It has been suggested that reamed intramedullary nailing of the femur should be avoided in some patients with multiple injuries. We have studied prospectively the effect of femoral reaming on the inflammatory process as implicated in the pathogenesis of acute respiratory distress syndrome (ARDS) and multiple-organ failure (MOF). We studied changes in the levels of serum interleukin-6 (IL-6) (proinflammatory cytokine), neutrophil CD11b (C3) receptor expression (activated neutrophil adhesion molecule), serum soluble intracellular adhesion molecule (s-ICAM-1), serum soluble E-selectin (the soluble products of endothelial adhesion molecules) and plasma elastase (neutrophil protease) in a series of patients with femoral fractures treated by nailing. We have also compared reamed nailing with unreamed nailing.

We found that the levels of serum IL-6 and elastase rose significantly during the nailing procedure indicating a measurable ‘second hit’. There was no clear response in leukocyte activation and no difference in the release of endothelial adhesion molecule markers. There was no significant difference between groups treated by reamed and unreamed nailing. Although clinically unremarkable, the one patient who died from ARDS was shown to be hyperstimulated after injury and again after nailing, suggesting the importance of an excessive inflammatory reaction in the pathogenesis of these serious problems.

Our findings have shown that there is a second hit associated with femoral nailing and suggest that the degree of the inflammatory reaction may be important in the pathogenesis of ARDS and MOF.

Stabilisation of the skeleton is an essential part of the early management of patients with multiple injuries and has been shown to decrease morbidity and mortality. Early fixation of femoral fractures reduces the incidence of acute respiratory distress syndrome (ARDS) and multiple-organ failure (MOF). While the association of fat embolism with ARDS is well established, it is now generally agreed that the development of ARDS is related to an uncontrolled inflammatory response to injury. This is characterised by a cascade of inflammatory reactions that may lead to an exaggerated systemic inflammatory response syndrome (SIRS) and then to ARDS and MOF.

Two inflammatory models have been proposed. The ‘one-hit model’ postulates that the initial massive injury and shock may produce an intense systemic inflammation resulting in early end-organ injury. In the ‘two-hit model’, patients enter a less intense state of SIRS but the inflammatory system is primed and vulnerable to a secondary insult which can amplify SIRS to precipitate late MOF. Several elements of the immune system have been implicated in this process including proinflammatory cytokines, activated polymorphonuclear leukocytes (PMN) and leukocyte-endothelial cell interactions.

Over the last few years the beneficial effects of the early stabilisation of fractures of the femoral shaft by intramedullary nailing, especially in the presence of a concomitant pulmonary contusion, have been challenged and an association between early reamed femoral nailing and a higher risk of ARDS/MOF suggested. It was proposed that reamed femoral nailing was a significant second hit. While this view remains controversial, there is no doubt that the previously injured lung is at risk and that reaming of the femur causes high intramedullary pressures which result in embolisation of marrow constituents to the lung. This may lead to pulmonary impairment and possibly ARDS.


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In traumatised patients the potential of surgical procedures to act as a second hit is now well recognised. Waydhas et al. reported that secondary operations may act as a second insult and precipitate late MOF if performed on patients with multiple injuries with ongoing post-traumatic inflammation.

Our aim was to consider femoral nailing, both reamed and unreamed, as a second hit and to determine its effect on those inflammatory mediators known to be implicated in the pathogenesis of ARDS.

### Materials and Methods

Between April 1995 and September 1996, we studied prospectively 32 adult patients after musculoskeletal injury in three hospitals in our area. The centres used similar protocols for the early fixation of fractures of long bones and delivery of perioperative care, but differed in the preferred method of fixation. At centres 1 and 2, fractures of the femoral shaft were stabilised by reamed intramedullary nailing in 15 patients (RFN group) while at centre 3, unreamed nailing was used in the remaining 17 patients (URFN group). This allowed us to assess the effects of femoral nailing and to compare the two techniques.

