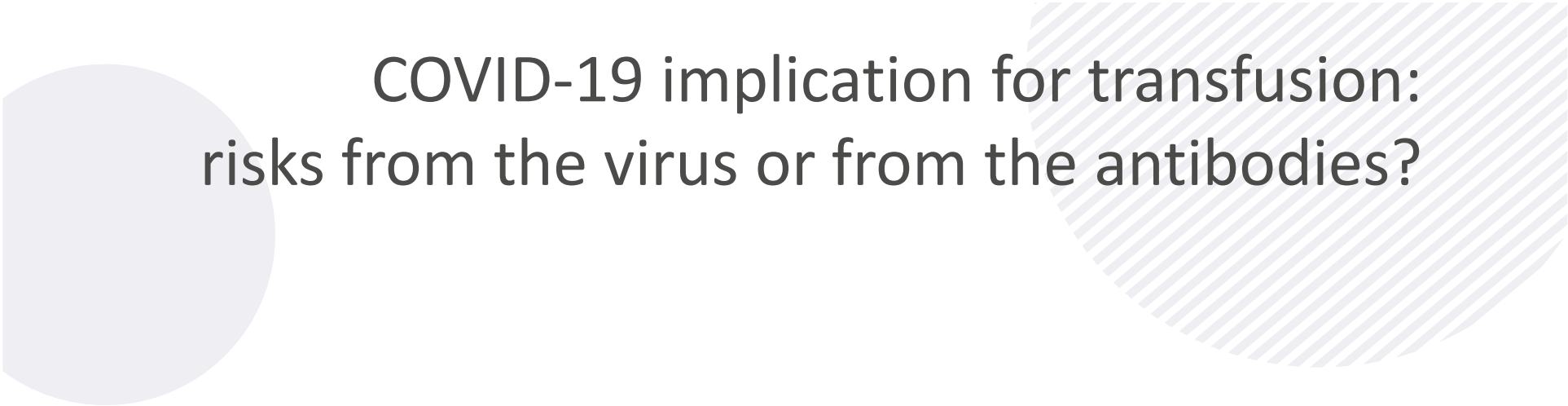




COVID-19 implication for transfusion: risks from the virus or from the antibodies?

Pierre Tiberghien

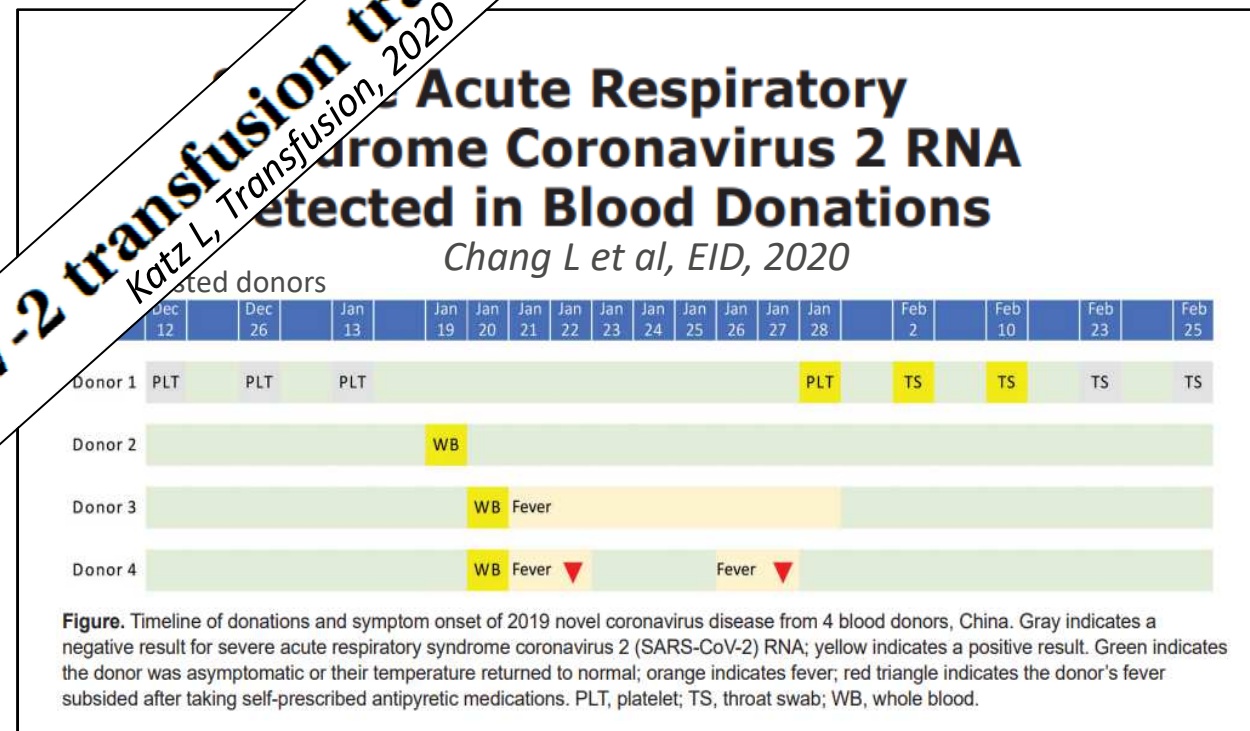
Etablissement Français du Sang,
Université de Franche-Comté

IPFA /PEI 28th International Workshop on Surveillance and
Screening of Blood-borne Pathogens
September 21th, 2022

COVID-19 implication for transfusion: risks from the virus?

- Respiratory viruses such as influenza do not typically cause viraemia.
- Detection of viral RNA in the blood after a respiratory infection is common.
- With the notable exception of « novel » coronavirus such as SARS-CoV (1) and MERS-CoV-2

Is SARS-CoV-2 transfusion transmitted?
Katz L, Transfusion, 2020



No evidence of SARS-CoV-2 transfusion transmission despite RNA detection in blood donors showing symptoms after donation

Cappy et al. Blood 2020

- Investigation of 354 donations collected early 2020; including 311 associated with a **post-donation information** (PDI) and 43 **trace-back donations** (blood components transfused to 4 immunocompromised COVID-19 patients).
- 4/311 (1.3 %) PDI samples tested positive** for SARS-CoV-2 RNA with **high Ct values** (Ct > 36), suggesting low viral loads. **Viral culture were negative** (3/4).

Table 1. Results of biological investigations of the four SARS-CoV-2 positive blood donations

DETECTION BY REAL TIME PCR ON NEAT PLASMA						DETECTION BY REAL TIME PCR AFTER UC			RT-PCR ³ / SEQUENCING		VIRUS CULTURE	
n positive/n tested (Ct)						n positive/n tested (Ct)						
R-GENE® PCR1 ¹		R-GENE® PCR2 ¹		NRC rtPCR ²		R-GENE® PCR1		R-GENE® PCR2				
# donation	N	RdPd	E	RdPd target 1	RdPd target 2	N	RdPd	E	plasma	after UC	plasma	after i-UC
1	POS 1/1 (37.9)	NEG 0/1	NEG 0/1	NEG 0/4	POS 2/4 (37.0; 37.1)	POS 1/1 (35.3)	POS 1/1 (41.6)	NEG 0/1	NEG	POS 1/1	NEG	NEG
2	POS 1/1 (37.5)	NEG 0/1	NEG 0/1	POS 1/3 (39.3)	POS 1/3 (37.4)	POS 1/2 (37.0)	NEG 0/2	NEG 0/1	NEG	POS 1/2	NEG	NEG
3	POS 1/1 (38.0)	NEG 0/1	POS 1/1 (38.4)	NEG 0/2	NEG 0/2	POS 1/2 (38.3)	NEG 0/2	POS 1/1 (42.2)	NEG	NEG 0/2	NEG	NEG
4	POS 1/1 (36.91)	NEG 0/1	NEG 0/1	POS 2/3 (36.7; 39.5)	POS 2/3 (36.9; 38.4)	Nd	Nd	Nd	POS 1/1	Nd	Nd	Nd

N: nucleocapsid gene, RdPd: polymerase gene, E: envelope protein, Ct: cycle threshold, UC: ultracentrifugation (12 mL of plasma), i-UC: ultracentrifugation on iodixanol pad (10.5 mL of plasma)

POS: positive, NEG: negative, Nd: Not done

¹ SARS-CoV-2 R-GENE[®] assay (BioMérieux)

² real time PCR from the National Reference Center for respiratory viruses (NRC rtPCR)

³ RT-PCR primers: 5'-GCTTTTCAAACGTCAAACC-3' and 5'-GGTGAGGGTTTCTACATCA-3'; hemi-nested PCR primers: 5'-TGCTGTGCTAAGGGTTTCT-3' and 5'-GGTGAGGGTTTCTACATCA-3'.

