Stimulation of the local femoral inflammatory response to fracture and intramedullary reaming

A PRELIMINARY STUDY OF THE SOURCE OF THE SECOND HIT PHENOMENON

We have undertaken a prospective study in patients with a fracture of the femoral shaft requiring intramedullary nailing to test the hypothesis that the femoral canal could be a potential source of the second hit phenomenon. We determined the local femoral intramedullary and peripheral release of interleukin-6 (IL-6) after fracture and subsequent intramedullary reaming.

In all patients, the fracture caused a significant increase in the local femoral concentrations of IL-6 compared to a femoral control group. The concentration of IL-6 in the local femoral environment was significantly higher than in the patients own matched blood samples from their peripheral circulation. The magnitude of the local femoral release of IL-6 after femoral fracture was independent of the injury severity score and whether the fracture was closed or open.

In patients who underwent intramedullary reaming of the femoral canal a further significant local release of IL-6 was demonstrated, providing evidence that intramedullary reaming can cause a significant local inflammatory reaction.

In the ‘two hit’ model of the inflammatory response to injury proposed by Bone, the initial injury causes a release of inflammatory mediators and a systemic inflammatory response. As a result, priming of the inflammatory system occurs which is thought to result in the increased sensitivity to further stimuli, such as surgery or infection. After a moderate primary inflammatory reaction, a secondary response has been shown to occur after surgery in already traumatised patients. This is known as the ‘second hit’ phenomenon which, if not controlled, may lead to complications such as the systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). The effects of the second hit phenomenon, including significant morbidity and mortality, have been described in previous studies.

The release of inflammatory mediators, particularly interleukin-6 (IL-6), after surgery in the traumatised patient has been shown to be a good marker for measuring the magnitude of the second hit response. A significant increase in the systemic concentration of IL-6 has been demonstrated after femoral intramedullary nailing. The source of the pro-inflammatory cytokine IL-6 detected in the peripheral circulation as part of the second hit phenomenon after femoral intramedullary nailing has not been established. The central hypothesis of this research was that femoral shaft fracture and intramedullary nailing produce a local femoral inflammatory reaction characterised by the release of IL-6, which spreads into the peripheral circulation, resulting in the second hit phenomenon.

Patients and Methods
Skeletal mature patients who had sustained a diaphyseal fracture of the femur requiring an intramedullary nail between 2001 and 2004 were asked to participate in the study. Local ethical committee approval was granted and informed consent or assent was obtained from the patient or their representative prior to their inclusion in this investigation. Those with a history of malignancy, age > 70 years, autoimmune disease or pregnancy were excluded. Details concerning the age, gender, the mechanism of injury and all associated injuries were recorded, and the Injury Severity score (ISS) determined.

Management of the fracture and the technique of nailing. Patients underwent primary stabilisation of the fracture, either by intramedullary nailing or by external fixation as part of a ‘damage control’ approach, within 24 hours of injury. Primary nailing was carried out in patients who were clinically stable using either...
a reamed or an unreamed technique with insertion of a solid femoral intramedullary nail (Synthes, West Chester, Pennsylvania). The decision as to which technique was used was made by the consultant responsible for the management of the patient. Routine antibiotic prophylaxis of 1.5 g intravenous cefuroxime was given to all patients at induction of anaesthesia. Reaming was performed using reamers (Synthes) in 0.5 mm increments to at least 1.5 mm above the selected diameter of the nail, with radiological screening to assess reduction of the fracture and insertion of the nail.

A damage control approach was taken when patients had either a thoracic injury (abbreviated injury scale (AIS) ≥ 3) in addition to a femoral fracture, bilateral femoral fractures, or were clinically unstable. The decision to use this approach was made by the surgeon (PVG). Primary external fixation was carried out in these patients using the Hoffmann II external fixator (Stryker, Kalamazoo, Michigan). They then had femoral nailing when they were clinically stable following resuscitation. Their clinical course was monitored daily and specific complications, including ARDS, sepsis, MODS and death, were recorded using established diagnostic criteria and scoring systems.