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Table I. Details of the 32 patients and their injuries

<table>
<thead>
<tr>
<th>Case</th>
<th>ISS</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Delay theatre (hours)</th>
<th>Injuries*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unreamed nailing</td>
<td>1</td>
<td>22</td>
<td>26</td>
<td>M</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>73</td>
<td>M</td>
<td>4</td>
<td>#Femur, hip, #pelvis, urethral lacerations, #tibia, HI</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>35</td>
<td>M</td>
<td>6</td>
<td>#Femur, #fibial plateau, lacerations knee</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>75</td>
<td>F</td>
<td>7</td>
<td>#Femur</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>40</td>
<td>M</td>
<td>9</td>
<td>#Femur, #fibial plateau, #wrist, dislocation shoulder, lacerations</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>70</td>
<td>F</td>
<td>6</td>
<td>#Femur</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>18</td>
<td>M</td>
<td>12</td>
<td>#Femur, # wrist, lacerations shin</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>83</td>
<td>F</td>
<td>7</td>
<td>#Femur</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>87</td>
<td>F</td>
<td>9</td>
<td>#Femur, #olecranion</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>24</td>
<td>M</td>
<td>3</td>
<td>#Open femur, #humerus, #ulna, HI</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>46</td>
<td>M</td>
<td>12</td>
<td>#Femur, #1st metacarpal</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>80</td>
<td>F</td>
<td>10</td>
<td>#Femur, laceration scalp</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>70</td>
<td>F</td>
<td>6</td>
<td>#Femur, #wrist</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>30</td>
<td>M</td>
<td>11</td>
<td>#Femur, #pelvis, HI</td>
</tr>
<tr>
<td>15</td>
<td>17</td>
<td>33</td>
<td>F</td>
<td>6</td>
<td>#Femur, #acetabulum, #forearm, HI, lacerations</td>
</tr>
<tr>
<td>16</td>
<td>26</td>
<td>18</td>
<td>M</td>
<td>4</td>
<td>#Femur, #iliac blade, #clavicle, right haemothorax, lacerations</td>
</tr>
<tr>
<td>17</td>
<td>27</td>
<td>28</td>
<td>F</td>
<td>3</td>
<td>#Femur, lung contusion, HI</td>
</tr>
</tbody>
</table>

Mean: 13.5 49 7.5 (95% CI 5.8 to 9.1)

Reamed nailing

18 | 10 | 25 | M | 6 | #Femur, #wrist, lacerations |
| 19 | 10 | 30 | M | 11 | #Femur, #clavicle, lacerations |
| 20 | 22 | 26 | M | 10 | #Femur, #pneumothorax, HI |
| 21 | 10 | 34 | M | 7 | #Femur, lacerations |
| 22 | 9 | 20 | F | 9 | #Femur |
| 23 | 9 | 27 | M | 6 | #Bilateral femurs (diseased) |
| 24 | 9 | 50 | M | 12 | #Femur |
| 25 | 10 | 36 | M | 12 | #Femur, laceration scalp |
| 26 | 9 | 80 | F | 11 | #Femur |
| 27 | 9 | 83 | F | 8 | #Femur |
| 28 | 11 | 32 | M | 7 | #Femur, #tibia, #forearm, lacerations |
| 29 | 27 | 57 | M | 10 | #Femur, #fibial plateau, #pelvis, lung contusion, HI, lacerations |
| 30 | 13 | 27 | M | 4 | #Femur, # wrist, #ankle, HI |
| 31 | 9 | 81 | F | 7 | #Femur |
| 32 | 11 | 18 | M | 9 | #Femur, #ankle, #pelvis, #wrist, lacerations |

Mean: 11.9 42 8.6 (95% CI 7.3 to 9.9)

* # = fracture; HI = head injury

In traumatised patients the potential of surgical procedures to act as a second hit is now well recognised. Waydhas et al. reported that secondary operations may act as a second insult and precipitate late MOF if performed on patients with multiple injuries with ongoing post-traumatic inflammation.

Our aim was to consider femoral nailing, both reamed and unreamed, as a second hit and to determine its effect on those inflammatory mediators known to be implicated in the pathogenesis of ARDS.

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In Table I, the details of the 32 patients and their injuries are presented. The table shows the case number, ISS, age, gender, delay in theatre, and injuries sustained. The groups were similar in regard to age and injury severity score (ISS) (Table I).

