mindray

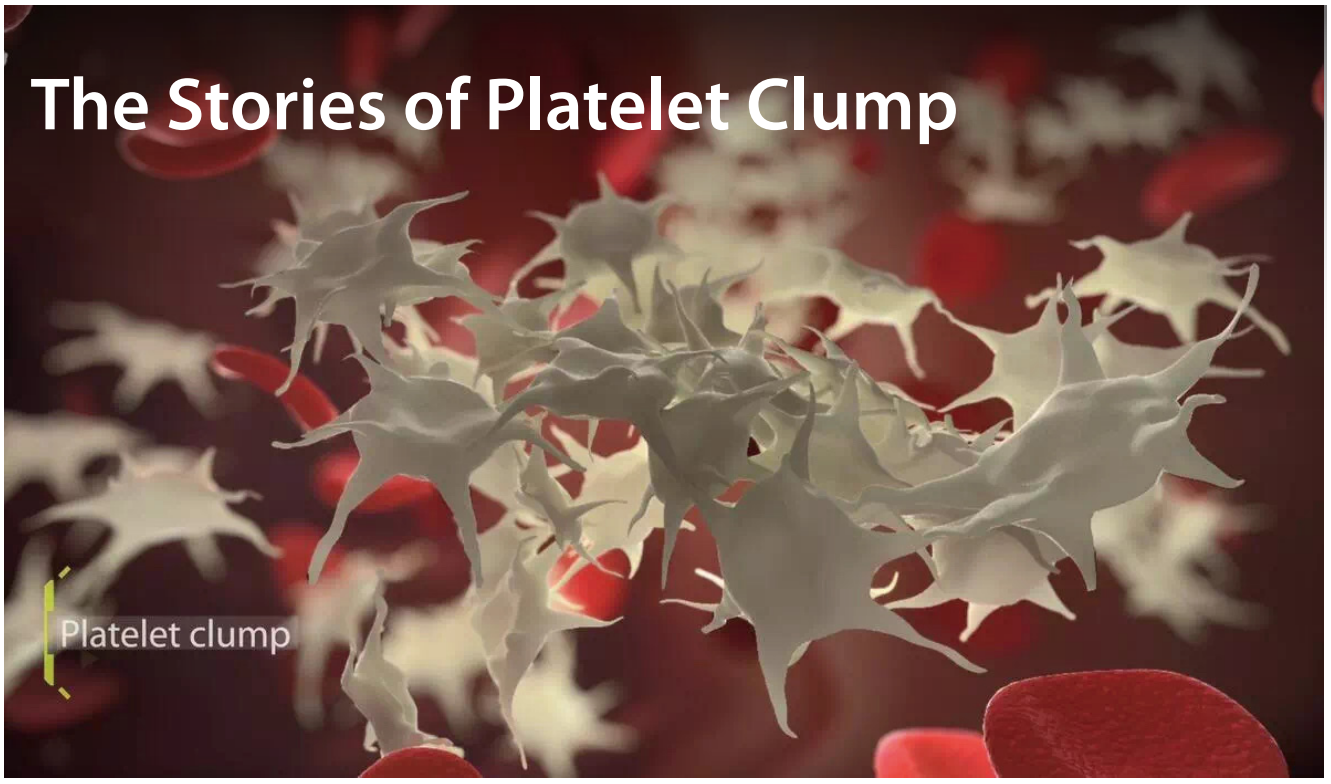
HemaBook

Discover the latest clinical applications and technologies about Mindray hematology

Content

Platelet Series

COVID-19 Series



Thrombocytopenia is a condition characterized by abnormally low levels of platelets in the blood. However, falsely low platelet counts, or pseudothrombocytopenia (PTCP), though easily unrecognized, are found in clinical cases. It is an in vitro phenomenon caused by platelet clumping that results in reporting of a spuriously low platelet count by automatic hematology analyzers.

Resolving platelet clumping has been a headache for laboratory technicians. **Is there a hassle-free solution?**

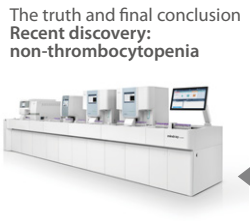
Let's look at two clinical cases that happened during the COVID-19 pandemic.

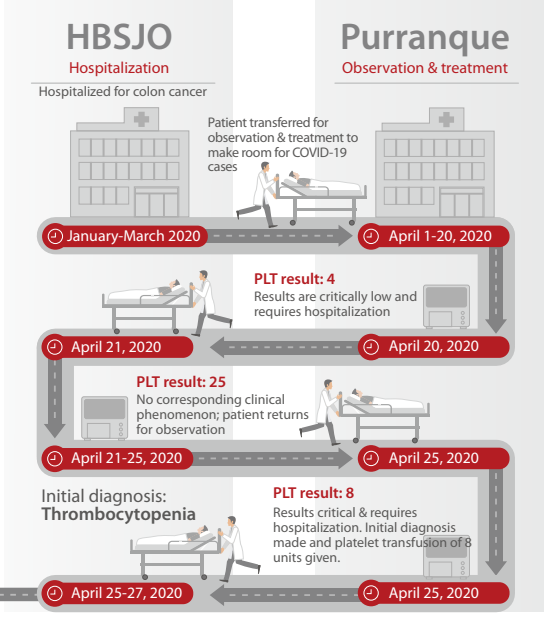
A Tale of Two Hospitals

The first case presented here was courtesy of San José Osorno Base Hospital in Chile. A patient had spent prolonged periods at the San José Osorno Base Hospital (HBSJO) between January and March 2020 without any history of thrombocytopenia. While at HBSJO, the patient needed care for an acute gastric ulcer bleed and a post-surgical infection. But as COVID-19 cases rose, so did the tensions and hospitalizations at HBSJO.

For their safety, all non-COVID-19 patients, including this patient, needed to be transferred to other hospitals. He was then moved to Purranque Hospital for continued treatment.

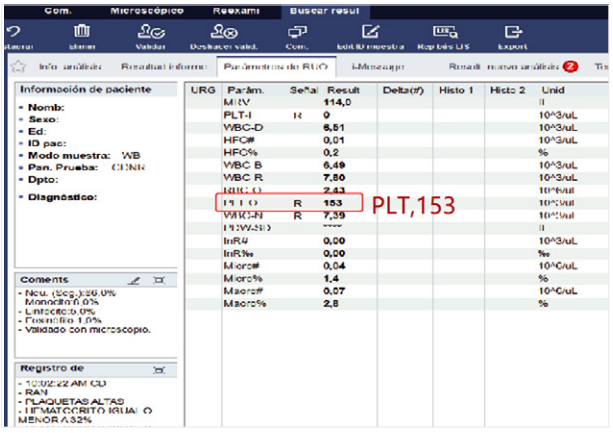
Purranque Hospital,while safely away from COVID-19, could not provide the same comprehensive services as HBSJO if an urgent clinical case were to arise. So, we go from one patient's story to the tale of two hospitals.



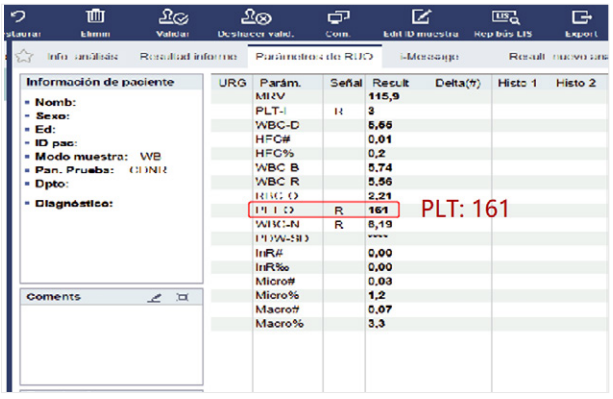


As can be seen, the patient' s clinical symptoms were not consistent with the test results that were given. In order to study the interference of anticoagulants on platelet counts, the laboratory staff from HBSJO collected two samples from the patient and conducted a study on Mindray CAL 8000 Cellular Analysis Line to test two different types of anticoagulants - EDTA and sodium citrate (3.2%). And the result was as follows:

CAL 8000 result		
Sample ID	4270198 (EDTA)	427199 (sodium citrate 3.2%)
First measurement	PLT-I: 1000/ul	3000/ul
Second measurement	PLT-I: 0 PLT-O: 153,000/ul	PLT-I: 3000/ul PLT-O: 161,000/ul



4270198(EDTA): Second measurement of PLT-I and PLT-O result



427199(sodium citrate 3.2%): Second measurement of PLT-I and PLT-O result

A PLT-I result was reported after the first measurement. After opening the RET channel, a PLT-O result was reported at the second measurement.

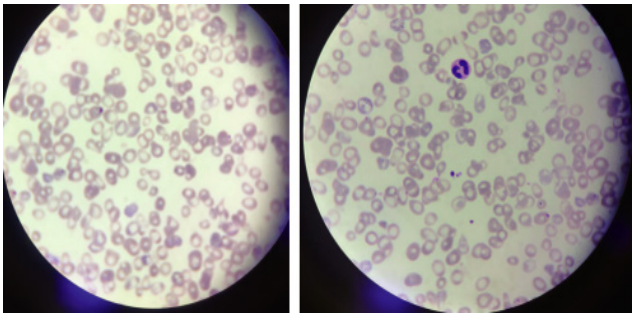
Looking at the results under a microscope, the truth was found. After an unforgettable back- and-forth between hospitals, the patient was finally given the right diagnosis and the correct medical treatment.

A Tricky but Solvable Medical Issue

PLT aggregation can lead to false diagnoses of thrombocytopenia, but it's not unsolvable.

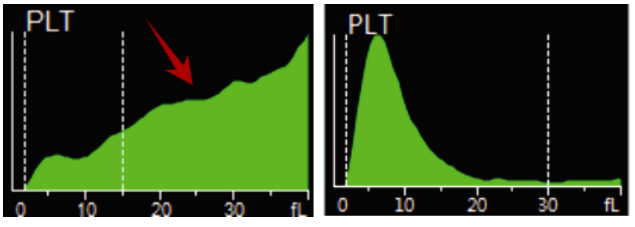
Here, on the other side of the planet, we had a case of a 23-year old male from China who was admitted to the Infectious Disease Department of a Chinese Hospital.

The patient had a CBC performed on Mindray CAL 8000 Cellular Analysis Line. According to the records, the platelet counts for this patient were usually 100+, but currently they were only at 61 which triggered a flag for "platelet clump" by CAL 8000. Under the microscope, however, no PLT aggregation was seen.



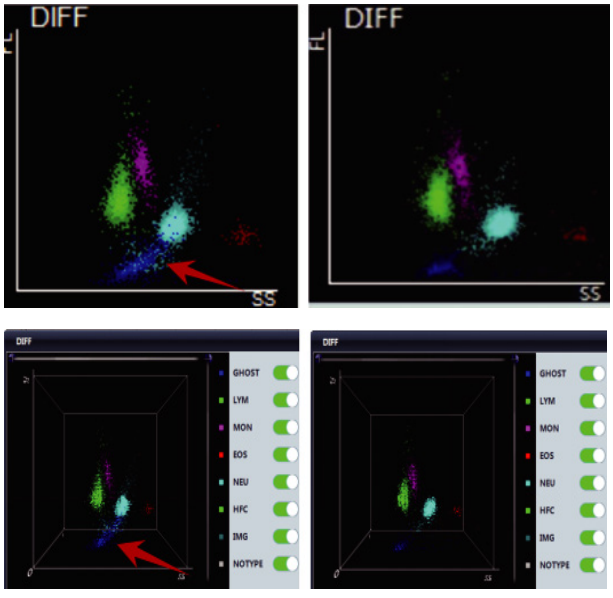
The laboratory technician did the first observation under a low-power lens, where no platelet aggregation was found.

Using an oil microscope, the number of platelets were evaluated again. And there was still no noticeable evidence of clumped PLT.

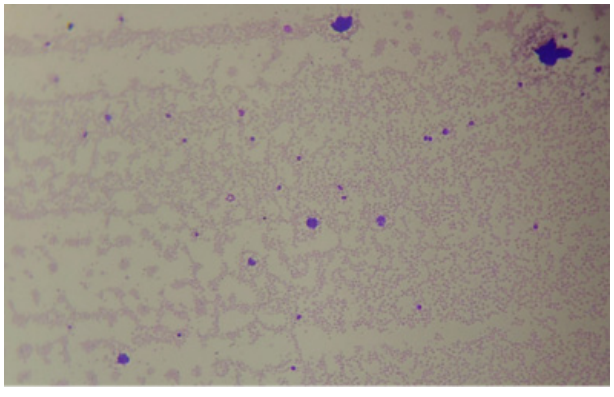


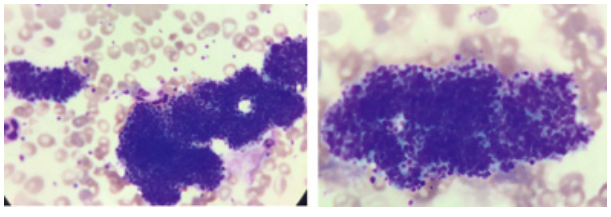
Histogram of the sample (left) and normal histogram (right)

Looking at the histogram of the sample, it should indicate there were small red blood cells or fragments. Compared to the normal scatter plot, the sample' s scatter plot had a cluster of blue particles, which indicated there was platelet aggregation.



The technicians kept looking for clues, all the way at the edge of the microscope slide.





The RET channel on CAL 8000 was opened and a PLT-O result was reported.

WBC		6.45	2.720	3.73	6.95	10^9/L
Neu#	R	3.86	1.560	2.30	5.55	10^9/L
Lym#		2.16	0.970	1.19	0.89	10^9/L
Mon#		0.31	0.120	0.19	0.46	10^9/L
Eos#	R	0.10	0.060	0.04	0.02	10^9/L
Bas#	R	0.02	0.010	0.01	0.03	10^9/L
IMG#		0.19	0.190	0.00	0.06	10^9/L
Neu%	R	59.9	-1.70	61.6	79.9	%
Lym%		33.6	1.70	31.9	12.9	%
Mon%		4.8	-0.30	5.1	6.6	%
Eos%	R	1.5	0.40	1.1	0.2	%
Bas%	R	0.2	-0.10	0.3	0.4	%
IMG%		3.0	2.90	0.1	0.9	%
RBC		3.56	0.750	2.81	3.22	10^12/L
HGB	L	90	20.0	70	80	g/L
HCT	L	30.1	7.20	22.9	26.7	%
MCV		84.5	2.90	81.6	83.0	fL
MCH	L	25.2	0.10	25.1	25.0	pg
MCHC	L	298	-9.0	307	301	g/L
RDW-CV	H	23.7	2.80	20.9	21.4	%
RDW-SD	H	71.0	10.10	60.9	63.2	fL
PLT	&R	193			178	10^9/L
MPV		****		9.2	****	fL
PDW		****		15.9	****	
PCT		****		0.172	****	%
P-LCC		****		56	****	10^9/L

PLT: 193

MRV		90.8			90.6	fL
PLT-I	R	60	-126.0	186	218	10^9/L
WBC-D		6.58	2.920	3.66	7.07	10^9/L
HFC#		0.01	0.010	0.00	0.00	10^9/L
HFC%		0.1	0.10	0.0	0.1	%
WBC-B		6.45	2.720	3.73	6.95	10^9/L
WBC-R		7.96			8.05	10^9/L
RBC-O		3.63			3.26	10^12/L
PLT-O	R	193			178	10^9/L
PDW-SD		****		17.6	****	fL
InR#		0.00	0.000	0.00	0.00	10^9/L
InR‰		0.00	0.000	0.00	0.00	‰
Micro#		0.45	0.080	0.37	0.40	10^12/L
Micro%		12.5	-0.90	13.4	12.4	%
Macro#		0.11	0.070	0.04	0.07	10^12/L
Macro%		3.0	1.50	1.5	2.1	%
RHE	L	27.1			27.1	pg

PLT-I: 60

PLT-O: 193

Based on the data, checking the PLT-O numbers can make it easy to identify a falsely low PLT reading caused by PLT aggregation - and easy to avoid a misdiagnosis.

