Intramedullary Nailing as a ‘Second Hit’ Phenomenon in Experimental Research

Lessons Learned and Future Directions

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Abstract

Background The ‘second hit’ phenomenon is based on the fundamental concept that sequential insults, which are individually innocuous, can lead to overwhelming physiologic reactions. This response can be expressed in several organic systems and can be examined by measurement of several parameters.

Purposes The purpose of this study was to evaluate the incidence of systemic effects of intramedullary nailing and the role of concurrent head and thoracic injuries as they have been recorded in vivo. We also wanted to determine what would be the optimal animal model for future research and what variables should be investigated.

Methods We reviewed the available literature of animal studies that used surgery, and particularly nailing, as a

second hit. The reviewed studies were retrieved through an electronic search of the MEDLINE database. We analyzed the methods of creating the first and second hits (nailing), the characteristics of the animal models, the variables examined, and the pathophysiologic responses, which appeared after the second hit.

Results Second hit reamed intramedullary nailing was found to provoke consumption of coagulation factors, whereas the effect of unreamed nailing on coagulation factors appears inconsistent. Hemodynamic factors were affected only transiently by the second hit, whereas the pulmonary function was affected only when the first hit included lung injury.

Conclusions Thoracic and head injuries predispose to an aggravated second hit. Primate animal models are considered to be closer to clinical reality and should be preferred for future studies. Future studies should include measurements of proinflammatory and antiinflammatory markers.

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Introduction

The stress response to trauma is the summation of the physiologic response to the injury (the first hit) followed by the response to any ongoing physiologic disturbance or subsequent intervention (the second hit) [21]. The two-hit concept, as introduced by Moore and Moore [40], has been one of the most compelling between the different theories and pathways for the development of posttraumatic complications [22, 41, 53, 65, 67].

The technical, financial, and ethical aspects of clinical trauma research and the multiplicity of variables and factors affecting the outcome on humans lead researchers toward in vivo animal studies [13]. Animal models have been used widely to provide answers through

postethanasia tissue analysis and experimental treatment alternatives that otherwise would be unavailable. Several parameters have been evaluated as markers in these *in vivo* experiments such as coagulation factors (fibrinogen, factor V, antithrombin-III, D-dimers) [23, 26, 48, 54], lung endothelial permeability [48, 50–52, 54], hemodynamic measurements [20, 35, 48–52, 60, 68–71], and certain cytokines such as interleukins (IL-6, IL-10) and tumor necrosis factor- α (TNF- α) [16].

Moreover, various insults and conditions have been studied as second hit, including intraperitoneal injections of lipopolysaccharide and saline [62, 63], focal polymicrobial contamination (peritonitis, pneumonia, myocardial inflammation) [4, 29, 44], and surgical procedures, mostly in the form of intramedullary nailing [16, 20, 23, 26, 35, 39, 45, 48–52, 54, 60, 68–71].

A careful review of the literature was done to investigate the existing evidence regarding: (1) the influence of second hit surgery (intramedullary nailing) on the coagulative, pulmonary, or hemodynamic functions in experimental animals; and (2) the role of head and thoracic injuries, as first hits, in the sequel of events that follow a surgical second hit. Additionally, we aimed to assess which of the available animal models of surgical two-hit phenomenon would be more relevant to clinical reality [31] and, finally, which variables (cytokines, physiology markers) would optimally monitor the two-hit inflammatory sequel.

Materials and Methods

A systematic review of the literature was conducted to identify all publications referring to intramedullary nailing as a second-hit phenomenon in the experimental setting. An electronic search of the MEDLINE database (up to July 2009) was conducted entering the following terms and Boolean operators: “animal” AND “nailing,” which identified 625 studies. Another double search was conducted using the 625 studies initially retrieved as a search pool. The following terms and Boolean operators were used for each search: (1) first search: “coagulation” OR “embolism” OR “haemodynamic” OR “hemodynamic” OR “pulmonary; and (2) second search: “injury” AND “lung” OR “head.” The term “second hit” (or 2nd hit) was not used as a search criterion. This was done for two reasons. First, it might not be used universally to describe the phenomenon, so using it in a search engine would result in missing articles and second, it was established as a term only in the mid1990s [40], so earlier studies not using this descriptive term would be excluded.

Only papers in English and German were retrieved. Articles were considered eligible if they met the following inclusion criteria: (1) only animal experimental studies; (2)

a two-hit sequel should exist; (3) the second hit consisted of a surgical nailing procedure; and (4) the values examined were relevant to the pathophysiologic state of the subject resulting from the second hit. Exclusion criteria included studies that examined multiple hit phenomena and/or studies in which the two-hit scenario was not clear, usually because of the doubtful effect of a weak first hit (eg, isolated osteotomy of long bones). Review papers, expert opinion articles, editorials, letters to the editor, publications on congress proceedings, and manuscripts with incomplete documentation or final results and outcome were excluded (Fig. 1). The quality of the reviewed manuscripts was evaluated by two assessors (NGL, NKK) according to the scientific content and their suitability for the presented review. The names of the institutions, authors, and journals were concealed on all manuscripts to minimize the risk of bias during the review.

Of the 625 papers initially selected, 20 met the eligibility criteria [20, 23, 24, 26, 35, 39, 42, 43, 45, 48–52, 54, 60, 68–71] (Table 1). The sheep model was the most common species used in 17 studies [20, 23, 24, 26, 35, 42, 43, 45, 48–52, 54, 68, 70, 71]. Other species used were dogs [39, 60] and mice [69]. The six conditions that were used as first hit were: hemorrhagic shock [16, 23, 24, 35, 42, 43, 48–52, 71], lung contusion [20, 23, 26, 48–52, 54], high-energy trauma/fracture generation [24, 39, 43, 45, 68, 69], vasogenic brain edema [35], fat embolization [60], and an acute respiratory distress syndrome (ARDS)-like condition induced by chemical agents [70]. All the models used reamed femoral nailing (RFN), or unreamed femoral nailing (UFN) as the second hit [20, 23, 26, 35, 39, 45, 48–52, 54, 60, 68, 70, 71]. Control groups treated by plate osteosynthesis [45, 48, 60], external fixators [24, 26, 51], and tibia intramedullary nailing [71] could be evaluated and were used for comparison. The variables that were used as markers for quantification of the second-hit phenomena included factors related to coagulation, hemodynamic, pulmonary function and permeability, and incidence of fat embolism (Table 1).

