



## COVER SHEET

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**Ray, Michael and Hatcher, Stephen and Whitehouse, Sarah L. and Crawford, Scott W. and Crawford, Ross W. (2005) Aprotinin and epsilon aminocaproic acid are effective in reducing blood loss after primary total hip arthroplasty – a prospective randomized double-blind placebo-controlled study. Journal of Thrombosis and Haemostasis 3(7):pp. 1421-1427.**

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Ray M, Hatcher S, **Whitehouse SL**, Crawford S, Crawford R. Aprotinin and epsilon aminocaproic acid are effective in reducing blood loss after primary total hip arthroplasty – a prospective randomised double blind placebo controlled study. *J Thromb Haemost* 2005; 3:1421-1427

**Increased CD 40 ligand expression on platelets is associated with increased cardiac events before and after total hip arthroplasty.**

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The aim of this study was to determine if pre-operative levels of CD 40 Ligand (L) in patients undergoing non cardiac surgery were associated with the development of adverse post operative cardiovascular events. **Methods:** Patients undergoing elective primary, unilateral cemented total hip arthroplasty (THR) under general anaesthesia were consented to participate in the study in accordance with the hospital Research and Ethics Committee. A BD FACScan flow cytometer with a 488 nm argon-ion laser performed 3-colour analysis. CD 40L on the platelet surface was measured with CD154. An adverse cardiovascular event was defined as death, acute myocardial infarction or the onset of a new atrial or ventricular arrhythmia. **Results:** The study included 45 patients, mean age  $69 \pm 10.9$  yrs of which 51% were male. In all cases the operation was considered a technical success. There were no signs of deep vein thrombosis detected by ultrasound 5 days after surgery in all patients. Cardiovascular events of ischaemia or atrial fibrillation post hip replacement occurred in 6 patients. These consisted of non ST elevation myocardial infarction (NSTEMI) plus atrial fibrillation in 2 patients, atrial fibrillation in 2 patients and NSTEMI and atrial fibrillation in 2 patients. CD 40L expression prior to THR was associated with the development of adverse post operative adverse cardiac events ( $p < 0.01$  CI 0.956-6.597), non ST elevation myocardial infarction ( $p = 0.064$ ) and atrial fibrillation ( $p = 0.043$ ). Six weeks after surgery, history and clinical assessment revealed no evidence of thrombosis or infection. There was one case of wound ooze and haematoma that required washout and resuturing 15 days after surgery.

**Conclusion :** CD 40L expression may be useful in predicting adverse cardiovascular events in patients undergoing non cardiac surgery.

## **Introduction**

CD40 ligand (CD40L) is expressed on platelets that have become activated <sup>1</sup> and increased levels have been demonstrated on the surface of platelets from patients with chronic heart failure <sup>2</sup>. Once CD40L has been translocated to the platelet surface, it is cleaved to the soluble form (sCD40L) which induces an inflammatory response from endothelial cells as well as promoting thrombosis <sup>3</sup>.

Cardiac complications after total hip arthroplasty (THA) are significant. The mortality rate three months from cardiac events has been reported as 0.16%, higher than that from pulmonary embolus (0.11%) <sup>4</sup>.

This study aimed to investigate the association of CD40L expressed on the platelets before THA with both the patients' history of cardiac disease and adverse cardiac events after the surgery.

## **Method**

### *Patient population*

A total of 45 patients undergoing primary, unilateral, cemented THA were studied. These patients were treated with aprotinin, EACA or placebo as described previously in a randomised study to study the effectiveness of these two antifibrinolytic agents in the reduction of bleeding <sup>5</sup>. All patients consented to participate in the study including having blood samples for flow cytometric analysis taken via an arterial line with the approval of the hospital Research and Ethics Committee. The study was conducted in accordance with the World Medical Association Declaration of Helsinki.

Patients were excluded if they had a previous history of pulmonary embolus, stroke, deep vein thrombosis, transient ischaemic attacks, malignancy, or renal failure (creatinine >0.15 mmol/L).

After the surgery all patients received 150 mg of aspirin daily as prophylaxis. Below knee thromboembolic stockings (Thrombexin Climax, MediBayreuth, Germany) and foot pumps (Impulse, Novamedix, Mansfield, UK) were employed.

#### *Blood sampling*

Two samples were taken, one before the administration of any study drug and the second one hour after the end of surgery. 2.7 mL of blood was added to a Vacutainer tubes (Becton Dickinson, NJ, USA) containing 0.109 M buffered trisodium citrate and immediately stained and tested by flow cytometry.

#### *Flow cytometry analysis*

This analysis was performed using a BD FACScan flow cytometer with a 488 nm argon-ion laser. Platelets were selected by forward scatter and side scatter as well as by positive staining with CD42a (BD Biosciences (BD) (San Jose, California, USA)) that targets GP IX and GP 1b-IX-V that are common to all platelets. CD40 ligand on the platelet surface was measured with CD154 (BD) <sup>6</sup>. A matched isotype was used as a negative control. The CELLQuest program was used to analyse the data.

Quality control of the flow cytometer was ensured throughout the study by using CaliBRITE 3 beads (BD).