Control groups. A control group was established to define the cytokine content of the normal femur. This femoral control group consisted of patients undergoing elective total hip replacement (THR) for osteoarthritis (OA). Patients with auto-immune conditions such as rheumatoid arthritis (RA) or a history of malignancy were excluded. A cemented THR (Charnley Prosthesis, Depuy, Leeds, United Kingdom) was carried out on these patients through a lateral approach with an osteotomy of the greater trochanter. The femoral neck was divided and the cemented THR (Charnley Prosthesis, Depuy, Leeds, United Kingdom) was carried out on these patients through a lateral approach with an osteotomy of the greater trochanter. The femoral neck was divided and blood samples were acquired as detailed below, prior to the preparation of the femoral canal.

A second control group of healthy volunteers attending orthopaedic clinics was also used. Peripheral venous IL-6 concentrations had previously been measured in these patients. This was designated the peripheral control group.

Blood sample acquisition. Peripheral and femoral blood samples were collected in Vacutainer bottles (Becton Dickinson, Oxford, United Kingdom) containing potassium ethylenediamine tetra-acetic acid (EDTA), to allow the preparation of plasma samples. Peripheral blood samples were taken on admission to hospital and throughout surgery at specific points in time. In the operating theatre, samples were taken at the induction of anaesthesia to establish a baseline, at the initial entry into the femoral canal, after reaming if performed, and at the end of the nailing procedure. Peripheral blood samples were also collected on days 1, 3, 5, and 7 after surgery.

Femoral blood samples were obtained using a Levin-type Gastro duodenal feeding tube (Vygon, Girencester, United Kingdom). These tubes were 125 cm long with a diameter of 10 Fr (French gauge). They had a closed distal tip, facilitating non-traumatic passage of the tube, and two opposed lateral eyes that allowed sampling. Samples were taken from the femoral diaphysis at least 10 cm from the entry point in the piriform fossa.

Femoral blood sampling was carried out on entry into the femoral canal, after reaming, prior to insertion of the nail. Matched peripheral blood samples were taken at the same time. A similar sampling procedure was carried out in the femoral control group, in which peripheral blood was taken at the induction of anaesthesia, on entry to the femoral canal, and at the end of the operation. Samples were taken immediately after the femoral neck had been divided and the head removed. The femoral canal was then opened and blood samples were taken from the diaphysis.

All peripheral and femoral blood samples were processed immediately after collection. The EDTA Vacutainer tubes containing blood were centrifuged at 400 g (Mistral 3000; MSE Scientific Instruments, Crawley, United Kingdom) for ten minutes. The plasma collected was placed into 1.8 ml test tubes and initially frozen at -20ºC. Samples were then transferred to a freezer at -80ºC after 24 hours for longer term storage.

Analysis of samples. For the detection of IL-6 in the plasma samples a Human Cytokine Antibody 10-plex Bead Kit (Biosource, Paisley, United Kingdom) or enzyme-linked immunosorbent assay kit (ELISA) (R&D systems, Minneapolis, Minnesota) was used. Luminex and ELISA assays correlate closely, with the ELISA value being equivalent to the Luminex value multiplied by a correction factor (1.02 in the case of IL-6, from Biosource data).

The majority of samples from the fracture and the femoral control group (n = 25) were analysed using the Cytokine Antibody Bead Kit technique on the Luminex 100 IS System running StarStation Software (Applied Cytometry Systems, Sheffield, United Kingdom).

Blood samples from six of the patients with fractures were acquired after samples had been analysed using the Luminex system. As the results of the ELISA and Luminex techniques are comparable for economic reasons the determination of IL-6 concentrations in these six patients was performed by the ELISA technique. A human IL-6 ELISA kit (R&D systems) was used and samples were analysed on an Opsys microplate reader (Dynatech Technologies, Horsham, Pennsylvania). The ELISA technique had previously been used for the determination of IL-6 in the historical peripheral control group using commercially-available IL-6 kits (R&D Systems).

Statistical analysis. As not all the data were normally distributed, a non-parametric test was used for all comparisons in order to maintain consistency. Statistical analysis of non-parametric data was made using the Mann-Whitney U-test and Spearman’s rank correlation. GraphPad Instat 3 statistical software (San Diego, California) was used for processing and analysis of data. A two-tailed p-value of < 0.05 was considered significant.
Results

There were 28 patients with fractures of the femoral shaft included in the study. However, only 27 femoral samples were analysed, as one sample from patient number 19 in the reamed group was spoiled during the running of the samples. An unreamed nailing was carried out in 16 patients and reaming in 12. The demographic data are shown in Tables I and II. Demographically, no statistically significant difference was demonstrated between the groups (Table III). Patients who had a damage control approach applied to their management are highlighted in Tables I and II (n = 4 in unreamed group, n = 1 in reamed group). Two patients who had a reamed femoral nail developed ARDS. Both received respiratory support and went on to make a full recovery. Another five patients, all with a high ISS (≥ 27), were admitted to the intensive care unit for respiratory support as they had associated chest injuries, but none developed ARDS. No patients developed MODS or died.