Approval was obtained from the Research Ethical Committee and all the patients or their representatives gave verbal or written informed consent before beginning the study. The patient arrived in hospital within two hours of injury and all had a fracture of the femoral shaft either isolated or in combination with other injuries. They were inpatients for at least seven days and were operated on within 12 hours of admission. Venous blood samples were collected on arrival in the trauma room. Subsequent blood samples were taken at the induction of anaesthesia, at nail insertion, at 1, 4 and 24 hours after operation and then at 3, 5 and 7 days.

We also collected venous blood samples from 20 (11 women and 9 men) healthy uninjured volunteers, randomly selected from the orthopaedic outpatient clinics, who formed the control group. They had a median age of 54 years (23 to 85).

We assessed the following immune system markers: interleukin-6 (IL-6), a known proinflammatory cytokine; soluble E-selectin (s-E-selectin) and soluble ICAM-1 (s-ICAM-1), endothelial adhesion molecules; and leukocyte activation, by measurement of plasma elastase release (E-αPI) and expression of surface receptor CD11b.
Operative technique. In centres 1 and 2 the femur was reamed. Using a traction table, the femur was reamed to at least 1 mm greater than the diameter of the nail. In centre 3 an unreamed technique was used. The femoral canal was opened with a drill before insertion of the nail.

Sample handling. Serial blood samples were collected into two types of endotoxin-free vacutainer tubes, one containing EDTA and the other clot activator. They were placed on ice and processed within two hours of collection. Tubes containing clot activator were centrifuged at 1000 rpm (Mistrall 3000; MSE Scientific Instruments, Crawley, UK) for ten minutes. Serum and plasma were collected and stored at -70°C. Flow cytometric studies were performed on 100 µl of blood from the samples in the EDTA tubes.

Flow cytometry of polymorphonuclear leukocytes (PMNs). Polymorphonuclear leukocytes were stained using fluorescent monoclonal antibodies (CALTAG Laboratories, Burlingame, California) in a whole-blood assay as previously described. Immunofluorescence was determined on a Coulter Epics (Luton, UK) flow cytometer. We measured the forward angle light scatter (FALS) and 90° light scatter (LS). At least 10,000 events were counted on each sample within a gated region of polymorphonuclear leukocytes. The collected data were analysed using software for the Coulter Epics XL.

Determination of IL-6, s-E-selectin, s-ICAM-1 and E-α1PI levels. We measured the levels of serum IL-6, s-ICAM-1 and s-E-selectin using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc, Minneapolis, Minnesota). The plasma elastase release was determined using an indirect sandwich ELISA assay recalibrated against the currently commercially available Merk elastase assay. All measurements were carried out in duplicate.

Statistical analysis. Calculations were performed on a personal computer using SPSS version 6.0 (London, UK). Data were expressed as means with 95% confidence intervals (CI). Variation with time and differences between the two treatment groups were assessed by repeated-measures two-way analysis of variance (ANOVA). Values for the patient who died were compared with those of survivors using a single sample t-test. The differences were considered to be statistically significant at p < 0.05.

Results

Effect of femoral nailing. On admission in all 32 patients the levels of serum IL-6, plasma elastase and CD11b expression (Figs 1a, 2a, 3a) were significantly raised above the control levels (p < 0.0001) and slowly returned towards them over the study period. The levels of s-ICAM-1 (Fig. 4a) and s-E-selectin (Fig. 5a) were slightly increased initially and continued to rise with time, reaching significance above the control values after day 3.

The levels of serum IL-6 and plasma elastase showed a further increase as a result of the nailing procedure (Figs 1a and 2a). We noted no overall difference in leukocyte activation and no difference in the release of endothelial adhesion molecules.

The effect of reaming. There was a trend towards higher levels of serum IL-6, plasma elastase, CD11b expression and s-ICAM-1 in the RFN group than in the URFN group, but this did not reach statistical significance (for IL-6, plasma elastase, s-ICAM-1, p > 0.05; for CD11b p = 0.052; Figs 1b, 2b, 3b and 4b). The levels of s-E-selectin were similar between groups (Fig. 5b).

Mortality. One patient in the RFN group with isolated, bilateral diaphyseal femoral fractures, no chest injury and an ISS score of nine died as a result of ARDS 33 hours after surgery. At initial presentation his immunoinflammatory system had been massively stimulated as illustrated by his IL-6 level of 272 pg/ml (mean of survivors 97.7 pg/ml, CI 89 to 106 pg/ml) and an elastase level of 325 µg/l (mean of...
Figure 2a – Mean plasma elastase concentration after femoral nailing in all 32 patients. The control group is represented by the dotted line and the 95% CI is shown. Figure 2b – Mean plasma elastase concentration after reamed and unreamed femoral nailing. The URFN group is represented by the dotted line and the 95% CI is shown.

