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Posted Date: 5 September 2024

doi: 10.20944/preprints202409.0477.v1

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Article

Shedding Light on tRNA-Derived Fragment Repertoire in FACS Blood Cells

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Abstract: tRNA-derived fragments function as markers additionally to playing the role key of signalling molecules in a number of disorders. It is known that the repertoire of these molecules differs greatly in different cell types and varies depending on the physiological condition. The aim of our research was to compare the pattern of tRF expression in the main blood cell types and to determine how the composition of these molecules changes during COVID-19-induced cytokine storm. Erythrocytes, monocytes, lymphocytes, neutrophils, basophils, and eosinophils from control donors and patients with severe COVID-19 were obtained by fluorescence sorting. We extracted RNA from FACS sorted cells and performed NGS of short RNAs. The composition of tRNA-derived fragments was analysed applying a semi-custom bioinformatic pipeline. In this study, we assessed the length and type distribution of TRFs and reported the 150 most prevalent TRF sequences across all cell types. Additionally we demonstrate a significant ($p < 0.05$, fold change > 16) change in the pattern of tRFs in erythrocytes (21 downregulated, 12 upregulated), monocytes (53 downregulated, 38 upregulated) and lymphocytes (49 upregulated) in patients with severe COVID-19. Thus, different blood cell types exhibit a significant variety of TRFs and react to cytokine storm by dramatically changing their differential expression patterns. We suppose that the observed phenomenon occurs due to the regulation of nucleotide modifications and alterations in activity of various Rnases.

Keywords: tRNA fragment; blood cells; severe COVID-19; sRNA NGS; RBC; lymphocytes; monocytes

1. Introduction

tRNA-derived fragments (tRFs) are a relatively recently discovered class of small RNA molecules that are shown to be involved in the pathogenesis of various diseases, particularly cancer and viral infections. Processed from transfer RNAs, tRFs regulate gene expression through various mechanisms like miRNA-like silencing of target mRNAs [1–4].

There are several types of classified tRFs based on the cleavage site within the tRNA molecule: tRF-1: Cleaved from the 3' trailer sequence of pre-tRNAs. tRF-2: Cleaved from the D-loop of mature tRNAs. tRF-3: Cleaved from the 3' end of mature tRNAs. tRF-5: Cleaved from the 5' end of mature tRNAs. i-tRF: Internal tRFs derived from the internal region of mature tRNAs [5].

Different tissues and organs possess a different set of tRNA fragments [2]. Nevertheless, it has not been studied how the pattern of these molecules differs in different blood cells. Supposably, the repertoire of these molecules changes in correspondence with changes in the immunological state of patients. In particular, it is not clear how these molecules will change in blood cells during the cytokine storm induced by COVID-19.

It has been previously reported by Wu et. al. that tRFs were the most significantly affected small non-coding RNAs in nasopharyngeal swabs of COVID-19 patients, they had also observed that SARS-

CoV-2-infected airway epithelial cells exhibit the same tendency [4]. In an attempt to additionally shed light on tRNA-derived fragments and their involvement in host-virus interactions it seems relevant to study tRFs in different pathophysiological circumstances. In the case of this study our aim was to reveal the differential expression of tRFs in fluorescence activated sorted cells of healthy control donors and SARS-CoV-2 infected patients, as well as to observe and characterise tRFs during viral infections.

2. Materials and Methods

2.1. Patients and Data Collection

Six healthy donors and five RT-PCR confirmed SARS-CoV-2 positive patients made up the two groups of research participants. Three patients were hospitalised to the critical care unit and two patients were moved to the infectious disease unit. Severe patients satisfied the following criteria for admission to the intensive care unit (ICU): body temperature ≥ 39 °C, respiration rate ≥ 30 /min, and oxygen saturation (SpO₂) $\leq 93\%$.

2.2. Cell Sorting

Cell sorting was performed on a MoFlow Astrios EQ device (Beckman Coulter). Erythrocytes were isolated using antibodies against CD235 protein (Beckman Coulter, IM2212U). To isolate leukocytes, erythrocytes were first lysed with VersaLyse lysing solution (Beckman Coulter). Granulocytes (neutrophils, basophils and eosinophils) were isolated using duraclone IM granulocyte antibody panel (B88651, Beckman Coulter). Lymphocytes and monocytes were isolated using antibodies CD45 (A79392 Beckman Coulter), CD16 (6607118 Beckman Coulter), CD14 (A70204 Beckman Coulter, USA). Cell sample purity for all cell populations was $>95\%$ according to flow cytometry data. A detailed description of cell sorting protocols is provided in the following article [6].

2.3. RNA Separation, Library Preparation and Next Generation Sequencing

RNA was isolated \extracted using ExtractRNA reagent (Evrogen) according to the provided protocol. After extraction, RNA was dissolved in 10 μ l RNase free water. The quality of the obtained RNA was checked on a TapeStation (Agilent). Only samples with RIN >5 were taken for sequencing. Short RNA libraries were prepared using the Small RNA Library Prep Kit (BGI, 1000006383). Sequencing was performed on a DNBSEQ-G400 instrument (BGI)

2.4. Bioinformatics

A general design of the bioinformatic processing pipeline is presented in Figure 1.