In both clinical cases, pseudothrombocytopenia (PCTP) was caused by platelet aggregation. While platelet aggregation is caused by both in vivo and in vitro factors, these cases focused on in vitro.

platelet aggregation in vitro

- **EDTA-PTCP**
  Occurence of this in hospitals is about 0.07 - 0.21%
- **Blood sampling**
  Commonly found in capillary blood
- **Heparin-induced**
  Happens to about 4% of patients treated
- **Citrate causes**
  Occurrence percent not available

EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) induced by EDTA anticoagulants is a common laboratory phenomenon. So when it does happen and isn't quickly identified, it creates misinformation which may lead to a misdiagnosis and, ultimately, the wrong medical treatment for the patient.

The good news is that, with technological advancements in laboratory medicine, more and more parameters can be added to the hematology analyzer to avoid situations like that. Currently, platelet testing can be done through PLT-I (based on DC sheath flow impedance) and PLT-O (based on nucleic acid fluorescent staining and done on the RET channel). When there is a blood sample with a high possibility of platelet aggregation, the PLT-O detection technology by the Mindray BC-6000 Series Auto Hematology Analyzer and the CAL8000/6000 Cellular Analysis Line can effectively correct PLT counts - especially when it comes to blood samples with pseudo-platelet reduction due to EDTA.

References:

[1] PLT case study. LABORATORIO HBSJO CHILE, Dra. Sandra.

[2] Can you see the "coastline"? Liuzhou Municipal Liutie Central Hospital, zhenni Lu,xiaoyong Liu, jiajia Huang.

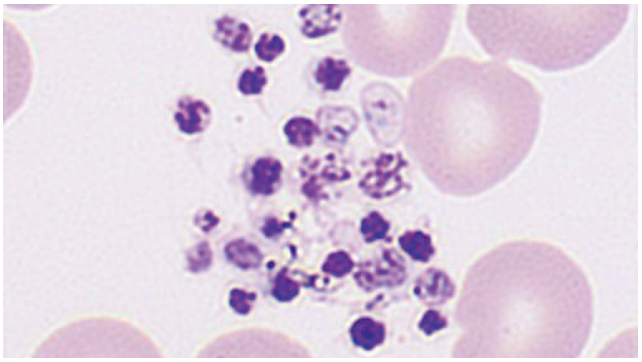
[3] PLT-O, getting a More Accurate Result for EDTA-PTCPPatients. Department of Laboratory Medicine at the Second People's Hospital inNeijiang, Sichuan Province. Zhenzhong Zhou.

One More Option to Solve EDTA-PTCP?

Ethylenediaminetetraacetic Acid - Pseudothrombocytopenia (EDTA-PTCP) is a laboratory artifact that may lead to an incorrect evaluation and unnecessary treatment of patients.

Which of the following method (or methods) would you take to correct platelet counts in case of an EDTA-induced platelet aggregation in thrombocytopenia?

- Recheck with blood smear and estimate the platelet count
- Recheck with addition of amikacin
- Recheck after warming at 37°C
- Recheck immediately dilution without any anticoagulant
- Reexamine on another hematology device
- Recheck with other anticoagulants



Professional clinical laboratory doctors pursue accuracy and truth with the highest sense of responsibility. Nowadays, some clinical studies have been conducted to explore EDTA-PTCP solutions, suggesting that Mindray hematology systems with SF Cube technology would be an option to effectively assist lab technicians in identifying correct platelet counts.

Mindray SF-Cube technology: An effective way for correcting platelet count in individuals with EDTA dependent pseudo thrombocytopenia

A Clinical Case Report

The patient here is a 32-year-old female with infertility. After the patient's EDTA-anticoagulated blood was drawn, it was analyzed within fifty-five minutes, and it showed a low platelet count (28×10^9/L). The test was done by the impedance method (PLT-I) on a popular brand's hematology system (device A). Platelet aggregation was confirmed by microscopic examination of the smear, indicating pseudothrombocytopenia (PTCP). Shortly after, a reexamination of this sample was done using the CDR (PLT-O) method on the Mindray BC-6800Plus. The results showed a markedly higher platelet count with a value of 180×10^9/L.

It's suspected that the low platelet count obtained by device A was due to EDTA-induced PTCP. So, the patient was asked to consent for an additional test using a blood sample tube with sodium citrate this time. Thirty minutes after the sample was collected it was then analyzed. The resulting platelet parameters of the blood samples run through various testing methods and devices are listed in Table 1.

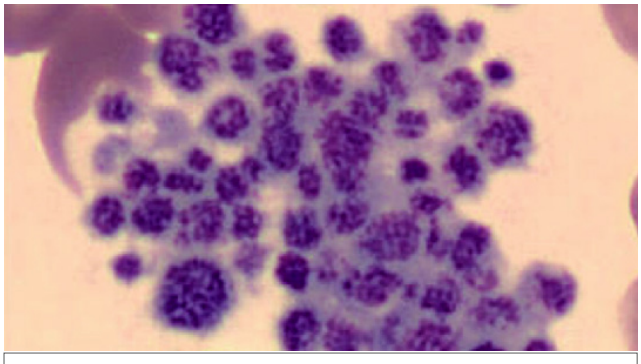


Table 1
Comparison of platelet parameters in EDTA and sodium citrate anticoagulant measured with different methods.

Platelet parameters	EDTA -anticoagulated		Sodium citrate-anticoagulated	
	PLT-I on device A	CDR (PLT-O) of Mindray BC-6800 Plus	PLT-F on device A	Neubauer chamber
Count (x 10^9/L)	28	180	166	176
MPV (fL)	10.9	12.2	11.3	-
PDW (fL)	12.8	17.6	16.0	-

-: Not detected; MPV: mean platelet volume; PDW: platelet distribution width.

Further Comparison of Other Samples

Under microscopic evaluation of the blood smear, the EDTA-anticoagulated blood showed platelet aggregation while the sodium citrate-anticoagulated blood showed none. The blood samples were analyzed within four hours from the time of collection, according to the manufacturer's instructions. In addition to that patient, the data from an additional five cases of EDTA-PTCP were collected and assessed (Table 2).

In the end, the author concludes: In patients with known or suspected EDTA-PTCP Mindray SF-Cube technology is a straightforward and effective way of determining the platelet count in EDTA-anticoagulated blood.

Table 2
Data on the different compared samples.

Cases	Platelet count (x10^9/L)			
	EDTA -anticoagulated		Sodium citrate-anticoagulated	
	PLT-I on device A	CDR (PLT-O) of Mindray BC-6800 Plus	PLT-F on device A	Neubauer chamber
1	86	322	292	300
2	12	133	195	173
3	29	345	334	326
4	14	192	179	185
5	77	227	236	260
Mean	44	244	247	249

Correction of spurious low platelet counts by optical Fluorescence platelet counting of BC-6800 hematology analyzer in EDTA-dependent pseudo thrombocytopenia patients

Identification and Characteristics of EDTA-PTCP Samples

Samples that triggered the "PLT aggregation" flag on the hematology analyzer showed a typical serrated irregularity and a zigzag tail (Figure 1) on the platelet histogram. Also, under microscopic evaluation, the presence of platelet satellitism, or giant platelets, is not seen.

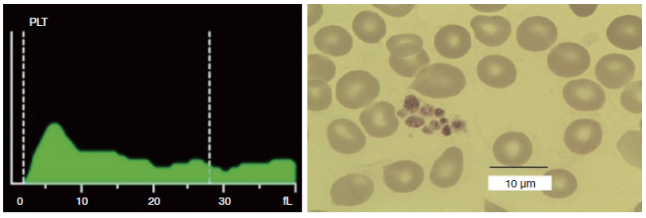


Figure 1: Typical platelet histogram and microscopic pictures of EDTA-PTCP samples. EDTA-PTCP, ethylene diamine tetraacetic acid-dependent pseudo thrombocytopenia.

Spurious Low Platelet Counts of BC-6800 by Optical Platelet Counting (PLT-O)

Twenty-three EDTA-PTCP samples in EDTA tubes (with platelet aggregation) were tested in the impedence (PLT-I (EDTA)) and reticulocyte channel of the Mindray BC-6800 (PLT-O (EDTA)). Interestingly, the PLT-O (EDTA) results were comparable to) the platelet counts of the re-collected samples in citrate tubes (PLT-I (Citrate)), which proves that the PLT-O (EDTA) method can accurately adjust for the platelet aggregation issue caused by EDTA (Figure 2).

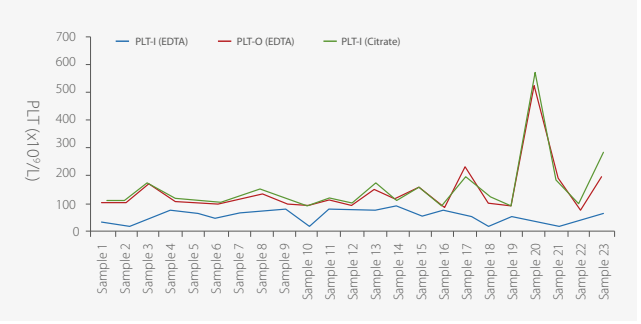


Figure 2: BC-6800's optical fluorescence platelet counts of EDTA-PTCP samples in EDTA tubes were comparable with impedance platelet counts of EDTA-PTCP samples in citrate tubes. PLT-I (EDTA), impedance platelet counts of EDTA-PTCP samples in EDTA tubes; PLT-O (EDTA), optical fluorescence platelet counts of EDTA-PTCP samples in EDTA tubes; PLT-I (citrate), impedance platelet counts of EDTA-PTCP samples in citrate tubes; EDTA-PTCP, ethylene diamine tetraacetic acid-dependent pseudo thrombocytopenia.

One Mainstream Brand's Hematology System's EDTA-PTCP Dissociation Effect: Available with Fluorescent Dye Staining?

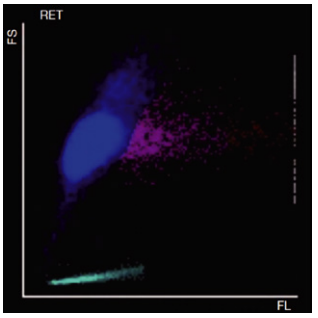
Optical fluorescence platelet counting is available in both high-end hematology analyzers (device B) and Mindray BC-6000 series hematology analyzers. In this method, a fluorescent dye is used to stain the nucleic acids in platelets, allowing the recognition of large platelets and excluding non-platelet particles such as erythrocyte debris, micro erythrocytes, or leukocyte debris.

To verify whether the dissociation effect of optical fluorescence platelet counting was dependent on fluorescent dye staining, 17 of those 23 EDTA-PTCP samples in EDTA tubes were also tested on device B' s reticulocyte channel and impedance channel., It was found that here was no significant difference between the platelet counts of reticulocyte channel and impedance channel (Figure 3). Only one of the 17 EDTA-PTCP samples showed a dissociation rate of more than 80%, with an average dissociation rate of 56% among all 17 EDTA-PTCP samples (Figure 3).

Figure 3: No dissociation effect of device B's optical fuorescence platelet counting on EDTA-PTCP patients. PLT-I (EDTA), impedanceplatelet counts of EDTA-PTCP samples in EDTA tubes; PLT-O (EDTA), optical fluorescence platelet counts of EDTA-PTCP samplesin EDTA tubes; PLT-I (citrate), impedance platelet counts of EDTA-PTCP samples in citrate tubes; EDTA-PTCP, ethylene diaminetetraacetic acid-dependent pseudo thrombocytopenia.

Conclusion

In summary, the author concludes: Optical fluorescence platelet counting of the BC-6800 hematology analyzer is effective for the correction of spurious low platelet counts in EDTA-PTCP patients, and its dissociation effect on EDTA-PTCP samples is independent of fluorescent dye staining.



SF Cube

In the busy daily work of the laboratory, PTCP is an inevitable trouble. Mindray SF Cube technology provides PLT-O (based on nucleic acid fluorescent staining and done on the RET channel) to correct PLT counts when there is pseudo-platelet reduction due to EDTA. PLT-O is available on Mindray BC-6000 Series Auto Hematology Analyzer and the CAL 8000/6000 Cellular Analysis Line.

References:

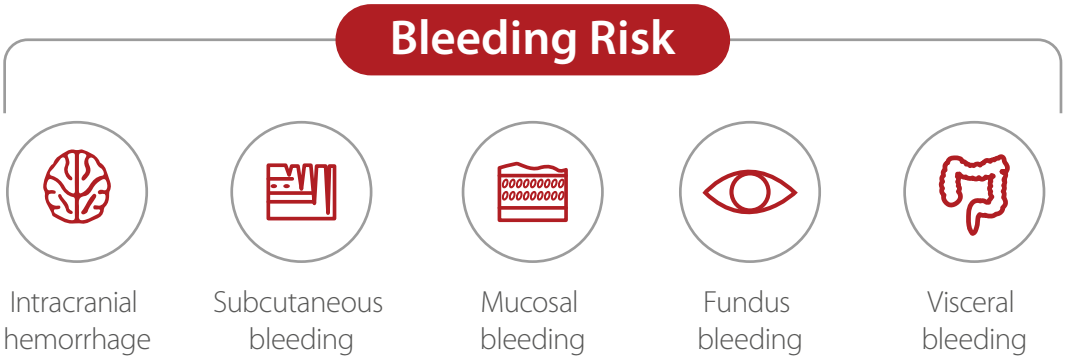
[1] J. Deng, et al. Mindray SF-Cube technology: An effective way for correcting platelet count in individuals with EDTA dependent pseudo thrombocytopenia. Clinica Chimica Acta 502 (2020) 99–101

[2] Y. Bao, et al. Correction of spurious low platelet counts by optical fluorescence platelet counting of BC-6800 hematology analyzer in EDTA-dependent pseudo thrombocytopenia patients. Transl Cancer Res 2020;9(1):166-172

Low Value? High Risk?