- None of the tested traced-back repository samples tested positive for SARS-CoV-2 RNA.

Risk of transmission of severe acute respiratory syndrome coronavirus 2 by transfusion: A literature review

Leblanc et al, Transfusion, 2020

Viral Cultures for Coronavirus Disease 2019 Infectivity Assessment: A Systematic Review

Jefferson et al, CID, 2020

- RNAemia, when present, is close to the limit of detection of rRT-qPCR with high cycle threshold (Ct) values (> 30)
- RNAemia tends to be associated with more severe disease
- When present, RNAemia appears to occur early in the course of disease (peak within 10 days of symptom onset), and duration of RNAemia is short
- RNAemia does not indicate the presence of infectious virions
- Overall, detecting infectivity in blood is unsuccessful
- In respiratory samples, Ct values above 34 are no longer contagious

Risk of transmission of severe acute respiratory syndrome coronavirus 2 by transfusion: A literature review

Leblanc et al, Transfusion, 2020

Viral Cultures for Coronavirus Disease 2019 Infectivity Assessment: A Systematic Review

Jefferson et al, CID, 2020

- RNAemia, when present, is close to the limit of detection of rRT-qPCR with high cycle threshold (Ct) values (> 30)
- RNAemia tends to be associated with more severe disease
- When present, RNAemia appears to occur early in the course of disease (peak within 10 days of symptom onset), and duration of RNAemia is short
- RNAemia does not indicate the presence of infectivity
- Overall, detecting infectivity in blood is unsuccessful
- In respiratory samples, Ct values above 34 are not

Sterile RNAemia?

- Viral load below a given threshold?
- Inhibitory factors in the blood?
- Defective viral genomes?
- RNA debris resulting from immune cell uptake and degradation?

Minipool testing for SARS-CoV-2 RNA in United States blood donors

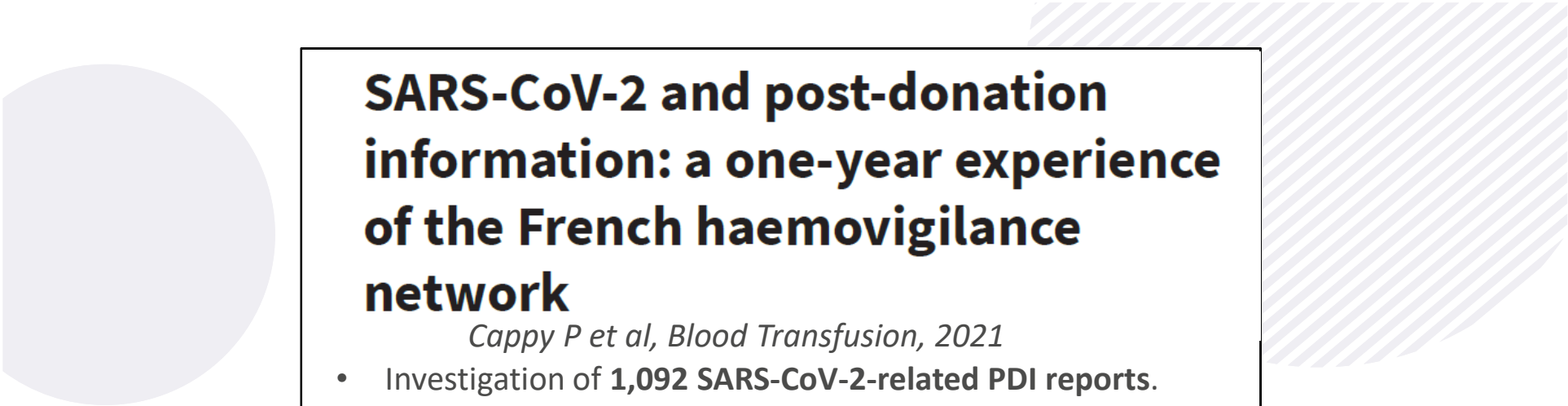
Bakkour S, et al. Transfusion, 2021

- **17,995 minipools** (6 or 16), ≈258,000 donations, tested for vRNA between March and September 2020.
- **Three confirmed reactive** minipools of 16
- **Estimated prevalence** of vRNA reactive donations: **1.16/100,000** (95% CI 0.40, 3.42).
- The vRNA-reactive samples were **non-reactive for Ab**
- Estimated viral loads of the positive donations ranged from <1000 to <4000 copies/ml.
- When tested, **no infectivity** was observed in inoculated permissive cell cultures.

Low rate of RNAemia in blood donations collected during the first wave of COVID-19 in France

Lecam S, et al. Transfusion, 2021

- **2818 minipools** (4)(9672 random donations) tested for vRNA during the first wave
- **Five MP** were **weakly reactive**. One out of the 20 plasmas included in these MP4 **one exhibited low-level reactivity** (RT-PCR, Procleix SARS CoV-2), confirmed upon sequencing.
- **Estimated prevalence** of vRNA reactive donations: **1.03/10,000** (95% CI 0–3.1).
- All 20 plasmas **non-reactive for Ab**, **no cytopathic effects** in cell culture
- When recalled, the index-donor reported having had symptoms compatible with SARS-CoV-2 infection a few days after donation.
- Recipients (2 immunocompromised patients) of RBC and (pathogen-reduced) pooled whole blood platelet did not develop COVID-19.



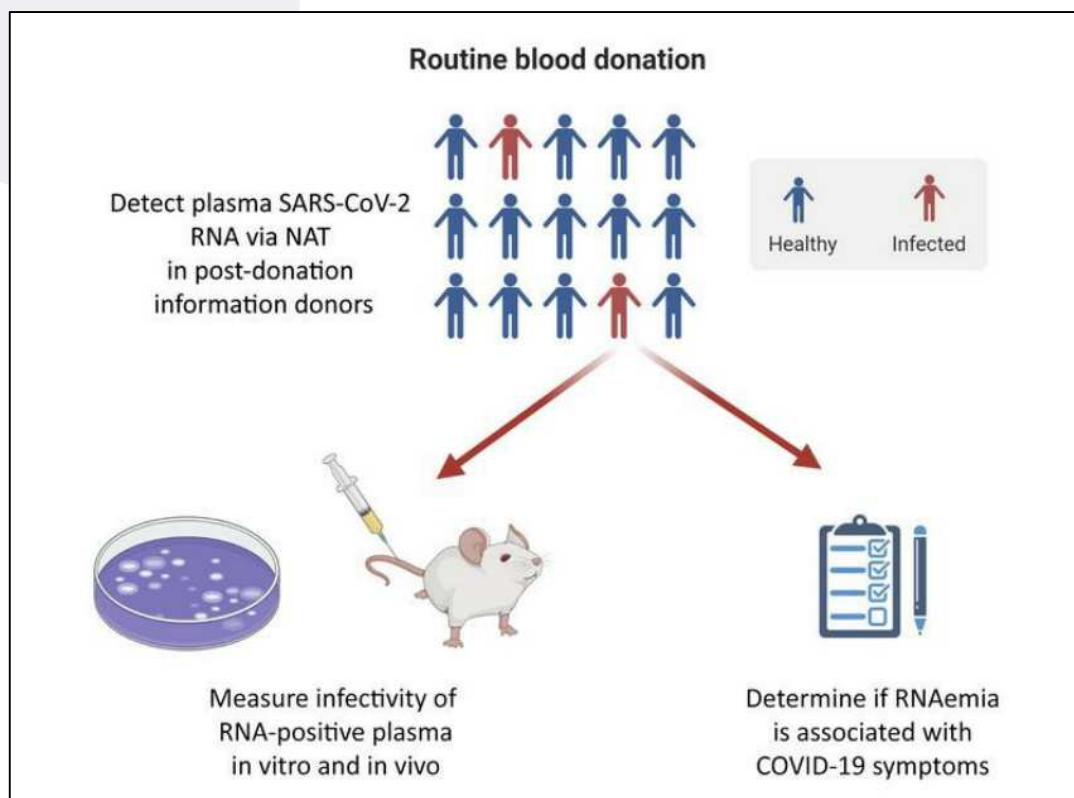
SARS-CoV-2 and post-donation information: a one-year experience of the French haemovigilance network

Cappy P et al, Blood Transfusion, 2021

- Investigation of **1,092 SARS-CoV-2-related PDI reports**.
- Sixty-eight percent reported a positive nasopharyngeal RT-PCR or antigenic testing and 22% of these also had symptoms at the time of testing.
- **Thirty-seven (3.4%) donations with PDI tested positive** for SARS-CoV-2 RNA (Ct between 36.5 and 42) confirming **very low viral loads**.
- There was no difference in demographic data or duration parameter (time elapsed between donation and onset of symptoms, positive RT-PCR or PDI) between RNAemic and non-RNAemic donors.
- Cell culture (Vero E6)(n=26) did not yield viral growth.