Results

As a second hit, reamed femoral nailing produced consistent alterations of the coagulation system (Table 2). The coagulation response has been evaluated by monitoring the levels of antithrombin III (AT-III), factor V, fibrinogen [23, 24, 26, 48], and D-dimers [26, 54]. AT-III and factor V levels were reduced in control groups in which RFN and UFN were performed without a preceding first hit [23, 68]. When a first hit was preintroduced, the effects of both types of nailing, in the decrease of these two factors, were even more intense [23, 48, 68]. Second-hit RFN considerably

Fig. 1 A flowchart illustrates our study selection process.

reduced the fibrinogen levels as well [23, 26, 48], whereas UFN provided inconsistent results. Measurable elevations of D-dimer levels were noted after second-hit RFN,

whereas they were not observed after UFN [26, 54] or when reaming-irrigating-aspirating (RIA) systems were used [54] to mimic the second hit. Neither plate fixation nor external fixation was found to alter any of the examined markers of the coagulation system [26, 48]. In general, reamed intramedullary nailing, either used as a first or, even more intensely, as a second hit procedure, was correlated with a significant consumption of the coagulation factors, whereas unreamed nailing effects were proven inconsistent.

The effect of RFN as a second hit on hemodynamic parameters was minor and appeared to depend on the resuscitation of the subjects (Table 2). First-hit RFN caused a transient increase in pulmonary arterial pressure (PAP), especially when performed in intact bones of sheep [20, 50, 51]. Whenever lung contusion or its combination with hemorrhage was induced, PAP declined, only to become elevated to baseline levels after RFN [51, 52, 71] or UFN [51]. However, it is notable that if PAP already had been reinstated, after adequate resuscitation, the effect of the second-hit nailing was negligible and did not further increase PAP. When the first hit (blunt thoracic trauma [45], fat embolism [60], or fracture stimulus [69]) caused a primary elevation of PAP levels, second-hit reamed nailing did not provoke an additional increase. Plate osteosynthesis or external fixation, as second-hit interventions, did not influence PAP levels [45, 51, 61]. Other hemodynamic factors such as median arterial pressure, heart rate, cardiac output, or central venous pressure were not affected by the second hit of nailing. With the exception of one study [42], neither RFN or UFN induced additional major hemodynamic responses other than those that already were implemented by the first hit [20, 68, 70, 71].

Measurable changes consistent with deterioration of pulmonary parameters were recorded mostly when RFN was used (Table 2). Pulmonary function/permeability has been assessed by different parameters such as pulmonary lymph flow and its protein clearance, polymorphonuclear leukocytes (PMNL) chemiluminescence or stimulator capacity, microvascular pressure, the alveolar-arterial pO_2 gradient, capillary filtration coefficient, PMNL-diapedesis for interstitial edema detection and by histologic tissue analysis. Second-hit RFN provoked a major decrease in PMNL stimulatory capacity [26, 50–52, 54, 69] indicating pulmonary cell exhaustion. In contrast, second-hit UFN increased PMNL stimulatory capacity [52, 69], whereas the use of external fixators or RIA systems [26, 51] did not cause any significant changes in PMNL. The pulmonary lymph flow and its protein clearance were increased by any first hit in the examined studies. Second-hit RFN was associated with even higher values [51, 52, 71], whereas UFN and external fixation did not provoke any additional changes to the first-hit impact [51, 52]. As happened with

Table 1. Description of the parameters of the reviewed studies

Study	Animal species/numbers	First hit	Second hit	Examined parameters	Observation period (hours)
Manning et al. [39]	15 dogs Group A: 12 (femoral # + RFN)* Group B: 3 (RFN)	Femoral fracture	RFN	TRG levels	Up to 15 minutes after the end of the nailing procedure
Pape et al. [50]	18 sheep Group A: (LC and HR + nailing)* Group B: 2 (nailing)	LC and hemorrhagic shock	Femoral nailing	1. Pulmonary artery pressure 2. Central venous TRG 3. PMNL chemiluminescence	72 hours since first hit, 2 hours after the second hit
Pape et al. [52]	19 sheep Group A: (LC and HR + UFN)* Group B: (LC and HR + RFN)*	LC and hemorrhagic shock	1. UFN 2. RFN	1. Pulmonary arterial pressure 2. PMNL stimulatory capacity 3. Pulmonary TRG levels 4. Pulmonary capillary permeability	72 hours since first hit, 2 hours after the second hit
Pape et al. [49]	25 sheep Group A: 8 (LC and HR + AO reamer)* Group B: 8 (LC and HR + Biomet reamer)* Group C: 6 (LC and HR + Howmedica reamer)* Group D: 4 (LC and HR)	LC and hemorrhagic shock	RFN	1. Pulmonary capillary permeability 2. Central venous TRG levels 3. 11-dhTXB2 levels 4. Pulmonary artery pressure	72 hours since first hit, 2 hours after the second hit
Wozasek et al. [71]	31 sheep Group A: 7 (IMN) Group B: 7 (HR + reamed nailing)* Group C: 11 (HR + reamed nailing + endotoxin) Group D: 6 (HR + endotoxin)	Hemorrhagic shock	Reamed nailing of intact femur and tibia	1. Pulmonary arterial pressure 2. Lymph flow 3. Protein clearance	96 hours since first hit, 72 hours after the second hit
Pape et al. [51]	29 sheep Group A: 9 (LC/HR + IMN)* Group B: 8 (IMN) Group C: 4 (LC/HR + Ex-Fix)*	LC and hemorrhagic shock	1. RFN 2. Femoral Ex-Fix	1. Pulmonary arterial pressure 2. TRG concentrations 3. PMNL chemiluminescence 4. Lymph flow 5. Microvascular pressure 6. Filtration coefficient 7. Pulmonary permeability	72 hours since first hit, 2 hours after the second hit
Lehmann et al. [35]	18 sheep Group A: 6 (brain edema) Group B: 6 (brain edema and HR) Group C: 6 (brain edema and HR + RFN)*	Vasogenic brain edema and traumatic hemorrhagic shock	RFN	1. Hemodynamic parameters 2. Intracranial pressure 3. Water content of the brain	6 hours