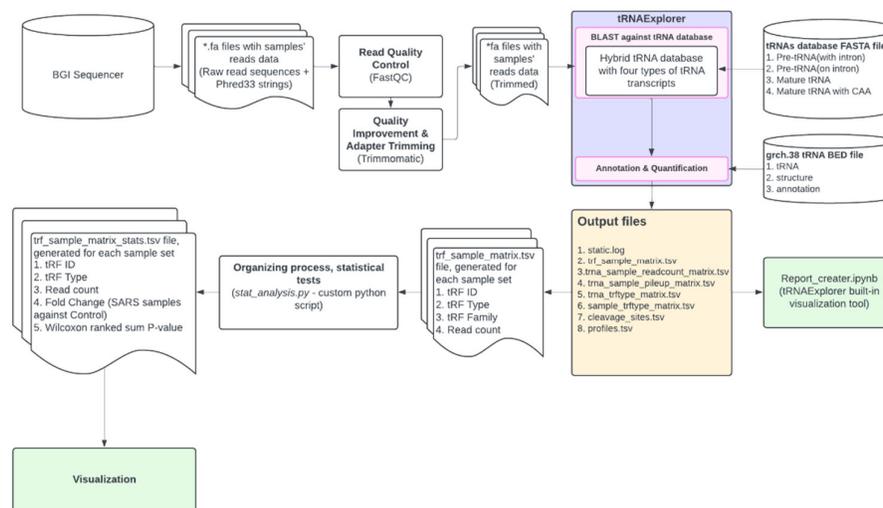


Figure 1. NGS data analysis pipeline.

Read Quality Control

For quality control we applied FastQC [7]. All datasets qualified quality control standards. Each FastQ file contained between 17 and 27 million reads.

Adapter Trimming

Adapter trimming was executed using Trimmomatic [8]. Long clipping sequence was set as AGTCGGAGGCCAAGCGGTCTTAGGAAGACAA. We opted to use optimal run settings for SE (single-end) reads (TruSeq3-SE:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:20) which allowed us to minimise execution time with no data loss. As an input, trimmomatic takes the adapter.fa file, in which our adapter sequence is registered. For each FastQ file from 50 to 68% of the input reads were marked as dropped after running Trimmomatic.

Read Mapping, Count and Normalisation

Full genome alignment and transcriptome-based pseudoalignment methods are not applicable for small-RNA-seq identification due to tRNA cleavage events, which led us to utilising "BLAST"(Blast Local Alignment Search Tool) [9].

We implemented BLAST through "tRNAExplorer" [10], a Python-based pipeline, optimised for tRF-profile analysis. In order to utilise tRNAExplorer, we needed a *.bed genome annotation file as a reference, which we would BLAST our reads *.fa files against. While there is an option to generate a custom annotation file, we used an already compiled grch.38 database which came within the tRNAExplorer package. To run the pipeline, we created project directories with sample lists individually for each type of cells and custom configuration file, with data directories paths and launch options. While tRNAExplorer supports trimming and QC, we omitted these steps due to previous data processing. As an input each run of the pipeline takes all the *.fa files with read data from the specified directory, list of samples, and path to database *.bed file. We automated these runs with another custom Python script. As a final result of data processing we obtained *.csv and *.tsv text files, containing BLAST run results for every sample, cleavage sites data and a collective read count table for all provided samples with annotations.

This data was already sufficient for differential expression analysis and visualisation, but we applied another extra step, running a custom Python script which organises data and performs statistical tests.

2.5. Statistics

Wilcoxon–Mann–Whitney test was used to perform statistical analysis on the data. P-values less than 0.05 were considered statistically significant. The p-values for the RNAseq volcano graphics were log converted to log₁₀ (1.31) for data visualisation.

3. Results

3.1. tRF Length Distribution in Main Cell Types

Analysing tRF length distribution in erythrocytes, monocytes, lymphocytes, neutrophils, basophils and eosinophils we observed a remarkable similarity between control and severe COVID-19 patient's cells (Figure 2). This data is additionally supported by our heatmap, which demonstrated a distinguishable pattern of tRF expression depending on cell type. Erythrocytes are presented as the most distinguishable group of cells in terms of tRF length and tRF expression patterns, which may be due to the fact that the erythrocyte is an inactive cell in terms of synthetic processes.

