Funkčný test stanovenia HIT prietokovou cytometriou

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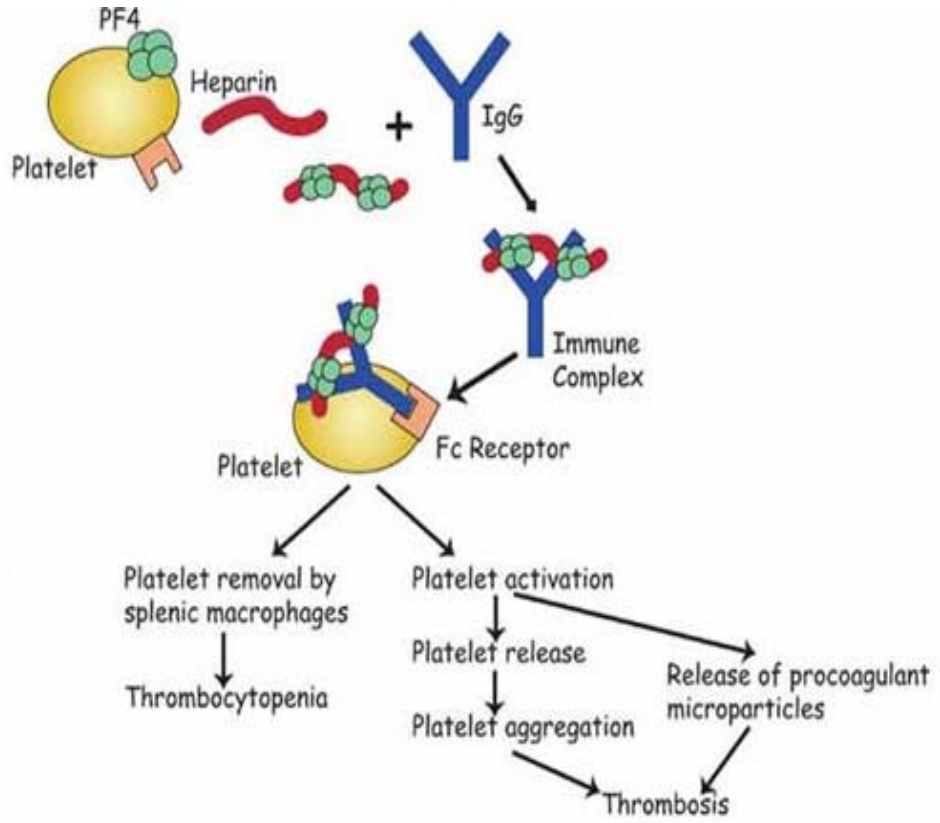
Stretnutie užívateľov prietokovej cytometrie - 13.-14.5.2018 Valeč

Univerzitná nemocnica Martin

Úvod

- Heparínom indukovaná trombocytopénia (HIT) – je protilátkami sprostredkovaná nežiadúca reakcia po podaní heparínu.
- Protilátky sa môžu vytvoriť až po expozícii heparínu a sú namierené proti doštičkovému faktoru 4 (PF4);
- Komplex heparín-PF4-protilátka sa viaže na doštičky cez FcγRII; spôsobuje ich aktiváciu a agregáciu (uvoľňujú mikropartikuly; zvýšená tvorba trombínu);
- Spôsobuje trombocytopéniu, arteriálnu alebo žilovú trombózu;
- Prevalencia je približne 0,5–5 % heparinizovaných pacientov.



•Warkentin T.E., et al Arch Intem Med. 2003; 163(20): 2518-2524

I. History and Physical Examination: Evaluating the Clinical Probability of HIT

A. Features of the history and physical examination that support a diagnosis of HIT

Feature	Comments
Fall in platelet count ≥ 50%	From highest platelet count after heparin exposure; platelet count fall is 30–50% in 10% of cases
Fall in platelet count begins 5–14 days after immunizing heparin exposure	Heparin administered during or soon after surgery is more likely to be immunizing
Fall in platelet count begins within 24 hours after heparin exposure	May occur in patients with previous heparin exposure within last 100 days
Nadir platelet count ≥ 20×10^9/L	Nadir may exceed lower limit of normal range (i.e. 150×10^9/L) in patients with high baseline platelet counts. May be < 20×10^9/L in cases associated with DIC
Venous or arterial thrombosis	Occurring ≥ 5 days after heparin exposure and up to 30 days after heparin cessation
Skin necrosis	At subcutaneous heparin injection sites
Anaphylactoid reaction	Within 30 minutes after intravenous heparin bolus or subcutaneous injection
Absence of alternative causes of thrombocytopenia	Such as infection, other medications known to cause thrombocytopenia, cardiopulmonary bypass within previous 96 hours, intra-aortic balloon pump, extracorporeal membrane oxygenation, etc.
Absence of petechiae and other mucocutaneous bleeding	Adrenal hemorrhage secondary to adrenal vein thrombosis may occur in association with HIT