Table 1. continued

Study	Animal species/numbers	First hit	Second hit	Examined parameters	Observation period (hours)
Neudeck et al. [45]	21 sheep	Blunt thoracic trauma and femoral fracture	1. UFN 2. RFN 3. Plating fixation	1. Femoral IM pressure 2. Fat embolization 3. Pulmonary response	5 hours
	Group A: 7 (thoracic trauma + RFN)*				
	Group B: 7 (thoracic trauma + UFN)*				
	Group C: 7 (thoracic trauma + plating)*				
Duwelius et al. [20]	30 sheep	LC	1. Femoral Osteotomy 2. Femoral Osteotomy and UFN 3. Femoral Osteotomy and RFN	1. Hemodynamic measurements (systemic BP, diastolic BP, cardiac output, pulmonary arterial pressure, wedge pressure) 2. Arterial blood gas. 3. Mixed venous blood gas. 4. Hemoglobin 5. Emboli quantification (intracardial ultrasound)	3 hours
	Group A1: 5 (femoral osteotomy)				
	Group A2: 5 (LC + femoral osteotomy)*				
	Group B1: 5 (femoral osteotomy and UFN)				
	Group B2: 5 (LC + femoral osteotomy and UFN)*				
	Group C1: 5 (femoral osteotomy and RFN) Group C2: 5 (LC + femoral osteotomy and RFN)*				
Schemitsch et al. [60]	41 dogs	FE induction and femoral fracture	1. RFN 2. UFN 3. Plate fixation	1. Pulmonary arterial pressure 2. Alveolar-arterial gradient 3. Histologic examination of lungs-brain-kidneys for evidence of intravascular fat	4 hours for Group B 24 hours for Group C
	Group A: 8 (FE)				
	Group B: 15 (FE + second hit at 4 hours)*				
	Group C: 18 (FE + second hit at 24 hours)* (Group B + C subdivision: 11 dogs had RFN, 11 dogs had UFN and 11 dogs had plating)				
Pape et al. [48]	21 sheep	LC and hemorrhagic shock	1. RFN 2. UFN 3. Plate fixation (on intact femur)	1. Pulmonary arterial pressure 2. Factor V 3. Protein C 4. AT-III 5. Fibrinogen 6. Pulmonary capillary permeability	72 hours since first hit, 2 hours after the second hit
	Group A: 8 (LC and HR + RFN)*				
	Group B: 7 (LC and HR + UFN)*				
	Group C: 6 (LC and HR + plating)*				
Wolinsky et al. [70]	12 sheep	IV infusion of Perila ketone (ARDS-like state induction)	Femoral Osteotomy and RFN	1. Central venous pressure 2. Pulmonary artery wedge pressure 3. Mean arterial pressure 4. Pulmonary arterial pressure 5. Cardiac output 6. Dynamic compliance (Cdyn)	5 hours
	Group A: 6 (femoral osteotomy and RFN) Group B: 6 (Perila ketone + RFN)*				
Willis et al. [69]	23 rats	Bilateral closed femoral fractures	1. RFN 2. UFN	1. Pulmonary capillary filtration 2. Pulmonary arterial pressure 3. Lung neutrophil content	N/A
	Group A: Control				
	Group B: (bilateral femoral #)				
	Group C: (bilateral femoral # + RFN)* Group D: (bilateral femoral # + UFN)*				

Table 1. continued

Study	Animal species/numbers	First hit	Second hit	Examined parameters	Observation period (hours)
Mousavi et al. [43]	24 sheep	Hemorrhagic shock ± bilateral femoral fractures	RFN	1. Fat intravasation	N/A
	Group A: 6 (HR and bilateral femoral # + RFN)*			2. Intracranial pressure	
	Group B: 6 (HR and intact femora + RFN)*	Hemorrhagic shock	RFN with AO or Howmedica reamer	3. Hemodynamic parameters	
	24 sheep			4. Respiratory parameters	
Mousavi et al. [42]	24 sheep	Hemorrhagic shock		1. Fat intravasation	N/A
	Group A: 6 (HR + AO reamer/high speed)*			2. Intramedullary pressure	
	Group B: 6 (HR + AO reamer/low speed)*			3. Central venous pressure	
	Group C: 6 (HR + Howmedica reamer/high speed)*			4. Mean arterial pressure	
	Group D: 6 (HR + Howmedica reamer/low speed)*			5. pH	
Hildebrand et al. [26]	24 sheep	LC	1. Femoral Ex-Fix 2. RFN	1. U/P ratio in the supernatant BALF	1 hour since first hit,
	Group A: 6 (sham thoracotomy + Ex-Fix)			2. PMNL capacity assessment	4 hours after the second hit
	Group B: 6 (LC + Ex-Fix)*			3. Histologic analysis (PMNL-diapedesis) for interstitial edema	
	Group C: 6 (sham thoracotomy + RFN)			4. Coagulation parameters (FIB, factor-V, AT-III, D-dimers)	
Pape et al. [54]	18 sheep	LC	1. RFN 2. RIA 3. UFN	1. D-dimers	4 hours
	Group A: 6 (LC + RFN)*			2. PMNL activity	
	Group B: 6 (LC + RIA)*			3. Pulmonary permeability	
	Group C: 6 (LC + UFN)*			4. Histologic evaluation	
White et al. [68]	21 sheep	High-energy injury to bone and soft tissue (fractures of both femurs and tibias)	Reamed nailing of femur and tibia	1. Central venous pressure	3–4 hours, tissues collected after 24 hours
	Group A: 7 (control group)			2. Pulmonary artery pressure	
	Group B: 7 (bilateral # of femurs and tibias)			3. Cardiac output	
	Group C: 7 (bilateral # of femurs and tibias + RN)*			4. Venous oxygen saturation	
Giannoudis et al. [23]	30 sheep	LC and hemorrhagic shock	1. RFN 2. UFN	5. FE (embolic scores and lung tissue histologic analysis)	7 hours
	Group A1: 5 (sham thoracotomy + RFN)			1. AT-III	
	Group A2: 5 (sham thoracotomy + UFN)			2. Factor V	
	Group B1: 5 (LC + RFN)*			3. Fibrinogen	
	Group B2: 5 (LC + UFN)*				
	Group C1: 5 (LC and HR + RFN)*				
	Group C2: 5 (LC and HR + UFN)*				