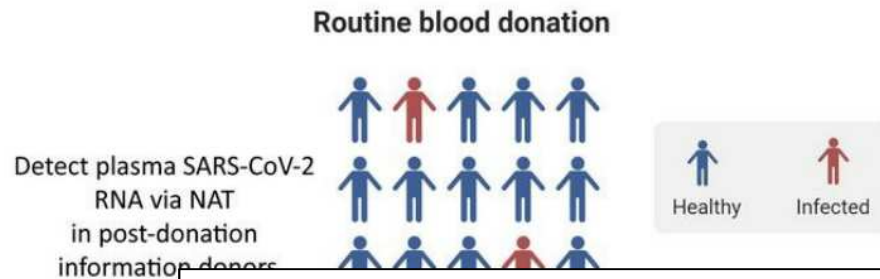
Frequent detection but lack of infectivity of SARS-CoV-2 RNA in presymptomatic, infected blood donor plasma

Saa et al (REDS-IV-P), JCI, 2022



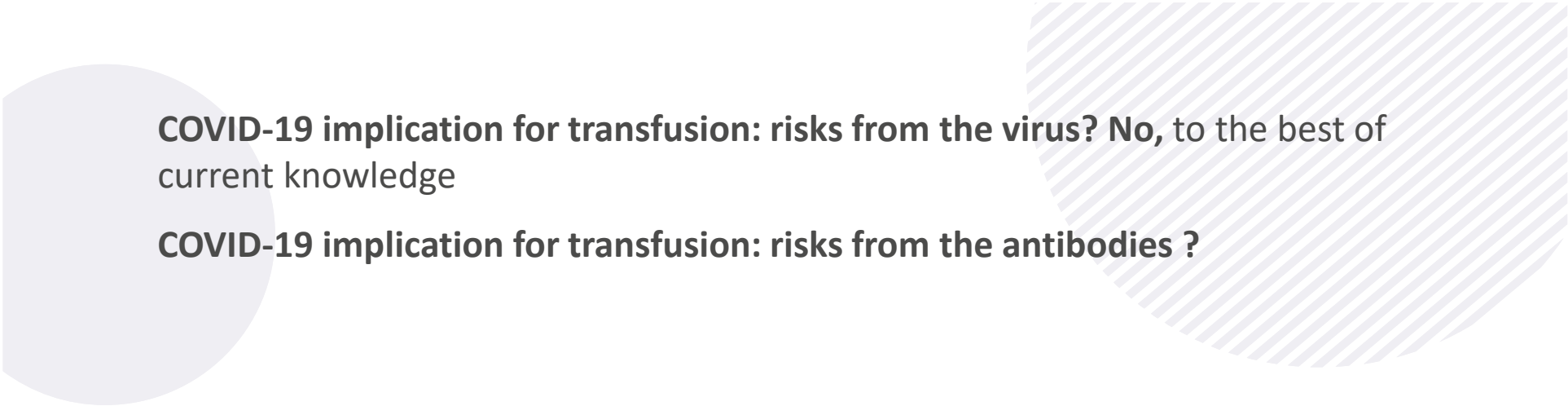
Frequent detection but lack of infectivity of SARS-CoV-2 RNA in presymptomatic, infected blood donor plasma

Saa et al (REDS-IV-P), JCI, 2022



Meas
RNA-
in v

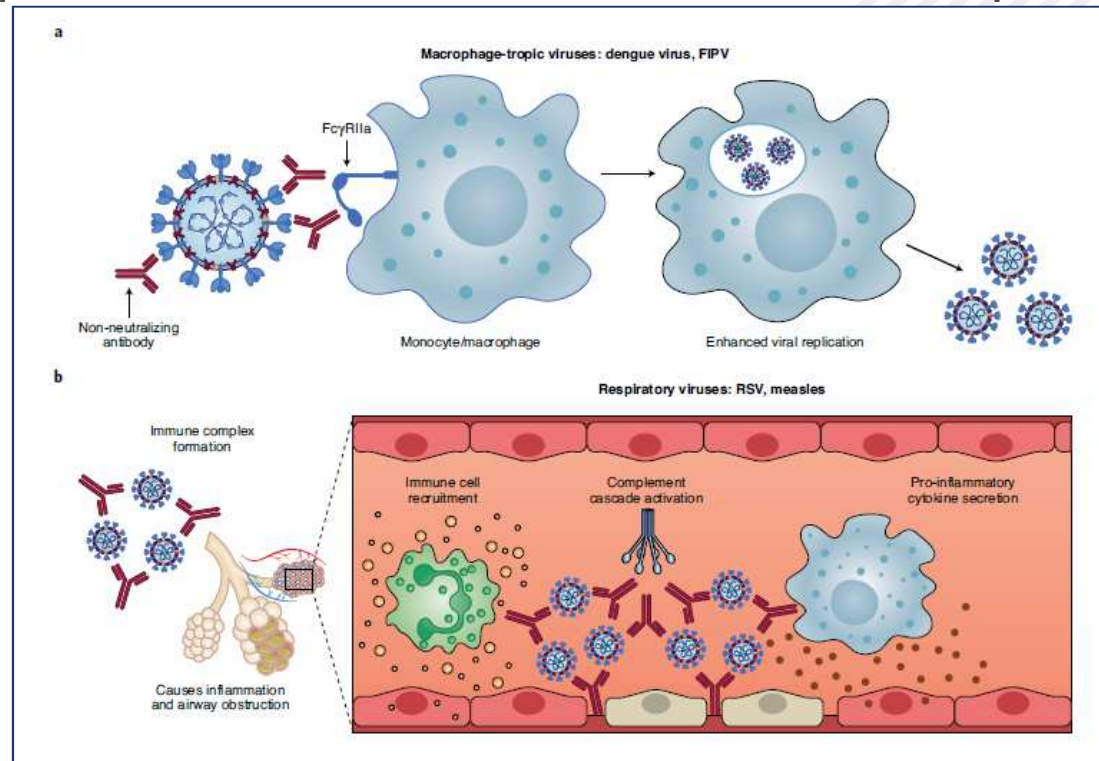
- Reports of symptoms or a diagnosis of COVID-19 after donation preceded or coincided with increased general population COVID-19 mortality.
- 2,250 blood donors who reported possible COVID-19–related post-donation information were tested for SARSCoV-2 RNA.
- **Detection of RNAemia peaked at 9%–15% of PDI donors** in late 2020 to early 2021 and **fell to approximately 4% after implementation of widespread vaccination.**
- RNAemic donors were 1.2- to 1.4-fold more likely to report cough or shortness of breath and 1.8-fold more likely to report change in taste or smell compared with infected donors without detectable RNAemia.
- **No infectious virus was detected** in plasma from RNAemic donors; inoculation of permissive cell lines produced less than 0.7–7 plaque-forming units (PFU)/mL and in susceptible mice less than 100 PFU/mL in RNA-positive plasma based on limits of detection in these models



COVID-19 implication for transfusion: risks from the virus? No, to the best of current knowledge

COVID-19 implication for transfusion: risks from the antibodies ?