2013 Clinical Practice Guideline on the Evaluation and Management of Adults with Suspected Heparin-Induced Thrombocytopenia(HIT)

B. The 4Ts: A clinical probability scoring system

4Ts	2 Points	1 Point	0 Points
Thrombocytopenia	Platelet count fall > 50% and platelet nadir ≥ 20 x 10^9/L	Platelet count fall 30–50% or platelet nadir 10-19 x 10^9/L	Platelet count fall < 30% or platelet nadir < 10 x 10^9/L
Timing of platelet count fall	Clear onset between days 5–14 or platelet fall ≤ 1 day (prior heparin exposure within 30 days)	Consistent with days 5–14 fall, but not clear (e.g. missing platelet counts) or onset after day 14 or fall ≤ 1 day (prior heparin exposure 30–100 days ago)	Platelet count fall ≤ 4 days without recent exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis at heparin injection sites; anaphylactoid reaction after IV heparin bolus	Progressive or recurrent thrombosis; Non-necrotizing (erythematous) skin lesions; Suspected thrombosis (not confirmed)	None
o**T**her causes of thrombocytopenia	None apparent	Possible	Definite

High probability (6–8 points), intermediate probability (4–5 points), low probability (≤3 points).

Adapted from Lo et al., *J Thromb Haemost* 2006;4:759. The 4Ts has not been compared with intuition-based diagnosis. It may be used as a guide for clinicians but should not substitute for clinical judgment. In a meta-analysis, the negative predictive value of a low probability 4T score was 99.8% (i.e. a low probability score reliably excludes HIT). The positive predictive values of intermediate and high probability scores were 14% and 64%, respectively (Cuker et al., *Blood* 2012;120:4160).