Table 1. continued

Study	Animal species/numbers	First hit	Second hit	Examined parameters	Observation period (hours)
Gray et al. [24]	24 sheep Group A: 6 (control group) Group B: 6 (bilateral # of femurs – trauma only) Group C: 6 (bilateral # of femurs + Ex-Fix)* Group D: 6 (bilateral # of femurs + RFN)*	Bilateral high-energy femoral fractures and hemorrhagic shock	1. Ex-Fix 2. RFN	1. Pulmonary embolic load (transesophageal echo) 2. Metabolic base excess 3. Plasma coagulation markers 4. Polymorphonuclear cell counts (bronchoalveolar lavage samples)	24 hours

* Indicates the two hit groups (groups in which both a first and a second hit were created) as defined by the criteria of this review article; # = fracture; ARDS = adult respiratory distress; AT-III = antithrombin III; BALF = bronchoalveolar fluid; BP = blood pressure; Cdyn = dynamic compliance; Ex-Fix = external fixation; FE = fat embolism; FIB = fibrinogen; HR = hemorrhage and resuscitation; IMN = intramedullary nailing; LC = lung contusion; N/A = not available; PMNL = polymorphonuclear leukocytes; RFN = reamed femoral nailing; RIA = reaming-irrigation-aspiration; RN = reamed nailing; TRG = triglyceride; U/P = urea/protein; UFN = unreamed femoral nailing; 11-dhTXB2 = 11-dehydro-thromboxane B2.

pulmonary lymph flow, pulmonary permeability and filtration coefficient increased when RFN was used as a first hit. Furthermore, when RFN was used as a second hit, the increase was nearly fivefold for the filtration coefficient and twofold for the pulmonary microvascular permeability [50–52, 54, 69]. Second-hit UFN also increased the pulmonary permeability, but less drastically [52, 69], whereas RIA did not elevate it further from the levels of the first hit [54]. Histologic findings of interstitial edema were more evident after reamed nailing [26, 54].

Second-hit intramedullary nailing created high embolic loads, but its effect on the coagulation or pulmonary system remains unclear (Table 2). Fat intravasation and fat embolization were examined in numerous animal studies [16, 20, 24, 39, 42, 43, 45, 49–52, 61, 68] in which intramedullary pressurization was performed either on intact or osteotomized bones. Central venous or pulmonary triglyceride (TRG) levels, transesophageal or intracardial ultrasound findings, and histologic examination of lung tissues were the usual methods of assessment. Reamed nailing, as a second hit, always created a transient increase in TRG levels additive to their increase after the first hit [39, 49–52]. The same pattern was recorded for unreamed nailing [52], whereas external fixation did not affect TRG [45]. Intracardial or transesophageal ultrasound revealed considerable amounts of fat particles after nailing [20, 24, 42, 43, 45] with the first reaming steps usually producing the highest amount of intravasation. It also was observed that reamers that are advanced rapidly into the medullary canal with low revolutions per minute resulted in higher embolic load than did the combination of slow advancement and high revolutions per minute [42]. External or plate fixation was not found to be responsible for production of embolic showers [24, 45]. Histologic tissue analysis was conducted in studies of different designs [20, 24, 26, 60, 61]. Lung, brain, and renal specimens of healthy animals that underwent reamed or unreamed nailing did not reveal evidence of fat emboli [20, 24, 60]. When induction of fat embolism or high-energy injury was used as the first hit, no difference in the amount of embolic fat could be detected in the lungs between control and study groups [24, 60, 68]. These results indicate second-hit nailing and especially reaming may provide a high embolic load detected by echocardiography. However, the deleterious effect of this fat embolization on systemic coagulation and pulmonary inflammation has not been proven.

The role of head and thoracic injuries as first hits seems to be important in the trauma surgery setting, although no definite conclusions have been extracted. Of the 20 reviewed studies, 11 [20, 23, 26, 45, 48–52, 54, 70] used first-hit lung injury models in the form of lung contusion [20, 23, 26, 48–52, 54], thoracic trauma [45], or chemically induced ARDS [70] (Tables 1, 2). It is interesting that in

Table 2. Description of the aims, conclusions, and measurable second hits of retrieved studies

