

NAT screening of PDMPs and blood components for non-enveloped viruses

- review of current screening algorithms

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Presentation plan

- recommendations for non-enveloped virus screening
- screening methodology
- quality control
- diagnostic algorithms
 - TMA
 - real-time PCR
- HAV, B19V and HEV NAT results (Poland)
 - epidemiological characteristics – frequency by age and gender
 - follow-up observations – implications for temporal donor deferral
 - non-enveloped virus transmission – observations and estimations
 - management of infected blood donors – problems and doubts



Non-enveloped viruses screening in Poland

	Requirements		Criteria for plasma discard	Time of screening	NAT results applied to qualification of cellular blood components
	domestic	Other			
HAV	not required	plasma for fractionation	confirmed infection	quality control before shipment to manufacturer	only if available
B19V	plasma dedicated for anti-D, and anti-HBs production, RBC for immunization	plasma for fractionation	VL> 1-1.6x10E5 IU/ml (ID)	quality control before shipment to manufacturer	only if available
HEV	not required	plasma dedicated for country with mandatory RNA HEV screening	confirmed infection	in paralel to RNA HCV, DNA HBV and RNA HIV screening	obligatory

NAT methodology for non-enveloped viruses in Poland (1)

transcription mediated amplification (TMA)

Marker	System	Assay	Format	Analytical sensitivity* 95% LOD (95% CI) in IU/mL
RNA HAV	Procleix Tigris	Procleix Parvo/HAV	MP 16	0.87 (0.74-1.48)
DNA B19V				306
RNA HAV	Procleix Panther	Procleix Parvo/HAV	MP 16	1.06 (0.90-1.30)
DNA B19V				325
RNA HEV	Procleix Panther	Procleix HEV Assay	MP 16	7.89 (6.63-9.83)



Procleix Tigris System
2013-16



Procleix Panther System
Since 2016

* Data from instruction manual

NAT methodology for non-enveloped viruses in Poland (2)

real time PCR



Cobas s201
since 2013

Marker	Platform	Assay	Format	Analytical sensitivity* 95% LOD (95% CI) in IU/mL
RNA HAV	cobas s201	cobas TaqScreen s201	MP 96	1.06 (0.94-1.24)
DNA B19V				11.48 (10.56-12.91)
RNA HAV	cobas 6800/8800	cobas DPX	MP 96	1.1 (0.9-1.3)
DNA B19V				13.9 (11.7-17.4)
RNA HEV	cobas 6800/8800	cobas HEV	MP 24	18.6 (15.9-22.6)



Cobas 6800/8800
since 2018

* Data from instruction manual

Quality control for NAT in Poland non-enveloped viruses

Preliminary evaluation of method (at IHTM prior to implementation)

- analytical sensitivity: multiple testing of six IS dilutions
- clinical sensitivity assessment (polymorphic forms, if available)
- proper identification of infected donations in MP testing (n=16-96) including high VL samples: risk of contamination and false results

Procedure validation (at RBTC, prior to implementation and every 12 months)

- several coded positive (including high VL) and negative samples tested in MPs
- several coded positive (including low VL) and negative samples tested individually

External quality control program (at RBTC, at least every 12 months)

e.g. LabQuality, EDQM, QCMD

IHTM -- Institute of Haematology and Transfusion Medicine, reference lab in Warsaw

RBTC – Regional Blood Transfusion Center, screening lab

Validation of NAT screening systems for non-enveloped viruses (Poland)

Reference material

- HAV – 2nd IS WHO HAV, NIBSC (00/562)
- HEV - PEI IS 6329/10
- B19V - B19V WHO Reference Reagent 1st WHO International Reference Panel for B19 Genotypes for NAT based assays, NIBSC (09/110)

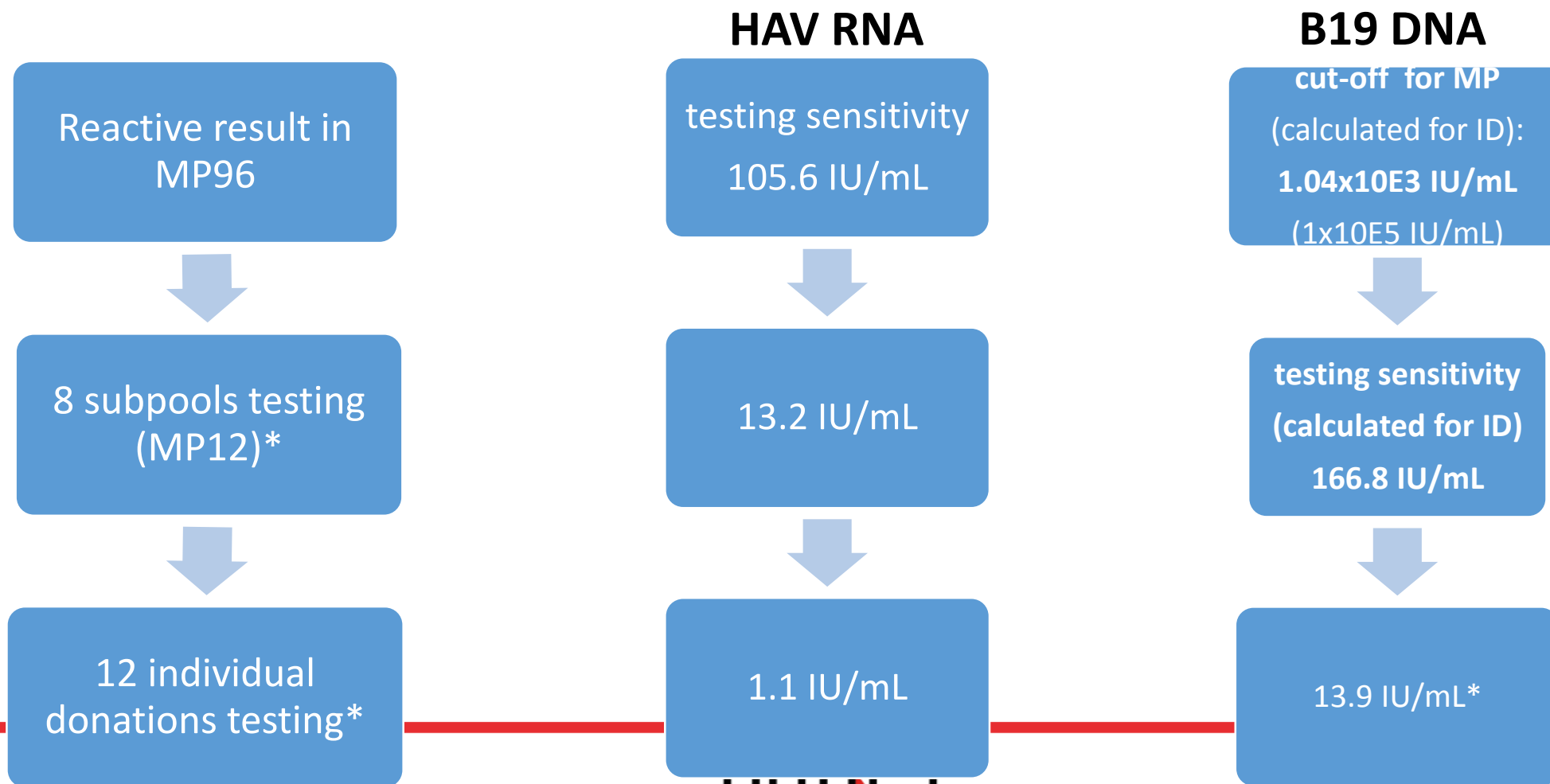
Polish positive plasma

(specific antibodies, VL, genotype)