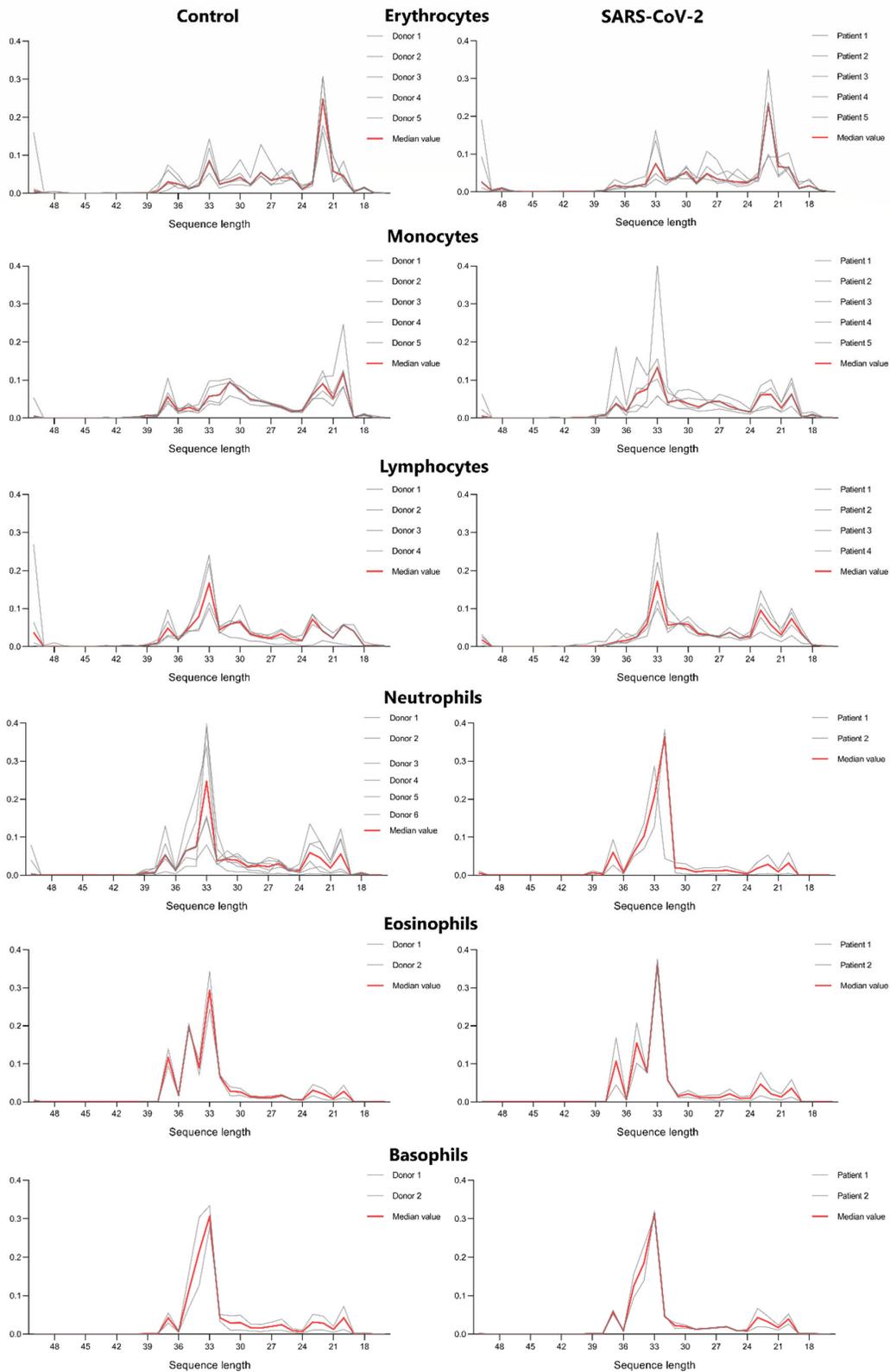


Figure 2. tRF length distribution in main cell types (red line represents median value).

3.2. tRF Type Composition in Main Cell Types

Analysing the main tRF types in sorted lymphocytes of control donors and severe COVID-19 patients we observed the prevalence of tRF-5 in 7 out of 8 samples. 1 severe COVID-19 patient exhibited dominating above all presence of i-tRF and tRF-3 types (Figure 3). It is worth noting that this patient had extremely high levels of IL-6 - 2 398 (pg/ml) on the same day that this sample was taken.

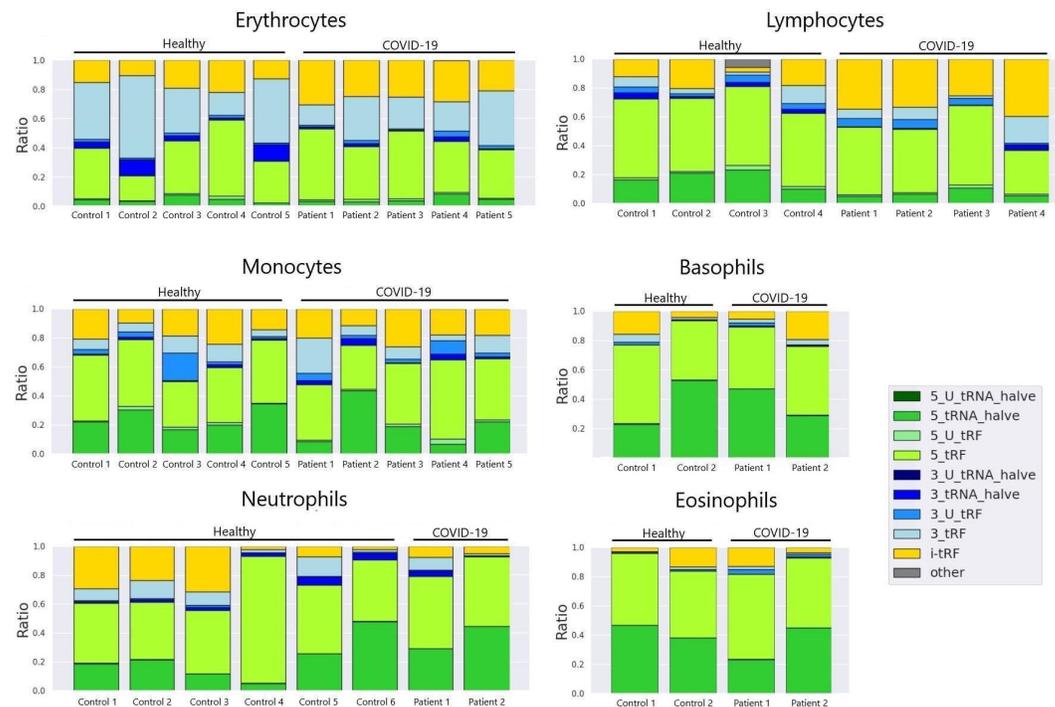


Figure 3. tRF type composition in main cell types (colour matches tRF types).

3.3. tRF Expression in Control Donors and Severe COVID-19 Patients

To compare tRF expression in different cell types we calculated the 150 most abundant tRFs based on RAW read counts, log normalised for data presentation. The heatmap demonstrates a relatively vivid frontier for erythrocytes (Figure 4).

downregulation, however, there is no data on the involvement of this fragment in any known biological processes.