Study	Animal species/numbers	Aim of study	Results/conclusion	Measured second hit postoperatively [†]
Manning et al. [39]	15 dogs Group A: 12 (femoral # + RFN)* Group B: 3 (RFN)	To investigate the contribution of marrow embolization from fracture to posttrauma pulmonary dysfunction	TRG statistically significant increase postoperatively were observed only when reaming was performed in intact femur; no second hit was detected in the fractured femurs after reaming	TRG : YES (intact femurs) NO (fractured femurs)
Pape et al. [50]	18 sheep Group A: (LC and HR + nailing)* Group B: 2 (nailing)	To compare the effects of isolated IMN with those of nailing after lung contusion and hemorrhagic shock	Nailing created a significant transient increase in PAP and TRG irrelevantly of a previous first hit (LC + HR); in contrast in the presence of previous LC and HR definite lung damage from IMN was detected	1. PAP: — 2. TRG: — 3. Lung F (QI- Kf): Group A
Pape et al. [52]	19 sheep Group A: (LC and HR + UFN) *Group B: (LC and HR + RFN)*	To investigate how RFN and UFN affect pulmonary damage after trauma	IMN after severe shock and lung contusion causes lung damage as a result of PMNL activation and TRG embolism; these effects are significantly less if nailing is performed without prior reaming	1. PAP: — 2. PMNL stimulation: Group B 3. TRG: Group B 4. Lung F. (PCP): Group B
Pape et al. [49]	25 sheep Group A: 8 (LC and HR + AO reamer)* Group B: 8 (LC and HR + Biomet reamer)* Group C: 6 (LC and HR + Howmedica reamer)* Group D: 4 (LC and HR)	To investigate how different types of reamers influence fat mobilization and pulmonary dysfunction	RFN can cause transient pulmonary hemodynamic and mediator effects and increased pulmonary capillary permeability; this was evident in the AO reamer group in this study	1. PAP: Group A and Group B 2. Dh-TXB2: all groups 3. TRG: Group A 4. Lung F. (PCP): Group A
Wozasek et al. [71]	31 sheep Group A: 7 (IMN) Group B: 7 (HR + reamed nailing)* Group C: 11 (HR + reamed nailing + endotoxin) Group D: 6 (HR + endotoxin)	To study the pathophysiologic events of IMN in the lungs of sheep with chronic instrumentation	Severe fat embolism alone caused by IMN does not change pulmonary permeability in sheep; only additional HR shock, even when additionally resuscitated leads to lung disturbance postoperatively; the subsequent endotoxin challenge does not aggravate lung injury	1. PAP: Group A 2. Lung F. (QI—Protein clearance): Group B
Pape et al. [51]	29 sheep Group A: 9 (LC/HR + IMN)* Group B: 8 (IMN) Group C: 4 (LC/HR + Ex-Fix)*	To determine if IMN affects lung function and microvascular permeability whether the lung already is injured; if so, whether a different method of fixation would diminish the effect	Intramedullary nailing causes additional damage to lungs after lung damage and hemorrhagic shock in sheep; this can be avoided if (in the presence of additional injuries) alternative methods are used	1. PAP: Group A 2. TRG: Group A 3. Lung F. (QI, PCP, Kf): Group A

Table 2. continued

Study	Animal species/numbers	Aim of study	Results/conclusion	Measured second hit postoperatively [†]
Lehmann et al. [35]	18 sheep Group A: 6 (brain edema) Group B: 6 (brain edema and HR) Group C: 6 (brain edema and HR + RFN)*	To investigate the incidence of femoral IMN after brain injury and hemorrhagic shock)	Brain edema and ICP are increased by reamed femoral nailing	ICP : Group C
Neudeck et al. [45]	21 sheep Group A: 7 (thoracic trauma + RFN)* Group B: 7 (thoracic trauma + UFN)* Group C: 7 (thoracic trauma + plating)*	To investigate whether the type of femur fixation influences the femoral intramedullary pressure, fat embolization, and pulmonary response after blunt thoracic trauma	IMN causes a higher increase of intramedullary pressure and more fat and bone marrow embolization than extramedullary ones; only minimal differences in the pulmonary hemodynamic response (PAP) were noted even in the presence of thoracic trauma	1. IMP: Group A, Group B 2. Echo emboli quantification: Group A, Group B 3. PAP : —
Duvelius et al. [20]	30 sheep Group A1: 5 (femoral osteotomy) Group A2: 5 (LC + femoral osteotomy)* Group B1: 5 (femoral osteotomy and UFN) Group B2: 5 (LC + femoral osteotomy and UFN)* Group C1: 5 (femoral osteotomy and RFN) Group C2: 5 (LC + femoral osteotomy and RFN)*	To investigate the association of RFN and UFN with pulmonary dysfunction; to investigate how RFN and UFN affects the relationship between embolization and pulmonary dysfunction in sheep with normal and contused lungs	Reaming before nailing of femoral fractures did not cause pulmonary dysfunction; pulmonary injury does not prime the lung for the damaging effects of FE	1. Hemodynamic parameters (systemic BP, diastolic BP, cardiac output, pulmonary wedge pres-sure): — 2. PAP : — 3. Histologic tissue analysis: — 4. Echo emboli quantification Group B2, Group C2
Schemitsch et al. [60]	41 dogs Group A: 8 (FE) Group B: 15 (FE + second hit at 4 hours)* Group C: 18 (FE + second hit at 24 hours)* (Group B + C subdivision: 11 dogs had RFN, 11 dogs had UFN and 11 dogs had plating)	To investigate if in the setting of induced FE, the method of fixation plays a role in additional compromise of pulmonary function	In the setting of induced FE, the method of fixation plays only a small role in the development of additional compromise of pulmonary function	1. PAP: — 2. Alveolar-arterial PO2 gradient: — 3. Histologic quantification of lungs-brain-kidneys for evidence of intravascular fat: —

