
33	Interleukin 1 family, member 9	1.1975	
34	Interleukin 5 (colony-stimulating factor, eosinophil)	1.1975	
35	ATP-binding cassette, sub-family F (GCN20). member 1	1.1173	
36	Chemokine (C-C motif) ligand 23	1.1173	
37	Chemokine (C-C motif) ligand 26	1.1173	
38	Chemokine (C-C motif) ligand 8	1.1173	

39	Chemokine (C-X-C motif) ligand 13	1.1173	
40	Interleukin 13 receptor, alpha 1	1.1173	
41	Interleukin 1 family, member 6 (epsilon)	1.1173	
42	Interleukin 8 receptor, beta	1.1173	
43	Interleukin 9 receptor	1.1173	
44	Secreted phosphoprotein 1	1.1173	
1	Chemokine (C-C motif) receptor 6		-1.1019
2	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-1.1019
3	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-1.1019
4	Interleukin 1 family, member 7 (zeta)		-1.1019
5	Interleukin 1 family, member 8 (eta)		-1.1019
6	Interleukin 1 receptor, type I		-1.1019
7	Chemokine (C motif) receptor 1		-1.1019
8	Chemokine (C-C motif) ligand 24		-1.181
9	Interleukin 1 family, member 10 (theta)		-1.181
10	Interleukin 8 receptor, alpha		-1.181
11	Chemokine (C-C motif) ligand 20		-1.2658
12	Chemokine (C-C motif) ligand 3		-1.2658
13	Chemokine (C-C motif) receptor 9		-1.2658
14	Chemokine (C-C motif) ligand 4		-1.3566
15	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-1.3566
16	CD40 ligand		-1.454
17	C-reactive protein, pentraxin-related		-1.5583
18	CCAAT/enhancer binding protein (C/EBP). beta		-1.7901
19	Chemokine (C-C motif) ligand 25		-1.9185
20	Chemokine (C-X-C motif) ligand 14		-1.9185

Table 22: The gene expression analysis of PIV infection in patient 2; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**2^-ΔC_t**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	**AVG ΔC_t**		**2^-ΔC_t**		**Fold Change**	**Up or Down-Regulation**
	Acu 2	Cont.2	Acu. 2	Cont.2	Acu 2/Cont.2	Acu 2/Cont.2
ABCF1	0.22	0.80	0.858565	0.574349	1.49	1.49
BCL6	5.32	1.20	0.025033	0.435275	0.06	-17.39
C3	0.42	0.90	0.747425	0.535887	1.39	1.39
C4A	-0.58	0.20	1.494849	0.870551	1.72	1.72
C5	0.42	6.10	0.747425	0.014579	51.27	51.27
CCL1	1.52	7.80	0.348686	0.004487	77.71	77.71
CCL11	1.12	1.90	0.460094	0.267943	1.72	1.72
CCL13	2.02	2.80	0.246558	0.143587	1.72	1.72
CCL15	0.82	1.80	0.566442	0.287175	1.97	1.97
CCL16	2.12	3.10	0.230047	0.116629	1.97	1.97

CCL17	1.22	2.10	0.429283	0.233258	1.84	1.84
CCL18	1.22	9.00	0.429283	0.001953	219.79	219.79
CCL19	1.12	1.10	0.460094	0.466516	0.99	-1.01
CCL2	0.32	7.40	0.801070	0.005921	135.30	135.30
CCL20	0.22	0.50	0.858565	0.707107	1.21	1.21
CCL21	0.82	1.40	0.566442	0.378929	1.49	1.49
CCL23	0.42	0.60	0.747425	0.659754	1.13	1.13
CCL24	6.22	1.00	0.013415	0.500000	0.03	-37.27
CCL25	-0.08	1.10	1.057018	0.466516	2.27	2.27
CCL26	8.42	3.80	0.002920	0.071794	0.04	-24.59
CCL3	0.12	0.80	0.920188	0.574349	1.60	1.60
CCL4	0.62	1.50	0.650671	0.353553	1.84	1.84
CCL5	1.12	1.70	0.460094	0.307786	1.49	1.49
CCL7	0.92	2.50	0.528509	0.176777	2.99	2.99
CCL8	0.12	0.70	0.920188	0.615572	1.49	1.49
CCR1	0.32	0.80	0.801070	0.574349	1.39	1.39
CCR2	1.52	4.60	0.348686	0.041235	8.46	8.46
CCR3	0.32	1.90	0.801070	0.267943	2.99	2.99
CCR4	0.42	2.30	0.747425	0.203063	3.68	3.68
CCR5	-0.48	2.00	1.394744	0.250000	5.58	5.58
CCR6	0.52	1.30	0.697372	0.406126	1.72	1.72
CCR7	1.02	2.60	0.493116	0.164938	2.99	2.99
CCR8	1.12	1.70	0.460094	0.307786	1.49	1.49
CCR9	0.22	0.70	0.858565	0.615572	1.39	1.39
CEBPB	5.42	2.20	0.023357	0.217638	0.11	-9.32
CRP	0.12	1.30	0.920188	0.406126	2.27	2.27
CX3CR1	2.42	8.80	0.186856	0.002244	83.29	83.29
CXCL1	1.42	6.20	0.373712	0.013602	27.47	27.47
CXCL10	0.32	0.90	0.801070	0.535887	1.49	1.49
CXCL11	0.62	1.60	0.650671	0.329877	1.97	1.97
CXCL12	1.42	1.80	0.373712	0.287175	1.30	1.30
CXCL13	1.32	6.90	0.400535	0.008373	47.84	47.84
CXCL14	0.12	1.00	0.920188	0.500000	1.84	1.84
CXCL2	2.22	8.30	0.214641	0.003173	67.65	67.65
CXCL3	1.62	2.20	0.325335	0.217638	1.49	1.49
CXCL5	0.62	1.10	0.650671	0.466516	1.39	1.39
CXCL6	0.42	1.60	0.747425	0.329877	2.27	2.27
CXCL9	0.82	1.40	0.566442	0.378929	1.49	1.49
CARD18	1.22	1.60	0.429283	0.329877	1.30	1.30
IFNA2	-0.48	10.00	1.394744	0.000977	1428.22	1428.22
IL10	0.22	5.30	0.858565	0.025383	33.82	33.82
IL10RA	0.42	0.60	0.747425	0.659754	1.13	1.13
IL10RB	1.42	2.80	0.373712	0.143587	2.60	2.60
IL13	0.92	1.10	0.528509	0.466516	1.13	1.13
IL13RA1	6.52	1.30	0.010896	0.406126	0.03	-37.27
IL17C	1.12	2.20	0.460094	0.217638	2.11	2.11
IL1A	-0.08	0.50	1.057018	0.707107	1.49	1.49
IL1B	-0.08	8.10	1.057018	0.003645	290.02	290.02

IL1F10	0.82	1.80	0.566442	0.287175	1.97	1.97
IL1F5	0.62	1.50	0.650671	0.353553	1.84	1.84
IL1F6	0.32	0.80	0.801070	0.574349	1.39	1.39
IL1F7	0.42	0.60	0.747425	0.659754	1.13	1.13
IL1F8	0.32	0.50	0.801070	0.707107	1.13	1.13
IL1F9	0.92	1.20	0.528509	0.435275	1.21	1.21
IL1R1	0.42	0.90	0.747425	0.535887	1.39	1.39
IL1RN	-0.28	0.60	1.214195	0.659754	1.84	1.84
IL22	1.92	2.00	0.264255	0.250000	1.06	1.06
IL5	0.42	0.70	0.747425	0.615572	1.21	1.21
IL5RA	1.42	1.80	0.373712	0.287175	1.30	1.30
IL8	0.22	0.60	0.858565	0.659754	1.30	1.30
IL8RA	1.12	2.00	0.460094	0.250000	1.84	1.84
IL8RB	0.42	1.10	0.747425	0.466516	1.60	1.60
IL9	1.82	2.30	0.283221	0.203063	1.39	1.39
IL9R	1.12	1.70	0.460094	0.307786	1.49	1.49
LTA	0.92	1.50	0.528509	0.353553	1.49	1.49
LTB	3.12	3.30	0.115023	0.101532	1.13	1.13
LTB4R	0.62	1.20	0.650671	0.435275	1.49	1.49
MIF	6.42	1.20	0.011679	0.435275	0.03	-37.27
SCYE1	3.82	2.40	0.070805	0.189465	0.37	-2.68
SPP1	1.02	1.80	0.493116	0.287175	1.72	1.72
TNF	1.22	7.10	0.429283	0.007289	58.89	58.89
CD40LG	0.92	1.50	0.528509	0.353553	1.49	1.49
TOLLIP	1.12	2.40	0.460094	0.189465	2.43	2.43
XCR1	1.12	2.70	0.460094	0.153893	2.99	2.99

Table 23: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after PIV infection in patient 2.

No.	Gene name	Up regulated genes	Down regulated genes
1	Interferon, alpha 2	1428.218	
2	Interleukin 1, beta	290.0183	
3	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	219.7928	
4	Chemokine (C-C motif) ligand 2	135.2983	
5	Chemokine (C-X3-C motif) receptor 1	83.2859	
6	Chemokine (C-C motif) ligand 1	77.7085	
7	Chemokine (C-X-C motif) ligand 2	67.6492	
8	Tumor necrosis factor (TNF superfamily, member 2)	58.892	
9	Complement component 5	51.2685	
10	Chemokine (C-X-C motif) ligand 13	47.8352	
11	Interleukin 10	33.8246	
12	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	27.4741	
13	Chemokine (C-C motif) receptor 2	8.4561	

14	Chemokine (C-C motif) receptor 5	5.579	
15	Chemokine (C-C motif) receptor 4	3.6808	
16	Chemokine (C-C motif) ligand 7	2.9897	
17	Chemokine (C-C motif) receptor 3	2.9897	
18	Chemokine (C-C motif) receptor 7	2.9897	
19	Chemokine (C motif) receptor 1	2.9897	
20	Interleukin 10 receptor, beta	2.6027	
21	Toll interacting protein	2.4284	
22	Chemokine (C-C motif) ligand 25	2.2658	
23	C-reactive protein, pentraxin-related	2.2658	
24	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	2.2658	
25	Interleukin 17C	2.114	
26	Chemokine (C-C motif) ligand 15	1.9725	
27	Chemokine (C-C motif) ligand 16	1.9725	
28	Chemokine (C-X-C motif) ligand 11	1.9725	
29	Interleukin 1 family, member 10 (theta)	1.9725	
30	Chemokine (C-C motif) ligand 17	1.8404	
31	Chemokine (C-C motif) ligand 4	1.8404	
32	Chemokine (C-X-C motif) ligand 14	1.8404	
33	Interleukin 1 family, member 5 (delta)	1.8404	
34	Interleukin 1 receptor antagonist	1.8404	
35	Interleukin 8 receptor, alpha	1.8404	
36	Complement component 4A (Rodgers blood group)	1.7171	
37	Chemokine (C-C motif) ligand 11	1.7171	
38	Chemokine (C-C motif) ligand 13	1.7171	
39	Chemokine (C-C motif) receptor 6	1.7171	
40	Secreted phosphoprotein 1	1.7171	
41	Chemokine (C-C motif) ligand 3	1.6021	
42	Interleukin 8 receptor, beta	1.6021	
43	ATP-binding cassette, sub-family F (GCN20). member 1	1.4948	
44	Chemokine (C-C motif) ligand 21	1.4948	
45	Chemokine (C-C motif) ligand 5	1.4948	
46	Chemokine (C-C motif) ligand 8	1.4948	
47	Chemokine (C-C motif) receptor 8	1.4948	
48	Chemokine (C-X-C motif) ligand 10	1.4948	
49	Chemokine (C-X-C motif) ligand 3	1.4948	
50	Chemokine (C-X-C motif) ligand 9	1.4948	
51	Interleukin 1, alpha	1.4948	
52	Interleukin 9 receptor	1.4948	
53	Lymphotoxin alpha (TNF superfamily, member 1)	1.4948	
54	Leukotriene B4 receptor	1.4948	
55	CD40 ligand	1.4948	
56	Complement component 3	1.3947	
57	Chemokine (C-C motif) receptor 1	1.3947	
58	Chemokine (C-C motif) receptor 9	1.3947	
59	Chemokine (C-X-C motif) ligand 5	1.3947	

60	Interleukin 1 family, member 6 (epsilon)	1.3947	
61	Interleukin 1 receptor, type I	1.3947	
62	Interleukin 9	1.3947	
63	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	1.3013	
64	Caspase recruitment domain family, member 18	1.3013	
65	Interleukin 5 receptor, alpha	1.3013	
66	Interleukin 8	1.3013	
67	Chemokine (C-C motif) ligand 20	1.2142	
68	Interleukin 1 family, member 9	1.2142	
69	Interleukin 5 (colony-stimulating factor, eosinophil)	1.2142	
70	Chemokine (C-C motif) ligand 23	1.1329	
71	Interleukin 10 receptor, alpha	1.1329	
72	Interleukin 13	1.1329	
73	Interleukin 1 family, member 7 (zeta)	1.1329	
74	Interleukin 1 family, member 8 (eta)	1.1329	
75	Lymphotoxin beta (TNF superfamily, member 3)	1.1329	
1	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-2.6759
2	CCAAT/enhancer binding protein (C/EBP). beta		-9.3179
3	B-cell CLL/lymphoma 6		-17.3878
4	Chemokine (C-C motif) ligand 26		-24.59
5	Chemokine (C-C motif) ligand 24		-37.2715
6	Interleukin 13 receptor, alpha 1		-37.2715
7	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-37.2715

Table 24: The gene expression analysis for Adv infection in patient1; the gene symbol, the average change of C_t values ($\mathbf{AVG\ \Delta C_t}$) between the acut and control samples, the level of gene expression ($\mathbf{2^{-\Delta C_t}}$) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 1	Cont.1	Acu. 1	Cont.1	Acu 1/Cont.1	Acu 1/Cont.1
ABCF1	1.56	0.56	0.339151	0.678302	0.50	-2.00
BCL6	1.26	1.36	0.417544	0.389582	1.07	1.07
C3	1.76	1.06	0.295248	0.479632	0.62	-1.62
C4A	0.66	0.76	0.632878	0.590496	1.07	1.07
C5	11.06	1.16	0.000468	0.447513	0.00	-955.43
CCL1	2.56	2.16	0.169576	0.223756	0.76	-1.32
CCL11	2.56	1.46	0.169576	0.363493	0.47	-2.14
CCL13	4.06	2.46	0.059954	0.181747	0.33	-3.03
CCL15	2.36	1.56	0.194791	0.339151	0.57	-1.74
CCL16	2.96	2.36	0.128514	0.194791	0.66	-1.52
CCL17	2.36	2.16	0.194791	0.223756	0.87	-1.15
CCL18	9.96	2.06	0.001004	0.239816	0.00	-238.86

CCL19	2.56	1.06	0.169576	0.479632	0.35	-2.83
CCL2	1.86	4.76	0.275476	0.036906	7.46	7.46
CCL20	1.76	0.36	0.295248	0.779165	0.38	-2.64
CCL21	1.96	1.06	0.257028	0.479632	0.54	-1.87
CCL23	1.26	0.66	0.417544	0.632878	0.66	-1.52
CCL24	1.06	0.56	0.479632	0.678302	0.71	-1.41
CCL25	1.06	0.16	0.479632	0.895025	0.54	-1.87
CCL26	4.16	3.66	0.055939	0.079110	0.71	-1.41
CCL3	1.16	0.66	0.447513	0.632878	0.71	-1.41
CCL4	1.26	0.96	0.417544	0.514057	0.81	-1.23
CCL5	0.76	1.66	0.590496	0.316439	1.87	1.87
CCL7	2.36	2.16	0.194791	0.223756	0.87	-1.15
CCL8	1.66	0.26	0.316439	0.835088	0.38	-2.64
CCR1	1.76	0.76	0.295248	0.590496	0.50	-2.00
CCR2	2.76	1.76	0.147624	0.295248	0.50	-2.00
CCR3	1.46	0.46	0.363493	0.726986	0.50	-2.00
CCR4	1.86	5.06	0.275476	0.029977	9.19	9.19
CCR5	0.76	3.26	0.590496	0.104386	5.66	5.66
CCR6	1.66	1.66	0.316439	0.316439	1.00	-1.00
CCR7	2.36	5.06	0.194791	0.029977	6.50	6.50
CCR8	2.46	1.56	0.181747	0.339151	0.54	-1.87
CCR9	1.56	1.06	0.339151	0.479632	0.71	-1.41
CEBPB	8.96	1.96	0.002008	0.257028	0.01	-128.00
CRP	1.16	1.16	0.447513	0.447513	1.00	-1.00
CX3CR1	4.06	5.16	0.059954	0.027970	2.14	2.14
CXCL1	1.66	3.86	0.316439	0.068869	4.59	4.59
CXCL10	1.86	2.36	0.275476	0.194791	1.41	1.41
CXCL11	1.86	0.26	0.275476	0.835088	0.33	-3.03
CXCL12	2.56	1.46	0.169576	0.363493	0.47	-2.14
CXCL13	9.56	1.26	0.001325	0.417544	0.00	-315.17
CXCL14	1.36	0.46	0.389582	0.726986	0.54	-1.87
CXCL2	4.26	2.86	0.052193	0.137738	0.38	-2.64
CXCL3	3.66	2.06	0.079110	0.239816	0.33	-3.03
CXCL5	1.66	1.06	0.316439	0.479632	0.66	-1.52
CXCL6	1.86	1.16	0.275476	0.447513	0.62	-1.62
CXCL9	2.46	1.76	0.181747	0.295248	0.62	-1.62
CARD18	8.56	0.76	0.002650	0.590496	0.00	-222.86
IFNA2	1.06	3.76	0.479632	0.073812	6.50	6.50
IL10	1.76	4.76	0.295248	0.036906	8.00	8.00
IL10RA	0.96	0.26	0.514057	0.835088	0.62	-1.62
IL10RB	2.26	1.06	0.208772	0.479632	0.44	-2.30
IL13	1.86	0.86	0.275476	0.550953	0.50	-2.00
IL13RA1	2.76	2.26	0.147624	0.208772	0.71	-1.41
IL17C	1.66	1.06	0.316439	0.479632	0.66	-1.52
IL1A	1.46	0.46	0.363493	0.726986	0.50	-2.00
IL1B	1.06	4.16	0.479632	0.055939	8.57	8.57
IL1F10	1.96	1.26	0.257028	0.417544	0.62	-1.62
IL1F5	2.06	1.96	0.239816	0.257028	0.93	-1.07

IL1F6	1.96	0.16	0.257028	0.895025	0.29	-3.48
IL1F7	1.86	0.26	0.275476	0.835088	0.33	-3.03
IL1F8	1.76	0.26	0.295248	0.835088	0.35	-2.83
IL1F9	2.26	0.36	0.208772	0.779165	0.27	-3.73
IL1R1	1.56	0.06	0.339151	0.959264	0.35	-2.83
IL1RN	1.46	0.66	0.363493	0.632878	0.57	-1.74
IL22	3.16	1.86	0.111878	0.275476	0.41	-2.46
IL5	1.66	0.26	0.316439	0.835088	0.38	-2.64
IL5RA	2.56	2.26	0.169576	0.208772	0.81	-1.23
IL8	1.16	3.46	0.447513	0.090873	4.92	4.92
IL8RA	1.86	1.96	0.275476	0.257028	1.07	1.07
IL8RB	13.86	1.46	0.000067	0.363493	0.00	-5404.70
IL9	3.46	1.36	0.090873	0.389582	0.23	-4.29
IL9R	2.76	1.06	0.147624	0.479632	0.31	-3.25
LTA	2.16	0.96	0.223756	0.514057	0.44	-2.30
LTB	2.16	2.16	0.223756	0.223756	1.00	-1.00
LTB4R	1.76	0.36	0.295248	0.779165	0.38	-2.64
MIF	1.06	1.16	0.479632	0.447513	1.07	1.07
SCYE1	4.36	2.36	0.048698	0.194791	0.25	-4.00
SPP1	2.46	1.26	0.181747	0.417544	0.44	-2.30
TNF	1.96	4.66	0.257028	0.039555	6.50	6.50
CD40LG	3.26	2.56	0.104386	0.169576	0.62	-1.62
TOLLIP	1.76	2.46	0.295248	0.181747	1.62	1.62
XCR1	2.66	2.96	0.158220	0.128514	1.23	1.23

Table 25: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after Adv infection in patient 1.

No.	Gene name	Up regulated genes	Down regulated genes
1	Chemokine (C-C motif) receptor 4	9.1896	
2	Interleukin 1, beta	8.5742	
3	Interleukin 10	8	
4	Chemokine (C-C motif) ligand 2	7.4643	
5	Chemokine (C-C motif) receptor 7	6.498	
6	Interferon, alpha 2	6.498	
7	Tumor necrosis factor (TNF superfamily, member 2)	6.498	
8	Chemokine (C-C motif) receptor 5	5.6569	
9	Interleukin 8	4.9246	
10	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	4.5948	
11	Chemokine (C-X3-C motif) receptor 1	2.1435	
12	Chemokine (C-C motif) ligand 5	1.8661	
13	Toll interacting protein	1.6245	
14	Chemokine (C-X-C motif) ligand 10	1.4142	
15	Chemokine (C motif) receptor 1	1.2311	
1	Chemokine (C-C motif) ligand 17		-1.1487

2	Chemokine (C-C motif) ligand 7		-1.1487
3	Chemokine (C-C motif) ligand 4		-1.2311
4	Interleukin 5 receptor, alpha		-1.2311
5	Chemokine (C-C motif) ligand 1		-1.3195
6	Chemokine (C-C motif) ligand 24		-1.4142
7	Chemokine (C-C motif) ligand 26		-1.4142
8	Chemokine (C-C motif) ligand 3		-1.4142
9	Chemokine (C-C motif) receptor 9		-1.4142
10	Interleukin 13 receptor, alpha 1		-1.4142
11	Chemokine (C-C motif) ligand 16		-1.5157
12	Chemokine (C-C motif) ligand 23		-1.5157
13	Chemokine (C-X-C motif) ligand 5		-1.5157
14	Interleukin 17C		-1.5157
15	Complement component 3		-1.6245
16	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-1.6245
17	Chemokine (C-X-C motif) ligand 9		-1.6245
18	Interleukin 10 receptor, alpha		-1.6245
19	Interleukin 1 family, member 10 (theta)		-1.6245
20	CD40 ligand		-1.6245
21	Chemokine (C-C motif) ligand 15		-1.7411
22	Interleukin 1 receptor antagonist		-1.7411
23	Chemokine (C-C motif) ligand 21		-1.8661
24	Chemokine (C-C motif) ligand 25		-1.8661
25	Chemokine (C-C motif) receptor 8		-1.8661
26	Chemokine (C-X-C motif) ligand 14		-1.8661
27	ATP-binding cassette, sub-family F (GCN20). member 1		-2.0000
28	Chemokine (C-C motif) receptor 1		-2.0000
29	Chemokine (C-C motif) receptor 2		-2.0000
30	Chemokine (C-C motif) receptor 3		-2.0000
31	Interleukin 13		-2.0000
32	Interleukin 1, alpha		-2.0000
33	Chemokine (C-C motif) ligand 11		-2.1435
34	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)		-2.1435
35	Interleukin 10 receptor, beta		-2.2974
36	Lymphotoxin alpha (TNF superfamily, member 1)		-2.2974
37	Secreted phosphoprotein 1		-2.2974
38	Interleukin 22		-2.4623
39	Chemokine (C-C motif) ligand 20		-2.639
40	Chemokine (C-C motif) ligand 8		-2.639
41	Chemokine (C-X-C motif) ligand 2		-2.639
42	Interleukin 5 (colony-stimulating factor, eosinophil)		-2.639
43	Leukotriene B4 receptor		-2.639
44	Chemokine (C-C motif) ligand 19		-2.8284

45	Interleukin 1 family, member 8 (eta)		-2.8284
46	Interleukin 1 receptor, type I		-2.8284
47	Chemokine (C-C motif) ligand 13		-3.0314
48	Chemokine (C-X-C motif) ligand 11		-3.0314
49	Chemokine (C-X-C motif) ligand 3		-3.0314
50	Interleukin 1 family, member 7 (zeta)		-3.0314
51	Interleukin 9 receptor		-3.249
52	Interleukin 1 family, member 6 (epsilon)		-3.4822
53	Interleukin 1 family, member 9		-3.7321
54	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-4.0000
55	Interleukin 9		-4.2871
56	CCAAT/enhancer binding protein (C/EBP). beta		-128.00
57	Caspase recruitment domain family, member1 18		-222.861
58	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)		-238.856
59	Chemokine (C-X-C motif) ligand 13		-315.173
60	Complement component 5		-955.426
61	Interleukin 8 receptor, beta		-5404.7

Table 26: The gene expression analysis of Adv infection in patient 2; the gene symbol, the average change of C_t values (**AVG ΔC_t**) between the acut and control samples, the level of gene expression (**$2^{-\Delta C_t}$**) for each acut and control and the fold change for each gene after normalization of gene level of acute gene to control gene.