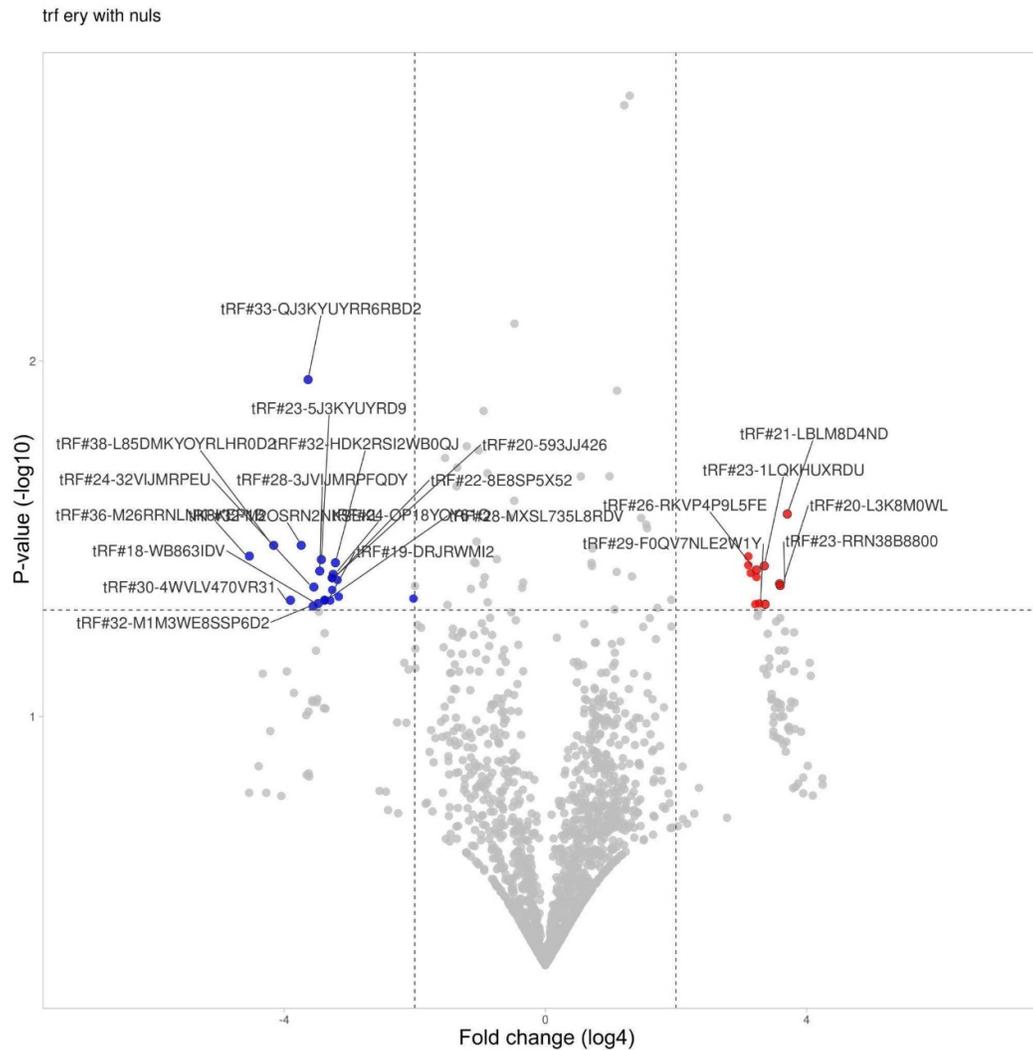


Figure 5. tRF differential expression in erythrocytes (For all volcano plots we applied \log_4 (instead of \log_2) for fold change presentation.).

Table 1. List of differentially expressed tRNA-derived fragments in erythrocytes during severe COVID-19.

Table 21.	Change	Fold change	P-value
tRF#21-LBLM8D4ND	Increased	170	0,027
tRF#23-RRN38B8800	Increased	146	0,043
tRF#20-L3K8M0WL	Increased	144	0,042
tRF#29-F0QV7NLE2W1Y	Increased	106	0,048
tRF#23-1LQKHUXRDU	Increased	104	0,038
tRF#21-SRRN2EK8B	Increased	94	0,048
tRF#26-RKVP4P9L5FE	Increased	88	0,039
tRF#17-M3WE8SN	Increased	88	0,040
tRF#21-8PR9DM3WE	Increased	86	0,048
tRF#23-6KK87SIRD4	Increased	78	0,039
tRF#33-73NK7F6Z2DNLDW	Increased	74	0,035
tRF#31-79MP9P9MH57SD	Increased	74	0,037
tRF#36-M26RRNLNK8KEP1B	Decreased	536	0,035

tRF#38-L85DMKYOYRLHR0D2	Decreased	320	0,033
tRF#30-4WVLV470VR31	Decreased	224	0,047
tRF#32-M2OSRN2NKSEKL	Decreased	178	0,033
tRF#33-QJ3KYUYRR6RBD2	Decreased	154	0,011
tRF#32-M1M3WE8SSP6D2	Decreased	138	0,049
tRF#24-32VIJMRPEU	Decreased	136	0,043
tRF#18-WB863IDV	Decreased	124	0,048
tRF#28-3VIJMRPFQDY	Decreased	120	0,039
tRF#23-5J3KYUYRD9	Decreased	116	0,036
tRF#19-DRJRWMI2	Decreased	108	0,047
tRF#28-MXSL735L8RDV	Decreased	108	0,047
tRF#29-YP9LON4VN1EM	Decreased	96	0,047
tRF#20-593JJ426	Decreased	92	0,041
tRF#22-8E8SP5X52	Decreased	92	0,041
tRF#20-PW55VP9N	Decreased	92	0,044
tRF#24-OP18YOY61Q	Decreased	90	0,040
tRF#32-HDK2RSI2WB0QJ	Decreased	86	0,037
tRF#21-1MQ8YUY60	Decreased	82	0,041
tRF#37-WYL1M3WE8S68L52	Decreased	80	0,046
tRF#28-3K76IR3DR2DV	Decreased	16	0,047

3.5. tRF Differential Expression in Lymphocytes

tRFs in lymphocytes demonstrated significant upregulation with 49 upregulated fragments, and zero downregulated, which vividly sets lymphocytes apart from other cells (Table 2). We applied log₄ transformation for data presentation (Figure 6).