Antibody-dependant enhancement / Enhanced respiratory disease



Lee et al, Nature Microbiol, 2020

Two main antibody-dependent enhancement (ADE) mechanisms in viral disease.

a: For **macrophage-tropic viruses** such as dengue virus, **non-neutralizing or sub-neutralizing antibodies** cause increased viral infection of monocytes or macrophages via **FcγRIIa-mediated endocytosis**, resulting in more severe disease

B: For **non-macrophage-tropic viruses** such as RSV and measles, non-neutralizing antibodies can **form immune complexes** with viral antigens inside airway tissues, resulting in the secretion of **pro-inflammatory cytokines**, immune cell recruitment and **activation of the complement** cascade within lung tissue. The **ensuing inflammation** can lead to airway obstruction and can cause **acute respiratory distress syndrome** in severe cases.

Early evidence suggested that immune complex formation, complement deposition and local immune activation present the most likely ADE in COVID-19.

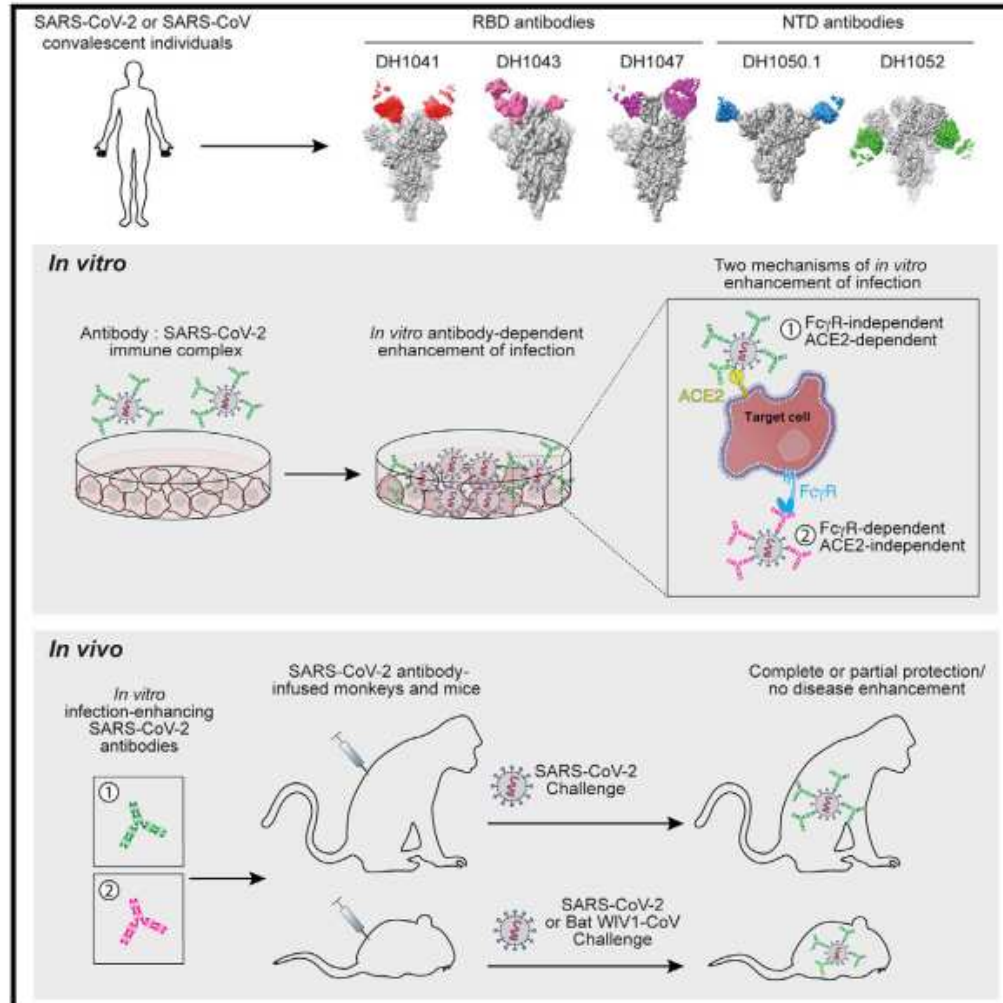
Severe pandemic 2009 H1N1 influenza disease due to pathogenic immune complexes

Monsalvo et al, Nature Medicine, 2011

- Pandemic influenza viruses often cause severe disease in **middle-aged adults without preexisting comorbidities**.
- **Preexisting serum Ab** that cross-react with, but **do not protect** against, 2009 H1N1 influenza virus were found in middle-aged adults.
- High titers of serum Ab of **low avidity** for H1-2009 Ag, and low-avidity **pulmonary immune complexes** against the same protein, were identified in severely ill individuals.
- **C4d deposition**—a marker of **complement activation** mediated by **immune complexes**—was present in lung sections of fatal cases.

***In vitro* and *in vivo* functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies**

Li et al, Cell, 2021



- Isolation of Abs against the receptor-binding domain (RBD) or the N-terminal domain (NTD) of SARS-CoV-2 spike from individuals with acute or convalescent SARS-CoV-2
- **RBD and NTD antibodies exhibit function-specific modes of binding.**
- Select RBD Abs demonstrated **Fc receptor- (FcγR)-dependent infection enhancement *in vitro***, while several non-neutralizing NTD antibodies **mediated FcγR-independent infection enhancement.**
- Both types of infection-enhancing Ab **protected from SARS-CoV-2 replication *in vivo***
- **Three of 46** monkeys infused with enhancing Abs had **higher lung inflammation scores** compared to controls. One monkey had alveolar edema and elevated bronchoalveolar lavage inflammatory cytokines.

CCP transfusion in hospitalized COVID-19 patients (French CCP monitored access program)

Occurrence of acute, often transient, increase in oxygen requirement early after CCP transfusion in a limited number of patients (<5%)

Convalescent plasma for COVID-19 complicated by ARDS due to TRALI

Wang KY et al, BMJ Case Rep, 2021

Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19

Hueso T et al, Blood, 2020

Convalescent plasma improves overall survival in patients with B-cell lymphoid malignancy and COVID-19: a longitudinal cohort and propensity score analysis

Hueso T et al, Leukemia, 2021

CCP transfusion in hospitalized COVID-19 patients (French CCP monitored access program)

Occurrence of acute, often transient, increase in oxygen requirement in patients (< 24h)

Convalescent plasma
ARDS due to COVID-19
Wang et al, 2020

A multicenter randomized open-label clinical trial for convalescent plasma in patients hospitalized with COVID-19 pneumonia

Christina Avendano-Sola et al, JCI, 2021

- **350 patients** were randomly assigned to either CP (n = 179) or SOC (n = 171).
- CCP infusion-related events were reported in 10 patients; **5 were cases of severe worsening of dyspnea, 3 of which were reported as suspected TRALI**. TRALI was ruled out after a full assessment. **All patients recovered** without sequelae except one patient who died on day 31 due to underlying disease
- Mortality at day 28 in favor of CCP: **HR 0.46, 95% CI 0.19–1.14**

Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19

Hueso T et al, Blood, 2020

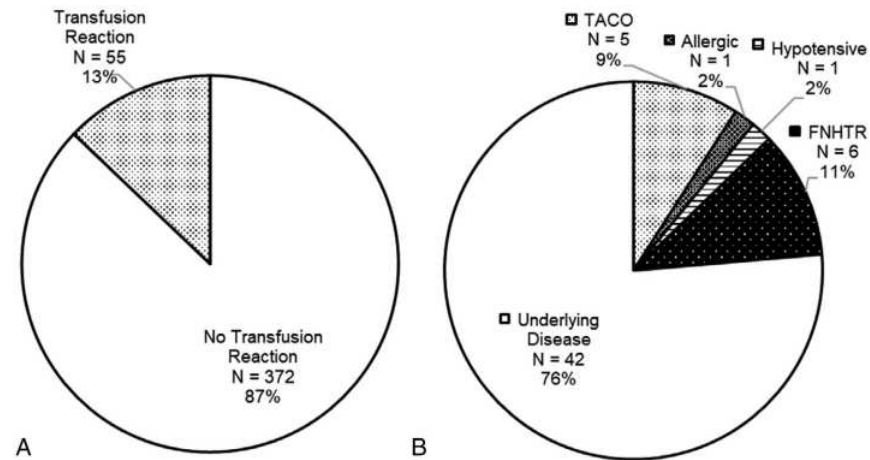
Convalescent plasma improves overall survival in patients with B-cell lymphoid malignancy and COVID-19: a longitudinal cohort and propensity score analysis

