The peptidylglycine alpha-amidating monooxygenase, PAM, is differentially expressed in viral co-infections.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY 11730

Co-infection is defined by the infection of a host or cell by more than one pathogen^{1,2}. Infections from the novel coronavirus severe acute respiratory syndrome coronavirus 2, SARS-CoV-2³, have resulted in the death of more than 200,000 Americans⁴ and over 1,000,000 people worldwide⁵ in less than one year. An upcoming influenza season raises the prospect of potential complications arising from co-infections involving SARS-CoV-2⁶. We mined published microarray data⁷ to discover transcriptional features of viral co-infection in the blood of human patients. We identified the peptidylglycine alpha-amidating monooxygenase, PAM, as among the genes whose expression was most different in the blood of patients with a variety of viral co-infections, including co-infection of human coronavirus OC43 and influenza A. Transcriptional changes in PAM appeared to be specific to co-infection settings. PAM may be of relevance to fundamental transcriptional biology underlying viral co-infections of human blood.

Keywords: viral co-infection, PAM, SARS-CoV-2, coronavirus, influenza virus, systems biology of viral infection.

1 The most basic relationship between host and pathogen is a simple infection between one
2 pathogen and one host or cell. Re-infection, or a second infection of a host with the same
3 pathogen despite an initial immune response can occur⁸. Super-infection, a process by which a
4 bacterial infection complicates an existing viral infection, such as those found in the respiratory
5 tract, can occur^{9,10}. A third type of complex infection setting is known as co-infection, a process
6 by which a cell or organism already infected with a virus is then infected with a second, different
7 virus^{1,2}. The novel coronavirus SARS-CoV-2 has resulted in the death of over 200,000
8 Americans in less than one year^{3,4}. The upcoming influenza season raises the prospect of
9 complications resulting from influenza and SARS-CoV-2 co-infection. A recent study of patients
10 in Northern California found that of 116 patients testing positive for a SARS-CoV-2, 24 (20.7%)
11 possessed a co-infection⁶. Little is known about the transcriptional changes that accompany
12 coronavirus co-infection. We mined published microarray data⁷ to understand in an unbiased
13 fashion and at the systems-level the most significant gene expression changes in the blood of
14 patients with coronavirus co-infections, as well as in the blood of patients with other viral co-
15 infections not involving a human coronavirus. We observed significant, recurrent transcriptional
16 changes in PAM that were among the largest gene expression changes transcriptome-wide in the
17 blood of patients with viral co-infection.

25 **Methods**

26 We used dataset GSE68310⁷ for this differential gene expression analysis of the blood in
27 patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0
28

1 expression beadchip technology; analysis was performed using platform GPL10558. In the
2 study from which this microarray data was obtained, investigators collected peripheral blood
3 from patients at baseline, day 0 of infection, correlating with a visit to the clinic upon infection
4 with a respiratory illness; day 2, day 4, day 6, and day 21 of infection following the clinic visit,
5 as well as in the season following infection (“Spring”). We compared the blood of $n=1$ patient
6 with respiratory syncytial virus A (RSVA) to the blood of $n=1$ patient with co-infection of RSVA
7 and human rhinovirus. We compared the blood of $n=1$ patient with influenza A infection (ID:
8 JR0021) with the blood of $n=1$ patient with human coronavirus HCoV OC43 and influenza A co-
9 infection (ID: JR1829). We compared the blood of $n=4$ patients with influenza A infection only
10 at baseline, day 0, day 2, day 4, day 6, day 21, and Spring (IDs: JR0021, JR0023, JR0035 and
11 JR0053). We compared the blood of $n=4$ patients with influenza B infection (IDs: JR1412,
12 JR1491, JR1939 and JR1978) to the blood of $n=5$ patients with influenza B and Human
13 Rhinovirus co-infection (ID: JR1053, JR1120, JR1275, JR1672, and JR1717). We compared the
14 blood of $n=2$ patients with HCoV HKU1 and human rhinovirus co-infection (IDs: JR0207 and
15 JR1702) at baseline, day 0, day 2, day 4, day 6, and day 21. We compared the blood of $n=3$
16 patients with influenza B infection only (IDs: JR1412, JR1491, JR1939) at baseline, day 0, day
17 2, day 4, day 6, day 21 and Spring (comparison across time points). We compared the blood of
18 patients with $n=6$ patients with rhinovirus infection only (IDs: JR0148, JR0154, JR0223,
19 JR0228, JR0276, JR0287) at baseline, day 0, day 2, day 4, day 6, day 21 and Spring. The
20 Benjamini and Hochberg method of p -value adjustment was used for ranking of differential
21 expression but raw p -values were used for assessment of statistical significance of global
22
23
24
25
26
27
28

