REX
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Updates and Information from Rex Pathology Laboratory
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Heparin Induced Thrombocytopenia

Introduction: Unfractionated heparin (UFH) has been widely used for over 50 years to prevent or treat venous thrombosis. It is routinely used to coat or flush vascular catheters to minimize the chance of forming clots. Heparin anticoagulation is essential in cardiac surgery, percutaneous cardiac catheterization and numerous other situations (see table 1). It is estimated that one-third of hospitalized patients have some heparin exposure yearly. Unfortunately, the increasing use of heparin has increased the rate of heparin induced thrombocytopenia (HIT). The rate of increase of HIT is ameliorated in part by the introduction of low molecular weight heparin (LMWH), which has a lower risk of HIT.

Table 1: Uses of Heparin

- Deep vein thrombosis (DVT)
- Pulmonary embolus (PE)
- Arterial and venous catheters
- Extracorporeal circulation (hemodialysis, cardiac bypass surgery)
- Vascular surgery
- Percutaneous coronary catheterization
- Acute coronary syndromes

What is heparin induced thrombocytopenia (HIT)? Heparin induced thrombocytopenia may develop in two distinct forms. Type I HIT, also called heparin associated thrombocytopenia (HAT) is a non-immunologic response to heparin therapy. HAT typically occurs in the first two or three days of therapy with a drop in platelets to 100,000/ul followed by recovery in four to five days without discontinuing heparin. This disorder is incidental, non-immune and has no harmful effects. There is no laboratory test to diagnose type I HIT and there is no increased risk of thrombosis.

On the other hand, type II HIT (HIT-II) is a serious immune mediated thrombocytopenia. It usually develops after five to 10 days of therapy and affects one to three percent of patients receiving unfractionated heparin. HIT-II can occur two to three days after heparin therapy, as an amnestic response in patients previously exposed to heparin. Typically the platelet count falls 30 to 50%, decreasing below 100,000/ul. Vascular thrombosis, arterial or venous, occurs in 30 to 80% of patients with HIT-II. Complications include deep venous thrombosis (DVT), pulmonary embolism (PE), acute myocardial infarction, skin necrosis, venous limb gangrene and possible death. The most common scenario for HIT-II is a postoperative patient on antithrombotic prophylaxis with UFH.

Complications of HIT-II: The mild thrombocytopenia associated with HIT-II usually does not cause bleeding. The thrombotic complications on the other hand are significant and can be devastating. The thrombosis may be venous or arterial. If the disease is not recognized, the complications can be life threatening. A list of complications is listed in Table 2 below.

Table 2: Complications of HIT-II

- DVT/PE
- Myocardial Infarction
- Occlusion of limb arteries
- Cerebrovascular accident or TIA
- Skin necrosis
- End organ infarction
- Death

Adapted from Chong.

How can HIT-II thrombocytopenia cause thrombosis? Thrombotic complications developing in a patient with thrombocytopenia is counterintuitive. An immune mediated antibody is responsible for the hypercoagulable state. Circulating heparin binds to platelet factor 4 (PF4), forming a highly reactive antigenic complex on the surface of platelets and on endothelial cell surfaces. Susceptible patients then develop an antibody (IgG) to the heparin-PF4 antigenic complex. Once produced, immunoglobulins bind to the heparin-PF4 immune complex on the platelet and endothelial cell surface. The bound complex activates the platelets. Thrombocytopenia develops as the reticuloendothelial system consumes the activated platelets. Most devastating, however, is the thrombotic state that develops as a result of platelet activation, the generation of procoagulant microparticles, and an additional increase in thrombin generation (Figure 1).

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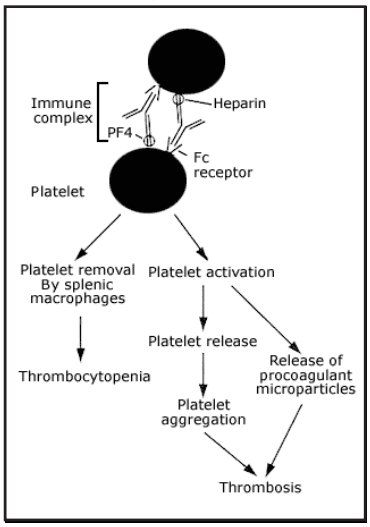
Figure 1: Pathogenesis of heparin-induced thrombocytopenia (HIT)



Adapted from Chong.