Gene Symbol	AVG ΔC_t		$2^{-\Delta C_t}$		Fold Change	Up or Down-Regulation
	Acu 2	Cont.2	Acu. 2	Cont.2	Acu 2/Cont.2	Acu 2/Cont.2
ABCF1	1.32	-0.96	0.400535	1.945310	0.21	-4.86
BCL6	1.62	0.04	0.325335	0.972655	0.33	-2.99
C3	1.82	-0.36	0.283221	1.283426	0.22	-4.53
C4A	0.62	-0.86	0.650671	1.815038	0.36	-2.79
C5	1.42	5.24	0.373712	0.026461	14.12	14.12
CCL1	2.52	5.94	0.174343	0.016289	10.70	10.70
CCL11	2.42	0.94	0.186856	0.521233	0.36	-2.79
CCL13	3.62	0.94	0.081334	0.521233	0.16	-6.41
CCL15	1.72	0.54	0.303549	0.687771	0.44	-2.27
CCL16	2.72	-0.56	0.151774	1.474269	0.10	-9.71
CCL17	2.22	1.04	0.214641	0.486327	0.44	-2.27
CCL18	2.42	7.34	0.186856	0.006172	30.27	30.27
CCL19	2.22	-0.76	0.214641	1.693491	0.13	-7.89
CCL2	0.92	5.54	0.528509	0.021493	24.59	24.59

CCL20	1.32	5.34	0.400535	0.024689	16.22	16.22
CCL21	1.82	-0.56	0.283221	1.474269	0.19	-5.21
CCL23	1.42	-1.06	0.373712	2.084932	0.18	-5.58
CCL24	0.92	-1.26	0.528509	2.394957	0.22	-4.53
CCL25	0.92	-1.26	0.528509	2.394957	0.22	-4.53
CCL26	4.52	1.54	0.043586	0.343885	0.13	-7.89
CCL3	1.32	-1.16	0.400535	2.234574	0.18	-5.58
CCL4	1.42	0.04	0.373712	0.972655	0.38	-2.60
CCL5	1.62	3.44	0.325335	0.092142	3.53	3.53
CCL7	2.22	1.14	0.214641	0.453760	0.47	-2.11
CCL8	3.62	7.34	0.081334	0.006172	13.18	13.18
CCR1	0.52	-0.66	0.697372	1.580083	0.44	-2.27
CCR2	3.02	0.84	0.123279	0.558644	0.22	-4.53
CCR3	1.62	0.14	0.325335	0.907519	0.36	-2.79
CCR4	1.92	-0.16	0.264255	1.117287	0.24	-4.23
CCR5	0.52	5.94	0.697372	0.016289	42.81	42.81
CCR6	1.82	0.04	0.283221	0.972655	0.29	-3.43
CCR7	2.22	6.34	0.214641	0.012344	17.39	17.39
CCR8	1.92	0.24	0.264255	0.846745	0.31	-3.20
CCR9	1.12	-0.16	0.460094	1.117287	0.41	-2.43
CEBPB	2.62	4.24	0.162668	0.052922	3.07	3.07
CRP	1.12	-0.56	0.460094	1.474269	0.31	-3.20
CX3CR1	7.52	11.34	0.005448	0.000386	14.12	14.12
CXCL1	1.02	4.44	0.493116	0.046071	10.70	10.70
CXCL10	0.52	2.94	0.697372	0.130308	5.35	5.35
CXCL11	1.72	6.04	0.303549	0.015198	19.97	19.97
CXCL12	2.12	5.44	0.230047	0.023035	9.99	9.99
CXCL13	2.32	3.04	0.200267	0.121582	1.65	1.65
CXCL14	0.92	-1.06	0.528509	2.084932	0.25	-3.94
CXCL2	4.52	7.14	0.043586	0.007090	6.15	6.15
CXCL3	3.52	6.24	0.087171	0.013230	6.59	6.59
CXCL5	1.42	-0.16	0.373712	1.117287	0.33	-2.99
CXCL6	1.72	-1.06	0.303549	2.084932	0.15	-6.87
CXCL9	1.92	3.84	0.264255	0.069830	3.78	3.78
CARD18	9.12	1.44	0.001797	0.368567	0.00	-205.07
IFNA2	0.52	4.14	0.697372	0.056720	12.30	12.30
IL10	1.02	3.14	0.493116	0.113440	4.35	4.35
IL10RA	0.72	-1.66	0.607097	3.160165	0.19	-5.21
IL10RB	2.42	-1.56	0.186856	2.948538	0.06	-15.78
IL13	1.72	5.14	0.303549	0.028360	10.70	10.70
IL13RA1	2.62	-0.26	0.162668	1.197479	0.14	-7.36
IL17C	1.82	-0.56	0.283221	1.474269	0.19	-5.21
IL1A	1.02	-0.96	0.493116	1.945310	0.25	-3.94
IL1B	0.52	5.34	0.697372	0.024689	28.25	28.25
IL1F10	1.62	-1.26	0.325335	2.394957	0.14	-7.36
IL1F5	1.72	-1.06	0.303549	2.084932	0.15	-6.87
IL1F6	12.82	0.84	0.000138	0.558644	0.00	-4039.61
IL1F7	1.32	4.74	0.400535	0.037421	10.70	10.70

IL1F8	1.22	-1.06	0.429283	2.084932	0.21	-4.86
IL1F9	1.82	-1.26	0.283221	2.394957	0.12	-8.46
IL1R1	1.22	3.04	0.429283	0.121582	3.53	3.53
IL1RN	-1.08	-0.46	2.114036	1.375542	1.54	1.54
IL22	3.22	6.54	0.107321	0.010746	9.99	9.99
IL5	1.52	5.24	0.348686	0.026461	13.18	13.18
IL5RA	2.42	0.24	0.186856	0.846745	0.22	-4.53
IL8	0.82	6.44	0.566442	0.011518	49.18	49.18
IL8RA	1.62	-0.36	0.325335	1.283426	0.25	-3.94
IL8RB	0.32	0.94	0.801070	0.521233	1.54	1.54
IL9	12.62	2.34	0.000159	0.197510	0.00	-1243.34
IL9R	2.22	0.04	0.214641	0.972655	0.22	-4.53
LTA	1.62	5.24	0.325335	0.026461	12.30	12.30
LTB	3.12	0.34	0.115023	0.790041	0.15	-6.87
LTB4R	1.92	0.34	0.264255	0.790041	0.33	-2.99
MIF	1.42	-2.86	0.373712	7.260153	0.05	-19.43
SCYE1	3.82	0.64	0.070805	0.641713	0.11	-9.06
SPP1	2.42	0.24	0.186856	0.846745	0.22	-4.53
TNF	2.02	4.24	0.246558	0.052922	4.66	4.66
CD40LG	3.22	0.14	0.107321	0.907519	0.12	-8.46
TOLLIP	1.82	0.44	0.283221	0.737135	0.38	-2.60
XCR1	2.42	1.24	0.186856	0.423373	0.44	-2.27

Table 27: The arrangement of fold change from upper to lower value for both upregulated genes and down regulated genes after Adv infection in patient 2.

No.	Gene name	Up regulated genes	Down regulated genes
1	Interleukin 8	49.18	
2	Chemokine (C-C motif) receptor 5	42.8137	
3	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	30.2738	
4	Interleukin 1, beta	28.2465	
5	Chemokine (C-C motif) ligand 2	24.59	
6	Chemokine (C-X-C motif) ligand 11	19.9733	
7	Chemokine (C-C motif) receptor 7	17.3878	
8	Chemokine (C-C motif) ligand 20	16.2234	
9	Complement component 5	14.1232	
10	Chemokine (C-X3-C motif) receptor 1	14.1232	
11	Chemokine (C-C motif) ligand 8	13.1775	
12	Interleukin 5 (colony-stimulating factor, eosinophil)	13.1775	
13	Interferon, alpha 2	12.295	
14	Lymphotoxin alpha (TNF superfamily, member 1)	12.295	
15	Chemokine (C-C motif) ligand 1	10.7034	
16	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	10.7034	

17	Interleukin 13	10.7034	
18	Interleukin 1 family, member 7 (zeta)	10.7034	
19	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	9.9866	
20	Interleukin 22	9.9866	
21	Chemokine (C-X-C motif) ligand 3	6.5887	
22	Chemokine (C-X-C motif) ligand 2	6.1475	
23	Chemokine (C-X-C motif) ligand 10	5.3517	
24	Tumor necrosis factor (TNF superfamily, member 2)	4.6589	
25	Interleukin 10	4.3469	
26	Chemokine (C-X-C motif) ligand 9	3.7842	
27	Chemokine (C-C motif) ligand 5	3.5308	
28	Interleukin 1 receptor, type I	3.5308	
29	CCAAT/enhancer binding protein (C/EBP). beta	3.0738	
30	Chemokine (C-X-C motif) ligand 13	1.6472	
31	Interleukin 1 receptor antagonist	1.5369	
32	Interleukin 8 receptor, beta	1.5369	
1	Chemokine (C-C motif) ligand 7		-2.114
2	Chemokine (C-C motif) ligand 15		-2.2658
3	Chemokine (C-C motif) ligand 17		-2.2658
4	Chemokine (C-C motif) receptor 1		-2.2658
5	Chemokine (C motif) receptor 1		-2.2658
6	Chemokine (C-C motif) receptor 9		-2.4284
7	Chemokine (C-C motif) ligand 4		-2.6027
8	Toll interacting protein		-2.6027
9	Complement component 4A (Rodgers blood group)		-2.7895
10	Chemokine (C-C motif) ligand 11		-2.7895
11	Chemokine (C-C motif) receptor 3		-2.7895
12	B-cell CLL/lymphoma 6		-2.9897
13	Chemokine (C-X-C motif) ligand 5		-2.9897
14	Leukotriene B4 receptor		-2.9897
15	Chemokine (C-C motif) receptor 8		-3.2043
16	C-reactive protein, pentraxin-related		-3.2043
17	Chemokine (C-C motif) receptor 6		-3.4343
18	Chemokine (C-X-C motif) ligand 14		-3.9449
19	Interleukin 1, alpha		-3.9449
20	Interleukin 8 receptor, alpha		-3.9449
21	Chemokine (C-C motif) receptor 4		-4.2281
22	Complement component 3		-4.5315
23	Chemokine (C-C motif) ligand 24		-4.5315
24	Chemokine (C-C motif) ligand 25		-4.5315
25	Chemokine (C-C motif) receptor 2		-4.5315
26	Interleukin 5 receptor, alpha		-4.5315
27	Interleukin 9 receptor		-4.5315
28	Secreted phosphoprotein 1		-4.5315
29	ATP-binding cassette, sub-family F (GCN20).		-4.8568

	member 1		
30	Interleukin 1 family, member 8 (eta)		-4.8568
31	Chemokine (C-C motif) ligand 21		-5.2054
32	Interleukin 10 receptor, alpha		-5.2054
33	Interleukin 17C		-5.2054
34	Chemokine (C-C motif) ligand 23		-5.579
35	Chemokine (C-C motif) ligand 3		-5.579
36	Chemokine (C-C motif) ligand 13		-6.4086
37	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)		-6.8685
38	Interleukin 1 family, member 5 (delta)		-6.8685
39	Lymphotoxin beta (TNF superfamily, member 3)		-6.8685
40	Interleukin 13 receptor, alpha 1		-7.3615
41	Interleukin 1 family, member 10 (theta)		-7.3615
42	Chemokine (C-C motif) ligand 19		-7.8899
43	Chemokine (C-C motif) ligand 26		-7.8899
44	Interleukin 1 family, member 9		-8.4561
45	CD40 ligand		-8.4561
46	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)		-9.0631
47	Chemokine (C-C motif) ligand 16		-9.7136
48	Interleukin 10 receptor, beta		-15.7797
49	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)		-19.4271
50	Caspase recruitment domain family, member 18		-205.074
51	Interleukin 9		-1243.34
52	Interleukin 1 family, member 6 (epsilon)		-4039.61

Table 28: The most 19 prominent and common up regulated genes (with fold change between 121.35 and 4.5) in all patients infected with IAV within the first 48h with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value and Frequency.	Gene function
1	Hs.624	Interleukin 8 (IL8)	121.35	-3.061 ($P \leq 0.002$) Frequency:100%	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus.
2	Hs.845	Interleukin 13 (IL13)	72.36	-3.061 ($P \leq 0.002$) Frequency:87.5%	Immune system process, cellular process, and response to stimulus.
3	Hs.75703	Chemokine (C-C motif) ligand 4	57.15	($P \leq 0.004$) Frequency:87.5%	Immune system process, defense response, inflammatory response, immune response, signal transduction, cell-cell signaling

4	Hs.143961	Chemokine(C-C motif) ligand 18 (pulmonary andactivation-regulated)	47.4	($P \leq 0.012$) Frequency:62.5%	G-protein- coupled receptor binding, cytokine activity, chemokine activity
5	Hs.514821	Chemokine (C-C motif) ligand 5 (CCL5)	34.58	-3.059 ($P \leq 0.002$) Frequency:100%	Immune system process, cellular process, biological adhesion, response to stimulus, biological regulation.
6	Hs.72918	Chemokine (C-C motif) ligand 1	27.02	($P \leq 0.004$) Frequency:75%	G-protein- coupled receptor binding, receptor binding, cytokine activity, chemokine chemokine receptor binding.
7	Hs.211575	Interferon, alpha 2	25.95	($P \leq 0.003$) Frequency:37.5%	Receptor binding, cytokine activity, interferon-alpha/beta receptor binding, protein, protein binding
8	Hs.494997	Complement component 5 (C5)	24.60	-2.197 ($P \leq 0.028$) Frequency:75%	Immune system process, metabolic process, cellular process, developmental process, response to stimulus, and biological regulation
9	Hs.789	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (CXCL1)	23.71	-3.061 ($P \leq 0.002$) Frequency:75%	Immune system process, cellular process, multicellular organism process, response to stimulus, biological regulation
10	Hs.126256	Interleukin 1, beta (IL1B)	18.85	-3.061 ($P \leq 0.002$) Frequency:87.5%	Immune system process, metabolic process, cellular process, gene expression, developmental process, and response to stimulus.
11	Hs.54460	Chemokine (C-C motif) ligand 11	16.9	-2.864, ($P< 0.004$) Frequency: 75%	G-protein- coupled receptor binding, receptor binding, cytokine activity, protein binding, chemokine activity, chemokine receptor binding.
12	Hs.1722	Interleukin 1, alpha	16.86	($P \leq 0.004$) Frequency:75%	Signal transducer activity Receptor binding, cytokine activity, Interleukin 1 receptor binding molecular transducer activity.
13	Hs.632586	Chemokine (C-X-C motif) ligand 10 (CXCL10)	16.1	-3.061 ($P \leq 0.002$) Frequency:75%	Pleiotropic effects, including stimulation of monocytes, natural killer and T-cell migration, and modulation of adhesion molecule expression within cytokine-cytokine interaction pathway.

14	Hs.303649	Chemokine (C-C motif) ligand 2 (CCL2)	12.85	-3.061 ($P \leq 0.002$) Frequency:75%	Immune system process, cellular process, viral reproduction, biological adhesion, developmental process and response to stimulus.
15	Hs.78913	Chemokine (C-X3-C moti) receptor1 (CX3CR1)	12.81	-2.119 ($P \leq 0.006$) Frequency:87.5%	Cellular process, biological adhesion, and response to stimulus.
16	Hs.193717	Interleukin 10 (IL10)	11.81	-2.433 ($P \leq 0.002$) Frequency:75%	Immune system, cellular process, gene expression, developmental process, and response to stimulus.
17	Hs.241570	Tumor necrosis factor (TNF superfamily, member 2) (TNF)	10.78	-3.061 ($P \leq 0.002$) Frequency:87.5%	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation.
18	Hs.306974	Interleukin 1 family, member 10 (theta)	10.03	($P \leq 0.006$) Frequency:75%	Immune system process, defense response, inflammatory response, immune response, response to stimulus.
19	Hs.251526	Chemokine (C-C motif) ligand 7 (CCL7)	4.5	-2.824 ($P \leq 0.007$) Frequency:87.5%	Immune system process, cellular process, response to stimulus, and biological regulation.

Table 29: The most 13 prominent and common downregulated genes in all patients infected with IAV after 48h of infection with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value and Frequency.	Gene function
1	Hs.301921	Chemokine (C-C motif) receptor 1	-250.14	-2.826, (P< 0.005) Frequency: 75%	Immune system process, defense response, inflammatory response, immune response G-protein signaling.
2	Hs.272493	Chemokine (C-C motif) ligand 15	-8.1	-2.903, (P< 0.004) 25%Frequency	Chemotactic for T cells and monocytes, induces changes in intracellular calcium concentration in monocytes. and is thought to act through the CCR1 receptor
3	Hs.546294	Chemokine (C-C motif) ligand 17	-6.5	-2.866, (P< 0.004) Frequency : 37.5%	Immune system process, defense response, inflammatory response, immune response, cell-cell signaling, G-protein signaling, cell-cell signaling.
4	Hs.414629	Chemokine (C-C motif) ligand 13	-6.1	-2.510, (P< 0.012) Frequency : 37.5%	G-protein- coupled receptor binding, chemokine activity, cytokine activity, molecular transducer

					activity. Chemokine receptor binding, molecular transducer activity
5	Hs.632592	Chemokine (C-X-C motif) ligand 11	-4.93	-2.983, (P< 0.003) Frequency :62.5%	Regulate cell trafficking of leukocytes , immune response,
6	Hs.278911	Interleukin 17C	-3.9	-2.491, (P< 0.013) Frequency : 37.5%	Receptor binding, cytokine activity, protein binding.
7	Hs.534847	Complement component 4A (Rodgers blood group)	-2.8	-2.864, (P< 0.004) 25%Frequency	Enzyme inhibitor activity, endopeptidase inhibitor activity, protein binding, enzyme regulator activity, protease inhibitor activity.
8	Hs.591680	Small inducible cytokine subfamily E,	-2.68	-2.629, (P< 0.009) Frequency : 37.5%	Control of angiogenesis, inflammation, wound healing, involved in the stimulation of inflammatory responses after proteolytic cleavage in tumor cells.
9	Hs.590921	Chemokine (C-X-C motif) ligand 2	-2.63	-3.059, (P< 0.002) Frequency : 37.5%	Immune system process, viral reproduction, biological adhesion, response to stimulus.
10	Hs.846	Interleukin 8 receptor, beta	-2.20	-3.059, (P< 0.002) Frequency : 37.5%	Signal transducer activity, transmembrane receptor activity, Interleukin 8 receptor activity, CXC chemokine receptor activity.
11	Hs.247838	Chemokine (C-C motif) ligand 24	-2.12	-1.609, (P< 0.108) Frequency : 37.5%	Immune system process, cellular process, response to stimulus
12	Hs.278909	Interleukin 1 family, member 8 (eta)	-2.07	-2.595, (P< 0.009) 50%Frequency	Immune system process, defense response, inflammatory response, immune response, response to stimulus.
13	Hs.75498	Chemokine (C-C motif) ligand 20	-2.06	-2.981, (P< 0.003) Frequency : 37.5%	Formation and function of mucosal lymphoid tissue via chemo- attraction of lymphocytes and dendritic cells towards the epithelial cells surrounding these tissues.

Table 30: The most 25 peominent and common up regulated genes in PIV infected samples after 48h of infection with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.211575	Interferon, alpha 2	752.42	-2.944, (P< 0.003)	Receptor binding, cytokine activity, interferon-alpha/beta receptor binding, protein, protein binding
2	Hs.126256	Interleukin 1, beta	154.57	-3.061 ($P \leq 0.002$)	Immune system process, gene expression, response to stimulus.
3	Hs.143961	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	110.44	-2.511, (P< 0.012)	G-protein- coupled receptor binding, cytokine activity, chemokine activity
4	Hs.303649	Chemokine (C-C motif) ligand 2	72.76	-3.061, (P< 0.002)	G-protein- coupled receptor binding, signal transducer activity, chemokine receptor activity, cytokine binding.
5	Hs.72918	Chemokine (C-C motif) ligand 1	54.41	-3.061, (P< 0.002)	G-protein- coupled receptor binding, receptor binding, cytokine activity, chemokine chemokine receptor binding.
6	Hs.1722	Interleukin 1, alpha	51.30	-2.943, (P< 0.003)	Signal transducer activity Receptor binding, cytokine activity, Interleukin 1 receptor binding molecular transducer activity.
7	Hs.494997	Complement component 5	51.26 (1Frequency)	-2.197, (P< 0.028)	Immune system process, metabolic process, cellular process, developmental process, response to stimulus, and biological regulation
8	Hs.789	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	46.62	-2.983, (P< 0.003)	Immune system process, cellular process, multicellular organism process, response to stimulus, biological regulation
9	Hs.78913	Chemokine (C-X3-C motif) receptor 1	42.5	-2.119, (P< 0.034)	Cellular process, biological adhesion, and response to stimulus
10	Hs.241570	Tumor necrosis factor (TNF superfamily, member 2)	38.38	-3.061, (P< 0.002)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation
11	Hs.590921	Chemokine (C-X-C motif) ligand 2	35.11	-3.059, (P< 0.002)	Immune system process, viral reproduction, biological adhesion, response to stimulus.