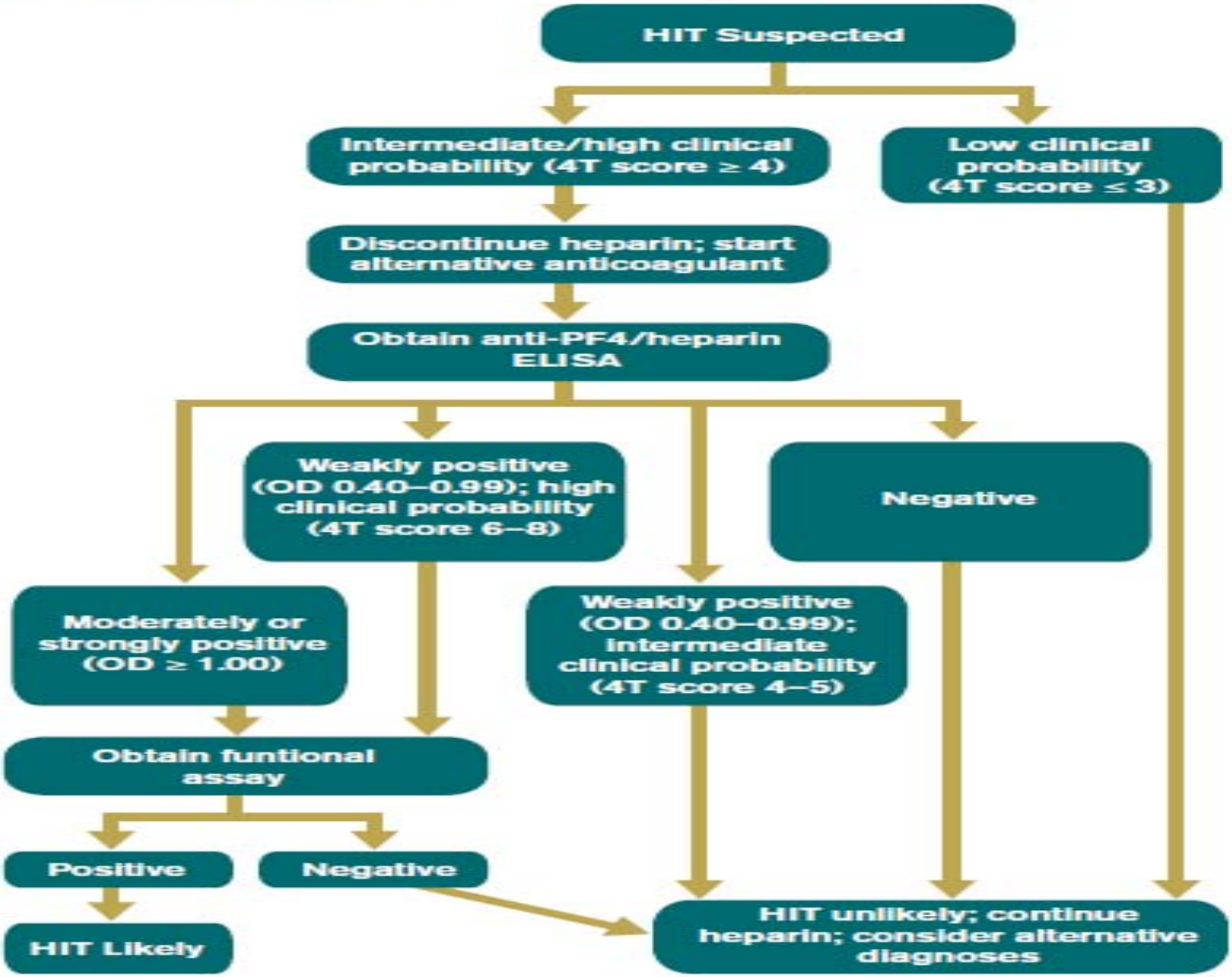
II. Laboratory Diagnosis

Assay category	Mechanism	Examples	Sensitivity	Specificity	Comments
Immunologic	Detects antibodies against PF4/heparin, regardless of their capacity to activate platelets	1. Polyspecific ELISA 2. IgG-specific ELISA 3. PaGIA	>95%	50–89%	OD of ELISA result correlates with clinical probability of HIT and odds of a positive functional assay
Functional	Detects antibodies that induce heparin-dependent platelet activation	1. SRA 2. HIPA	90–98%	90–95%	Not widely available; requires referral to a reference laboratory

PF4, platelet factor 4; PaGIA, particle gel immunoassay; OD, optical density; SRA, serotonin release assay; HIPA, heparin-induced platelet activation assay.

III. Diagnostic and Initial Treatment Algorithm

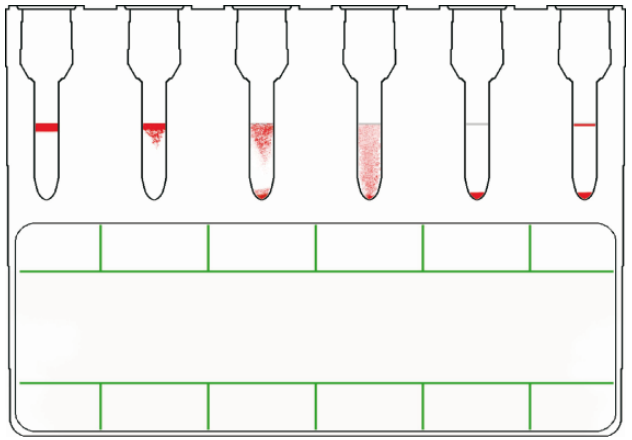
Adapted from Cuker et al., *Blood* 2012;119:2209. OD, optical density.