Table 2. continued

Study	Animal species/numbers	Aim of study	Results/conclusion	Measured second hit postoperatively [†]
Pape et al. [48]	21 sheep Group A: 8 (LC and HR + RFN)* Group B: 7 (LC and HR + UFN)* Group C: 6 (LC and HR + plating)*	To investigate whether the type of femur fixation influences the coagulatory response and the pulmonary permeability in a sheep model of severe trauma	In the presence of severe trauma, RFN is associated with a higher level of pathologic activation of the coagulation cascade and increased pulmonary permeability when compared with UFN or plate osteosynthesis	1. Factor V: Group A 2. Protein C: Group A 3. AT-III: Group A 4. FIB: Group A 5. Lung F. (PCP): Group A
Wolinsky et al. [70]	12 sheep Group A: 6 (femoral osteotomy and RFN) Group B: 6 (Perila ketone + RFN)*	To determine the effect of reamed femoral nailing on pulmonary function after chemically induced (Perila ketone) pulmonary damage	PK infusion had a significant effect on pulmonary function, but RFN had no further effect; the fat embolization that occurs during RFN in an appropriately resuscitated sheep has no clinically significant effect on pulmonary function, even in the setting of a severe pulmonary dysfunction	1. Hemodynamic parameters (CVP, mean BP, CO, Cdyn): — 2. Pulmonary arterial wedge pressure: — 3. PAP: —
Willis et al. [69]	23 rats Group A: control Group B: (bilateral femoral #) Group C: (bilateral femoral # + RFN)* Group D: (bilateral femoral # + UFN)*	To determine the effect of femoral fracture or femoral fracture and IM fixation on pulmonary microvascular integrity and function	Femoral fracture alone does not cause an increase in pulmonary microvascular permeability; femoral fracture and IMN cause lung capillary leak, which is not increased by reaming the femoral canal	1. Lung F. (PCP): Group C, Group D 2. PAP: — 3. Lung neutrophil content: Group D
Mousavi et al. [43]	24 sheep Group A: 6 (HR and bilateral femoral # + RFN)* Group B: 6 (HR and intact femora + RFN)*	To examine the correlation between intramedullary nailing and intracranial pressure	Reaming should be performed at a low DS and high RPM to minimize the risk of fat intravasation and the subsequent ICP increase; the existence of a femoral fracture did not show a significant influence on changes in ICP	1. Fat intravasation: Group A 2. Intracranial pressure: — 3. Hemodynamic parameters: — 4. Respiratory parameters: —
Mousavi et al. [42]	24 sheep Group A: 6 (HR + AO reamer/high speed)* Group B: 6 (HR + AO reamer/low speed)* Group C: 6 (HR + Howmedica reamer/high speed)* Group D: 6 (HR + Howmedica reamer/low speed)*	To study the correlation between reaming of the intramedullary canal with modified parameters and different reamer head designs and pressure induced fat intravasation in sheep that had normal pulmonary function	Intramedullary reaming and nailing should be done soon after hemodynamic stabilization; the surgeon is advised to use the slowest possible driving speed with high revolution rates per minute and to use smaller core reamer head designs to produce the lowest possible pressure increase during reaming and to minimize the risk of fat intravasation and pulmonary dysfunction	1. Fat intravasation: — 2. Intramedullary pressure: — 3. Central venous pressure: Groups A and C 4. Mean arterial pressure: Groups A and C 5. Ph: Groups A and C

Table 2. continued

Study	Animal species/numbers	Aim of study	Results/conclusion	Measured second hit postoperatively [†]
Hildebrand et al. [26]	24 sheep Group A: 6 (sham thoracotomy + Ex-Fix) Group B: 6 (LC + Ex-Fix) *Group C: 6 (sham thoracotomy + RFN) Group D: 6 (LC + RFN)*	To investigate the effect of femoral reaming after lung contusion injury	Reaming of the femoral canal should be avoided in polytrauma patients with severe chest trauma as it could act as an additional stimulus for adverse outcome; temporary Ex-Fix appears to be a safe method for fracture stabilization until inflammatory and coagulatory disturbances after trauma have been normalized	1. Lung F. (PCP, U/P ratio): Group D 2. PMNL capacity: Group D 3. Histologic analysis (PMNL-diapedesis) for edema: Group D 4. Coagulation parameters: FIB : Group D Factor-V : — AT-III : — D-dimers: Group D
Pape et al. [54]	18 sheep Group A: 6 (LC + RFN)* Group B: 6 (LC + RIA)* Group C: 6 (LC + UFN)*	To investigate if pulmonary embolization can be minimized by use of a reamer system that provides simultaneous irrigation and aspiration of intramedullary contents.	In the presence of a unilateral pulmonary injury, the systemic effects of intramedullary reaming of an intact femur can be minimized with use of a modified reamer design that simultaneously irrigates the canal and removes debris	1. D-dimers: Group A 2. PMNL activity: Group A 3. Lung F. (PCP): Group A 4. Histologic evaluation (edema) : Group A
White et al. [68]	21 sheep Group A: 7 (control group) Group B: 7 (bilateral # of femurs and tibias) Group C: 7 (bilateral # of femurs and tibias + RN)*	To investigate the (1) hemodynamic; (2) embolic; and (3) coagulation responses to high energy fracture of femur and tibia; to determine how this response was modified by intramedullary reaming and nailing.	High-energy fractures and soft tissue injuries resulted in a significant tropotropic (depressor) cardiovascular response, transcardiac embolism of medullar contents and activation of the coagulation system; subsequent stabilization of the fractures using IMN did not significantly exacerbate any of these responses	1. Central venous pressure: — 2. PAP: — 3. Cardiac output: — 4. Venous oxygen saturation: — 5. Fat embolism (embolic scores and lung histologic analysis): — 6. Coagulation response: —
Giannoudis et al. [23]	30 sheep Group A1: 5 (sham thoracotomy + RFN) Group A2: 5 (sham thoracotomy + UFN) Group B1: 5 (LC + RFN)* Group B2: 5 (LC + UFN)* Group C1: 5 (LC and HR + RFN)* Group C2: 5 (LC and HR + UFN)*	To investigate whether coagulopathy worsened during femoral IMN in the presence of LC and HR and whether reamed or unreamed nailing influenced these results	The magnitude of the injury appears equally important as the type of subsequent surgery and should be considered in planning for fracture fixation in patients at high risk for complications	1. AT-III: Groups B1-B2-C1-C2 2. Factor V: Groups B1-B2-C1-C2 3. Fibrinogen: Groups C1-C2