Hueso T et al, Leukemia, 2021

Transfusion reactions associated with COVID-19 convalescent plasma therapy for SARS-CoV-2

Nguyen et al, Transfusion, 2020

FIGURE 1 A, Distribution of COVID-19 convalescent plasma transfusion events (total of 427) split by outcome of transfusion vs no transfusion reactions. B, Distribution of transfusion reaction events (total of 55) split by type of transfusion reactions include underlying disease, TACO, TRALI, allergic, hypotensive, and FNHTR. FNHTR, febrile nonhemolytic transfusion reaction; TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury



Transfusion reactions associated with COVID-19 convalescent plasma therapy for SARS-CoV-2

Access to and safety of COVID-19 convalescent plasma in the United States Expanded Access Program: A national registry study

Senefeld et al, Plos Medicine, 2021

PLOS MEDICINE

Expanded access to COVID-19 convalescent plasma

95,000 patients

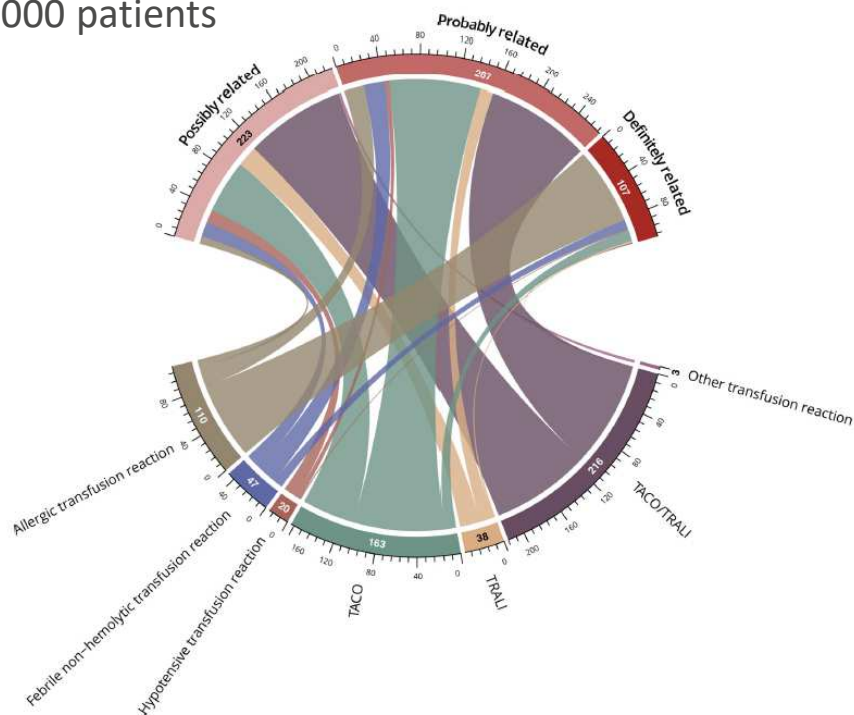
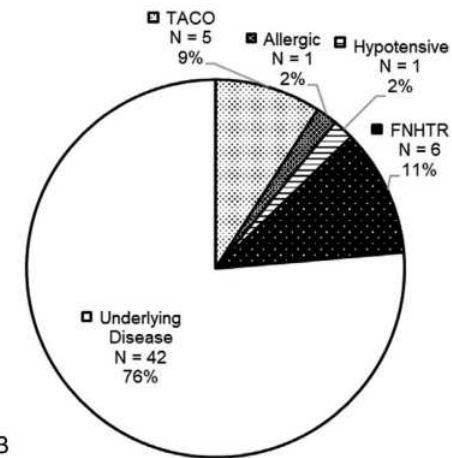


Fig 8. Chord diagram of the attributions associated with serious transfusion reactions. Attribution to a category of relatedness is depicted by a line connecting from each serious transfusion reaction type. The width of each line and the circumferential axis indicate the number of patients in each combined serious transfusion reaction and relatedness category group. Other transfusion reactions that were adjudicated to be possibly related to the transfusion (n = 3) included leukomoid reaction (n = 1), pulmonary embolism (n = 1), and red cell dilution (n = 1). TACO, transfusion-associated circulatory overload; TRALI, transfusion-related acute lung injury.

<https://doi.org/10.1371/journal.pmed.1003872.g008>

usion, 2020



B

sed reporting to ANSM

9 D'IMPUTABILITE POSSIBLE, PROBABLE OU CERTAINE,
E DE PSL, ENQUETE TERMINEE / 100 000 BC

les L*	CGR*	Plaquettes*	Plasma*
100.6	125.5	76.2	1.5

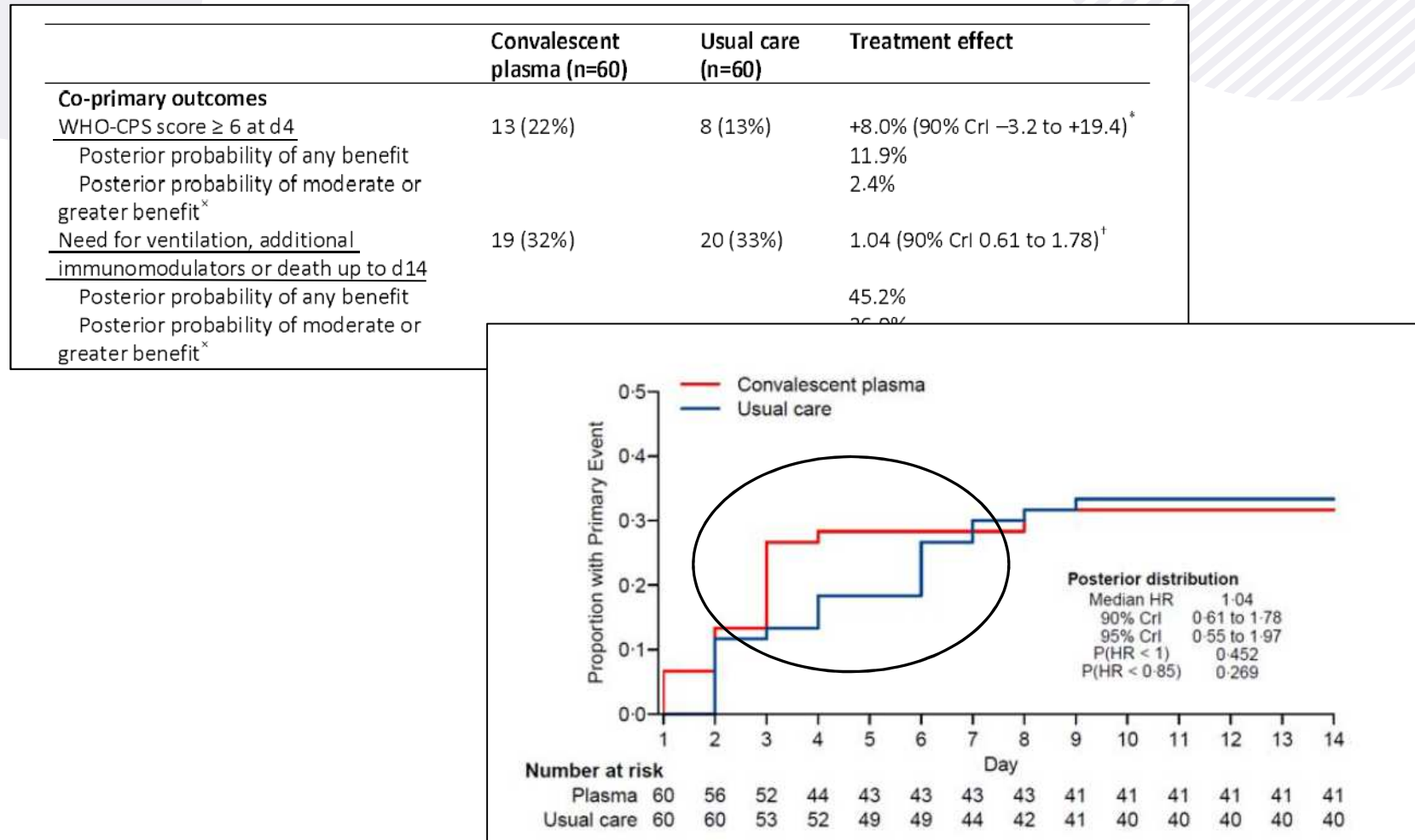
Orientation diagnostique
Allergie
CÉdème pulmonaire et surcharge
Hémolyse autre
Hémolyse