- High viral load B19V
- B19V genotype 2 (from patients)
- HEV genotype 3
- HAV genotype 1A (available since 2018)

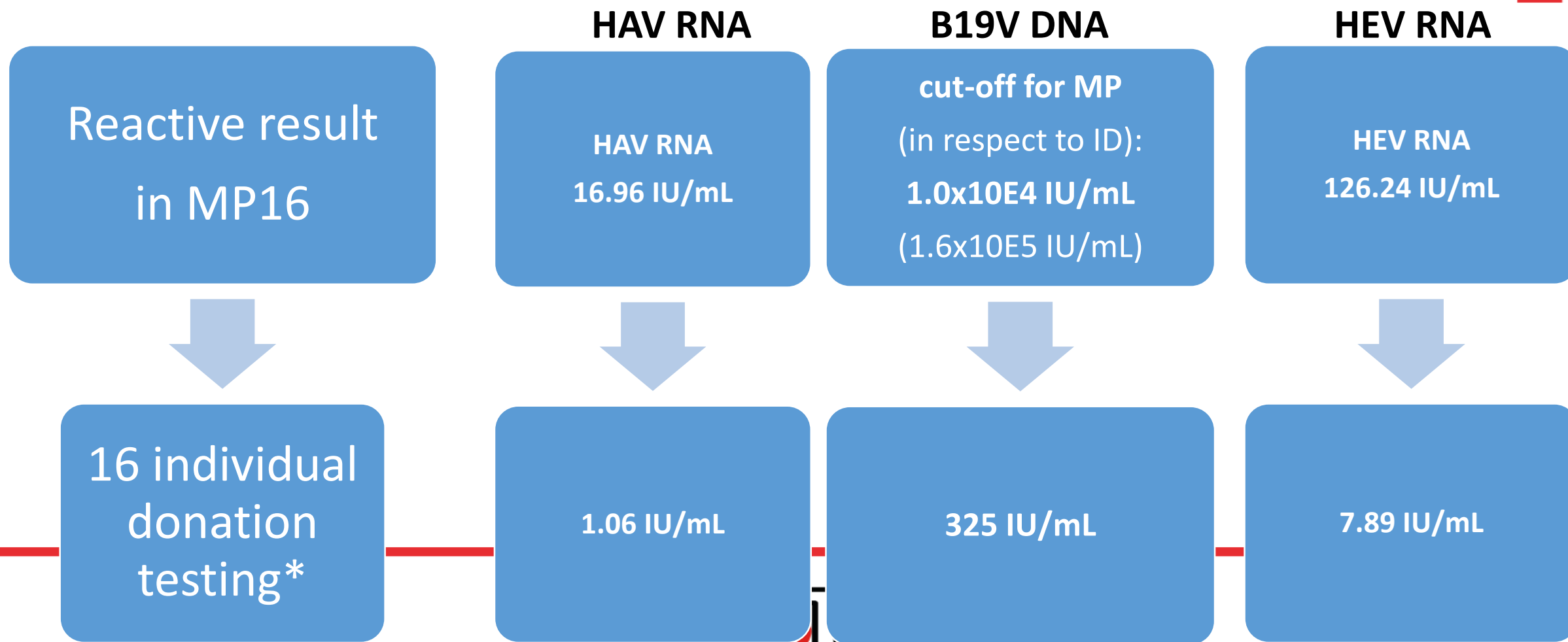
Real-time PCR screening algorithm

LODs (cut-off) for screening and resolution of reactive MPs



TMA screening algorithm

LODs (cut-off) for screening and resolution of reactive MPs



*LODs for Procleix Assays run on Procleix Panther based on instruction manual

Identification of reactive donation laboratory procedures, IHTM

Marker	Confirmatory testing (assay, 95% LOD)	Supplementary testing (assay)	follow-up	look-back
RNA HAV	IDT, in previously unopened tube* (RealStar HAV RT-PCR kit 1.0, Altona Diagnostics; 95% CI:115 IU/mL)	Anti-HAV IgG and IgM (Architect HAV Ab-IgG, Abbott, HAV Ab-IgM; Abbott)	Yes (serology+NAT)	Yes (serology+NAT)
DNA B19V	Not required	Not required	Yes (quantitative NAT)	No
RNA HEV	IDT, in previously unopened tube* (RealStar HEV RT-PCR kit 2.0, Altona Diagnostics; 95% LOD: 50 IU/mL)	Anti-HEV IgG and IgM (Wantai HEV-IgG ELISA, Wantai HEV-IgM ELISA)	Yes (serology+NAT)	Yes (serology+NAT)

*if negative->multiple testing

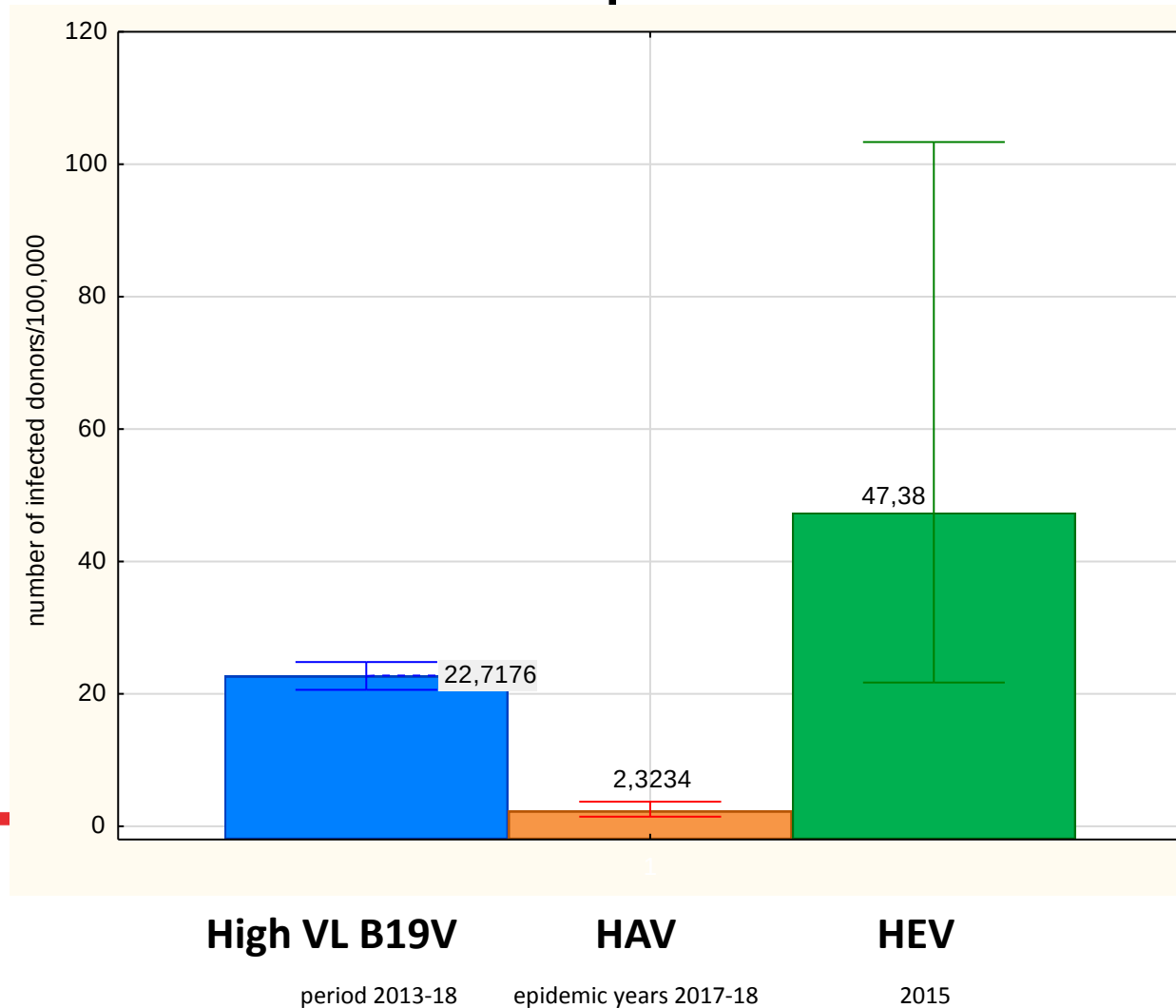
Identification of positive* donation procedures for donors and blood components