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 PAM gene expression between the blood of a patient with a single infection and the blood of a patient with co-infection, or between the blood of patients with co-infection over time, using a two-tailed, unpaired t-test with Welch's correction. All statistical tests were performed with PRISM 8.4.0 (455).

Results

We mined published microarray data⁷ to determine in an unbiased fashion and at the systems-level the most significant transcriptional changes in the blood of patients with viral co-infection.

PAM is differentially expressed and at decreased levels in the blood of a patient with co-infection of influenza A and human coronavirus OC43.

We identified the gene encoding the peptidylglycine alpha-amidating monooxygenase, PAM, as among the most differentially expressed in the blood of a patient with co-infection of influenza A and HCoV OC43 when compared to the blood of a patient with infection of influenza A only (Table 1). When sorting each of the genes expressed in the blood during influenza A and HCoV OC43 co-infection based on significance of change in expression as compared to influenza A infection only, PAM ranked 14 out of 47254 total transcripts (Table 1). Differential expression of PAM in influenza A and HCoV OC43 co-infection was statistically

1 significant (Table 1; $p=1.65E-09$). PAM was expressed at significantly lower levels in the blood
2 during co-infection of influenza A and HCoV OC43 as compared to that of a patient with
3 infection of influenza A only (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.8992
4 ± 0.0103 in PAM levels in the blood during co-infection of influenza A and HCoV OC43 (Table
5 1).
6
7

8
9 PAM is differentially expressed and at increased levels in the blood of a patient with co-infection
10 of respiratory syncytial virus A (RSVA) and human rhinovirus.

11 We asked whether differential expression of PAM could be observed in co-infections not
12 involving a human coronavirus. We again found that PAM was among the most differentially
13 expressed genes in the blood during co-infection, here in the blood of a patient with co-infection
14 of RSVA and human rhinovirus when compared to the blood of a patient with infection of
15 RSVA only (Table 2). When sorting each of the genes expressed in the blood during RSVA and
16 human rhinovirus co-infection based on significance of change in expression as compared to
17 RSVA infection only, PAM ranked 5 out of 47254 total transcripts (Table 2). Differential
18 expression of PAM in RSVA and human rhinovirus co-infection was statistically significant
19 (Table 2; $p=7.06E-09$). PAM was expressed at significantly higher levels in the blood during co-
20 infection of RSVA and human rhinovirus as compared to that of a patient with infection of
21 human rhinovirus only (Figure 2; $p<0.0001$). We calculated a mean fold change of $1.0624 \pm$
22 0.0125 in PAM levels in the blood during co-infection of RSVA and human rhinovirus (Table 2).
23
24
25
26
27
28

1 PAM is differentially expressed and at decreased levels in the blood of a patient with co-infection
2 of influenza B and human rhinovirus.