12	Hs.193717	Interleukin 10	30.45	-2.433, (P< 0.015)	Immune system, cellular process, gene expression, developmental process, and response to stimulus.
13	Hs.654593	Interleukin 10 receptor, beta	30.45	-2.136, (P< 0.033)	Signal transducer activity, interleukin receptor activity, and growth factor binding, cytokine binding, and interleukin-10 binding, molecular transducer activity.
14	Hs.100431	Chemokine (C-X-C motif) ligand 13	24.88	-2.046, (P< 0.041)	G-protein- coupled receptor binding, receptor binding, cytokine activity, chemokine activity, chemokine receptor binding
15	Hs.632586	Chemokine (C-X-C motif) ligand 10	18.5	-3.061, (P< 0.002)	Pleiotropic effects; stimulation of monocytes, natural killer T-cell migration, modulation of adhesion molecule and expression within cytokine-cytokine interaction pathway
16	Hs.624	Interleukin 8	10.2	-3.061, (P< 0.002)	Immune system process, viral reproduction, response to stimulus.
17	Hs.511794	Chemokine (C-C motif) receptor 2	8.45 (1Frequency)	-2.669, (P< 0.008)	G-protein- coupled receptor binding, chemokine receptor binding, molecular transducer activity.
18	Hs.54460	Chemokine (C-C motif) ligand 11	7.62	-2.864, (P< 0.004)	G-protein- coupled receptor binding, receptor binding, cytokine activity, protein binding, chemokine activity, chemokine receptor binding.
19	Hs.414629	Chemokine (C-C motif) ligand 13	4	-2.510, (P< 0.012)	G-protein- coupled receptor binding, chemokine activity, cytokine activity, molecular transducer activity. chemokine receptor binding, molecular transducer activity.
20	Hs.376208	Lymphotoxin beta (TNF superfamily, member 3)	3.9	-2.312, (P< 0.021)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation, tumor necrosis factor receptor binding.
21	Hs.514821	Chemokine (C-C motif) ligand 5	3.31	-3.059, (P< 0.002)	Immune system process, response to stimulus. Biological regulation
22	Hs.184926	Chemokine (C-C motif) receptor 4	2.57	-2.756, (P< 0.006)	G-protein- coupled receptor binding, chemokine receptor binding, cytokine activity, peptide binding, molecular

					transducer activity.
23	Hs.368527	Toll interacting protein	2.42 1Frequency	-2.845, (P< 0.004)	Signal transducer activity. Toll receptor binding, protein binding, molecular transducer activity.
24	Hs.287369	Interleukin 22	2.39 1Frequency	-2.981, (P< 0.003)	Receptor binding, cytokine activity, interleukin-22 receptor binding.
25	Hs.370036	Chemokine (C-C motif) receptor 7	2.22	-2.981, (P< 0.003)	G-protein- coupled receptor binding, chemokine receptor binding, cytokine activity, peptide binding, molecular transducer activity,

Table 31: The common three down regulated genes in PIV infected samples with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.407995	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	19.30	-2.120, (P< 0.034)	Immune system process, response to stimulus, biological regulation
2	Hs.247838	Chemokine (C-C motif) ligand 24	19.25	-1.609, (P< 0.108	Immune system process, cellular process, response to stimulus.
3	Hs.517106	CCAAT/enhancer binding protein (C/EBP). beta	5.56	-1.569, (P< 0.117)	Immune system response, cellular process, gene expression, response to stimulus.

Table 32: The common 11 up regulated genes in Adv infected samples after 48h of infection with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.624	Interleukin 8	27.51	-3.061, (P< 0.002)	Immune system process,viral reproduction, biological adhesion,response to stimulus.
2	Hs.450802	Chemokine (C-C motif) receptor 5	24.42	-2.936, (P< 0.003)	Immune system process response to stimulus, biological regulation.
3	Hs.126256	Interleukin 1, beta	18.42	-3.061 ($P \leq 0.002$)	Immune system process, gene expression, response to stimulus.
4	Hs.370036	Chemokine (C-C motif) receptor 7	11.75	-2.981, (P< 0.003)	G-protein- coupled receptor binding, chemokine receptor binding, cytokine activity, molecular transducer activity.

5	Hs.211575	Interferon, alpha 2	9.43	-2.944, (P< 0.003)	Receptor binding, cytokine activity, interferon-alpha/beta receptor binding, protein, protein binding
6	Hs.78913	Chemokine (C-X3-C motif) receptor 1	8.17	-2.119, (P< 0.034)	Cellular process, biological adhesion, and response to stimulus
7	Hs.789	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	7.81	-3.061, (P< 0.002)	Immune system process, cellular process, multicellular organism process, response to stimulus, biological regulation.
8	Hs.303649	Chemokine (C-C motif) ligand 2	6.71	-3.061, (P< 0.002)	G-protein- coupled receptor binding, signal transducer activity, chemokine receptor activity, cytokine binding.
9	Hs.193717	Interleukin 10	6.17	-2.433, (P< 0.015)	Immune system, cellular process, gene expression, developmental process, and response to stimulus.
10	Hs.241570	Tumor necrosis factor (TNF superfamily, member 2)	5.52	-3.061, (P< 0.002)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation.
11	Hs.514821	Chemokine (C-C motif) ligand 5	2.65	-3.059, (P< 0.002)	Immune system process, response to stimulus. Biological regulation

Table 33: The most 17 prominent and common down regulated genes in Adv infected samples with statistical analysis, fold change, and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.278910	Interleukin 1 family, member 6 (epsilon)	-2021.5	-2.118, (P< 0.034)	Receptor binding, cytokine activity, Interleukin 1 receptor binding.
2	Hs.960	Interleukin 9	-621.5	-2.119, (P< 0.034)	Receptor binding, cytokine activity, Interleukin 9 receptor binding.
3	Hs.56279	Caspase recruitment domain family, member 18 (Iceberg caspase 1 inhibitor)	-214.11	-1.334, (P< 0.182)	Defense response, inflammatory response, regulation of apoptosis, inhibits IL1B by interacting with caspase 1.
4	Hs.278909	Interleukin 1 family, member 8 (eta)	-7.30	-2.595, (P< 0.009)	Immune system process, defense response, inflammatory response, immune response, response to stimulus.

5	Hs.516301	Interleukin 1 family, member 5 (delta)	-6.86 1Frequency	-2.492, (P< 0.013)	Immune system process, defense response, inflammatory response, immune response, response to stimulus.
6	Hs.376208	Lymphotoxin beta (TNF superfamily, member 3)	-6.86 1Frequency	-2.312, (P< 0.021)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation, tumor necrosis factor receptor binding.
7	Hs.591680	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	-6.5	-2.629, (P< 0.009)	Control of angiogenesis, inflammation, wound healing, involved in the stimulation of inflammatory responses after proteolytic cleavage in tumor cells.
8	Hs.211238	Interleukin 1 family, member 9	-6.11	-2.315, (P< 0.021)	Signal transducer activity, receptor binding, cytokine activity, interleukin-1 receptor inhibitor activity, molecular transducer activity.
9	Hs.10458	Chemokine (C-C motif) ligand 16	-5.5	-2.394, (P< 0.017)	G-protein- coupled receptor binding, receptor binding, cytokine activity, protein binding, chemokine activity.
10	Hs.50002	Chemokine (C-C motif) ligand 19	-5.44	-2.433, (P< 0.015)	Immune system process, defense response, inflammatory response, immune response, signal transduction, response to virus.
11	Hs.592244	CD40 ligand	-5.11	-2.446, (P < 0.014)	Expressed on the surface of T cells, regulates B cell function by engaging CD40 on the B cell surface.
12	Hs.131342	Chemokine (C-C motif) ligand 26	-4.9	-1.726, (P< 0.085)	Immune system process, defense response, inflammatory response, immune response signal transduction, cell-cell signaling.
13	Hs.414629	Chemokine (C-C motif) ligand 13	-4.6	-2.510, (P< 0.012)	G-protein- coupled receptor binding, chemokine activity, cytokine activity, molecular transducer activity. Chemokine receptor binding, molecular transducer activity
14	Hs.306974	Interleukin 1 family, member 10 (theta)	-4.51	-2. 748, (P< 0.006)	Immune system process, defense response, inflammatory response, immune response, response to stimulus.
15	Hs.496646	Interleukin 13 receptor, alpha 1	-4.39	-2.353, (P< 0.019)	Cell communication, signal transduction, cellular process.
16	Hs.89714	Chemokine (C-X-C motif) ligand 5	-4.25	-2.746, (P< 0.006)	Immune system process, defense response, inflammatory response, immune response, signal

					transduction.
17	Hs.164021	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)	-4.15	-2.433, (P< 0.015)	G-protein binding, inflammatory response, immune response, signal transduction.

Appendix B - Figures

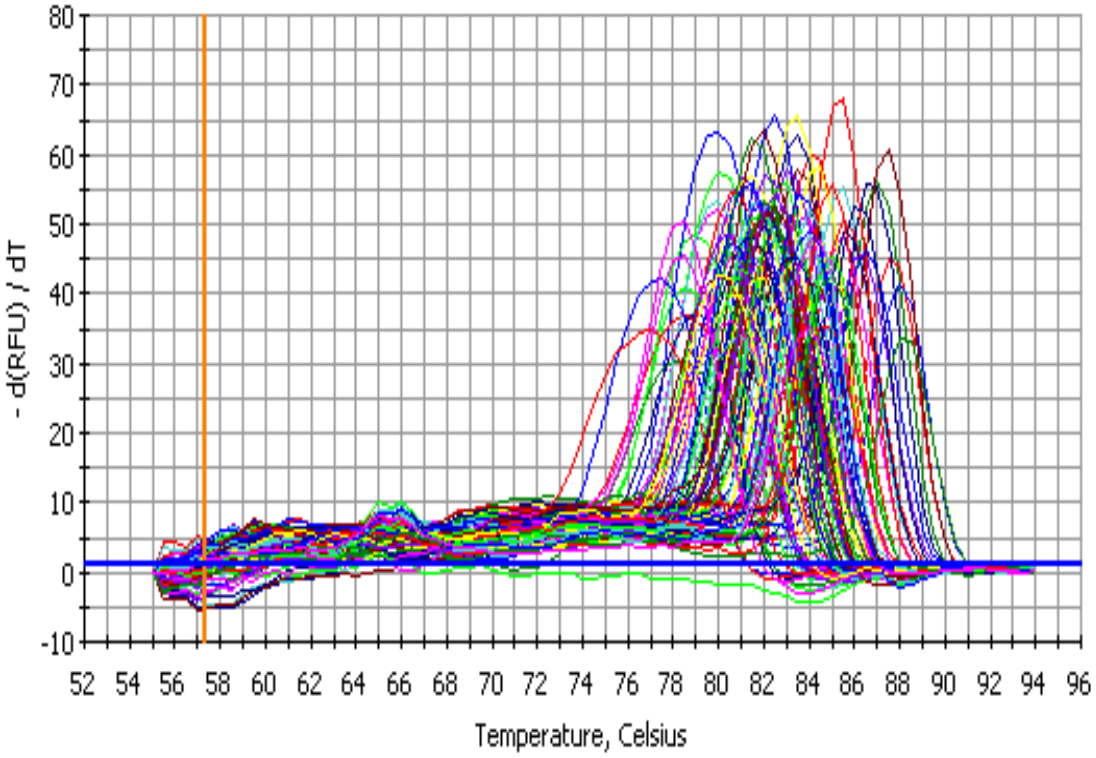

Figure 1: The melting curve plot with only on peak at each well, this indicates that each well contains only one DNA (specific gene) with no contamination with other external DNA and this enhance the results of experiment.

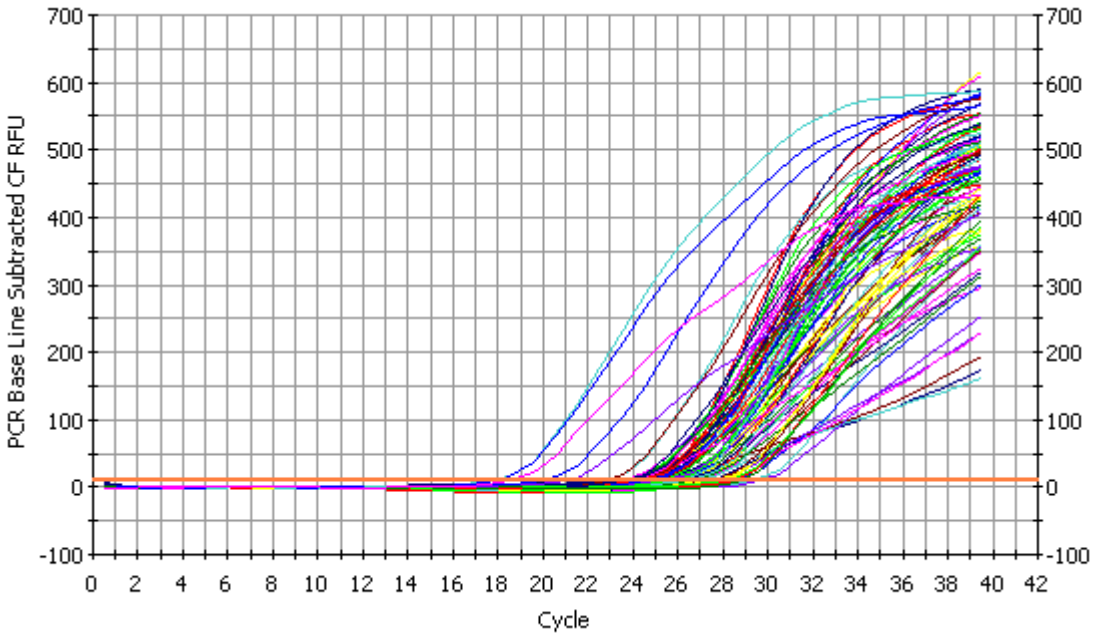

Figure 2: The amplification of 84 inflammatory genes and the determination of the baseline (in this plot almost at the log 10 of y axis) in all samples enhance the standardization of all samples at the same base line and this revial the differences in gene expression precisely.

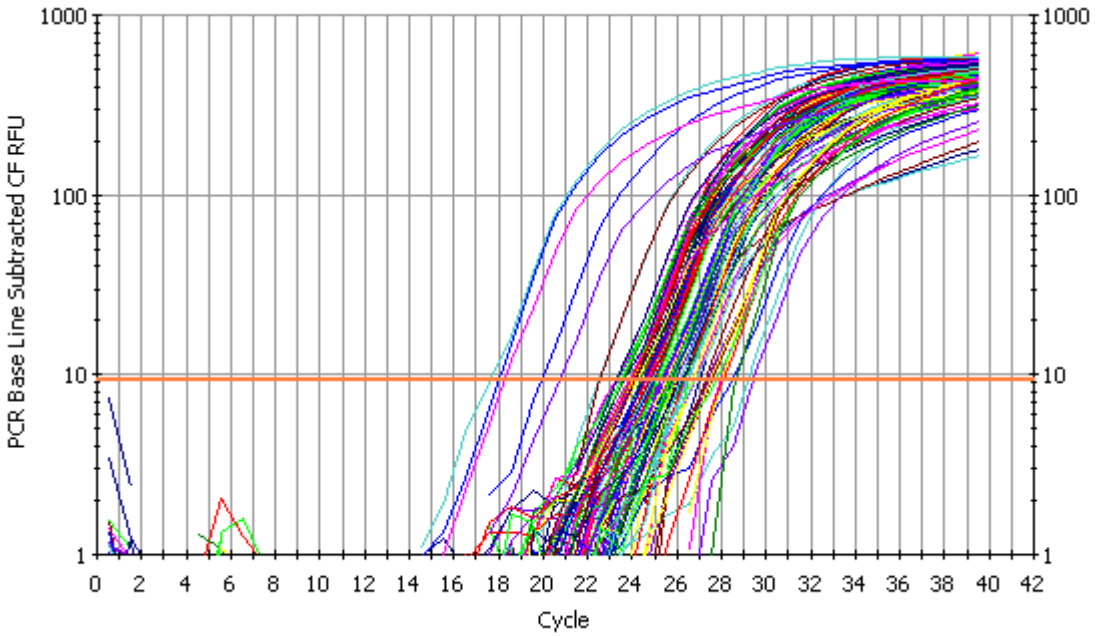

Figure 3: The amplification of 84 inflammatory genes and the determination of the threshold value (in this plot at cycle 16) in all samples to enhance the standardization of all samples at the same threshold value and this revial the differences in gene expression precisely.

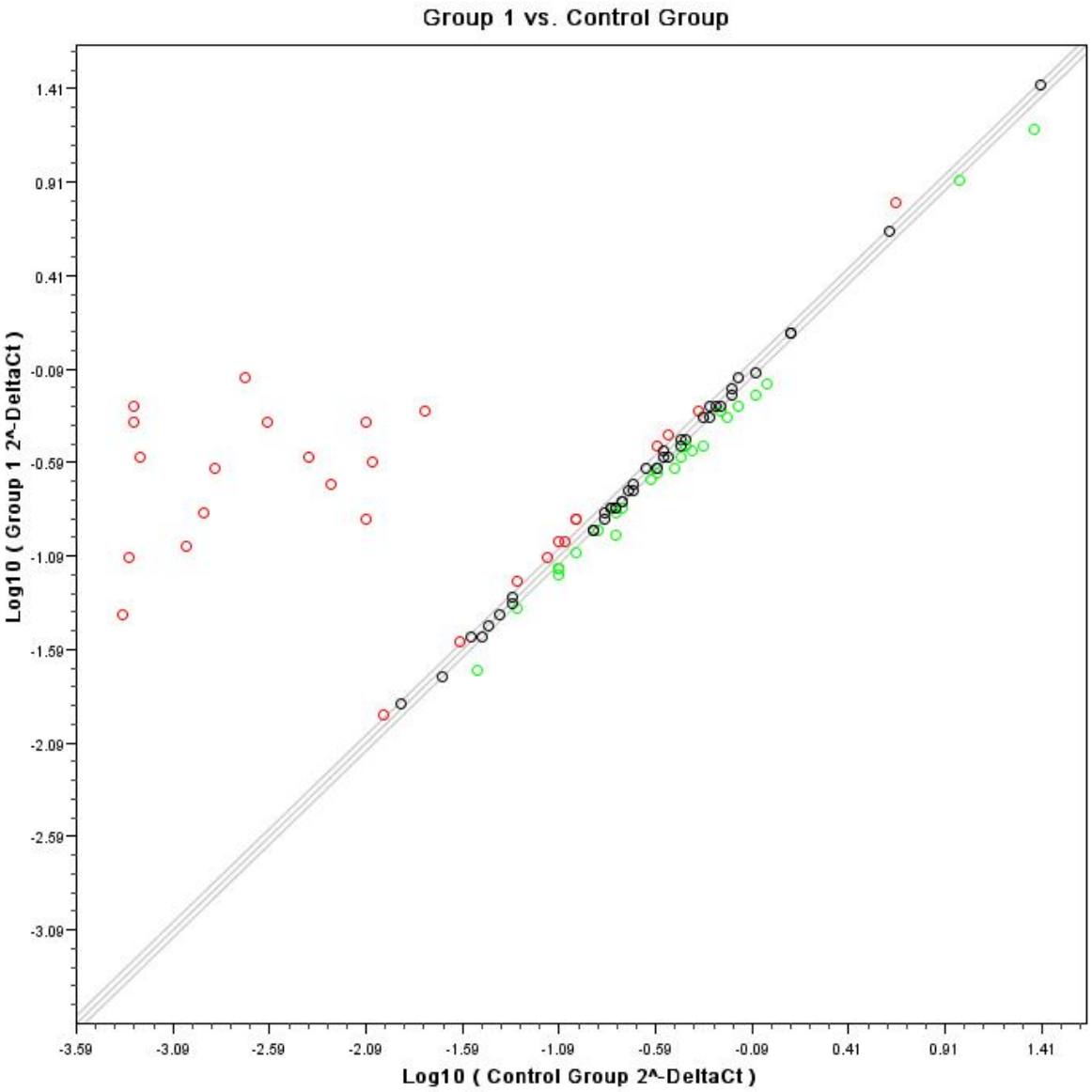

Figure 4: The up and down regulated immuno-inflammatory genes in patient 1 after 48 h of IAV infection. The upper triangular represents the change of 26 (30.95%) upregulated genes (red color) with log10. The 23 (27.38%) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

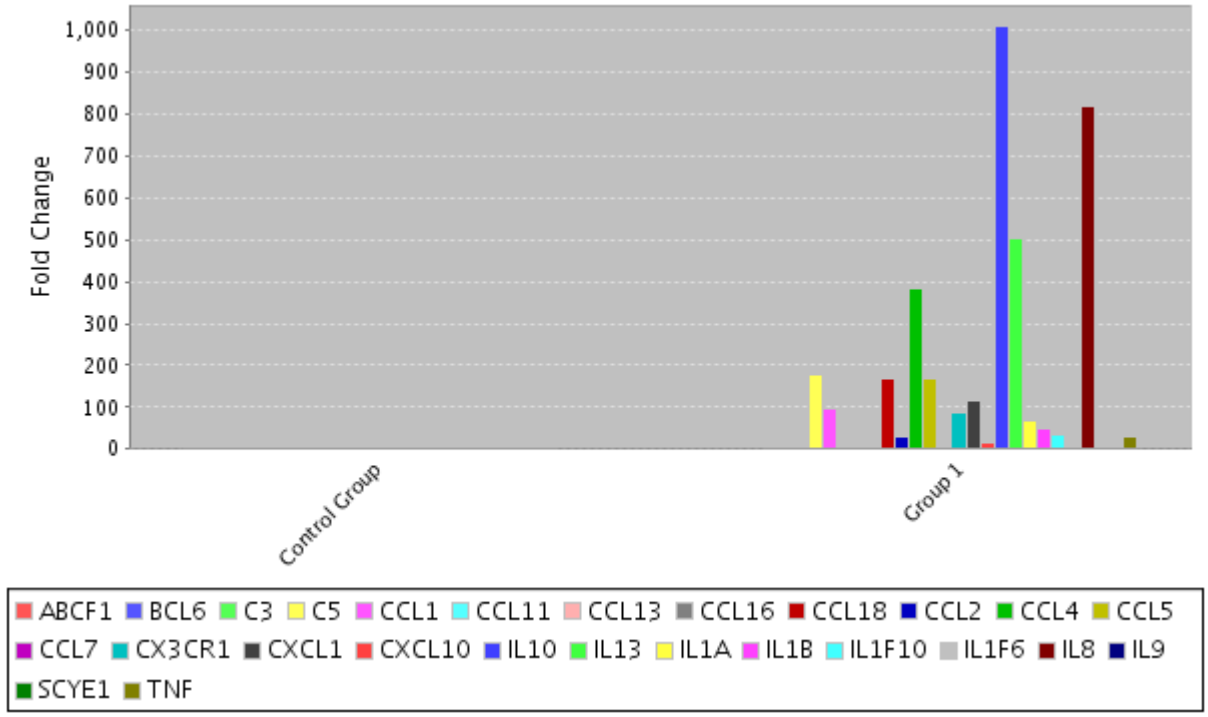

Figure 5: The up regulated 26 genes after 48 h of IAV infection in patient 1. The upper upregulated gene is IL10 (1009 fold change) and the lower upregulated genes are BCL6, CCL7, IL1F6 and SCYE1(1.13 fold change).