	Minimal period of temporary deferral	cellular blood components	criteria for donor restoration	notification donor	surveillance system	referral to doctor
HAV	4 months	discarded ,if available	negative IDT NAT	Yes	Yes	Yes
HEV	12 months	discarded	negative IDT NAT	Yes	Yes	Yes
B19V	12 months	discarded, if available	IDT VL <10 ³ IU/mL**	Yes	No	No

* confirmed

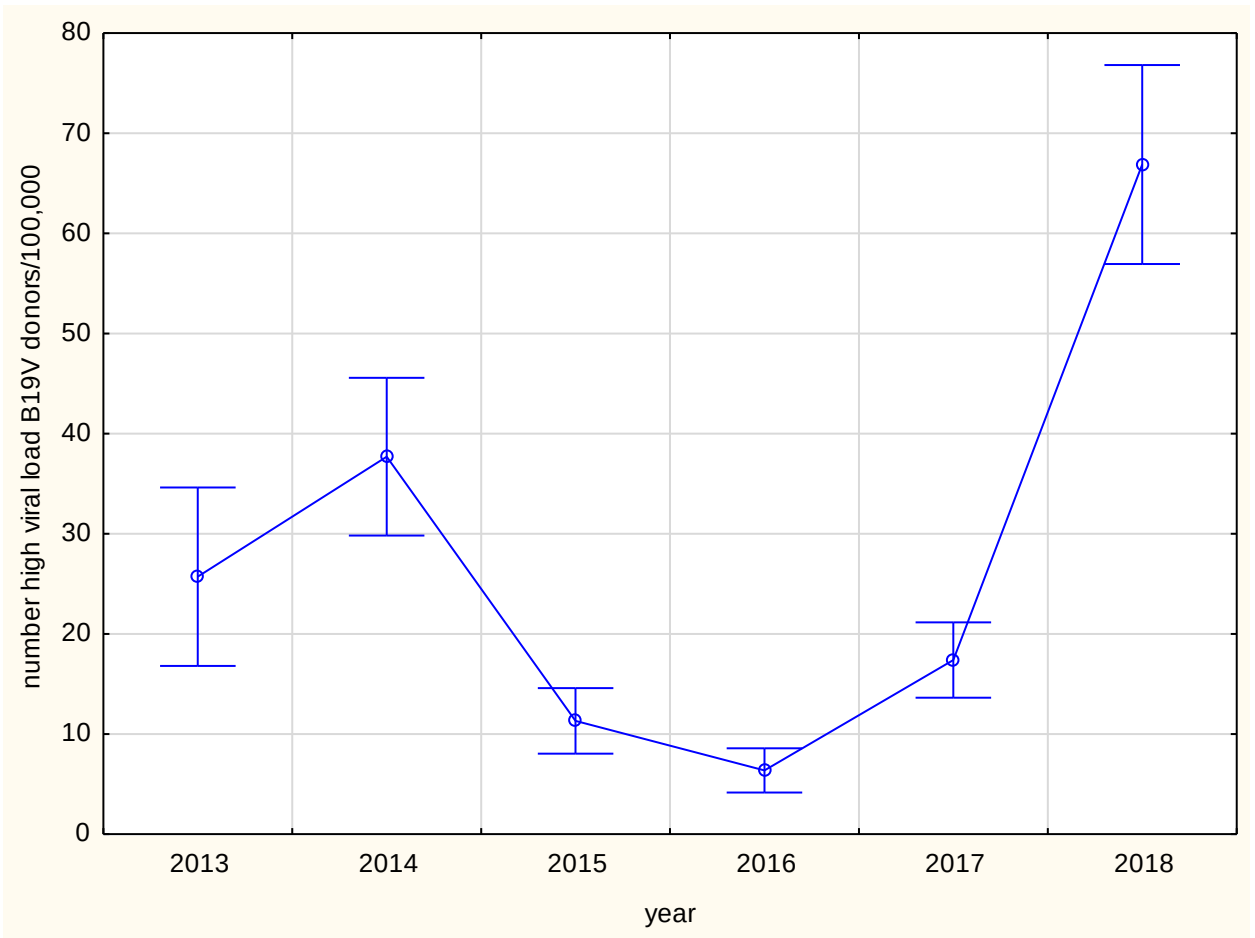
**in case of cellular blood components for clinical use, in case of plasma for fractionation according to the manufacturer recommendation

Frequencies of non-enveloped viruses in Polish blood donors comparison



based on *Transfusion* 2018 May;58(5):1245-1253.

Frequency of high viral load parvovirus B19 infections in Polish blood donors, 2013-2018