Table 2. continued

Study	Animal species/numbers	Aim of study	Results/conclusion	Measured second hit postoperatively [†]
Gray et al. [24]	24 sheep Group A: 6 (control group) Group B: 6 (bilateral # of femurs – trauma only) Group C: 6 (bilateral # of femurs + Ex-Fix) [*] Group D: 6 (bilateral # of femurs + RFN) [*]	To compare the physiologic effects of external femoral fixation with those of intramedullary stabilization during the first 24 hours after femoral fracture	A higher pulmonary embolic load can be expected during early intramedullary femoral fracture stabilization compared with primary external fixation; however, the degree of stimulation to systemic coagulation and pulmonary inflammation by each type of surgery is comparable	1. Pulmonary embolic load: Group D 2. Metabolic base excess: — 3. Plasma coagulation markers: Groups B, C, D 4. Polymorphonuclear cell counts: — (bronchoalveolar lavage samples)

^{*} Indicates the two hit groups as defined by the criteria of this review article; [†] next to the examined variables are the groups in which a measurable second hit was recorded; — indicates no second hit was measured in any group of the study; # = fracture; ARDS = adult respiratory distress; AT-III = antithrombin III; BALF = bronchoalveolar fluid; BP = blood pressure; Cdyn = dynamic compliance; CVP = central venous pressure; DS = drive speed; Ex-Fix = external fixation; FE = fat embolism; FIB = fibrinogen; HR = hemorrhage and resuscitation; ICP = intracranial pressure; IMN = intramedullary nailing; IMP = intramedullary pressure; Kf = filtration coefficient; LC = lung contusion; N/A = not available; PAP = pulmonary arterial pressure; PCP = pulmonary capillary pressure; PK = perila ketone; PMNL = polymorphonuclear leukocytes; QI = lymph flow; RFN = reamed femoral nailing; RIA = reaming-irrigation-aspiration; RN = reamed nailing; TRG = triglyceride; U/P = urea/protein; UFN = unreamed femoral nailing; 11-dhTXB2 = 11-dehydro-thromboxane B2.

some studies that compared lung injury groups with control subjects, postsecond-hit impaired lung function was detected only in cases in which the physiopathologic reaction had been activated by a first-hit pulmonary trauma [26, 50, 51]. Nonetheless, other studies showed that the fat embolization that occurs during RFN in appropriately resuscitated animals has no clinically significant effect on pulmonary function, even in the setting of a severe pulmonary dysfunction [20, 70]. First-hit head injury was used in only one study [35] of those that fulfilled our inclusion criteria. In this study, there was strong correlation between head trauma as first-hit and postsurgery brain edema, which is indicative of the influence of preexisting head injury in the postsurgery inflammatory processes.

There is a relative consistency on the existing animal models of second-hit phenomena as to the animal species [20, 23, 24, 26, 35, 42, 43, 45, 48–52, 54, 68, 70, 71], whereas more variance was recorded for the assessed variables (Tables 1, 2).

Discussion

Trauma and subsequent interventions initiate a cascade of pathophysiologic events in the affected individual. Several in vivo studies have investigated the posttraumatic sequel by examining a large number of variables related to coagulative, pulmonary, and hemodynamic function. To date, most studies have used animal models to obtain information under well-defined conditions that mimic specific clinical scenarios. Nonetheless, their results occasionally have been controversial and the absence of a summation of the existing evidence led us to conduct this review of the literature. We aimed to provide conclusive answers to specific questions related to the influence of surgery, and specifically intramedullary nailing, on coagulation, pulmonary, and hemodynamic parameters and to the potential special role that head and thoracic injuries may pose.

We perceived several weaknesses in this systematic review. The existing evidence consists of numerous studies that differ in species, presence or absence of associated injuries, type of surgical impact, systemic and pulmonary parameters, and the grading of damage. The nature of the collected information and existing discrepancies (Table 3) does not allow a comparative statistical analysis of the data. Thus, our review can offer only descriptive information. Additionally, only short-term (between 2 and 7 hours after the second hit) postoperative physiologic disturbances were monitored. Current studies do not provide definitive answers regarding potential long-term reactions, like late ARDS. Another shortcoming of the existing evidence is the lack of data related to the role of head trauma

Table 3. Deficits of the review resulting from study characteristics

Deficit	Reason
Lack of total homogeneity of examined species	Sheep model used in 17 studies [20, 23, 24, 26, 35, 42, 43, 45, 48–52, 54, 68, 70, 71], dog and rat models used in three studies [39, 60, 69]
No adequate examination of proinflammatory agents	Proinflammatory cytokines (interleukin-6 and tumor necrosis factor- α) were not assessed in any animal study examining second-hit nailing
Lack of realistic polytrauma scenarios	In the studies that used high-energy long bone fractures as a first hit [24, 39, 43, 68, 69], this was not combined with lung contusion or hemorrhage
Lack of head trauma first hit scenarios	Only one study [35] examining the effects of head trauma in relation to early fixation was found
Lack of comparison between early and late fixation groups	Although many of the relevant clinical studies focus on the timing of fixation by separating patients into early and late fixation groups, this was not translated to relevant animal studies
Lack of homogeneity in the intervals between first and second hits	The intervals between the first and second hits were not always comparable in studies examining the same variables after similar first-hit implementation
Short observation periods	In half of the studies, the observation period after the surgical second hit did not exceed 4 hours; in only three studies, the observation periods expanded to 24 hours or more [24, 60, 71]

as a first hit [35], which is a major component of the polytrauma spectrum.