/ 100 000 BC

Nombre FIP			Tous les PSL*	CGR*	Plaquettes*	Plasma*
Plasma	Tous les PSL	%	109.6	125.5	76.3	4.5
28	40	56.3%	53.1	55.3	75.1	4.1
1	26	36.6%	31.0	9.1	127.3	113.7
0	2	2.8%	10.2	6.2	47.3	0.8
0	2	2.8%	9.7	11.3	2.1	4.1
1	1	1.4%	7.9	9.3	4.0	0.0
30	71	100%	3.0	2.8	6.1	1.1
11.3	2.3	.	2.5	2.4	3.4	1.9
			2.5	2.7	2.4	1.5
			1.5	0.3	11.3	0.0
transfusionnelle						
Hémosidérose			38	1.2	1.6	0.0
Hémolyse drépanocytaire			26	0.9	1.1	0.0
Mésocytose			88	0.3	0.9	0.0
n	Allergy (/ 100 000)	TACO, TRALI (/ 100 000)	Ratio Allergy / TACO, TRALI	0.4	1.2	0.8
				0.4	0.6	1.1
	114	6	22,8	0.1	1.8	0.0
				0.0	0.0	0.4
	10,5	(<) 1	10,5	0.0	0.0	0.0
				0.0	0.0	0.0
				0.0	0.0	0.0
				0.0	0.3	0.0
	116	483	0,2	229.6	359.3	134.4

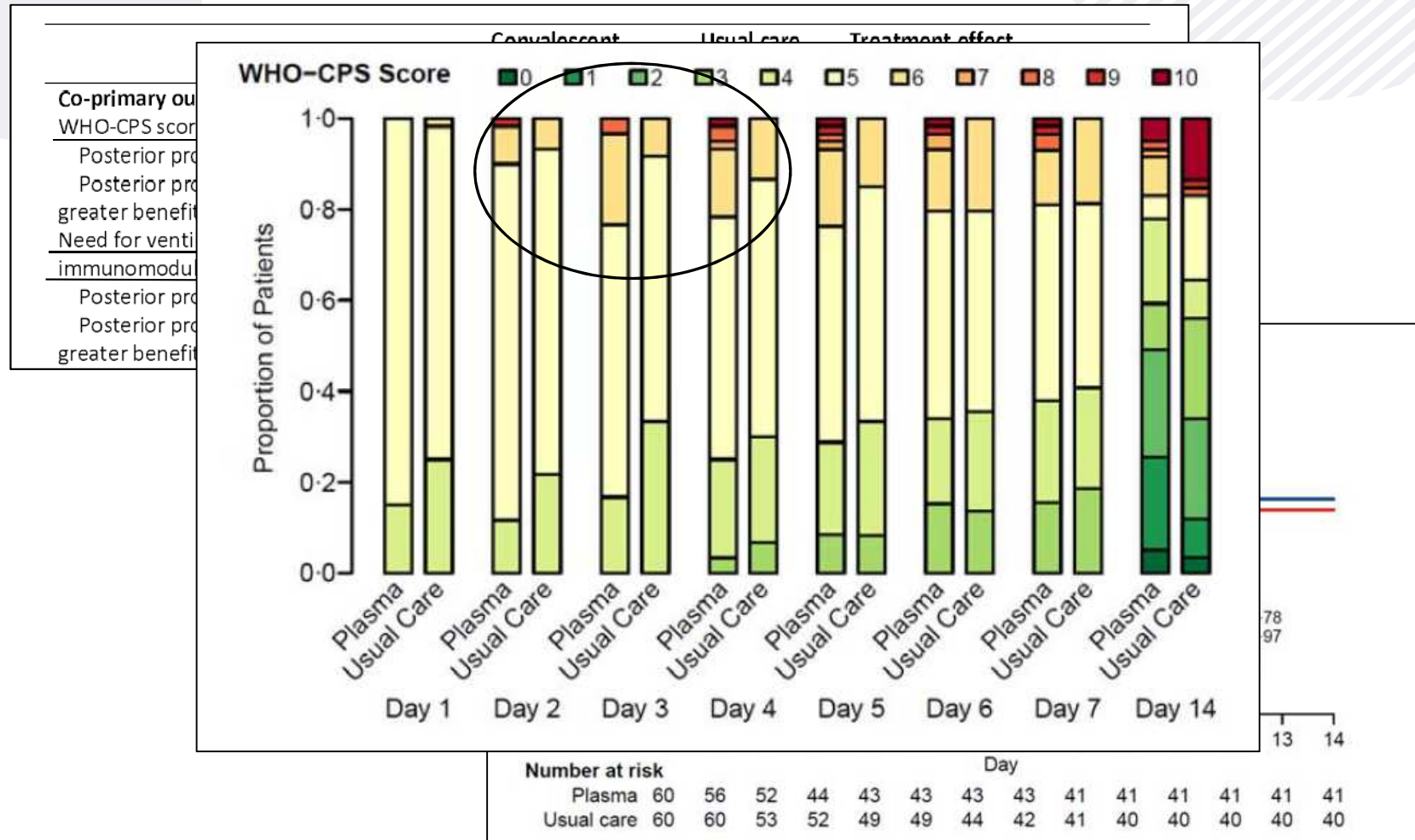
Efficacy and safety of convalescent plasma to treat hospitalised COVID-19 patients with or without underlying immunodeficiency: a randomized clinical trial

Lacombe et al, medRxiv, 2022



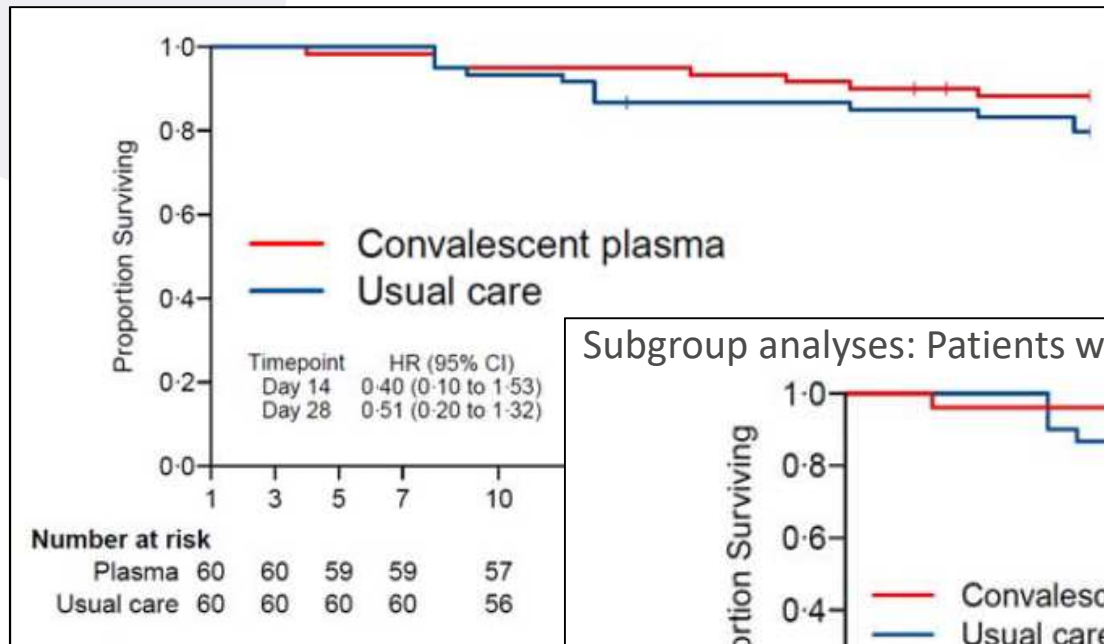
Efficacy and safety of convalescent plasma to treat hospitalised COVID-19 patients with or without underlying immunodeficiency: a randomized clinical trial

