Linda M. Scott, Peter J. Campbell, E. Joanna Baxter,
Tony Todd, Philip Stephens, Sarah Edkins, Richard Wooster,
Michael R. Stratton, P. Andrew Futreal, and Anthony R. Green

Correspondence: Anthony R. Green, Department of Haematology, Cambridge Institute for Medical Research, Hills Road, Cambridge CB2 2XY, United Kingdom; e-mail: arg1000@cam.ac.uk.

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To the editor:

Replacement of unfractionated heparin by low-molecular-weight heparin for postorthopedic surgery antithrombotic prophylaxis lowers the overall risk of symptomatic thrombosis because of a lower frequency of heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is a prothrombotic immune-mediated adverse drug reaction that usually begins 5 or more days after starting heparin.[1] One randomized clinical trial reported a significant difference in HIT between unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) in postoperative orthopedic patients.[2] To corroborate that LMWH is safer than UFH in this patient population, we performed a "before-after" prospective cohort study in which 231 consecutive patients over a 9-month period received UFH (Liquemin [Roche, Basel, Switzerland], 5000 IU 3 times/day subcutaneously) following hip or knee replacement. During the subsequent 12-month period, 271 patients received LMWH (enoxaparin [Clexane; Sanofi-Aventis, Frankfurt, Germany], 40 mg/d subcutaneously). Both anticoagulants were started the night before surgery. The primary study end point was HIT, defined a priori as a positive test for HIT antibodies (platelet activation assay[3]) plus a 30% or greater fall in the platelet count between postoperative days 4 to 16, and/or symptomatic, objectively documented thrombosis (venous or arterial). We predefined thrombotic events occurring between days 5 to 16 as potentially HIT related. Routine testing for HIT antibodies was also performed in most patients at end of study (day 16). Stored sera were also tested posthoc for anti-PF4/polyanion antibodies (GTI, Brookfield, WI).[4] Clinical events were adjudicated by 2 investigators by consensus. The study was approved by the ethics committee of the University of Greifswald. The patients we report overlap somewhat with those included in a previous publication[5] reflecting the participating surgeons' perspective; however, that report included an additional 130 nonstudy patients, used a different definition of HIT (> 50% platelet count fall), and did not report systematic serologic investigations.

We found a higher frequency of HIT in patients who received UFH (5.2%; 95% confidence interval [CI], 2.7%-8.9%), compared with LMWH (0%; 95% CI, 0.0%-1.4%); $P < .001$ (Table 1). In addition to the 6 patients who had thrombosis associated with HIT, 5 patients had

thrombosis without HIT (3 in the UFH group, 2 in the LMWH group). Thus, total thrombosis (both HIT and non-HIT) was significantly more frequent in UFH-treated patients (Table 1). Two other findings deserve comment. First, among the 431 patients undergoing serologic testing who did not develop HIT manifesting as a platelet count fall, there was a strong association between symptomatic thrombosis after day 4 and a positive platelet activation test for HIT antibodies: 3 (50.0%) of 6

Table 1. Frequency of HIT, thrombosis (HIT, non-HIT associated, total), and HIT antibodies in before-after prospective cohort study comparing UFH and LMWH after orthopedic surgery

	UFH, n = 231 (%)	LMWH, n = 271 (%)	P
Patients with HIT*	12 (5.2)	0 (0)	< .001
All symptomatic thrombotic events, HIT and non-HIT associated	9 (3.9)	2 (0.7)	.028
All symptomatic thrombotic events before or on day 4†	1 (0.4)	1 (0.4)	.910
All symptomatic non-HIT-associated thrombotic events after day 4‡	2 (0.9)	1 (0.4)	.597
All symptomatic HIT-associated thrombotic events after day 4 (HIT)§	6 (2.6)	0 (0)	.009
HIT antibody status at day 16/platelet activation assay	25/202 (12.4)	13/238 (5.5)	.010
HIT antibody status at day 16/immunoassay	46/196 (23.5)	19/228 (8.3)	< .001

*The 12 patients who met the a priori definition for HIT included 6 with platelet count fall (> 30%) alone, 3 with platelet fall (> 30%) plus symptomatic thrombosis, and 3 with symptomatic thrombosis alone; all HIT-associated symptomatic thrombi occurred after day 4. (Six of the 12 HIT patients had a platelet count fall more than 50%, 2 with thrombosis.)

†One UFH-treated patient developed pulmonary embolism (day 1), and one LMWH-treated patient developed cardiac shock (day 2).

‡Two UFH-treated patients developed pulmonary embolism (both day 10), and 1 LMWH-treated patient developed acute coronary syndrome (day 7).

§Four UFH-treated patients developed deep vein thromboses (DVTs; days 8, 8, 10, and 16), and 2 developed pulmonary embolism (days 7 and 15).

patients with thrombosis tested antibody positive, compared with 26 (6.1%) of 425 patients without thrombosis (odds ratio [OR], 15.3 [95% CI, 2.94-25.23]; P = .005). This supports previous suggestions[6] that formation of platelet-activating HIT antibodies can be associated with thrombosis even in the absence of a significant platelet count fall. Second, among the 87.7% of study patients who underwent serologic testing for HIT antibodies, there was approximately a 60% lower seroconversion rate with LMWH compared with UFH (55.6% and 64.7% by the platelet activation assay and immunoassay, respectively). Nevertheless, despite this moderate reduction in frequency of HIT antibody formation, there was complete avoidance of clinical HIT using LMWH (0 versus 12 cases). This underscores the importance of considering HIT to be a largely preventable adverse drug reaction, at least in postorthopedic surgery patients.