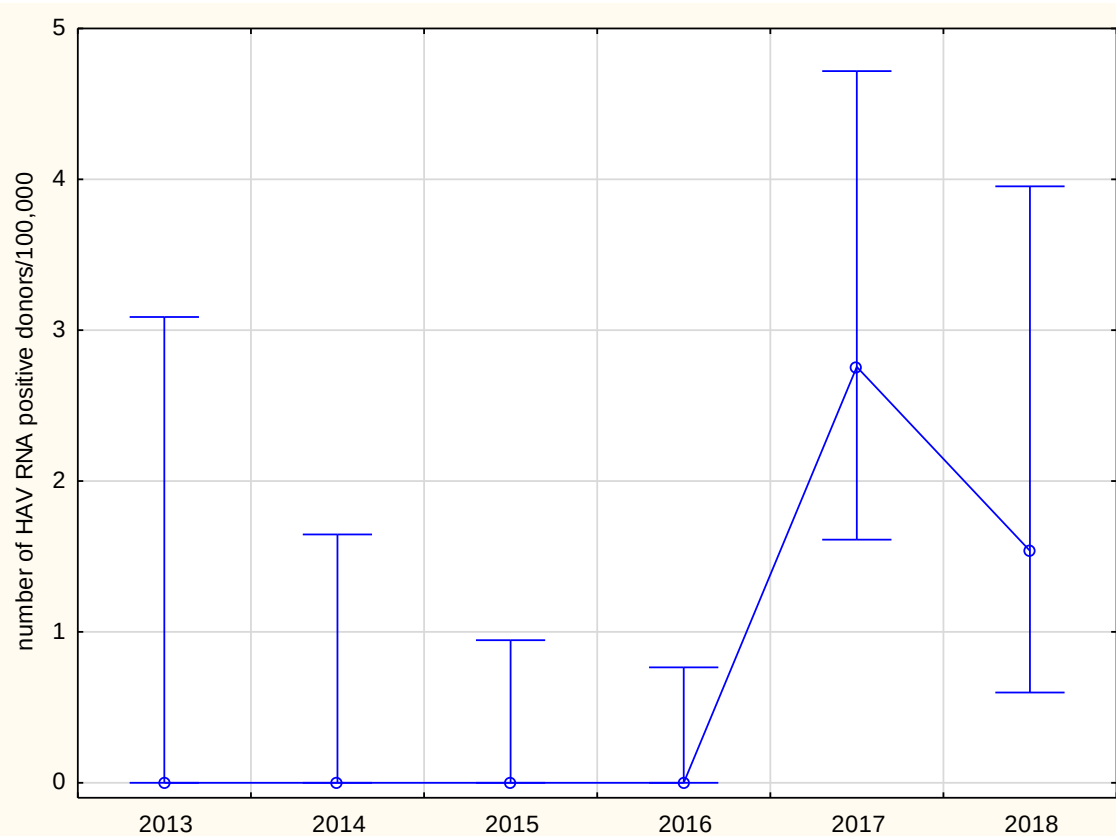


relative risk for 2016 vs 2018 = 10,5 (95% CI: 7.2-15.3),
p<<<0.05

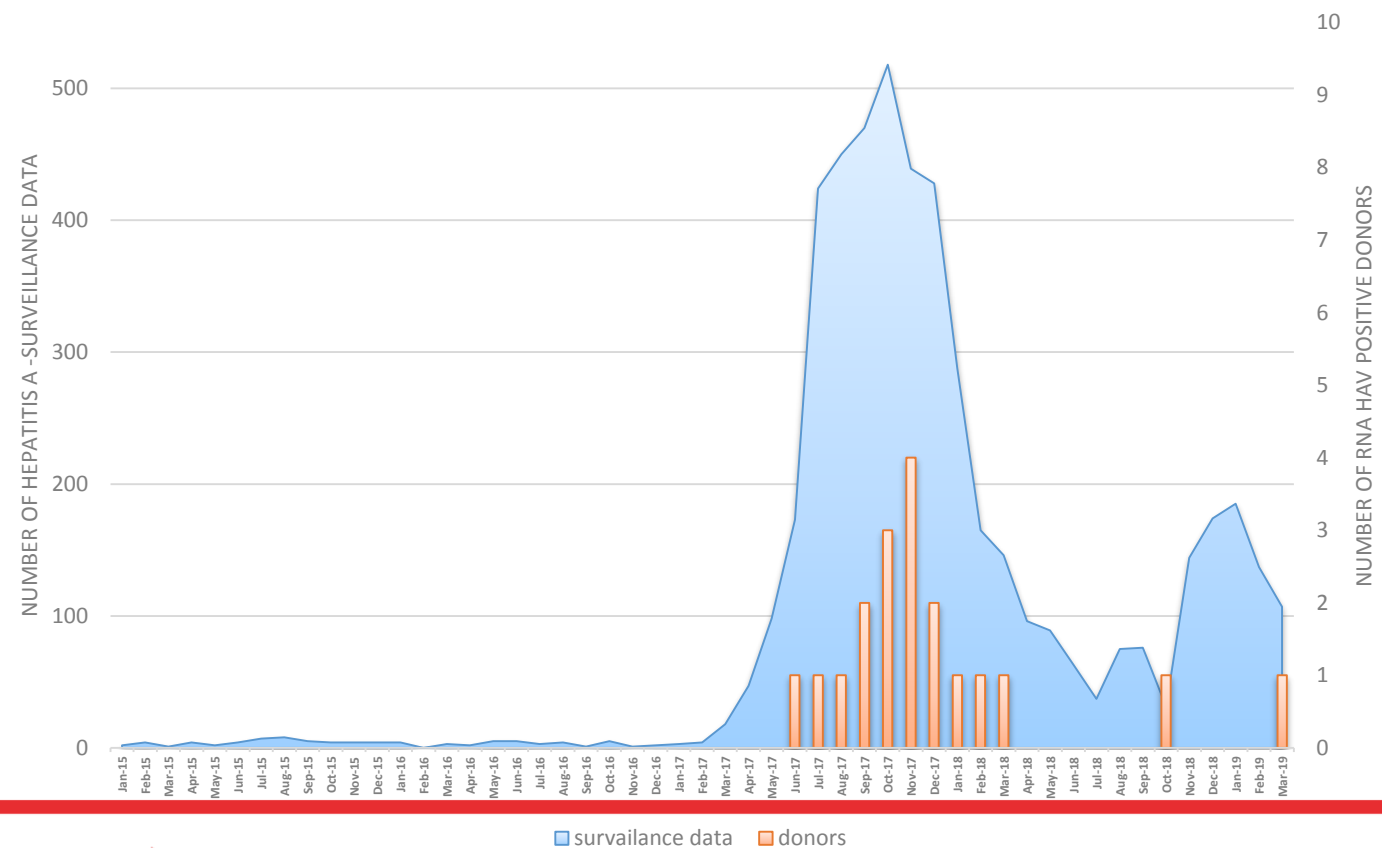
period	donors number	
	tested	high VL B19V+
2013-18	1.998.451	454
2013	124.425	32
2014	233.452	88
2015	406.673	46
2016	502.513	32
2017	471.536	82
2018	260.164	174

Hepatitis A virus incidence in Polish blood donors 2013-2018

frequency (95% CI) in blood donors*



number of infected blood donors vs surveillance data**



*data reported for donors tested until the end of 2018

**infected donors and surveillance data reported until the end of April 2019

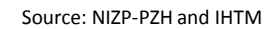


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Source: PZH-NIZP

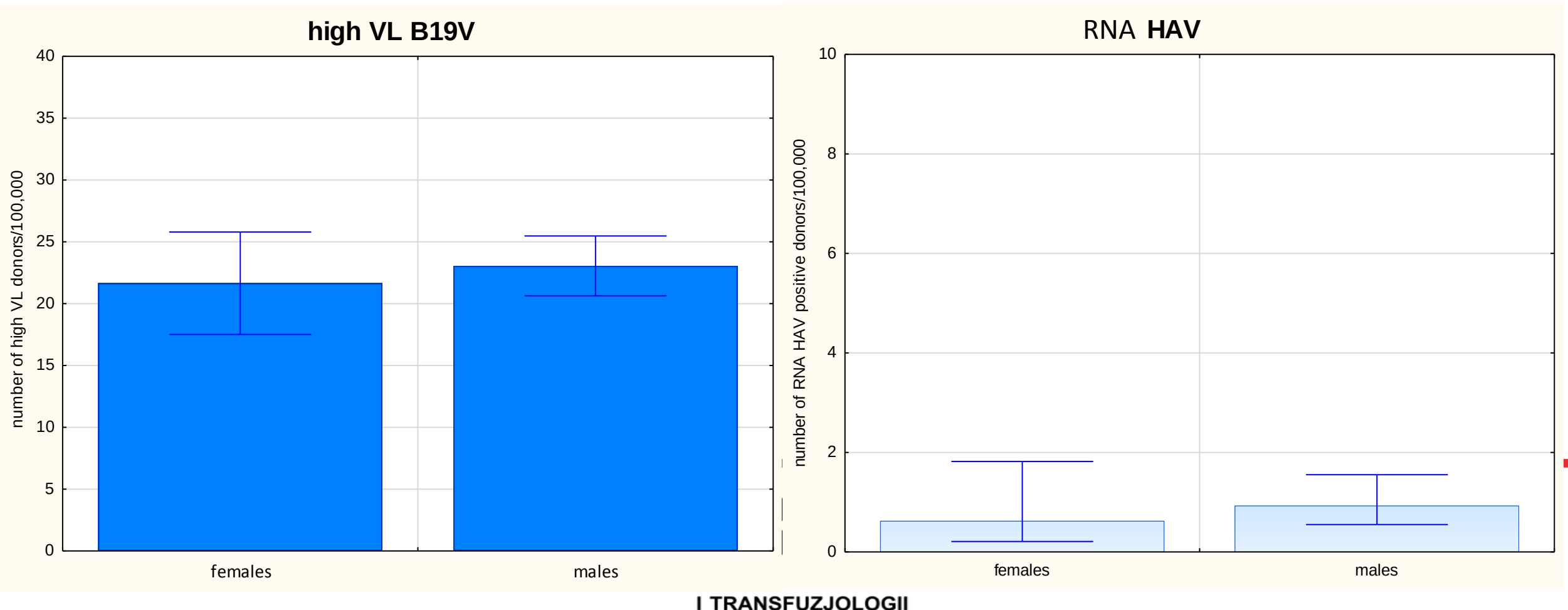
IHTM

WWW.IHIT.WAW.PL



Characteristics of non-enveloped virus infections in blood donors (1)