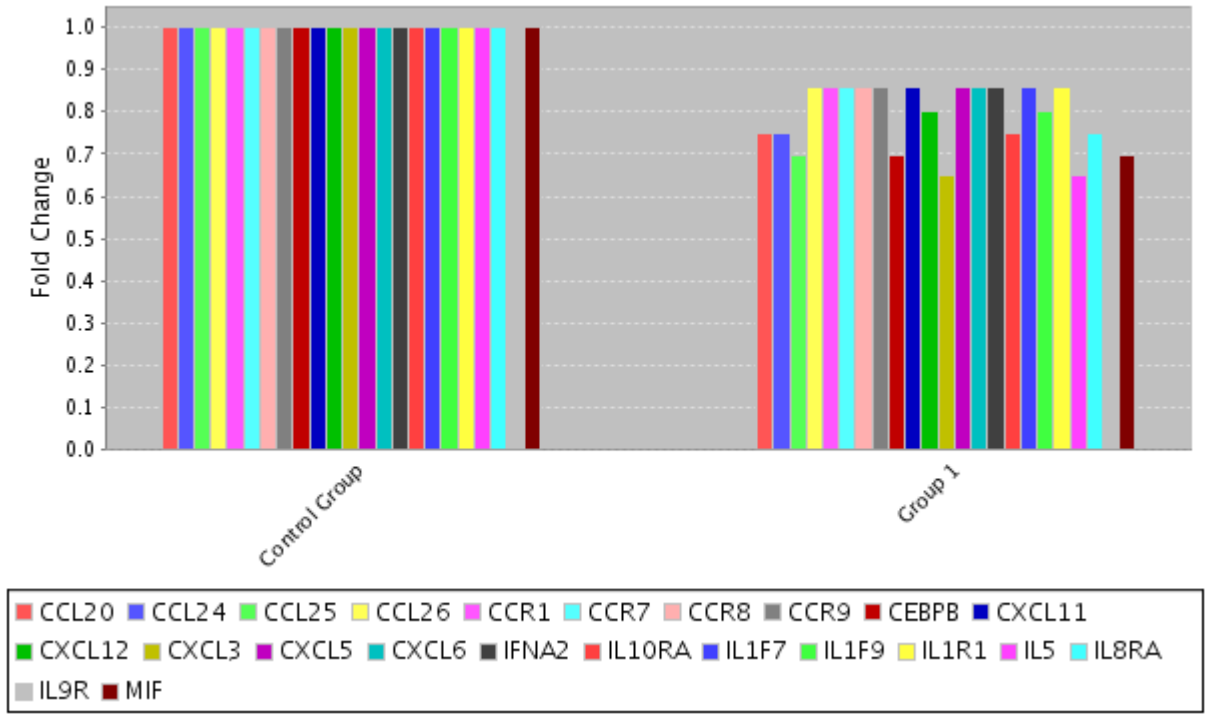

Figure 6: The down regulated 23 genes after 48 h of IAV infection in patient 1. The upper downregulated gene is IL5 (-1.5 fold change) and the lower downregulated gene is CCL26 (-1.16 fold change).

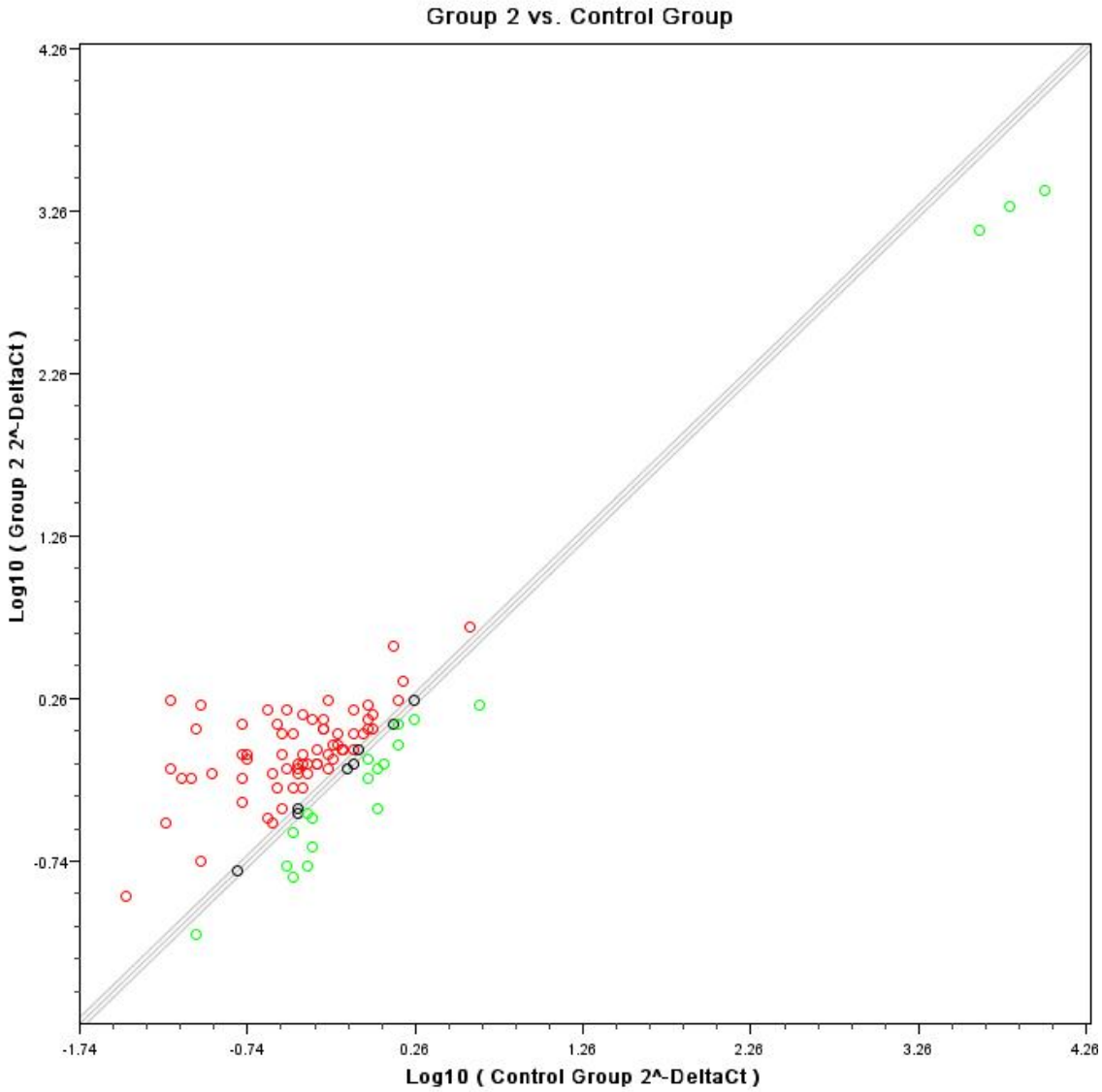

Figure 7: The up and down regulated genes after 48 h of IAV infection in patient 2. The upper triangular represents the change of 62 (73.80%) upregulated genes (red color) with log10. The 15 (17.85 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

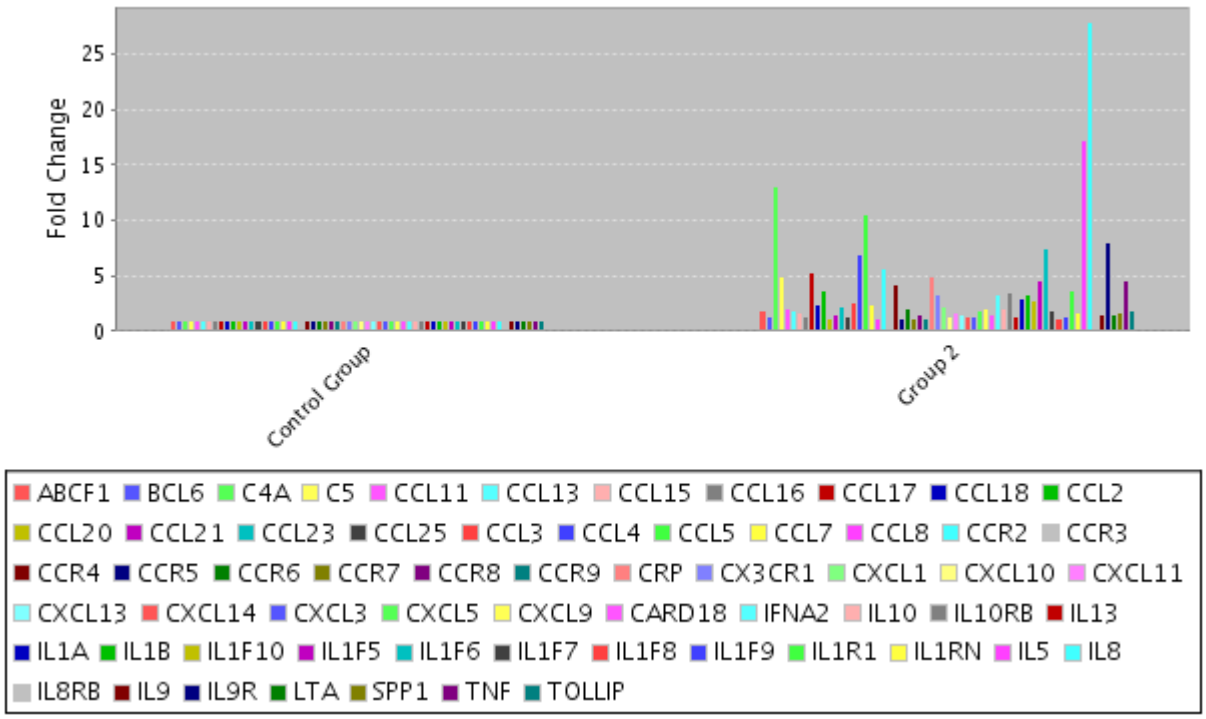

Figure 8: The up regulated 62 (73.80%) genes after 48 h of IAV infection in patient 2. The most upregulated gene is IL8 gene (27.85 fold change) and the lower upregulated genes are IL1F8, CCR9 genes (1.148 fold change).

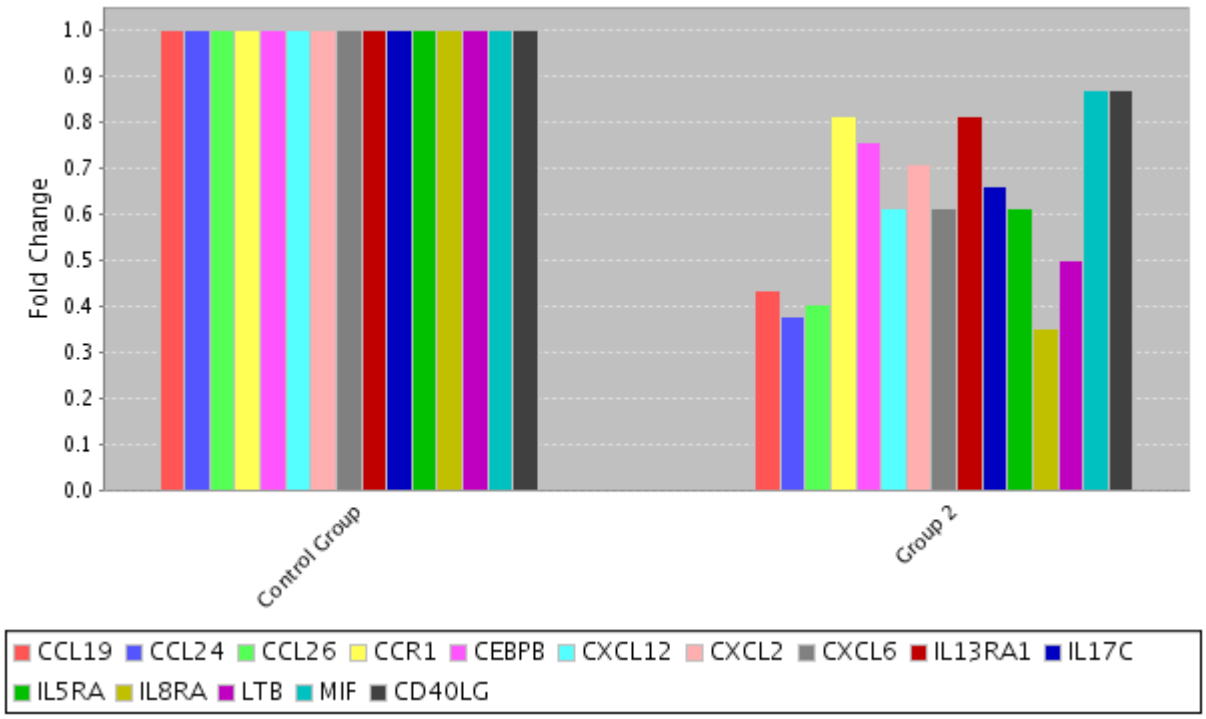

Figure 9: The down regulated 15 genes in patient 2 after 48 h of IAV infection. The upper downregulated gene is IL8RA gene (-2.80 fold change) and the lower downregulated gene is MIF gene (-1.14 fold change).

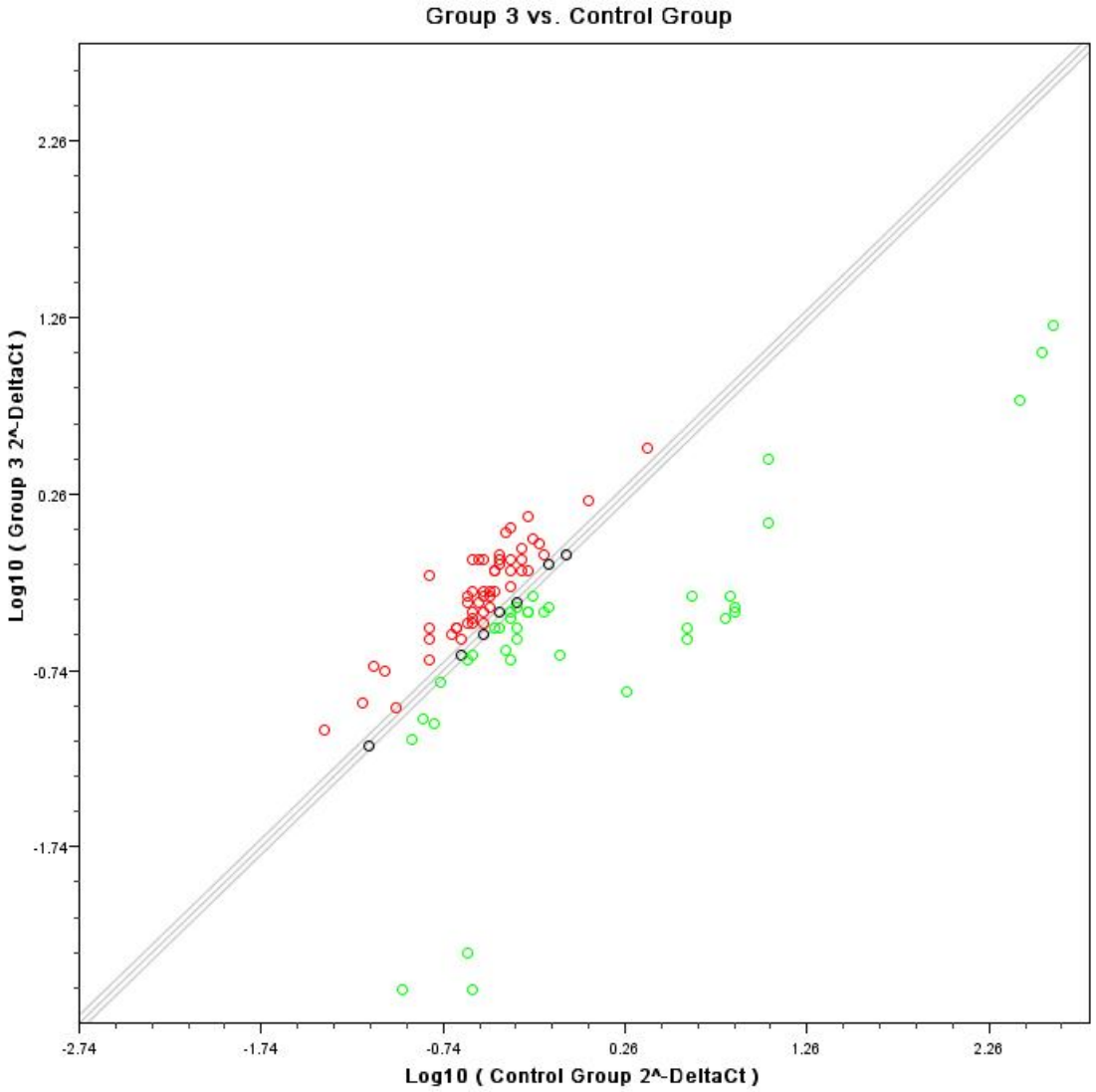

Figure 10: The up and down regulated genes in patient 3 after 48 h of IAV infection. The upper triangular represents the change of 49(58.33%) upregulated genes (red color) with log10. The 29 (34.52 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

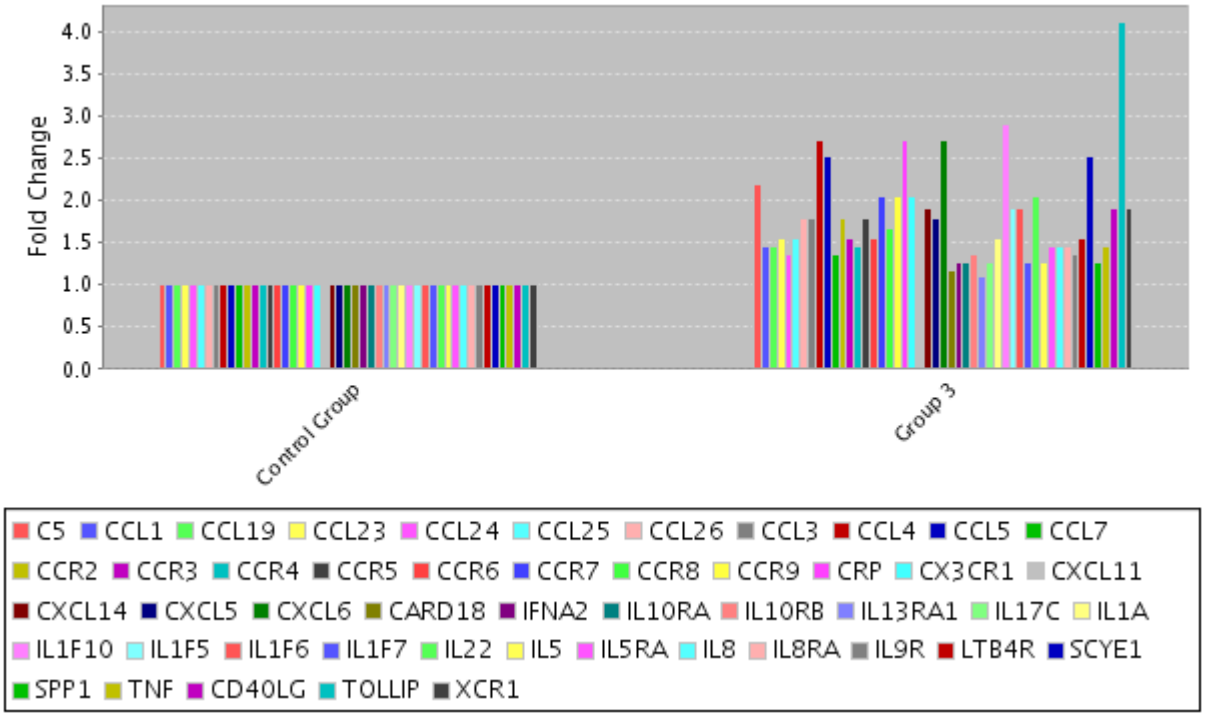

Figure 11: The up regulated 49 genes in patient 3 after 48 h of IAV infection. The most upregulated gene is TLLIP gene (4.11 fold change) and the lower upregulated gene is IL13RA1 gene (1.10 fold change).

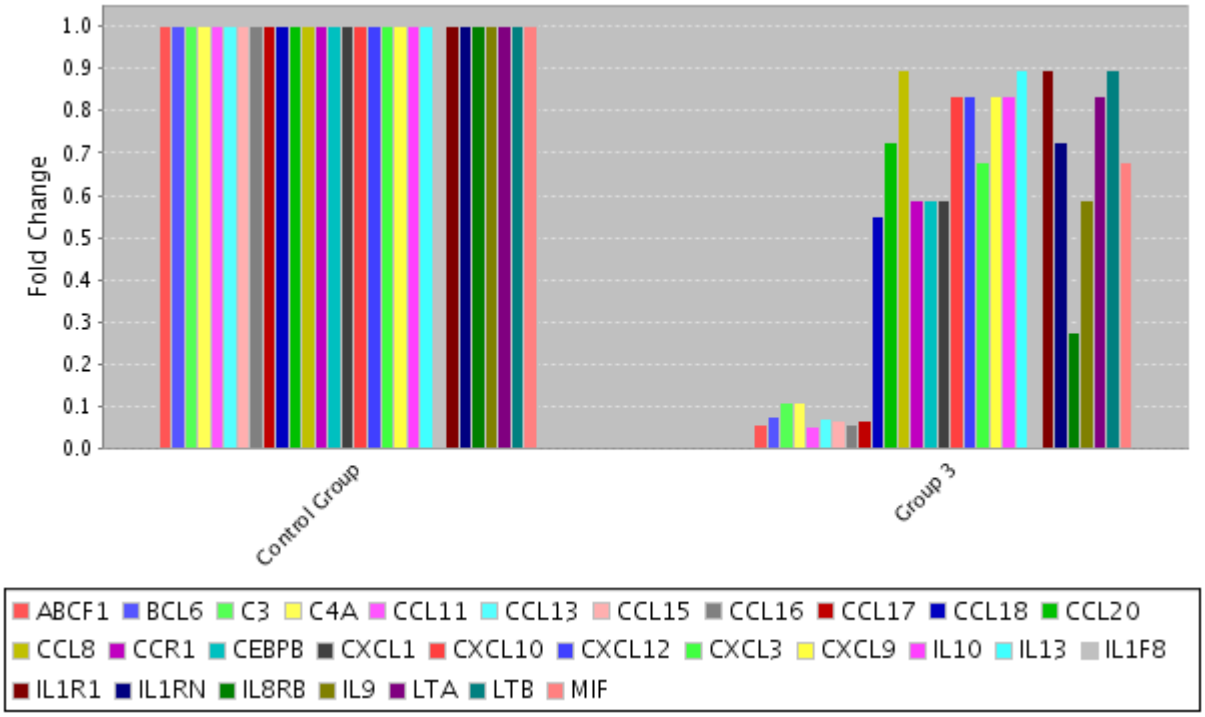

Figure 12: The down regulated 29 genes in patient 3 after 48 h. of IAV infection. The upper downregulated gene is CCL11gene (-19.15 fold change) and the lower downregulated gene is CCL8 gene (-1.11 fold change).