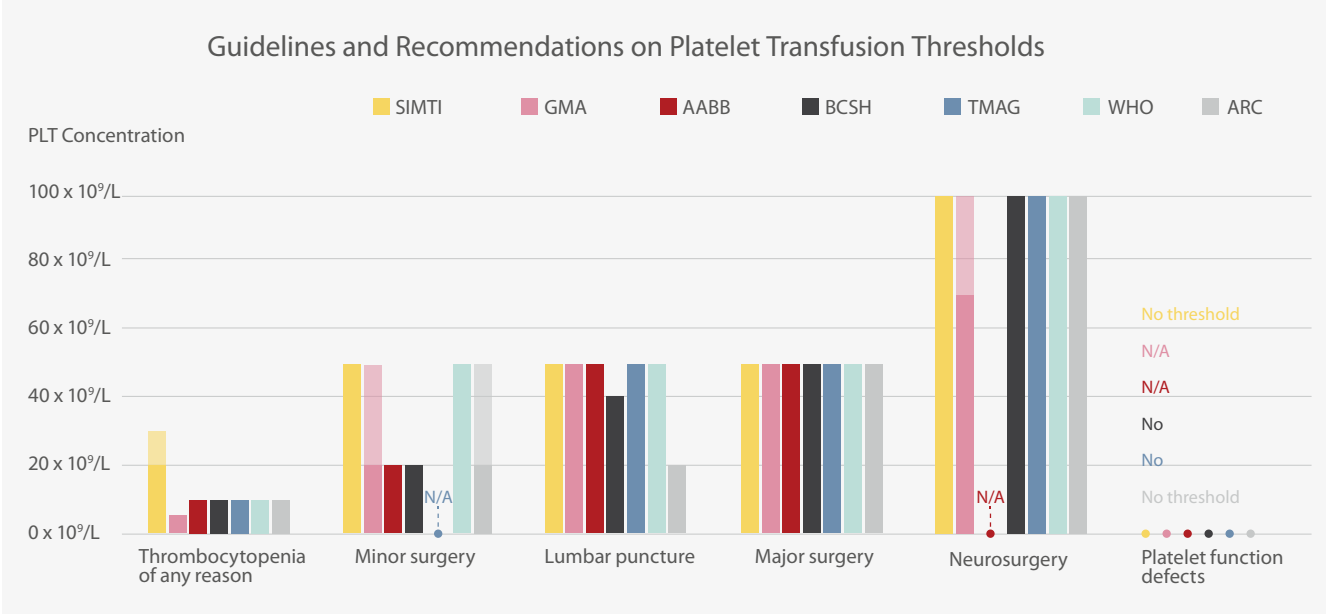
Resolve Your Concerns about the Risk of Low-value Platelet Measurements

When the platelet value is low, patients are at risk of bleeding. Bleeding in vital organs is life-threatening.



According to Giuseppe Lippi and other professors, there is no universal agreement on the definition of platelet transfusion thresholds.[1] However, the degree of accuracy and imprecision of the vast majority of fully automated hematological analyzers appears unsatisfactory, especially in the lower thrombocytopenic range, i.e.,<50× 10^9/L .

The current guidelines and recommendations show that there is no consensus on the low PLT infusion threshold in several specific clinical situations, as shown in the following table. [1]



Abbreviations: SIMTI, Italian Society of Transfusion Medicine and Immunohaematology; GMA, German Medical Association; AABB, American Association of Blood Banks; BCSH, British Committee for Standards in Haematology; TMAG, British Columbia Transfusion Medicine Advisory Group; WHO, World Health Organization; ARC, American Red Cross; N/A, not available.

*Only in case of perioperative bleeding.

Low-value platelet counts also play an important role in evaluating the effectiveness of platelet transfusion. In the most common platelet transfusion formulas, including the post-transfusion platelet increment (PPI), the percentage platelet recovery (PPR), and the corrected count increment (CCI), the absolute count of platelet is one of the key calculation factors.[2] Among them, most clinicians use an estimate of transfused platelet content and average body surface area to calculate the CCI, and an absolute platelet increment of greater than 10×10^9/L at 1 or 24 hours is considered a successful transfusion, which is consistent with the previous formula.[3]

The platelet count results are still regarded as the mainstay for driving platelet transfusion practices, but the hematology analyzers, as the method, show different analytical performance. The Italian Working Group on Diagnostic Hematology of the Italian Society of Clinical Chemistry, Clinical Molecular Biology (WGDH-SIBioC) has conducted a multicenter study based on international guidelines, to verify the analytical performance of nine different types of hematology analyzers (HAs) in the automated platelet analysis. Let's take a look at the report below.

ORIGINAL ARTICLE

WILEY

Multicenter evaluation of analytical performances of plateletcounts and platelet parameters: Carryover, precision, and stability

Four hundred and eighty-six peripheral blood samples (PB), collected in K3EDTA tubes, were analyzed by ABX Pentra, ADVIA2120i, BC-6800, BC-6800Plus, Cell-DYN Sapphire, DxH800, XE-2100, XE-5000, and XN-20 with PLT-F App.

TABLE 1 Hematology analyzers and methods of platelet counts

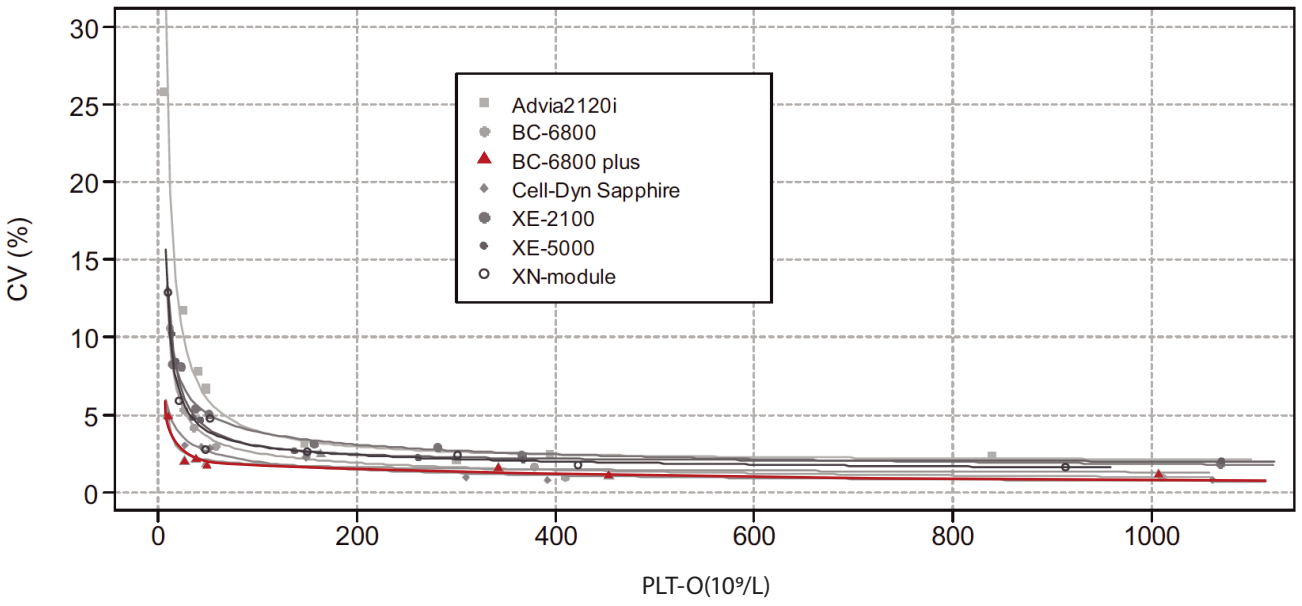
Methods of platelet counts	Hematology analyzer								
	ABX Pentra	ADVIA 2120i	BC-6800	BC- 6800 Plus	Cell-DYN Sapphire	DxH800	XE-2100	XE-5000	XN-20 module with PLT-F App
Impedance method (PLT-I)	X		X	X	X	X	X	X	X
Optical method (PLT-O)		X	X	X	X		X	X	X
Fluorescence method (PLT-F)									X

TABLE 2 Carryover (CO) and low limit of quantification (LoQ)

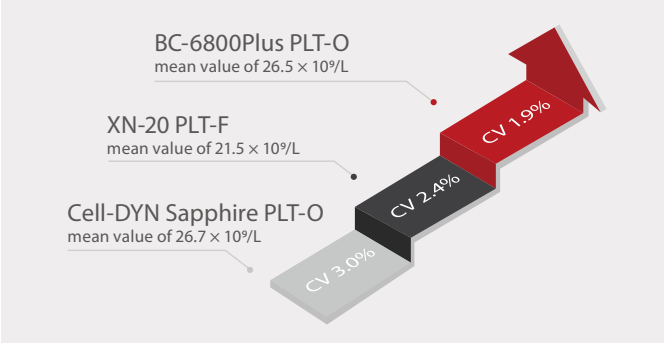
	ABX Pentra		ADVIA2120i		BC-6800		BC-6800 Plus		DxH800		Cell-DYN Sapphire		XE-2100		XE-5000		XN-20 module	
	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ	CO	LoQ
PLT-F ($x10^9$/L)	//	//	//	//	//	//	//	//	//	//	//	//	//	//	//	//	0.00%	4.0
PLT-I ($x10^9$/L)	0.00%	16.0	//	//	0.00%	19.0	0.00%	23	0.05%	8.0	0.03%	5	0.09%	18.6	0.05%	14.6	0.35%	9.0
PLT-O (x 10^9/L)	//	//	0.50%	2.50	0.00%	11.0	0.00%	4	//		0.09%	2	0.07%	11.5	0.07%	13.9	0.36%	12.0
IPF absolute value(a)($x10^9$/L)	//	//	//	//	//	//	//	//	//	//	//	//	0.00%	6.0	0.00%	11.8	0.00%	1.0
IPF relative value (%)	//	//	//	//	0.00%	//	0.00%	//	//	//	//	//	0.00%	//	0.00%	//	0.00%	//
PCT (%)	0.00%	//	//	//	0.00%	//	0.00%	//	0.040%	//	0.00%	//	0.05%	//	0.00%	//	0.00%	//
P-LCR or large PLT count (%)		//	0.30%	//	0.00%	//	0.00%	//	//	//	//	//	0.00%	//	0.00%	//	0.00%	//

Note: The PLT limit of acceptability for carryover is 0.5% according to Vis et al.[31]

The carry-over (CO) rates of BC-6800 and BC-6800Plus both meet the requirements, the lowest in the group. The limit of quantification (LoQ) of PLT-O of BC-6800 and BC-6800Plus is lower than that of PLT-I, and the LoQ of PLT-O of BC-6800Plus is as low as 4x10⁹/L, which is lower than PLT-O of XN-20, which is equivalent to PLT-F of XN-20. Interestingly, LoQ of PLT-I of XN-20 is lower than that of PLT-O.



Generally speaking, the imprecision (%CV) increases as the platelet count decreases. In the segment of chart B (PLT-O precision), BC-6800Plus shows the lowest CV at all concentration levels. In the abstract, it' s described as: No HAs showed desirable CVAPS for PLT counts less than 50.0 × 10⁹/L, with the exception of Cell-DYN Sapphire (CV 3.0% with PLT-O mean value of 26.7 × 10⁹/L), XN-20 (CV 2.4% with PLT-F mean value of 21.5 × 10⁹/L), and BC-6800Plus (CV 1.9% with PLT-O mean value of 26.5 × 10⁹/L).



Among the cells of peripheral blood, platelets are the smallest, but as a population, the concentration of platelets plays an important role in hemostasis. To account for the risk of bleeding caused by low platelets, Mindray has introduced multi-fold PLT-O platform (SF Cube) to provide the correct count for thrombocytopenia samples. PLT-O is available in Mindray BC-6000 Series Auto Hematology Analyzers and the CAL 8000/6000 Cellular Analysis Lines.

References:

[1] Platelet Transfusion Thresholds: How Low Can We Go in Respect to Platelet Counting? Lippi G, Favaloro EJ, Buoro S. Semin Thromb Hemost. 2019 Sep 28. doi: 10.1055/s-0039-1696943. [Epub ahead of print]

[2] Rebulla P. Formulae for the definition of refractoriness to platelet transfusion. Transfus Med 1993;3(1):91–3.

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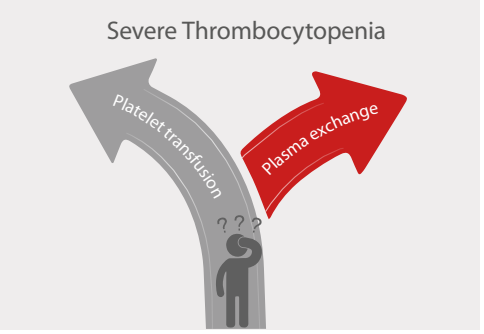
Is Platelet Transfusion Always Required in Thrombocytopenia?

When the platelet (PLT) count is extremely low, is platelet transfusion really required? Let's analyze the following case, which tells us a different answer.



Case background

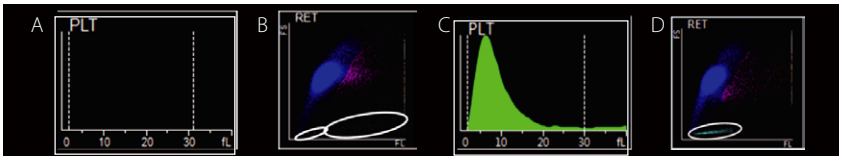
A 49-year-old woman went to the emergency department due to transient cognitive disorder. She had obvious paroxysmal headaches, with vomiting and epigastric pain. Brain CT revealed no abnormalities. The laboratory results revealed anemia and severe thrombocytopenia. What should be expected?

▸ Blood Test

Blood test (Figure 1) showed low RBC count of 3.07×10^{12}/L and low HGB concentration, which indicated anemia. PLT-O count was 15×10^{9}/L (CDR mode, Mindray BC-6200). Such a low PLT count and abnormal RBC-related parameters caught our attention: Should platelet transfusion be initiated?