Andreas Greinacher, Petra Eichler, Theresia Lietz, and Theodore E. Warkentin

Correspondence: Andreas Greinacher, Institute for Immunology and Transfusion Medicine, Ernst-Moritz-Arndt University, Sauerbruchstraße, 17489 Greifswald, Germany; e-mail: greinach@uni-greifswald.de

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To the editor:

A Portuguese patient homozygous for the −25G>A mutation of the *HAMP* promoter shows evidence of steady-state transcription but fails to up-regulate hepcidin levels by iron

Mutations of hepcidin are a rare cause of juvenile hemochromatosis (JH). We report a homozygous −25G>A mutation in the hepcidin 5′ untranslated region (UTR) that generates a new start codon with a consequent frameshift. In this patient with a rare coincidental association of JH, Turner syndrome, and absolute lymphopenia, the absence of normal hepcidin synthesis was expected. Surprisingly, the patient had detectable hepcidin, suggesting that the start of translation was maintained at the original ATG with some normal protein production. However, hepcidin was not appropriately induced by an iron challenge test, supporting role of hepcidin on the clinical expression of iron overload in this case.

The hepatic peptide hepcidin is a key regulator of iron balance.[1] Mutations of hepcidin are a rare cause of juvenile hemochromatosis (JH),[2-3] and include nonsense, frameshift,[2] and missense mutations C70R and C78T affecting conserved cysteines.[3-5]

Recently a −25G>A mutation in the *HAMP* 5′UTR was described in 2 Portuguese siblings with iron overload and absence of urinary hepcidin.[6] Here, the same mutation was found in a different Portuguese family where the proband shows coincidental association of JH, Turner syndrome, and absolute lymphopenia.[7] Although no comparative haplotype analysis was performed, geographic and historical tracking does not indicate any relationship with the previously described family. In the proband, no mutations in the coding regions of *HAMP* and hemojuvelin genes were found by sequencing.[3,8] However, in the 5′UTR region of *HAMP*, a G>A point mutation was identified at position −25 from the canonical ATG. This was confirmed by WAVE (Transgenomic, Omaha, NE) denaturing high-performance liquid chromatography (DHPLC; heteroduplexes were formed by heat denaturation at 94°C for 3 minutes and cooling to 25°C for 45 minutes; the mixture was analyzed at a melting temperature of 64.1°C, with a linear acetonitrile gradient: Start: 44.3% Buffer A, 55.7% Buffer B; Stop: 35.3% Buffer A, 64.7% Buffer B). In a family study performed with written informed consent, both parents and one brother were heterozygous for the mutation. Elevated serum ferritin levels in these subjects were likely due to the additional effect of regular high alcohol intake.

The G>A substitution changes GTG to ATG and creates a new start site for translation. Since this mutation introduces an earlier initiation codon with a shifted reading frame, it would be expected to encode a different peptide. Surprisingly, urinary hepcidin, analyzed in first morning voided samples,[9] was detected in the proband at a concentration of 12 ng/mg creatinine with a serum ferritin concentration of 19 ng/mL at the time of evaluation. The result was confirmed by Western blot analysis. The production of hepcidin could be explained by the maintenance of the start of translation at the original ATG, a phenomenon also observed with other genes.[10] In this case, urinary hepcidin was evaluated when iron depletion had been already achieved by phlebotomy treatment. To explain the initial severe phenotype of the patient, we hypothesized that hepcidin was not appropriately induced by iron and performed an iron challenge test with urinary hepcidin determination 24 hours after the ingestion of 80 mg iron as ferrous sulfate.[9] No increase in urinary hepcidin was observed in the patient, in contrast with a 2- to 3-fold increase observed in 2 control subjects (Figure 1). Of interest, a reduction in serum
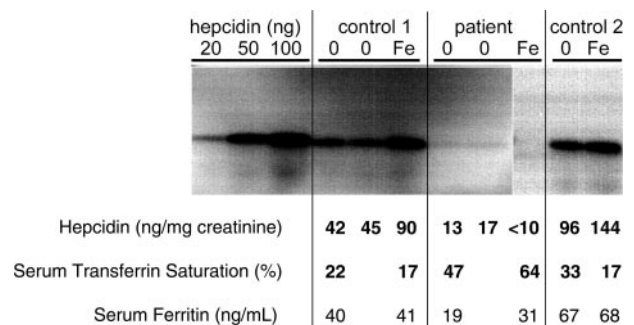

hepcidin (ng) 20 50 100	control 1 0 0 Fe	patient 0 0 Fe	control 2 0 Fe

	control 1		patient		control 2	
Hepcidin (ng/mg creatinine)	42	45	90	13	17	<10	96	144
Serum Transferrin Saturation (%)	22		17	47		64	33	17
Serum Ferritin (ng/mL)	40		41	19		31	67	68

Figure 1. Urinary hepcidin levels in 2 controls and the proband, before and after ingestion of 80 mg iron as ferrous sulfate. Synthetic hepcidin standards (20, 50, and 100 ng) and urinary extracts equivalent to 0.5 mg creatinine were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting with rabbit anti–human hepcidin antibody. 0 = urine collected before iron ingestion; Fe = urine collected 24 hours after iron ingestion. Measures of urinary hepcidin, serum transferrin saturation, and serum ferritin are given for each subject at the indicated test times.