Platelet flow cytometry -

what the ISTH-SSC says and what is done in Switzerland

Hämostaseclub der SGH

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Flow cytometry is an important tool for the investigation of platelets

- surface receptors (e. g., GT, BSS)
- abnormalities of collagen (GPVI, GPIa/IIa) or thrombin receptors (PAR-1)
- storage pool disorders
- activation of platelets
- monitoring of antiplatelet drugs

Often preceded by functional assays such as aggregometry (Harrison P. Hämostaseologie 2009; Gresele P. JTH 2014)

Several advantages

- Samples with low platelet count (e. g. BSS)
- Small sample volume (children)
- Analysis in a physiological state
- Insignificant preactivation of platelets
- Simultaneous determination of resting and activated platelets

Technical guidance and standardization is scarce

Consensus protocol of the European Working Group on Clinical Cell Analysis (Schmitz G. TH 1998):

- Type of specimen (whole blood, platelet concentrates)
- Specimen collection preparation
- Anticoagulant (usually sodium citrate)
- Selection of antibodies (monoclonal) and conjugates
- Buffers for incubation and washing, fixatives
- Instrumentation and software
- Staining, washing, controls
- Analysis, data acquisition and evaluation, report, QC

ISTH collaborative project (SSC on platelet physiology)

„*Standardization of flow cytometry for the assessment of inherited and acquired disorders of platelet number and function*“

- Beginning 2017, duration1 year (SSC report 2018)
- Aim: to provide guidance for the use of platelet flow cytometry to diagnose hereditary and acquired PFD
- Little evidence
  - expert panel and consensus
  - based on literature review

ISTH-SSC guidance on the diagnosis of inherited PFD

(Gresele P. JTH 2014)

- Flow cytometry among the first and second of 3 diagnostic steps
- 1. screening
  - GPIIb/IIIa or GPIIa, GPIb/IX or GPIb, GPIIb/IIIa activation (PAC-1)
- 2. extension
  - GPIa/Iia (CD29/49b)
  - GPIV (CD36)
  - GPVI
  - Granule content (CD62P, CD63)
  - Procoagulant activity (annexin V binding)

Screening panel should be carried out

- On resting platelets using AB to GPIIb/IIIa (CD41/61), GPIb/IX (CD42a/b)
- On activated platelets using AB to GPIIb/IIIa activation epitope (PAC-1)

Explanded panel should evaluate

- GPIa/IIa (CD29/49b), GPIV (CD36) and GP VI, as well as granule marker (CD62P, CD63) to identify rarer abnormalities
- Assessment of platelet procoagulant activity (i. e. annexin V binding) → disorders with enhanced (Stormorken syndrome) or impaired (Scott syndrome) procoagulant activity

Current practice in Switzerland

- Survey by the WPH-SGH covering major hospitals and one private laboratory (not pediatric)
- Platelet flow cytometry is available only in 5 Swiss university hospitals
    - CHUV
    - HUG
    - Inselspital Bern
    - UHBS
    - USZ
- Differences regarding panels, methods, equipment, and place in the diagnostic hierarchy

		L1	L2	L3	L4	L5
Accepted samples		internal	internal + external	internal	internal + external	internal + external
Test frequency		Routine: 1-2/week (60/y) Research: daily	on demand (<10/y)	weekly (4x1)	1/month	weekly (2x2)
Diagnostic hierarchy		2. line	2. line GP screen in macrothrombocytopenia or suspected secretion defect (α granules)	1. line	2. line	2. line
Apparatus		BD Accuri C6	BD Accuri C6	BD FACSCanto	BD FACSCanto II	BC Navios
Blood sampling	Tubes	Buffered citrate (PFA tubes)	Citrated vacutainer	Citrated monovettes (Sarstedt)	K2-EDTA monovettes (Sarstedt)	Citrated monovettes (Sarstedt)
	Sample delivery	Courier	Courier	Courier	Courier	Courier
Specimen		Diluted PRP (activation/analysis) Gel-filtered (research)	Whole blood (GP screen) PRP (α granule testing)	PRP (150 G/l)	PRP	Whole blood

		L1	L2	L3	L4	L5
Panel	Surface receptors	GPIIb/IIIa (CD41/61) GPIb/IX (CD42a/b) GPVI, GPIa (CD49b)	GPIIIa (CD61), GPIb (CD42b), GPIa (CD49b) = GP screening	GPIIb/IIIa (CD41/61) GPIb (CD42b)	GPIIb (CD41) GPIb (CD42b)	GPIIb/IIIa (CD41/61) GPIb/IX (CD42a/b) GPVI, GPIa/IIa (CD29/CD49b) (collagen receptors)
	Activation markers	CD62P, PAC-1, Annexin V	CD62P	CD62P		PAC-1, CD62P, CD63
	Contents	CD62P, Mepacrine	CD62P (α granule secretion)	CD62P , Mepacrine		CD62P, CD63
	Microparticels	No	No	No	No	No
Isotype control		yes	yes	-	yes	yes
Colours		FITC, PE	FITC, APC, PE	FITC, PE	FITC, APC	FITC, PE, PerCP, APC

		L1	L2	L3	L4	L5
Stimulation	TRAP or thrombin	Different thrombin concentrations	TRAP-6	Different thrombin concentrations	-	TRAP-6
	ADP	Different conc.	-	Different conc.	-	(on demand)
	Others	Convulxin (different conc.)	-	Convulxin (different conc.)	-	-
Fixation	pre-activation	-	Only for GP screen	-	yes (if analysis not within 4 h)	-
	post-activation	-	-	-	-	yes
Definition of negativity		isotype, normal control	?	normal control	normal control	isotype, normal control
External QC		-	-	-	-	-
Accreditation		-	-	yes	yes	yes

		L1	L2	L3	L4	L5
Remarks		ongoing developments (intracellular cations + anions, phosphorylation state of signalling proteins)	flow used most often for VASP (53/2015)			VASP available

Summary

- Screening panel available in all Swiss university hospitals as a routine method
- 3 of 5 institutions offer an extended panel
- Major differences regarding sample preparation, specimen, methodology, and test frequency
- Guidance and standardization are pending

Thank you

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