Name	XXX						
Gender	Female						
Age	49-year-old						
Items	Resluts	Reference Range	Unit	Items	Resluts	Reference Range	Unit
ABO	B			HCT	25.3	35-45	%
Rh	+			MCV	82.6	82.0-100.0	fL
WBC	6.08	3.5-9.5	10^9/L	MCH	26.8	27.0-34.0	pg
NEUT%	60.30	40-75	%	MCHC	324	316-354	g/L
LYMPH%	29.50	20-50	%	RDW-CV	14.5	11.0-16.0	%
MONO	8.20	3-10	%	RDW-SD	42.2	35.0-56.0	fL
EO%	1.80	0.4-8.0	%	PLT	15	125-350	10^9/L
BASO%	0.20	0.0-1.0	%	MPV	....	6.5-12.0	fL
NEUT#	3.67	1.8-6.3	10^9/L	PDW	....	9.0-17.0	%
LYMPH#	1.79	1.1-3.2	10^9/L	PCT	....	0.108-0.282	%
MONO#	0.50	0.1-0.6	10^9/L	P-LCR	....	19.1-46.6	%
EO#	0.11	0.02-0.52	10^9/L	RET#	124.6	22.4-82.9	10^9/L
BASO#	0.01	0-0.06	10^9/L	RET#	4.6	0.67-1.92	%
RBC	3.07	3.8-5.1	10^{12}/L	IRF	14.4	2.4-17.5	%
HGB	82.00	115-150	g/L	LFR	85.6	89.4-99.5	%

Figure 1. Blood test showed low PLT count, RBC count, and increased RET%



The PLT histogram was far from the normal ones. We then checked RET scattergram, in which few PLT dots were observed and RET increased (Figure 2).

Figure 2. Comparison of PLT-I & PLT-O results (A/B: the case; C/D: normal)

These abnormal results triggered the re-exam rule, and then the peripheral blood smear was reviewed (Figure 3). The result demonstrated evidence of intravascular hemolysis, which included schistocytes, a small number of spherocytes, helmet cells, and thrombocytopenia.

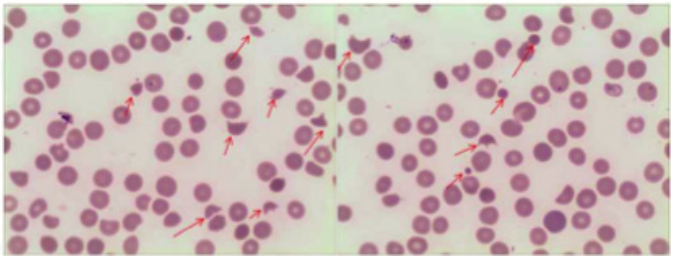


Figure 3. PLT was rarely seen, and schistocytes, small numbers of spherocytes and helmet cells were found. (100x)

▸ Bone Marrow Examination

Erythrocytic cell lines 44%, Granulocytic cell lines 40%, G/E=0.91/1.

Undifferentiated erythroblasts were common in the bone marrow smear, and Howell-Jolly body were found. A total of 257 megakaryocytes were found in the smear, and 50 were differentiated, including 3 megakaryoblasts, 44 promegakaryocytes and 3 naked nucleus megakaryocytes. PLT was rarely seen. Such bone marrow smear showed a megakaryocytic thrombocytopenia, increased function of erythroid differentiation.

▸ Chemistry Test

In the chemistry test, the result of total bilirubin (TBIL) increased, indirect bilirubin (IBIL) increased, which showed hemolytic disease.

Name Gender Age	XXX Female 49		
Items	Resluts	Unit	Reference Range
TBIL	60.50	umol/L	2.0-20.4
DBIL	14.70	umol/L	0.0-6.80
IBIL	45.80	umol/L	3.40-14.00
AST	27 00	U/L	7-40
AST	33.00	U/L	13-35
ALT/AST	1.22		0.23-247
v-Glutamyl transferase	8.00	U/L	7-45
Alkaline Phosphatase	76.00	U/L	40-150
Total Bile Acid	8.00	umol/L	0.00-10.00
Glycocholic Acid	1.39	mg/L	0-2.7
Cholinesterase	8924.00	U/L	4500-13000
Total Protein	65.60	g/L	60.00-95.00
Albumin	39.50	g/L	35.00-55.00
Globulin	26.10	g/L	20.00 40.00
Albumin/Globulin	1.51		1.20-2.40
Glucose	9.15	mmol/L	3.61-6.11
Urine Nitrogen	8.27	mmol/L	1.79-7.14
Creatinine	99.00	umol/L	40-120

Figure 4. Chemistry testing results

Considering the cases with typical symptoms: (1) hemolytic anemia (schistocytes); (2) thrombocytopenia; (3) neurologic symptoms (transient mental disorder), thrombotic thrombocytopenia (TTP) disease is likely. The results and TTP diagnostic suggestion were transferred to doctors immediately. And Further ADAMTS13 testing confirmed TTP. Finally, plasma exchange instead of platelet transfusion was taken.

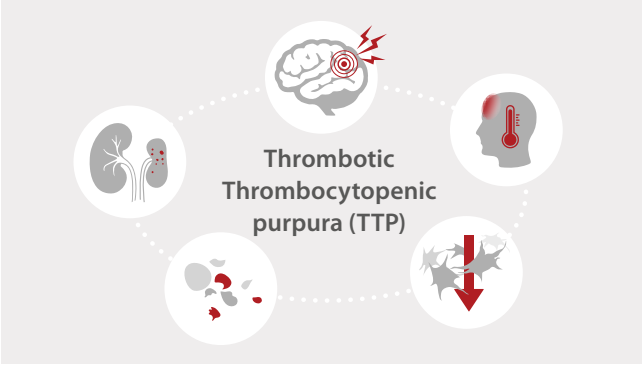


Figure 5. Clinical symptoms of TTP disease

Five medical signs[2]	In this case
Fever	No
Hemolytic Anemia	Yes
Thrombocytopenia	Yes
Transient neurologic symptoms	Yes
Reduced kidney function	No

Conclusion

If the PLT level is lower than the decision-making threshold, experienced doctors will immediately recheck whether the specimen is qualified, the histogram and scattergram are normal or not, and any other abnormal cell counting results or significant flag messages, etc. Further confirmation will be carried out by blood smear examination under the microscope. Finally, after considering the patient's symptoms and medical history information, the laboratory can report the results and provide possible diagnosis.

Mindray's automatic 8x PLT-O counting (SF Cube technology) provides accurate and stable counting for thrombocytopenia samples. Together with high quality blood smear from SC-120 Auto Slide Maker, Mindray hematology solutions support efficient management of thrombocytopenia. PLT-O is available in Mindray BC-6000 Series Auto Hematology Analyzers, and the CAL 8000/6000 Cellular Analysis Lines.

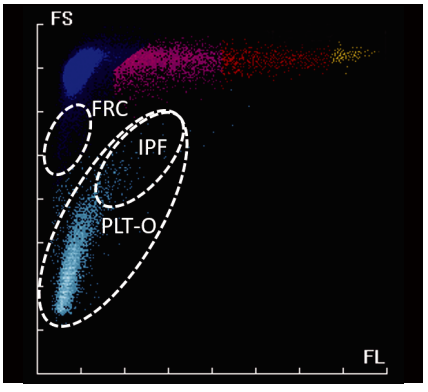


Figure 6. PLT-O scattergram

Extension: Why is platelet transfusion not recommended in thrombotic thrombocytopenia?

Common causes of thrombocytopenia include decreased production in bone marrow, increased destruction in peripheral blood, and medication induced[1].

Thrombotic thrombocytopenia, including TTP in this case, is a kind of disease caused by increased PLT destruction. Because of von Willebrand factor (VWF) cleavage protease (ADAMTS13) deficiency, VWF cannot be cut off normally, and ultra-large VWF (ULVWF) accumulate, resulting in abnormal PLT aggregation, microthrombosis and fragmented RBC[2]. Under such condition, platelet transfusion may accelerate thrombosis, leading to worsening symptoms[3]. So for thrombotic thrombocytopenia, the main treatment should be plasma exchange.

Acknowledgement

We would like to extend our thanks to Dr. Xiao Zuomiao, Dr. Chen Xianchun, Dr. Xiao Dejun and Dr. Luo Shi from Ganzhou People's Hospital, China, for providing the case information.

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[1] What Is Thrombocytopenia? - NHLBI, NIH". www.nhlbi.nih.gov. Retrieved 2015-05-01.

[2] Tsai, Han-Mou(2010). "Pathophysiology of thrombotic thrombocytopenic purpura." International journal of hematology vol. 91,1: 1-19. doi:10.1007/s12185-009-0476-1.

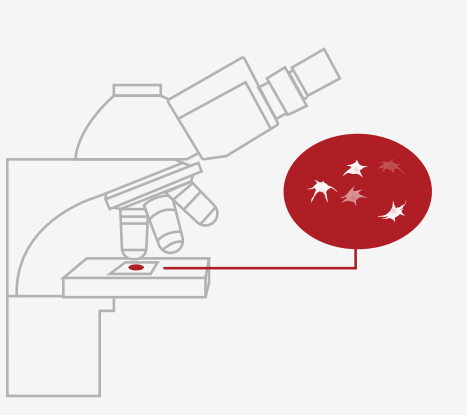
[3] Joly, BS; Coppo, P; Veyradier, A (2017). "Thrombotic thrombocytopenic purpura." Blood. 129 (21): 2836–2846. doi:10.1182/blood-2016-10-709857.

How Mindray Counts Low Platelets Correctly

Clinical Significance

Derived from megakaryocytes, platelets (PLT) are produced and matured in the bone marrow. Besides widely known thrombosis and wound repair, PLT also plays important roles in inflammation, immunity and cancer biology[1]. Normal reference intervals of PLT in peripheral blood varies in the range of 150-400×10⁹/L. When the PLT value is lower than 100×10⁹/L, a common clinical problem identified as thrombocytopenia (low PLT) may be the result[2].

There are several causes of thrombocytopenia including decreased PLT production, increased PLT destruction, increased splenic sequestration and dilution[3]. Currently, complete blood count (CBC) and blood smear reviews are essential diagnostic methods for the initial evaluation of thrombocytopenia samples[2]. Therefore, counting low PLT correctly by an auto hematology analyzer might be a prospective approach which will greatly reduce the blood smear rate, and save laborious time in the diagnostic lab, rapidly screening out thrombocytopenia samples.

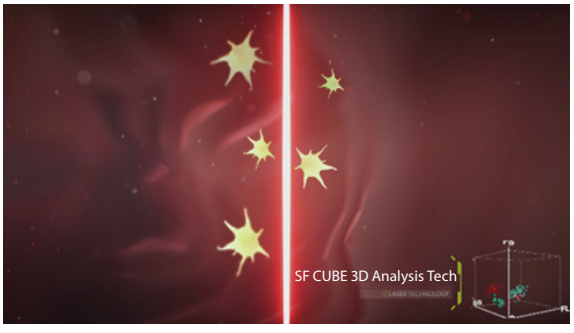


However, counting low PLT correctly is not a simple process. How does Mindray's high-end auto-hematology analyzer deal with low PLT samples?

Mindray Solution

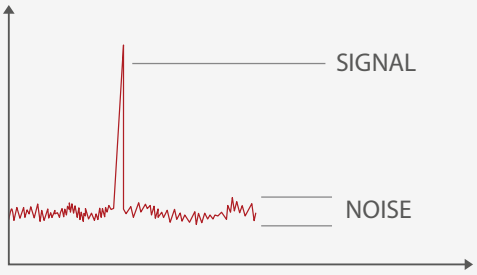
▸ Tech 1. Highly specific fluorescent staining

Fluorescent staining dye (FR dye) has been specially designed with the ability to identify target cells immediately. Next, the fluorescent molecule captures nucleic acid in PLT cells while avoiding interferences from small RBCs, RBC /WBC fragments and other small particles. PLT stained with specific fluorescent dye subsequently goes through the laser detector for optical measurement. The high specificity and affinity of fluorescent dye ensures that the binding inside PLT cells is stable enough for cells to flow through the laser. This ensures that even low amounts of PLT can be mapped and counted in the SF CUBE 3D scattergram accurately.



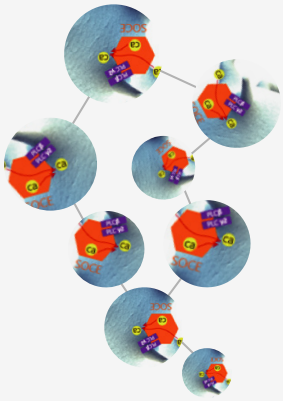
▸ Tech 2. High-resolution optical detection

Also, Mindray's high-end hematology analyzer combines reflective light suppression technology with SiPM (Silicon photomultiplier), which is highly sensitive to fluorescence signals while simultaneously minimizing the background noise during optical detection. This greatly improves the detection limit of particles, and the lower limit for small particles reaching up to 1um (a diameter of 2 um is defined as small PLT), ensuring that the sample results are not interfered by small PLT or particles.

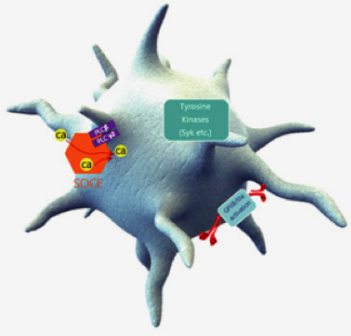


▸ Tech 3. PLT de-aggregation

Pseudothrombocytopenia is caused by in vitro platelet clumping in EDTA-anticoagulated blood tube, which may lead to a falsely low PLT count[4]. Mindray's R&D conducted preliminary studies focusing on PLT's activation mechanisms, which is mainly regulated by calcium channel, tyrosine kinase pathway and GPIIb/IIIa. And then antagonists are applied in DR dilluent to directly block the binding sites on the surface of PLT which greatly minimizes the formation of PLT clumps.More detailed PLT de-aggregation mechanism will be released in the next HemaBook Chapter.

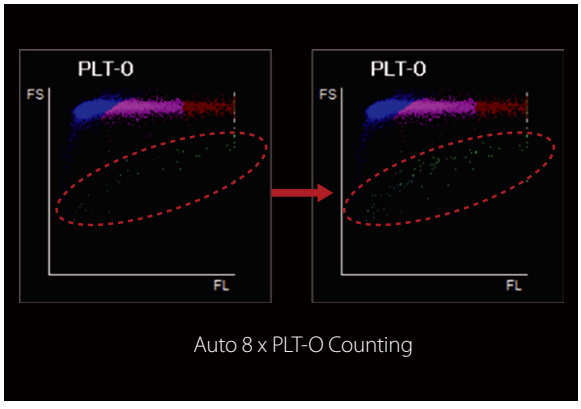


PLT Clump



▸ Tech 4. Auto 8×PLT-O counting

Mindray is in the process of a patent application (see below picture) for Auto 8×PLT-O Counting Technology. To begin with, PLT-I value obtained from impedance channel is firstly compared with a default value (50×10⁹/L). If this is lower than the cutoff value, the analyzer can automatically prolong counting time up to 8-fold in order to collect more PLT particles for further analysis. In addition, Auto 8×PLT-O Counting Tech can eliminate other interference factors (e.g. fragmented RBC/WBC) which are often easily miscounted as PLT by the impedance channel. No additional sampling, no manual intervention, no additional channels and reagents required – 8×PLT-O counting is both efficient and effective in counting low PLT correctly .