Specific clinical features of HIT-II: There are three characteristic clinical features of HIT-II that are relatively specific.

(1) Heparin-induced skin lesions occur in about 10 to 20% of patients who receive subcutaneous UFH or LMWH. Lesions appear at the site of the injection and can be necrotizing.

(2) An acute systemic reaction may occur immediately following intravenous bolus of heparin. The circulating antibodies to heparin trigger an abrupt response of fever, chills, flushing, tachycardia, hypertension and dyspnea. A drop in platelet count accompanies this reaction. This reaction has similar features of a febrile transfusion reaction.

(3) Hemorrhagic adrenal necrosis occurs in three to five percent of HIT patients (due to adrenal vein thrombosis) and may produce abdominal or flank pain. When necrosis is bilateral and sudden, an adrenal (Addisonian) crisis can result. Death can be avoided with timely use of corticosteroids.

Diagnosis: Although the thrombocytopenia of HIT-II is mild or moderate with minimal bleeding risk, the high risk for development of thrombosis (arterial or venous) may be limb or life threatening. This phenomenon of thrombocytopenia occurring with thrombosis has been termed heparin induced thrombocytopenia with thrombosis (HITT). Clinically, it is often difficult to distinguish between HIT-I and HIT-II, as well as other etiologies of thrombocytopenia occurring in heparinized patients. However, the development of new or progressive thrombosis is one defining clinical feature of HIT-II.

Discontinuation of heparin with associated recovery and a positive heparin-PF4 antibody test are necessary to make a definitive diagnosis. Functional assays for HIT-II antibody that involve platelet aggregation or secretion of serotonin or adenosine triphosphate (ATP) are difficult to perform and not routinely available. The enzyme-linked immunosorbent assay (ELISA) for platelet factor 4 is practical and readily available through Mayo Medical Laboratories for clinical use. It is important to note that a patient may have HIT-II with negative laboratory results. ***If the diagnosis is suspected clinically, discontinue heparin immediately.*** One should **not** wait for the laboratory results because the adverse sequelae that may occur could be life threatening.

Heparin-PF4 antibody (HIT) test: The ELISA method of HIT-II antibodies is very sensitive but relatively nonspecific for the diagnosis of HIT-II. The test is available through Mayo Reference Laboratory as "Heparin-PF4 antibody", requires one ml. of serum and has a turn-around time of 48 hours. The results are reported as the percent of reactivity with antigen and the percent of heparin inhibition. A negative result has a 90% negative predictive value for exclusion of HIT-II. If the serum is tested prior to a detectable rise in antibody, a negative result may be a false negative. Repeat testing in one or two weeks may establish the diagnosis.

The *specificity* of the ELISA method for the diagnosis of HIT-II is only 20 to 50%. Up to 50% of surgical patients and up to 20% of medical patients treated with UFH may develop detectable antibodies. Only a small proportion (1 – 5%) will develop clinical HIT-II. A positive test result does not make the diagnosis of HIT-II without associated thrombocytopenia. As is true with most laboratory tests, the result must be considered in conjunction with the clinical findings.

Interpretation of lab results: The typical patterns of results and interpretation are depicted in table 3 below. The percent of reactivity of the serum with heparin-PF4 antigen complex is a function of quantity and avidity of the antibody to the antigen. An excess of heparin is added to the serum to confirm the percentage of reactivity with the antigen as a result of the heparin-dependent antibodies. The excess heparin binds the antibody and essentially removes the antibody. Results are given as a percent of heparin inhibition. Interpretations of the two results (% of reactivity and heparin inhibition) are given as positive, negative or equivocal.