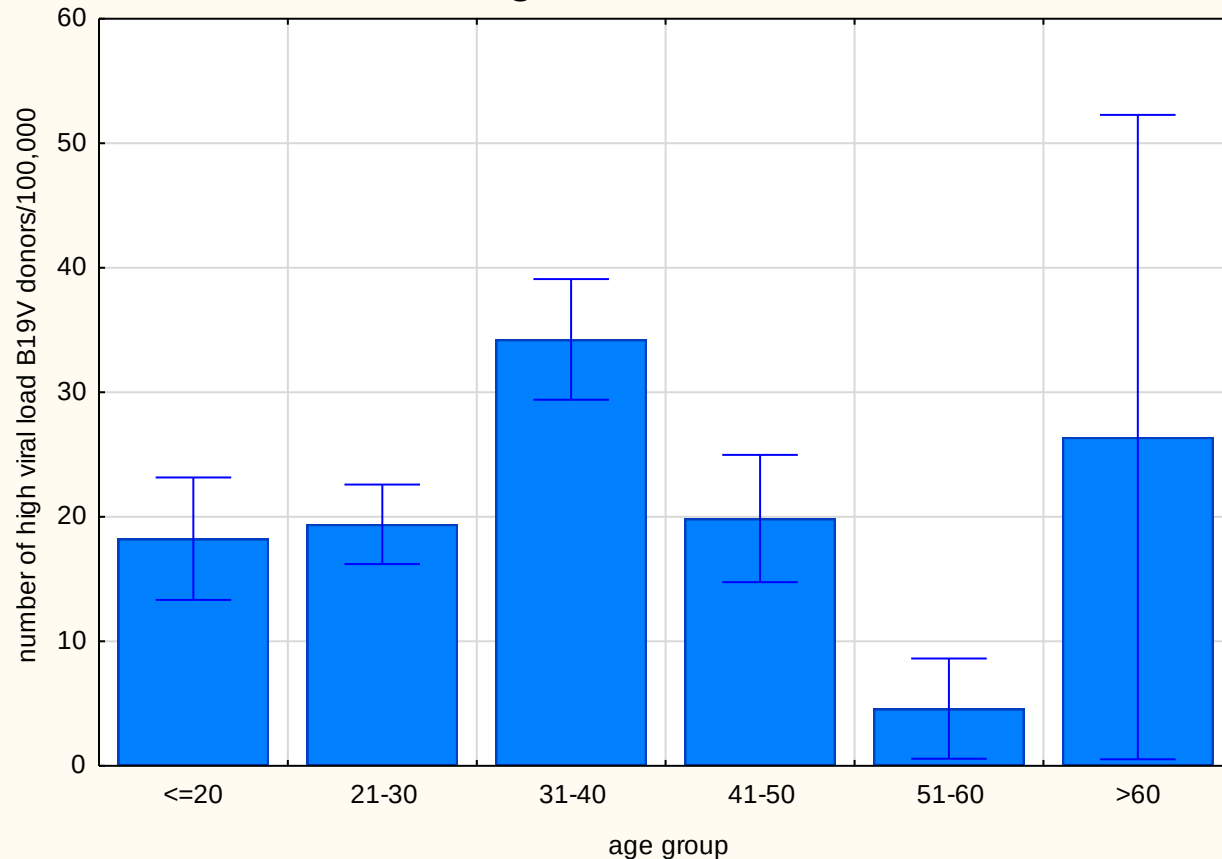
high viral load B19V and HAV frequency in females vs males,
Poland, 2013-2018



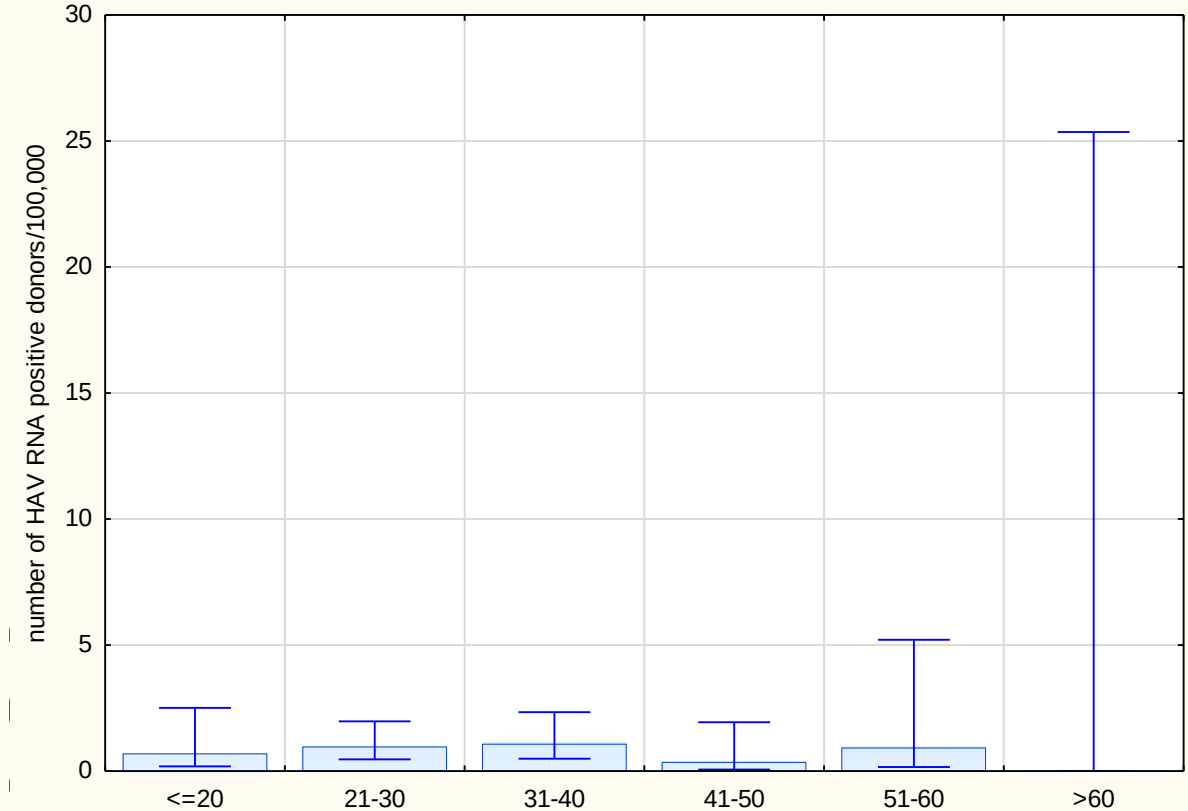
Characteristics of non-enveloped virus infections in blood donors (2)