Auto 8 x PLT-O Counting

IPC No.

G01N 15/10 (2006.01) G01N 33/48 (2006.01) G01N 15/14 (2006.01)

Title: BLOOD DETECTION METHOD AND DEVICE

Prepare a first detection solution using a blood sample

↓

Control the first detection solution to pass through an impedance detection area

↓

Perform platelet detection on the first detection solution in the impedance detection area with an impedance method to obtain a first platelet detection result

↓

Prepare a second detection solution using the blood sample when the first platelet detection result is less than a preset platelet detection threshold

↓

Control the second detection solution to pass through the impedance detection area

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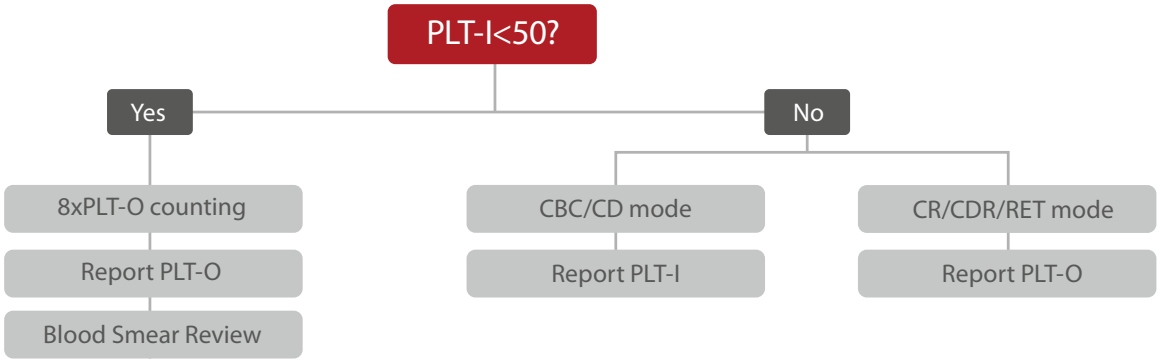
Perform platelet detection on the second detection solution in the impedance detection area with the impedance method to obtain a second platelet detection result



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[3] Thrombocytopenia: an update, K. J. Smock, et al, Int. Jnl. Lab. Hem. 2014, 36, 269–278

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Mindray's Solution for Solving In-vitro PLT Aggregation

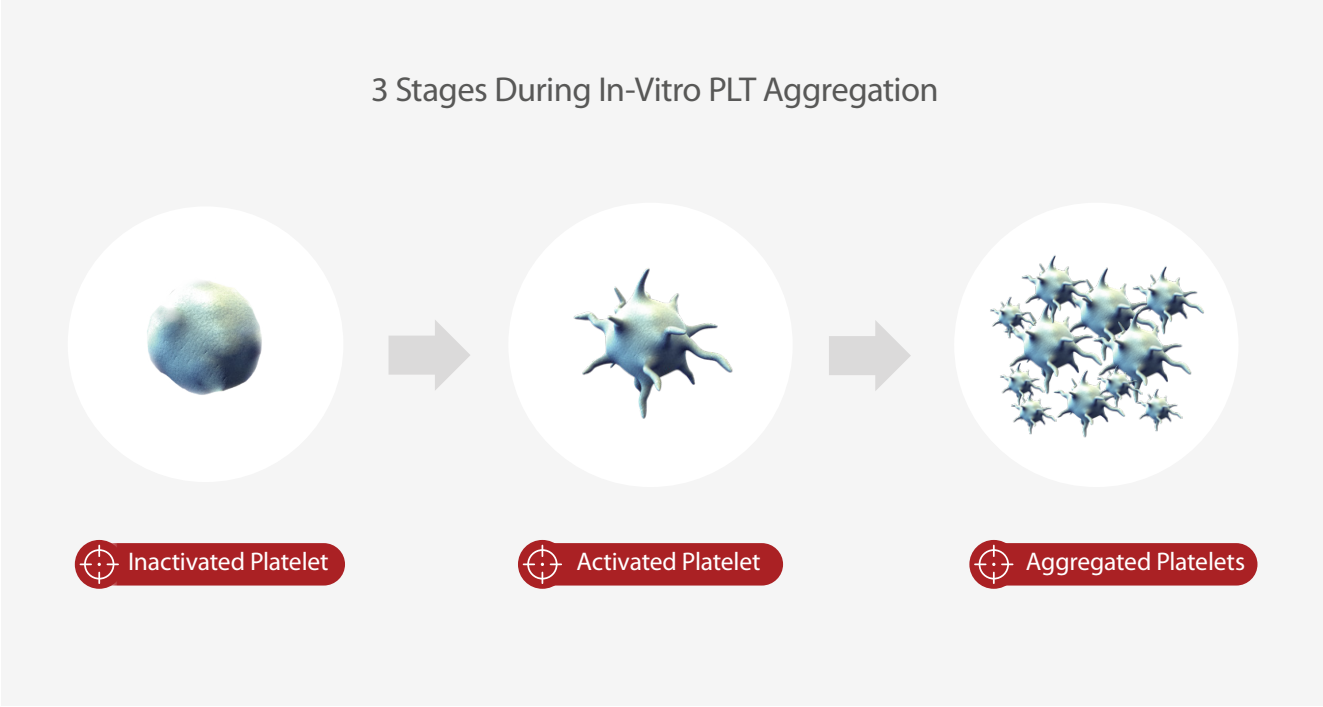
EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) induced by EDTA anticoagulants is a common laboratory phenomenon. It is caused by in-vitro PLT aggregation and may lead to a low PLT result and, ultimately, misdiagnosis and wrong medical treatment for the patient.

In the previous chapter (The Stories of Platelet Clump), we looked at two clinical cases which initially had incorrect low PLT results using the traditional PLT measurement principle. After re-running the samples in the Mindray hematology analyzer using RET mode, PLT-O showed a more reliable result and finally gave the correct diagnosis.

Today, let's explore how the Mindray solution solves the in-vitro PLT aggregation problem.

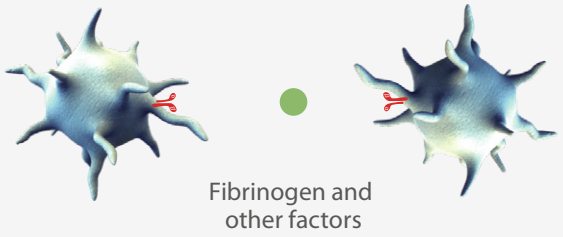
What are the critical factors of in-vitro PLT aggregation?

During PLT aggregation, PLT has three stages: inactivated PLT/activated PLT/ aggregated PLT. PLT activation is the most critical step for PLT aggregation.[1]

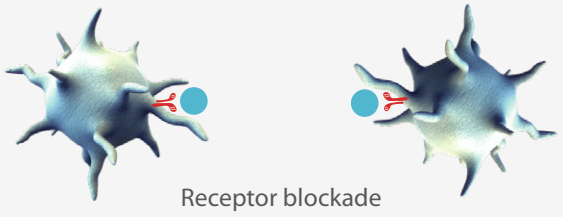


Thus, the PLT aggregation problem may be solved by blocking the PLT activation process. In order to discover the mechanism of PLT activation, we carried out a further exploration into the subcellular structure and cellular signaling pathway, and found there were three main regulatory pathways during PLT activation: the calciumion pathway, Tryosine kinases pathway, and glycoprotein GPIIb/IIIa pathway. [2]

In addition, the corresponding receptor antagonist research has been done to block the regulatory pathway to achieve the aim of PLT de-aggregation. Several different kinds of receptor antagonists have been tried and found to perform well in blocking PLT aggregation.

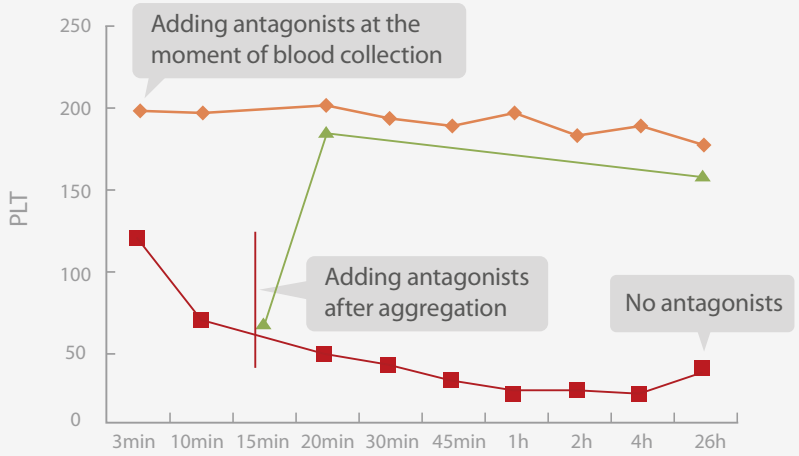


Without receptor antagonist (2 PLT gather together)



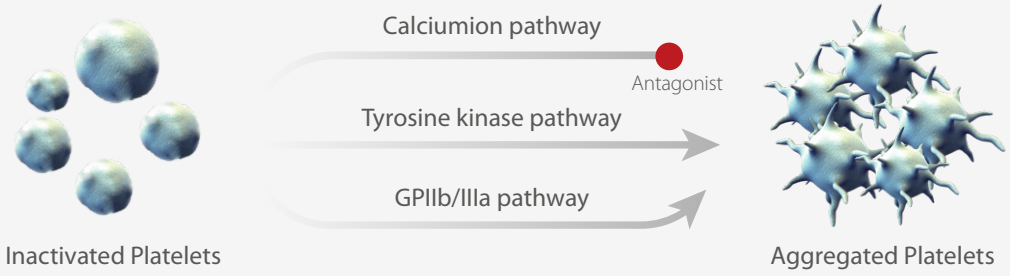
With receptor antagonist (cannot successfully aggregate)

As the data shown below, adding antagonists to samples sensitive to receptor antagonists before aggregation can prevent aggregation from happening; adding antagonists after aggregation can realize PLT de-aggregation as well.

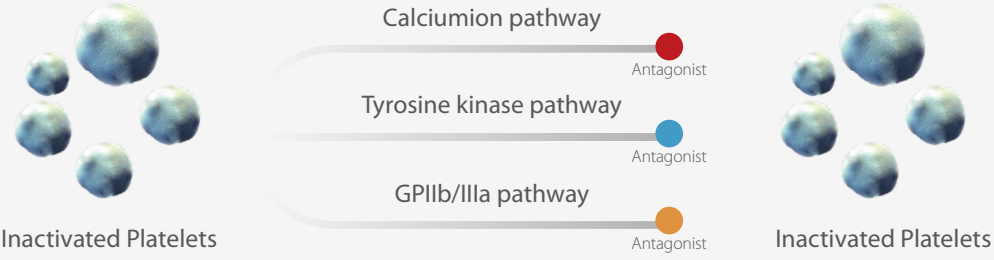


However, after testing different kinds of samples, there are still some samples that are not sensitive to these antagonists. This is because these antagonists can only target single regulatory pathway and have a limited effect on the other two pathways.

A further study was done by combining the molecular biology method to explore the molecular mechanism and different radical group characteristics. Ultimately, from thousands of potential chemical compounds, a series of optimal compounds were found which contain specific radical groups which are highly efficient at blocking the three regulatory pathways.

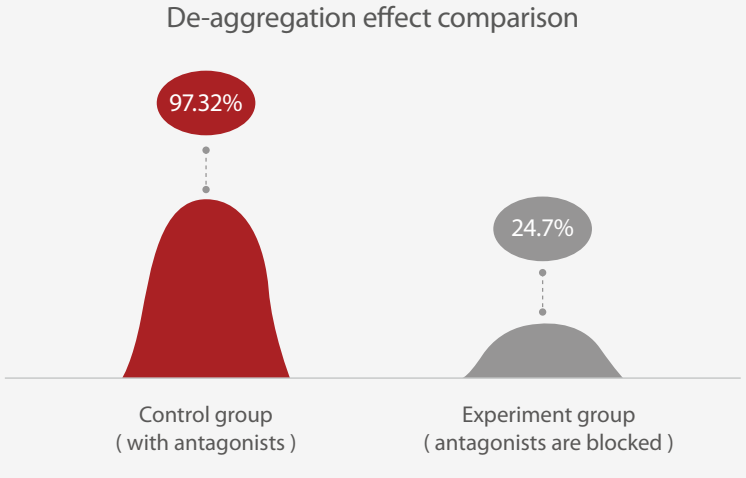


Traditional solution: single target antagonist



Mindray solution: a series of optimal compounds which can block the three pathways

In order to further prove their effects on PLT de-aggregation using those antagonists, a comparison experiment was performed between control group and the group where antagonists were blocked. The experiment results are shown below:



From the experiment, we have found that antagonists containing specific radical groups have an obvious effect on platelet de-aggregation.

Besides, there are also 3 critical factors (appropriate pH, temperature, mechanical mixing) that enhance the PLT de-aggregation. The superposition effect of these factors in de-aggregation is obvious. Thanks to the joint effects of multiple factors, the aggregation platelets are de-aggregated, and a reliable platelet value is obtained.

The PLT de-aggregation function was used in BC-6800/BC-6200/BC-6800Plus/CAL 6000/CAL 8000 in RET mode.

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How D-dimer in Coagulation Correlates with COVID-19?

D-dimer in COVID-19

As COVID-19 continues to swipe around the world, rapid diagnosis as well as prognosis and treatment of the COVID-19 patients has become an equally important topic among clinicians. Recently scientists have discovered that COVID-19 has a host cell receptor, Angiotensin Converting Enzyme II [1] or ACE2. With the help of ACE2, COVID-19 invades the human body rapidly by reproducing on its own at a massive rate, destroying normal cell, tissue and microvascular system, finally causing acute lung injury, multiple organ failure [2-4], and intravascular coagulation which occurs in 71.4% of patients who died from COVID-19 [5]. It is widely known that D-dimer is a significant bio-marker which correlates with hypercoagulability. More clinical studies have also revealed the relationship between D-dimer and COVID-19.

As published on Jama by Zhi Yong's Group, in the patients' death(non-survivor) group of novel coronavirus pneumonia, the D-dimer level initially increased as the disease developed, until the 7th day when the D-dimer level broke through the normal range, and finally plateaued at a high level [Figure 1 A] [6]. In comparison, the survivor group remained within the normal range consistently. Another article published in the Lancet also claims that there is a close correlation between the D-dimer level and the mortality rate of victims [Figure 1 B] [7]. The same conclusion was also drawn in Shah' s research, which utilized a systematic meta-analysis method (including results from 18 articles and a total of 3,682 patients) to draw the forest plots [Figure 1 C, D] [8]. To sum up, whether in severe or dead COVID-19 patients, the D-dimer level was higher than that which was found in non-severe or surviving patients.