The femoral control group consisted of three patients (median age 31 years; 18 to 74). The peripheral control group comprised 20 healthy uninjured patients (median age 54 years; 23 to 85).

An analysis of the second hit phenomenon: the peripheral IL-6 response to fracture of the femoral shaft and intramedullary nailing. Patients with a fracture had a significantly elevated peripheral IL-6 concentration on admission to hospital compared to the peripheral control group (p < 0.0001). Their median IL-6 concentration on admis-

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| Table I. Demographic data, including the injury severity score and the mechanism of injury, in patients who had unreamed femoral nailing. Highlighted patients had a damage control approach to their management, as detailed in Materials and Methods |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Gender | Male | Male | Male | Female | Male | Female | Male | Female | Male | Female | Male | Male | Male | Male | Female |
| Age (yrs) | 48 | 54 | 57 | 67 | 21 | 17 | 58 | 67 | 58 | 35 | 40 | 18 | 16 | 16 | 56 | 24 |
| Mechanism of injury | RTA | RTA | RTA | Crush injury | RTA | RTA | RTA | RTA | Fall | Crush injury | RTA | RTA | RTA | RTA | RTA | RTA |
| Injury severity score | 13 | 29 | 34 | 18 | 9 | 13 | 15 | 16 | 9 | 33 | 9 | 16 | 9 | 9 | 32 | 16 |
| Abbreviated injury score | Head & neck | - | - | - | - | - | - | 2 | - | - | 1 | - | - | - | - | - |
| Face | 2 | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - |
| Thorax | - | 3 | 3 | 3 | - | 2 | - | - | - | 4 | - | - | - | - | 4 |
| Extremity | 3 | 4 | 5 | 3 | 3 | 3 | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 4 |
| Abdomen | - | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Unreamed nail | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Complication | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Timing of secondary nailing from injury (hrs) | 288 |

* RTA, road traffic accident

| Table II. Demographic data, including the injury severity score and the mechanism of injury, in patients who had unreamed femoral nailing. The highlighted patient had a damage control approach to their management, as detailed in Materials and Methods |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| Gender | Male | Female | Female | Male | Male | Male | Female | Male | Male | Female | Male |
| Age (yrs) | 22 | 16 | 18 | 35 | 18 | 34 | 38 | 47 | 55 | 22 | 50 | 61 |
| Mechanism of injury | RTA | RTA | RTA | RTA | RTA | RTA | RTA | RTA | RTA | RTA | RTA |
| Injury severity score | 27 | 9 | 9 | 9 | 16 | 9 | 11 | 18 | 19 | 26 | 10 |
| Abbreviated injury score | Head & neck | - | - | - | - | - | - | 1 | - | - | - |
| Face | 3 | - | - | - | - | - | 1 | - | - | 1 | - |
| Thorax | - | 3 | 3 | 3 | 4 | 3 | 4 | 3 | 3 | 3 | 3 |
| Extremity | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 |
| Abdomen | - | 2 | - | - | - | 1 | - | - | - | 1 | - |
| Open or closed | Open | Closed | Closed | Closed | Open | Closed | Closed | Closed | Closed | Open | Closed |
| Type of nail | Reamed | Reamed | Reamed | Reamed | Open | Reamed | Reamed | Reamed | Reamed | Closed | Reamed |
| Complication | - | UTI | ARDS | ARDS | - | - | - | - | - | - | ARDS |
| Timing of secondary nailing from injury (hrs) | 288 |

* RTA, road traffic accident
† UTI, urinary tract infection
‡ ARDS, acute respiratory distress syndrome
sion was 169 pg/ml (72 to 3205). The peripheral IL-6 concentration remained significantly elevated throughout the seven days of the study (p < 0.0001). There was no statistically significant difference between the median peripheral IL-6 concentrations in the unreamed and reamed groups (Fig. 1).