#### *Adverse Cardiac Events*

The cardiologist was blinded to all aspects of the study patients and assessed the patients' charts after their discharge. An adverse event was defined as two of the three events of chest pain, ECG changes and elevation of troponin I.

### **Results**

#### *Demographics*

Three of the 15 patients who received aprotinin, 3 of the 15 who received EACA and none of the placebo group experienced adverse postoperative cardiac events. There was no statistically significant relationship between the administration of aprotinin or EACA and the occurrence of postoperative cardiac events.

The other demographics describing the patient population are shown in Table 1. Patients with adverse postoperative cardiac events were more commonly diabetic and stayed in hospital longer.

Table 1. Demographics of patients.

	Postoperative cardiac events	No postoperative cardiac events
n	6	39
History of cardiac events	3	6
Hypertension	1	13
Diabetes	2 #	1
Recent history Viox	2	6
Male:female	2:4	21:18
Age (years)	74	71
Hospital stay (days)	11.5 #	7.0
Preoperative haemoglobin (g/L)	133	132
Preoperative platelet count ( $\times 10^9/L$ )	303	275
Preoperative platelet aggregation (ohm)	4.5	5.0

Values represent number or median value

#  $p = <0.05$

#### *CD40 ligand expression on platelets*

Antifibrinolytic therapy had no effect on platelet activation in this group of patients 5. Before or after THA there was no difference in CD40L expression on platelets between the aprotinin, EACA or placebo groups, so the results of all patients were considered together.

CD40L expression was markedly decreased during THA surgery (Fig. 1).

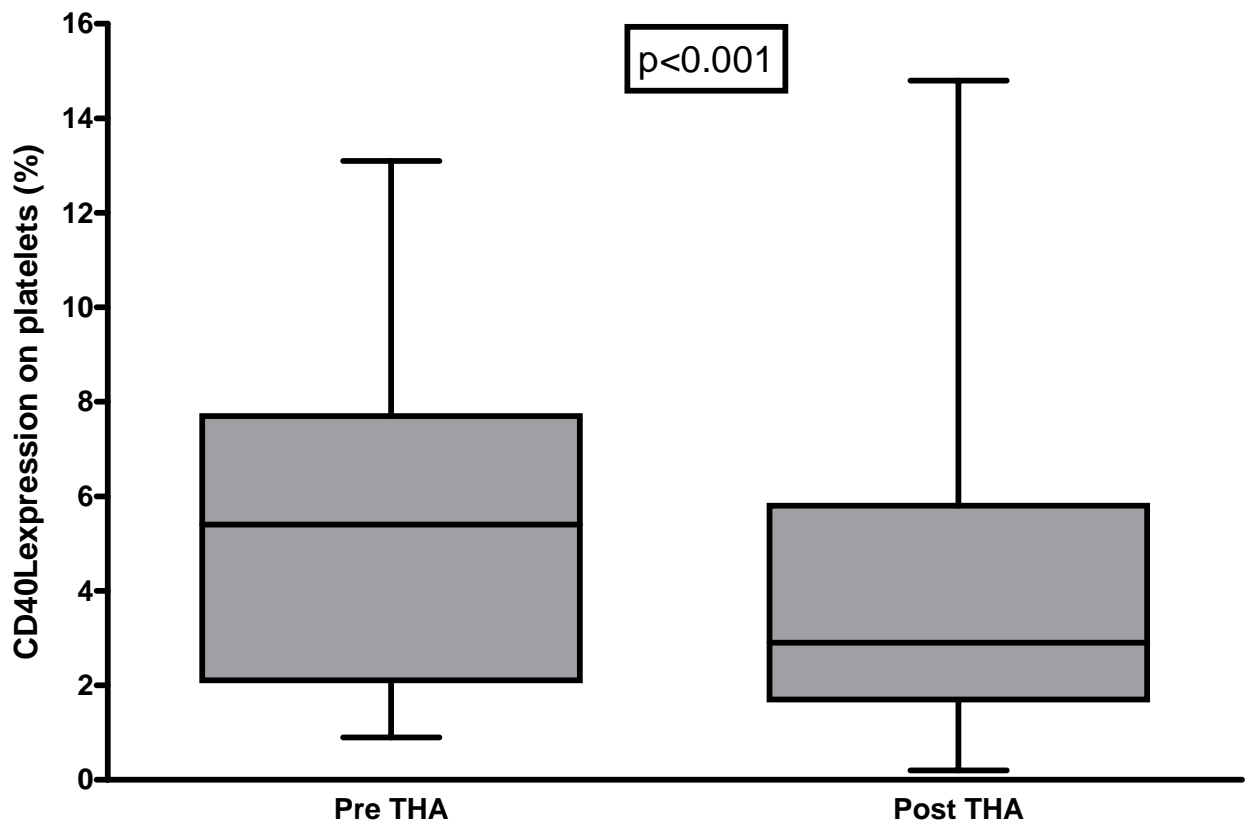


Fig. 1 The expression of CD40L on patients' platelets before and one hour after the completion of THA. The horizontal bar represents the median value, the box the 25<sup>th</sup> and 75<sup>th</sup> quartiles and the whiskers the range of values.

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