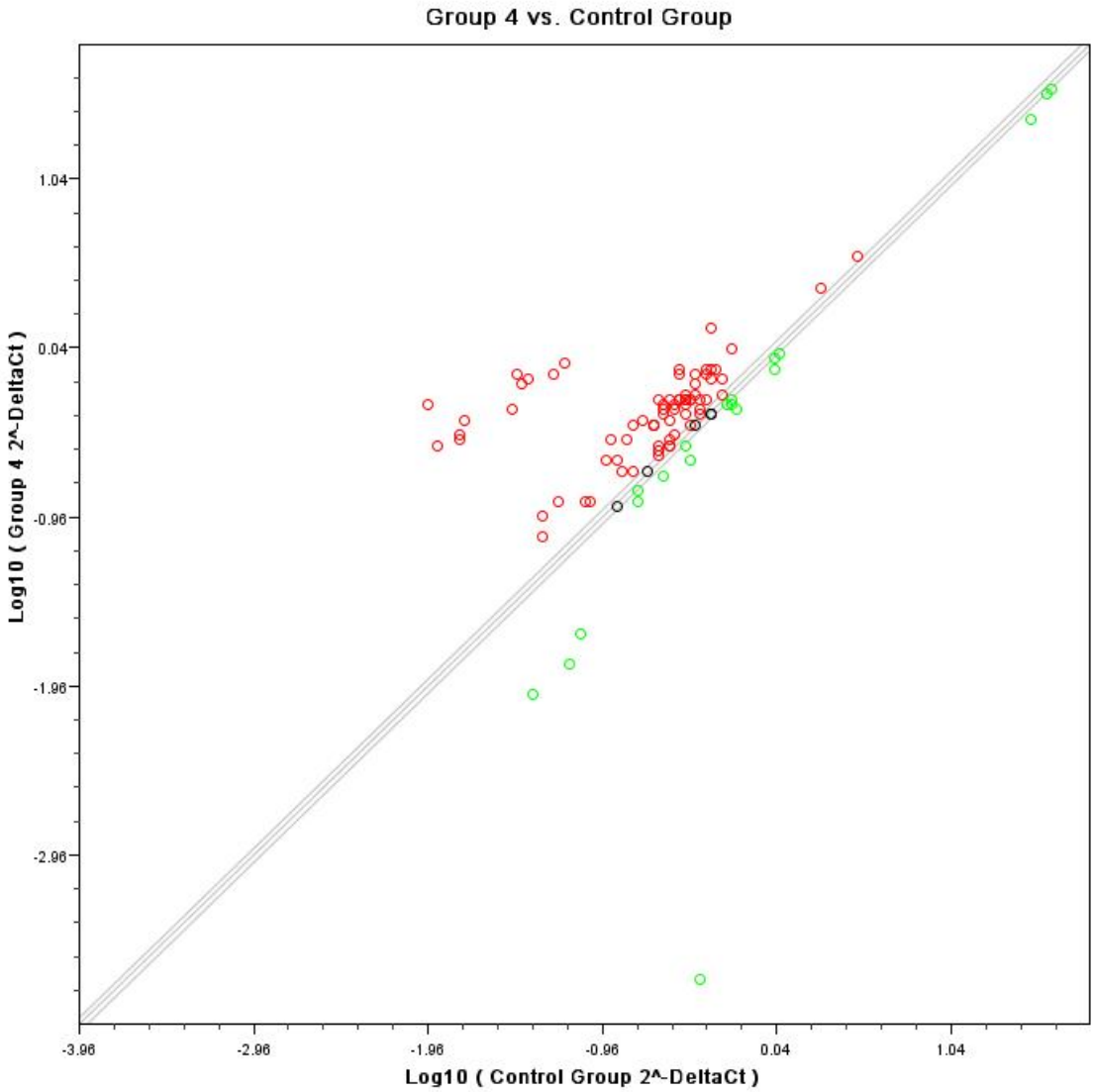

Figure 13: The up and down regulated genes in patient 4 after 48 h of IAV infection. The upper triangular represents the change of 70 (83.33%) upregulated genes (red color) with log10. The 11(13.09 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

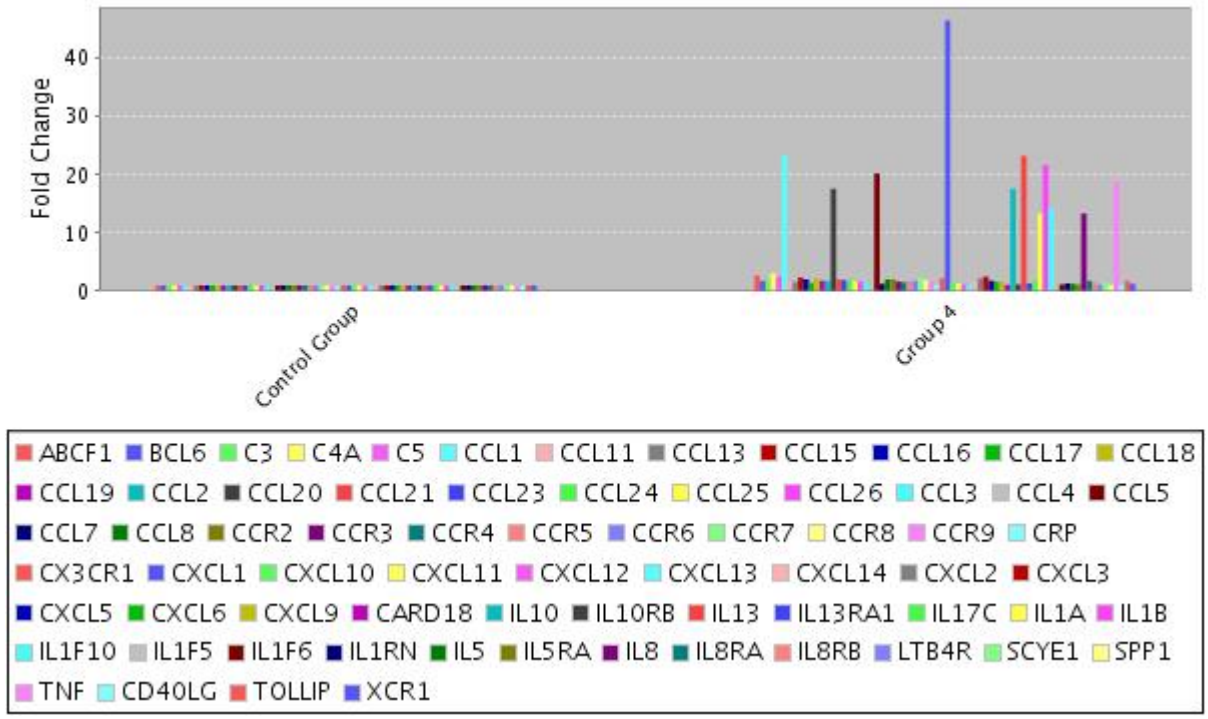

Figure 14: The up regulated 70 genes in patient 4 after 48 h of IAV infection. The most upregulated gene is CXCL1 gene (46.52 fold change) and the lower upregulated gene is CD40LG gene (1.10 fold change).

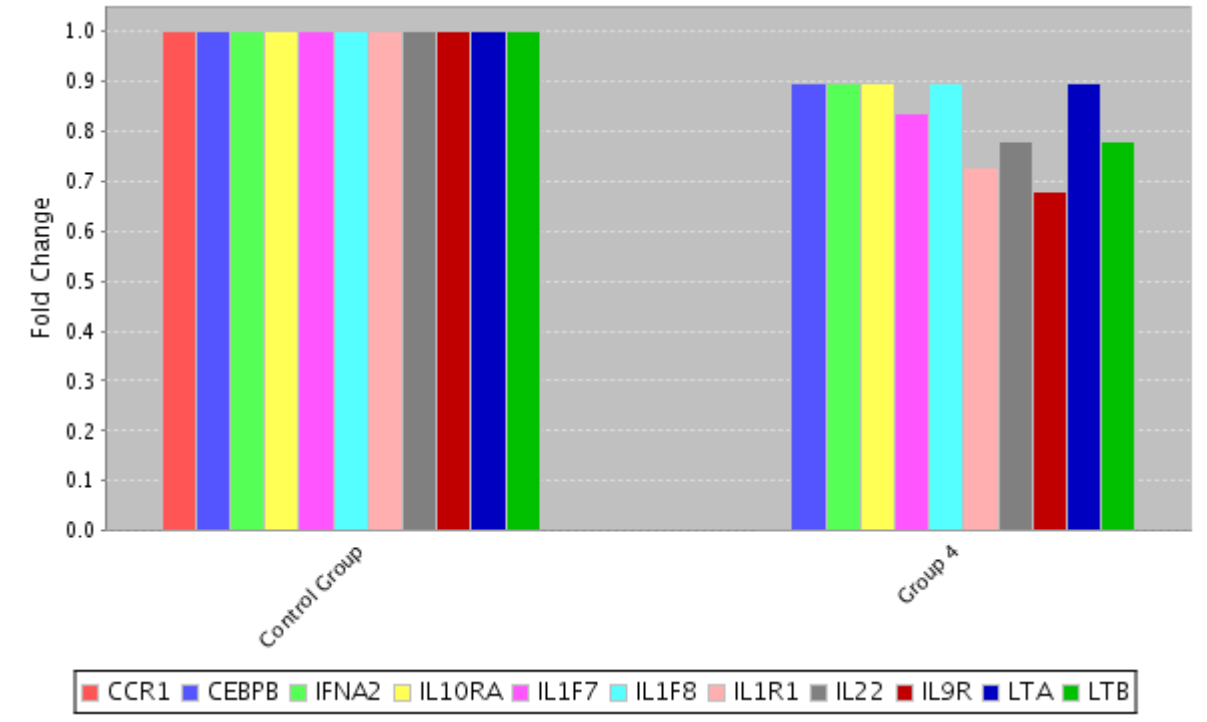

Figure 15: The down regulated 11 genes in patient 4 after 48 h of IAV infection. The upper downregulated gene is CCR1gene (-1992 fold change) and the lower downregulated gene is CEBPB gene (-1.11 fold change).

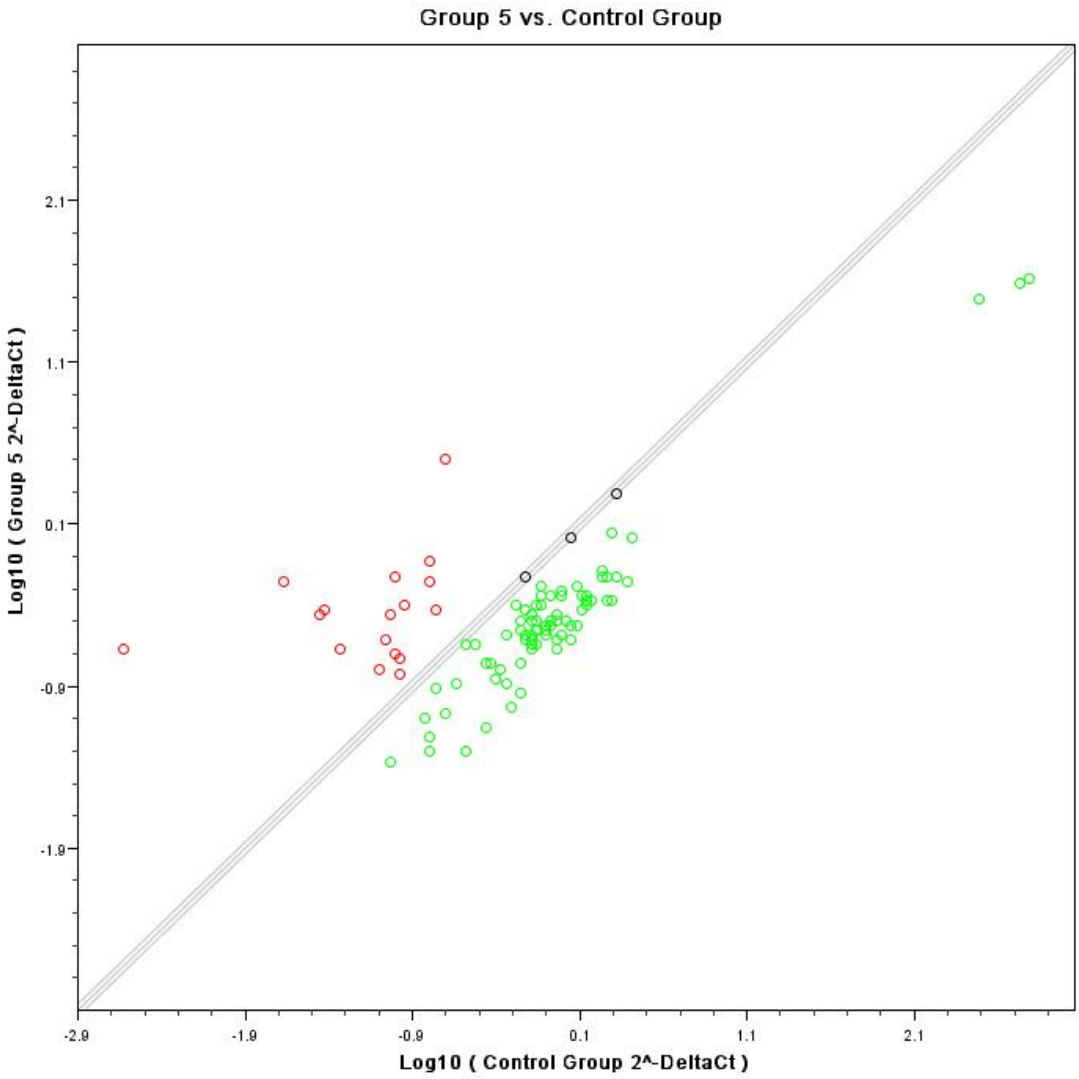

Figure 16: The up and down regulated genes in patient 5 after 48h of IAV infection. The upper triangular represents the change of 16 (19.04%) upregulated genes (red color) with log10. The 66 (78.57 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line

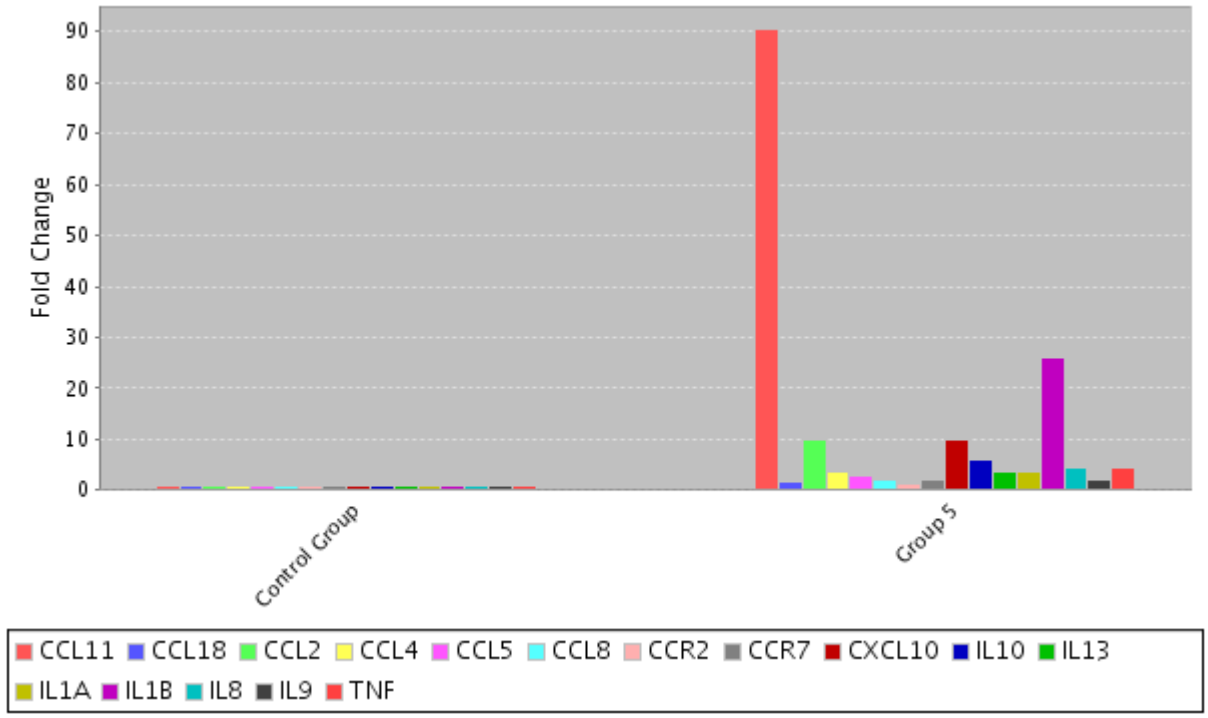

Figure 17: The up regulated 16 genes in patient 5 after 48 h infection with IAV. The most upregulated gene is CCL11 gene (90.51 fold change) and the lower upregulated gene is CCR2 gene (1.14 fold change).

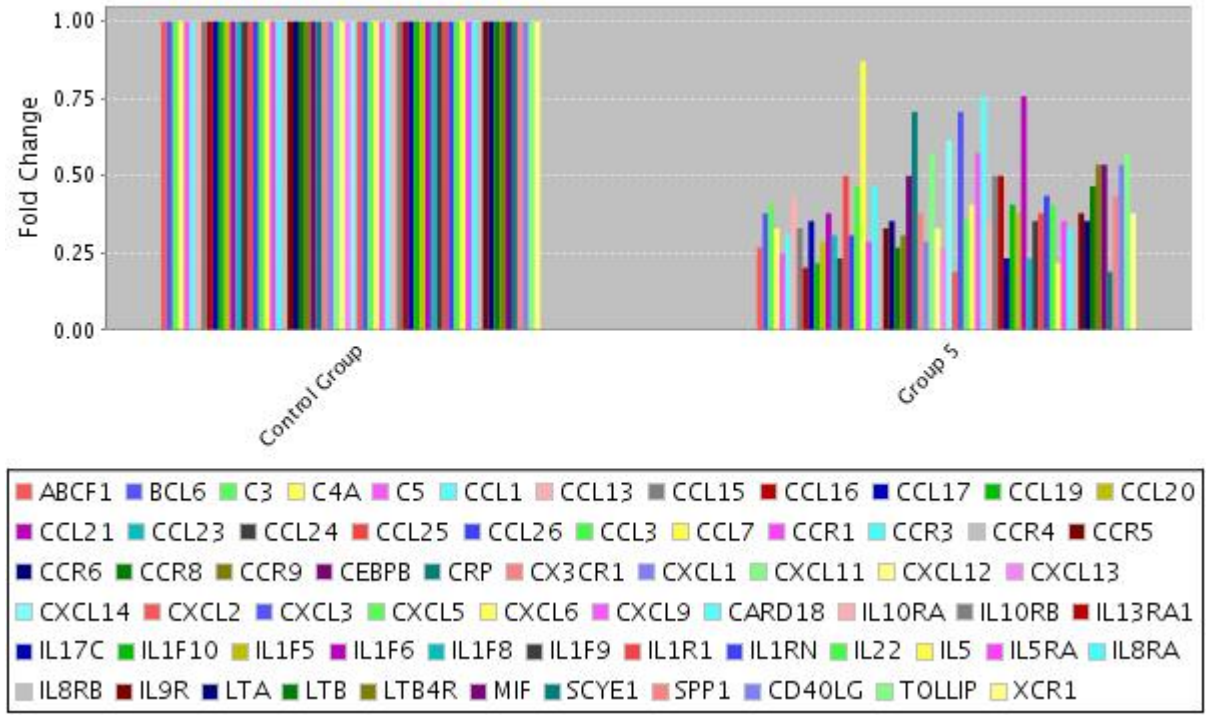

Figure 18: The down regulated 66 genes in patient 5 after 48 h of infection with IAV. The upper downregulated gene is CXCL2 gene (- 5.27 fold change) and the lower downregulated gene is SCYE1 gene (-1.14 fold change).