There was a small rise in the peripheral IL-6 concentration immediately after reaming, although this change was not statistically significant. The median concentration before reaming was 186 pg/ml (61 to 297), and afterwards was 201 pg/ml (67 to 329). At 24 hours after operation the median peripheral IL-6 concentration was higher in the reamed than in the unreamed group, but this difference was not statistically significant.

The effect of the fracture on the local femoral and peripheral IL-6 concentrations. Following the fracture there was a significant elevation in the median intramedullary IL-6 concentration compared with the femoral control group (p = 0.0005). The median femoral IL-6 concentration on entry into the canal in patients with a fracture (n = 27) was 3947 pg/ml (128 to 25 689). The median femoral IL-6 concentration in the control group (n = 3) was 8 pg/ml (5 to 11) (Fig. 2). The assay sensitivity was 3 pg/ml.

The median IL-6 concentration in the peripheral circulation after fracture was significantly elevated compared with the peripheral control group (p < 0.0001). In the patients with a fracture (n = 27) the median peripheral IL-6 concentration at the time of entry into the femoral canal was
215 pg/ml (60 to 1797). The median peripheral IL-6 concentration in the peripheral control group (n = 20) was 4 pg/ml (3 to 5).

The median femoral intramedullary IL-6 concentration was significantly elevated compared with the median peripheral IL-6 concentration at the time of entry into the femoral canal in patients with a fracture (p < 0.0001) (Fig. 3).

No statistically significant difference in the median local femoral IL-6 concentration at the time of entry into the canal was demonstrated between patients who had an open or closed fracture of the femoral shaft. No correlation was established between the median peripheral and femoral IL-6 concentrations (Spearman’s r value = 0.05, p-value not significant, Fig. 4).

The effect of the ISS on peripheral and local femoral concentrations of IL-6. A correlation between an increasing ISS and an increasing peripheral concentration of IL-6 was demonstrated (Spearman’s r value = 0.77, p < 0.0001). This has been shown previously. No relationship was established between the ISS and the femoral concentration of IL-6 at the time of entry into the femoral canal (Spearman’s r value = -0.05; p-value not significant, Fig. 5).

The effect of femoral intramedullary reaming on local femoral concentrations of IL-6. In the patients who had a reamed femoral nail (n = 11), reaming caused a significant elevation in the local femoral concentration of IL-6 (p = 0.01). The median IL-6 concentration in the femoral canal before reaming was 3699 pg/ml (923 to 18299) and after was 15903 pg/ml (1854 to 44922) (Fig. 6). There was no significant increase in the peripheral IL-6 concentration after femoral reaming or at 24 hours after the nailing procedure.

Discussion

Trauma, including fracture of the shaft of the femur, has previously been shown to cause significant stimulation of the inflammatory response system, resulting in the release of cytokines and mediators such as IL-6 which are responsible for driving the inflammatory response. The pro-inflammatory cytokines and mediators released after injury have been implicated in the development of the potentially fatal complications ARDS and MODS. A further increase in the systemic concentration of IL-6 has been demonstrated after surgical procedures, including intramedullary nailing of the femur.

In this study, the concentrations of IL-6 within the femur were significantly elevated compared with the femoral controls following fracture. As well as a local increase in IL-6 concentration, a significant elevation in the peripheral IL-6 concentration compared to peripheral controls (p < 0.0001) was found in patients after injury, which agrees with other published studies. However, the local femoral elevation in IL-6 concentration was significantly greater than the elevation in the peripheral circulation (p < 0.0001), providing support for the view that a local reaction in the femoral canal is responsible for the rise in IL-6 found in the femur. One potential source might be the osteoblasts, which are known to produce IL-6.

The local femoral intramedullary inflammatory response to trauma has not previously been described. Previous research has documented the inflammatory constituents present in the haematoma at the fracture site after injury. A massively elevated local concentration of IL-6 (mean 12538 pg/ml) has been reported in haematoma sampled at the time of surgery for open reduction and fixation of frac-
tures of long bones.\textsuperscript{23} A dramatic increase in the local concentration of IL-6, significantly greater than that in the peripheral circulation, has been shown to occur in other local inflammatory conditions, including bacterial peritonitis (mean 2595 pg/ml (SD 1360)) and in the peritoneal fluid of patients with burns and an associated abdominal compartment syndrome (mean 6462 pg/ml (SEM 2052)).\textsuperscript{24,25}