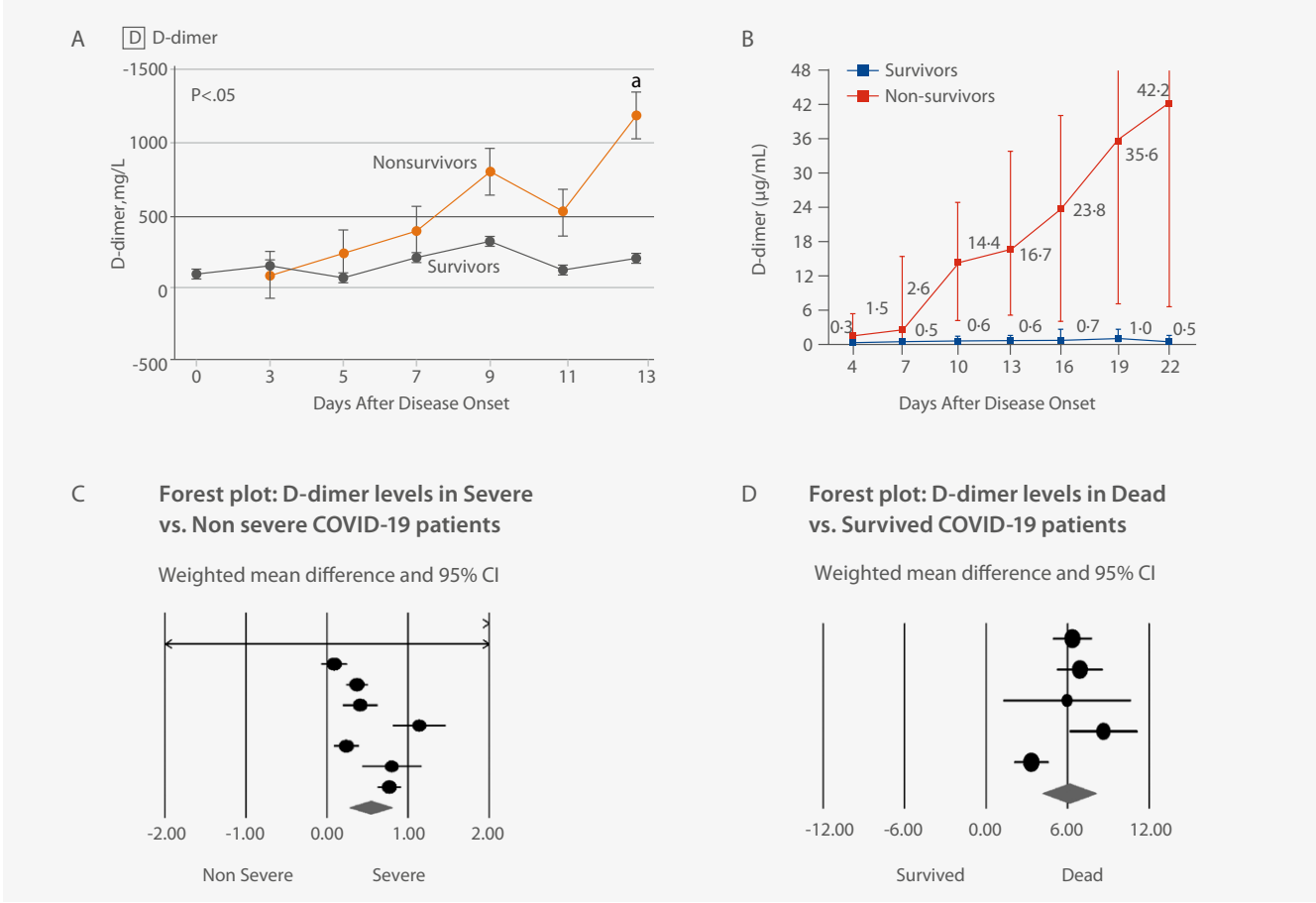


Figure 1: Correlation between D-dimer and COVID-19

Application of D-dimer in COVID-19 Prognosis

According to the study by Zhang' s group, D-dimer among all parameters tested in patients with COVID-19 had the highest C-index, which indicates that it has the highest prediction coincidence rate in routine lab testing methods [Figure 3A]. In addition, they also found the 2 μg/ml of D-dimer could be the cut-off value of mortality risk of COVID-19, as DD > 2 μg/ml the survival probability will decrease dramatically [Figure 2B]. Consequently, they based the evaluation of this value and manifested that when 2 μg/ml was set as the cut-off value, 92.3% of sensitivity and 83.3% of specificity is the optimum in all groups [Figure 2C] [9].

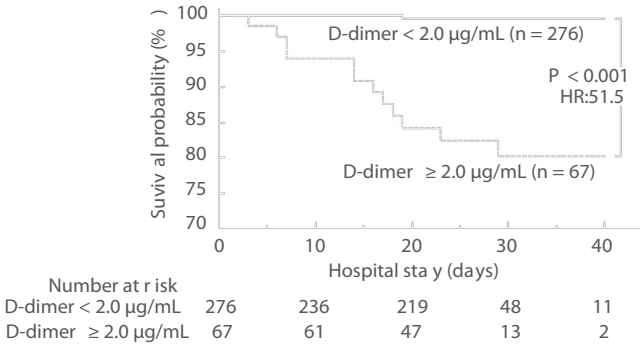
There has been evidence regarding an increased incidence of venous thromboembolic events (VTE) including deep vein thrombosis (DVT) and pulmonary embolism (PE), in patients with severe COVID-19 infection [9], and D-dimer can also be used as a monitoring indicator of VTE and PE with a cut-off value of 0.55 μg/ml. Furthermore, Yao not only found that patients with over 2 μg/ml D-dimer needed intensive care and early intervention, but suggested a cut-off value of 1 μg/ml could help doctors identify patients with a poor prognosis [10].

A

Routine Laboratory Tests	C-index	95% CI
D-dimer	0.883	0.842-0.916
Lymphocyte	0.872	0.832-0.906
Prothrombin time	0.858	0.814-0.895
C-reaction protein	0.844	0.799-0.882
Platelet	0.781	0.734-0.824
Neutrophil	0.773	0.725-0.817
White blood cell	0.625	0.571-0.676
Hemoglobin	0.583	0.528-0.635
Creatinine	0.567	0.510-0.623

Abbreviation: CI, confidential interval.

B



Number at r isk

D-dimer < 2.0 μg/mL	276	236	219	48	11
D-dimer ≥ 2.0 μg/mL	67	61	47	13	2

C

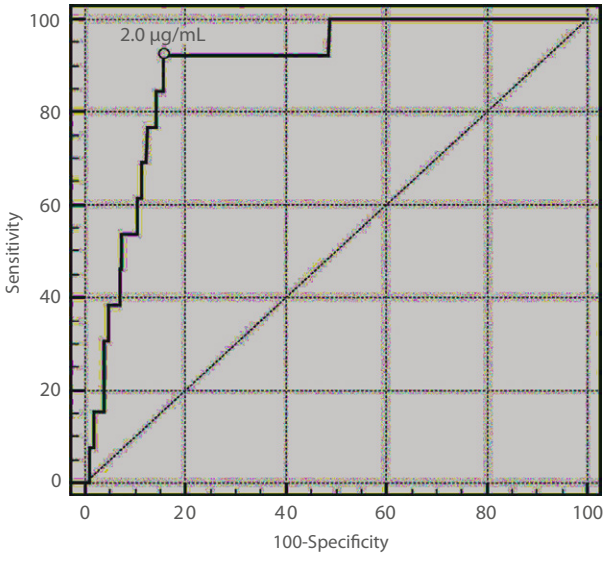


Figure 2: Numberic Results of D-Dimer by Zhang's Group

In conclusion, D-dimer has enormous clinical values in the treatment and prognosis of COVID-19 as a sensitive monitoring index. In consideration of disordered coagulation micro-environment in patients infected with COVID-19 or at high risk of VTE induced by reduced activity, increased bed time, or in people being quarantined for hospitalization, testing of D-dimer on a regular basis is necessary for rapid monitoring of disease treatment. While a cut-off value of over 2 ug/ml has been proved by many researchers monitoring patients' treatment, laboratories are still advised to set their own standard so the variation in demographics can be taken into account.

Mindray's Coagulation D-dimer Solution

Mindray' s auto-coagulation analyzers C3100 & C3510 are equipped with both classic mechanical and optical detection mechanisms. The mechanical methodology is insensitive to interference from icteric, lipemic, chylus and hemolytic samples. Moreover, the patented VRIM(VLin-Rate Integrative Method) algorithm has also been developed to combine "Two Point End Method" at a low D-dimer concentration together with "Rate Method" at a higher level [Figure 3]. This has enabled a much wider linearity range of D-dimer results compared with other models on the market [Figure 4].

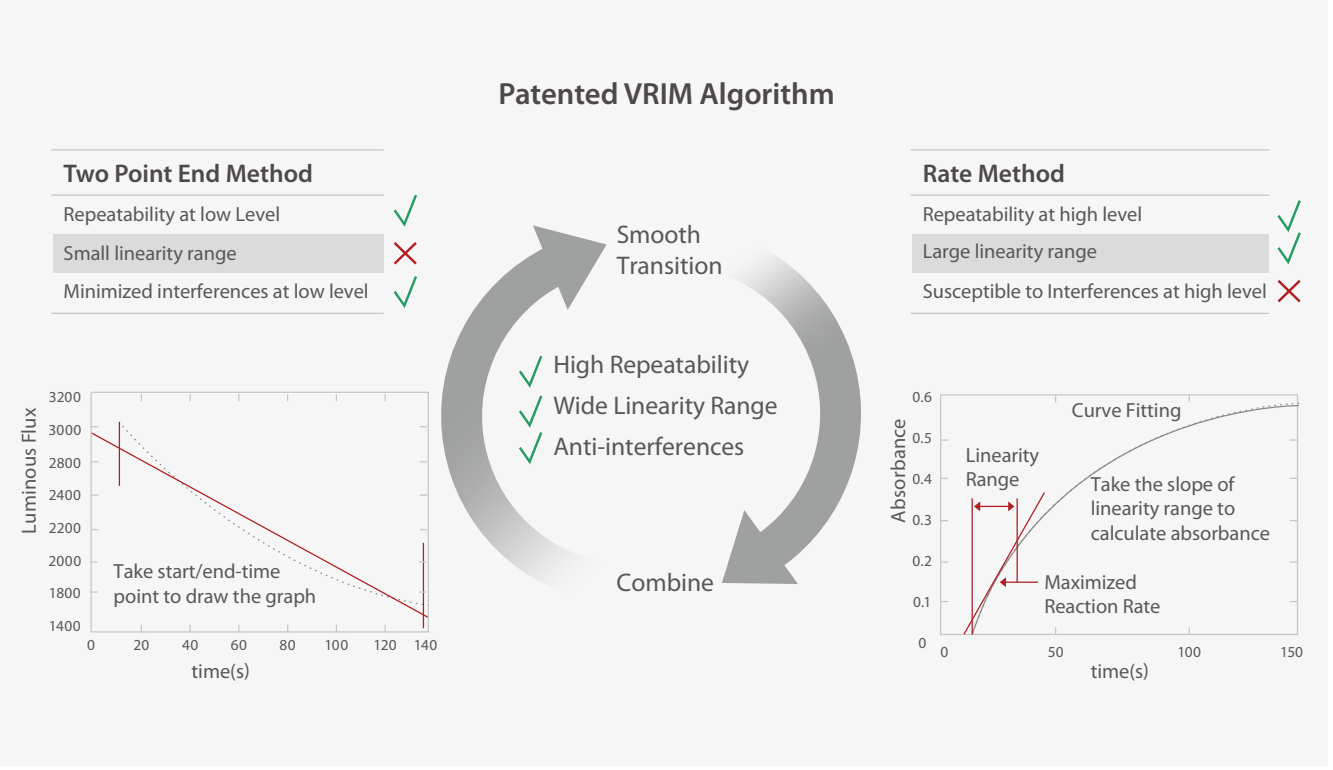


Figure 3: Mindray's patented VRIM Algorithm for D-dimer testing

Manufacturer	Algorithm	Linearity Range (μg/ml)
Mindray	VRIM	0.20~8.0
Brand A	Rate	0.17~4.4
Brand B	Two Point End	0.15~3.7
Brand C	Two Point End	0.22~3.0

Figure 4: Comparison of Linearity Range (without dilution) between Mindray and other brands

In addition, Mindray' s coagulation solution to D-dimer testing is less susceptible to common interferents. As is shown in [Figure 5], when the serum samples are added with bilirubin, hemoglobin, triglycerides and rheumatoid factors at respective concentration, D-dimer results remain at constant levels as before. The Comparison study with Sysmex CS5100 has also shown a good correlation with R2> 97% with interferents added.

Interferent (Concentration)	Before Adding	After Adding
Bilirubin (40 mg/dL)	2.43	2.38
Hemoglobin (200 mg/dL)	2.31	2.36
Triglycerides (1800 mg/dL)	2.39	2.25
Rheumatoid Factor (1300 IU/mL)	1.54	1.55

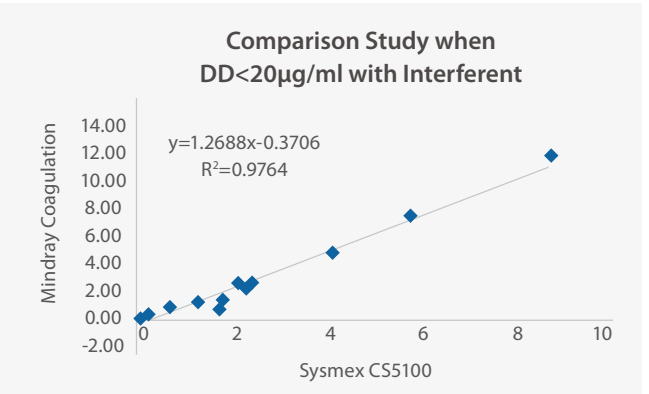


Figure 5: Comparison study with interferents

Figure 6: Mindray's D-dimer coagulation reagents

Mindray's D-dimer coagulation reagents are all manufactured in a bottled liquid state which are ready to use [Figure 6], while the majority of coagulation testing kits are made into powder. Simply by opening the cap and loading D-dimer reagents onto the analyzer, preparation can be set up rapidly with ease on Mindray' s coagulation analyzers.

C3100

- Throughput: up to **200** test/h (PT), up to **44** test/h (D-dimer)
- D-dimer tested with special optical channel
- **61** sample position, 11 reagent position
- **12** incubation channel, 4 mechanical testing channel
- Separate sample/reagent probe ensure low carry over

C3510

- Throughput: up to **300** test/h(PT), up to **91** test/h(D-dimer)
- D-dimer tested with special optical channel
- **80** sample position, 24 (cooling) + 4 reagent position
- **16** incubation channel, 4 mechanical+6 optical testing channel
- Separate sample/reagent probe ensure low carry over

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Have You Noticed the Changes of Red Blood Cells in COVID-19?