Lacombe et al, medRxiv, 2022

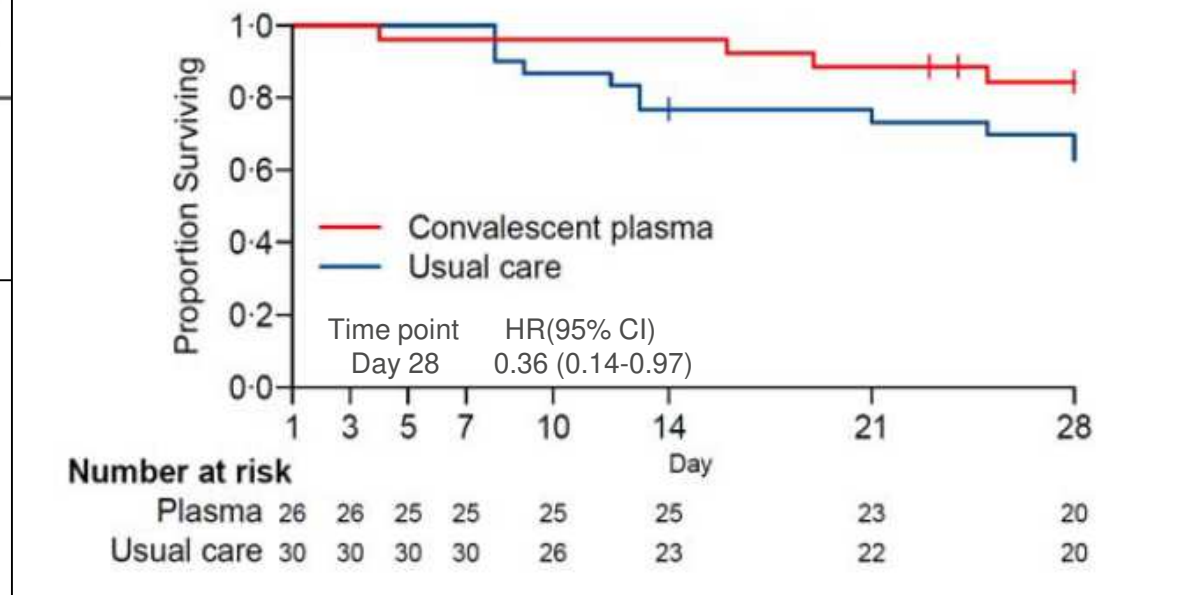


Efficacy and safety of convalescent plasma to treat hospitalised COVID-19 patients with or without underlying immunodeficiency: a randomized clinical trial

Lacombe et al, medRxiv, 2022

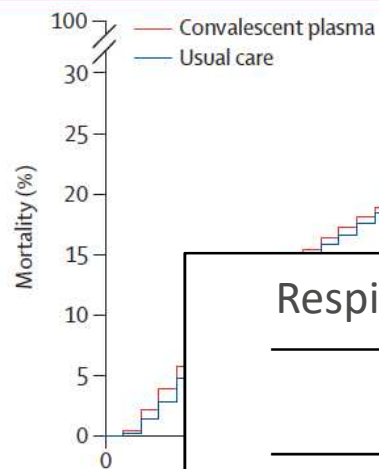


Subgroup analyses: Patients with underlying immunodeficiency



Convalescent plasma in patients admitted to hospital with COVID-19 (RECOVERY): a randomised controlled, open-label, platform trial

RECOVERY Collaborative Group, Lancet, 2021



Number at risk
Convalescent plasma 5795
Usual care 5763

Figure 2: Effect of allocation to c

Respiratory adverse events up to 72 hours

	Convalescent Plasma (n=5795)	Usual Care (n=5763)
Number with form completed	5267	5128
Sudden worsening in respiratory status		
No additional support required	66 (1%)	68 (1%)
New or increased use of O ₂	681 (13%)	660 (13%)
New non-invasive respiratory support	447 (8%)	435 (8%)
New invasive mechanical ventilation	163 (3%)	160 (3%)
Other	32 (1%)	22 (<1%)
Total: Any sudden worsening in respiratory status	1130 (21%)	1132 (22%)
Persistent worsening	729 (14%)	725 (14%)

Early Outpatient Treatment for Covid-19 with Convalescent Plasma

Sullivan et al, NEJM, 2022

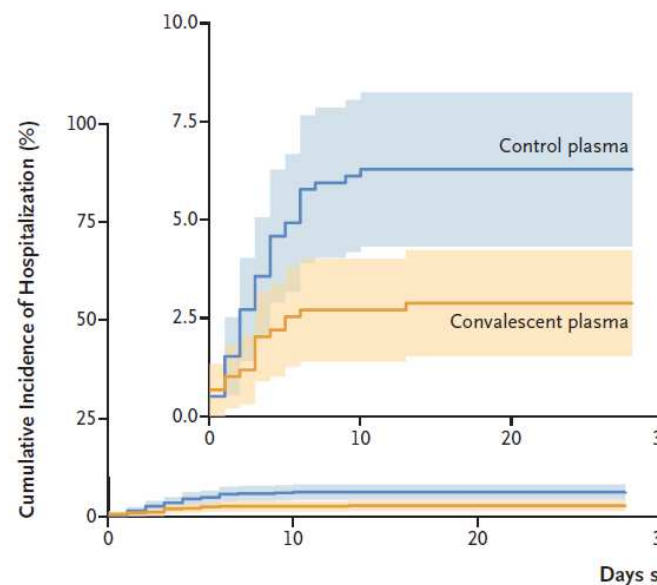
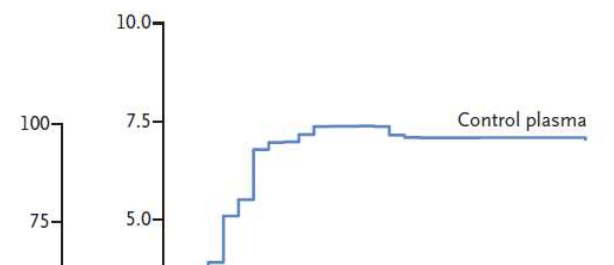


Figure 2. Cumulative Incidence of Coronavirus Disease 2019–Related Hospitalization. On the left, the results of the unadjusted analysis are shown. Shaded areas represent confidence intervals according to the adjusted targeted minimum loss–based estimation.

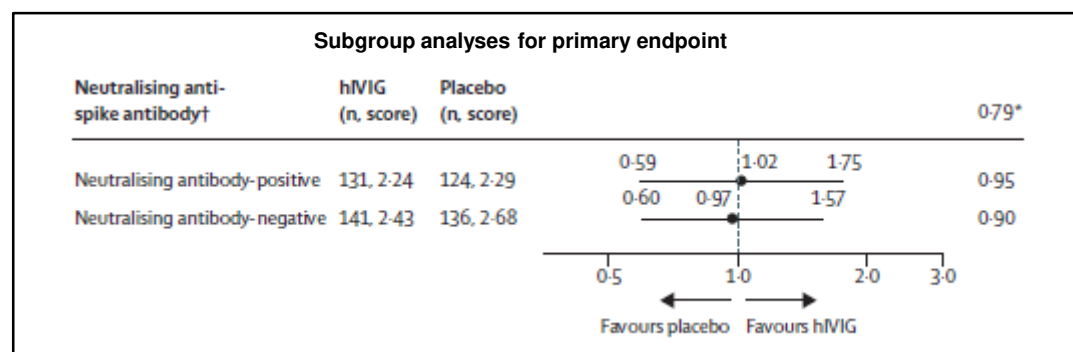


	Control (N=589)	Convalescent (N=592)	P-value ²
Number randomized, n	615	610	
Number transfused, n	589	592	
Number of grade 3 or 4 adverse events, n	55	34	0.13
Dyspnea	1 (1.8%)	0 (0%)	
Hypoxia	3 (5.5%)	0 (0%)	
Pneumonitis	2 (3.6%)	0 (0%)	

Hyperimmune immunoglobulin for hospitalised patients with COVID-19 (ITAC): a double-blind, placebo-controlled, phase 3, randomised trial

