1 **Differential expression of cathepsins in the blood of patients with viral co-infections.**

2
3 Shahan Mamoor¹
4 ¹shahanmamoor@gmail.com
5 East Islip, NY USA

6 We mined a published microarray dataset¹ to identify the most significant changes in the blood
7 of patients with viral co-infections. We found significant differential expression of multiple
8 cathepsin proteins in patients with viral co-infections, suggesting the process of peptide
9 cleavage for antigen processing may potentially be altered during viral co-infection.
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

25 Keywords: cathepsin, viral co-infection, systems biology of viral infection.
26
27
28

Co-infections are defined by the concurrent infection of a host or cell with more than one pathogen. The Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has resulted in the death of over 180,000 Americans in less than one year²; co-infection of patients with SARS-CoV-2 involving multiple pathogens has been described³, but our understanding of the transcriptional biology of viral co-infection is limited. We found significant differential expression of multiple cathepsin proteins in the blood of four patients with viral co-infections. Cathepsins have roles in proteolytic cleavage of proteins for antigen presentation during antigen-specific immune defense and during the apoptotic response⁴, and may be relevant to processes underlying viral co-infection.

Methods

We used dataset GSE68310¹ for this differential gene expression analysis of the blood in patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0 expression beadchip technology; analysis was performed with platform GPL10558. We compared the blood of $n=1$ patient with HCoV HKU1 infection (ID: JR0336) to the blood of $n=3$ patients with HCoV HKU1 and Human Rhinovirus co-infection (IDs: JR207, JR611, JR1702). We compared the blood of $n=1$ patient with HCoV NL63 infection (ID: JR1447) to the blood of $n=1$ patient with HCoV NL63 and Human Rhinovirus co-infection (ID: JR1446). We compared the blood of $n=1$ patient with Influenza A infection (ID: JR0080) to the blood of $n=1$ patient with Influenza A and HCoV OC43 co-infection (ID: JR1829). We compared the blood of $n=1$ patient with Enterovirus infection (ID: JR0471) to the blood of $n=1$ patient with Enterovirus and Rhinovirus infection (ID: JR0209). In the study from which this microarray data was obtained, investigators collected peripheral blood from patients at baseline, day 0 of infection, correlating with a visit to the clinic upon infection with a respiratory illness; day 2, day 4, day 6, and day 21

of infection following the clinic visit, as well as in the season following infection (“Spring”).

The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was used to evaluate the significance of difference in cathepsin gene expression between the blood of a patient with a single infection and the blood of a patient or patient(s) with co-infection using either a two-tailed, unpaired t-test with Welch’s correction, or a one-way ANOVA with Dunnett’s multiple comparisons test. All statistical tests were performed with PRISM 8.4.0 (455).

Results

We mined published microarray data¹ to discover genes associated with viral co-infection in humans.

Cathepsin B is differentially expressed in the blood of a patient with co-infection of HCoV NL63 and Human Rhinovirus.

We observed significant differential expression of the gene encoding cathepsin B, CTSB, in the blood of a patient with co-infection of HCoV NL63 and Human Rhinovirus. When sorting all of the genes expressed in the blood of a patient with HCoV NL63 and Rhinovirus co-infection based on significance of change in expression as compared to a patient with infection of HCoV NL63 only, CTSB ranked 150 out of 47254 total transcripts. Differential expression of CTSB in the blood in HCoV NL63 / Human Rhinovirus co-infection was statistically significant (Table 1; $p=1.52E-06$).

Cathepsin C is differentially expressed in the blood of three patients with co-infection of HCoV HKU1 and Human Rhinovirus.

After finding differential expression of CTSB in HCoV NL63 co-infection, we asked

whether dysregulated expression of cathepsin genes could be observed in other human coronavirus co-infections. We observed significant differential expression of the gene encoding cathepsin C, CTSC, in the blood of three patients with co-infection of HCoV HKU1 and Human Rhinovirus. When sorting all of the genes expressed in HCoV HKU1 and Rhinovirus co-infected patient blood based on significance of change in expression as compared to that of a patient with infection of HCoV NL63 only, CTSC ranked 233 out of 47254 total transcripts. Differential expression of CTSC in the blood in HCoV HKU1 / Human Rhinovirus co-infection was statistically significant (Table 2; $p=6.19E-04$).