As of January 26th, 2021, the coronavirus disease 2019 (COVID-19) pandemic has affected over 100 million people worldwide. Vaccination would help improve the situation in future. However, at this time, identifying patients at highest risk for severe disease is important. In order to facilitate early intervention and to manage local hospital resources to mitigate the critical care crises, our smart doctors conduct research in routine, low-cost, and suggestive parameters to assist with COVID-19 prognosis and the identification of severe cases[1] [2] [3].

Figure 1. Applications of routine blood tests.

Inflammatory parameters, such as white blood cell count (WBC), neutrophil count, neutrophil-to-lymphocyte ratio (NLR) could support COVID-19 diagnosis and prognosis. How about red blood cells?

Observed erythrocytes changes in critically ill patients

Dr. Wang compared hematological results from the good and poor outcome groups and found the best single parameter for predicting the prognosis of severe patients is RDW-SD[4,7]. What's more, combined parameters Lym# & RDW-CV as well as Lym# & RDW-SD are better for predicting the prognosis of severe COVID-19 (Figure 2)[7].

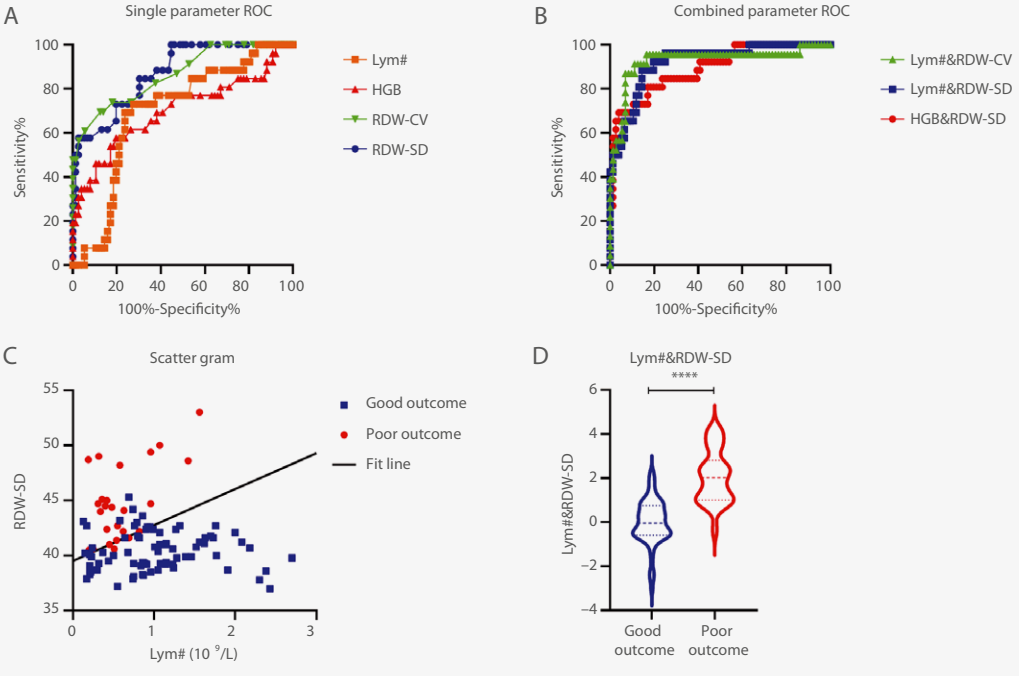


Figure 2. Prediction analysis of hematology parameters and the outcomes of patients with severe COVID-19.
(A) ROC curve, the single parameter for predicting the prognosis of ill patients; (B) ROC curve, joint parameters for predicting the prognosis of ill patients; (C) the linearly fitted schematic scatter plot for Lym# & RDW-SD; (D) comparison of Lym# & RDW-SD in ill patients with different prognoses. Lym# & RDW-SD: joint parameter generated after linear fitting of Lym# and RDW-SD. ****, P<0.0001.

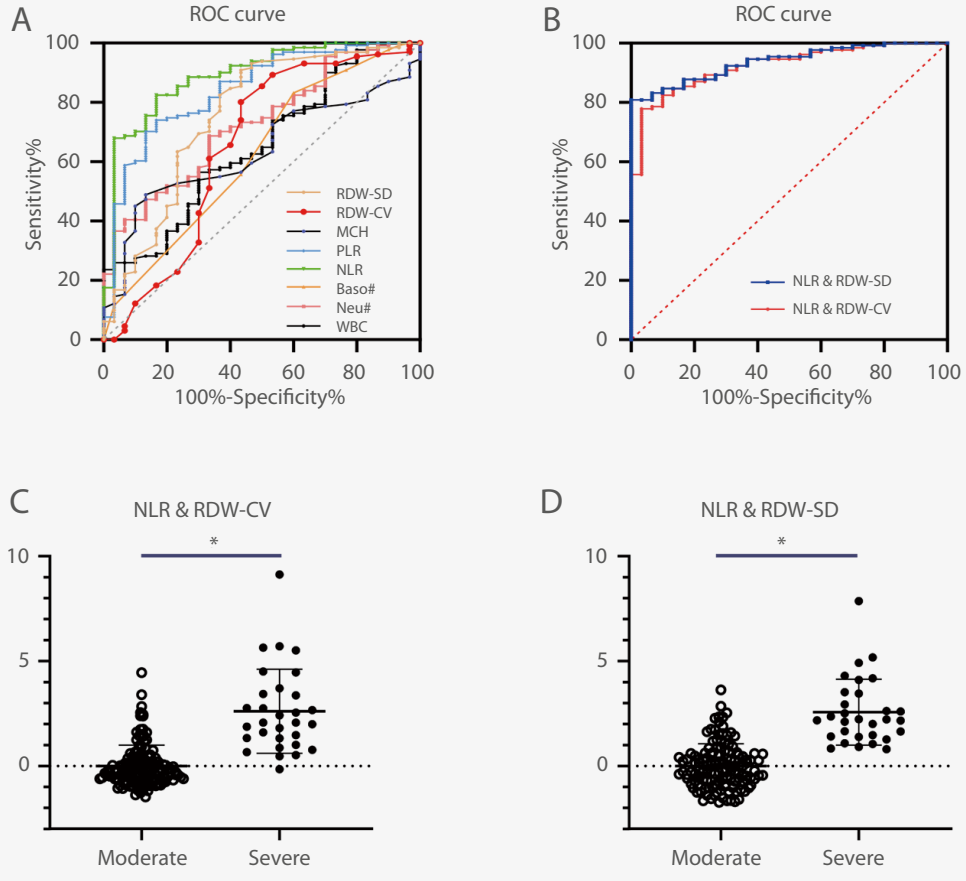
Dr. Zhang has found that HGB is lower in the severe group than in the moderate group[5]. New joint parameters Lym% & HGB have the best sensitivity and specificity (Table1). So Lym% & HGB can be used as indicators of disease prognosis..

Table 1. Receiver operating characteristic analysis results for the three parameters

Parameter	AUC	95% CI	Cutoff	Sensitivity	Specificity	Predict value (+)	Predict value (-)
Lym (%)	0.89	0.88-0.91	18.8	85.6%	77.5%	0.83	0.81
HGB (g/L)	0.79	0.76-0.81	116	71.1%	77.2%	0.80	0.68
Lym% & HGB	0.92	0.91-0.94	0.481	88.9%	79.8%	0.85	0.85

AUC, area under the ROC; Lym%, percentage of lymphocytes; HGB, hemoglobin.

Another article from Dr. Wang[6] described that many hematological parameters changed as the disease progressed, including NLR, RDW-CV, RDW-SD. The combined parameters of NLR & RDW-SD, as generated by linear fitting, had the better diagnostic efficiency (AUC =0.938), which was the best one among single parameters (Figure 3). When the cut-off value was 1.046, the sensitivity for distinguishing the severe cases from the moderate cases of COVID-19 was 90.0% while the specificity was 84.7%.



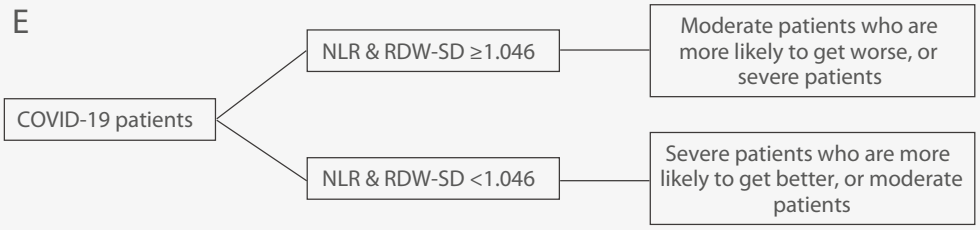


Figure 3. ROC analysis using single and combined parameters in the diagnosis of severe cases of COVID-19. Differentiated diagnosis of moderate and severe COVID-19 patients using different parameters. The positive sample is the blood routine result of the severe patient, and the negative sample is the blood routine result of the moderate patient. Panel A is an ROC plot that uses a single parameter to identify severe from moderate patients. Panel B is an ROC plot that uses the combined parameters of NLR and RDW-SD and NLR and RDW-CV to identify patients; Panels C and D are scatter plots that use the combined parameters for comparison between the two groups; Panel E is a recommendation management strategy for COVID-19 patients. "*" standing for significant deviation.

Why did these erythrocyte changes happen in critically ill patients?

It's been found that the increase in RET may contribute to elevated RDW (Figure 4). As the disease progressed, MFR and HFR increased, so did RDW-SD. The increased RET in peripheral blood may cause an increase in anisocytosis.

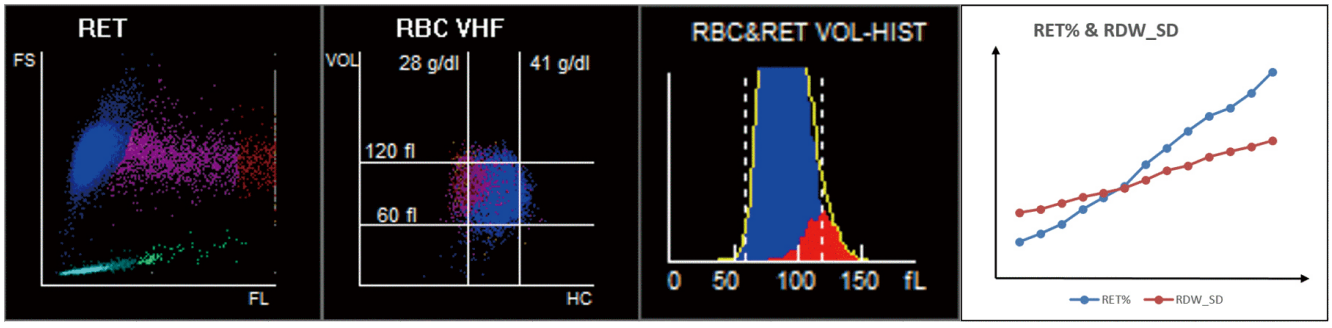


Figure 4. Dynamic monitoring of RET scattergram in critical COVID-19 cases. RET scattergram is from Mindray BC-6800Plus.

Increased RDW-SD

1. As the disease progressed, MFR and HFR increased, RDW-SD increased.

2. As the infection symptoms worsen, the level of oxidative stress in the body increases, oxygen free radicals increase. Insufficient circulating nutrients in patients may also lead to an increase in RBC membrane instability, followed by increased RDW.

Decreased HGB

1. Long-term hypoxia leads to increased synthesis of erythropoietin and active erythropoietin hyperplasia.

2. On the contrary, HGB synthesis is prevented in critically ill patients due to malnutrition or iron deficiency, resulting in low HGB and low-HC (hemoglobin concentration) RETs.

Figure 5. Mechanism of RDW-SD and HGB change in COVID-19[6].

How can we observe these erythrocyte changes in the hematology analyzer?

When we look at the 9-square scattergram, the RBC volume/hemoglobin concentration (V/HC) scattergram showed that the magenta scatters of critical patients were significantly left-skewed, indicating that RETs with a low HC (hemoglobin concentration) increased significantly, which may represent a unique pattern of erythroid hyperplasia in critical COVID-19 patients (Figure 6A)[7]. However, whether such low-HC RETs could be a diagnostic marker of critical COVID-19 still requires further investigation[7].

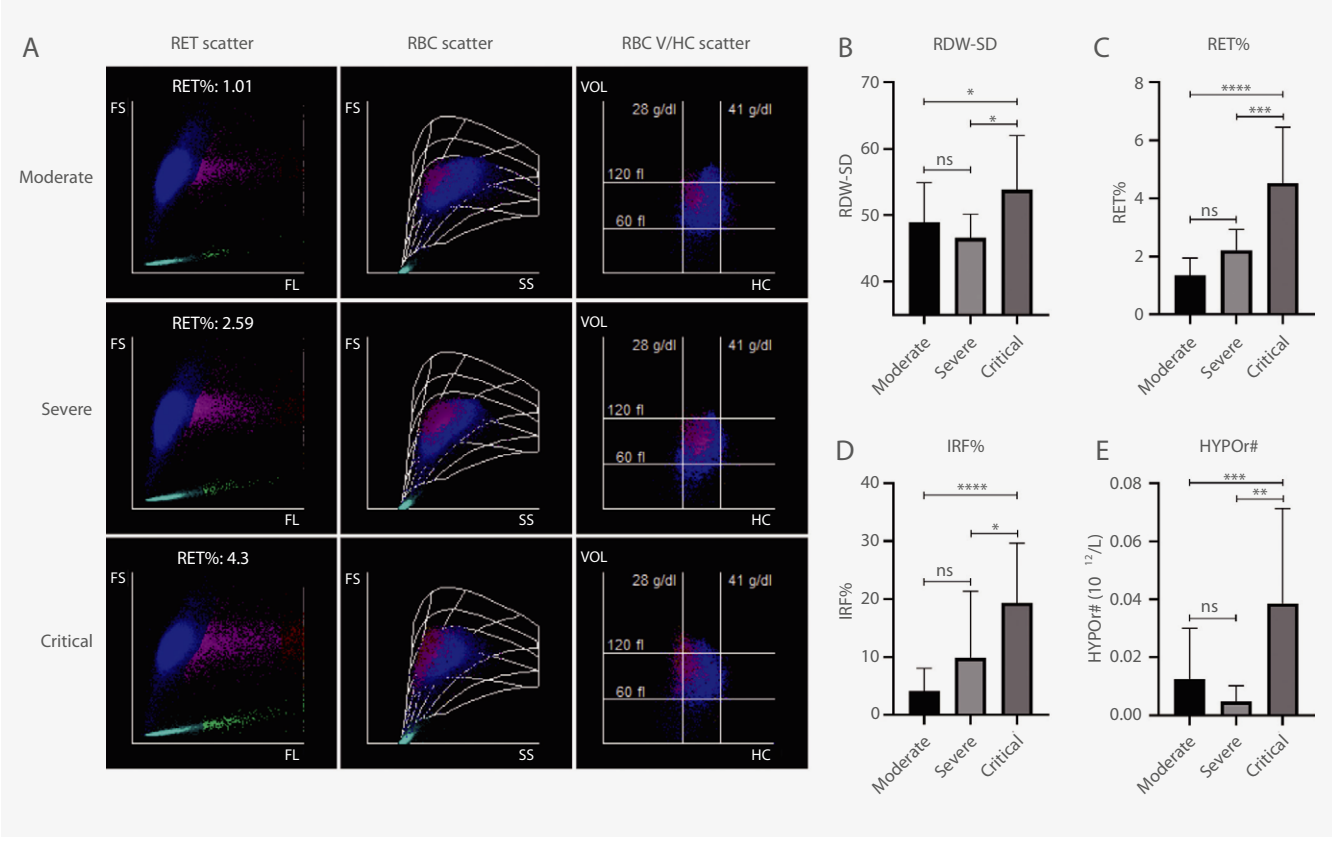


Figure 6. Differences of RET scattergram results in patients with different severity of COVID-19. (A) Scattergram of RET channel data from Mindray BC-6800 hematology analyzer. Blue scatters are RBCs, magenta, and the red scatter is the RETs, and cyan scatters are PLTs. (B,C,D,E) Comparison of parameters obtained from the RET channel for patients with different severities of COVID-19. Data are shown as the mean ± SD. ****, P<0.0001; ***, P<0.001; **, P<0.01; *, P<0.05. FS, forward scatter; SS, side scatter; FL, fluorescence; HC, hemoglobin concentration; VOL, volume; ns, nonsignificant.