Table 2. List of differentially expressed tRNA-derived fragments in lymphocytes during severe COVID-19.

tRF id	Change	Fold change	P-value
tRF#23-N93VUIRI0Q	Increased	557	0,024
tRF#19-881X3SKQ	Increased	280	0,039
tRF#33-HM21YP4LR45D0Q	Increased	265	0,009
tRF#31-R29P4P9L5HJVE	Increased	263	0,040
tRF#34-RLXN4VZ87HFKJJ	Increased	247	0,033
tRF#34-HM21YP4LR45DKQ	Increased	245	0,030
tRF#29-MEF91SS2PMER	Increased	233	0,046
tRF#19-4N4OEZ07	Increased	233	0,045
tRF#29-53N86SBHH90V	Increased	207	0,005
tRF#17-23KYY8M	Increased	203	0,039
tRF#19-VKS4I71Z	Increased	190	0,043
tRF#37-5QKDN6QQ1362NZO	Increased	188	0,040
tRF#19-7383RPEE	Increased	183	0,005
tRF#19-4KRQ59J4	Increased	170	0,030
tRF#20-X29X3PMZ	Increased	170	0,010
tRF#38-5QKDN6QQ1362NZ0H	Increased	165	0,046
tRF#28-6XQ6S8V0J8DR	Increased	150	0,037
tRF#19-0HRF2LFQ	Increased	150	0,001
tRF#22-RKIP4OI3H	Increased	143	0,004
tRF#22-9X0UD394N	Increased	140	0,037
tRF#28-I89NJ4S2I7DM	Increased	133	0,001
tRF#21-YRXXE8QZE	Increased	123	0,036
tRF#23-34HWH3RX0B	Increased	123	0,044
tRF#22-46S9Y8RHP	Increased	118	0,037
tRF#19-P7M84I2Q	Increased	115	0,037
tRF#19-6XQ6S82X	Increased	115	0,031
tRF#20-1QKS3W2V	Increased	113	0,049
tRF#19-MIF91S2H	Increased	113	0,039
tRF#21-WRD81H93E	Increased	110	0,043
tRF#21-9L5H52NLO	Increased	110	0,035

tRF#22-W60XY9BIQ	Increased	108	0,031
tRF#23-R9J89O9N9	Increased	105	0,035
tRF#19-8NWE6WIZ	Increased	105	0,035
tRF#28-Z3R918VBY9DV	Increased	105	0,032
tRF#23-9M8O9Q4DZ	Increased	103	0,049
tRF#22-Z3FJ6KEWH	Increased	103	0,049
tRF#23-9N1QKS3WD1	Increased	103	0,033
tRF#22-7EMQ18Y31	Increased	98	0,034
tRF#21-7O3B1NR8E	Increased	93	0,030
tRF#22-282K63ZNQ	Increased	90	0,033
tRF#18-7383RP7	Increased	90	0,031
tRF#23-RPM8309M0F	Increased	88	0,030
tRF#22-6LQ6S8V02	Increased	88	0,030
tRF#35-S4I7LZM3Q01M3K	Increased	85	0,029
tRF#25-QSD2NSWWDZ	Increased	83	0,031
tRF#23-1E6SF8WOD9	Increased	83	0,029
tRF#22-2EJ1OWZIQ	Increased	80	0,029
tRF#34-5QKDN6QQ1362HQ	Increased	44	0,026
tRF#35-5QKDN6QQ1362NZ	Increased	25	0,030