3 Further analysis of patient blood during viral co-infection revealed that PAM was among
4 the most differentially expressed genes in the blood of a patient with co-infection of influenza B
5 and human rhinovirus when compared to the blood of a patient with infection of influenza B only
6 (Table 3). When sorting each of the genes expressed in the blood during influenza B and human
7 rhinovirus co-infection based on significance of change in expression as compared to influenza B
8 infection only, PAM ranked 65 out of 47254 total transcripts (Table 3). Differential expression of
9 PAM in influenza B and human rhinovirus co-infection was statistically significant
10 (Table 3; $p=1.1E-06$). PAM was expressed at significantly lower levels in the blood during co-
11 infection of influenza B and human rhinovirus as compared to that of a patient with infection of
12 human rhinovirus only (Figure 3; $p<0.0001$). We calculated a mean fold change of $0.9601 \pm$
13 0.0115 in PAM levels in the blood during co-infection of influenza B and human rhinovirus
14 (Table 3).

15 After finding that PAM was among the most differentially expressed genes in the blood
16 during three separate types of viral co-infections, we asked whether differential expression of
17 PAM was specific to viral co-infection or could be observed in patient blood during the course of
18 infection with a single virus.

19 PAM is not differentially expressed in patient blood during infection with influenza A.

20 We probed microarray data from the blood of four patients with influenza A infection

only, comparing total gene expression at baseline, day 0, day 2, day 4, day 6, day 21 and the following Spring, to determine if expression of PAM was significantly modulated during the course of influenza A infection in the absence of co-infection. PAM was not differentially expressed in human blood during influenza A infection (Table 4; $p=9.92E-01$). When sorting each of the genes expressed in the blood during the course of influenza A infection based on significance of change in expression as compared to baseline, PAM ranked 46991 out of 47254 total transcripts (Table 4).

PAM is not differentially expressed in patient blood during infection with influenza B.

We also microarray data from the blood of three patients with influenza B infection only, comparing total gene expression at baseline, day 0, day 2, day 4, day 6, day 21 and the following Spring, to determine if expression of PAM was significantly modulated during the course of influenza B infection in the absence of co-infection. PAM was not differentially expressed in human blood during influenza B infection (Table 5; $p=2.36E-01$). When sorting each of the genes expressed in the blood during the course of influenza B infection based on significance of change in expression as compared to baseline, PAM ranked 18537 out of 47254 total transcripts (Table 5).

PAM is not differentially expressed in patient blood during infection with human rhinovirus.

We also probed microarray data from the blood of six patients with infection of human rhinovirus only, comparing total gene expression at baseline, day 0, day 2, day 4, day 6, day 21

1 and the following Spring, to determine if expression of PAM was significantly modulated during
2 the course of human rhinovirus infection in the absence of co-infection. PAM was not
3 differentially expressed in human blood during human rhinovirus infection (Table 6;
4 $p=9.96E-01$). When sorting each of the genes expressed in the blood during the course of human
5 rhinovirus infection based on significance of change in expression as compared to baseline, PAM
6 ranked 47051 out of 47254 total transcripts (Table 6).
7
8

9
10 Together, the data indicated that PAM was differentially expressed in the blood of
11 patients with three different types of viral co-infections, including co-infections involving
12 influenza A, influenza B, and human rhinovirus, but not during the course of influenza A,
13 influenza B, or human rhinovirus infections in the absence of co-infection. Though these data
14 argued that differential expression of PAM was specific to viral co-infections in the blood, and
15 although PAM was among the genes most differentially expressed in the blood of patients with
16 viral infection when compared to that of patients with single viral infections, it is difficult to
17 draw definite conclusions from comparison of patient blood due to inherent variability in gene
18 expression from patient to patient. Thus, we asked one further question: does expression of PAM
19 significantly differ, across time, in the blood of patients during the course of viral co-infection?
20
21

22 PAM is significantly differentially expressed in the blood of patients with co-infection of human
23 coronavirus HKU1 and human rhinovirus.
24

25
26 To answer this question, we queried microarray data from the blood of two patients with
27 co-infection of HCoV HKU1 and human rhinovirus, comparing total gene expression at baseline,
28