Cathepsin S is differentially expressed in the blood of a patient with co-infection of Enterovirus and Human Rhinovirus.

After finding differential expression of cathepsin genes in two separate patients with human coronavirus infections, we asked whether we could observe changes in cathepsin expression in co-infections not involving a coronavirus. We observed significant differential expression of the gene encoding cathepsin S, CTSS, in the blood of a patient with co-infection of Enterovirus and Human Rhinovirus. When sorting all of the genes expressed in Enterovirus and Rhinovirus co-infected patient blood based on significance of change in expression as compared to that of a patient with infection of Enterovirus only, CTSS ranked 111 out of 47254 total transcripts. Differential expression of CTSS in the blood in Enterovirus / Rhinovirus co-infection was statistically significant (Table 3; $p=2.19E-07$).

Cathepsin H is differentially expressed in the blood of a patient with co-infection of Influenza A and HCoV OC43.

Analysis of a fourth case of viral co-infection revealed significant differential expression of the gene encoding cathepsin H, CTSH, in the blood of a patient with co-infection of the Influenza A virus and HCoV OC43. When sorting all of the genes expressed in Influenza A and HCoV OC43 co-infected patient blood based on significance of change in expression as

1 compared to that of a patient with infection of Influenza A infection only, CTSH ranked 10 out
2 of 47254 total transcripts. Differential expression of CTSH in the blood in Influenza A / HCoV
3 OC43 co-infection was statistically significant (Table 4; $p=3.85E-08$).

4
5 Cathepsin B is expressed at lower levels in the blood of a patient with HCoV NL63 and Human
6 Rhinovirus co-infection when compared to that of a patient with Rhinovirus infection only.

7 We obtained exact mRNA expression levels of cathepsin B in the blood from a patient
8 with co-infection of HCoV NL63 and Human Rhinovirus as well as from a patient with infection
9 of Rhinovirus only to determine exactly how cathepsin B levels changed in viral co-infection
10 settings. Cathepsin B was expressed at significantly lower levels in the blood of a patient with
11 HCoV NL63 and Rhinovirus co-infection when compared to that of a patient with Rhinovirus
12 infection only (Figure 1); this difference was statistically significant (Figure 1; $p<0.0001$).

13
14
15 Cathepsin C is expressed at higher levels in the blood of a patients with HCoV HKU1 and
16 Human Rhinovirus infection when compared to that of a patient with Rhinovirus infection only.

17 We obtained exact mRNA expression levels of cathepsin C in the blood of patients with
18 co-infection of HCoV HKU1 and Human Rhinovirus as well as from a patient with infection of
19 Rhinovirus only to determine exactly how cathepsin C levels changed in viral co-infection
20 settings (Figure 2). Cathepsin C was expressed at significantly higher levels in three patients
21 with HCoV HKU1 and Rhinovirus co-infection when compared to that of a patient with
22 Rhinovirus infection only; in two out of three patients, this difference was statistically significant
23 (Figure 2; $p=0.0001$, $p=0.1099$, and $p=0.0119$).

24
25 Cathepsin S is expressed at higher levels in the blood of a patient with co-infection of
26 Enterovirus and Human Rhinovirus when compared to that of a patient with Enterovirus
27 infection only.

28 We obtained exact mRNA expression levels of cathepsin S in the blood of a patient

1 with co-infection of Enterovirus and Human Rhinovirus as well as from a patient with infection
2 of Enterovirus only to determine exactly how cathepsin S levels changed in viral co-infection
3 settings (Figure 3). Cathepsin S was expressed at significantly higher levels in the blood of a
4 patient with Enterovirus and Rhinovirus co-infection when compared to that of a patient with
5 Enterovirus infection only; this difference was statistically significant (Figure 3; $p<0.0001$).
6

7 Cathepsin H is expressed at higher levels in the blood of a patient with co-infection of Influenza
8 A and HCoV OC43 when compared to the blood of a patient with Influenza infection only.

