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Metagenomics analysis of the virome of 300 concentrates from a Swiss platelet bank

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SUMMARY

Background and objectives: Platelet concentrates are frequently transfused to patients with reduced immunity. An exhaustive description of their viral content is needed to prevent unwanted infection.

Material and methods: To track viral sequences, a shotgun metagenomics approach was used on a bank of 300 platelets concentrates. Sequences were analysed through the diagnostics-oriented pipeline ezVIR.

Results: We only observed viruses commonly described in healthy individuals.

Conclusion: Herein is reported the first viral landscape of a platelet concentrates bank.

Keywords

next generation sequencing; platelet concentrates; transfusion-transmissible infections

Using metagenomics, we recently reported on the virome of red blood cell concentrates and fresh frozen plasmas [1]. Platelet concentrates are frequently transfused to immunosuppressed patients. However, due to their limited availability for research, their viral landscape has not been reported. In order to assess the risk of transfusing pathogenic viruses, we performed viral screening of 300 platelet concentrates manufactured at the blood transfusion center of Geneva (Switzerland).

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1. Material and methods

Platelet concentrates manufacturing

Three hundred platelet concentrates were collected from single donors in 2016 containing, on average, 39% of donor's plasma, and 0.23×10^6 residual leukocytes/unit. Four ml were derived from each concentrate before clinical use. Samples were not pathogen-reduced.

Nucleic acid extraction and Sequencing

Extraction was made using the QIAamp Circulating Nucleic Acid Kit (Qiagen) without carrier RNA. Pools were made of 30 extracts each. Pool libraries were prepared as previously described [1]. As positive controls, two libraries, one DNA (DNA B19V) and one RNA (RNA HRV14), were prepared from one spiked single concentrate with 10^5 copies/ml of *Parvovirus B19* (B19V) and *Human Rhinovirus* 14 (HRV-14), respectively. Negative controls consisted of empty tubes submitted to the entire process. Libraries were run on the HiSeq 2500, producing paired-end, 100 nt long reads.

Library Analysis

Libraries were analysed with an updated version of ezVIR [2]. Non-human data were assembled using IDBA-UD, and scaffolds were classified with DIAMOND, using NR as the database. Cross-contamination was described as previously reported in [3]. Briefly, for each sequencing lane, a percentage of reads can be misattributed to other libraries. For each lane, we compared the libraries with most abundance of each virus, against the other libraries. If the ratio was below 0.24%, they were considered a contaminants.

2. Results and discussion

Twenty-four libraries were produced: 10 DNA pools, 10 RNA pools, 2 positive controls, and 2 negative controls (DNA and RNA). After removing low quality and complexity reads, DNA libraries were comprised of an average of 91.2% human content and 8.8% non-human content, while RNA libraries had an average of 89.5% human content and 10.5% non-human content. The spiked viruses – *Parvovirus B19* (B19V) and *Human Rhinovirus 14* (HRV-14) were found with almost complete coverage (99.99% and 99.98% respectively).

Five virus families were identified (figure 1): Anelloviridae (*Torque Teno Virus* - TTV), Herpesviridae (H*erpesvirus 6* - HHV6), Papillomaviridae (*Papillomavirus)*, Polyomaviridae (*Polyomavirus* and *Merkel Cell Polyomavirus*) and Flaviviridae (*human Pegivirus* - HPgV). The percentage of genome covered and number of reads for each virus are shown in table 1. These signals are consistent with studies from other blood products, namely viruses reported to commonly circulate in the blood of healthy individuals: TTV, *Pegivirus*, HHV6, *Polyomavirus* and *Papillomavirus*. TTV is detected in blood with a prevalence between 1.9% to 62% and a tropism for peripheral mononuclear cells, liver and bone marrow [7]. *Pegivirus* infects 1/6 of the global population and is known to be transmissible through transfusion [8,9], with a tropism for bone marrow, spleen, and plasma [8]. While *Herpesvirus 6* viraemia has been described in the blood virome, the signal may also be due to HHV6 integrated in the genome [10]. While associated with skin flora, Polyomavirus and Papillomavirus have

also been reported in the blood of healthy individuals [10]. These observations suggest virus transmission to the product from the donor's blood. HHV6 reads were also detected in the negative control, mapping to a region common to several HHV6 strains (a repeat region in the first 3000nt of the genome). Since no hits for other species were found for these reads, we discarded the possibility of a reagent contaminant or read misclassification. It could be a case of cross-contamination from sample 10, though the low abundance of reads does not allow us to confirm it. Finally, known reagent contaminants are found: *Parvo-like hybrid virus*, *Kadipiro virus*[4,5] and *Xenotropic murine leukemia-related virus* [6].

Herein is reported a novel insight into the viral landscape of a platelet concentrates bank. It contains less diversity of viral signatures than reported for the blood virome, probably due to the plasma/leukocyte reduction process applied to blood products manufacturing, and the high level of pooling in this study. Despite this, we were able to find several commensal viruses also present in other blood products. Finding signatures of two DNA viruses in RNA libraries suggest a replication phase releasing RNA or incomplete DNase efficacy during library preparation. No uncommon viruses were found, unlike our previous analysis in red blood cells and plasma [1], where we found an *astrovirus MLB2* linked to meningitis in immunocompromised patients [11]. As generally observed in high throughput sequencing, unspecific background signal was systematically present, and must be carefully identified and removed. This is achieved by controlling for specific reagents, using double indexing, and complementing the analysis with robust molecular methods, respectively [3–6]. Thus, a metagenomic approach offers an attractive option for the exhaustive screening of platelet concentrate banks, as it allows for the detection of viruses which aren't part of standard diagnostics, and also can lead to follow up association studies in larger platelet banks and/or defined donor populations [11,12].