high viral load B19V and HAV frequency in age groups,
Poland, 2013-2018

high viral B19V

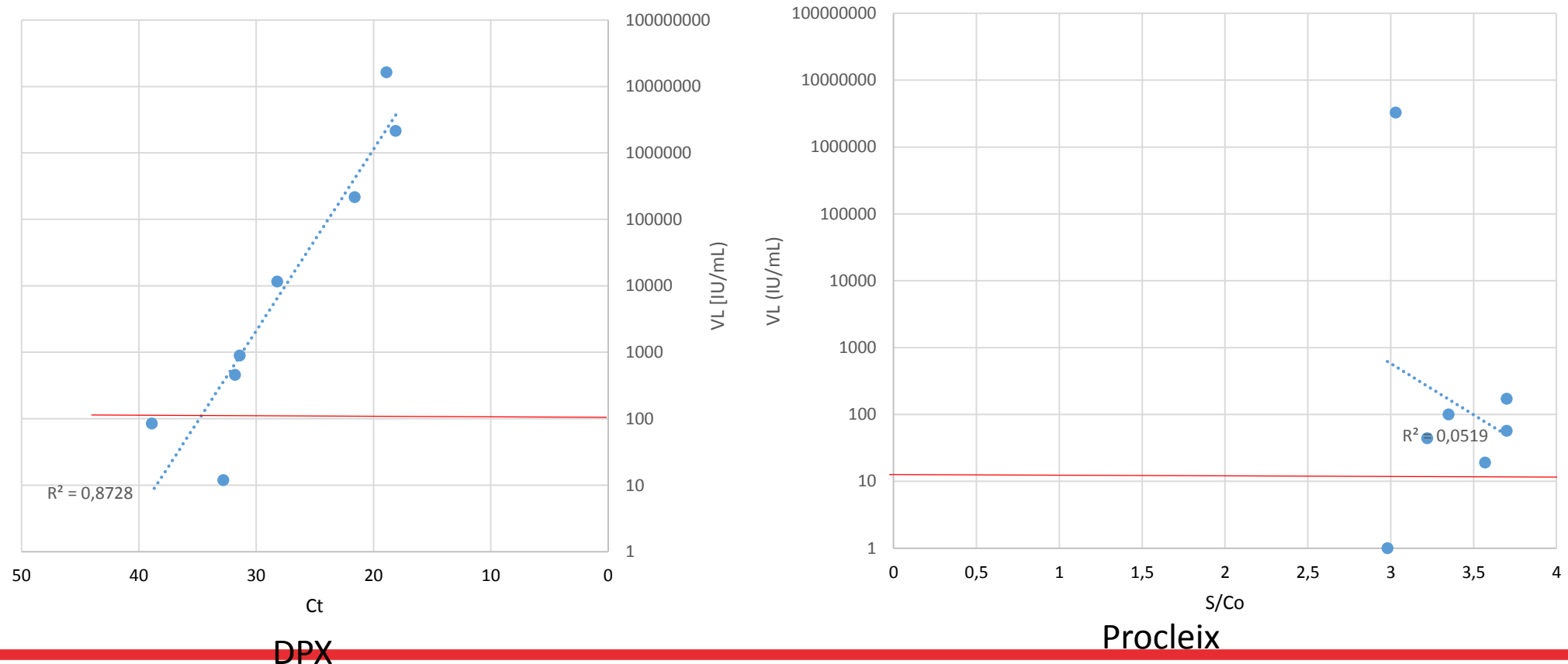


HAV



Virological characteristics of HAV infected donors

viral load in index donations



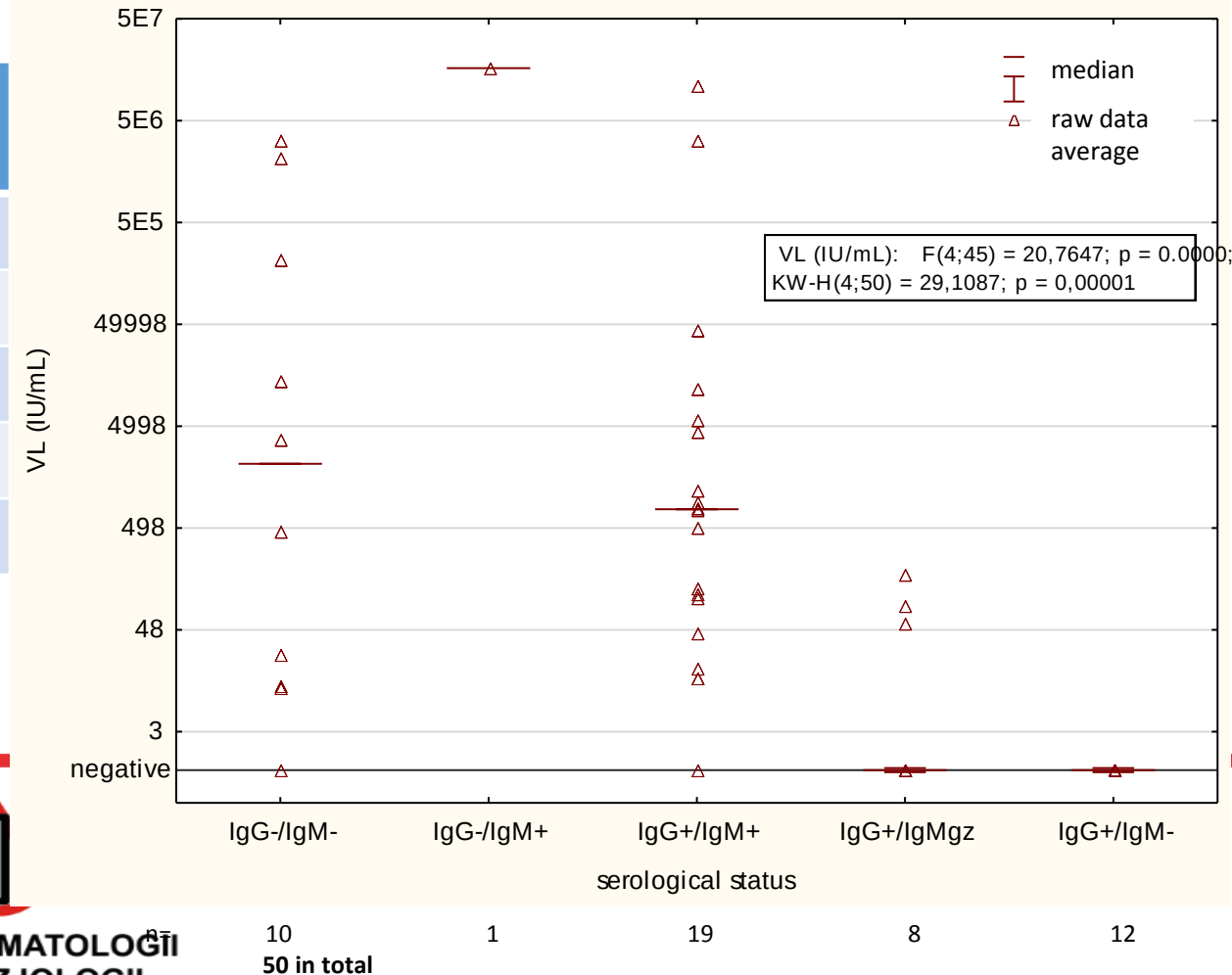
Virological characteristics of HAV infected donors

viral load and serological status

index samples (n=20)