With advanced technologies, the newly combined hematological parameters, such as Lym% & RDW-SD, Lym# & HGB and NLR & RDW-SD, have been found as supportive predictors during COVID-19 prognostics. More and more covariates can be studied and developed on the Mindray BC-6000 series analyzers. Especially on BC-6800Plus, the RET channel can detect the number, size, and hemoglobin concentration of RBCs and RETs highly sensitive laser scattering technology. Thus, it's recommended to start using self-defined parameters for COVID-19 prognosis now.

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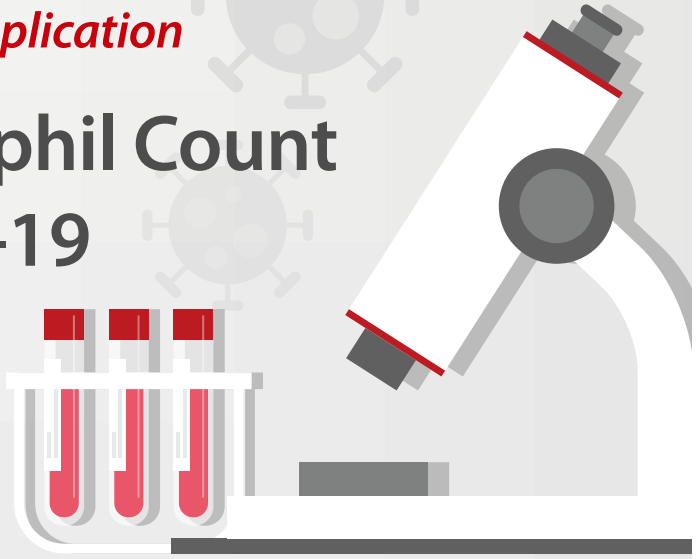
Common parameter, new application

How Does Eosinophil Count Change in COVID-19 Patients?



Is prophylactic anticoagulant therapy a common treatment for clinicians to deal with thrombotic events in COVID-19?

Is there a connection between the eosinophil count and anticoagulation monitoring in COVID-19 patients?

Thrombotic events in COVID-19 patients

Thrombosis has emerged as an important complication among hospitalized patients with COVID-19. A prothrombotic state induced by SARS-Cov-2 can manifest in venous thromboembolism (VTE), arterial thrombosis and disseminated intravenous coagulation (DIC). [1]

In 28 studies including 2928 patients, thrombotic complications occurred in 34% of ICU patients, deep venous thrombosis (DVT) reported in 16.1% and pulmonary embolism in 12.6% of patients, and were associated with high mortality. [2]

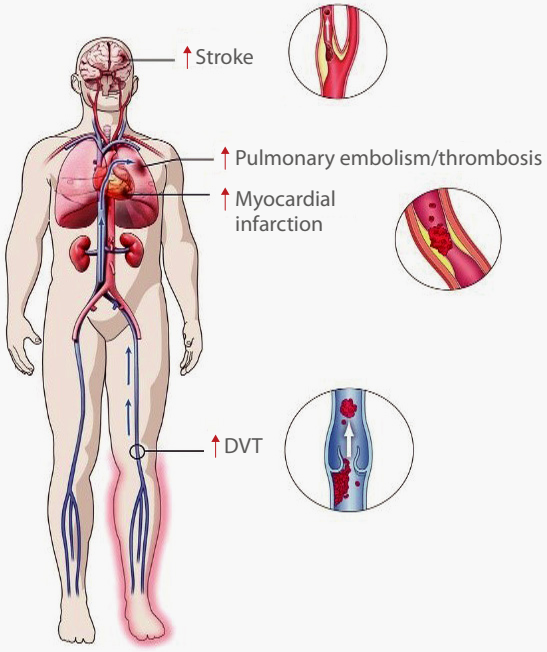


Figure 1. Possible venous and arterial thrombotic complications associated with COVID-19. [1]

Antithrombotic treatment of low molecular weight heparin in COVID-19 patients

Low molecular weight heparin (LMWH) and unfractionated heparin (UFH) are recommended by the international society for thrombosis on hemostasis (ISTH), American Society of Hematology (ASH) for the treatment of thrombotic events associated with SARS-CoV-2 infection. Particularly, LMWH has a stronger antithrombotic effect than UFH.

LMWH dose monitoring

LMWH predominantly acts on factor Xa. For this reason, LMWH activity is monitored using serum anti-factor Xa activity (AFXa) levels instead of activated Partial Thromboplastin Time (aPTT) (Figure 2). [3]

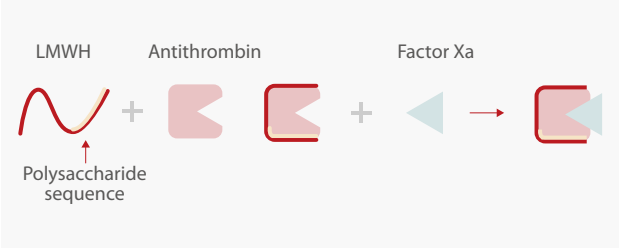


Figure 2. The antithrombotic mechanism of LMWH. [3]

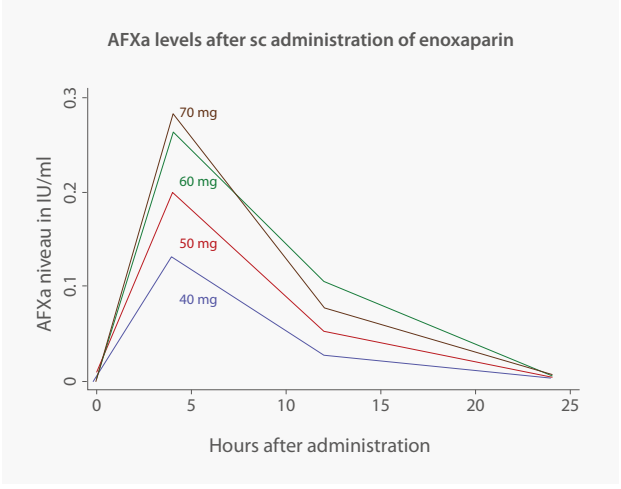


Figure 3. Variation in AFXa over time for each dose of enoxaparin. [4]

Enoxaparin is one of the most important LMWH. The AFXa level reached peak 3-5 hours after administration. The AFXa levels below 0.2 IU/mL may increase the risk of VTE in COVID-19 patients, due to the hypercoagulability. [4]

Eosinophil counts in antithrombotic treatment to COVID-19 patients

Dr. Selma Ari has found the increased eosinophil count is associated with the level of subprophylactic anticoagulation in COVID-19 patients. [5]

Table 1 Results of laboratory parameters at admission

Variable	Subprophylactic anticoagulation group (13 patients) anti-factor Xa < 0.2 IU/mL	Prophylactic anticoagulation group (67 patients) anti-factor Xa > 0.2 IU/mL	*p* value
WBC X10³ /mL	5.91 ± 1.31	5.54 ± 1.89	0.51
Neutrophil	3.57 ± 1.27	3.51 ± 1.71	0.91
Lymphocyte	1.76 ± 0.60	1.54 ± 0.66	0.25
Eosinophil (%)	2.96 ± 2.55	0.90 ± 1.28	*0.001*
Eosinophil count	168.42 ± 147.25	50.32 ± 73.42	*0.001*
Platelet X10³ /mL	232.00 ± 62.21	197.57 ± 57.87	0.06
CRP (mg/L)	12.18 ± 16.66	25.12 ± 31.04	0.08
Fibrinogen (mg/dl)	367.08 ± 134.97	410.00 ± 117.34	0.24
D-dimer (µgr/mL)	0.57 ± 0.38	1.21 ± 3.35	0.50
PT	11.55 ± 0.91	11.82 ± 1.92	0.62
aPTT (s)	23.25 ± 3.24	25.62 ± 8.45	0.32
INR	0.95 ± 0.06	0.96 ± 0.19	0.89
Baseline anti-factor Xa level (IU/mL)	0.18 ± 0.06	0.43 ± 0.23	*<0.001*

Parameters whose p<0.05 are written in italics

In the laboratory results, only eosinophil counts and AFXa are significantly different between subprophylactic anticoagulation group and prophylactic anticoagulation group when the patients are admitted to hospital (Table 1). [5]

Table 2 Results of laboratory parameters before discharge

Variable	Subprophylactic anticoagulation group (13 patients) anti-factor Xa < 0.2 IU/mL	Prophylactic anticoagulation group (67 patients) anti-factor Xa > 0.2 IU/mL	*p* value
WBC X10³ /mL	6.25 ± 0.82	5.55 ± 1.95	0.08
Neutrophil	3.81 ± 1.14	3.26 ± 1.58	0.08
Lymphocyte	1.81 ± 0.69	1.79 ± 0.78	0.52
Eosinophil (%)	3.06 ± 1.49	2.07 ± 1.92	*0.001*
Eosinophil count	182.49 ± 95.81	112.18 ± 102.54	*0.009*
Platelet X10³ /mL	264.42 ± 117.14	226.94 ± 89.08	0.25
CRP (mg/L)	8.54 ± 11.47	19.45 ± 35.44	0.19
Fibrinogen (mg/dl)	377.33 ± 145.03	416.98 ± 148.71	0.31
D-dimer (µgr/mL)	0.72 ± 0.77	0.78 ± 1.08	0.91
PT	11.72 ± 0.59	11.93 ± 1.28	0.65
aPTT (s)	22.34 ± 1.38	24.38 ± 3.58	0.01
INR	0.96 ± 0.05	0.98 ± 0.11	0.46
Control anti-factor Xa level (IU/mL)	0.16 ± 0.04	0.53 ± 0.26	*<0.001*

Parameters whose p<0.05 are written in italics

Laboratory analysis collected before the discharge of patients revealed that eosinophil counts in subprophylactic anticoagulation group were higher than in prophylactic anticoagulation group , whereas AFXa were lower in subprophylactic anticoagulation group (Table 2). [5]

Eosinophils and thrombosis

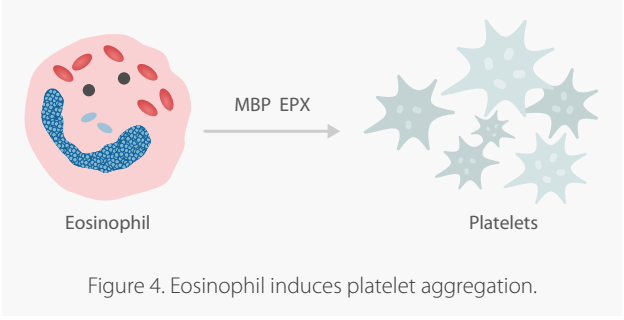


Figure 4. Eosinophil induces platelet aggregation.

Eosinophil induces platelet aggregation and thrombus formation through the production of major basic protein (MBP) and eosinophil peroxidase (EPX). [6]

Enzymes released from eosinophils (peroxidases, cationic proteins, and neurotoxins) may decrease the anticoagulant activity of heparin. [7]

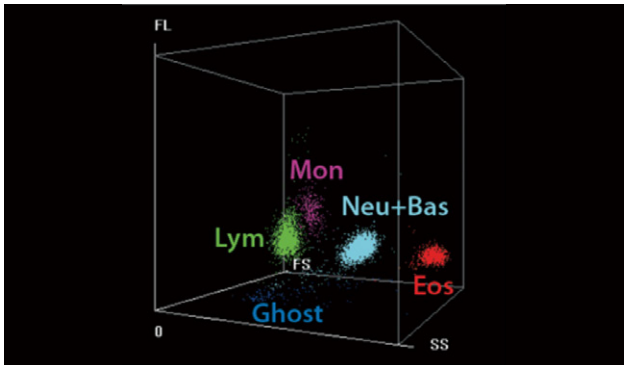


Figure 5. SF Cube on Mindray BC-6800.

In this study, in subprophylactic anticoagulation group, high eosinophil levels had lower anticoagulant activity in COVID-19 patients. Eosinophil counts were examined with Mindray BC- 6800 auto hematology analyzer. Its SF Cube analysis technology can produce three-dimensional scattergram which can help doctors better identify and differentiate blood cell populations, especially to reveal abnormal cell population undetected by other techniques.

Nowadays a large number of parameters on BC-6800 can be used in clinical diagnosis and scientific research. There fore, clinicians are welcome to do more research on COVID-19 on Mindray BC-6200/BC-6800/BC-6800Plus/ CAL 6000/CAL 8000.

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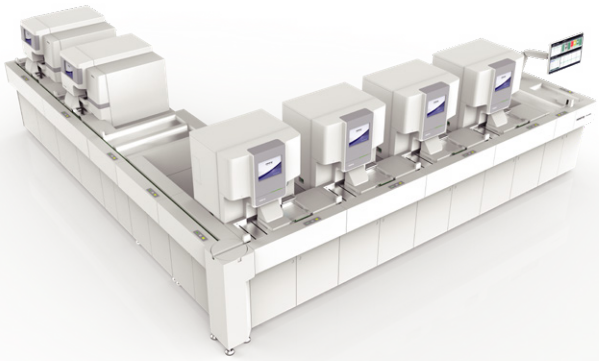

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