The ITAC (INSIGHT 013) Study Group, Lancet, 2022

	hIVIG (n=295)	Placebo (n=284)	OR, RRR, or HR (95% CI)	p value
Efficacy outcomes				
Primary				
Ordinal outcome at day 7*	NA	NA	1.06 (0.77–1.45)	0.72
Secondary				
Number reaching one of the two most favourable categories (categories 1 and 2)†	178	160	1.11 (0.91–1.35)	0.30
Number discharged from hospital or reached most favourable category (category 1)‡	268	252	1.07 (0.92–1.26)	0.37



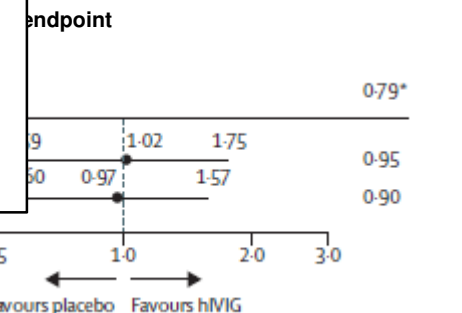
Hyperimmune immunoglobulin for hospitalised patients with COVID-19 (ITAC): a double-blind, placebo-controlled, phase 3, randomised trial

The ITAC (INSIGHT 013) Study Group, Lancet, 2022

Figure S2. Composite safety outcome (death, SAE, grade3 or 4 events, organ failure or serious infection) through Day 7 subgroup analyses

Subgroup	hIVIG No.	hIVIG %	Placebo No.	Placebo %	Odds Ratio (hIVIG/Placebo) and 95% CI	P-val.	Int. P-val.*
Days since symptom onset							0.78
< 6 days	82	22	52	19.2	1.27	0.62	
6-7 days	65	30.8	62	29	1.21	0.64	
8-9 days	68	25	70	28.6	0.72	0.43	
10-12 days	80	20	100	22	0.96	0.91	
Neutralizing anti-spike antibody**							0.001
nAb Positive	133	26.3	128	16.4	2.21	0.02	
nAb Negative	141	22.7	137	34.3	0.51	0.02	

“Most safety events were of a respiratory nature, including increasing dyspnea, increasing oxygen requirements and respiratory failure”



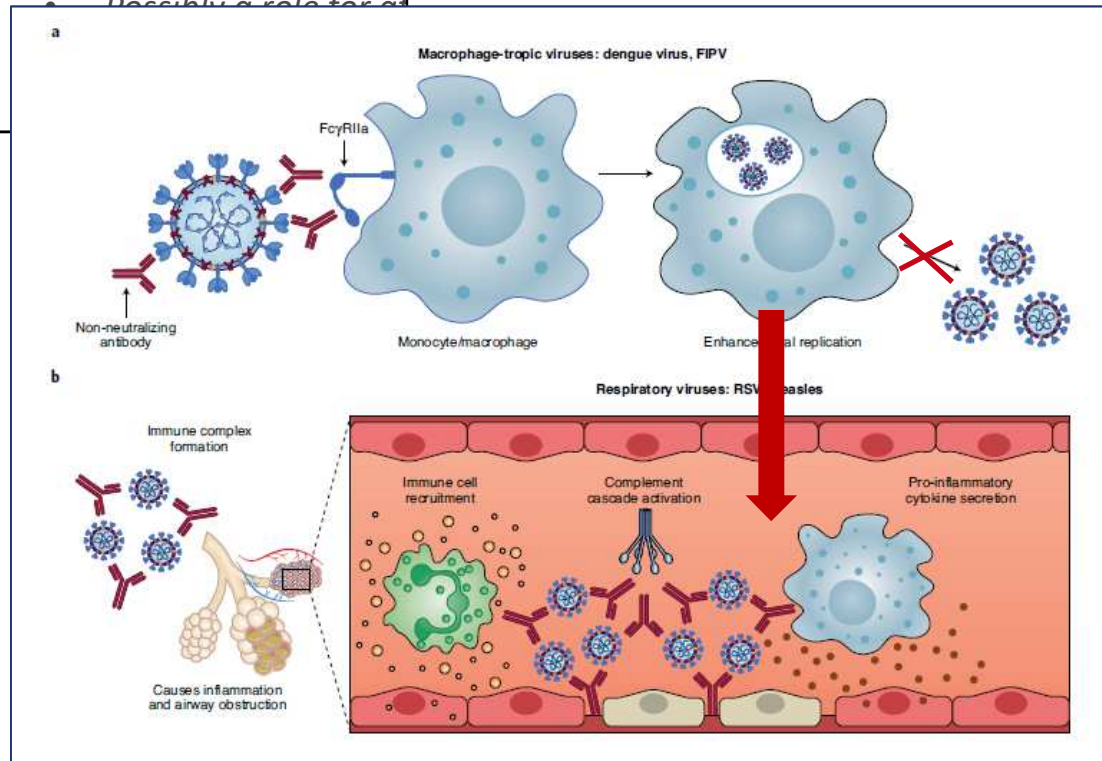
FcγR-mediated SARS-CoV-2 infection of monocytes activates inflammation

Junqueira et al, Nature, 2022

- Antibody-mediated SARS-CoV-2 uptake by monocytes/macrophages triggers inflammatory cell death that aborts production of infectious virus but causes systemic pathology.
- Vaccine recipient platelets enhance infection
- Possibly a role for...

Inflammasome activation in infected macrophages drives COVID-19 pathology

Sefik et al, Nature, 2022



masomes, release IL-1

on SARS-CoV-2

2.

n leads to the release of
ophages.

n through the
s and suicide by
ral cycle.

COVID-19 implication for transfusion: risks from the virus? No, to the best of our knowledge today

COVID-19 implication for transfusion: risks from the antibodies ? Maybe

Transient lung worsening early after COVID-19 convalescent plasma transfusion

- Only in COVID-19 patients with lung disease / high viral load only?
- Occurring infrequently
- Difficult to distinguish from other causes of ARDS: COVID-19 disease worsening *per se*, TRALI/ALI, or even TACO
- Does/would not prevent a favorable outcome

Further studies are needed to assess:

- Occurrence, severity and characteristics
- Relation with immunization status and with outcome
- Involved COVID-19 convalescent plasma / antibodies
- Impact of vaccination in addition to convalescence:
 - In CCP donors
 - In blood and plasma donors: potential for adverse events in recipients of blood components and IvIg, notably (exclusively?) in SARS CoV-2 infected recipients

Many thanks to the contributors

Karine Lacombe, APHP St Antoine / Sorbonne Université

Thomas Hueso, IGR / Paris Saclay

Anne-Lise Beaumont, Anne-Sophie Godron, APHP St Antoine

Sophie Grabar, Sorbonne Université

And all the involved clinicians

Pascal Morel

Pascale Richard

France Pirenne

Anne François

Stéphane Bégue

Thibaud Bocquet

Sophie Lecam

Brigitte Bonneaud

Lucile Malard

Saadia Jbilou

Anne-Marie Fillet

Syria Laperche

Cathy Bliem

And all EFS colleagues
ensuring the collection,
testing and issuing of CCP

Etablissement
Français du
Sang

Fabrice Cognasse, EFS / Université de St Etienne

Pierre Gallian, EFS / IHU Méditerranée Infection

Xavier de Lamballerie, IHU Méditerranée Infection

Olivier Adotevi, CHU Besançon / Université de Franche-Comté (UFC)

Eric Toussiro, CHU Besançon / UFC

Maxime Desmarests, CHU Besançon / UFC

Antoine Durrbach, APHP Mondor, Paris – Saclay

Pierre Cappy, APHP Henri Mondor

Paul Bastard, IHU Imagine

Laurent Abel, IHU Imagine

Jean-Laurent Casanova, IHU Imagine

Yazdan Yazdanpanah, APHP Bichat / REACTing

Hubert Schrezenmeier, German Red Cross

Lise Estcourt, NHSBT

Dave Roberts, NHSBT

Bart Rjinders, Erasmus MC UMC, Rotterdam

Ellen Van der Schoot, Sanquin

Heli Harvala, NHSBT

Christian Erikstrup, Aarhus University Hospital

Gaia Mori, EBA

Dragoslav Domanovic, EBA

And last but not the least!, our blood donors

With support from Fondation de la Recherche Médicale, Reacting, Sorbonne Université,
l'Etablissement Français du Sang and the European Union (ESI, H2020)