- 2013 Clinical Practice Guideline on the Evaluation and Management of Adults with Suspected Heparin-Induced Thrombocytopenia (HIT)

Metóda

- a) imunologický test ID-PaGIA Heparin/PF4 (particle gel immunoassay);
- Imunologický test je založený na reakcii partikúl a príslušnej protilátky v sére pacienta metódou stĺpcovej aglutinácie;
- Sérum sa inkubuje so špecifickými časticami. Po centrifugácii gélu sa na častice naviažu protilátky proti komplexu heparín / PF4;



Metóda

- b) funkčný test – kit HITAlert;
- Inkubácia PRP (trombocytov darcu) so sérom pacienta bez, s nízkou a vysokou dávkou heparínu
- Stanovenie aktivovaných doštičiek darcu (CD41+; Annexin V+). Annexin V rozpoznáva fosfatidylserín na povrchu aktivovaných doštičiek

Cieľ

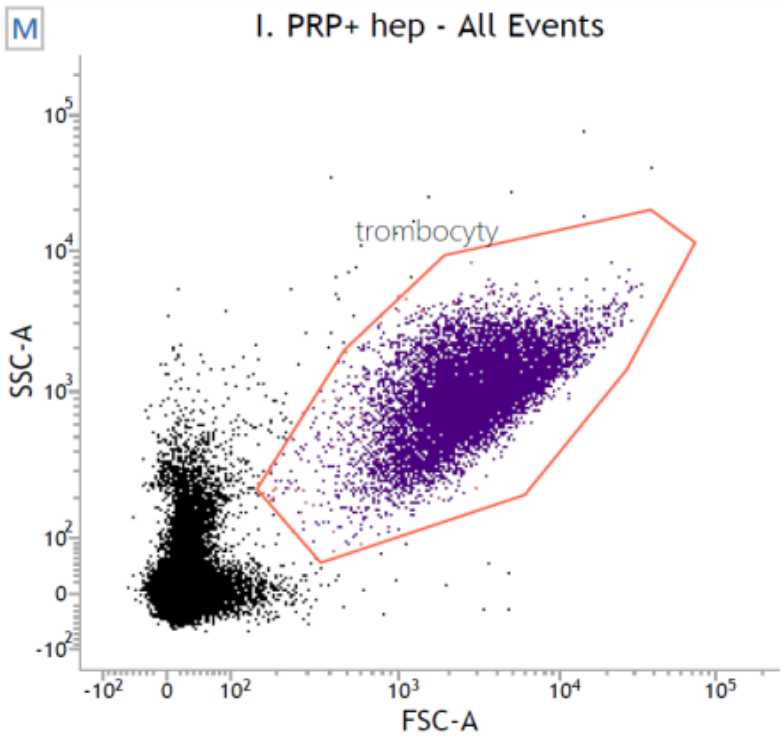
- Cieľom práce bolo poukázať na výhody, úskalia a možnosti aplikácie funkčného testu HIT Alert pomocou prietokovej cytometrie.

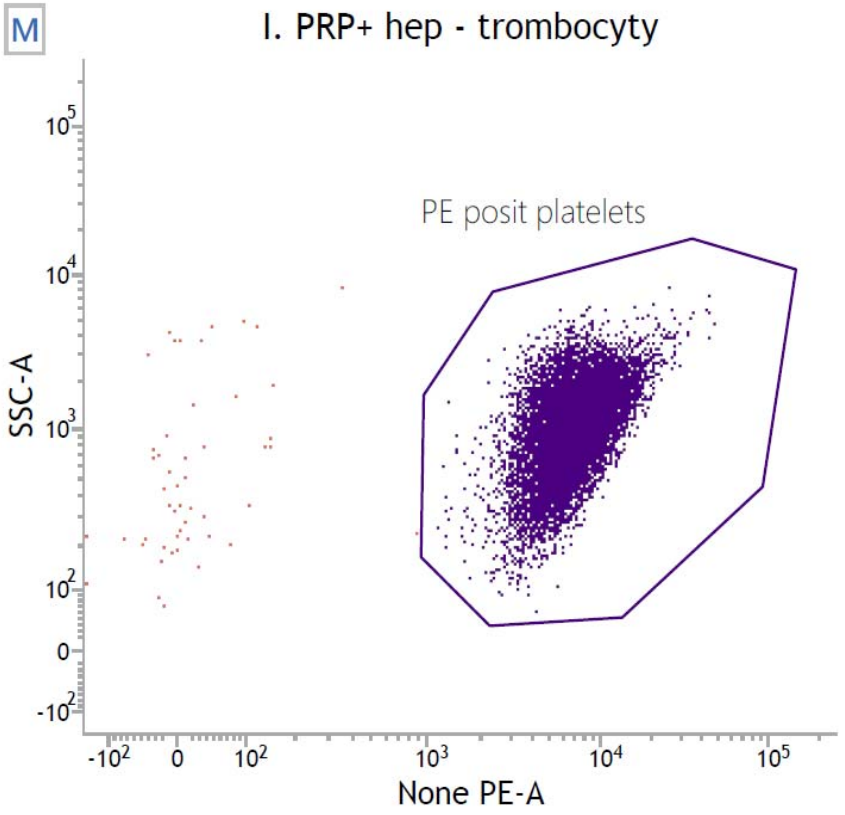
Výsledky

- Analýzou 14 vzoriek pacientov s podozrením HIT imunologickým testom ID-PaGIA Heparin/PF4 sme dostali 13 pozitívnych výsledkov.