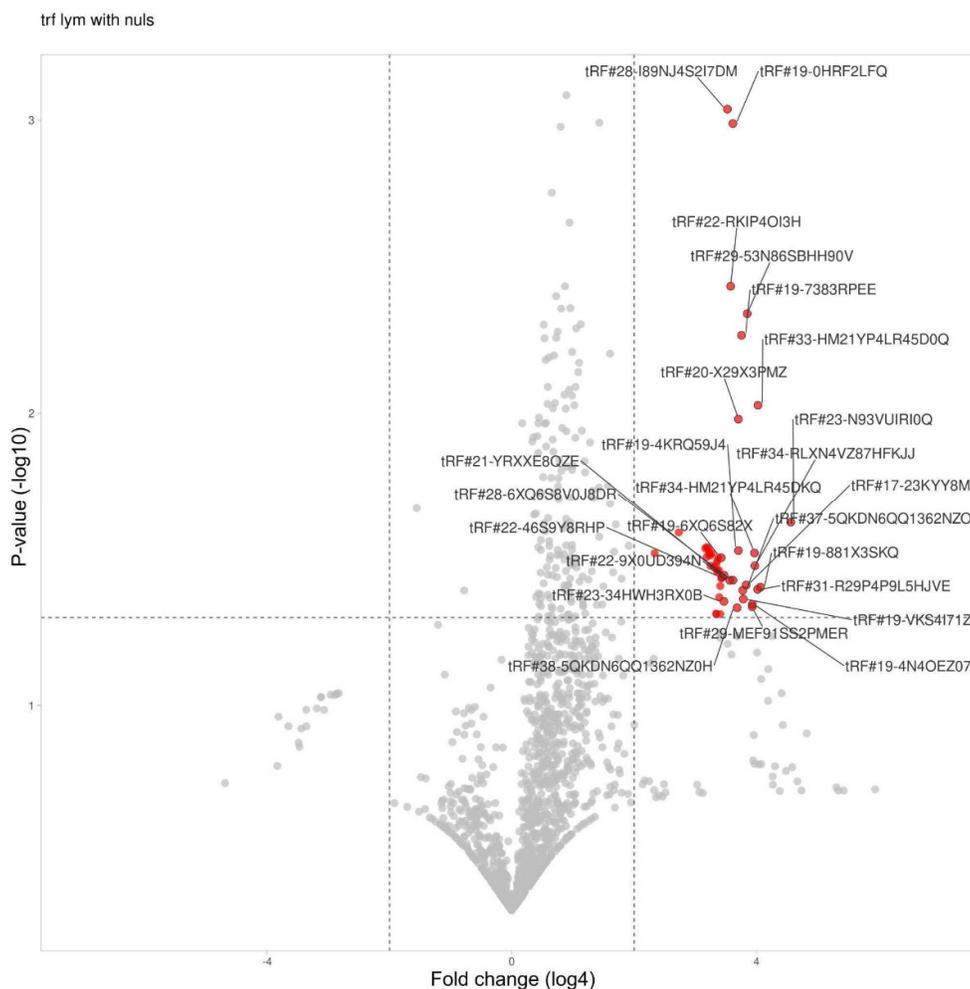


Figure 6. tRF differential expression in lymphocytes.

3.6. tRF Differential Expression in Monocytes

Monocytes were presented with the largest overall amount of significant hits. 38 upregulated tRFs and 53 downregulated (Figure 7, Table 3).

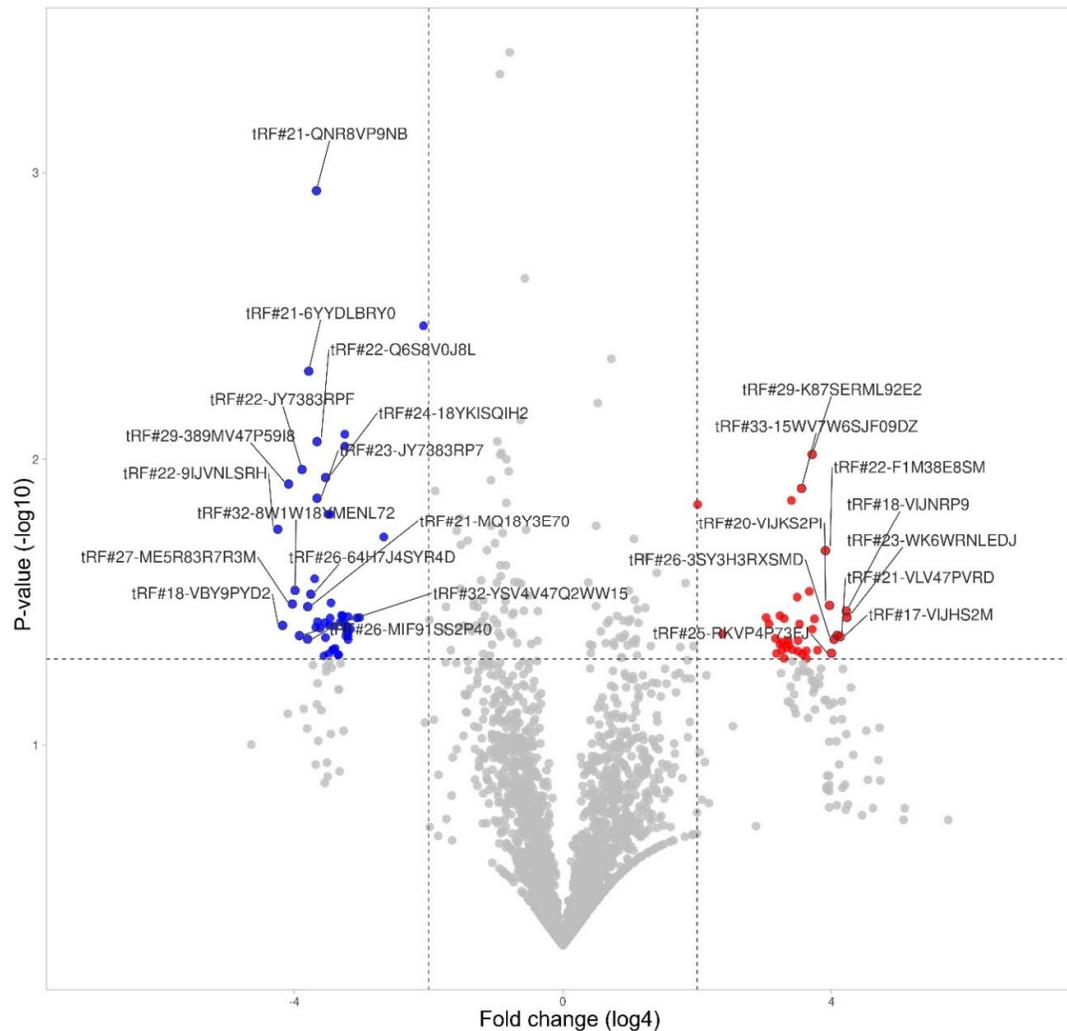


Figure 7. tRF differential expression in monocytes.

Table 3. List of differentially expressed tRNA-derived fragments in monocytes during severe COVID-19.

tRF id	Change	Fold change	P-value
tRF#23-WK6WRNLEDJ	Increased	352	0,036
tRF#18-VIJNRP9	Increased	348	0,034
tRF#17-VIJHS2M	Increased	306	0,042
tRF#21-VLV47PVRD	Increased	288	0,041
tRF#26-3SY3H3RXSMD	Increased	270	0,043
tRF#25-RKVP4P73FJ	Increased	256	0,048
tRF#20-VIJKS2PI	Increased	246	0,033
tRF#22-F1M38E8SM	Increased	226	0,021
tRF#44-K8HJ83ML5F82NZD7HY	Increased	192	0,047
tRF#25-RKILQ673FJ	Increased	180	0,036
tRF#28-R1RXQ678Y2D8	Increased	172	0,039
tRF#29-K87SERML92E2	Increased	172	0,010