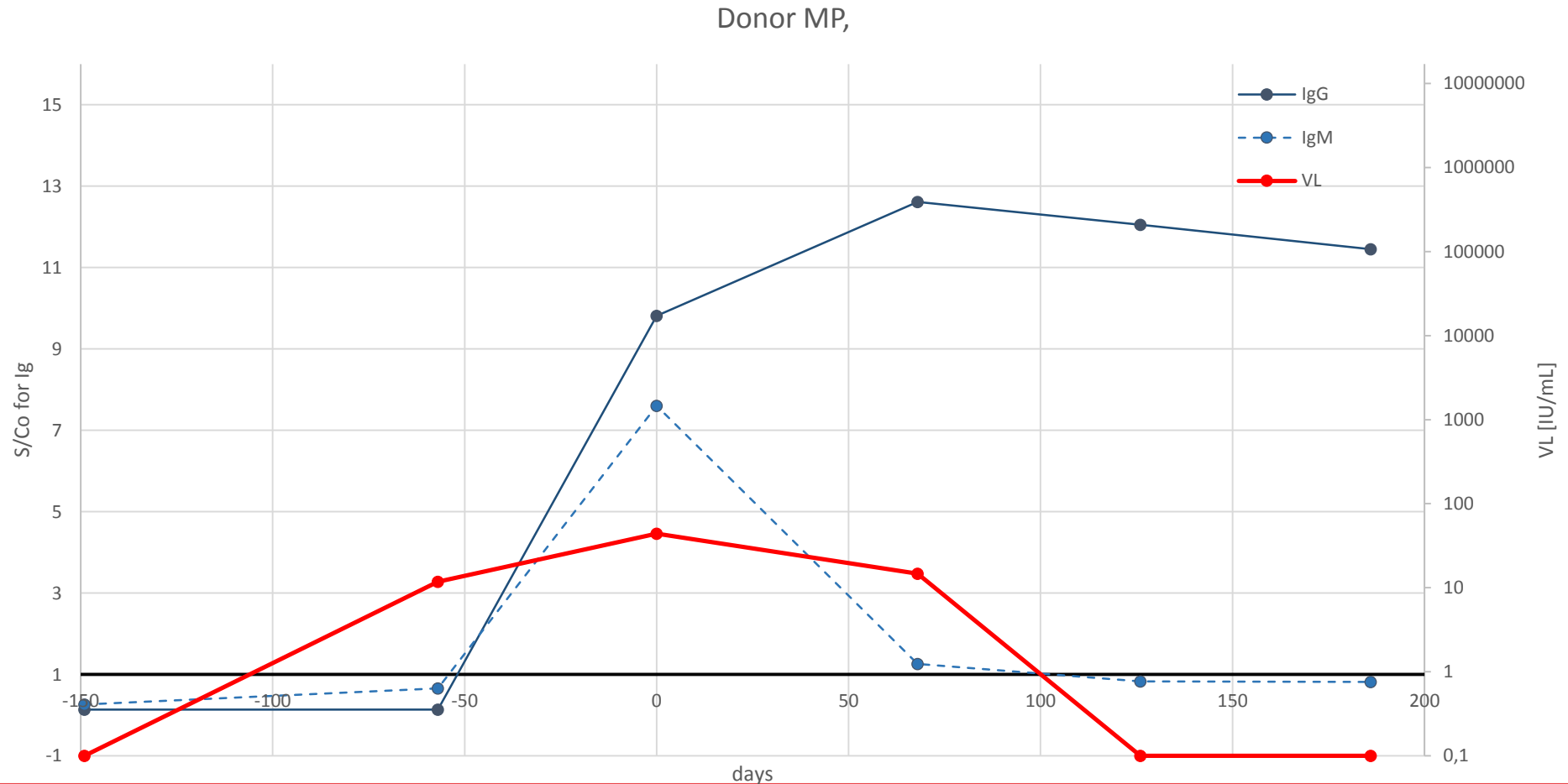
IgG	IgM	number (%)	median VL in IU/ml (range)
negative	negative	7 (35%)	3,810 (12-3,280,000)
negative	positive	1 (5%)	16,400,000
positive	positive	9 (45%)	898 (19-11,300,000)
positive	gray zone	3(15%)	85 (57-172)
		20	679 (12-16,400,000)

index samples+ follow-up samples (n=50)



HAV infection in blood donors

maximum period of RNA HAV detection - 125 days (4.1 months)

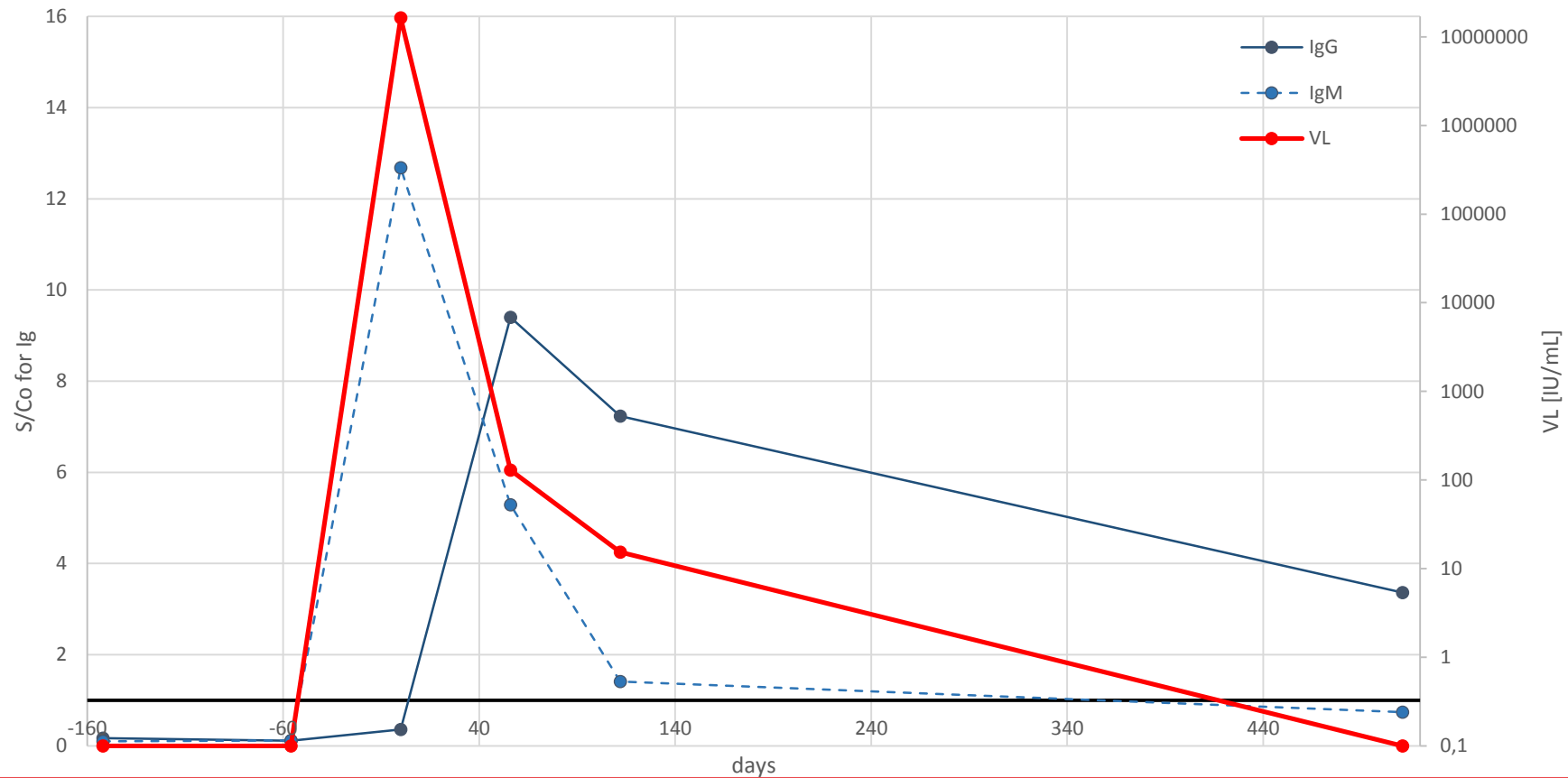


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HAV infection in blood donors