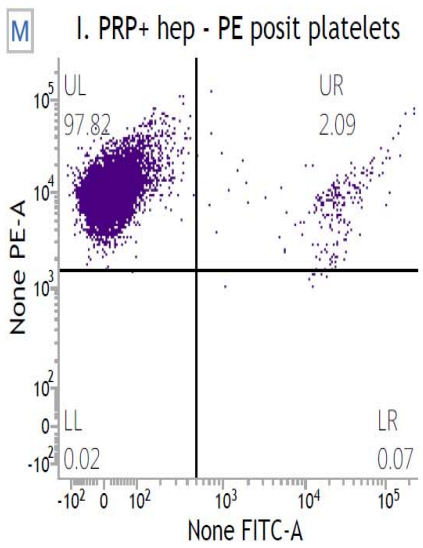
- Ďalším testovaním týchto vzoriek prietokovou cytometriou-kitom HITAlert malo 7 vzoriek pozitívny a 6 vzoriek negatívny výsledok.

Analýza

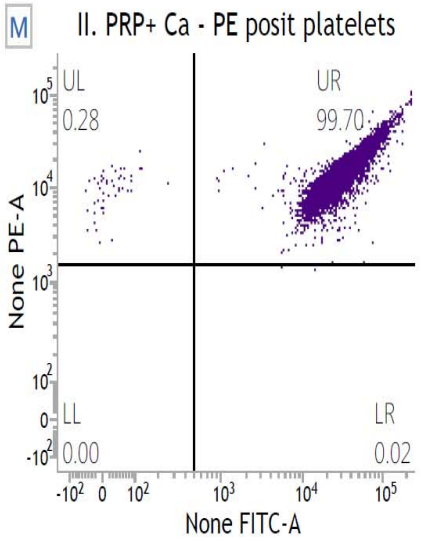

M
I. PRP+ hep - All Events
trombocyty
SSC-A
FSC-A


M
I. PRP+ hep - trombocyty
PE posit platelets
SSC-A
None PE-A

Negatívna kontrola

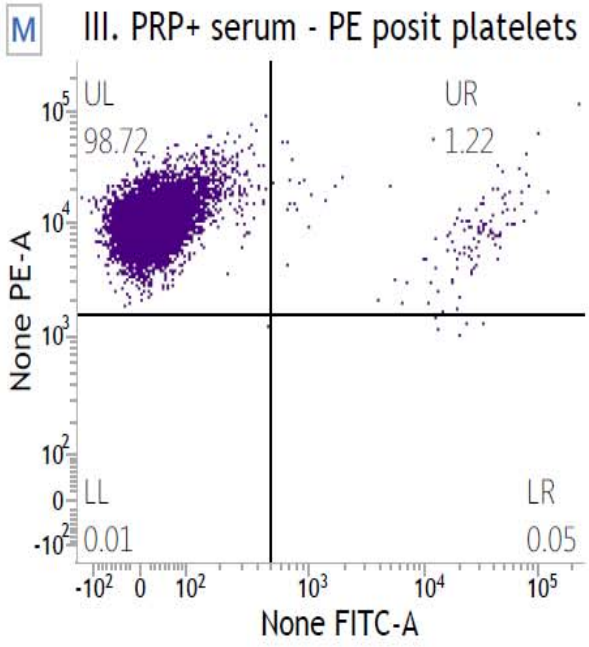