Table 3 Interpretation of possible results:

Result	Reactivity (%)	Heparin (%) Inhibition	Interpretation
Normal	<20	not done	negative
Positive	>40	>50	positive
Equivocal	20-40	>50	equivocal
Equivocal	20-40	<=50	equivocal
Equivocal	>40	<=50	equivocal

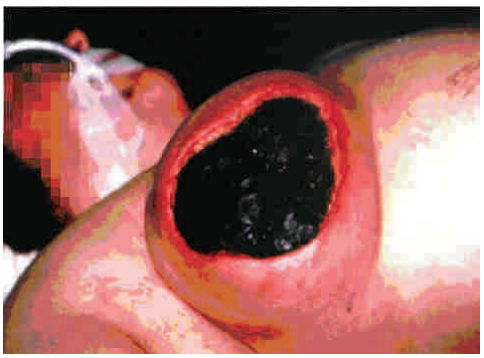
Mayo Medical Laboratory Interpretation Book

Delayed onset and/or recovery of HIT-II: . Patients with delayed onset HIT-II may be difficult to diagnose. Typically, delayed onset HIT-II presents as patients with thrombosis and thrombocytopenia who return to the hospital after being exposed to heparin. At this time the association with recent heparin administration is made and diagnosis of HIT-II is suspected. These patients with delayed onset HIT-II have high titer heparin-PF4 antibodies by the ELISA method.

Sometimes HIT-II resembles an autoimmune disorder, either because the onset of thrombocytopenia begins after heparin has been stopped or the thrombocytopenia persists for a week or more despite stopping all heparin. The median time to platelet count recovery is four days after discontinuing heparin. Ninety percent of patients return to the normal range within one week. Some patients may take weeks to recover a normal platelet count, thus raising the suspicion of an autoimmune disorder.

Serologic evidence of persisting high levels of antibody supports the diagnosis of HIT-II in this setting.

Coumadin-induced (Warfarin) skin necrosis: It is routine to initiate anticoagulation in a patient with thrombosis with heparin, followed by coumadin (warfarin). The overlap of these therapies is designed to prevent the potential hypercoagulable state induced by coumadin. Coumadin depletes Vitamin K dependent coagulation factors (II, VII, IX and X) and prolongs the prothrombin time (INR). In addition coumadin depletes two fibrinolytic proteins (protein C and S). Protein C is a vitamin K-dependent naturally occurring plasma protein that is converted to the activated form by the thrombin-thrombomodulin complex. Activated protein C functions as an anticoagulant by inactivating the cofactors of the coagulation cascade, factors Va and VIIIa. Coumadin (warfarin)-induced skin necrosis is thought to be due to a rapid elimination of protein C relative to other vitamin K-dependent factors during the initial phase of oral anticoagulation. Patients with HIT-II are capable of inactivating the antithrombotic effects of heparin and are susceptible to skin necrosis induced by coumadin. Accordingly, *when heparin is discontinued, coumadin therapy should never be given in HIT-II patients.* Coumadin is ineffective in treating hypercoagulable states such as HIT-II because it does not inhibit thrombin generation but rather impairs only the synthesis of vitamin K dependent factors. A photograph of the devastating effects of coumadin skin necrosis of the breast is depicted in figure 2.



Warfarin induced skin necrosis (www.jvascbr.com.br)

Treatment of HIT-II: The principles for treatment of HIT-II are listed in table 4 below. Stopping heparin is important but does not prevent onset, progression or recurrence of thrombosis in HIT-II. According to the 2004 Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy it is important to administer a rapidly acting non-heparin anticoagulant when HIT-II is the likely diagnosis even before results of HIT-PF4 antibody test are available.

Table 4: Six treatment principles of HIT

Two Do's
- Stop heparin
- Start alternative, non-heparin anticoagulant

Two Don'ts
- Avoid or postpone coumarin pending substantial platelet count recovery (give intravenous Vit. K if coumarin already given when HIT is recognized)
- Avoid platelet transfusions

Two Diagnostics
- Test for HIT antibodies
- Investigate for lower-limb DVT (duplex ultrasonography)

(Adapted from Warkentin)

There are two reasons to give intravenous vitamin K after coumadin has been given in a patient when HIT-II is recognized. First, it may reduce the risk of coumadin-induced thrombosis and second, it reduces the risk of under-dosing of direct thrombin inhibitor therapy because of prolongation of the APTT by coumadin.