1 day 0, day 2, day 4, day 6, day 21 and the following Spring, to determine if PAM was
2 differentially expressed in the blood of patients with a viral co-infection across time, and not
3 based on comparison between a patient with co-infection versus a patient with infection with a
4 single virus. We found significant differential expression of PAM in the blood during co-
5 infection with HCoV HKU1 and human rhinovirus; when sorting each of the genes expression in
6 the blood during HCoV HKU1 and human rhinovirus co-infection based on significance of
7 change in expression across infection time points as compared to baseline, PAM ranked 364 out
8 of 47254 total transcripts (Table 7), equating to 99.2% differential expression. Differential
9 expression of PAM in the blood during the course of viral co-infection with HCoV HKU1 and
10 human rhinovirus was statistically significant (Table 7; $p=0.0124033$).

15 Thus, together, we conclude that the gene encoding the peptidylglycine alpha-amidating
16 monooxygenase, PAM, is among the genes whose expression changes most significantly
17 transcriptome-wide in the blood of patients with viral co-infections, including co-infections with
18 human coronavirus OC43 and influenza A, RSVA and human rhinovirus, and influenza B and
19 human rhinovirus. Differential expression of PAM is specific to viral co-infection settings, as it
20 does not occur, across the course of infection in the blood of patients with single viral infection
21 of influenza A, influenza B, or human rhinovirus, but does occur across the course of infection in
22 the blood of patients with co-infection of human coronavirus HKU1 and human rhinovirus.
23
24
25
26
27
28

Discussion

Peptidylglycine α -amidating monooxygenase, PAM, was identified from the porcine pituitary gland, as an enzyme with the ability to convert peptides ending in glycine to des-glycine peptide amide in a two-step reaction known as amidation^{11,12}. PAM is a type I membrane protein and catalytically bifunctional, performing both steps of the amidation reaction with unique catalytic domains: first, α -hydroxylation using its hydroxylation domain, referred to as PHM, and subsequent α -amidation using its lyase domain, referred to as PAL^{12,13}. This reaction requires copper, ascorbate, and molecular oxygen¹². It has been reported that over half of all peptides require carboxy-terminal α -amidation for activation of biological function: these peptides include neurokinin, calcitonin, gastrin I, amylin, GHRF, oxytocin, substance P, and vasopressin¹². There are two splice variants of PAM produced; PAM-1 and PAM-2. PAM-1 is cleaved, generating two fragments: a membrane-bound fragment containing the PAL, and a soluble fragment containing the PHM; the membrane-bound PAL-containing fragment is then subject to a second round of proteolysis which produces a second soluble PAM fragment known as sf-CD as well as two intermediate fragments, a 19 kilodalton (kDa) fragment and a 22 kDa fragment¹³. Residing between the PHM and PAL regions of the non-cleaved PAM transcript is a linker region; this linker region contains a histidine cluster that has been described to function as a pH sensor in the secretory pathway¹³. However, it is difficult to draw conclusions regarding functionality as a pH sensor and phenotypic consequences of mutating this histidine cluster (as found in the PAMH3A mutant), as the PAMH3A mutant when expressed in AtT-20 cells fails to produce the second

1 soluble PAM fragment, sf-CD, as is evidenced when wild-type PAM is expressed in AtT-20 cells
2 treated with the MG132 proteasome inhibitor¹³. Thus, although the PAM H3A mutant is
3 defective in responsiveness to changes in pH due to loss of imidazole side chains contained in
4 histidine, it also appears to be defective in the production of sf-CD¹³.

5
6 PAM is expressed in the central nervous system of adult mice, and in the pancreas, the
7 heart, adrenal glands, and lungs¹⁴. In mice, PAM deficiency is embryonic lethal between
8 embryonic days 14.5 and 15.5 (E14.5-E15.5)¹⁵. Lethality is thought to be a result of
9 cardiovascular defects and is associated, in the embryo, with severe edema with fluid
10 accumulation in aorta, lungs and liver observable, thinning of the arterial walls, as well as
11 changes in thickness of the epithelial cell of the developing bronchi¹⁵. No PHM or PAL activity
12 can be detected in PAM-null mutants, suggesting that PAM is the sole source of amidation during
13 murine development¹⁵. PAM heterozygous mice suffer from mild glucose intolerance¹⁵.