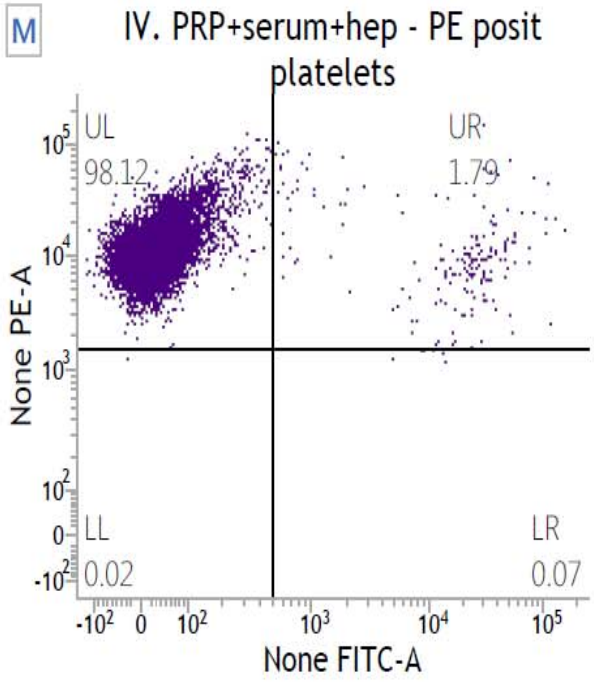
Poztívna kontrola

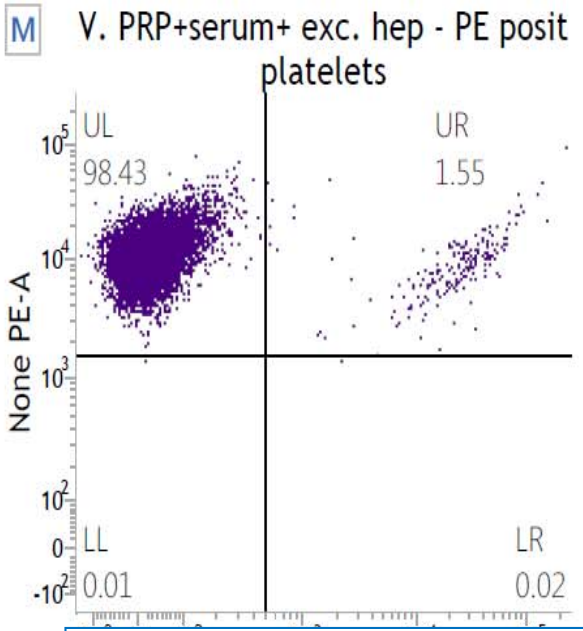


- **Kritériá interpretácie**
- **HIT negat. HIT pozit.**
- **I. < 5 % < 5 %**
- **II. 80-100 % 80-100 %**
- **III. < 5 % aktivácia menej ako**
- **polovica vzorky IV**
- **IV. < 8 % > 8 %**
- **V. < 5 % aktivácia menej ako**
- **polovica vzorky IV**

Vyšetrovaná vzorka:

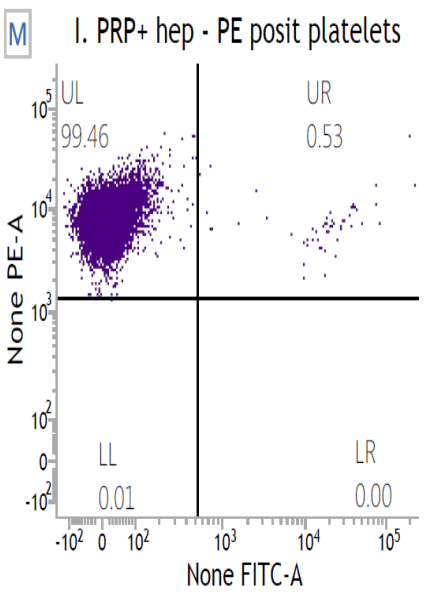




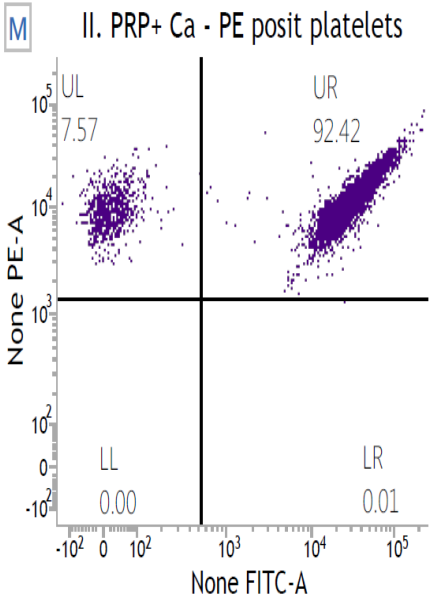


Výsledok testu: NEGATÍVNY

Negatívna kontrola



Pozitívna kontrola



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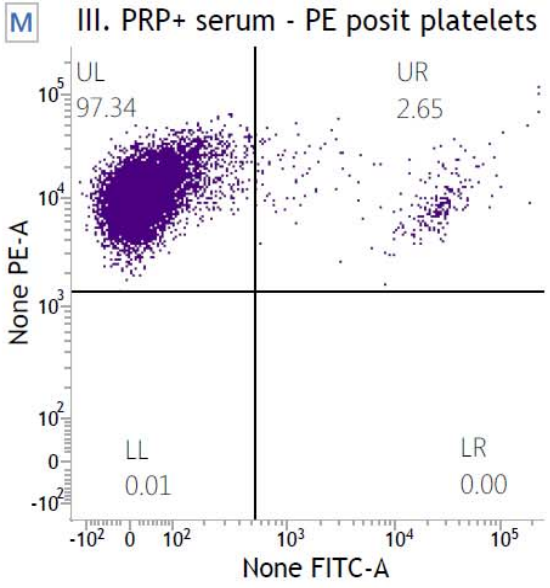

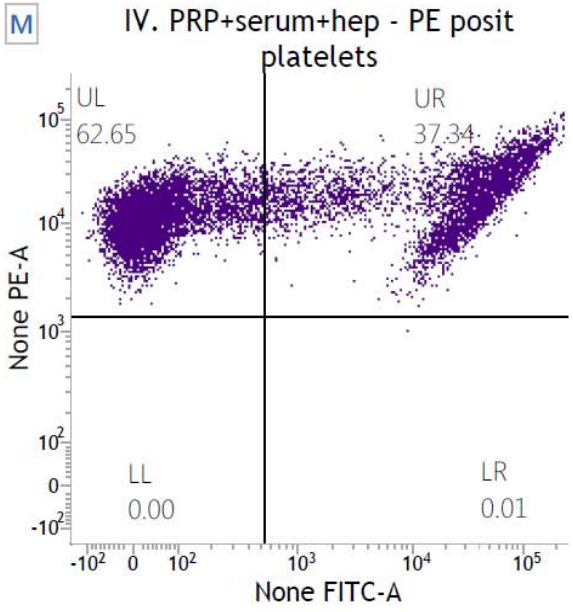

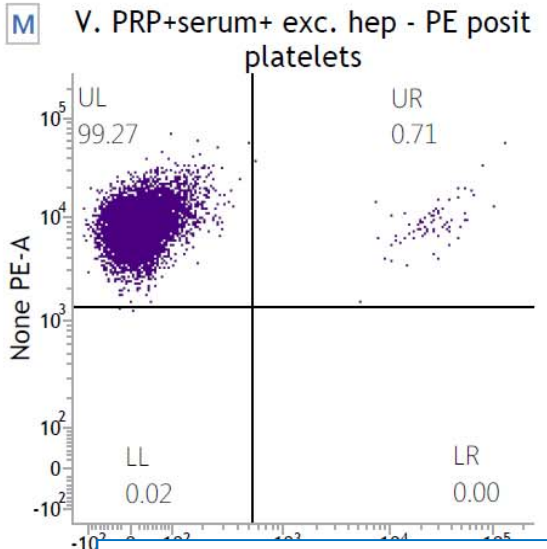

Výsledok testu: POZITÍVNY

Záver

- Na základe našich doterajších skúseností aplikáciou funkčného testu stanovenia HIT pomocou prietokovej cytometrie môžeme povedať, že metóda má svoje:
- **výhody** (CE/IVD funkčný test, relatívne krátky čas analýzy- 110 minút, nerádioaktívny, vysoká senzitivita a špecificita)
- **úskalia** (vyžaduje darcovské doštičky, špecializované pracoviská s odbornou znalosťou a prístrojovým vybavením prietokovej cytometrie).

Poďakovanie

- Táto práca bola podporená projektami:
- APVV – 16-0020;
- Vega 1/0168/16, Vega 1/0187/17;
- Centra excelentnosti pre perinatologický výskum (CEPV II, ITMS 26220120036);
- Centra excelentnosti pre výskum v personalizovanej terapii (CEVYPET).