Activation of coagulation has been shown to occur after bone injury in animals [25, 48] and in human patients [58]. It also has been proposed that the magnitude of this effect is dependent on the degree of the injury sustained [23, 33]. Second-hit reamed nailing negatively influences the coagulative system by reducing FIB, AT-III, and factor V and increases D-dimer levels, whereas unreamed nailing causes little to no effect [23, 24, 26, 48]. Hemodynamic variables were altered minimally and transiently by the second hit of reamed or unreamed nailing [20, 68, 70, 71]. The reported cause-and-effect relationship between fat embolism resulting from nailing and the development of lung injury [26, 48, 50–52] has been questioned in numerous animal [20, 45] and clinical [8, 11, 66] studies. It is an issue requiring additional investigation with either standard reaming techniques or the latest advances of the RIA system [54].

Chest trauma, the most frequent injury in polytraumatized patients with an incidence of approximately 80% [27, 46], has been evaluated extensively in clinical [5, 7, 8, 15, 47] and in vivo models in the form of controlled lung contusion [20, 23, 26, 48–52, 54]. Investigators have examined the relationship of ARDS with femoral fracture stabilization and concluded the injury to the chest cavity was the primary predictor of the development of ARDS and not the timing of femoral fracture stabilization [6, 12]. Research has shown the ability and importance of the central nervous system to orchestrate diverse systemic inflammatory response mechanisms [55, 59, 64]. Neuro-hormonal signaling to the corresponding receptors of immune cells has been suggested to suppress cytokine production and thus impair a competent immune regulatory cell–cell interaction [14, 38]. Head trauma has not been studied adequately in the in vivo setting of second-hit

trauma research [35]. There is related evidence that intramedullary nailing (second hit) increases intracranial pressure only in the presence of brain edema (first hit) [35, 43].

In the majority of the reviewed studies [20, 23, 24, 26, 35, 42, 43, 45, 48–52, 54, 68, 70, 71], sheep were selected as the most appropriate animal species. However, if relevance to the clinical setting is the target [17, 32], baboons seem to be more advantageous because they are similar to humans in nearly all physiologic and immunologic aspects [32] and can be monitored using human analytical procedures [36, 57]. They are defective, however, to sheep in terms of costs and availability [19], and perhaps this explains the lack of baboon models in the studies retrieved for this review.

Concerning the first hit, its accuracy and reproducibility are critical because it can influence the immune response of the subject to the subsequent second-hit phenomena [9, 10, 72]. The use of specialized first hits, like hemorrhagic shock by venesection [16, 23, 24, 35, 42, 43, 48–52, 71] or lung contusion [20, 23, 26, 48–52, 54], which we repeatedly found during this review, although easily controllable, include physiologic limitations and do not totally reflect the clinical analog of the incidence of polytrauma. Hemorrhage, in the absence of injury, has been shown to result in a markedly different and less severe physiologic response [37, 56]. Moreover, skin is an important reservoir of preformed cytokines and the soft tissue component of the injury produces an important stress response, which may substantially influence outcome [33, 34]. The inductions of blunt trauma in anesthetized animals, taking into account that femoral fractures and thoracic or head injury should be included in the spectrum of sustained injuries, would provide beneficial realistic scenarios. Such violent clinically alike models should be supported by renowned funding bodies and research committees for the potential

investigators to avoid facing problems obtaining permission from local ethical committees. Similar animal models have been used successfully in previous studies [39, 43, 45, 60, 68, 69] exploiting existing instrumentation for the creation of the injuries. Concerning the selection of the second hit, in all the reviewed studies use of femoral nailing [20, 23, 24, 26, 35, 39, 42, 43, 45, 48–52, 54, 60, 68–71] reflects its feasibility in animal models. Femoral nailing induces marrow intravasation, which predisposes to more evident and detectable second-hit phenomena than any other type of surgical intervention. It is interesting that by reviewing the literature, we found only one study that examined the trauma surgery two-hit phenomenon that did not use femoral nailing as a second hit [16].

The focused selection of the examined variables adds to effectiveness and clarity of measured outcomes. Among the variety of variables examined in the studies of this review, inflammatory cytokines and new coagulative factors have a significant role to play in the description of the trauma surgery sequel. Proinflammatory and antiinflammatory agents (IL-1b, IL-6, TNF- α) frequently are examined in the clinical setting [1–3] because their early posttraumatic release contributes to the development of multiple organ distress syndrome (MODS) and ARDS [22, 30, 62]. Nonetheless, this review revealed their absence from experimental studies examining nailing as a second hit. Regarding the coagulation parameters, future investigations should include D-dimers [26, 54]. The serum concentrations of these breakdown products of fibrin are believed to be sensitive indicators of fibrinolytic cascade function [28] and of the severity of osseous and soft tissue injury [18]. Their high clinical prognostic value was confirmed by this review because it has been proved that the effect of reamed nailing during a lung contusion state could be precisely monitored postoperatively by D-dimer levels [26, 54]. The prognostic value of this marker also has been used to detect patients at high risk for postoperative complications such as ARDS and MODS after femoral nailing [28, 58].

The concept of analyzing the second hit phenomenon specializing in the trauma surgery setting allowed us to reach the following conclusions. Second hit RFN produces consumption of coagulation factors without affecting the hemodynamic parameters. The large embolic loads resulting from intramedullary nailing produce an insignificant effect on systemic coagulation and pulmonary inflammation. Lung and head injuries may predispose to an aggravated second hit and require additional experimental investigation.

This review has led us to the following suggestions for future studies. Blunt trauma (including chest, head injury, and femoral fractures) as a first hit and intramedullary nailing (RFN or RIA) as a second hit to a primate

anthropoid (eg, baboons) is probably the optimal in vivo model to approximate clinical reality. Different end points (eg, 6, 12, or 24 hours) between trauma and surgery should be used in order for the timing and sequence of events to reflect the clinical reality and not to mislead a study towards the best or worse case scenario [23]. Detection of specific inflammatory (IL-6, TNF- α) and coagulative (D-dimers) factors could clarify the etiology of ARDS and organ failure and identify early subjects likely to experience considerable postoperative respiratory complications. Observation of the postsurgery events in animal models should be expanded to the limits of the survivorship of the species so that late complications also can be recorded.

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