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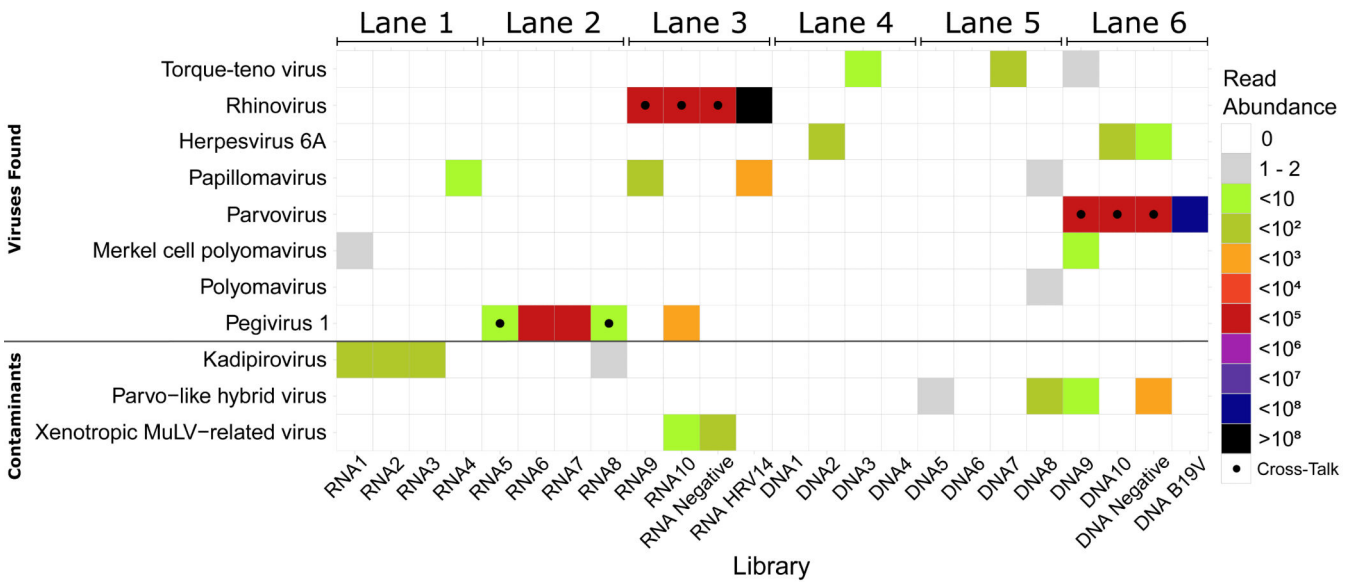

Figure 1:

Viral signatures in each individual pool sorted by sequencing lane and sequencing type. The dotted libraries represent cross-talk contaminations.

Table 1:

Quantitative values of virus signals found by ezVIR.

Library	Virus Family	*Genome Name*	ID	% covered	Genome length	Total mapped reads
DNA 1	none	none				
DNA 2	Herpesvirus 6A	*Human herpesvirus 6A AJ*	KP257584.1	0.54	156714	16
DNA 3	TT virus	*Torque teno virus 1*	NC 002076.2	5.19	3852	3
DNA 4	none	none				
DNA 5	none	none				
DNA 6	none	none				
DNA 7	TT virus	*Torque teno midi virus 2 MD2–013*	AB290919.1	11.96	3’253	14
DNA 8	Polyomavirus	*Polyomavirus HPyV6 607b*	HM011561.1	2.42	4’926	2
	Papillomavirus	*Human papillomavirus type 20*	U31778.1	1.33	7’757	2
DNA 9	Merkel cell polyomavirus	*Merkel cell polyomavirus MCC350*	EU375803.1	6.48	5’387	4
	TT virus	*Torque teno virus 1*	NC 002076.2	2.60	3’852	2
DNA 10	Herpesvirus 6A	*Human herpesvirus 6A AJ*	KP257584.1	0.55	156’714	12
DNA negative	Herpesvirus 6A	*Human herpesvirus 6A GS*	KJ123690.1	0.06	156’864	3
DNA B19V	Parvovirus	*Human parvovirus B19*	NC 000883.2	99.98	5’596	65’895’350
RNA 1	Merkel cell polyomavirus	*Merkel cell polyomavirus MCC350*	EU375803.1	3.71	5’387	2
RNA 2	none	none				
RNA 3	none	none				
RNA 4	Papillomavirus	*Human papillomavirus type 107*	EF422221.1	1.32	7’562	4
RNA 5	none	none				
RNA 6	Pegivirus 1	*Hepatitis GB virus C GT110*	D90600.1	96.66	9’395	32’787
RNA 7	Pegivirus 1	*Hepatitis GB virus C GT110*	D90600.1	97.79	9’395	32’322
RNA 8	none	none				
RNA 9	Papillomavirus	*Human papillomavirus type 107*	EF422221.1	1.32	7’562	11
RNA 10	Pegivirus 1	*Hepatitis G virus strain HGV*	AF081782.1	53.73	9’373	485
RNA negative	none	none				
RNA HRV 14	Rhinovirus	*Human rhinovirus type 14*	K02121.1	99.99	7’212	102’745’995
	Papillomavirus	*Human papillomavirus type 18*	X05015.1	29.50	7’857	175