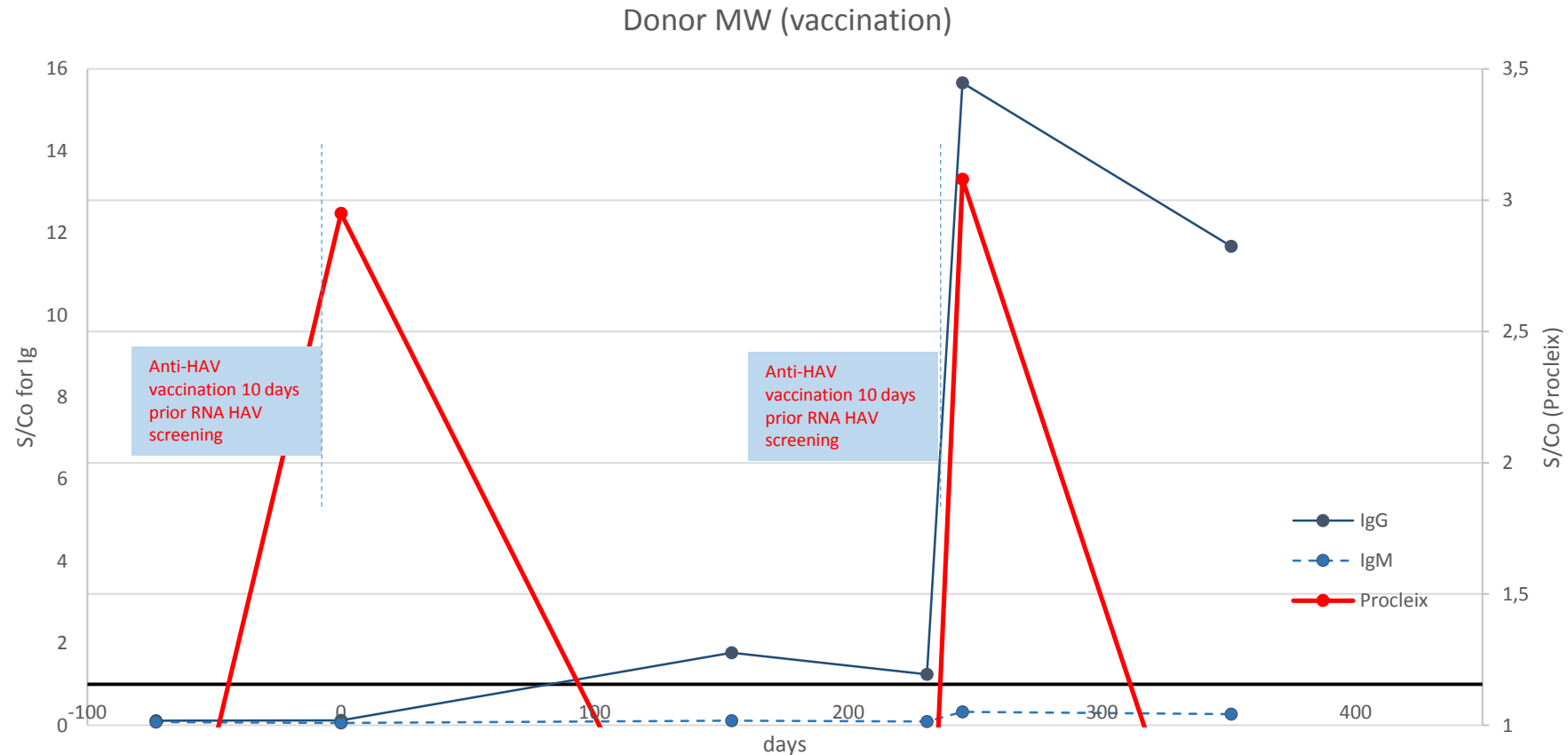
maximum period of specific IgM detection - 113 days (3.8 months)

donor: MSz



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Special case: RNA HAV repeat reactive results in Procleix Parvo/HAV assay 10 days after anti-HAV vaccination



RNA HAV positive blood components follow-up (1)



RNA HAV positive blood components follow-up (2)

	15 donations		
blood components	red blood cells (RBC)	platelets (PLT)	plasma
Total no. prepared 40	15	10	15
Total no. transfused 19 (47.5%)	13 (86.7%)	6 (60%)	0 (0%)

RNA HAV positive blood components follow-up (3)

	TRANSFUSION						
BLOOD COMPONENTS HAV VL serological status: IgG/IgM	RBC 12 IU/mL neg/neg	RBC 11,600 IU/mL pos/pos	RBC 100 IU/mL pos/pos	RBC 172 IU/mL pos/pos	RBC 26 IU/mL neg/neg	RBC 3,280,000 IU/mL neg/neg	PLT 217,000 IU/mL neg/neg
No. of days between transfusion and HAV markers testing in recipient	48 days	43 days	292 days	210 days	390 days	12 days	53 days
RECIPIENTS HAV VL serological status: IgG/IgM	neg pos/neg	neg pos/neg	neg pos/neg	neg pos/neg	neg pos/neg	2,860,000 pos/neg*	54,600 pos/pos*
Transmission	unlikely	unlikely	indeterminable/indeterminable/indeterminable	indeterminable/indeterminable/indeterminable	indeterminable/indeterminable/indeterminable	probable	probable

*64 year-old patient /neurological ward, no symptoms of hepatitis A after transfusion

**30 year-old oncological patient with bile duct cancer, died due to exacerbation of basic illness, assessment of clinical significance of HAV infection in process

Non-enveloped viruses TTI per year estimation

	HAV	HEV	high VL B19V
Frequency per 100,000	2.7	47.4	10-65
Extrapolated no. of infected donors per 600,000 (per ~1 year)	16	284	60-390
Estimated infectivity	2/7 (29%)*	42%***	22.6%**
Estimated no. of highly infected donors per year	4-5	119	14-88

* **current study; previous reports :infectivity range 8.3%-16.7%** (Gallian P et al. Hepatitis A: an epidemiological survey in blood donors, France 2015 to 2017. Euro Surveill. 2018 May;23(21). doi: 10.2807/1560-7917.ES.2018.23.21.1800237; Kim MJ et al. Identification of transfusion-transmitted hepatitis A through postdonation information in Korea: results of an HAV lookback (2007-2012). Vox Sang. 2018 Jul 13. doi: 10.1111/vox.12672)

**B19V infectivity estimated for seronegative recipients receiving high VL B19V blood components from seronegative blood donors (frequency of seronegative blood donors (IHTM data) with high VL X frequency of average seronegativity in general population Siennicka J, [Seroprevalence study of parvovirus B19 in Poland]. Przegl Epidemiol. 2006;60(3):571-80. – $48\% \times 47\% = 22.6\%$).

*** Hewitt PE et al. Hepatitis E virus in blood components: a prevalence and transmission study in southeast England. Lancet. 2014 Nov 15;384(9956):1766-73

Questions to consider

- **clinical significance of transfusion-transmitted HAV and high VL B19V infections**

Still limited number of TTI observations

- **real infectivity of B19V and HAV via blood components**

Role of VL and neutralizing antibodies in donors and recipients

- **HAV/B19V plasma test always to be performed immediately after collection (?)**

Significant proportion of cellular blood components are transfused despite infection identified in screening for manufacturers (delay)

- **solutions to be discussed:**
 - inclusion of HAV/HEV testing in HCV/HBV/HIV multiplex
 - obligatory testing in epidemic periods

Thank you for your attention!



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