Direct thrombin inhibitors: A relatively new class of drugs that directly inhibit the action of thrombin is useful in the treatment of HIT-II. Heparin is also a thrombin inhibitor but acts indirectly as an accelerator of antithrombin III. The direct thrombin inhibitors that are FDA approved for use in HIT-II are Argatroban and Fondaparinux. Both are unrelated to heparin and do not cross-react with heparin antibodies. The APTT is used to monitor Argatroban but is insensitive to Fondaparinux. Fondaparinux requires the anti-Xa assay. Both the APTT and anti-Xa assay are available at Rex Hospital Laboratory with same day turn-around-times.

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References:
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2. Warkentin, T.E., Think of HIT, American Society of Hematology Education Program Book, December 2006, p. 408-414.
3. Mayo Medical Laboratories Test Catalog, Serum heparin-PF4 Antibody (HIT), 2006, p. 269
4. Mayo Medical Laboratories Interpretive Handbook, Heparin-PF4 Antibody (HIT), 2006 p. 400 – 402.
5. Lewis, B.E., Wallis, D.E, Leya, F. Hursting, M.J., Kelton, J.G. Argatroban anticoagulation in patients with heparin-induced thrombocytopenia. Archives of Internal Medicine. 2003;163:1849-1856.
6. Warkentin, T.E., Greinacher, A. Heparin-induced thrombocytopenia: recognition, treatment and prevention. The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004; 126 (3 Supplement): p. 311S-337S.

Reference Range Changes

The chart below indicates recent reference range changes resulting from a change in methods or revision in the reference range provided by the vendor. The units change in testosterone will provide easier comparison of total testosterone (performed at Rex) with free testosterone (performed at Mayo Medical Laboratories).

(Thanks to laboratory patron Mark Jalkut, M.D. for prompting a review of selected reference ranges.)

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Test	New Normal Range	Units	Old Normal Range	Units
CEA	0.8 - 3.4	ng/mL	1.0-4.4	ng/mL
hsCRP	0 - 3 Low Risk: < 1.0 Average Risk: 1.0-3.0 High Risk: > 3.0	mg/L	0 - 2.3	mg/L
Folate	3.0 - 15.0	ng/mL	3.0 - 17.0	ng/mL
FSH	Males: 0.1 - 3 years: ND - 5.5 4 - 9 years: ND - 1.9	mIU/mL	 Not listed Not listed	
FT3 (free T3)	1.8 - 4.2	pg/mL	1.5 - 4.4	pg/mL
intact PTH (EDTA tube)	16 - 87	pg/mL	11 - 83.0	pg/mL
Progesterone	Females: Cord Blood: 465 - 755 1.1 - 9 years: ND - 1.4 Adult Females: Follicular phase: ND - 1.13 Midcycle: 0.48 - 1.72 Luteal phase: 0.95 - 21 Midluteal (day 7-8): 6.0 - 24		 485 - 755 Not listed 0.32 - 2.0 0.77 - 2.3 1.19 - 21.6 4.4 - 28	
Testosterone	Males: 20 - 49 years: 262 - 1593 > 50 years: 181 - 760 Females: Ovulating: ND - 80 Postmenopausal: ND - 62	ng/dL	 0.0 - 11.0 0.9 - 7.4 ND - 0.81 ND - 0.74	ng/mL
T3 (triiodothyronine)	84-172	ng/dL	75 - 182	

ND = not detected

2007 Antibiogram

Enclosed in this issue of the lab bulletin is the 2007 Rex Laboratory Antibiogram, based on antimicrobial susceptibility results for 2006. We hope you find this information helpful.