14
15
16 Nevertheless, PAM functions in carboxy-terminal α -amidation, which is important for
17 biologic activity of amidated peptides. We found significant changes in expression of PAM in
18 the blood of patients with viral co-infections, and these changes were among the most significant
19 gene expression changes transcriptome-wide when compared to the blood of patients with
20 infection with a single virus. PAM may be relevant to biological processes underlying viral co-
21 infection, including viral co-infections with influenza viruses and human coronaviruses.
22
23
24
25
26
27
28

References

1. Alter, M.J., 2006. Epidemiology of viral hepatitis and HIV co-infection. *Journal of hepatology*, 44, pp.S6-S9.
2. Heng, M.C., Heng, S.Y. and Allen, S.G., 1994. Co-infection and synergy of human immunodeficiency virus-1 and herpes simplex virus-1. *The Lancet*, 343(8892), pp.255-258.
3. Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. and Garry, R.F., 2020. The proximal origin of SARS-CoV-2. *Nature medicine*, 26(4), pp.450-452.
4. World Health Organization. United States of America Situation. <https://covid19.who.int/region/amro/country/us>.
5. World Health Organization. Coronavirusdisease (COVID-19) pandemic. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>.
6. 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*.
7. 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.
8. Lambers, F.A., Prins, M., Thomas, X., Molenkamp, R., Kwa, D., Brinkman, K., van der Meer, J.T., Schinkel, J. and MOSAIC (MSM Observational Study of Acute Infection with Hepatitis C) Study Group, 2011. Alarming incidence of hepatitis C virus re-infection after treatment of sexually acquired acute hepatitis C virus infection in HIV-infected MSM. *Aids*, 25(17), pp.F21-F27.
9. Hament, J.M., Kimpen, J.L., Fleer, A. and Wolfs, T.F., 1999. Respiratory viral infection predisposing for bacterial disease: a concise review. *FEMS Immunology & Medical Microbiology*, 26(3-4), pp.189-195.
10. Navarini, A.A., Recher, M., Lang, K.S., Georgiev, P., Meury, S., Bergthaler, A., Flatz, L., Bille, J., Landmann, R., Odermatt, B. and Hengartner, H., 2006. Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. *Proceedings of the National Academy of Sciences*, 103(42), pp.15535-15539.

- 1 11. Bradbury, A.F., Finnie, M.D.A. and Smyth, D.G., 1982. Mechanism of C-terminal amide
2 formation by pituitary enzymes. *Nature*, 298(5875), pp.686-688.
- 3 12. Kim, K.H. and Seong, B.L., 2001. Peptide amidation: Production of peptide hormones in vivo
4 and in vitro. *Biotechnology and Bioprocess Engineering*, 6(4), pp.244-251.
- 5 13. Vishwanatha, K., Bäck, N., Mains, R.E. and Eipper, B.A., 2014. A histidine-rich linker region
6 in peptidylglycine α -amidating monooxygenase has the properties of a pH sensor. *Journal of*
7 *Biological Chemistry*, 289(18), pp.12404-12420.
- 8 14. Braas KM, Harakall SA, Ouafik L, Eipper BA, May VI. Expression of peptidylglycine alpha-
9 amidating monooxygenase: an in situ hybridization and immunocytochemical study.
10 *Endocrinology*. 1992 May 1;130(5):2778-88.
- 11 15. Czyzyk, T.A., Ning, Y., Hsu, M.S., Peng, B., Mains, R.E., Eipper, B.A. and Pintar, J.E., 2005.
12 Deletion of peptide amidation enzymatic activity leads to edema and embryonic lethality in the
13 mouse. *Developmental biology*, 287(2), pp.301-313.
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25
- 26
- 27
- 28