tRF#31-ZPQR16ZSIJ7KE	Increased	162	0,029
tRF#22-WEK6S1852	Increased	152	0,047
tRF#23-K8HJ83MLDS	Increased	152	0,050
tRF#18-VIJKS2DU	Increased	140	0,048
tRF#33-15WV7W6Sjf09DZ	Increased	138	0,013
tRF#25-P21MNKYUPR	Increased	132	0,038
tRF#38-Y2R79K3BEE4O3Q03	Increased	128	0,043
tRF#24-PS5U8918JP	Increased	128	0,047
tRF#21-W60XY9BIE	Increased	126	0,030
tRF#30-6Q46D6PUMZQZ	Increased	114	0,046
tRF#22-9LON4VN11	Increased	112	0,014
tRF#34-IEWS7YRR50SRIZ	Increased	106	0,045
tRF#18-HSQSD2D2	Increased	104	0,043
tRF#32-ZPQR17NSRJ7KQ	Increased	102	0,046
tRF#26-R3HJ83RPFQE	Increased	96	0,036
tRF#19-K876IR19	Increased	96	0,050
tRF#37-9EUK46S9Y8RH93Q	Increased	92	0,044
tRF#20-9VWVEH93	Increased	92	0,047
tRF#28-WS3V2VR0PSDZ	Increased	88	0,035
tRF#20-SVKKN27F	Increased	88	0,044
tRF#32-FN5KYUSRYWRSJ	Increased	82	0,048
tRF#27-WN1Q18Y3HRK	Increased	80	0,042
tRF#20-3K7SIR3D	Increased	70	0,038
tRF#22-WEPSJR852	Increased	66	0,036
tRF#21-MXSL73VLE	Increased	27	0,041
tRF#25-QNR8ZP9LON	Increased	16	0,014
tRF#22-9IJVNLSRH	Decreased	360	0,018
tRF#18-VBY9PYD2	Decreased	326	0,038
tRF#29-389MV47P59I8	Decreased	288	0,012
tRF#27-ME5R83R7R3M	Decreased	266	0,032
tRF#32-8W1W18YMENL72	Decreased	252	0,029
tRF#32-YSV4V47Q2WW15	Decreased	230	0,042
tRF#22-JY7383RPF	Decreased	218	0,011
tRF#26-MIF91SS2P40	Decreased	194	0,043
tRF#21-MQ18Y3E70	Decreased	194	0,033
tRF#21-6YYDLBRY0	Decreased	190	0,005
tRF#26-64H7J4SYR4D	Decreased	182	0,030
tRF#21-MUWL47PE	Decreased	168	0,026
tRF#21-R84QPYVMD	Decreased	164	0,039
tRF#21-QNR8VP9NB	Decreased	162	0,001
tRF#22-Q6S8V0J8L	Decreased	160	0,009
tRF#23-JY7383RP7	Decreased	160	0,014
tRF#33-1MN0YU09FKRFD2	Decreased	158	0,037
tRF#22-9P9NH57SJ	Decreased	150	0,039
tRF#23-RXSINH4DV	Decreased	138	0,049
tRF#24-941QKS3WF8	Decreased	136	0,037
tRF#20-J87383RP	Decreased	134	0,042
tRF#24-18YKISQIH2	Decreased	134	0,012
tRF#38-ML5F924ZDRJKW4DZ	Decreased	128	0,016
tRF#21-9P4P9L5HE	Decreased	126	0,048
tRF#24-Z3RQ18YJFH	Decreased	124	0,038
tRF#23-J87383RP7	Decreased	122	0,036
tRF#20-RK9P4P9L	Decreased	122	0,016
tRF#22-MQ18Y3E7M	Decreased	120	0,032
tRF#28-RUPLQVNRDF0E	Decreased	116	0,047
tRF#22-73H3RXPLM	Decreased	114	0,046

tRF#24-94SX73V2KK	Decreased	112	0,038
tRF#21-MIF91SS20	Decreased	110	0,046
tRF#24-04SXQ3V2KJ	Decreased	104	0,048
tRF#21-9LV470JPD	Decreased	102	0,048
tRF#20-1PSJPM17	Decreased	98	0,037
tRF#34-JY7383RPD9W1JV	Decreased	96	0,035
tRF#24-YDLBRY73JL	Decreased	94	0,035
tRF#21-3P47M26YB	Decreased	92	0,037
tRF#22-6YR29P4PP	Decreased	90	0,008
tRF#21-WB8689SVD	Decreased	90	0,009
tRF#21-WLV47PU9E	Decreased	88	0,039
tRF#21-N1EH6KK80	Decreased	88	0,042
tRF#18-SR99RHD2	Decreased	86	0,038
tRF#33-5F924ZDRJKW4DZ	Decreased	86	0,039
tRF#22-5721V98B3	Decreased	84	0,036
tRF#26-MY73H3RXPL0	Decreased	84	0,040
tRF#22-J4S2I7L7M	Decreased	84	0,042
tRF#28-RKVP4P9L5F0Q	Decreased	84	0,043
tRF#26-94SL735FV10	Decreased	80	0,039
tRF#18-6M0Y1MY	Decreased	70	0,036
tRF#24-MY73H3RXII	Decreased	66	0,036
tRF#33-1N3KYUSR681SD2	Decreased	40	0,019
tRF#25-R8VP9N9QFY	Decreased	18	0,003

4. Discussion

This pilot study aimed at demonstrating a distinguishable tRF pattern for different sorted cell types. Given the fact that tRFs have been discovered relatively recently, research aimed at understanding their origin can help elucidate their biological purposes.

Analysing **tRF length distribution** in erythrocytes, monocytes, lymphocytes, neutrophils, basophils and eosinophils we observed a remarkable similarity between control and severe COVID-19 patient's cells (Figure 2). This data is additionally supported by our heatmap (Figure 4), which demonstrated a distinguishable pattern of tRF expression depending on cell type. Erythrocytes are presented as the most distinguishable group of cells in terms of tRF length and tRF expression patterns, which may be due to the fact that the erythrocyte is an inactive cell in terms of synthetic processes.