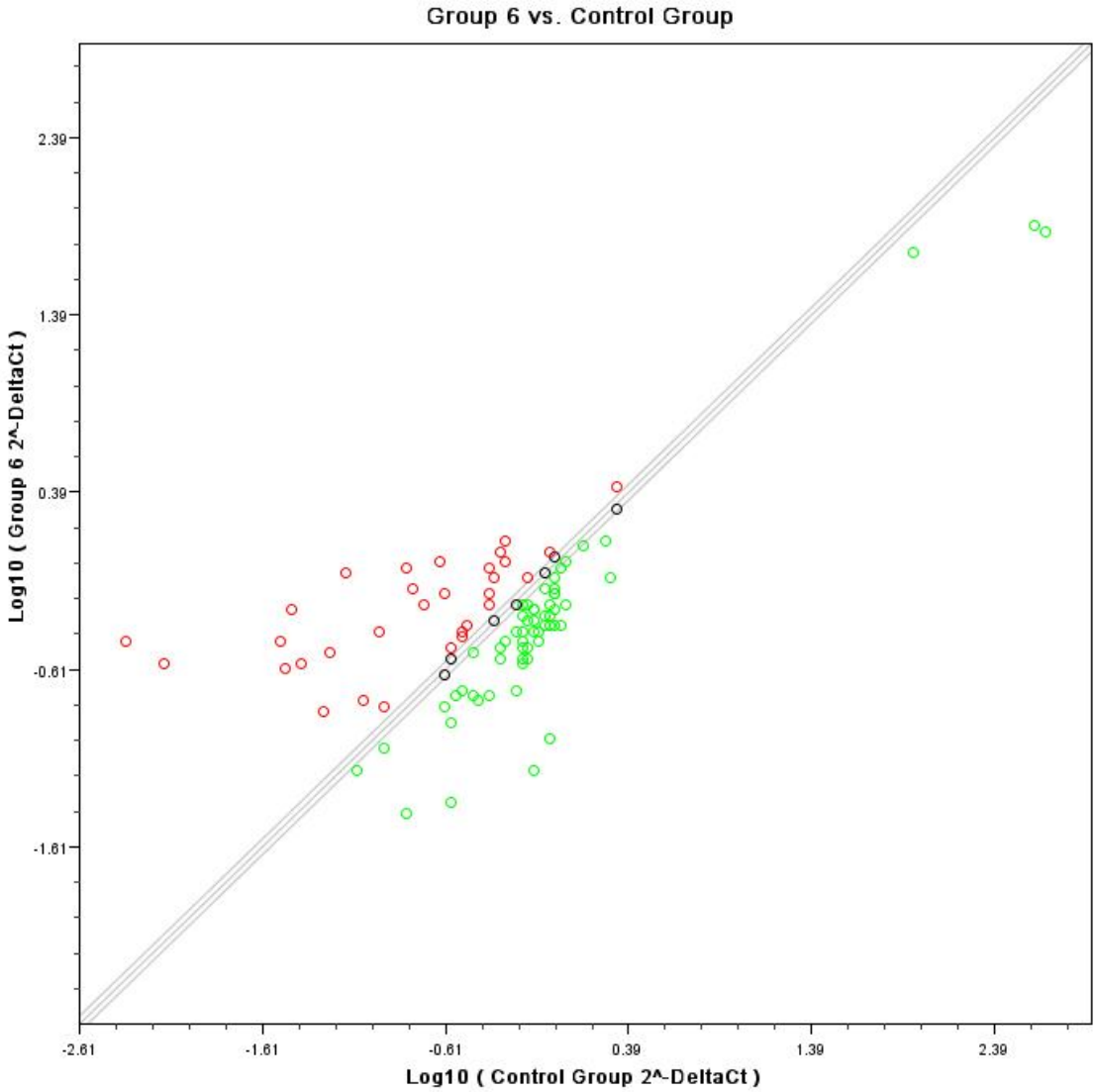

Figure 19: The up and down regulated genes in patient 6 after 48 h infection with IAV. The upper triangular represents the change of 27 (32.14%) upregulated genes (red color) with log10. The 48 (57.14 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line

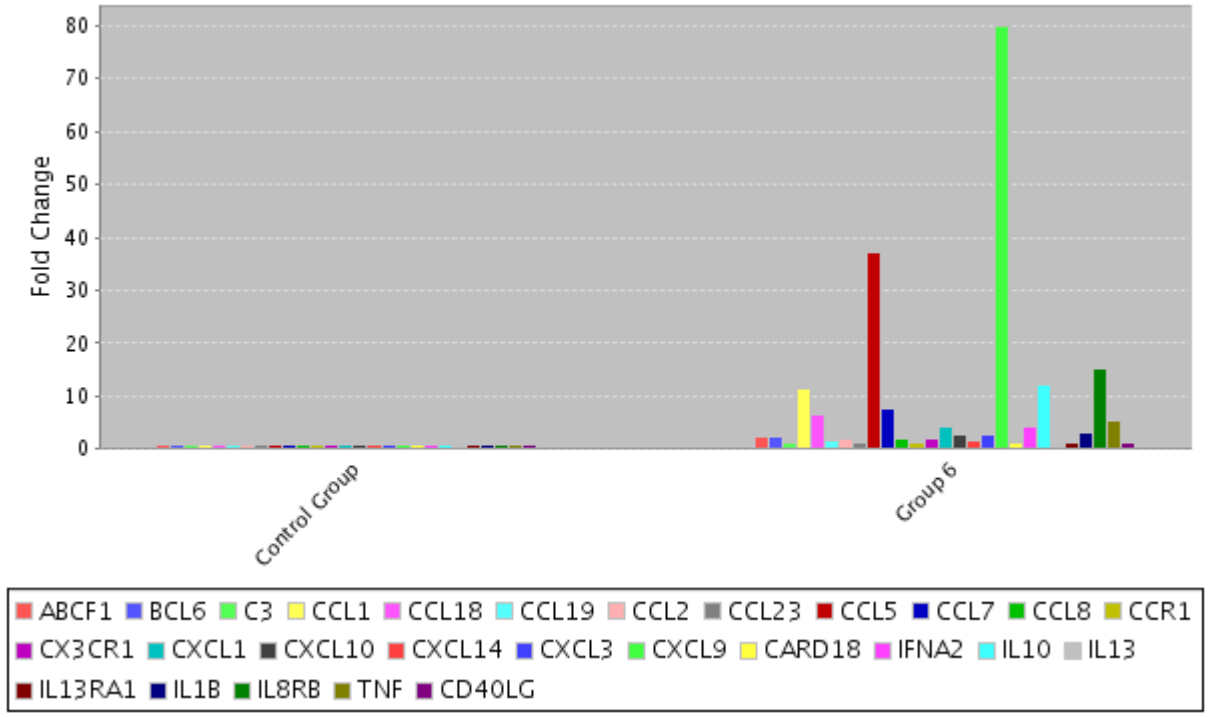

Figure 20: The up regulated 27 genes in patient 6 after 48 h of infection with IAV. The most upregulated gene is CXCL9 gene (79.89 fold change) and the lower upregulated gene is CCR1 gene (1.16 fold change).

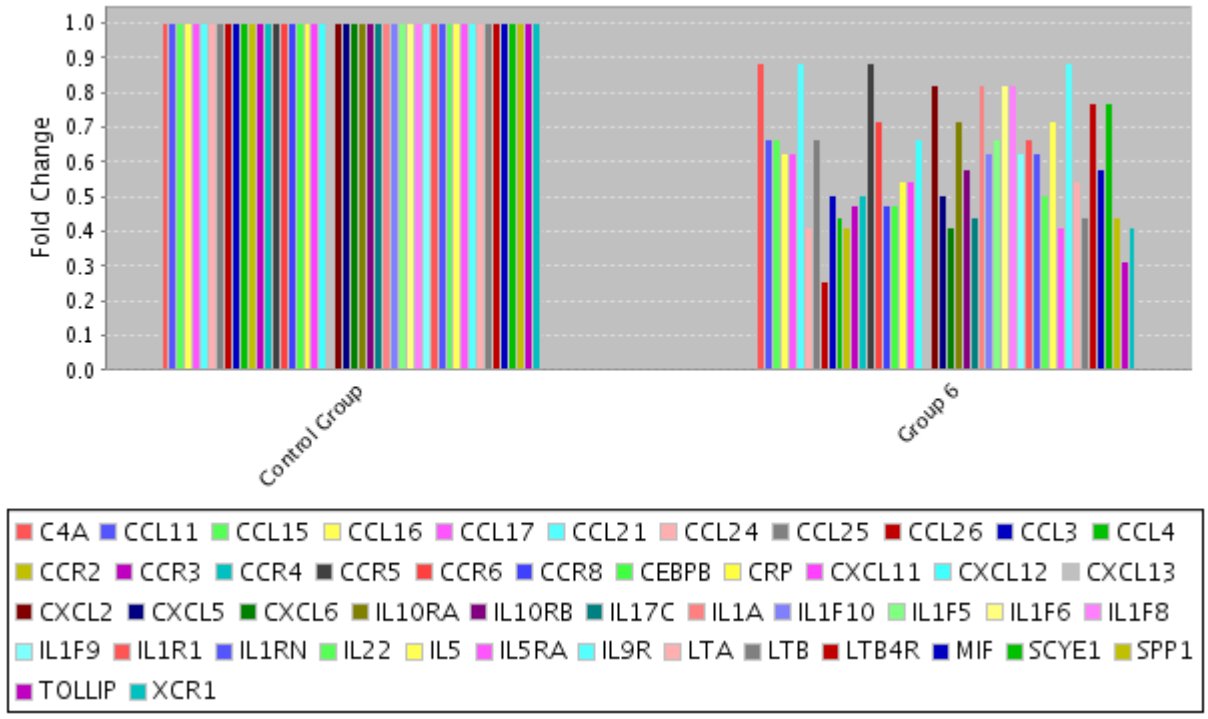

Figure 21: The down regulated 48 genes in patient 6 after 48 h of IAV infection. The upper downregulated gene is CCL26 gene (- 3.94 fold change) and the lower downregulated gene is C4A gene (-1.13 fold change).

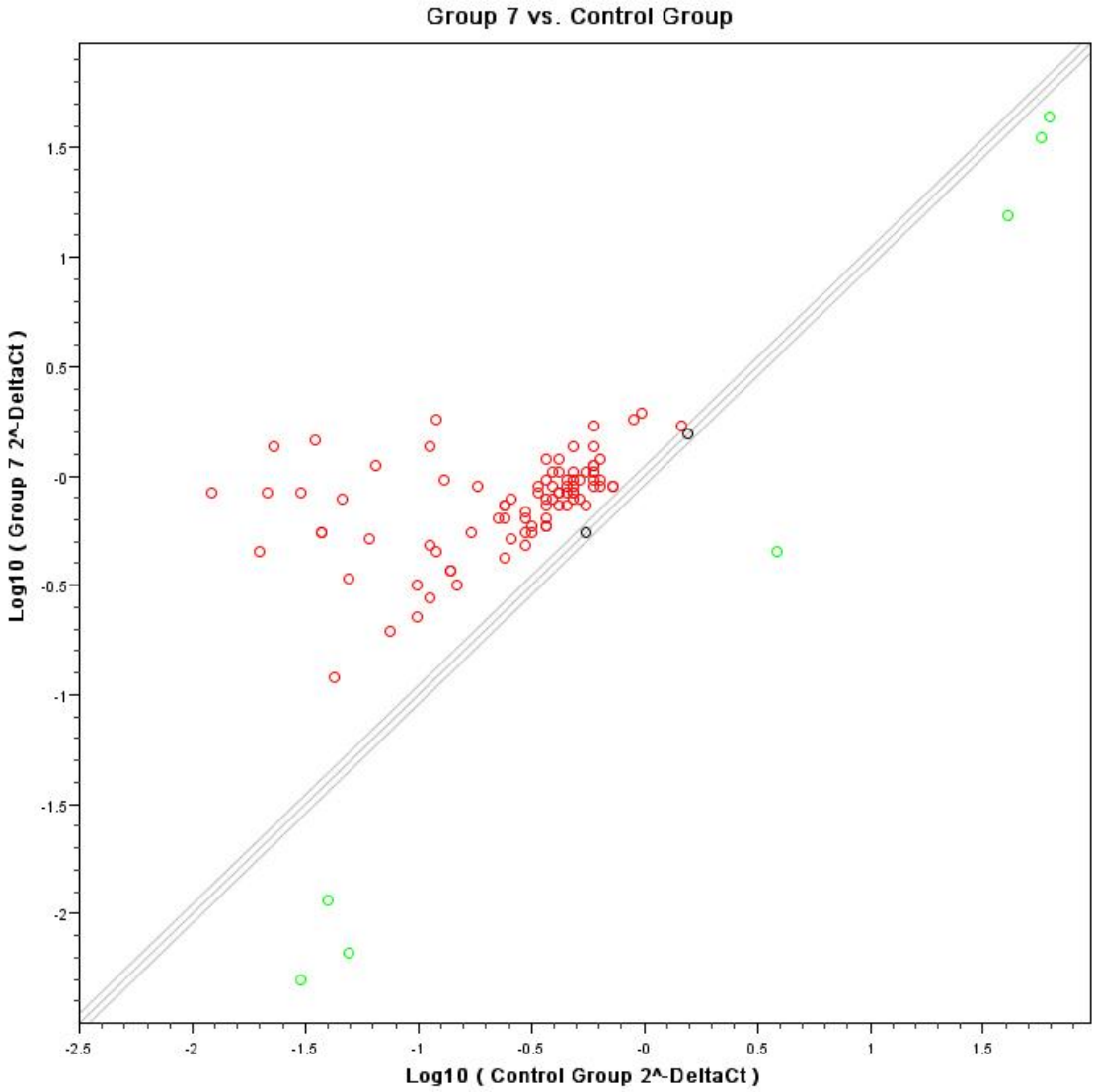

Figure 22: The up regulated 83 genes in patient 7 after 48 h of infection with IAV. The upper triangular represents the change of 83 (98.80%) upregulated genes (red color) with log10. No downregulated genes (the green circles represent the control genes) in the lower triangular. The unchanged genes (black color) represented on the middle line

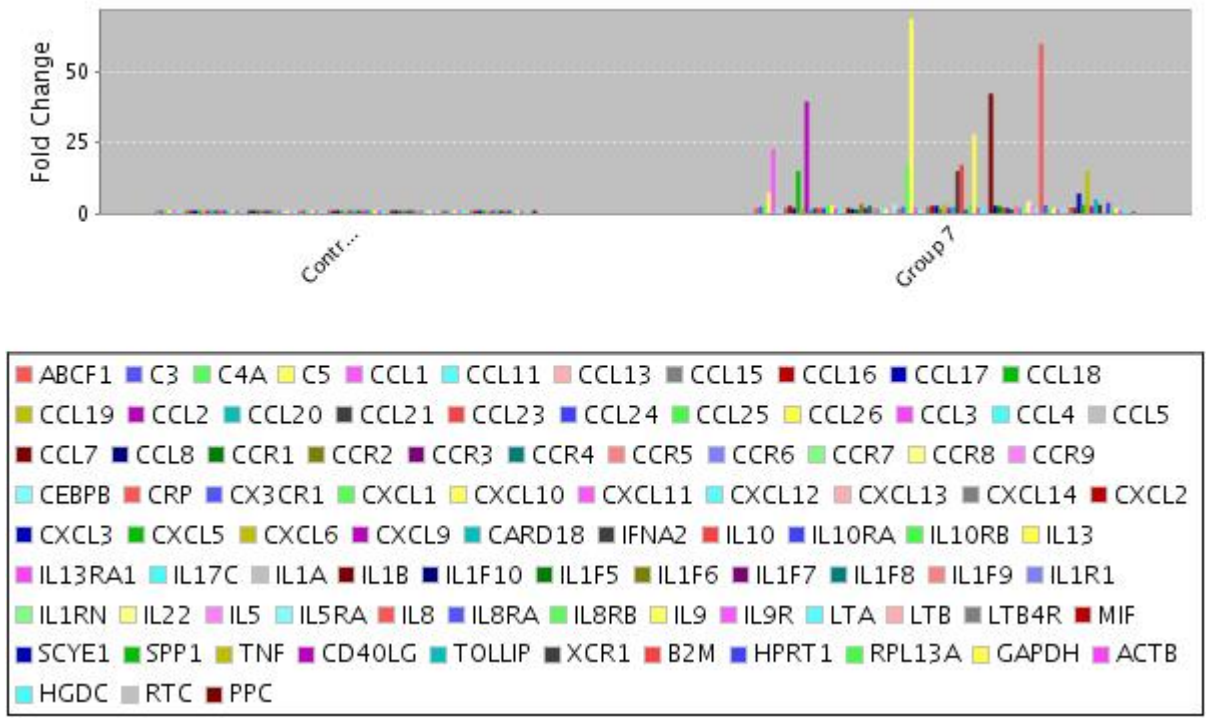

Figure 23: The up regulated 83 genes in patient 7 after 48 h infection with IAV. The most upregulated gene is CXCL10 gene (68.59 fold change) and the lower upregulated gene is IL10R gene (1.23 fold change).

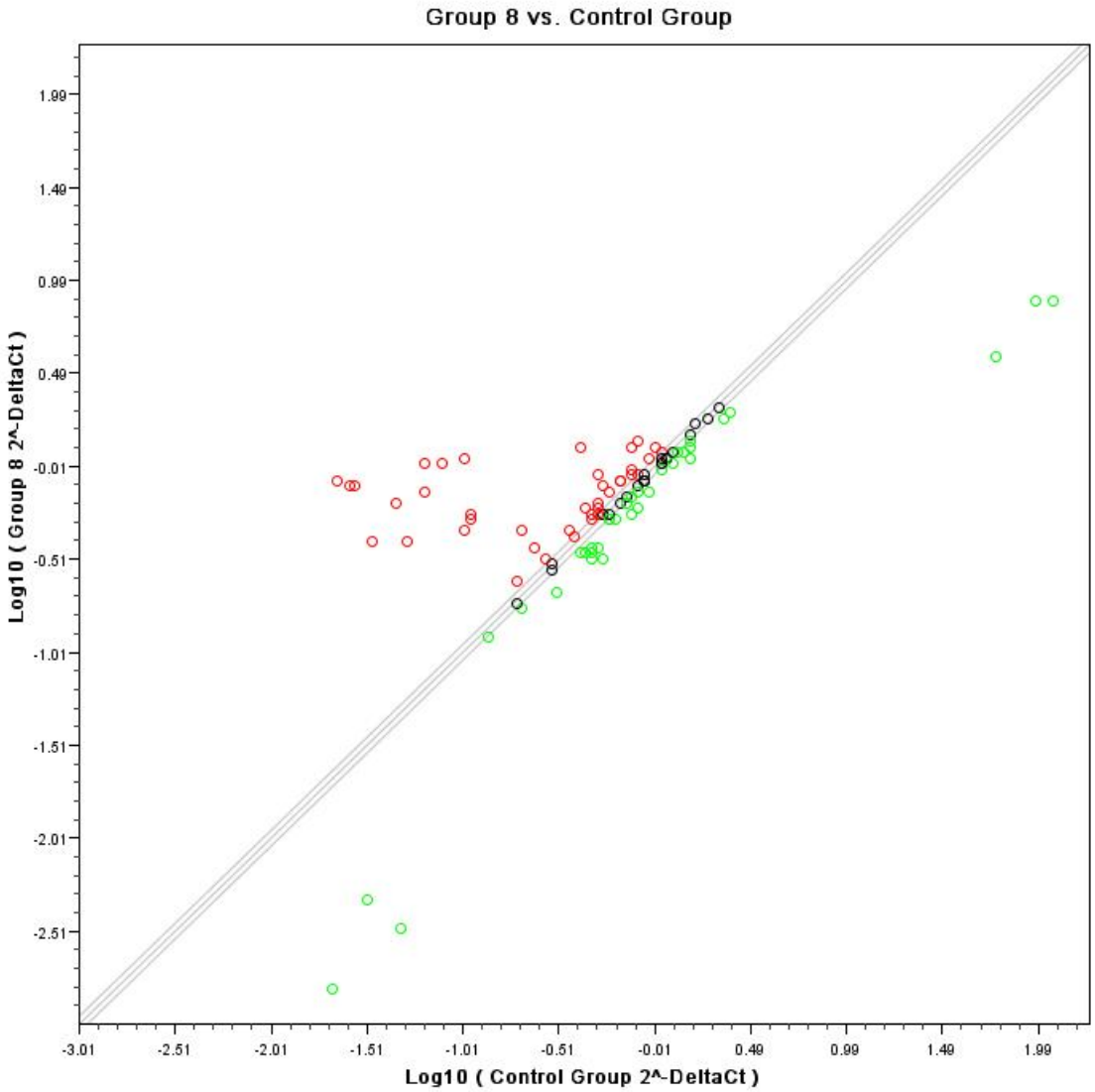

Figure 24: The up and down regulated genes in patient 8 after 48 h infection with IAV. The upper triangular represents the change of 38 (45.23%) upregulated genes (red color) with log10. The 24 (28.57 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line

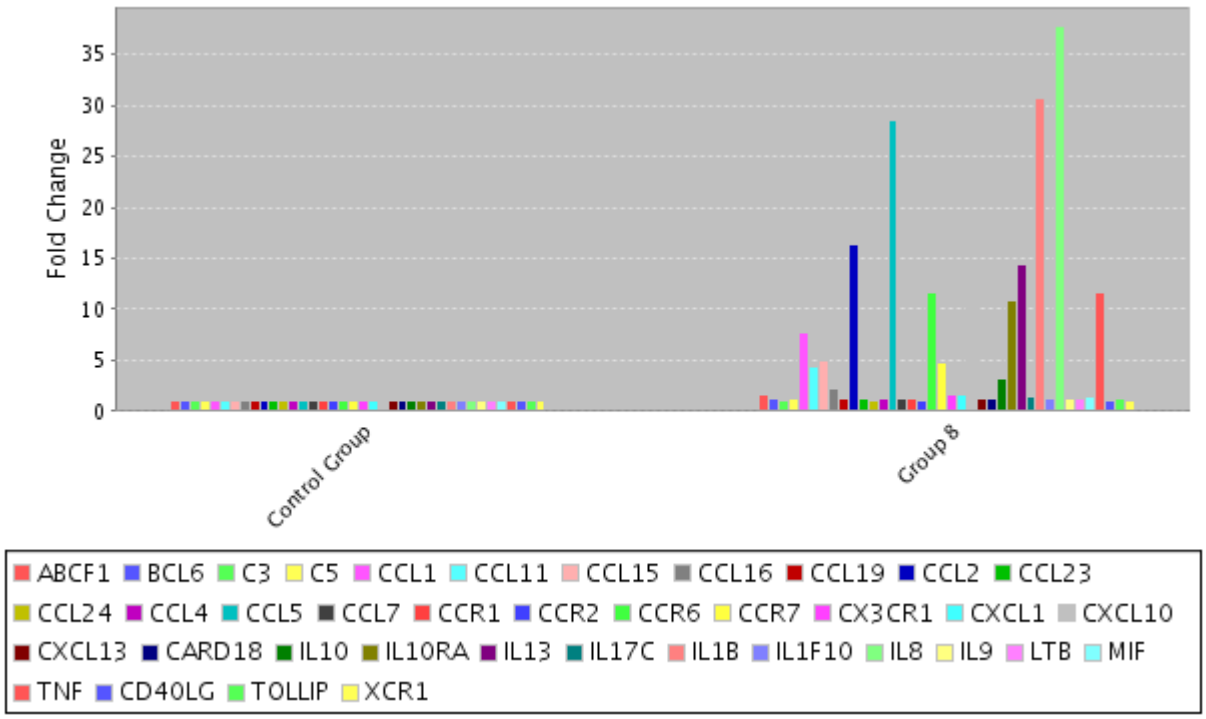

Figure 25: The up regulated 38 genes in patient 8 after 48 h infection with IAV. The most upregulated gene is IL8 gene (37.79 fold change) and the lower upregulated gene is CCR1gene (1.10 fold change).