9
10 Finally, we also obtained exact mRNA expression levels of cathepsin H in the blood of a
11 patient with co-infection of Influenza A and HCoV OC43 as well as from a patient with infection
12 of Influenza A only to determine exactly how cathepsin H levels changed in viral co-infection
13 settings (Figure 4). Cathepsin H was expressed at significantly higher levels in the blood of a
14 patient with Influenza A and HCoV OC43 co-infection when compared to that of a patient with
15 Influenza A infection only; this difference was statistically significant (Figure 4; $p<0.0001$).
16

17 Thus, by mining published microarray data, we discovered significant differential
18 expression of multiple cathepsin genes in the blood of patients with viral co-infections,
19 including three human coronaviruses co-infections: this included cathepsin B, cathepsin C,
20 cathepsin H and cathepsin S.

21 **Discussion**

22
23 Cathepsins are a family of primarily cysteine proteases that reside within the lysosome
24 and include cathepsins B, C, F, H, K, L, O, S, V, X and W. Of the cathepsins we identified as
25 differentially expressed in patients with viral co-infection, cathepsins B and H possess both
26 exopeptidase and endopeptidase function, cathepsin C functions as an exopeptidase only and
27 cathepsin S functions as an endopeptidase only⁴.
28

1 Cathepsin B can prevent cell death in cytotoxic T-lymphocytes and natural killer (NK)
2 cells occurring upon degranulation⁵, and in mice, either cathepsin B or cathepsin L are required
3 for reovirus entry into fibroblasts⁶.

4 Cathepsin C is described as important for control of murine cytomegalovirus in some
5 organs, but cytolytic function of cytotoxic T-cells and NK cells from cathepsin C-deficient mice
6 was indistinguishable from that of wild-type mice⁷.

7 Cathepsin S can function in degradation of the invariant chain (Ii) in processing of class
8 II invariant chain major histocompatibility complexes, and in a mouse model of pulmonary
9 inflammation induced by nebulized immunodominant ovalbumin (OVA) following intraperitoneal
10 delivery of OVA conjugated to aluminum hydroxide, administration of a cathepsin S inhibitor
11 LHVS nearly eliminated eosinophil infiltration to the lungs⁸.

12 One report describes an important role for a cathepsin in SARS-CoV pathogenesis.
13 Cathepsin L is described as required for membrane fusion of SARS-CoV-1 using its spike (S)
14 glycoprotein. Accordingly, a small molecule inhibitor of cathepsin L, MDL28170 demonstrated
15 effectiveness in blocking infection of 293T cells with by HIV (SARS S) pseudovirion⁹.

16 We discovered significant differential expression of cathepsin B, cathepsin C, cathepsin
17 H and cathepsin S in the blood of patients with viral co-infections, three of which involved
18 patients with human coronavirus co-infections. Cathepsin function may be important to
19 cellular defense or to the viral life cycle during co-infections in the blood.
20
21
22
23
24
25
26
27
28

References

1. Zhai, Y., Franco, L.M., Atmar, R.L., Quarles, J.M., Arden, N., Bucasas, K.L., Wells, J.M., Nino, D., Wang, X., Zapata, G.E. and Shaw, C.A., 2015. Host transcriptional response to influenza and other acute respiratory viral infections—a prospective cohort study. *PLoS Pathog*, 11(6), p.e1004869.
2. World Health Organization. United States of America Situation. <https://covid19.who.int/region/amro/country/us>.
3. Kim, D., Quinn, J., Pinsky, B., Shah, N.H. and Brown, I., 2020. Rates of co-infection between SARS-CoV-2 and other respiratory pathogens. *Jama*.
4. Conus, S., 2010. Cathepsins and their involvement in immune responses. *Swiss medical weekly*, 140(2930).
5. Balaji, K.N., Schaschke, N., Machleidt, W., Catalfamo, M. and Henkart, P.A., 2002. Surface cathepsin B protects cytotoxic lymphocytes from self-destruction after degranulation. *The Journal of experimental medicine*, 196(4), pp.493-503.
6. Ebert, D.H., Deussing, J., Peters, C. and Dermody, T.S., 2002. Cathepsin L and cathepsin B mediate reovirus disassembly in murine fibroblast cells. *Journal of Biological Chemistry*, 277(27), pp.24609-24617.
7. Andoniou, C.E., Fleming, P., Sutton, V.R., Trapani, J.A. and Degli-Esposti, M.A., 2011. Cathepsin C limits acute viral infection independently of NK cell and CD8+ T-cell cytolytic function. *Immunology and cell biology*, 89(4), pp.540-548.
8. Riese, R.J., Mitchell, R.N., Villadangos, J.A., Shi, G.P., Palmer, J.T., Karp, E.R., De Sanctis, G.T., Ploegh, H.L. and Chapman, H.A., 1998. Cathepsin S activity regulates antigen presentation and immunity. *The Journal of clinical investigation*, 101(11), pp.2351-2363.
9. Simmons, G., Gosalia, D.N., Rennekamp, A.J., Reeves, J.D., Diamond, S.L. and Bates, P., 2005. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences*, 102(33), pp.11876-11881.