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2007 ANTIBIOGRAM

(January -December 2006 Results for Rex Inpatients)

Gram-Negative Organisms	NON-URINE SOURCES															URINES			
	PCNs				Cephalosporins 1st	Cephalosporins 3rd		Cephalosporins 4th	Quin	Aminogly			Miscellaneous						
Gram-Negatives	**# *Isolates***	Ampicillin	Amp/Sulbactam	Piperacillin/Tazo	Cefazolin	Ceftazidime	Ceftriaxone	Cefipime	Levofloxacin	Amikacin	Gentamicin	Tobromycin	Aztreonam	Imipenem	TMP/SMX	**# *Isolates***	Levofloxacin	Nitrofurantoin	TMP/SMX
Acinetobacter baumannii	19	*	100	*	*	58	0	58	58	100	89	100	*	100	89	8	62	*	75
Citrobacter koseri	5	*	*	*	*	100	80	100	100	100	100	100	80	100	100	12	100	83	100
Citrobacter freundii	11	*	*	*	*	100	100	100	100	100	100	100	100	100	100	30	80	93	83
Enterob. aerogenes	12	*	*	100	*	100	100	100	100	100	100	100	100	100	100	19	89	0	89
Enterob. cloacae	27	*	*	100	*	86	89	100	89	100	100	100	96	100	96	22	82	32	86
E. coli	197	60	63	99	88	93	94	97	80	99	86	88	94	100	83	806	72	94	78
Klebsiella oxytoca	22	0	90	100	64	95	95	95	100	100	100	100	95	100	100	17	88	76	82
Klebsiella pneumoniae	52	0	88	98	92	98	98	98	98	100	98	96	98	100	94	201	96	35	94
Proteus mirablis	71	92	96	100	97	99	99	99	77	100	96	99	99	*	89	111	76	0	86
Pseudomonas aeruginosa	118	*	*	89	*	68	*	74	51	97	74	84	*	87	*	72	52	*	*
Serratia marcescens	18	*	*	100	0	100	100	100	100	100	100	88	100	100	100	6	83	0	100
Stenotrophomonas maltophilia	8														78	5			100

Numbers reflect the percent susceptible based on achievable blood levels of antimicrobials.
A blank = not tested; an asterisk = not reportable or alternative testing recommended.

ESBL (Extended-Spectrum Beta Lactamase) data:

E.coli	20 isolates, 2% of all *E. coli* isolates
K. oxytoca	3 isolates, 8% of all *K oxytoca*
K. pneumoniae	10 isolates, 4% of all *K. pneun*

Gram-Positive Organisms	NON-URINE SOURCES													URINES		
		Penicillins			Miscellaneous											
Gram-Positives	***# Isolates***	Ampicillin	Oxacillin	Penicillin	Clindamycin	Cefotaxime *non-CSF	Ceftriaxone *non-CSF	Erythromycin	Levofloxacin	Vancomycin	Tetracycline	TMP/SMX	***# Isolates***	Levofloxacin	Nitrofurantoin	Tetracycline
Enterococcus faecalis	104	99	*	99	*			*	*	97	*		205	*	99	
Enterococcus faecium	32	22	*	19	*			*	*	34	*		25	*	8	*
Staph. aureus	675	*	38	2	*			28	50	100	94	98	78	30	95	91
Staph aureus (MSSA)	199	*	100	2	*			63	86	100	94	98			100	
Staph aureus (MRSA)	275	*	0	0	49			5	24	100	95	97			98	
Coagulase negative Staph	64	*	41	10	*			44	47	100	85		8	12	100	75
Staph. epidermidis	104	*	25	5	*			36	28	100	88		102	12	99	85
Staph lugdunensis	20	*	90	24	*			76	100	100	100		3	100	100	100
Strep. agalactiae (Group B)	92	100	*	100	*			49	100	100	*		30	100	*	*
Strep. pneumoniae	46			48		96	96	60	100	100	83	73	0			

Numbers reflect the percent susceptible based on achievable blood levels of antimicrobials.
A blank = not tested; an asterisk = not reportable or alternative testing recommended.

Strep agalactiae (Group B) data is for both inpatients and outpatients.

MRSA/MSSA isolate numbers are for a 6 month period

MRSA Clindamycin data based on 27 isolates tested for inducible resistance starting in 12/06.

As of 12/06 all MRSA and Group B Streptococci requiring susceptibilities will be screened for inducible clindamycin resistance by the "D" test.

43% of *Strep. Pneumoniae* tested intermediate to penicillin.