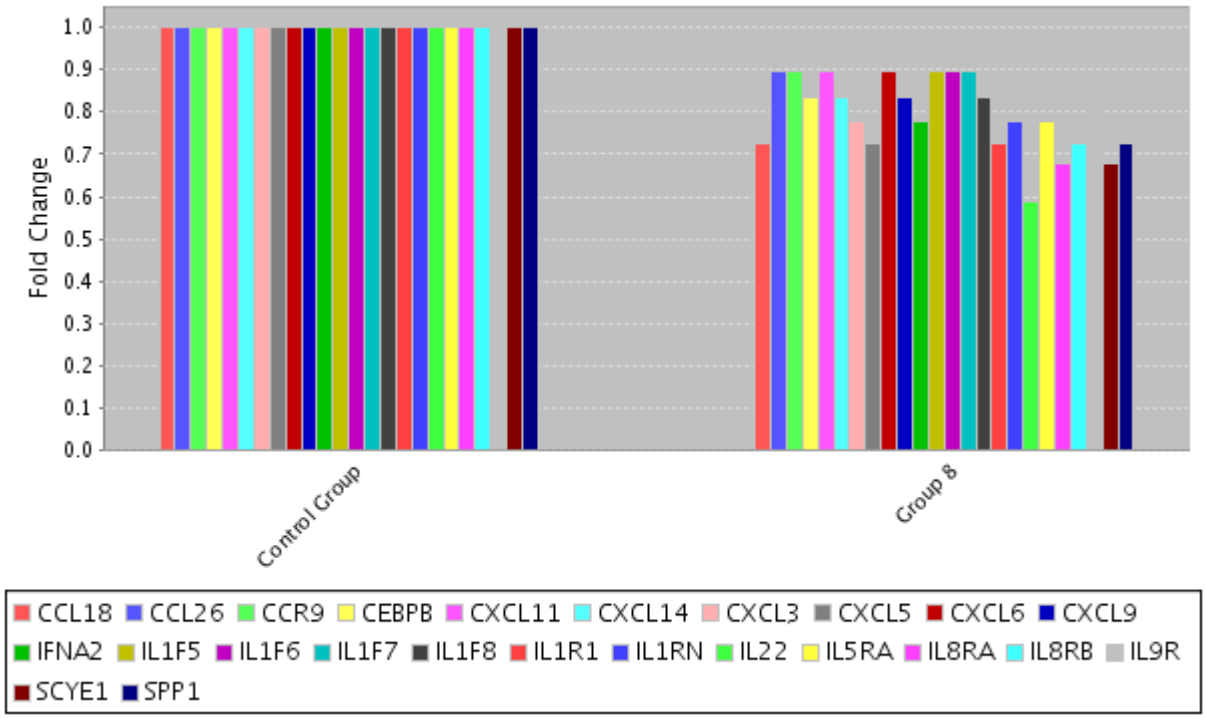

Figure 26: The down regulated 24 genes in patient 8 after 48 h infection with IAV. The upper downregulated gene is CCL18 gene (- 1.37 fold change) and the lower downregulated gene is CCL26 gene (-1.11fold change).

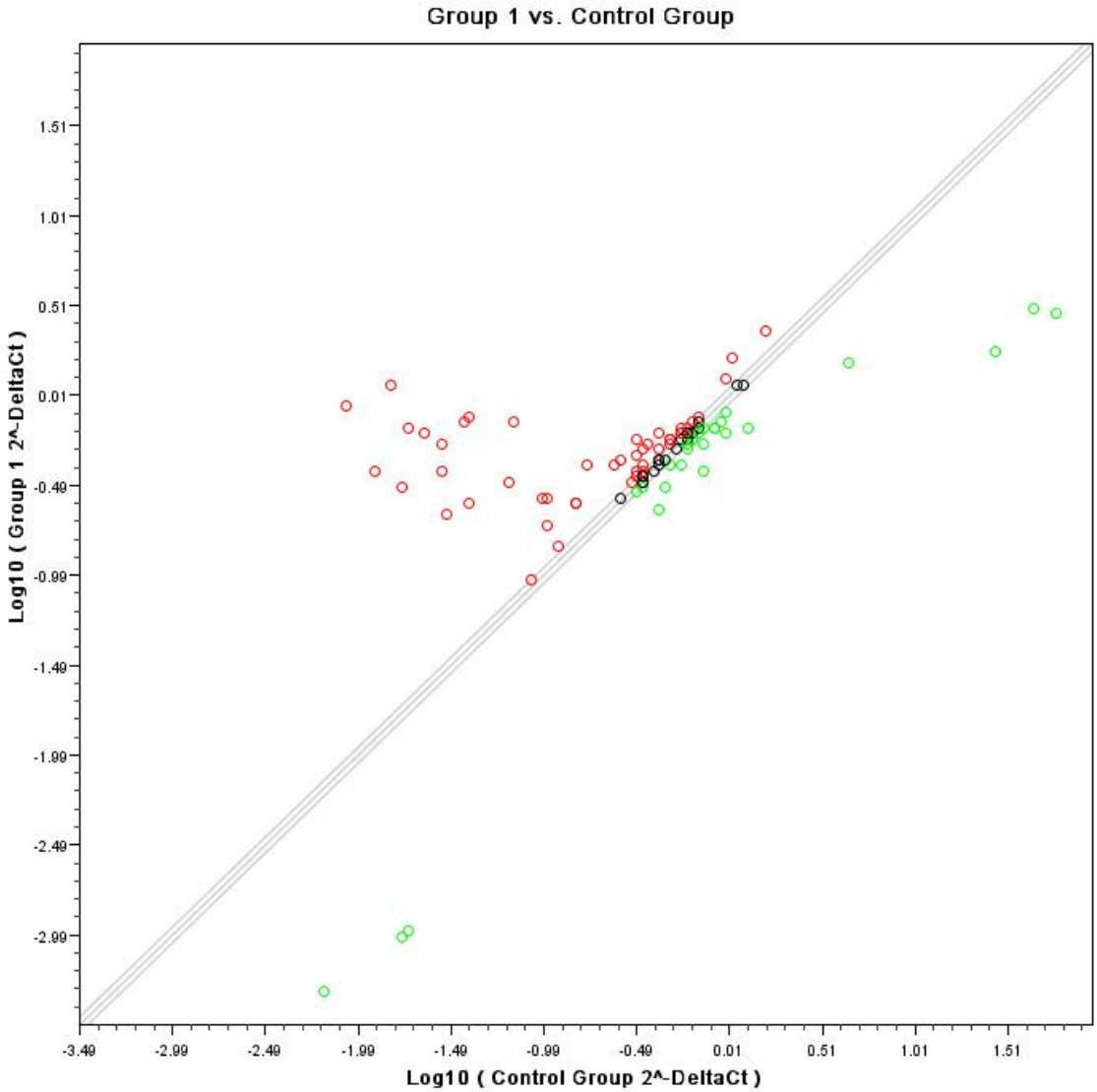

Figure 27: The up and down regulated genes in patient 1 after 48 h of PIV infection. The upper triangular represents the change of 44 (52.38%) upregulated genes (red color) with log10. The 20 (23.80 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line

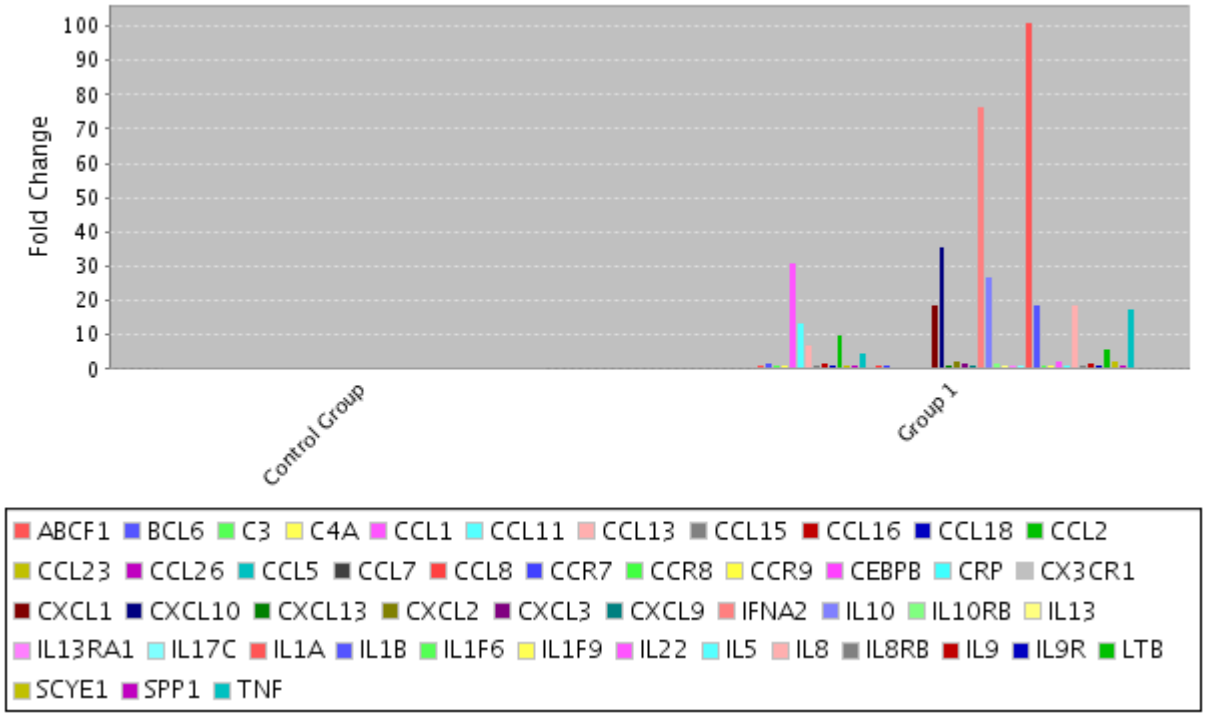

Figure 28: The up regulated 44 genes in patient 1 after 48 h of PIV infection. The most upregulated gene is IL1A gene (101.13 fold change) and the lower upregulated gene is SPP1 gene (1.11 fold change).

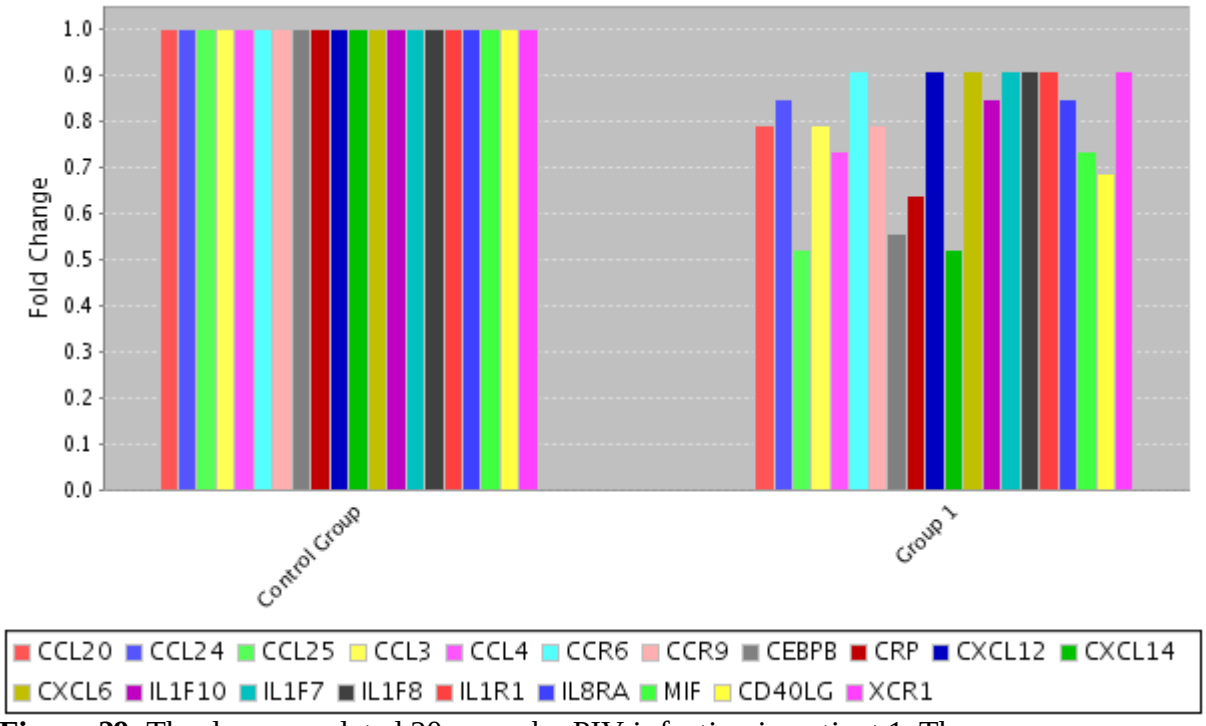

Figure 29: The down regulated 20 genes by PIV infection in patient 1. The upper downregulated gene is CXCL14 gene (-1.91 fold change) and the lower downregulated gene is CCR6 gene (-1.11fold change).

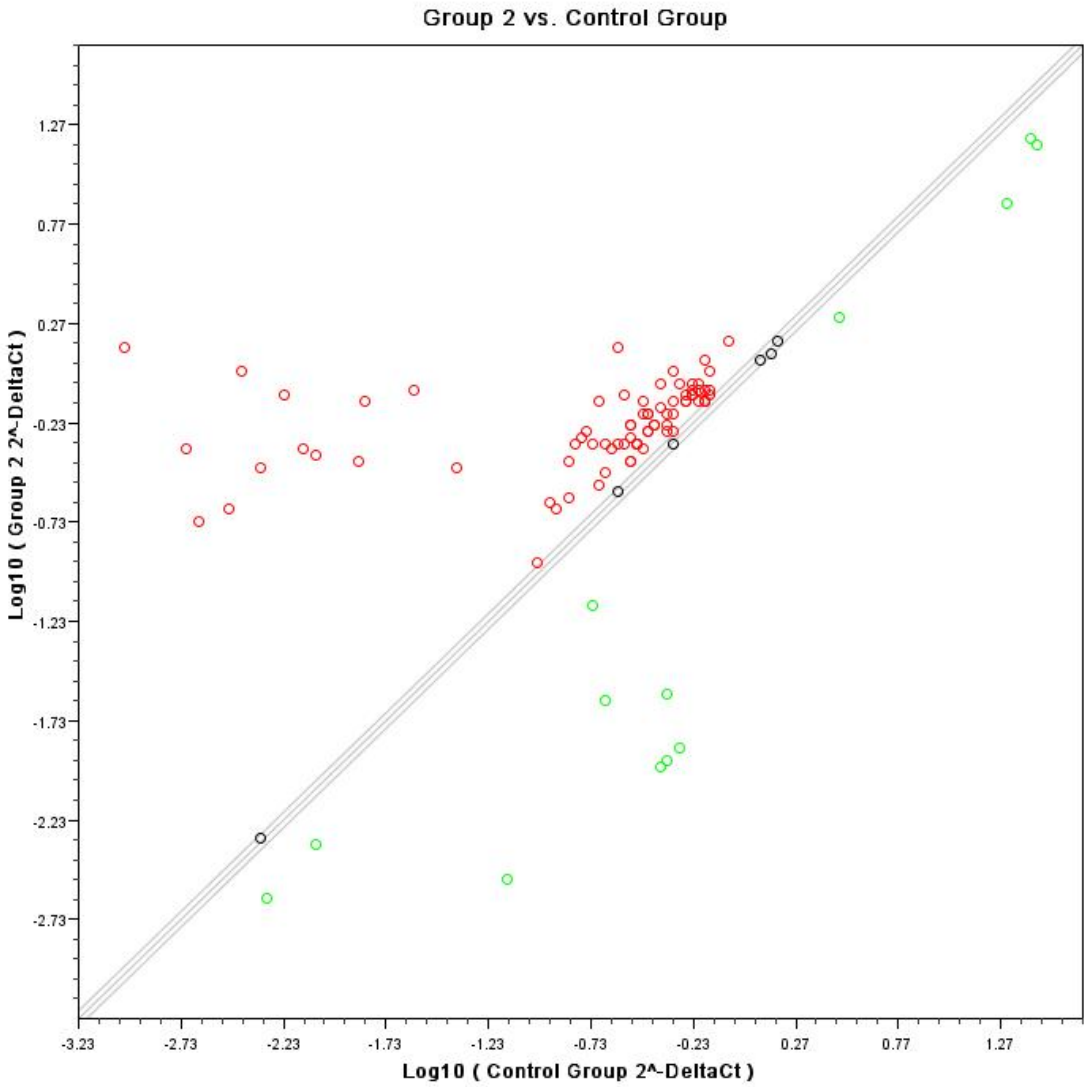

Figure 30: The up and down regulated genes in patient 2 after 48 h of PIV infection. The upper triangular represents the change of 75 (89. 28%) upregulated genes (red color) with log10. The 7 (8.33 %) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line

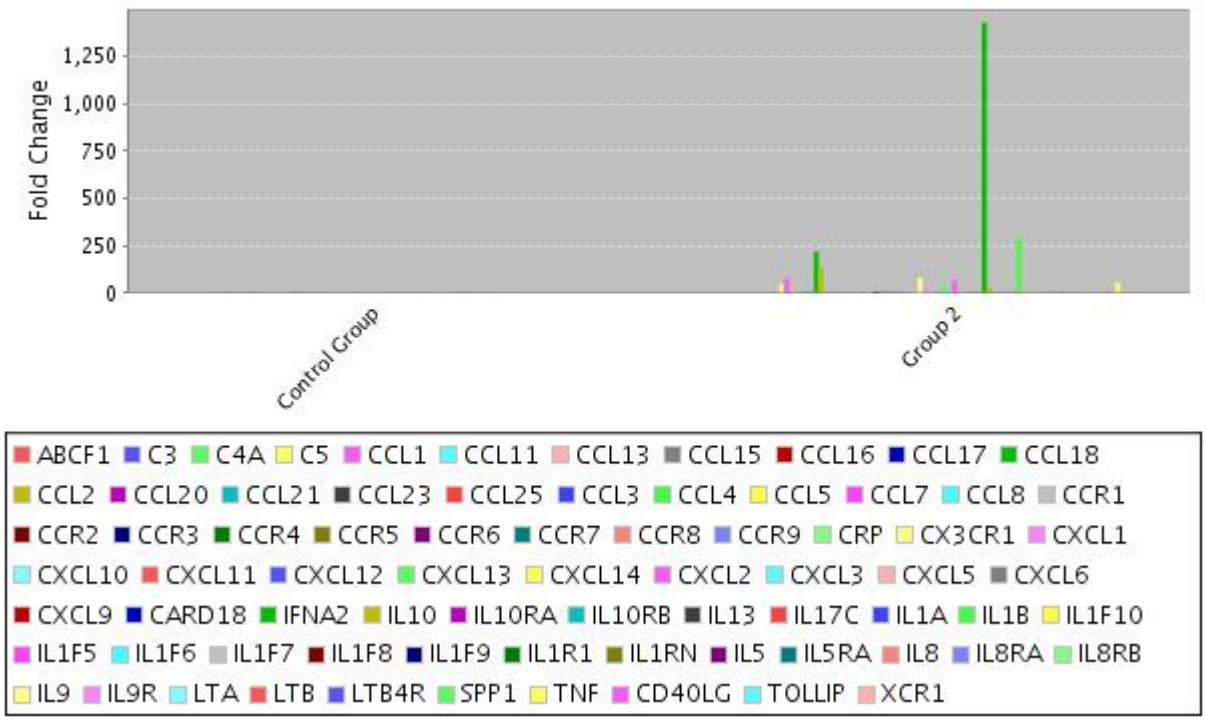

Figure 31: The up regulated 75 genes in patient 2 after 48h of PIV infection. The most upregulated gene is IFNA2 gene (1428.22 fold change) and the lower upregulated gene is LTB gene (1.13 fold change).

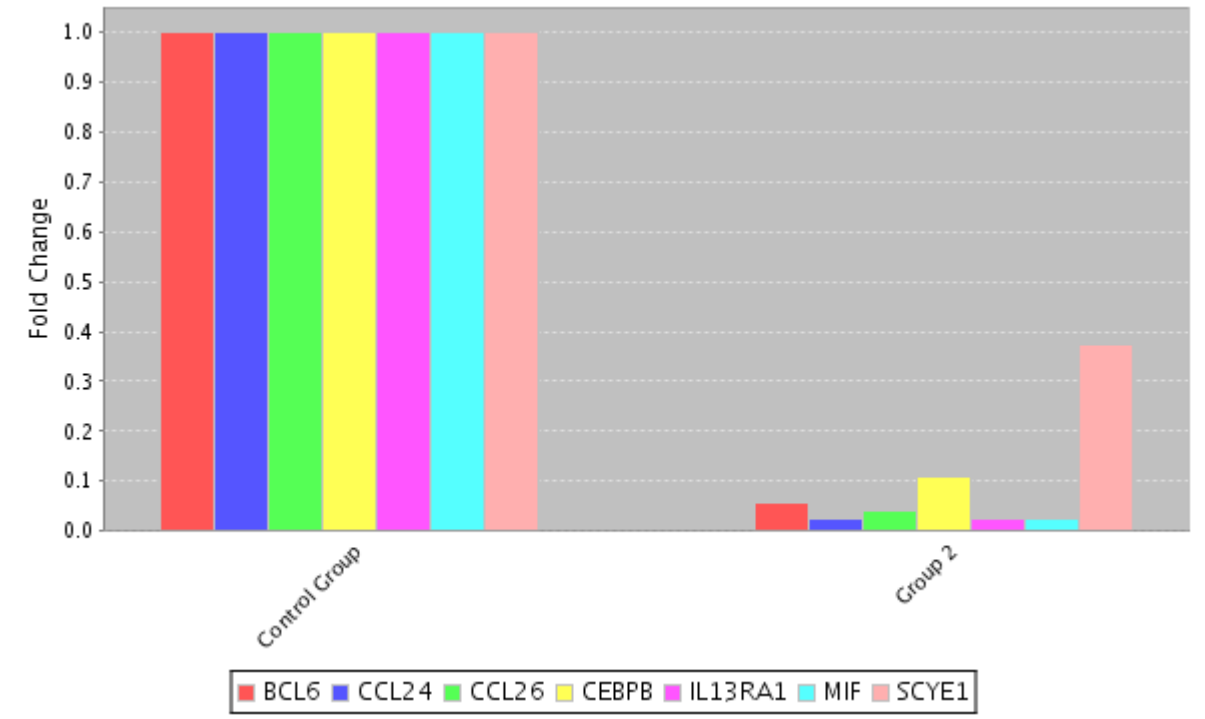

Figure 32: The down regulated 7 genes in patient 2 after 48 h of PIV infection. The upper downregulated gene is IL13RA1 and MIF genes (-37.27 fold change) and the lower downregulated gene is SCYE1gene (- 2.67 fold change).