The finding that both trauma and infection can cause dramatically elevated local concentrations of pro-inflammatory cytokines raises the question as to why these cytokines are there. The local environment may be acting as a cell-priming or stimulating zone. For example, the femur has a rich vascular supply, and after a traumatic insult circulating cells involved in the inflammatory response such as macrophages and leucocytes, will pass through the femoral environment, where they may be stimulated or primed by the pro-inflammatory cytokines present. The inflammatory cells may then leave the local environment in a primed or stimulated state, which may lead to either a local or a systemic effect by mechanisms previously discussed. An alternative explanation could be that the local reservoir of IL-6 sets up a pro-inflammatory cytokine gradient, where cytokines may travel from a local zone of higher concentration to one of a lower concentration in the systemic circulation, thereby modulating the inflammatory response.\textsuperscript{23} These two hypotheses are not mutually exclusive, and it is conceivable that a combination of these processes could occur.

The rise in the peripheral IL-6 concentration correlated in a linear fashion with the ISS.\textsuperscript{16} However, no such relationship was found between the rise in local femoral IL-6 concentration and the ISS, and there was correlation between the peripheral and femoral IL-6 concentrations at the time of entry to the femoral canal. Patients with both closed and open fractures of the femoral shaft were included in this study. It was thought that patients with an open fracture might have a lower intramedullary concentration of IL-6, as IL-6 could be lost to the external environment both at the time of and after injury, but there was no significant difference. Prior to reaming, the local femoral IL-6 concentration in patients with a fracture was significantly elevated compared to the femoral control group \((p = 0.006)\). Reaming produced a further significant increase in the local femoral concentration of IL-6 \((p = 0.01)\) but did not have a similar effect on the peripheral concentration. We were not able to compare the effect of reamed with unreamed nailing on the local femoral IL-6 concentration. After insertion of the solid nail the femoral canal was full and so did not allow further sampling of the blood.

Femoral reaming has been shown to have beneficial local effects, with improved times to fracture union compared to unreamed nailing.\textsuperscript{26} This may be a result of the improved stability of a reamed nail with a larger diameter. The reamings may also have osteogenic potential as does bone graft, and come to be deposited at the fracture site. Our finding of increased local concentrations of IL-6 after reaming raises the question as to whether additional local action of IL-6 may be to contribute to bone remodelling, but further investigation of this hypothesis is required.

Reaming of the femoral canal normally takes less than 30 minutes to perform. Up-regulation of mRNA for IL-6 in inflammatory cells after stimulation has been shown to take two to four hours, with maximal production of IL-6 occurring after four to six hours.\textsuperscript{27} Our finding of a significant increase in local IL-6 concentrations within 30 minutes...
after intramedullary reaming poses the question as to the possible molecular mechanisms responsible.

We found that the peripheral concentration of IL-6 was significantly elevated at 24 hours after intramedullary nailing compared to healthy volunteer controls (p < 0.0001). When the concentrations of IL-6, 24 hours after operation in both the unreamed and reamed groups were compared to the pre-operative concentrations, no significant difference was seen. No significant further elevation or second hit phenomenon was demonstrated. The inflammatory state that was demonstrated pre-operatively persisted after surgery and was characterised by a significant elevation of peripheral IL-6 concentrations throughout the study. In previous similar investigations femoral intramedullary nailing has been shown to produce a second hit phenomenon. It is possible that the failure to demonstrate a significant second hit phenomenon in our study could have been a cause of a type II statistical error.7,9

Having established that the IL-6 concentration is increased in the femoral canal following fracture and intramedullary reaming, we wished to determine whether the femoral canal could be the source of the second hit phenomenon. However, we were not able to determine whether the IL-6 detected in the peripheral circulation originated from the femoral canal or was produced in the peripheral circulation. Sampling directly from the venous drainage could have been achieved by cannulating the femoral vein on the fractured side and sampling peri- and postoperatively. However, this additional procedure could have inflicted further morbidity on the already traumatised patient and it was decided not to undertake this.

A further way to study the processes occurring during femoral intramedullary nailing would be to use a large animal model. Previous researchers have used sheep, allowing the study of local and systemic changes during intramedullary nailing.24,29 The use of such a model could allow continuous monitoring of the venous outflow from the femur both during and after femoral nailing and intramedullary reaming to determine whether IL-6 and other pro-inflammatory cytokines were being released into the systemic circulation, generating the second hit phenomenon.

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References


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