Figure 3a – Mean CD11b expression after femoral nailing in all 32 patients. The control group is represented by the dotted line and the 95% CI is shown. Figure 3b – Mean CD11b expression after reamed and unreamed femoral nailing. The URFN group is represented by the dotted line and the 95% CI is shown.

Figure 4a – Mean s-ICAM-1 levels after femoral nailing in all 32 patients. The control group is represented by the dotted line and the 95% CI is shown. Figure 4b – Mean s-ICAM-1 levels after reamed and unreamed femoral nailing. The URFN group is represented by the dotted line and the 95% CI is shown.
survivors 168 µg/ml, CI 151 to 185 µg/l). After injury he also showed a significantly increased response (second hit) illustrated by his peak IL-6 level of 293 pg/ml (mean of survivors 172 pg/ml, CI 161 to 197 pg/ml) and an elastase level of 600 µg/l (mean of survivors 198 µg/l CI 173 to 238 µg/l).

Discussion

Several authors have reported stimulation of the inflammatory system after trauma and proposed that loss of control of this normal response may be responsible for the widespread tissue destruction characteristic of ARDS and MOF.21-23 The ‘two-hit model’ suggests that ARDS and MOF may be precipitated by a secondary insult and that certain surgical procedures can act as important inflammatory insults.24,25

The potential systemic effects of the reaming process have led to fierce controversy,26,27 but the basic science of this phenomenon has not been adequately characterised. The reaming procedure has been shown to cause an increase in intramedullary pressure, embolisation of bone-marrow contents into the lung and release of inflammatory mediators.28 The potential of triglycerides to damage alveolar architecture is well described.6 Hyperstimulation of the inflammatory system, either by single or multiple ‘hits’, is considered by many to be the key element in the pathogenesis of ARDS and MOF.29 To our knowledge, there have been no previous studies which have measured the first- and second-hit responses and assessed the role of femoral nailing in this.

We have confirmed that the inflammatory system is stimulated by trauma. All 32 patients studied after injury showed significantly (p < 0.0001) increased levels of IL-6, plasma elastase and CD11b expression when first seen. Levels of endothelial adhesion molecules in the peripheral blood progressively increased, reaching statistical significance (p < 0.03) three days after injury. This increased activity represents the first-hit phenomenon of the inflammatory cascade and confirms that in the early events after trauma, IL-6 is released, neutrophils are activated and there are interactions between neutrophils and endothelial adhesion molecules.

Our study has shown the second hit due to femoral nailing, with clear responses in both the levels of serum IL-6 and plasma elastase, but the response of the levels of s-ICAM-1 and CD11b to nailing was not as clear. While a higher trend was observed in the reamed group in all of these markers, this did not reach statistical significance; the p value, however, for s-ICAM-1 was 0.08 and that for the CD11b measurements 0.052 (ANOVA). When considered together with the nature of these molecules, representing activity on both sides of the neutrophil-endothelial binding site, this finding could be considered as a type-II statistical error and this difference may indeed be real.

Our results should be considered along with those of Pape et al15 who reported a significant rise in elastase levels in the central venous blood of patients who had reamed femoral nailing compared with those with unreamed nailing. They also observed that during reaming the stimulatory capacity of polymorphonuclear leukocytes measured by chemiluminescence was higher in RFN patients than in the URFN group.

Perhaps the most potentially important element of our study is the assessment of the patient who developed severe ARDS and died three days after bilateral reamed nailing. While there were no obvious additional clinical risk factors, his inflammatory profile showed a massive first hit which was significantly restimulated by the nailing procedure. This may reflect his initial degree of injury or an idiosyncratic hyperstimulation response. This phenomenon has not been measured before.

We believe strongly in the critical value of skeletal stabilisation in the management of patients with multiple injuries but this case suggests that if individual hyper-
stimulation can be identified, then management could be modified to avoid the stimulating procedure or block the appropriate molecules.

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References