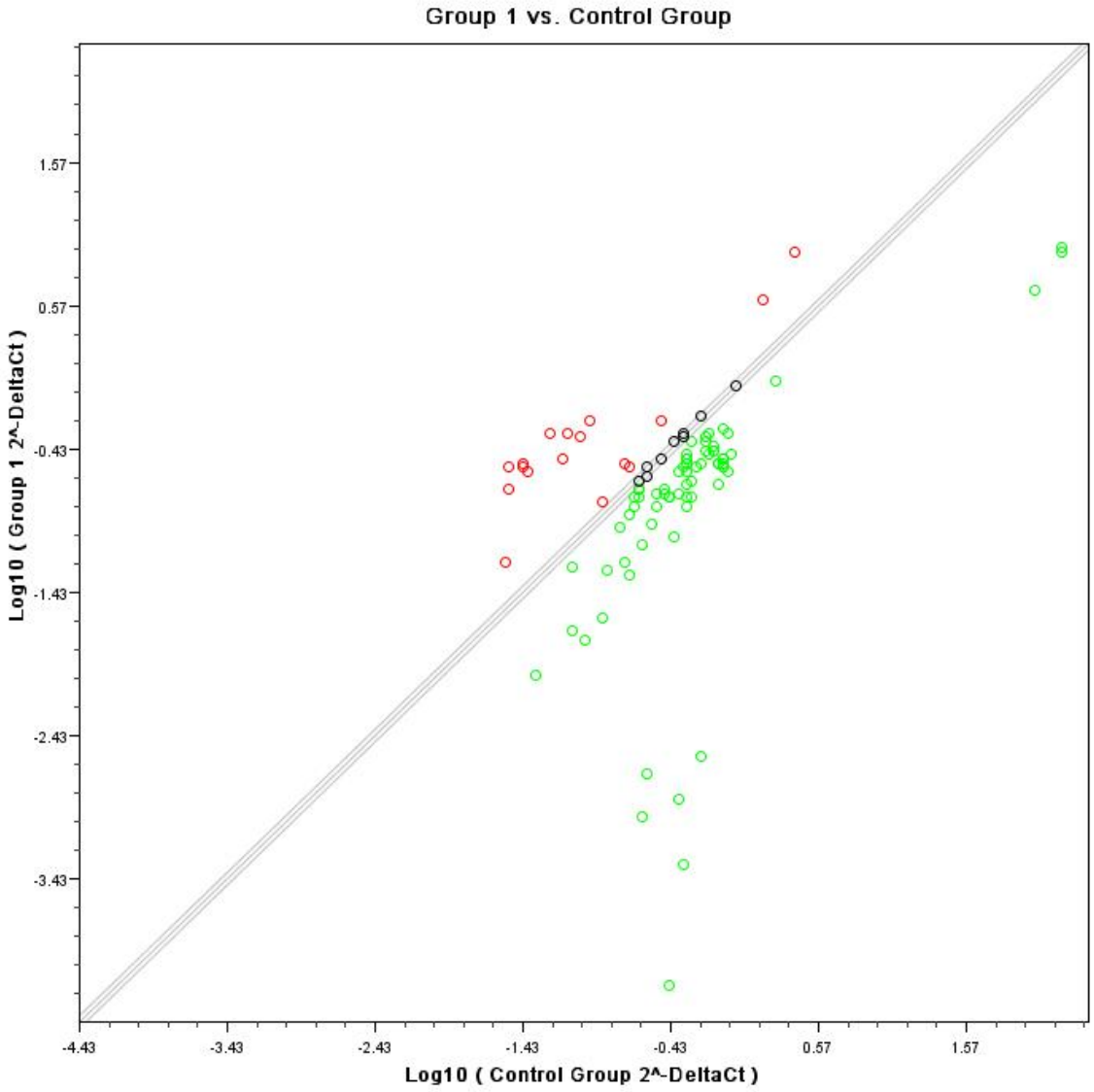

Figure 33: The up and down regulated genes in patient 1 after 48 h of Adv infection. The upper triangular represents the change of 15 (17.85%) upregulated genes (red color) with log10. The 61 (72.61%) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

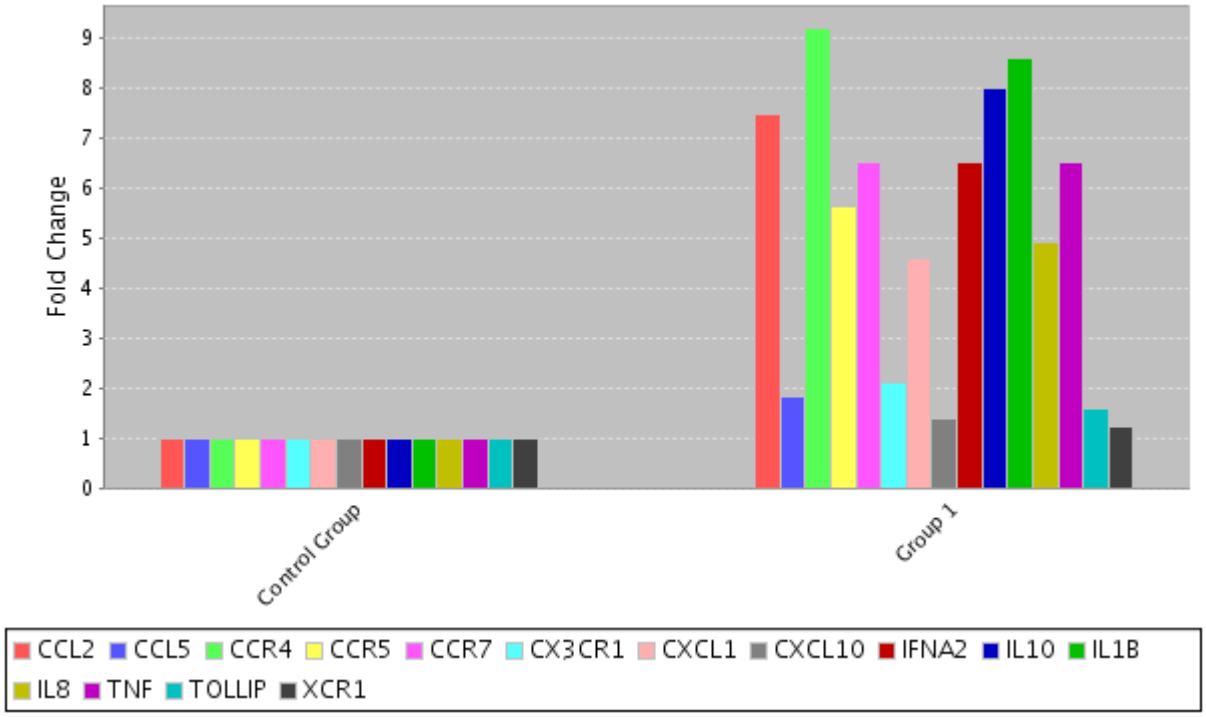

Figure 34: The up regulated 15 genes in patient 1 after 48 h of Adv infection. The most upregulated gene is CCR4 gene (9.18 fold change) and the lower upregulated gene is CCR1 gene (1.23 fold change).

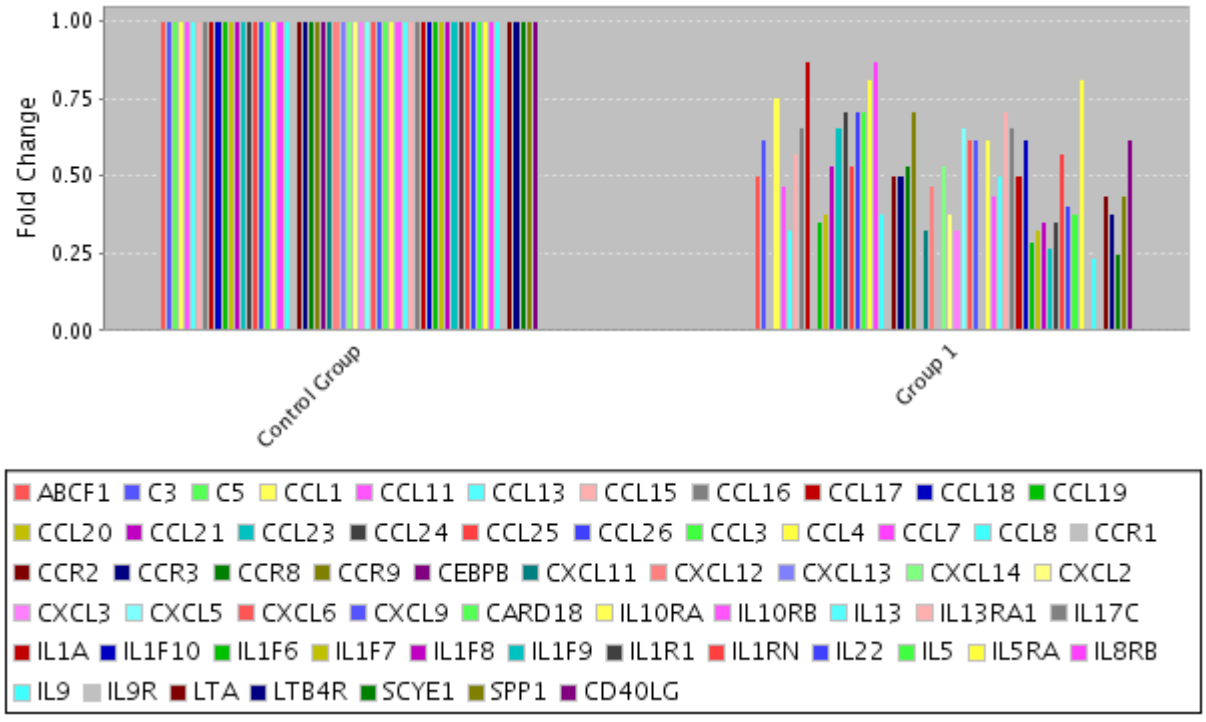

Figure 35: The down regulated 61 genes in patient 1 after 48 h of Adv infection. The upper downregulated gene is IL8RB gene (-5404 fold change) and the lower downregulated gene isCCR17 and CCL7 genes (-1.14 fold change).

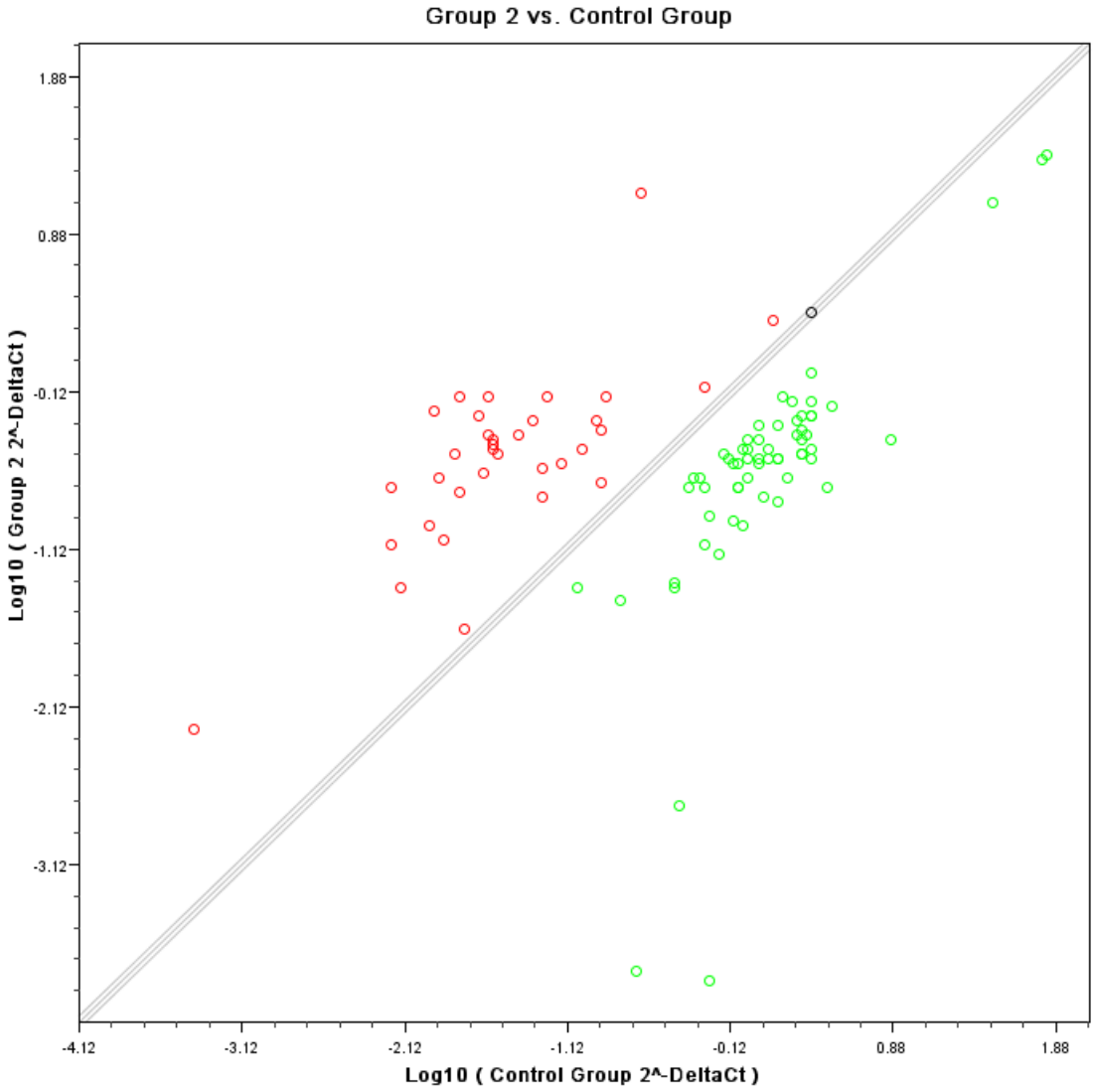

Figure 36: The up and down regulated genes in patient 2 after 48 h of Adv infection. The upper triangular represents the change of 32 (38.09%) upregulated genes (red color) with log10. The 52 (61.90%) downregulated genes (green color) in the lower triangular. The unchanged genes (black color) represented on the middle line.

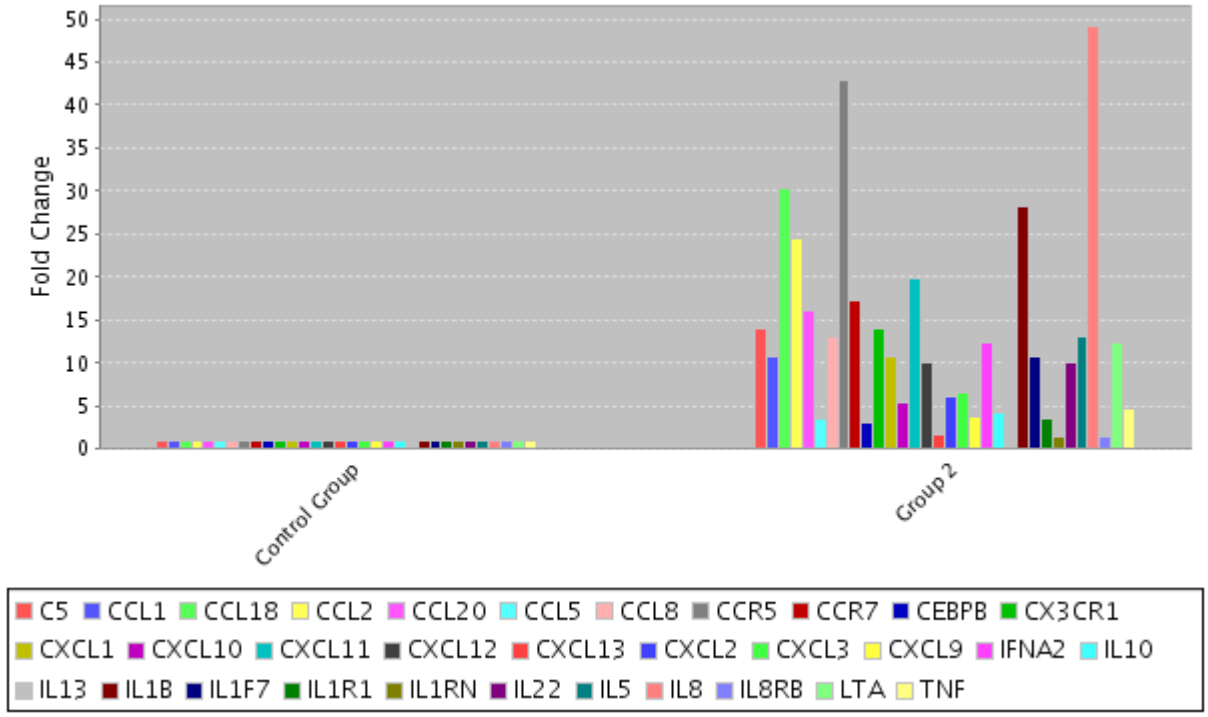

Figure 37: The up regulated 32 genes in patient 2 after 48 h of Adv infection. The most upregulated gene is IL8 gene (49.18 fold change) and the lower upregulated gene is IL8RB gene (1.53 fold change).

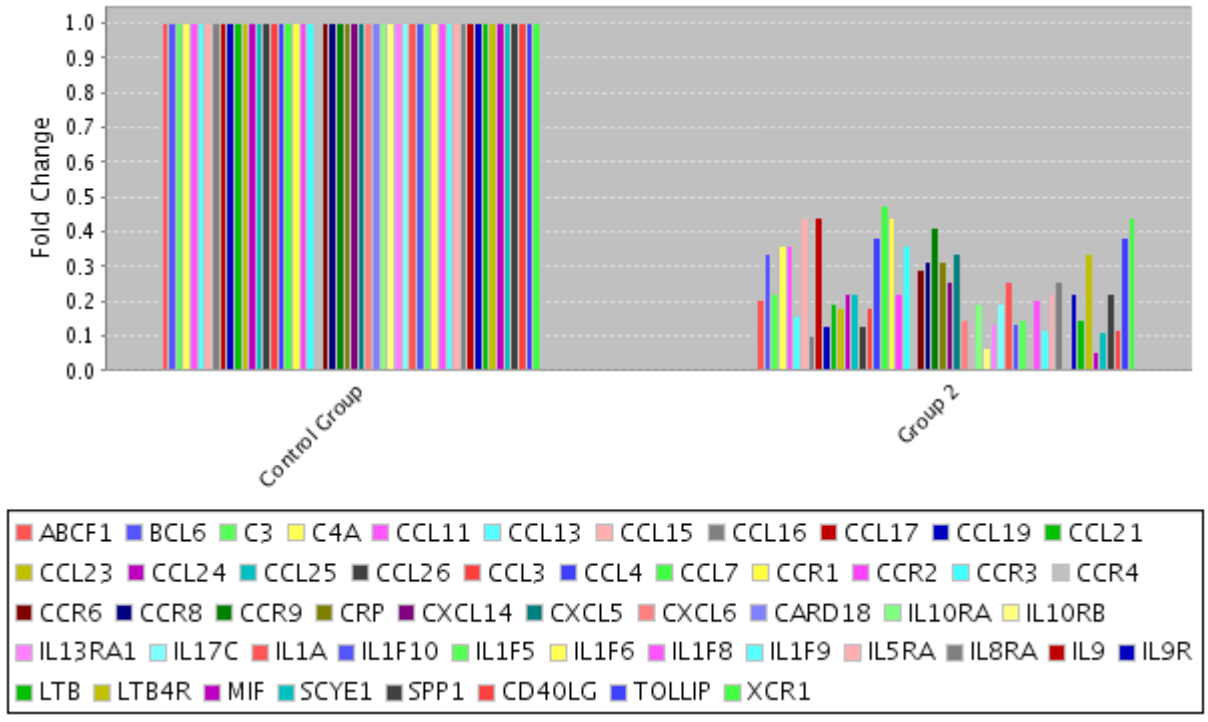

Figure 38: The down regulated 52 genes in patient 2 after 48 h of Adv infection. The upper downregulated gene is IL1F6 gene (-4039.60 fold change) and the lower downregulated gene is CCL7 gene (-2.11 fold change).

تاثير الاصابة بالفايروسات التنفسية :فايروس النزلة الوافدة وفايروس نظيرالنزلة الوافدة وفايروس الحمى الغدية على التعبير الجيني المناعي لكريات الدم البيضاء في الانسان

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الدكتوره رلى فائق الخزاعي

ملخص

تسبب فايروسات النزلة الوافدة وفايروس شبه النزلة الوافدة وفايروس الحمى الغدية اصابات تنفسية حادة وبمعدلات مرضية ونسبة وفيات عالية في كل انحاء العالم. تعتبر معرفة كيفية قيام هذه الفيروسات بموائمة الاستجابة الخلوية (الخلية المضيف) ذات اهمية كبيرة لدراسة العلاقة بين الفيروسات المرضية والمضيف عند المستوى الجزيئيي. الهدف من هذه الدراسة هو اولا لتحديد التغيرات الحاصلة بالتعبير الجيني لأربعة وثمانين جين التهابي داخل كريات الدم البيضاء والتي عزلت بشكل مركز من عينات دم المرضى. الهدف الثاني هو لتحديد العلاقة بين التعبير الجيني لهذه الجينات الالتهابية واحتمالية حصول حالة الربو(الازمة) المرضية بسبب الاصابة بهذه الفيروسات. تم فحص تسعين عينة دم لمرضى يعانون اعراض الانفلونزا بعد يومين من ظهور اعراض الاصابة وباعمارتتراوح بين 35 و40 سنة. وباستخدام فحص الاليزا المرتبط مع الاضداد المناعية النوعية لهذه الفايروسات الثلاثة تم تشخيص ثمانية مرضى مصابين بفايروس النزلة الوافدة ومريضين مصابين بفايروس شبه النزلة الوافدة اضافة لمريضين مصابين بفايروس الحمى الغدية. تم تحليل التعبير الجينى لخلايا كريات الدم البيضاء للمرضى المصابين باستخدام تقنية Real-time RT-PCR-Array.

اظهرت النتائج ان خلايا كريات الدم البيضاء للمرضى المصابين بفايروس النزلة الوافدة استجابت بزيادة نسخ جزيئات الحامض النووي الرايبوزي لأثنين وعشرين جين التهابي بمستوى معنوية عالية وبمعدل تضاعفي يتراوح بين 121 و 1.01 ضعف مقارنة مع عينات السيطرة في حين حصل انخفاض بمعدل جزيئات الحامض النووي الرايبوزي لواحد وثلاثين جين. الاصابة بفايروس شبه النزلة الوافدة لأثنين من المرضى سبب زيادة في التعبير الجيني لثمانية واربعين جين وانخفاض في التعبير الجيني لثلاثة جينات فقط. أما الاصابة بفايروس الحمى الغدية لأثنين من المرضى فقد سبب زيادة في التعبير الجيني لأحد عشر جين وانخفاض بالتعبير الجيني لأربعة واربعين جين التهابي. من النتائج نستنتج حصول تبدلات في التعبير الجيني للعديد من الجينات الالتهابية في كريات الدم البيضاء بعد 48 ساعة من الاصابة بالفايروسات الثلاثة.كما ان العلاقة بين الفيروس والخلية المصابة قد تكون بشكل استجابة خلوية كما في حالة الاصابة بفايروس النزلة الوافدة وفايروس شبه النزلة الوافدة او قد تكون العلاقة بشكل تاثير فايروسي كما في حالة الاصابة بفايروس الحمى الغدية.بالاضافة لذلك فان تسعة من الجينات التي حصل لها زياده بالتعبير الجيني نتيجة الاصابة بالفايروسات الثلاثة هي من الجينات المسببة لحالة الربو (الازمة) الفايروسية. ان المرضى الاثني عشر المصابين بهذه الفيروسات يحتمل انهم اصيبوا بانواع فايروسية مصلية متقاربة. هذه النتائج قد تساهم في فهم العلاقة بين الفايروسات المرضية والخلايا المضيفة عند المستوى الجزيئي.