Rank	ID	p-value	t	B	FC	Gene	Gene name
150	ILMN_1696360	1.52E-06	-7.9967769	5.59547	0.9418 ± 0.0100	CTSB	cathepsin B

Table 1: Differential expression of Cathepsin B in the blood of a patient with HCoV NL63 and Rhinovirus co-infection.

The rank of differential expression relative to all transcripts measured, the probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change of CTSB expression in patient with co-infection when compared to single infection, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
233	ILMN_2242463	6.19E-04	3.9211827	-0.58503	P1: 1.0455 ± 0.0144 P2: 1.0196 ± 0.0096 P3: 1.0293 ± 0.0169	CTSC	cathepsin C

Table 2: Differential expression of Cathepsin C in the blood of three patients with co-infection of HCoV HKU1 and Human Rhinovirus.

The rank of differential expression relative to all transcripts measured, the probe ID, p -value with respect to global differential expression, t , a moderated t -statistic, B , the log-odds of differential expression between the two groups compared, fold change of CTSC expression in patient with co-infection when compared to single infection, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
111	ILMN_1743032	2.19E-07	9.4078679	7.464881	1.0660 ± 0.0139	CTSS	cathepsin S

Table 3: Differential expression of Cathepsin S in the blood of a patient with co-infection of Enterovirus and Human Rhinovirus.

The rank of differential expression relative to all transcripts measured, the probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change of CTSS expression in patient with co-infection when compared to single infection, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
10	ILMN_2390853	3.85E-08	11.0043788	8.90855	1.1023 ± 0.0198	CTSH	cathepsin H

Table 4: Differential expression of Cathepsin H in the blood of a patient with co-infection of Influenza A and HCoV OC43.

The rank of differential expression relative to all transcripts measured, the probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change of CTSS expression in patient with co-infection when compared to single infection, gene and gene name are listed in this chart.

Figure 1: Cathepsin B is expressed at significantly lower levels in the blood of a patient with HCoV NL63 and Rhinovirus co-infection when compared to that of a patient with HCoV NL63 infection only.

The mRNA expression level of CTSB in $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection is graphically depicted with mean CTSB level marked and the result of a statistical test evaluating significance of difference in CTSB expression between patients, a p-value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 2: Cathepsin C is expressed at significantly higher levels in the blood of three patients with HCoV HKU1 and Rhinovirus co-infection when compared to the blood of a patient with HCoV HKU1 infection only.

The mRNA expression level of CTSC in $n=1$ patient with HCoV HKU1 infection and $n=3$ patients with HCoV HKU1 and Rhinovirus co-infection is graphically depicted with mean CTSC level marked and the result of a statistical test evaluating significance of difference in CTSC expression between patients, a p-value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and Rhinovirus co-infection is lacking baseline sample.

Figure 3: Cathepsin S is expressed at significantly higher levels in the blood of a patient with co-infection of Enterovirus and Human Rhinovirus when compared to that of a patient with Enterovirus.

The mRNA expression level of CTSS in $n=1$ patient with Enterovirus infection and $n=1$ patient with Enterovirus and Rhinovirus co-infection is graphically depicted with mean CTSS level marked and the result of a statistical test evaluating significance of difference in CTSS expression between patients, a p-value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 4: Cathepsin H is expressed at significantly higher levels in the blood of a patient with co-infection of Influenza A and HCoV OC43 when compared to that of a patient with Influenza A infection only.

The mRNA expression level of CTSH in $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV OC43 co-infection is graphically depicted with mean CTSH level marked and the result of a statistical test evaluating significance of difference in CTSH expression between patients, a p-value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.