Rank	ID	p-value	t	B	FC	Gene	Gene name
14	ILMN_2313901	1.65E-09	-1.4E+01	11.87322	0.8992 ± 0.0103	PAM	peptidylglycine alpha-amidating monooxygenase

Table 1: Differential expression of PAM in the blood of a patient with co-infection of RSVA and human rhinovirus as compared to that of a patient with RSVA infection.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the patients compared, fold change of PAM expression in the blood of a patient with RSVA and human rhinovirus co-infection relative to that of a patient with infection of RSVA only, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
5	ILMN_2313901	7.06E-09	12.7086516	10.422	1.0624 ± 0.0125	PAM	peptidylglycine alpha-amidating monooxygenase

Table 2: Differential expression of PAM in the blood of a patient with co-infection of influenza A and human coronavirus OC43 as compared to that of a patient with influenza A infection.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the patients compared, fold change of PAM expression in the blood of a patient with influenza A and HCoV OC43 co-infection relative to that of a patient with infection of influenza A only, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
65	ILMN_2313901	1.1E-06	-5.4283747	4.89056	0.9601 ± 0.0115	PAM	peptidylglycine alpha-amidating monooxygenase

Table 3: Differential expression of PAM in the blood of a patient with co-infection of influenza B and human rhinovirus as compared to that of a patient with influenza B infection.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the patients compared, fold change of PAM expression in the blood of a patient with influenza B and human rhinovirus co-infection relative to that of a patient with infection of influenza B only, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
46991	ILMN_2313901	9.92E-01	0.1248	PAM	peptidylglycine alpha-amidating monooxygenase

Table 4: PAM is not differentially expressed during the course of infection in the blood of patients with infection of influenza A.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
18537	ILMN_2313901	2.36E-01	1.5199	PAM	peptidylglycine alpha-amidating monooxygenase

Table 5: PAM is not differentially expressed during the course of infection in the blood of patients with infection of influenza B.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
47051	ILMN_2313901	9.96E-01	0.10047	PAM	peptidylglycine alpha-amidating monooxygenase

Table 6: PAM is not differentially expressed during the course of infection in the blood of patients with infection of human rhinovirus.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
364	ILMN_2313901	0.0124033	6.36148	PAM	peptidylglycine alpha-amidating monooxygenase

Table 7: PAM is differentially expressed during the course of infection in the blood of patients with co-infection of human coronavirus HKU1 and human rhinovirus.

The rank of differential expression relative to all transcripts measured, probe ID, *p*-value with respect to global differential expression, F statistic, gene and gene name are listed in this chart.

Figure 1: PAM is expressed at significantly lower levels in the blood of a patient with RSVA and human rhinovirus infection as compared to that of a patient with RSVA infection only.

Messenger RNA levels of PAM are graphically depicted in the blood of a patient with RSVA infection (left) and in the blood of a patient with co-infection of RSVA and human rhinovirus (right), with the result of a statistical test evaluating significance of difference in PAM expression, a *p*-value, listed above.

Figure 2: PAM is expressed at significantly higher levels in the blood of a patient with influenza A and HCoV OC43 infection as compared to that of a patient with influenza A infection only.

Messenger RNA levels of PAM are graphically depicted in the blood of a patient with influenza A infection (left) and in the blood of a patient with co-infection of influenza A and HCoV OC43 infection (right), with the result of a statistical test evaluating significance of difference in PAM expression, a *p*-value, listed above.

Figure 3: PAM is expressed at significantly lower levels in the blood of a patient with influenza B and human rhinovirus infection as compared to that of a patient with influenza B infection only.

Messenger RNA levels of PAM are graphically depicted in the blood of a patient with influenza B infection (left) and in the blood of a patient with co-infection of influenza B and human rhinovirus (right), with the result of a statistical test evaluating significance of difference in PAM expression, a *p*-value, listed above.