Regarding **tRF type composition**, It has been previously noted that 3-tRFs are produced in response to various cellular stresses like oxidative stress, hypoxia, and viral infection [11]. Some 3-tRFs can inhibit viral replication by interfering with viral gene expression or packaging. They can act as signalling molecules to mediate stress responses [12,13].

Given the fact that IL-6 is a key mediator of the "cytokine storm" that leads to acute respiratory distress syndrome (ARDS) and multi-organ dysfunction in severe COVID-19 [14], it is assumable that such distribution of tRF types may be due the condition the patient underwent. Other cell types did not exhibit any significant alterations in tRF composition.

Overlooking **tRF expression in control donors and severe COVID-19 patients**, it is worth noting how the expression is increased in the majority of tRF in granulocytes (neutrophils, basophils, eosinophils). A similar tendency to increased expression is observed in monocytes. Granulocytes and monocytes both originate from CFU-GM (Colony Forming Unit–Granulocyte–Macrophage), also known as granulocyte–macrophage progenitor (GMP) [15].

The aforementioned suggests that tRFs may dominantly persist from the progenitor stages of cell development, and are only slightly modified by environmental or physiological factors.

Most knowledge today regarding tRFs is concentrated around cancer research [2,16–18].

In our study we encountered fragments which have been previously associated with several types of cancer.

Lymphocytic tRFs: It is noted that tRF#31-R29P4P9L5HJVE previously has been acknowledged as a marker for lung cancer prediction among smokers 10 years prior to being diagnosed [19].

tRF#19-VKS4I71Z has been mentioned as an abundant novel-trf in a 2016 study of RNA-seq data from human prostate tissue [20].

In a 2023 research dedicated to studying tRFs in cancer, a high level tRF#34-5QKDN6QQ1362HQ has been mentioned as a predictor of breast cancer improved survival [2].

Monocytic tRFs: tRF#22-WEK6S1852 in previous research was found to be significantly downregulated and associated with human malignant mesothelioma [16].

A 2019 colon cancer study revealed significantly differentially expressed tRFs between colon cancer tissues and peritumor tissues, whereas another upregulated tRF that we observed - tRF#22-9LON4VN11 was mentioned, demonstrating downregulation in colon cancer tissues with a log2 fold change of -1.26 [17].

Another fragment we observed - tRF#18-HSQSD2D2, downregulation of which has been previously associated with early-stage breast cancer [2].

In a study devoted to examining the dysregulation of different tRFs in chronic lymphocytic leukemia - tRF-20-RK9P4P9L was amongst the top 15 differentially expressed sRNAs in aggressive chronic lymphocytic leukemia vs. normal controls, with the linear fold change being -76.64 and -258.08, respectively. Samples were composed of CD5+/CD19+ B cells. It is worth noting that in our study, the same tRF was also significantly downregulated but only in CD14++ CD16- monocytes [18].

tRF#22-WEPSJR852 was found in peripheral blood of fibromyalgia patients in a dissertation dedicated to the search of morphological substrate to fibromyalgia [21].

Erythrocytes appeared to be the only cell type which showed differential expression of tRFs that have not previously been associated with any type of cancer or disease. The fact that the erythrocyte is an inactive cell in terms of synthetic processes may elucidate these findings, however, regarding different cell types, tRFs and their interrelations with the conditions mentioned earlier it is more likely to suppose a coincidence, rather than some significant finding.

Differences in the length, type, and composition of specific tRFs between different cells are supposedly due to the specificity of the tRNA cleavage system. The same can be said about changes that occur during cytokine storm. The regulation system of tRNA processing is still poorly understood.

Nonetheless, we can say that it occurs at several "levels". First of all - the regulation of nucleotide modifications in certain molecules [22]. Another stage is dependent on the regulation of RNases that cleave tRNAs at specific sites.

The main types of nucleases involved in tRF biogenesis are: Dicer - cleaves 5' ends of mature tRNAs. Angiogenin (ANG) - cleaves mature tRNAs at anticodon loops. RNase Z/ELAC2 - cleaves 3' trailer sequences of pre-tRNAs [11,23,24].

RNase P: excision of external transcribed spacer (ETS) and internal transcribed spacers (ITS) from pre-tRNA transcripts [25].

Regulation of these enzymes can occur at the level of transcription initiation, as well as post-translational modifications of the enzyme

It is also likely that tRNA cleavage is regulated by proteins that bind this molecule [26] and make certain sites inaccessible for the aforementioned process. Unfortunately, we cannot state by what mechanism the difference between tRFs in blood cells is regulated.

In summary, it should be noted that TRF profiles significantly differ in different types of blood cells and demonstrate dramatic differential expression (sometimes more than 500-fold) during cytokine storm. Such profound differences suggest a major role of tRNA-derived fragments in the functioning of blood cells.

5. Conclusions

The composition of tRFs in erythrocytes monocytes, lymphocytes, neutrophils, basophils and eosinophils was analysed on the basis of sRNA-SEQ. We demonstrate notable alterations in the length

and types of these molecules in main blood cell populations. We additionally observed a significant change in the profile of tRFs in the erythrocytes, monocytes and lymphocytes of patients infected with SARS-CoV-2.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. All RAW read data and nucleotide composition of tRFs are presented in two supplementary files: “Control cells” for control donors and “COVID cells” for patients with severe COVID-19.

Funding: This work was supported by St. Petersburg State University, project ID: 95412780.

Institutional Review Board Statement: This study was conducted according to the guidelines of the declaration of Helsinki and approved by the ethics committee.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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